31

WBC

WORLD BUIATRICS CONGRESS

SEPTEMBER 4TH TO 8TH

MADRID 2022

www.wbc-madrid2022.com

ABSTRACT BOOK



31st World Buiatrics Congress

September 4th to 8th, 2022

Madrid, Spain

Organizers:

The National Association of Spanish Specialists in Bovine Medicine (ANEMBE)

The World Association for Buiatrics (WAB)

WBC 2022 Congress Technical Secretariat:

GRUPOPACIFICO
The power of meeting

Marià Cubí, 4, 08006,
Barcelona, Spain.
Email: wbcmadrid2022@pacifico-meetings.com

LIST OF THE BIENNIAL "WORLD BUIATRICS CONGRESSES"

	Year	Venue		Year	Venue
1	1960	Hannover (Germany)	17	1992	St Paul (USA)
2	1962	Vienna (Austria)	18	1994	Bologna (Italy)
3	1964	Copenhagen (Denmark)	19	1996	Edinburgh (Scotland)
4	1966	Zurich (Switzerland)	20	1998	Sydney (Australia)
5	1968	Opatija (Yugoslavia)	21	2000	Punta del Este (Uruguay)
6	1970	London (UK)	22	2002	Hannover (Germany)
7	1972	Philadelphia (USA)	23	2004	Quebec (Canada)
8	1974	Milan (Italy)	24	2006	Nice (France)
9	1976	Nice (France)	25	2008	Budapest (Hungary)
10	1978	Mexico City (Mexico)	26	2010	Santiago (Chile)
11	1980	Tel Aviv (Israel)	27	2012	Lisbon (Portugal)
12	1982	Amsterdam (The Netherlands)	28	2014	Cairns (Australia)
13	1984	Durban (South Africa)	29	2016	Dublin (Ireland)
14	1986	Dublin (Ireland)	30	2018	Sapporo (Japan)
15	1988	Palma de Mallorca (Spain)	**31**	**2022**	**Madrid (Spain)**
16	1990	San Salvador da Bahia (Brazil)			

WORLD ASSOCIATION FOR BUIATRICS

President
Emile Bouchard, Canada

Vice-presidents
Luis Poo, Chile
Motoshi Tajima, Japan

President Elect
Arcangelo Gentile, Italy

Secretary General
Gatz Riddell, JR., USA

Treasurer
David H. Black, UK

Honorary President
H.J. Breukink, The Netherlands

Honorary Secretary General
M. Stöber, Germany
P. Lekeux, Belgium

Honorary ExCo Members
R.G. Eddy, UK
K. Hamana, Japan
H.R. Han, Korea
W. Hofmann, Germany
A.D. Weaver, UK

Committee Members
Saïd Alali, Morocco
Ricardo Spacagna Jordao, Brazil
Francois Schelcher, France
Octavio Campuzano, Mexico
Juan V. Gonzalez-Martin, Spain
Przemyslaw Sobiech, Poland
Bo Han, China
Peter Heimberg, Germany
Norman Bruce Williamson, New Zealand
Thomas Wittek, Austria
Inhyung Lee, South Korea

WBC 2022 ORGANIZING COMMITTEE

Ángel Ávila Coya
Juan Manuel Cainzos Cagiao
Fernando Criado García
Gumersindo de la Riera Díaz
Jorge Eseverri Azcoiti
Manuel Ángel Fernández Sánchez
Juan Carlos González Fernández
Francisco Jose González Rodríguez
Juan Carlos Marco Melero
Joaquin Ranz Vallejo
Marco Aurelio Sánchez Moreiro

WBC 2022 SCIENTIFIC COMMITTEE

President: Gumersindo de la Riera
Honorific President: Alfonso Monge

Ángel Abuelo
Saïd Alali
Marta Alonso
Julio Álvarez
Miguel Ángel Aparicio Tobar
Rob Atwill
Ángel Ávila Coya
Mohamed Aziz Darghouth
Mário Balaro
Jesse Barandika
Pietro Baruselli
Joaquim Baucells
Lindert Benedictus
David Black
Javier Blanco Murcia
Gabriel Bo
Calvin W. Booker
Émile Bouchard
Sebastian Buczinski
Juan Manuel Cainzos
Luis Octavio Campuzano
Antonio Cantalapiedra
Jude L. Capper
Octavio Catalán
Ricardo Chebel
Peter-Henning Clausen
Jill Colloton
Fernando Criado García
Gumersindo de la Riera
André Desrochers
Albert DeVries
Trevor DeVries
Fernando Diaz Royón
Ronald Erskine
Jorge Eseverri
Manuel Ángel Fernández
Klibs Galvão
Álvaro García Guerra
Joseba Garrido
Tom Geary
Arcangelo Gentile
Giovanni Gnemmi
Arturo Gómez
Adrián González
Juan Carlos González
Francisco González Rodríguez
Marcello Guadagnini
María Guelbenzu-Gonzalo
Juan Pablo Gutiérrez
Bo Han
Peter Heimberg
Luis Miguel Jiménez Galán
Vicente Jimeno
José Luis Juaristi
Delia Lacasta Lozano
Alfonso Lago
Ryan Law
Inhyung Lee
Luis León
Jaime Llena
Fiona Lovatt
José Mallo
Xavier Manteca Vilanova
Juan Carlos Marco Melero
Daniel Martínez Bello
Alfonso Monge
Antonio Natale
Ian Ohnstad
Georgios Oikonomou
Luís Miguel Ortega
Simon Peek
Francisco Peñagaricano
Ana Pico
Luis Poo Kutscher
Luis Ángel Quintela
Joaquín Ranz Vallejo
Michela Re
Gatz Riddell
Carlos Risco
Juan Miguel Rodríguez
Allen Roussell
Jonathan Rushton
Marco Aurelio Sánchez
José Eduardo Santos
Ynte Schukken
Adlai Schuler
Geof Smith
Przemyslaw Sobiech
Odon Sobrino Abuja
Ricardo Spacagna Jordão
Motoshi Tajima
Aurora Villarroel
Robert Wijma
Norman Williamson
Peter Windsor
Ruth Zadoks
Daniel Zeledón

WBC 2022 SPONSORS

The WBC 2022 organizing Committee wishes to thank our sponsors for the support

Premium Partners

Boehringer Ingelheim

Ceva

Elanco

HIPRA

MSD Animal Health

Zoetis

Sponsor Major

Virbac

Sponsor Partners

Biogénesis Bagó

CALIER

FATRO

HUVEPHARMA

LIVISTO

syva

vetoquinol

ACHIEVE MORE TOGETHER

Exhibitors


ALIVIRA


BOLUS es
for farm animals


COLEGIO OFICIAL
DE VETERINARIOS
DE MADRID


DRAMIŃSKI
ultrasound scanners


ECBHM
European College of Bovine Health Management


exopol


fera
Original thinking... applied


GD
AHEAD IN ANIMAL HEALTH


GRUPO
asis


HORIBA


Humeco
Productos veterinarios


I.B Co., Ltd.


imv
imaging


MAYMO


nimrod


MULTIMIN
Solution for Injection for Cattle.


Norbrook


NOREL
ANIMAL NUTRITION


ProVet Scan
by New VeTec


Idee
Clevere Produkte
für erfolgreiche Tierhalter und Tierärzte


RANDOX
FOOD DIAGNOSTICS


smaXtec


s.p.®
s.p.veterinaria


TechMix
REDEFINING HYDRATION


uniform
agri

INDEX

VOLUME 1

KEYNOTE LECTURES 15

VIP PRESENTATIONS 17

K01 – K04 Animal Health 20
K05 – K08 Internal Medicine & Toxicology 21
K09 – K12 Immunology and Vaccines 24
K17 – K20 Buffaloes and Camelids 28
K21 – K24 Small Ruminants 31
K27 – K29 Reproduction in Dairy Cattle 33
K30 – K33 Udder Health and Mastitis 34
K38 – K41 Lameness 36
K42 – K45 Genetics and Breeding 39
K47 – K49 Teaching & Continuing Education 41
K51 – K53 Nutrition & Metabolism Diseases 42
K55 – K58 Infectious Diseases 43
K59 – K62 Cattle Welfare 46
K65 Feedlot 48
K67 – K70 Diagnostic Imaging 49
K71 – K74 Sustainable Cattle Productive Systems 51
K76 – K79 Surgery 53
K80 – K83 Biotechnology 56
K84 – K85 Pharmacology & Therapeutics 59
K87 – K90 Epidemiology 61
K91 – K93 Public Health, Food Security and Antimicrobial Resistance 64
K94 – K97 Parasitism 66

WORKSHOPS 71

W01 – W03 ECBHM WORKSHOP 73
W04 REPRODUCTION IN BEEF CATTLE WORKSHOP 74
W05 – W07 REPRODUCTION IN DAIRY CATTLE 75
W08 REPRODUCTION WORKSHOP 76
W09 GENETICS AND BREEDING WORKSHOP 77
W10 CONTINUING EDUCATION WORKSHOP 77
W11 – W12 UDDER HEALTH WORKSHOP (IN FARM) 78
W14 CATTLE WELFARE WORKSHOP 79
W15 DIAGNOSTIC IMAGING WORKSHOP 80
W16 – W18 NUTRITION AND BIOTECHNOLOGY WORKSHOP 81
W19 – W21 BIOTECHNOLOGY WORKSHOP 83
W22 EPIDEMIOLOGY WORKSHOP 85
W23 SURGERY WORKSHOP 86
W24 – W27 APPLICATION OF SYSTEMS THINKING DISCIPLINE IN BEEF CATTLE VETERINARY MEDICINE 87

PROTECTION WITH A FAIRY TALE ENDING



CALF SCOUR VACCINATION AND CALF EXCELLENCE DELIVERED TOGETHER

Fencovis is the most recently validated vaccine that provides the optimal combination of oil-free adjuvant and killed antigens against the commonest calf scour pathogens. Unlike previously available calf scour vaccines, Fencovis prevents diarrhoea caused by rotavirus and *E. coli F5* while also reducing the incidence and severity of diarrhoea caused by coronavirus.

Scan the QR code to see the product information

FENCOVIS®

ADVANCED PROTECTION AGAINST CALF SCOUR

FENCOVIS ABBREVIATED PRESCRIBING INFORMATION

Fencovis suspension for injection. Composition: Each 2 ml contains: inactivated *E. coli* with expression of adhesin F5 (K99), strain O9: K35 PR≥1. Inactivated bovine rotavirus, G6P1 serotype, tm-91 PR≥1 strain. Inactivated bovine coronavirus, strain C.197 PR≥1. Target species: Cattle (heifers and pregnant cows). Indications: Active immunisation of heifers and pregnant cows to stimulate the development of antibodies against bovine rotavirus, bovine coronavirus and *E. coli* with expression of adhesin F5 (K99) and to increase the level of passive immunity of calves against neonatal diarrhea caused by bovine rotavirus, bovine coronavirus and *E. coli* with adhesin expression F5 (K99). Contraindications: None. Use during pregnancy and lactation: It can be used during pregnancy. The effect of vaccination on lactation before or after parturition has not been studied. Adverse reactions: average body temperature increase of 1.0°C was observed very frequently in laboratory and field studies; in individual cases, the maximum increase could reach 2.1°C. Mild localised inflammation at the injection site, which resolves within two days, was frequently observed in field studies. Dosage: Route of administration: intramuscular. Dose: 2 ml during each pregnancy between 12 and 3 weeks before the expected time of delivery. Waiting times: Zero days. Conservation: Store and transport refrigerated (between 2°C and 8°C). Once opened, the vials should not be stored at more than 25°C. Do not freeze. Protect from light. Authorisation no.: 4097 ESP. Presentation: Plastic box with 2, 10 or 20 glass vials of 1 dose (2 ml). Cardboard box with 1 glass or plastic vial of 5 doses (10 ml). Plastic box with 5 or 10 glass or plastic vials of 5 doses (10 ml). Cardboard box with 1, 12 or 24 glass or plastic vials of 25 doses (50 ml). Cardboard box with 1 glass or plastic vial of 50 doses (100 ml). Owner: Boehringer Ingelheim Vetmedica GmBH 55216 Ingelheim/Rheim GERMANY. Drug subject to veterinary prescription. Administration under the control or supervision of the veterinarian. In case of doubt consult your veterinarian.

Date of preparation: August 2022.

#CattleFirst

Boehringer Ingelheim

ORAL PRESENTATIONS 91
Animal Health **(AH)** 93
Buffaloes and Camelids **(BC)** 116
Biotechnology **(BT)** 122
Cattle Welfare **(CW)** 125
Diagnostic Imaging **(DI)** 145
Epidemiology **(EP)** 153
Feedlot **(FE)** 158
Genetics and Breeding **(GB)** 164
Herd Health Management, Smart Farming and Economics **(HH)** 177
Infectious Diseases **(ID)** 212
Internal Medicine and Toxicology **(IM)** 255
Immunology and Vaccinology **(IV)** 267
Lameness **(LA)** 290
Nutrition and Metabolic Diseases **(NU)** 307
Parasitism **(PA)** 325
Public Health, Food Safety and Antimicrobial Resistance **(PH)** 336
Pharmacology and Therapeutics **(PT)** 351
Reproduction in Beef Cattle **(RB)** 354
Reproduction in Dairy Cattle **(RD)** 359
Sustainable Cattle Productive Systems **(SP)** 377
Small Ruminants **(SR)** 383
Surgery **(SU)** 403
Teaching and Continuing Education **(TE)** 406
Udder Health and Mastitis **(UH)** 412

WE SEE





WHAT YOU SEE

Dedicated to meeting your needs.

KEYNOTE LECTURES

VIP PRESENTATIONS

KEYNOTE LECTURES

K01 - K04	Animal Health
K05 - K08	Internal Medicine & Toxicology
K09 - K12	Immunology and Vaccines
K17 - K20	Buffaloes and Camelids
K21 - K24	Small Ruminants
K27 - K29	Reproduction in Dairy Cattle
K30 - K33	Udder Health and Mastitis
K38 - K41	Lameness
K42 - K45	Genetics and Breeding
K47 - K49	Teaching & Continuing Education
K51 - K53	Nutrition & Metabolism Diseases
K55 - K58	Infectious Diseases
K59 - K62	Cattle Welfare
K65	Feedlot
K67 - K70	Diagnostic Imaging
K71 - K74	Sustainable Cattle Productive Systems
K76 - K79	Surgery
K80 - K83	Biotechnology
K84 - K85	Pharmacology & Therapeutics
K87 - K90	Epidemiology
K91 - K93	Public Health, Food Security and Antimicrobial Resistance
K94 - K97	Parasitism



UNA VIDA MEJOR PARA ELLOS, UNA VIDA MEJOR PARA TODOS



En Elanco estamos comprometidos a crear una vida mejor para los animales.

Creemos que unos animales más sanos son la pieza fundamental para abordar asuntos globales como la seguridad alimentaria y la nutrición, salud, bienestar de los seres humanos y la sostenibilidad medioambiental.

Combinamos nuestra visión del mundo orientada a los objetivos con décadas de innovación científica para mejorar la salud de los animales, las personas y el planeta.

¡Fórmate e infórmate!

Descubre las iniciativas que hemos preparado para profesionales de salud animal como tú.

Elanco

Conoce más sobre nosotros, nuestros productos y servicios, nuestro compromiso

#ElancoHealthyPurpose

y mucho más.

Más información:

VetConecta
conectar para crecer

Espacio creado por y para los profesionales que amamos los animales y cuidamos de su salud y bienestar.

Descubre materiales, formaciones, noticias y soluciones para tu práctica diaria:

Más información:

THE VITAL 90 DAYS

¿Cómo hacer frente a los retos y oportunidades que surgen a los 90 días en torno al parto de la vaca lechera?

Encuentra información así como recursos de apoyo y una área técnica exclusiva para veterinarios en nuestra plataforma para el vacuno de leche que incluye personas, productos, servicios, herramientas de diagnóstico y análisis de datos.

Más información:

SUMANDO JUNTOS
Una iniciativa de Elanco

Estamos a tu lado defendiendo los beneficios que supone en las personas tener un acceso adecuado a proteína de alto valor biológico y facilitar su acceso a personas en situación de necesidad.

Un futuro mejor se consigue juntos. ¡Te invitamos a formar parte!

Más información:

¡Síguenos! **@SumandoJuntosElanco**

©Elanco PM-ES-22-0390

Monday, September 5th, 2022

Men-animals relations. Historic aspects and evolution

Bernard Vallat.

FICT, Paris, France.

The benefits of animal domestication for humanity evolution is widely recognized

Domestication has been based on commitment from animal owners to protect animals from predators, to provide conditions for appropriate feeding, disease prevention and treatment and housing when necessary . Men owners always exerted rights on their domestic animals. Indeed, domestication led to genetic selection linked with the needs of human communities: mainly work, protection and war, animal origin food, cultural events, soil fertilization.

A majority of domestic animal cannot survive without owners protection and care.

Many voices at global level are advocating for an evolution of the historic deal between men and domestic animal and some so called “antispecists”are proposing to ban the concept of man superiority and even the right to have domestic animals for any purpose.

In addition to philosophical controversies, antispecists are using other arguments linked with health and environment based on scientific publications often supported by activist NGOs. In front of these controversies the answer must be science based, using sources with full independence.

However, it is always recommended to recall the strong benefits of domestic animal for mankind.

- Food and high-quality nutrition.
- Soil fertilization versus chemical products.
- Cultural and sport key partners.
- Providers of leather, wool and skin clothes.
- Companions of billions of families.
- Key factor of poverty reduction in developing countries.
- Unavoidable element for scientific research for human and animal health.
- High genetic diversity.

Tuesday, September 6th, 2022

Why do many veterinarians no longer use the clinical examination of ruminants on a regular basis?

Walter Baumgartner.

University of Veterinary Medicine, Vienna, Austria.

Objectives: Clinical propaedeutics provides the preliminary knowledge for diagnosing diseases, their therapy and prophylaxis. In the clinic, the diseases are discussed and the aetiology (cause of the disease), pathogenesis (development of the disease) or pathophysiology, specific symptoms, diagnosis and differential diagnosis, prophylaxis and therapy are dealt with. This then results in the prognosis (prediction), which is important for the animal owner, i.e. the veterinarian‘s statement about the further course of the disease (duration, outcome, treatment costs).

Material and methods: Diseases in animals and humans are recognised due to particular changes in the state of life. These deviations from the physiological state are the disease manifestations or symptoms.

A distinction is made between 1. subjective symptoms, which the patient feels and reports to the doctor and 2. objective symptoms („signs“), which the doctor detects without the patient influencing them. Since animals usually lack the ability to communicate subjective sensations, the veterinarian has to deal mainly with symptoms that have to be objectively recorded.

Most misdiagnoses are not so much due to technical inability, lack of experience or wrong thinking, but simply due to forgetting to exhaust a number of diagnostic possibilities.

There are existing different types of diagnosis:

1. Aetiological diagnosis: not only an organ localisation, but also the cause and type of disease (e.g. inflammation, degeneration) could be established without difficulty.
2. Provisional diagnosis (tentative diagnosis): The disease cannot be identified with certainty.
3. Functional diagnosis: A dysfunction of one or more organs is established without proving the exact nature of the organ disease in question.
4. Organ diagnosis: Localisation of the disease in a specific organ.
5. Symptomatic diagnosis: Cause and affected organ could not be established, therefore only a specific, concise symptom is cited as diagnosis.

There are two principles regarding the type of examination:

Examination by organ system (gastrointestinal tract, respiratory tract, circulatory and lymphatic systems, etc.) and topographical examination (includes all organs and organ systems in each region of the body).

General examination: This covers all organ systems and should always be carried out at least during the initial examination of a patient. In intensive animal husbandry, the basic examination procedure is modified and the points of anamnesis and environment are dealt with in particular detail (checklist).

Special examinations: If the general examination leads to the suspicion that a certain organ system is affected (e.g. skin, sexual apparatus or nervous system), this organ system is examined in more detail in a special examination. The special examinations supplement the simple examinations with special diagnostic procedures that are usually technically complex and are only used in certain cases (e.g. urinalysis, blood tests, diagnostic imaging procedures, ECG).

Results and Conclusions: The importance of clinical examination of cattle directly in the barn during the first visit of a sick animal to determine the disease is shown with the help of some specific examples (including use of percussion to differentiate respiratory diseases; differentiation of all different forms of mastitis; differentiation between primary and secondary enteritis in calves, etc.).

Once the prognosis has been established, a targeted therapy (e.g. much less use of antibiotics and other drugs; more sustainable and cheaper way of treatment) can be carried out. The successful therapy process thus achieved cannot be expected from experts via telephone without a clinical examination (unfortunately, nowadays one finds widespread telephone service in many countries of the world).

References:

BAUMGARTNER, W., WITTEK, T. (2018): Klinische Propädeutik der Haus- und Heimtiere. 9. Aufl., Enke, Stuttgart.

BAUMGARTNER, W, WITTEK, T. (2018): Diagnostyka kliniczna zwierzat. 9th ed., Edra Urban & Partner, Wroclaw.

Controlling epidemic bovine diseases in the last decades: What can we learn from it?

Klaus Doll.

Clinic for Ruminants, University of Giessen, Germany.

Objectives: The main reason for the foundation of veterinary schools in the middle of the 18th century was, besides the need of veterinarians for the horse stocks of the armies, the control of the then raging infectious animal diseases. First and foremost, rinderpest threatened the economies of Europe. In many areas, the disease destroyed up to 75% of the cattle population. The Netherlands even lost almost its entire cattle population to the disease between 1711 and 1717. As a fierce and rapidly spreading disease, rinderpest usually occurred in the wake of large campaigns, as at that time the armies often carried large herds of oxen as living provisions. Nowadays, international trade in animals and animal products, the spread of vectors with other traded goods, and due to climate change, and international travel, threaten cattle populations. The aim of this paper is to show, with some examples, the measures taken in recent years in countries with intensive milk production to control or eradicate the main epidemic infectious diseases in cattle.

Materials and methods: The main focus is on the control of tuberculosis, foot-and-mouth disease, bovine viral diarrhea/mucosal disease, paratuberculosis and, as an example of an arthropod-borne viral disease, bluetongue, which was introduced into northwestern Europe in 2006. The influence of modern infection epidemiology, new diagnostic methods and new vaccines on the control of such infectious diseases is described, as well as the influence of non-medically indicated, but rather eco- and trade policy-driven choices of control measures. The causes of failures or new outbreaks are also discussed.

Results: Due to their zoonotic and/or economic importance, the control of the most important infectious diseases in cattle is now regulated by the World Organisation for Animal Health (WOAH / OIE), the European Union animal health law and other supranational and national regulations. Irrespective of this, the following basic principles should always be observed with regard to the infectious diseases to be controlled and the choice of control strategy: Zoonotic and economic importance of the infection and its prevalence, characteristics of the pathogen, the severity and course of the diseases, its etiology and transmission routes, potential reservoir of the pathogen (examples: paratuberculosis, tuberculosis), involvement of vectors (example: bluetongue), presence of latently or persistently infected animals, predictive value of available test methods, availability and efficacy of vaccines, prospects of success of control or eradication programs and their acceptance by livestock owners, and their cost-effectiveness. This is because the attainability of a control target is often based on overly optimistic assumptions, and likewise, verification of the efficacy of such intervention strategies has been rare. In addition to the existing infection prevention measures, breeding for disease resistance is likely to become much more important in the future in connection with marker-assisted selection in the cattle sector as well.

Conclusions: As in the days of the first modern veterinary schools, the control of animal diseases will remain an important veterinary task in the future. For despite all the progress made, there are still numerous challenges in this field that have not yet been satisfactorily resolved, such as the control of bovine tuberculosis or paratuberculosis in some countries. In addition, there is always the risk of pathogen introduction (example: foot-and-mouth disease) into disease-free countries or the emergence of new diseases. However, the field of infectious disease epidemiology as well as clinical buiatrics is obviously neglected at some veterinary schools in the course of shifting the focus of education to companion animals. But farm animal medicine is more than laboratory diagnostics and microbiology, because especially in the early detection of a disease outbreak, the recognition of the first clinical symptoms is and remains of crucial importance.

Wednesday, September 7th, 2022

Successful dairy farming

Jack Britt.

Jack H Britt Consulting, Etowah NC, USA.

Demand for milk and dairy products will double in the next 50 years according to the United Nations. Increased populations in Africa and South Asia will increase demand for milk and dairy products in regions where milk yield per cow is among the lowest worldwide. Climate change will make it more challenging to meet this demand, and cattle that are more heat tolerant will increase in warmer regions. Milk yield per cow globally needs to increase from 3,000 kg in 2020 to 6,000 kg in 2072. Half of this increase will be associated with improved genetics of dairy cattle, particularly through application of genomic technologies across breeds. This will require better records of ancestry and greater use of artificial insemination with semen from superior bulls. More genetic emphasis will be on changing milk constituents rather than volume produced per cow. Increased milk output will require improved feeding and management and an increase in average herd size, because yield increases as cows move into larger herds, which typically provide better feed and management. Novel breeds and crossbred cows will become more productive, particularly in areas where increased temperatures will subject cows to more stress. Health of dairy cow will improve through development of better vaccines and novel health products that will replace antibiotics. There will more focus on understanding and managing epigenetics of dairy cattle, with particular focus on body condition scores and specific periods, including development in utero, the first 3 months after birth, during the onset of puberty and during first pregnancy. All of this will evolve into components of genetic evaluation systems. There will be more emphasis on managing microbiomes of dairy cows, particularly microbiomes of the rumen, digestive tract, udder, and uterus. For example, specific microbes may be injected, using ultrasound-guided technology, into the amnionic sac of the fetus to provide it with the desired microbiome before birth. Technologies developed for dairy cows will be applicable to dairy buffalo, goats, sheep, and other dairy mammals. A major challenge to improving milk production worldwide will be changing the culture of dairy farming in countries where output per cow is low. This will require improved genetics, improved feeding, improved healthcare, and often will require dairy farmers to pool milk cows into jointly owned herds to acquire and apply more advanced technologies. More details are available at Britt et al. 2018. J. Dairy Sci. 101 (5): 3722-3741 and Britt et al. 2021. Animal 15 (2) 100298.

Animal Health

K01

Getting the right balance-managing the animals "health maintenance system"

Christopher Chase.

Department of Veterinary and Biomedical Sciences, South Dakota State University Brookings SD USA

Homeostasis is an important function of the immune response to maintain health. Homeostasis- the animals "health maintenance system" is dependent on the integration and regulation of the mucosa and lamina propria of the respiratory and gastrointestinal tract (GIT) along with continuous interaction with the microbiome. The mucosa epithelium of respiratory and gastrointestinal (GI) system is the largest immune organ of the body provides the barrier, "the kill zone" that eliminates 99.9% of all infections. The kill zone integrates all the components of the immune system; 1) barrier components (mucous and mucins, tight junctions); 2) innate immunity (macrophages, defensins, neutrophils, interferon, cytokines and 3) adaptive immunity (secretory IgA and IgG, and T and B lymphocytes). This system is very susceptible to dehydration and changes in microbial populations. In addition, the mucosa epithelium along with the lamina propria is the immune "fire wall", the immune regulatory system that provides "homeostasis" mechanisms that balance the immune system to provide a stable healthy internal environment to minimize inflammation. Once the mucosa epithelium is breached, the innate system is the first to be activated and responds almost immediately. The adaptive response follows up 10-14 days later in naïve animals. The immune system is regulated to prevent an over response (too much of a good thing). The cumulative effect of these anti-inflammatory response is to regulate the immune system, maintain homeostasis and to direct the immune response away from the memory response to the short-term antibody immune response. At the same time, over expression of pro-inflammatory cytokines from infectious agents, feed intake issues (acidosis, ketosis) and stress can result in immune dysfunction and an over reactive immune system that can result in immunopathology and disease. ". The microbiome- the microorganisms in the respiratory and GIT, is essential for immune development, immune response, and maximizing ruminant productivity. Microbiome provides essential signals to enhance the kill zone and the anti-inflammatory state. Stressors along with the intake of feed and hydration affect the microbiome and the intestinal epithelial cells resulting in important immune interactions.

K04

Precision technology in dairy calves: new technology with a new direction

David Renaud.

University of Guelph, Ontario, Canada.

There has been a tremendous increase in the number of technologies that have become available to dairy producers over the last few decades. This has led to the adoption of pedometers, rumination collars, and in-line milk sensors in dairy cows; however, there has also been precision technologies that have been developed and integrated in calves. Automated milk feeders have increased substantially over the last few years, where in a survey of Canadian farms, the number of automated milk feeders went from none being reported in 2007 to more than 16% of farms using an automated milk feeder in a 2015 survey. The main reasons for this change, as highlighted by producers by the survey, were it helped raise better calves and offer more milk to calves while reducing labor. Beyond these reasons, automated milk feeders can aid in disease identification, both diarrhea and respiratory disease, through reductions in the level of daily milk consumption, drinking speed, and number of rewarded and unrewarded visits. In addition, declines in these behaviours can often be seen in the days prior to the development of disease which is spurring on new research evaluating non-antimicrobial interventions to prevent disease or reduce the duration of disease. In fact, a recent study has found that providing colostrum at an automated milk feeder alarm led to a reduction in the development of respiratory disease. Beyond automated milk feeding machines, tracking calf activity through pedometers to predict disease has become a possibility. Specifically, differences in activity levels have been found in several studies where a greater lying time, fewer lying bouts, fewer step counts, and a smaller acceleration activity index have been observed in calves with respiratory disease compared to healthy controls. Collectively, this research highlights that activity monitors could play an important role in aiding producers to detect disease earlier. Temperature monitoring devices have also been assessed to identify disease in calves; however, additional research is needed to further understand their ability in a commercial setting. Precision technologies have great potential to be applied in the management of dairy calves, especially in early disease detection, and further research is ongoing on how to use changes in behaviour for early interventions to mitigate or prevent disease.

Internal Medicine & Toxicology

K05

Jejunal Hemorrhage Syndrome

Simon Peek.

University of Wisconsin, USA.

Session Objectives: To briefly review the literature on the condition covering the last 30 years (1). Provide retrospective information on approximately 100 cases seen at the University of Wisconsin in the last 20 years to help guide clinical decision making (2). To present information regarding risk factors, herd management and prevention (3).

Introduction: Jejunal hemorrhage syndrome (JHS) is now a worldwide disease of predominantly dairy cattle, first documented in the US in 1992. It has since been identified in other parts of North America, Europe, Asia, and the Middle East. It is characterized by rapid, occasionally substantial, obstructive jejunal clot formation that causes affected individuals to become colicky. The condition can clinically mimic other causes of small bowel obstruction. Most affected individuals will pass blood clots in feces over the ensuing 12-24-hour period but significant bowel devitalization, necrosis and peritonitis may accompany intraluminal hemorrhage and obstruction.

Etiology: Much interest regarding etiology has centered on *Clostridium perfringens* type A, based upon studies identifying the organism in blood clots within the jejunum and feces of affected cattle. Additionally, pathologic investigations have histologically identified large numbers of gram positive rods adjacent to the typical areas of intestinal necrosis. However, attempts to fulfill any of Koch's postulates with isolates obtained from clinical cases, even in immunocompromised experimental animals, have failed and it is worth remembering that this organism is a commensal. There has also been interest in a possible role for the mold *Aspergillus fumigatus*, commonly present in livestock environments and feedstuffs. Similarly, no definitive role has been proven for this organism but mold inhibitors are frequently used as feed additives.

Clinical Signs: Affected cattle usually present with peracute colic ranging from moderate to severe. Two retrospective studies have identified that cows tend to be in the 3rd to 5th month of lactation. Rarely, bulls, dry cows and beef animals are affected. Fecal production and character can be informative and helpful in distinguishing JHS from other causes of peracute colic; initially cattle will have scant to absent manure production but over a few hours often develop "tarry" feces with fresher clots mixed-in. Continued, complete absence of fecal production is uncommon but most cattle become visibly distended. Dilated loops of small intestine are visible on ultrasound examination, sometimes with detectable echogenic material consistent with intraluminal clots. Detectable small bowel distension on rectal examination is an inconsistent finding.

Treatment: Although occasional success using purely medical treatment with flunixin, catharctic laxatives or lubricants (usually Epsom salts or mineral oil), *Clostridium perfringens* type C and D antitoxin and antibiotics (procaine penicillin or ceftiofur) is reported, our approach is to combine medical therapy with surgery. Via a right flank approach, we employ aborad manual massage of the identified clots as the preferred treatment. In over 100 cases we have an approximately 60% discharge rate from the hospital (combining surgery with catharctics, high dose pencillin, flunixin, and fluid therapy). Intraoperatively, if cattle have extensive devitalized intestine, if the clots cannot be broken up and dislodged, or reform almost immediately - these are poor prognostic signs. Most cattle that do well post-operatively produce feces with clots within 1-2 hours (sometimes much less!) of surgery, and importantly continue to do so over the following 12-24 hours. Importantly, there is a lifetime recurrence rate of up to 25% in cattle followed long term.

Prevention: Given the uncertainty over etiology and known risk factors, success with prevention and management continues to be frustrating. Surveys of affected herds in the US reveal that cattle tend to be in the first 5 months post-partum and the median parity is the third lactation. We have observed an increased prevalence in Brown Swiss. Problem farms experience quiet periods interspersed with "outbreaks" when clusters of new cases occur. Energy and protein rich diets that are frequently being adjusted alongside immune and other physiologic stresses associated with early lactation are the predictable scapegoats for these outbreaks. Vaccines against *Clostridium perfringens* type A, commercial or autologous, and mold inhibitors have been used extensively but no controlled studies exist examining their efficacy. However, transition and early lactation cows undoubtedly benefit from optimizing intestinal health as consistently as possible whether one is referring to JHS or other enteric/metabolic diseases.

References: Available on request.

K06

Recent advances in the treatment of calf diarrhea

Geof Smith.

North Carolina State University, Raleigh, USA.

Calf diarrhea remains the leading cause of mortality in both beef and dairy calves prior to weaning. Although farmers need to focus on prevention, some diarrhea is inevitable. Therefore, veterinarians need to be able to work with producers to recognize disease promptly and institute successful treatment programs. Goals of therapy in the diarrheic calf are to **1) restore dehydration; 2) correct acid-base abnormalities; 3) replenish electrolytes and 4) provide nutritional support to prevent starvation**. Key principles of therapy are as follows:

1. A metabolic (or strong ion) acidosis is common in calves with diarrhea- Research has shown that calves produce significant amounts of D-lactate in their gastrointestinal tract during diarrhea and lose large amounts of sodium. Together these produce a decrease in blood pH (acidosis) which is one of the most significant reasons for mortality in diarrheic calves. In contrast, children don't frequently develop acid-base disturbances with diarrhea necessitating different approaches to treatment between calves and humans. Fluid therapy is criti-

cal and the primary goal should be to rehydrate and prevent/correct acidemia.

2. Oral electrolyte therapy is the backbone of a treatment program- Significant advances in our understanding of oral electrolytes has occurred in the past 10 years. We know now that oral electrolytes designed to treat calves with diarrhea should look significantly different than those used in children. We have learned a lot about the importance of including an alkalinizing agent (such as bicarbonate or sodium acetate) and having a product with a proper strong ion difference (SID).

3. Intravenous fluid therapy can be done easily on farms- Even with the widespread use of quality oral electrolytes, some calves will still need intravenous fluids to survive. While this is traditionally difficult to accomplish on farms, the advent of hypertonic saline and hypertonic sodium bicarbonate has simplified fluid therapy protocols. Veterinarians and producers can effectively rehydrate calves and restore a normal blood pH without placing intravenous catheters and administering smaller volumes (200-400 ml) of fluids IV.

4. Nutrition is important- Many calves with diarrhea starve to death. While milk is expensive, multiple studies have shown that increased rates of milk feeding within the first week of life are critical to not only reduce the incidence of diarrhea but reduce the duration of diarrhea and improve survival rates. Farmers can improve the plane of nutrition their calves receive either by increasing the volume of milk calves receive or by improving the quality (protein) of milk replacer (or both). Not only will calves be in better body condition and better able to tolerate diarrhea, but they have a more robust immune response to the pathogen causing disease.

5. Antibiotics have been overused with calf diarrhea in the past- For years the dairy industry would feed antibiotics such as tetracycline and neomycin to treat and/or prevent diarrhea. Although there is little scientific evidence to support this practice, most countries now prohibit the routine feeding of antimicrobials to young calves in an attempt to practice judicious use of drugs and limit the emergence of resistant bacteria. It is also important to point out that rotavirus and *Cryptosporidium parvum* remain the leading causes of diarrhea around the world and are not susceptible to antimicrobials. Therefore, most calves with diarrhea don't need antibiotics to be treated successfully. However, a certain percentage of calves with diarrhea will develop septicemia (usually E. coli), which can be a significant cause of mortality. In conclusion, antimicrobials are indicated only in selected calves that indicate diarrhea plus other clinical signs suggestive of septicemia (for example fever, blood in the manure, severe depression, etc).

6. Manipulating the gut-brain axis- The next frontier in treating calf diarrhea will be effectively exploiting the gut-brain axis to develop therapeutic strategies for preventing and/or treating diarrhea. Although probiotic research to this point hasn't been extremely successful in treating diarrhea, studies with fecal microbiota transplantation or feeding rumen fluid to calves have shown promise in reducing the severity and duration of diarrhea. Moving forward, research to figure out how to effectively and economically manipulate the gut-brain axis or microbiome of calves to limit diarrhea will be critical. Or said another way – what can I feed a calf to prevent or treat diarrhea effectively.

K07

Flotation Tank Use in the Management of the Down Cow

Simon Peek.

University of Wisconsin, USA.

Session Objectives: To briefly review the causes of recumbency and indications for flotation tank use in dairy cattle, providing context for downer cow management on US dairies (1). Provide retrospective information on approximately 200 recumbent dairy cattle managed by flotation tank over the last 20 years at the University of Wisconsin in the last 20 years (2).

Introduction: Over roughly the last two decades, flotation tanks have become more widely available as a management tool for non-ambulatory cattle. Multiparous dairy cattle are the most common candidates for flotation, in whom the well-known peri and post-parturient metabolic conditions are the most significant causes of recumbency. Dystocia related injury to the pelvis, spine and lumbosacral spinal cord, especially in primaparous heifers, in addition to musculoskeletal injury in cattle of any age are also potential causes of initial recumbency that can result in an individual becoming non-ambulatory. Whereas flotation tanks were once the exclusive province of university teaching hospitals they are now widely available through private practices, and commercial businesses, especially in dairy dense areas of the US. Their appeal lies in the buoyant, evenly distributed support that water provides. Of direct relevance to the topic of non-ambulatory cattle are concerns over animal welfare. Non-ambulatory dairy cattle can be a major challenge on any dairy farm, particularly on larger facilities where their housing and nursing needs make them labor intensive. Surveys on health and management practices across the United States highlight that whilst approximately 70% of all dairy operations had at least one down cow (defined as a non-ambulatory individual for greater than 24 hours), that proportion increased with size of operation; 81% of operations between 100 and 499 head, and 97% of farms with greater than 500 head had at least one individual so affected. Collated data from owner responses report that approximately 60% of cattle recumbent for more than 24 hours fail to survive, consistent with other studies that identify recumbency as not only a health and welfare issue but a significant contributor to culling losses.

Previous Literature and Retrospective Study: There have been two notable previous retrospective studies from North American teaching hospitals (Cornell University (Burton et al 2009) and University of Montreal (Puerto-Parada et al 2021) examining the use of flotation tanks in the management of down cows. These identified survival rates to discharge of 37% and 55% respectively with comparable referral dairy cattle caseloads to our own at the University of Wisconsin. Other important findings in these papers included the observation that cattle who could stand after the first flotation event were 5 times more likely to survive as those that could not (Burton et al 2009), alongside an increased odds of non-survival when cattle were referred only after an extended period of recumbency, or when they demonstrated specific clinical or clinicopathologic abnormalities suggestive of a worsened status including tachycardia, tachypnea, hypothermia, elevated muscle enzymes or increased creatinine (Puerta-Parada

2021). Our own recent investigation into almost 200 cases identified a comparable survival rate of 47% to discharge from our hospital. We were also able to identify differences in outcome according to cause, specifically cattle recumbent due to calving paralysis or metabolic derangements (hypocalcemia and ketosis for example) being approximately 10 and 22 times more likely to survive respectively than those with certain orthopedic/musculoskeletal conditions. Appetite and the ability to walk out of the flotation tank after the first floatation session were variables retained in a final multivariate variable model in our study.

Conclusion: In many countries worldwide, intensification of the dairy industry is leading towards a smaller number of larger dairy farms. The US experience suggest that this increases the likelihood that non-ambulatory cattle will be encountered – one method of management includes the use of flotation tanks. This equipment has dramatically increased in availability in the US within the last 25 years. Prompt and appropriate use, alongside timely identification of cattle with poor prognostic indicators can reduce unnecessary animal suffering and treatment costs. Large referral based studies suggest that survival rates of 40-55% are achievable, and that relatively easy to identify parameters within the first 24 hours of flotation can be used to guide decision making.

References: Available on request.

K08

BRD Diagnostic and Treatment Approaches in Dairy Calves

Geof Smith.

North Carolina State University, Raleigh, USA.

BRD remains an economically significant disease in dairy calves and multiple studies have shown that it has a significant long-term economic impact on the future productivity of that heifer. Calves that have been treated for pneumonia have a greater age at first calving, a higher cull rate and lower milk production than calves from the same herd that were not treated for BRD. While prevention of respiratory disease is critical, veterinarians need to be able to work with producers to recognize disease promptly and institute successful treatment programs. This presentation will highlight research over the past 10 years on key approaches to diagnosing and treating respiratory disease in the dairy calf. Key principles are as follows:

1. Early diagnosis is critical- The earlier BRD can be recognized, the higher the chances of treatment success. With early treatment we also hope to minimize the long-term damage done to lung tissue which may further reduce the animals' potential for milk production in the future. There have been a number of clinical scoring systems developed that farmers can use to identify calves that need treated. In addition, lung ultrasound of dairy calves has been developed which can easily be implemented on farm. Ultrasound can be utilized to answer several questions such as which calves need treated for pneumonia, how effective are farm employees at identifying pneumonia, what age is BRD beginning in the herd, are calves responding appropriately to treatment and which calves have chronic lung damage and need to be culled. Ensuring that veterinarians work with farmers to establish some type of system to recognize pneumonia early is critical to ensure a good outcome.

2. Culture and sensitivity data from nasal swabs or calves that have died from BRD are likely unreliable- For years we have used culture and sensitivity data to help establish treatment protocols for various farms which often come from nasal swabs or lung cultures taken from dead calves previously treated with antimicrobials. It's becoming clear that these data may be unreliable. Studies show that bacteria in the upper airway may be quite different than what is actually in the lungs and either transtracheal wash or bronchoalvelolar lavage techniques are much more reliable. Furthermore, the practitioner often finds resistant pathogens in lung cultures from calves treated with antimicrobials, however these organisms are often not representative of the actual bacteria causing disease within the herd. There is extremely limited data present in calves that shows diagnostic testing and susceptibility results are positively correlated with treatment outcome. While determining which pathogens are present in a herd is still helpful, establishing goals for treatment success and monitoring outcomes of therapy may be more effective than relying on susceptibility testing to design protocols.

3. We need to consider treating calves longer for BRD- Several studies using ultrasound have shown that many calves treated for BRD continue to have lung lesions despite an apparent resolution of clinical signs. There is also an increasing prevalence of *Mycoplasma bovis* found in dairy calves associated with BRD. We know that Mycoplasma is able to persist for weeks after calves are initially infected. It is able to evade the immune system and survive in necrotic areas of the lung. With the development on long-acting macrolide antibiotics, it has become common that calves receive a single antimicrobial treatment lasting 7-10 days. New research would suggest that many of these calves would do better with 2-3 weeks of therapy and perhaps as long as 30 days.

4. Minimizing stress is critical- There is a significant body of data showing that various causes of stress including weather, transport, nutrition, overcrowding and other diseases (like diarrhea) significantly increase the risk for BRD. Data also indicates that some calves are able to tolerate stress better than others. For example, one study demonstrated that calves with elevated cortisol concentrations upon arrival to a veal farm had a significantly increased risk of developing severe pneumonia as compared to calves with lower levels of cortisol upon arrival. We can use this information to limit stress but also as a way to potentially genetically select for calves that may be more resistant to developing BRD over time.

Immunology and Vaccines

K09

Bovine Immune System and the Implications for Disease Resistance

Ángel Abuelo Sebio.

Michigan State University, Dewitt, USA.

The ability of cows to resist the establishment of mastitis is related, in part, to the efficiency of their immune system. However, many aspects of bovine innate and acquired host defenses are suboptimal in the transition period that contributes to increased severity and duration of diseases. Health disorders occurring during this time may greatly impact the productive efficiency of dairy cattle in the ensuing lactation. Therefore, it is not surprising that considerable research efforts have focused on defining how host defenses change as a consequence of the lactation cycle and understanding those factors that may contribute to immune dysfunction during this critical period. The immune system consists of a variety of biological components and processes that serve to protect dairy cows from pathogens. The primary roles of the mammary immune system are to prevent microbial invasion, eliminate existing infections and other sources of cellular injury, and restore tissues to normal function. In cows, the immune system utilizes a multifaceted network of physical, cellular, and soluble factors to facilitate defense against a diverse array of microbial challenges. This integrated system of defense mechanisms is highly regulated to maintain a delicate balance between the activation of immunity needed to prevent the establishment of disease and resolution of activity once the threat of invasion has passed. This presentation will provide a brief overview of the bovine immune system, describe how suboptimal immune responses can fail to prevent infections, and outline current strategies to optimize immune responses in dairy cows during times of increased susceptibility to disease.

K10

Transcriptomics to define mechanisms of bovine respiratory disease (BRD) resistance

Amelia R. Woolums[1], Matthew Scott[2], Cypriana Swiderski[3], Andy Perkins[1], Bindu Nanduri[1], Brandi Karisch[1].

[1]Mississippi State University, USA; [2]Texas A&M University VERO, USA; [3]University of Arizona, USA.

Objectives: Our objectives were to define gene expression profiles identified in transcriptomes from whole blood collected from beef cattle at high risk for bovine respiratory disease (BRD) on the day they were received after purchase ("at arrival") and develop a disease prediction model from BRD occurrence within 28 days after arrival.

Materials and Methods: Seven groups of mixed-breed beef cattle weighing 182 – 273 kg were purchased from local auction markets between the years of 2015 and 2020. Blood was collected on arrival via jugular venipuncture and was stored Tempus Blood RNA Tubes at -80 C. Cattle received conventional management, with some groups receiving vaccines or antimicrobials at arrival for BRD control. Cattle were examined daily for clinical signs of BRD and were treated if needed per standardized protocols. After 60 – 85 days, cattle that had been treated 0, 1, or 2 or more times, or died or were euthanized due to BRD (Healthy, Treated_1, or Treated_2+) were identified, and a sample of each group (n = 5 to 115) was selected for RNA sequencing or NanoString nCounter gene expression profiling of banked blood samples. All RNA was isolated with Tempus Spin RNA isolation kits via manufacturer's instruction. RNA sequencing and NanoString probe-based expression analysis were performed with captured and prepared polyadentylated mRNA. After data processing of sequenced reads via a HISAT2/StringTie pipeline, differentially expressed genes (DEGs; FDR ≤ 0.05) between Healthy, Treated_1, and Treated_2+ cattle were identified using edgeR +/- DESeq2 likelihood ratio testing. NanoString gene expression output was normalized and statistically analyzed with nSolver Advanced Analysis Software v4.0 in accordance with the nSolver Gene Expression Data Analysis Guidelines ($p \leq 0.05$). The relationship between DEG and treatment group was evaluated with multiclass receiver operating characteristics (ROC) curve analysis, and development of a decision tree model based on expression of 6 genes (*HERC6, IFI6, ISG15, MX1, LOC100297044,* and *CFB)*.

Results: Over all seven groups of cattle, genes related to production of specific proresolving mediators (SPM) and antimicrobial peptide production were upregulated in Healthy cattle relative to Treated_1 and/or Treated_2+ cattle. Genes related to type I interferon production, complement factor B, and proinflammatory processes were upregulated in Treated_2 cattle relative to Healthy and/or Treated_1 cattle. Expression profiles in Treated_1 cattle were similar in some ways to Healthy, and in some ways to Treated_2+, suggesting that clinical diagnosis may have misclassified some Treated_1 cattle. The decision tree model classified Treated_2+ cattle with 90% accuracy.

Conclusions: Analysis of whole blood transcriptomes in cattle at arrival reveals differential expression of genes that consistently differentiate cattle that go on to stay healthy, or require BRD treatment. To date our analysis has most reliably predicated Treated_2+ cattle; this may be due to misclassification of some Healthy and Treated_1 by clinical diagnosis. In future research we plan to evaluate blood and airway gene expression simultaneously in cattle with BRD, or at risk for BRD, to refine our understanding of the relationship between DEG in blood and lung inflammation. Specific proresolving mediator expression may protect cattle from BRD; this hypothesis warrants further investigation.

K11

Impact of Oxidative Stress on Inflammation and Immunity

Ángel Abuelo Sebio.

Michigan State University, Dewitt, USA.

Increased incidence of several economically important diseases (i.e., mastitis, metritis, displaced abomasum and ketosis) cause significant pre-harvest production losses in transition dairy cattle and decreases the availability of safe and nutritious food for a growing global population. A major underlying factor responsible for the development of transition cow disorders is metabolic stress, which occurs when cows fail to adapt physiologically to an increase in energy requirements needed for parturition and the onset of copious milk synthesis and secretion. Metabolic stress can be characterized as resulting from the combined effects of altered nutrient metabolism, dysfunctional inflammatory responses, and oxidative stress. Together, these factors form destructive feedback loops that exacerbate metabolic stress and cause health disorders in transition cows. A better understanding of how nutrition and immunology interact to influence metabolic stress will facilitate the development of control programs to improve transition cow health. The ability to detect signs of metabolic stress early enough in the dry period to implement needed management adjustments prior to calving will be the key to successive monitoring and intervention programs. This presentation will focus on the impact of oxidative stress has on inflammation and immunity and how controlling oxidative stress can assist in improving dairy cattle health during the periparturient period.

K12

Sheep farmer-vet engagement - maximising mutual value

Fiona Lovatt.

Flock Health Ltd., Barnard Castle, UK.

With over 15 million breeding ewes in the UK, the sheep industry is a significant livestock sector that fits well on the marginal uplands or alongside arable rotations or other agricultural enterprises. However historically, there was the danger of a 'downward spiral' in which sheep farmers perceived that veterinarians were expensive and neither sufficiently interested nor expert enough to contribute to the success of the sheep enterprise. Further research suggested that many veterinarians did not have much contact with their sheep farmer clients, they do not believe themselves to be sufficiently knowledgeable or expert enough to be proactive and they do not necessarily believe that farmers will pay for their services.

Sheep farmers who did see the benefit of regular veterinary involvement, suggested that a proactive veterinarian might work with groups of smaller flocks and this initiated the launch of the concept of Flock Health Clubs.

Objective 1: Can this spiral could be reversed to the benefit of both sheep farmers and sheep vets.

Objective 2: Are Flock Health Clubs beneficial to farmer-vet relationships and flock health and welfare.

Materials and methods: Qualitative and quantitative data were collected from veterinary surgeons and sheep farmers by means of interviews and electronic surveys to assess the impact of Flock Health Clubs.

Results: Since May 2018, 115 veterinary surgeons have reported running an active Flock Health Club, predominantly in UK but also in Ireland, Canada and Norway.

Both farmers and veterinary surgeons are positive about the contribution their Flock Health Club has made towards improved farmer–veterinary surgeon relationships and communications with respect to sheep health and practices, resulting in more opportunities for veterinarians to work proactively with sheep farmers.

Positive changes in management practices included reducing flock lameness, increased farmer confidence in their ability to deal with lameness and a reduction in inappropriate medicine use.

Conclusions suggest that setting up a Flock Health Club within a practice has significant and measurable flock health benefits which result from its ability to transform the relationship between sheep farmers and veterinary surgeons to improve mutual confidence and motivation. They are considered to be constructive environments for both farmer peer to peer learning and veterinary-based knowledge exchange.

K13

Effect of Estrus Expression and Estradiol Exposure on pregnancy rates in Beef Cattle

Gabriel Bo.

Instituto de Reproducción Animal Córdoba (IRAC), Zona Rural General Paz, (5145) Córdoba, Argentina. Instituto de Ciencias Básicas, Medicina Veterinaria, Universidad Nacional de Villa María, Villa del Rosario, Córdoba, Argentina

Synchronization of estrus and the timing of fixed-time artificial insemination (FTAI) and ovulation are reproductive technologies that have been utilized successfully to improve reproductive performance in beef herds. The main advantage of FTAI in beef cattle has been the possibility of obtaining high pregnancy rates to AI (P/AI) without the necessity of estrus detection, which has been a challenge that has affected the widespread application of AI in beef cattle world-wide.

There are basically two types of FTAI protocols currently used in beef cattle; gonadotropin realizing hormone (GnRH)-based and estradiol-based, both of which are combined with the use of progesterone (P4) releasing devices. Although FTAI is usually done without estrus detection, low pregnancy rates have been reported when expression of estrus was low in beef cattle treated with GnRH-based protocols. In South America, estradiol/P4-based protocols are commonly used to synchronize follicular wave emergence and ovulation for FTAI in beef cattle. Ovulations are usually induced in these protocols with a low dose of estradiol cypionate (ECP) administered

at the time of removal of the P4 device. Although ECP may induce expression of estrus in some cattle, the occurrence of estrus at the time of FTAI after P4 device removal has been associated with greater P/AI. The failure to show behavioral estrus after the removal of the P4 device in cows induced to ovulate with ECP may be because of low production of estradiol associated with a small dominant follicle. Furthermore, elevated preovulatory estradiol concentrations have been reported to have a positive impact on subsequent conceptus development, with cows exhibiting estrus having a greater conceptus length when compared to those not exhibiting estrus. We performed three experiments to evaluate the effects of expression of estrus and gonadotropin releasing hormone (GnRH) treatment on pregnancies per AI (P/AI) in beef cattle that were treated with an estradiol/P4-based protocol for FTAI. In Experiment 1, ovulation, as determined by ultrasonography, occurred earlier in cows that showed estrus (68.0 ± 2.5 h) than in cows that did not (82.0 ± 2.1 h, $P < 0.05$). Furthermore, cows that received GnRH ovulated earlier (78.0 ± 2.6 h) than those that did not (86.0 ± 2.0, $P < 0.05$). Experiment 2, P/AI was greater ($P < 0.01$) in animals that were observed in estrus by 48 h (56.4%) than in those that did not show estrus (46.5%). Likewise, animals that did not show estrus but were treated with GnRH had greater P/AI (53.8%, $P < 0.04$) than those that did not receive GnRH (37.9%). Experiment 3 was designed to determine the effect of delaying GnRH treatment to the time of FTAI (at 56 h) in cows not showing estrus by 48 h after P4 device removal. Cows that showed estrus by 48 h or 56 h had greater P/AI (62.3%, $P < 0.05$) than those did not show estrus (51.5%). Furthermore, when cows that did not show estrus by 48 h were analyzed separately, P/AI were greater ($P < 0.05$) in those that received GnRH at 48 h and were FTAI by 56 h (64.9%) than in those that received GnRH concurrent with FTAI by 56 h after device removal (54.6%). Finally, another experiment evaluated the effect of expression of estrus on pregnancy rates to embryo transfer (P/ET) and pregnancy loses up to parturition in recipients synchronized with two estradiol-based protocols. In this experiment, pregnancy was determined at 30 and 60 d by ultrasonography, and all pregnant recipients were followed until parturition to determine pregnancy losses during gestation. Overall, 88.0% (357/407) of the recipients synchronized showed estrus and a greater P/ET (P = 0.05) was found in the recipients that showed estrus (39.0%) vs. those that did not show estrus (26.0%). Pregnancy losses were lower (P = 0.01) and the calving rate was higher (P = 0.01) in recipients that showed estrus (25.0% and 29.3%, respectively) than in those that did not (88.8% and 2.9%, respectively). In summary, expression of estrus was associated with earlier ovulations and resulted in greater P/AI. Furthermore, the studies performed in embryo recipients have shown that expression of estrus was also associated with a greater P/ET, lower embryo/fetal losses and greater calving rates.

K14

Presynchronization strategies in beef cattle: Optimizing hormonal response and fertility to FTAI

A Garcia-Guerra, E. Rojas Canadas, A. E. Crist

Department of Animal Sciences, The Ohio State University, Columbus, OH, USA.

In many countries, ovulation synchronization protocols for fixed timed artificial insemination (FTAI), rely solely on the utilization of GnRH for the control of follicle development. Development of GnRH based synchronization protocols hinges on the premise that administration of the initial GnRH will induce ovulation resulting in the emergence of a new follicular wave ~ 2 days later, and therefore, ensuring the presence of a new, synchronized, dominant follicle at the final GnRH administration. Results from research on suckled beef cows indicate that cows in which ovulation occurs after initial GnRH have a larger dominant follicle and increased circulating estradiol (E2) concentrations at the time of the final GnRH. Despite the aforementioned benefits, the proportion of cows that have ovulation in response to the initial GnRH rarely exceeds ~60% and can be as low as ~25%. Ovulatory response after GnRH administration in cattle is affected by follicular size and maturational status, as well as circulating concentrations of both progesterone (P4) and E2. In this regard, elevated circulating P4 reduces the magnitude of the GnRH-induced LH surge and the resulting ovulatory response. As a result, presynchronization strategies have been designed, mainly for dairy cattle, to improve ovulatory response to the initial GnRH by promoting the presence of a growing dominant follicle or reducing circulating P4. Thus, we conducted a series of studies to evaluate the effect of a presynchronization strategy designed to promote the presence of a growing dominant follicle and reduced circulating P4 in order to maximize ovulatory response to GnRH and consequently improve synchronization and fertility to FTAI. The presynchronization strategy utilized consists in the insertion of a P4 device for 6 days, and PGF administration at the time of P4 device removal 48 hours before initiation of a 6-day CO-Synch protocol (P4 Presynch + 6-day CO-Synch). The 6-day CO-Synch utilized was as follows: d-9 (insertion of a P4 device + GnRH); d-3 (P4 device removal + PGF); d0 (FTAI + GnRH). Utilization of the P4 Presynch + 6-day CO-Synch in suckled beef cows improved (P<0.01) pregnancies per AI (P/AI) compared to utilization of the 6-day CO-Synch without presynchronization (64.4% vs 54.8%, respectively), despite no differences (P>0.20) in estrous expression (65% vs 62%, respectively). Yearling beef heifers that were presynchronized had greater estrous expression (84.9% vs 72.4%; P<0.01) and tended to have greater P/AI (55.4% vs 47.5%; P=0.07) than heifers receiving the 6-day CO-Synch alone. Subsequently, we evaluated the effect of a simpler presynchronization strategy consisting of PGF 48 h before initiation of the 6-day CO-Synch (PGF Presynch + 6-day CO-Synch). Suckled beef cows and yearling heifers were randomly assigned to one of the following groups: P4 Presynch + 6-day CO-Synch; PGF Presynch + 6-day CO-Synch; or 6-day CO-Synch. Cows in the P4 Presynch + 6-day CO-Synch group had greater (P<0.01) P/AI (66.1%) than cows in the PGF Presynch + 6-day CO-Synch (56.7%) and 6-day CO-Synch (51.5%) groups. Simi-

larly, heifers in the P4 Presynch + 6-day CO-Synch group had greater (P=0.02) P/AI (67.6%) than heifers in the PGF Presynch + 6-day CO-Synch (53.4%) and 6-day CO-Synch (54.4%) groups. In conclusion, incorporation of presynchronization strategies using a P4 device and PGF before initiation of a CO-Synch treatment regimen significantly improves fertility to FTAI in suckled beef cows and yearling heifers.

K15

Using eCG to increase pregnancy rates in Beef Cattle

Gabriel Bo.

Instituto de Reproducción Animal Córdoba (IRAC), Zona Rural General Paz, (5145) Córdoba, Argentina. Instituto de Ciencias Básicas, Medicina Veterinaria, Universidad Nacional de Villa María, Villa del Rosario, Córdoba, Argentina.

Cattle are managed differently in different parts of the world. Pastoral systems (usually in New Zealand, Australia, some European countries, South America and beef cattle in North-America) usually require the maintenance of a seasonal calving interval of ~12 months. Cows that do not maintain this calving interval are usually culled as not pregnant, or if two mating seasons are used, they are often moved to next breeding period. Although this may seem straightforward, the reality is that it is difficult to impregnate cows shortly after calving so as to maintain a consistent calving interval without aggressive interventions. Several hormone treatment protocols have been developed to control the timing of the first breeding, especially in cows in anovulatory anestrus (AA). Postpartum cows that are AA have insufficient pulsatile release of LH to support the final stages of ovarian follicular development and ovulation, limiting the effectiveness of fixed-time AI (FTAI) protocols. Exogenous progestins have been shown to increase LH pulse frequency during and after treatment leading to ovulation; however, the efficacy of such treatments can be compromised in herds with a high proportion of AA cows and those with low body condition scores. Thus, gonadotropins are often included in synchronization protocols to improve FSH and LH support. One of the gonadotropins that has created much discussion is equine chorionic gonadotropin (eCG). This hormone is a large molecular weight glycoprotein produced by endometrial cups in the mare. In the mare, eCG has primarily an LH activity, but in the cow it has either FSH or LH activity, depending on the receptor populations in the ovary. Although eCG has been been used to induce superovulation in a variety of species, the application of eCG at the time of removal of a progesterone releasing device has been used extensively in fixed-time AI (FTAI) programs in Bos indicus (300 IU) and Bos taurus (400 IU) beef cattle. The most important effect of eCG is the stimulation of the growth of the dominant follicle which increases ovulation rate, especially in cows in postpartum anestrus and/or with low body condition scores (BCS). However, treatment with eCG also increases circulating progesterone concentrations in the subsequent luteal phase with an associated increased in CL diameter and increased progesterone production. It has been shown that treatment with eCG increases the expression of steroidogenic enzymes (P450scc, 3β-HSD and StAR) in the CL. Other studies have shown beneficial effects of eCG in fixed-time AI (FTAI) programs in Bos taurus (200 IU) and Bos indicus (200 IU) prepubertal heifers, and in grazing dairy cattle, administration of 400 IU eCG resulted in increased pregnancy rates after the first and second breeding, especially in AA cows. Although most of the reports on the use of eCG were in cows synchronized with estradiol and progesterone releasing devices, the use of eCG has also been reported to benefit standard GnRH-based synchrony programs in suckled beef cattle. The addition of eCG was shown to increase pregnancy rates in Bos indicus cows in postpartum anestrus treated in a Co-Synch plus progesterone protocol, in primiparous Bos taurus cows in Canada and in Bos taurus in Ireland during the spring breeding season. However, there was no improvement in pregnancy rates in older Bos taurus cows in good BCS. In another study performed by our group in Argentina, the use of eCG in a 5-day Co-Synch plus progesterone protocol was evaluated in comparison to the standard 8-day estradiol/progesterone protocol in 801 suckled beef cows in postpartum anestrus and 183 suckled beef cows that were cycling. Cows in the 5-day Co-Synch group were treated with two PGF and half of the cows received 400 IU of eCG at progesterone device removal. Cows in the estradiol-based treatment group received 400 IU of eCG and 1 mg of ECP at progesterone device removal on Day 8. Cows were FTAI 52 to 56 hours after device removal in the estradiol group and 72 hours after device removal in the 5-day Co-Synch groups. Although no differences in P/AI were observed in the cycling cows, P/AI in cows in postpartum anestrus were higher in the groups receiving eCG (5-day Co-Synch: 46.3%; estradiol: 54.5%) than in the 5-day Co-Synch group that did not receive eCG (26.8%).

K16

Doppler ultrasonography for pregnancy diagnosis: Setup and utilization in FTAI programs

A. Garcia-Guerra, S. R. Wellert.

Department of Animal Sciences, The Ohio State University, Columbus, OH, USA.

Reproductive management programs in beef cattle employ pregnancy diagnosis to identify animals which have failed to conceive in order to make culling decisions or commence re-breeding. A short interval between breeding and pregnancy diagnosis is essential to the intensive utilization of fixed time artificial insemination (FTAI) programs. The earlier non-pregnant animals are identified, the sooner steps can be implemented for re-insemination and thus increase overall reproductive efficiency. Direct methods of pregnancy diagnosis are based on the identification of conceptus components using transrectal palpation or B-mode ultrasonography. Conversely, indirect methods are based on the identification of hormones or conceptus-specific substances such as progesterone and pregnancy associated glycoproteins, respectively. Ultrasonographic evaluation of the corpus luteum (CL) and the identification of luteolysis can serve as an indirect method of non-pregnancy

diagnosis. The reduction in circulating progesterone characteristic of CL regression is temporally associated with a marked decrease in CL perfusion which can be identified using color flow Doppler ultrasonography (CFDU). Thus, CFDU of the CL has the potential to be used for early pregnancy diagnosis in order to improve reproductive efficiency and increase the use of FTAI in beef cattle. A series of experiments were performed to determine the diagnostic performance of CFDU in beef cattle and its utilization in a resynchronization program for FTAI. In experiment 1, beef heifers and cows were submitted to FTAI using a 5-day CO-Synch. At d21, post AI, CFDU of the CL was performed using different settings for Color Flow Mapping (CFM) and Power Doppler (PWD) which differed in pulse repetition frequency (PRF) as follows: CFM720 (720 Hz); CFM960 (960 Hz); CFM1500 (1500 Hz) and PWD960 (960 Hz). Cows and heifers were diagnosed pregnant if color pixels covered >10% of the periphery of the CL, and contained at least two, color internal tracts penetrating towards the center of the CL. Sensitivity and specificity of CFDU were not different (P>0.50) between different PRF settings, however, the sensitivity (100%) and specificity (87.7%) for CFM960 was numerically greater. In experiment 2, beef heifers were submitted to FTAI using a 5-day CO-Synch and randomized to receive a CIDR or remain as untreated controls on d15 post AI. Transrectal CFDU of the CL was performed at 15, 17, 20, and 22 d post AI to determine pregnancy status. There was no effect of treatment (P=0.49), day (P=0.99) or treatment by day interaction (P=0.99) on CFDU sensitivity (>95%). Conversely, CFDU specificity was different (P<0.01) between days, although no treatment (P=0.91) or treatment by day interaction (P=0.82) was identified. Specificity was less on d15 (20.5%) and increased to reach its maximum at d22 (74.4%). In experiment 3, we evaluated the reproductive performance of beef cattle submitted to an early resynchronization program for 2nd service using CFDU of the CL for early pregnancy diagnosis. Beef heifers and suckled cows were submitted to a 6-day CO-Synch for 1st service and assigned to begin resynchronization for 2nd service at d16 (Resynch-16) or d25 (Resynch-25). Cattle in the Resynch-16 group received a CIDR at d16 post 1st FTAI. On d22, CIDRs were removed, pregnancy diagnosis was performed using CFDU (CFM960) and non-pregnant cattle were administered PGF. Cattle in the Resynch-25 group received a CIDR and GnRH at d25 post 1st FTAI. On d31 CIDR were removed, pregnancy was diagnosed using B-mode ultrasonography and non-pregnant cattle were administered PGF. In both groups, non-pregnant cattle were submitted to 2nd FTAI and administered GnRH at 60 (heifers) or 72 h (cows) after CIDR removal. First service P/AI at d31 tended to be greater (P=0.07) for Resynch-25 (60.9%) than Resynch-16 (53.5%) cows, while there was no difference (P=0.52) between groups for heifers (47.2%). Fertility to 2nd service was greater (P=0.01) for cows submitted to Resynch-25 (28.6%) than Resynch-16 (10.4%), however, there were no differences (P=0.25) between groups for heifers (52.1%). Cumulative P/AI (1st and 2nd service) was greater (P<0.01) for Resynch-25 (72.1%) than Resynch-16 (57.0%) in cows and tended to be greater (P=0.09) for Resynch-25 (76.5%) than Resynch-16 (66.1%) in heifers. In conclusion, CFDU performed at d22 after FTAI has high sensitivity and moderate to high specificity. Although, use of CFDU and early resynchronization allows for faster re-insemination of non-pregnant cattle, overall fertility is less than with later resynchronization strategies

Buffaloes and Camelids

K17

Development of an alpacas breeding program in Peru

Juan Pablo Gutiérrez García.

UCM, Madrid, Spain.

Alpaca is a South American camelid specie bred mainly to produce noble fiber for the textile industry. Suri and Huacaya are the two genetic types clearly differentiated in their morphology and fiber characteristics, with a higher proportion of the second one due to its higher robustness. The Suri has long and lustrous fibers that hang down against its body, while Huacaya fiber is crimp and grows perpendicular to the skin. Pacomarca is an experimental farm founded to act as a selection nucleus from which basic genetic improvement of alpaca fiber can spread throughout the rural communities in the Peruvian Altiplano. State-of-art techniques in animal science, such as performance recording and genealogical control are applied to demonstrate their usefulness in improvement programs under the Altiplano conditions. Searching for new ways of improving the performance of alpacas both technically and scientifically is continuously carried out while training courses for farmers are organized. Pacomarca has developed useful software (PacoPro) to carry out the integral processing of production and genealogical data. Mating is carried out individually, and gestation is diagnosed via ultrasound. Mechanized shearing is carried out under a specific own developed normative that has been adopted as national norm, so as the animal is protected and the fleece value is maximized. Breeding values predicted from genetic evaluation are used for selection. Selection objective is decreasing or removing the prickling of the alpaca fiber, and decreasing fiber diameter has been used as the main selection criterion during the last decade. Morphological traits have also been partly considered as part of the selection criterion. Genetic parameters have been precisely estimated from the data concerning these and other candidate traits to selection as well as genetic correlations among them. Fiber traits heritabilities estimated were moderate to high, those for morphological traits being moderate, those for reproductive traits low, and those for weight traits high. Genetic correlations among different type of traits were not relevant except those between fiber and weight traits that were high and unfavorable, showing that both productive aptitudes are at odds. Successful genetic trend has been observed by a reduction of fiber diameter from 22.5 to 17.9 microns from 2007 to 2019. Selection objective continues being removing the prickling of the alpaca fiber, but selection criterion has been moved to reducing the percentage of medulated fiber in the last four years. After 20 years of intense selection, the variability among the best 36 sires was still very high concerning different proportions of medullation and fiber diameter, which results promising. Selection work continues while the farm remains involved in high research standards for alpaca genetics.

K18

Assisted reproductive technologies in buffaloes: techniques and outcomes

Pietro Baruselli Sampaio, Lígia Mattos Rebeis, Laís Ângelo De Abreu.

USP, Sao Paolo, Brasil.

Buffaloes present reproductive seasonality and become sexually active with decreasing days (short days) during the late summer to early autumn. The use of assisted reproductive technologies (ART) such as artificial insemination (AI) and embryo transfer (ET) are important alternatives for maximizing the genetic gain and productivity of this species. This review aims to elucidate some aspects of the ART and their outcomes in buffalo production system. The use of AI has already proved to be a reliable technology in increasing genetic progress in buffalo herds. However, the low efficiency of traditional estrus detection system makes this biotechnology difficult to be used in this species. Buffaloes present poor manifestation of the symptoms of estrus, mainly due to low frequency of homosexual behavior. The use of hormonal treatments based on GnRH plus PGF2a (Ovsynch protocol) for timed artificial insemination (TAI) present satisfactory results in cycling buffalo. However, studies have shown that anestrous buffaloes synchronized with Ovsynch have a lower P/AI when compared to cycling buffaloes (20.0 vs. 65.3%). In contrast, research carried out by our group showed that the use of protocols based on P4, E2 and eCG can present favorable P/AI both in the non-breeding (high presence of anestrous) and in the breeding season (66.7 vs. 62.7%), enabling the application of AI throughout the year. ET is an effective technique in accelerating maternal and paternal genetic gain with a consequent increase in herd productivity. In buffaloes, however, the production of ***in vivo*-derived** (IVD) embryo has low efficiency. Studies carried out by our group show that despite responding to superstimulation (average of 15 follicles > 8 mm at the end of FSH treatment) with a moderate ovulation rate (approximately 60%), the embryo recovery rate (number of embryos and ova recovered per ovulation) is low (34.8%). While IVD present low efficiency in buffalo, ***in vitro* production** (IVEP) has been studied as an alternative and positive results have been achieved in recent years. Historically, the association of follicular aspiration (OPU) with IVEP presented low results in buffalo when compared to cattle. However, recent studies show that is possible to select buffalo donors using different technologies to improve the ET efficiency. Antral follicle population (AFP) is directly associated with the number of oocytes recovered by OPU for IVEP and the number of embryos produced per procedure. Furthermore, strong relationship between AFP and anti-Mullerian hormone (AMH) was found in buffalo. Studies carried out by our group suggest that AMH can be considered an endocrine marker to predict the performance of IVEP in buffaloes. Recently, superstimulation with FSH prior to OPU has been used successfully in IVEP programs in buffalo, resulting in the increase of total embryos produced per OPU session. We found that FSH treatment prior to OPU increased the proportion of medium and large diameter follicles available for OPU procedure in all buffalo donor categories (heifers, primiparous and multiparous). Furthermore, FSH treatment increased the proportion of viable oocytes for culture and embryos produced per OPU-IVEP session, suggesting that the use of FSH treatment may be a strategy to improve the efficiency of OPU/IVEP in buffalo. In addition, another fact to be considered when evaluating the efficiency of IVEP is the bull effect. The semen of certain bulls shows high blastocyst rates and improve the efficiency of the ET programs. The use of calves as oocyte donors is an important strategy to accelerate genetic gain by decreasing generation intervals. We compared the embryo production of calves (2 to 4 months) in relation to prepubertal heifers (13 to 15 months) and lactating buffaloes. Although the embryo rate was lower in calves compared to prepubertal heifers and lactating buffaloes, the P/TE was similar between categories (around 35%), which demonstrates the feasibility of this technique for donor buffalo calves. Therefore, synchronization protocols are designed to control both luteal and follicular function and permit fixed-time AI with high pregnancy rates during the buffalo breeding (autumn-winter) and nonbreeding (spring-summer) season. In addition, the application of OPU/IVEP is showing promising results and has become an alternative to superovulation for *in vivo* embryo production. Therefore, nowadays AI and ET can be implemented commercially in buffalo farm to increase the meat and milk production.

K19

Diagnostic and surgical approaches in Italian mediterranean buffaloes in the field

Antonio Natale.

Freelance, Italy.

Objectives: In the last 70 years in Italy, precisely in Campania and in the DOP area "(Mozzarella di Bufala Campana DOP)" the management and managerial techniques of buffalo breeding (Italian Mediterranean Buffalo) have undergone an intense acceleration, leading to a profound transformation of the buffalo breeding, with a numerical increase and genetic improvement of this species, with appreciable both productive and reproductive results.

It should be noted that the dairy buffalo (Italian Mediterranean buffalo) and the dairy cow belong to the same suborder, but are to be considered as two DIFFERENT SPECIES. For this reason, the approaches to nutrition, reproduction and production require different strategies and operations.

Specifying that buffalo milk represents the most important economic aspect for the farmer, and bearing in mind that the milk itself has dairy processing as its sole destination, it has become necessary in recent years to improve both quantity and quantity in a uniform and substantial way.

To this end, to obtain the protein and lipid increase in milk, a higher yield, it was essential to make a substantial change in the forage / concentrate ratio in the diet, resorting more and more to the concentrates in the ration to meet the needs of the species (Italian Mediterranean buffalo).

This new nutritional concept has led to the appearance of

those metabolic pathologies typical of high-production dairy cattle in the buffalo species (Italian Mediterranean buffalo). That is, technopathies that lead to pathological situations resulting from the alteration of the normal homeostatic balance of the buffalo.

The topics covered by "(BuBoVet srl)" concern the most important pathologies of metabolic origin which have an evolution of surgical interest with the relative operative techniques to be applied in the field: breech pathologies (pododermatitis, phlegmon, white line diseases, etc.).) but above all left and right abomasal dislocation, dilation-dislocation of the cecum, post-partum uterine prolapse, prolapse of the rectum. Furthermore, "(BuBoVet srl)" describes the most commonly used surgical treatments such as: caesarean section, abdominal surgery in heifers and calves, resolution of distal limb fractures in buffaloes (Italian Mediterranean buffalo).

Conclusions: The topics covered by "(BuBoVet srl)" concern the most important pathologies of metabolic origin which have an evolution of surgical interest with the relative operative techniques to be applied in the field: breech pathologies (pododermatitis, phlegmon, white line diseases, etc.).) but above all left and right abomasal dislocation, dilation-dislocation of the cecum, post-partum uterine prolapse, prolapse of the rectum. Furthermore, "(BuBoVet srl)" describes the most commonly used surgical treatments such as: caesarean section, abdominal surgery in heifers and calves, resolution of distal limb fractures in buffaloes (Italian Mediterranean buffalo).

K20

Diagnostic and surgical approached in Italian Mediterranean Buffaloes in the field (II)

Antonio Natale.

Freelance, Italy.

Objectives: In the last 70 years in Italy, precisely in Campania and in the DOP area "(Mozzarella di Bufala Campana DOP)" the management and managerial techniques of buffalo breeding (Italian Mediterranean Buffalo) have undergone an intense acceleration, leading to a profound transformation of the buffalo breeding, with a numerical increase and genetic improvement of this species, with appreciable both productive and reproductive results.

It should be noted that the dairy buffalo (Italian Mediterranean buffalo) and the dairy cow belong to the same suborder, but are to be considered as two DIFFERENT SPECIES. For this reason, the approaches to nutrition, reproduction and production require different strategies and operations.

Specifying that buffalo milk represents the most important economic aspect for the farmer, and bearing in mind that the milk itself has dairy processing as its sole destination, it has become necessary in recent years to improve both quantity and quantity in a uniform and substantial way.

To this end, to obtain the protein and lipid increase in milk, a higher yield, it was essential to make a substantial change in the forage / concentrate ratio in the diet, resorting more and more to the concentrates in the ration to meet the needs of the species (Italian Mediterranean buffalo).

This new nutritional concept has led to the appearance of those metabolic pathologies typical of high-production dairy cattle in the buffalo species (Italian Mediterranean buffalo). That is, technopathies that lead to pathological situations resulting from the alteration of the normal homeostatic balance of the buffalo.

The topics covered by "(BuBoVet srl)" concern the most important pathologies of metabolic origin which have an evolution of surgical interest with the relative operative techniques to be applied in the field: breech pathologies (pododermatitis, phlegmon, white line diseases, etc.).) but above all left and right abomasal dislocation, dilation-dislocation of the cecum, post-partum uterine prolapse, prolapse of the rectum. Furthermore, "(BuBoVet srl)" describes the most commonly used surgical treatments such as: caesarean section, abdominal surgery in heifers and calves, resolution of distal limb fractures in buffaloes (Italian Mediterranean buffalo).

Conclusions: The topics covered by "(BuBoVet srl)" concern the most important pathologies of metabolic origin which have an evolution of surgical interest with the relative operative techniques to be applied in the field: breech pathologies (pododermatitis, phlegmon, white line diseases, etc.).) but above all left and right abomasal dislocation, dilation-dislocation of the cecum, post-partum uterine prolapse, prolapse of the rectum. Furthermore, "(BuBoVet srl)" describes the most commonly used surgical treatments such as: caesarean section, abdominal surgery in heifers and calves, resolution of distal limb fractures in buffaloes (Italian Mediterranean buffalo).

Small Ruminants

K21

Ovine anaplasmosis, an emerging disease in Europe

Delia Lacasta Lozano.

Universidad de Zaragoza, Spain.

Ovine anaplasmosis, an emerging disease in Europe, is being spread quickly through sheep flocks, causing relevant economic losses. This disease was described in tropical and subtropical countries as a mild disease that did not produce relevant clinical signs. However, when this bacterium enters a naive population, the clinical picture can be much more severe. Since 2014, when an outbreak of ovine anaplasmosis was diagnosed for the first time in Spain, the number of severe clinical cases of anaplasmosis has increased.

Anaplasma ovis is an obligate intraerythrocytic Gram-negative bacteria that belongs to the family Anaplasmataceae. This species is primarily transmitted by ticks, particularly the genera *Ixodes, Dermacentor, Rhipicephalus* and *Amblyomma* and can infect sheep, goats and wild ruminants. The bacterium replicates by binary fission within the erythrocyte and leaves it using a not well-defined mechanism, but apparently not lytic, to infect new erythrocytes. Thus, the haemolytic anaemia associated with this disease results from the immune response. During the acute phase, the number of infected erythrocytes doubles every 24-48 hours. However, this is a chronic process, and the destruction of the erythrocytes is slow and progressive, and the animal begins to show noticeable changes around 30-40 days after infection.

Depending on the naïve immune system, age, and other still unknown factors, there are animals in which anaemia goes unnoticed and others in which this disease causes severe clinical signs. The acute phase of the disease is characterised by nonspecific weakness, depression, a marked loss of body condition, fever peaks, progressive anaemia and finally, the death of the animals due to secondary infections. Severely affected animals that do not die often have to be culled due to lack of production. There are some flocks where the number of affected animals can be very high, causing significant economic losses, especially since the most commonly affected sheep are yearlings. The infected animals that survive the infection remain infected for life, maintaining a high bacterial load in the blood for at least seven years.

Until 2020 the disease had only been diagnosed in adult sheep in Spain, with the highest incidence in one or two-year-old animals. However, in 2020, an outbreak of ovine anaplasmosis was diagnosed for the first time in fattening lambs. After the slaughter of apparently healthy three-month-old lambs, 34.84% showed jaundiced carcasses. All tested lambs with icteric carcasses had severe regenerative anaemia just before slaughter and showed positive *A. ovis* PCR with a high bacterial load in blood. During the spring of 2020, similar clinical cases were diagnosed in different geographical areas of Spain, reaching percentages close to 2% of lamb carcasses condemnation due to jaundice at the Mercazaragoza slaughterhouse (Zaragoza, Spain), causing very relevant economic losses. In the spring of 2021, cases of jaundiced carcasses condemnations were again diagnosed at the slaughterhouse, reconfirming the presence of A. ovis in affected animals. The percentage of condemnation in affected flocks is usually close to 40% during late spring.

A study about possible antibiotic treatments to control anaplasmosis in affected lambs was carried out in 2021. Oral and injectable doxycycline and oxytetracycline were used in *A. ovis* infected lambs. It was concluded that injectable oxytetracycline and doxycycline reduce the bacterial load of *A. ovis* in blood. However, oral doxycycline does not reduce condemnations of jaundiced carcasses at the abattoir.

Further studies are needed in treatment and prevencion of this emerging disease.

K22

Responsible use of medicines in sheep flocks - antibiotics, anthelmintics, supplements

Fiona Lovatt.

Flock Health Ltd., Barnard Castle, UK.

Responsible use of medicines has been described as using the ‘right product for the right condition in the right animal in the right way and at the right time’ or ‘as little as possible but as much as necessary’.

However arguably, responsible use of medicines is not necessarily about how to use treatments at all but about how to ensure good health. To achieve this, the mantra Plan Prevent Protect (Lovatt et al., 2019) has been increasingly widely adopted to encourage responsible use of medicines. This is an encouragement to the veterinary surgeon and farmer to *plan* ahead, to *prevent* unnecessary disease, for example though the provision of a good environment, good hygiene and good nutrition and to *protect* the flock through appropriate vaccination or via the provision of sufficient quality colostrum to neonates.

Improvement in practical antimicrobial stewardship is essential, with clear motivations from both the One Health and human health perspective. The emergence of antimicrobial resistance (AMR) is widely recognised as a leading global health threat with significant implications for the future of both human and veterinary medicine.

It has been suggested (O Neill, 2016) that, by 2050, there will be 10 million global human deaths annually due to AMR. This projected figure is backed up by a Lancet report (Murray et al., 2022) that estimates that, in 2019, there were 1.27 million human deaths directly attributable to, and 4.95 million associated with, bacterial AMR.

Already we know that there are flocks and farms as well as individual people and animals that carry bacteria resistant to many of the commonly known antibiotics (eg. Duncan et al., 2019) and we know that AMR can pass between different bacteria with no respect of species barrier or geographical boundaries.

From a veterinary point of view, practitioners are increas-

ingly finding common conditions that are resistant to available antimicrobials with implications that compromise animal health and welfare and have consequential impacts on the mental well-being of veterinary practitioners, their wider practice team and their clients.

In the UK, the responsible use of antimicrobials throughout the livestock sectors have been a focus for the last six years, though arguably it has followed the sustainable use of parasiticides, which led the charge in 2003 via standards set by the multi-stakeholder group SCOPS (Sustainable Control of Parasites in Sheep).

Worm control on sheep farms is a particular challenge due to the ready availability of cheap anthelmintics. However, increasing anthelmintic resistance means that sustainable lamb production requires a more complicated model than worked in the past. Sheep farmers are no longer able to stick to rigid format of three weekly dosing with the cheapest drench and there is increasingly a need for them to work with trusted advisors such as veterinarians to future-proof their enterprise, to develop sustainable worm control plans and only to treat based on diagnostic evidence.

For sustainable progress in the responsible use of all medicines, it is essential that veterinarians, the gate-keepers of animal health and welfare, are empowered to make good decisions, to communicate well and to work collaboratively with both farmers and colleagues.

K23

Differential diagnosis of lower respiratory tract diseases in adult sheep

Delia Lacasta Lozano.

Universidad de Zaragoza, Spain.

Due to its particular anatomy and physiology, multiple diseases settle in the respiratory system of small ruminants, which entails wide and varied differential diagnoses of these disorders. This, coupled with the difficulties involved in differentiating the clinical signs, makes the differential diagnosis of respiratory diseases challenging. In this context, diagnostic imaging techniques become a very useful tool to reach the final clinical diagnosis of these conditions.

As most of the diseases that settle in the lung produce very similar clinical signs, it is crucial to perform a comprehensive clinical examination of the respiratory system. Depending on the severity of the process, mixed or expiratory dyspnea and tachypnea will be common clinical signs of all lower tract diseases. The cough and the adventitious sounds (crackles, rhonchi, and wheezes) will help us differentiate if it is a productive or non-productive disorder, which will be critical to guide a proper diagnosis.

PRODUCTIVE DISORDERS:

- **Ovine Respiratory complex (ORC)** is a complex disease process involving a range of host-pathogen-environment interactions (HPE). Several commensal microorganisms have been associated with ORC: *Mannheimia haemolytica, Pasteurella multocida, Bibersteinia trehalosi* and *Mycoplasma* sp., usually in mixed isolations. The ORC has three clinical forms of presentation: hyperacute, causing sudden deaths, acute, with fever, anorexia, depression, lethargy and severe respiratory clinical signs and the chronic form, causing mixed dyspnea, cough and chronic weight loss.
- **Ovine pulmonary adenocarcinoma** (OPA) is a contagious lung cancer of sheep caused by jaagsiekte sheep retrovirus (JSRV). OPA-affected sheep show signs of an afebrile respiratory condition associated with loss of weight. Initially, mixed dyspnoea and moist respiratory sounds (crackles and rhonchi), caused by the accumulation of fluid in the respiratory airways, are detected. In the final stages, variable amounts of frothy seromucous pulmonary fluid are discharged from the nostrils when the sheep's head is lowered (wheelbarrow test).
- **Aspiration (gangrenous) pneumonia** is caused by inhalation of foreign materials, producing inflammation and necrosis of the lung parenchyma. Most of the microorganisms producing gangrenous pneumonia are normal inhabitants of the nasopharynx or the environment, being *Trueperella pyogenes, Pasteurella multocida* and *Mycoplasma ovipneumoniae,* the most frequently isolated microorganisms. Respiratory signs may include coughing, mixed or expiratory dyspnea, crackles and rhonchi when the process progresses sufficiently; abnormal breath odour can be detected suggestive of the necrotising exudates.

NON-PRODUCTIVE DISORDERS:

- **Pulmonary form of lentivirosis.** The respiratory clinical form of lentivirosis (SRLV) is the most prevalent, and it is generally seen in adult animals (≥2 years old). Animals with pulmonary lentivirosis show dyspnea and tachypnea, weakness, loss of weight and delay in walking with the flock. No cough, nasal discharge or fever is observed in uncontaminated cases. However, multiple concurrent diseases such as OPA, CRO or gangrenous pneumonia can be present, thus complicating the clinical diagnosis of the disease.
- **Visceral form of Caseous lymphadenitis (CLA),** is caused by *Corynebacterium pseudotuberculosis*. In the visceral form, most of the affected animals had lesions in the respiratory system. For that reason, this disease is also included in the differential diagnosis of lower respiratory tract diseases. Respiratory clinical signs associated with CLA are often not readily recognised because only dyspnoea is detected without audible sounds of the thoracic cavity.
- **Verminous pneumonia.** The main pulmonary parasites of sheep are *Dictyocaulus filaria, Protostrongylus rufescens* and *Muellerius capillaris*.

In productive disorders with moist cough and adventitious sounds, the wheelbarrow test must be carried out to confirm or rule out OPA. In addition, the exhaled air after coughing must be smelled to detect necrotising processes associated with gangrenous pneumonia. If both diseases are ruled out, the most likely cause of the disease will be ORC. In the case

of non-productive disorders, the symptoms are pretty inapparent, and the clinical diagnosis is challenging. Ultrasonography is an advantageous diagnostic technique that can be easily applied in farm conditions. The etiological diagnosis can be made in living animals by bronchoalveolar lavage or after necropsy with an aseptic sampling of affected lung tissue and mediastinal lymph nodes.

K24

mRNA therapy to induce passive immunity in ruminants

Amelia R. Woolums[1], Merrilee Thoresen[1], E. Heath King[1], Santiago Cornejo[1], Hannah E. Peck[2], Daryll Vanover[2], Philip J. Santangelo[2].

[1]Mississippi State University, USA; [2]Emory University, USA.

Objectives: Our objective was to determine whether messenger RNA (mRNA) treatment of a ruminant mucosal surface could lead to rapid expression of antibody, which could provide passive immunity to infection within hours of treatment.

Materials and Methods: Initial studies were conducted in sheep. Yearling non-pregnant Kathadin-cross ewes were treated intravaginally with a topical spray of mRNA encoding a broadly neutralizing antibody to human immunodeficiency virus. Subsequently, Holstein calves weighing 127 – 172 kg were treated by respiratory aerosol with mRNA encoding a neutralizing antibody to influenza. At 24 hours or later times post treatment, animals were euthanized to evaluate expression of antibody on treated mucosal surfaces.

Results: In both sheep and cattle, expression of neutralizing antibody on treated mucosal surfaces was confirmed within 24 hours after treatment. In cattle treated by respiratory aerosol, expression was widely evident in the treated lung. Microscopic evaluation indicated that mucosal epithelial cells were transfected by the topically applied mRNA and then expressed antibody. Treated animals showed no adverse reactions during treatment, and no evidence of inflammation in treated tissues was evident postmortem.

Conclusions: While studies to date have evaluated expression of antibodies to human pathogens, they nevertheless demonstrate that ruminants can be induced to produce specific neutralizing antibodies rapidly after topical treatment of mucosal epithelia with mRNA. This approach merits further investigation as a method to provide rapid local protection from a variety of infectious agents.

Reproduction in Dairy Cattle

K27

The implications of spontaneous versus synchronized ovulations on the reproductive performance of high-producing dairy cows

Paul Fricke.

University of Wisconsin – Madison, USA.

In species classified as induced ovulators, the LH surge is induced by the act of coitus thereby precisely timing insemination relative to ovulation. Lactating dairy cows are spontaneous ovulators. Thus, the LH surge is induced by a cascade of endocrine events beginning with an increase in follicular estradiol in the absence of luteal progesterone, a GnRH surge from the hypothalamus is then followed by an LH surge from the anterior pituitary, followed by ovulation. Increased estradiol in the absence of progesterone causes behavioral estrus which is manifested by increased physical activity and standing to be mounted. Detection of behavioral estrus either visually, through the use of estrus detection aids, or via automated activity monitoring systems is widely used to determine timing of AI. Cow-related factors that limit service rates include the association of high milk production and duration of estrus, ovulation failure after estrus, ovulation in the absence of behavioral estrus, and anovular conditions which affect 20% to 30% of dairy cows at the end of the voluntary waiting period. One factor that limits fertility to spontaneous estrus is the high variation among cows in the timing of ovulation relative to increased activity resulting in imprecise timing of AI relative to ovulation. Another factor is the high rate of hepatic metabolism of estradiol and progesterone associated with high feed intake in high-producing cows. Under this endocrine milieu, development of preovulatory follicles from deviation until ovulation occurs in a prolonged low-progesterone environment marked by high frequency LH pulses that overstimulate the oocyte and compromise fertility similar to a persistent follicle. Development of the Ovsynch protocol turned dairy cows into induced ovulators, which allowed for increased service rates and precise timing of AI relative to ovulation. Subsequent modifications of the Ovsynch protocol, including presynchronization strategies and complete induction of luteal regression, lead to fertility programs that yield greater fertility than that of a detected estrus in high-producing dairy cows.

K29

Optimizing use of sexed and beef semen in dairy heifers

Paul Fricke.

University of Wisconsin – Madison, USA.

Our objective was to evaluate reproductive management programs for submission of Holstein heifers for first insemination with sexed semen. Nulliparous Holstein heifers (n = 736)

from three commercial farms were randomized within farm to one of three treatments for first AI with sexed semen: 1) **CIDR5** (d -6, GnRH +CIDR; d -1, PGF_{2a} -CIDR; d 0, PGF_{2a}; d 2, GnRH+TAI); 2) **CIDR6** (d -6, GnRH +CIDR; d -1, PGF_{2a}; d 0, PGF_{2a} -CIDR; d 2, GnRH+TAI); and 3) **EDAI** (PGF_{2a} on d 0 followed by once daily estrus detection and AI). Heifers detected in estrus 24 h before TAI (d 1) were inseminated and the protocol was discontinued. All heifers were inseminated with sexed semen (ABS Sexcel™ Sexed Genetics, DeForest, WI) from sires that were randomly allocated between treatments within each farm, and AI technicians were blind to treatment at AI. Heifers were followed for 84 d after first service to determine days to AI and pregnancy. Actual farm costs (US$) were used for hormonal treatments, detection of estrus, semen and AI, pregnancy diagnosis, and feed ($1.70/heifer/d) to calculate cost per pregnancy. Feed costs for nonpregnant heifers or heifers moved to a bull pen during the 84-d breeding period (n=112) were allocated to the feed costs for heifers that became pregnant during the 84-d breeding period. Pregnancy outcomes were analyzed using the GLIMMIX procedure of SAS with farm included as a random effect in the model. Costs were analyzed using the MIXED procedure of SAS with treatment as a fixed effect and farm as a random effect in the model. Delaying CIDR removal decreased early expression of estrus before scheduled TAI (0.004%vs. 27.8%); however, CIDR5 heifers tended to have more P/AI at 35 (52.9% vs. 45.3% vs. 45.8%) and 64 (51.8% vs. 44.8% vs. 44.9%) d after AI than CIDR6 and EDAI heifers, respectively. Overall, CIDR5 and CIDR6 heifers had fewer days to first AI and pregnancy than EDAI heifers which resulted in less feed costs than EDAI heifers due to fewer days on feed until pregnancy. Despite greater hormonal treatment costs for CIDR5 heifers, costs per pregnancy were $16.66 less for CIDR5 than for EDAI heifers. In conclusion, delaying CIDR removal by 24 h within a 5-CIDR-Synch protocol suppressed early expression of estrus before TAI, but tended to decrease P/AI for heifers inseminated with sexed semen. Further, submission of heifers to a 5-d CIDR-Synch protocol for first AI tended to increase P/AI and decrease the cost per pregnancy compared to EDAI heifers.

Udder Health and Mastitis

K30

How the milking machine influences mastitis

Ian Ohnstad.

The Dairy Group (UK).

Objectives: A fully functioning milking machine, correctly installed, maintained and operated will milk the vast majority of cows effectively, efficiently and with minimal adverse effects on udder health and cow behaviour.

Although we continually improve our understanding of the relationship between the cow, machine and operator, there remains room for improvement. Although the milking machine is often blamed for high somatic cell counts and clinical mastitis only 6 to 20% of new mastitis infections were related to the affects of the milking machine, either directly or indirectly (Mein, 2004).

Materials and Methods: However, poorly operating milking equipment can lead to sub-optimal teat conditions and, but not always, lead to udder health problems (IDF Bulletin, 1994). In addition, our scientific knowledge of the impact of machine milking and its use on udder health has often not been passed down to the milking staff.

The essential elements of a milking machine are the creation of vacuum, regulation of that vacuum at an appropriate level for the milking system and a means to alternate vacuum and atmospheric pressure in the pulsation chamber.

Continual exposure to vacuum leads to teats becoming congested with circulatory fluids leading to cow discomfort and compromised milk let down. The former leads to lowered resistance of the teat canal to bacterial invasion (Teat Club International, 2001). When atmospheric pressure is applied to the pulsation chamber the milking liner closes around the teat and vacuum at the teat end is relieved and the teat is massaged, resulting in the maintenance of blood circulation and minimising congestion. However, the closing liner applies a compressive load on the teat that can lead to excess keratin production near the teat end (Reinemann, ????), which in itself can impair the first line of the udder´s defence against bacteria.

When vacuum is applied to the pulsation chamber the liner opens and milk flows from a positive to negative pressure. A complete pulsation cycle is from the start of liner opening to the end of liner closing. The frequency varies between manufacturers, being typically 55 to 62 cycles per minute for milking cows.

Regardless of whether cows are milked in a high- or low-level system, the vacuum level at the teat end during peak flow rate should be in the range of 32.0 – 42.0 kPa (ISO 5707: 2007) to milk cows gently and efficiently.

How the milking machine influences udder health

Although the machine can cause short, medium or long-term changes in teat condition, environmental factors can also induce same. In practical situations, it will often be the combination of both that leads to increased levels of sub- and clinical mastitis.

The milking machine can affect mastitis infection rates by acting as a:

- Vector – liner slip causing teat end impacts or transferring bacteria from teat to teat (IDF Bulletin, 1987) often causing mastitis. Liner slip can be operator induced, such as by machine stripping or by incorrect matching of a liner to herd average teat size, incorrect vacuum level, inadequate effective reserve, poor cluster position and worn liners. Plus, liners can spread mastitis causing bacteria from cow to cow, if not disinfected. The machine can also spread bacteria if incorrectly cleaned and sanitised between milkings;
- Adversely affecting the first line of the udders defence. This includes physical teat damage by the milking machine or not maintaining teat skin in a soft and supple condition so influencing the first line of the udders defence.

Not only is it essential for the milking installation to be correctly installed, it must be correctly maintained and used properly. Many checks can be readily carried out by the operator on a daily and weekly basis (IDF Bulletin 396, 2005), with the plant fully serviced by a suitably trained engineer.

Monitoring teat condition and factors affecting it (as recommended by Teat Club International) has a great influence in minimising adverse udder health and leads to more effective and timeliness of the milking operation.

Conclusion: Udder health can be significantly improved by applying the knowledge we have of how the machine, cow and operator interact. Thorough and effective milk harvesting is a combination of correct operation of the milking machine and an effective milking routine, with benefits to udder health.

K31

What is milking efficiency? Why is it important?

Ronald Erskine[1], Rhyannon Moore-Foster[2].

[1]Michigan State University, USA; [2]Colorado State University, USA.

Objectives: This seminar will present potential pitfalls of stressing parlor efficiency as the overarching goal in a herd milking routine. The concept of milking efficiency will be discussed, especially as it relates to milk ejection and milking routine. Finally, the potential negative impact of bimodal (delayed) milk ejection on milk yield will be presented.

Background: Most dairy farms have their milking equipment evaluated and maintained on a routine basis. Although proper equipment function is necessary for milking performance, it does not guarantee it. Two management areas that can lead to poor milking efficiency are: 1) milking routines that don't achieve consistent milk letdown and 2) overmilking. Either one of these problems can leave cows 'high and dry' and expose teats to high vacuum levels.

During stimulation of teats before milking, a neuro-reflex arc stimulates the pituitary gland to release oxytocin into the blood, then to the udder. It takes about 1 to 2 minutes for oxytocin levels to increase in blood to optimally contract myoepithelial cells that surround the milk ducts, which then squeeze the milk down towards the teats. The two important points about this oxytocin release are enough stimulation (at least 10 seconds of actual *physical touching*) of the teats and the duration of the latency period, that is, the time interval between when teats are first stimulated until the cluster is attached—lag time is the time between unit attachment and milk ejection. Unfortunately, with increasing herd size, the number of cows that can be milked through parlors per hour, or parlor turnover rate, is often identified as one of the choke points of herd capacity. Thus, parlor efficiency, measured by cows milked per hour, milk produced per hour, parlor turnover per hour, etc., is emphasized at the expense of adequate milking preparation for the cow.

Results: Review of milking dynamics from over 60 Michigan dairy herds found that a mean of 25% of cows had bimodal milk ejection (range 0 to 75%) at the time of unit attachment. However, when the time of milk letdown after attachment (latency period) was compared to the milking unit on time in over 3,800 milkings, there was no correlation between these two variables Thus, milking time did not increase because of bimodal ejection.

In a follow up study of over 600 cows in a herd with a 50% frequency of bimodal milk ejection, delayed milk ejection of 30 to 60 seconds in duration resulted in a loss of 1.5 kg of milk per milking, and a delayed ejection of over 60 seconds resulted in a loss of more than 3 kg of milk per milking. This outcome was from a single milking for each cow on the trial, the impact of delayed milk ejection over longer periods, such as a week are not yet fully understood. However, preliminary work suggests that 1) for any given milking routine, there is a range of frequency of bimodal events between cows, and 2) cows that have bimodal milking ejection more frequently will also have greater losses in milk yield over time.

Conclusions: A proper premilking routine should allow for 90% of cows with immediate milk let down after cluster attachment, and milk should be flowing 95% of the time while the cluster is attached. Despite the drive to fully utilize a milking parlor in terms of cows per hour, if parlor efficiency is stressed to much over milking efficiency, it is possible to increase the amount of milk gained from a parlor each day. However, the law of diminishing returns comes into play at some point, where maximum is no longer the optimum, and the actual milk per cow per day is decreased.

K33

Who is teaching your employees?

Ronald Erskine.

Michigan State University, USA.

Objectives: This seminar will review and discuss the status and challenges of the changing employee structure in the dairy industry. Insight from employee and manager perspectives as to the gaps in traing will be reviewed, and a potential model of employee education presented.

Background: Many dairy operations worldwide are increasingly relying on hired labor, especially foreign-born workers. However, many dairy managers have limited human resource knowledge and experience; this often leads to frustration with protocol drift and a sense that employees are not motivated to enhance the success of the farm beyond prescribed instructions. Yet, a large majority of dairy managers state that hiring and retaining employees is a top priority of their dairy enterprise, even though employee turnover varies widely between dairy operations. Taken together, these gaps in dairy farm labor can be described as a cultural lag. That is, there is a gap between the human resource needs within the industry's labor force and the capacity of producers and managers to address them. Herds that believe that 'mastitis was a problem in their herd' or 'had difficulty with compliance of milking protocols', are more likely to have higher BTSCC. Thus, employee management and training, as well as producer values and attitudes regarding mastitis, are also related to milk quality.

Results: When herd owners or managers were asked in a survey, "Who trains new employees how to milk cows?", 90% responded that they perform the training. However, when the employees were asked the same question, only 23% stated they learned how to milk from the managers or owners; 78% said they learned from other employees, or they just "learned on the job". Employee responses examined by language (Spanish-speaking and English-speaking) showed that only 14% of Latino workers said they learned the milking protocols from managers or owners, which was lower than English-speaking workers. Additionally, focus group discussions found that employees strongly expressed their appreciation for education, which helped them better understand ***why*** they do their tasks and the importance of those tasks. Separately, dairy producers also noted the positive attitude of employees brought about by veterinarian-initiated education and cited several examples of improved interest and team effort on the part of the employees in the work they performed.

Conclusions: We believe that engaged employees take the initiative and work to get the desired result for the dairy operation, beyond just "doing the job." We further believe that "on-farm education", facilitated by "science teachers" who provide hands-on training, such as veterinarians, can positively impact employee engagement and thereby improve productivity on dairy operations. Proactive, routine, training, followed by 1) accountability and performance metrics, and 2) a farm culture that promotes employee feedback—such as milking efficiency analysis of a milking protocols as described in an earlier paper (Erskine) at this congress—can result in better employee engagement, more consistent protocol compliance, less employee turnover, and improved farm productivity.

Lameness

K38

Dairy cattle lameness; where does recent research take us?

Georgios Oikonomou.

University of Liverpool, UK.

Introduction: Lameness is undoubtedly one of the most critical challenges the dairy industry faces today. The condition is painful, and described as one of the clearest indicators of compromised welfare in dairy cattle (Bicalho and Oikonomou, 2013). This talk will be focused on how research currently conducted at the University of Liverpool and elsewhere is attempting to fill gaps in our understanding of lameness. We will discuss new research regarding the aetiopathogenesis of claw horn lesions (CHL), the role of the foot skin microbiome in the development of digital dermatitis lesions and developments in the field of automatic lameness detection.

Aetiopathogenesis of claw horn lesions: Despite the importance of CHL, their aetiopathogenesis is not yet fully understood. We have enrolled 2,352 Holstein dairy cows from four farms in the UK and recorded presence of foot lesions at four time points from before calving to late lactation. Cows were also genotyped. Measurements of foot angle, heel depth, backfat thickness, longissimus dorsi thickness, digital cushion thickness, and sole and foot skin temperature were taken from all studied animals to assess their role in CHL development. Additionally, measurements of the serum concentration of beta hydroxybutyrate, non-esterified fatty acids, insulin, relaxin, proinflammatory cytokines, and endotoxins have been obtained on a subset of animals as potential contributors to the aetiopathogenesis of the disease.

The role of the foot skin microbiome in the development of digital dermatitis.

Digital dermatitis is a painful, infectious, foot skin disease that compromises the welfare of hundreds of millions of production and wild ruminants. Disruption of the healthy skin microbiome has been associated with several diseases in humans. Associations between host genetics and skin integrity and/or skin microbiota profiles have also been described. We have recently showed intriguing interactions between host genetics and the foot skin microbiota profiles in cattle but the significance of host–microbiome-pathogen interactions in the development of DD lesions has not yet been studied in depth.

Automatic lameness detection.

Early lameness detection has been shown to be an important aspect of lameness management in dairy herds (Groenevelt et al., 2014) and yet for the most part relies on visual mobility/ locomotion scoring by farm staff or trained scorers. This process, albeit useful, can be time consuming and subjective even when agreement within the same experienced assessor is examined. CattleEye Ltd has recently developed and commercialised a system for automatic lameness detection. This system is the first to utilize inexpensive 2D surveillance cameras placed above the passageway exiting the milking parlour. Footage of cows exiting the milking parlour is sent directly to company servers where it is stored and processed.

The final result of the analysis is a number between 0 and 100, indicating the degree of lameness in relation to changes observed between reference points in each frame and changes observed between frames. We recently conducted a study to evaluate the performance of this video surveillance system for automatic detection of dairy cattle lameness. Our aim was to investigate the validity of the mobility scores provided by the system by comparing them against those recorded by two experienced assessors. Additionally, we examined the system's ability to detect cows with potentially painful foot lesions. Our study showed that the CattleEye system had a comparable performance to two experienced scorers when mobility score was used as a reference and outperformed the human scorer when lesion presence was used as the gold standard (Anagnostopoulos et al., 2021).

References:

Anagnostopoulos, A., B.E. Griffiths, Neary J, R.F. Smith, and G. Oikonomou. 2021. Initial validation of an intelligent video surveillance system for automatic detection of dairy cattle lameness. Page in BCVA Congress, 14-15 October 2021, Newport, South Wales.

Bicalho, R.C., and G. Oikonomou. 2013. Control and prevention of lameness associated with claw lesions in dairy cows. Livest. Sci.. doi:10.1016/j.livsci.2013.06.007.

Groenevelt, M., D.C.J. Main, D. Tisdall, T.G. Knowles, and N.J. Bell. 2014. Measuring the response to therapeutic foot trimming in dairy cows with fortnightly lameness scoring. Vet. J. 201:283–288. doi:10.1016/j.tvjl.2014.05.017.

K39

Sensible Treatment of Claw Disorders including Pain Management

Jan Shearer.

Iowa State University, USA.

Objectives: To review common approaches to treatment of claw disorders with emphasis on what therapies are useful and which are potentially counterproductive? To suggest a sensible treatment strategy that incorporates a comprehensive plan for pain management.

Materials and Methods: Information for this presentation has been derived from personal research and a review of pertinent literature on the subject of the treatment of claw disorders.

Results of a 2-Part ISU Study: *A survey of veterinarians and hoof trimmers in North America* on treatment practices indicated that topical treatments for claw horn lesions were used by 59% of veterinarians and 53% of hoof trimmers. The medication used most frequently was the soluble powder form of tetracycline or oxytetracycline (48% by veterinarians and 81% by hoof trimmers) followed by copper sulfate for veterinarians and ichthammol ointment (a sulphurous, tarry compound with mild antiseptic properties used primarily as a drawing agent) for trimmers (Kleinhenz, 2014).

Results of the survey prompted a follow-up study to determine the effect of topical treatment with oxytetracycline soluble powder or powdered copper sulfate on the rate of wound healing in 18 cows with claw lesions. Cows were randomly divided into a treatment group: treated topically with oxytetracycline soluble powder (7) or copper sulfate powder (3) and a bandage; and a control group (8): no topical treatment and a bandage. Photos of lesions were taken at 24 hours post treatment when the bandages were removed and again at day 21 following the day of treatment. Photos were evaluated by 2 independent observers who scored the lesions for the visual presence of granulation tissue and evidence of re-epithelization. Based upon observer scores at day 21, lesions topically treated with oxytetracycline or copper sulfate were more likely to have abnormal or exuberant granulation tissue (observer agreement on 15/18 observations, $p > 0.0054$) and less likely to have evidence of re-epithelization (observer agreement on 11/18 observations, $p > 0.0553$). Although the number of observations is admittedly small, these data suggest that topical treatment with oxytetracycline or copper sulfate may delay wound healing (Shearer et al., 2015).

Tetracyclines are known for their ability to cause tissue irritation when used intramuscularly and copper sulfate is corrosive to the skin and eyes. Information from the equine literature indicates that treatments with low pH and corrosive properties cause cellular toxicity, which is likely to interfere with cell migration and proliferation in the early stages of wound healing.

Use of a Bandage: Despite evidence of a therapeutic benefit, the use of a bandage for the treatment of claw lesions was cited as a routine procedure for 53% of both veterinarians and hoof trimmers alike. A well-bandaged foot simply looks better and leaves one with the sense of a more professional job. However, these esthetics are short-lived as the housing systems for most dairy cows requires them to parade through a footbath or enter a manure slurry covered floor. What started out as a clean medicated bandage soon becomes one soaked in footbath solutions and organic matter. Possibly, it is for these reasons in part that healing was delayed by the use of a bandage in three well-controlled studies (White et al., 1981; Pyman, 1997; Kluwitter et al, 2019).

Sensible Treatment of Claw Lesions: There is little dispute that corrective trimming and the use of a foot block to elevate an injured claw make good sense. However, based upon available literature and the studies cited above, there is little justification for the *routine* topical treatment in combination with the use of a bandage. More important than selecting a topical treatment or a what kind of bandage to use is having a plan that assures prompt examination and treatment of lame cows. Finally, claw lesions are painful; provisions for managing pain whether associated with therapeutic trimming or during the post treatment period are important considerations. A multimodal treatment regimen would include the following:

1. use of intravenous regional or ring block anesthesia for corrective trimming
2. sedative-analgesics to reduce the pain and anxiety
3. careful corrective trimming avoiding injury to adjacent healthy corium tissues,
4. use of an orthopedic foot block
5. administration of analgesics in the post treatment period

6. comfortable housing and attentive management of lame cows' post-treatment.

K40

Factors affecting claw lesion healing in cattle

Jan Shearer.

Iowa State University, USA.

Objective: Wound healing is a complex process generally described in terms of 4 overlapping phases including: *hemostasis, inflammation, proliferation* and *maturation*. The objective of this session is to describe wound healing in the context of claw lesions with emphasis on the factors that may interfere with these events.

Materials and Methods: Information for this presentation has been derived from research observations and a review of pertinent literature on the subject of wound healing.

Wound Healing as it applies to Claw Lesions: Restoration of a new layer of epithelium (new hoof horn) on the surface of exposed corium is the primary objective in claw lesion healing. Wounds that result in significant tissue loss or those that are heavily contaminated are generally left open to heal by 2nd intention. Wound edges cannot be opposed as with sutures and lesions are generally contaminated requiring closure of the defect by granulation, and eventually re-epithelization of the granulating tissue surface. Bridging the gap between wound margins, depending upon size and depth of the lesion may be prolonged.

The 4 Phases of Wound Healing.

Hemostasis: Hemorrhage plays a key role in wound repair as the source of blood platelets essential for blood clot formation. Within minutes, platelets enter the site of injury and begin to clump forming a clot. Coincident with the clotting process is the release of numerous cytokines and vasoactive mediators that cause vasoconstriction to reduce blood loss and activate inflammatory cells in preparation for the second phase of the healing process (Stadelmann et al, 1998; Auer and Stick, 2012).

Inflammation: The inflammatory phase is characterized by the influx of white blood cells that phagocytize bacteria and cellular debris within the site of injury. This phase is characterized by pain, swelling and loss of function at the wound site. Quite often, chronic lesions or wounds are those stuck (or stalled) in the inflammatory phase of healing.

Proliferation: The proliferative phase is characterized by angiogenesis, fibroplasia and granulation tissue formation and epithelialization. Any interference in these events has the potential to result in prolonged wound healing and a chronic lesion. The timing of topical treatments frequently coincides with key events that occur during this period.

Whereas hemostasis occurs within minutes and inflammation within minutes to hours after injury; the proliferative phase marked by the entry of fibroblasts and the formation of granulation tissue begins in 2-3 days of injury. Neovascularization supports the development of granulation tissue that fills the wound defect. Although less resistant to external factors than intact skin, granulation tissue provides an early, though imperfect, barrier to injurious agents from the environment (Stadelmann et al, 1998; Auer and Stick, 2012).

Re-epithelialization of the lesion is the ultimate objective in wound healing. The speed of re-epithelialization depends upon the severity and type of injury suffered. For example, re-epithelialization is rapid when the injury is superficial (i.e. such as an abrasion) and the basement membrane and basal cell layer are intact or minimally damaged. On the other hand, when a full thickness defect of the epithelium occurs the recovery process is prolonged. In this circumstance, residual keratinocytes at the wound site are not immediately available for recruitment to start the healing process. Instead, re-epithelialization must occur from the wound edges requiring centripetal movement of keratinocytes from the wound margins (O'Toole EA, 2001).

Persistent or Exuberant Granulation Tissue.

The clinical indication of an interference with wound healing is the presence of exuberant granulation tissue (Auer and Stick, 2012). This is corroborated by the observations that ulcers with excessive granulation tissue healed more slowly compared with lesions free of granulation tissue (van Amstel et al, 2003). Re-epithelialization of mild to moderate lesions requires somewhere around 21-30 days with more severe lesions requiring as much as 40 days and potentially as long as 60 days.

Causes of Non-Healing Claw Lesions.

One of the most frequent causes delayed claw lesion healing is infection of the exposed corium by organisms associated with digital dermatitis. The longer the corium remains exposed, the greater the likelihood of secondary infection. Complicating factors include bandaging and topical treatment of claw lesions among others. An understanding of the factors that affect claw lesion healing is important to assure a successful outcome from treatment.

K41

Dairy cattle lameness genetics; can we breed lameness out of our herds?

Georgios Oikonomou, Matthew Barden.

University of Liverpool, UK.

Genetic selection alone will not fully address the issue of dairy cattle lameness as environmental effects (i.e. housing, diet, foot trimming practices etc) have a strong influence on herd lameness prevalence. However, genetic selection can definitely complement efforts to decrease lameness prevalence through improved management and may actually have a more important role to play than previously thought.

The University of Liverpool (in collaboration with SRUC and the Royal Veterinary College) is currently conducting a large-scale study on the genetics of cattle lameness. 2,352 Holstein cows were enrolled on four dairy farms and subse-

quently assessed at four stages of a lactation cycle. At each stage, feet were inspected by a veterinary surgeon and all foot lesions were recorded and scored according to severity. Cows were genotyped and genetic indexes were assigned to each cow following national genetic evaluations. Combining data from all stages indicated that the heritability of the susceptibility to sole haemorrhages, sole ulcers, and white line lesions was 0.29, 0.35 and 0.10, respectively. Initial analyses on digital dermatitis suggest heritability estimates greater than 0.30. We also studied cows' ability to recover from sole lesions and showed a heritability of 0.27; this was only weakly correlated to sole lesion susceptibility. This suggests there is potential to selectively breed cows which can recover from sole lesions more effectively, but selecting for reduced susceptibility alone is unlikely to achieve this. Efforts to reduce the prevalence of chronically lame cows could be more successful if genetic selection for sole lesion recovery is considered alongside selection for reduced susceptibility. We also found that heritability estimates of digital cushion thickness ranged from 0.23 – 0.44 and 0.14 – 0.29 depending on the location of fat pad measurement. Our results clearly highlight the potential for genetic selection to improve resistance to lameness and the importance of accurate phenotyping. Genome-wide association analyses of claw horn lesions and digital cushion thickness traits revealed a polygenic background; candidate genes were identified with roles in immunity and inflammation, and in carbohydrate and lipid metabolism.

The same dataset was also used to evaluate the current performance of Lameness Advantage (UK lameness genetic evaluation) and the results were very promising (Barden et al., 2022). The odds ratios (95% confidence intervals) for one-point increase in the Lameness Advantage index were 0.79 (0.72 - 0.86), 0.68 (0.59 – 0.78), 0.94 (0.84 – 1.04), and 0.82 (0.74 – 91) for sole haemorrhage, sole ulcer, white line disease, and lameness, respectively. The same trends were present when the sire's Lameness Advantage index was evaluated in place of the animal's own, although the strength of this association was generally weaker. Effectively, for every two-point decrease in Lameness Advantage (worse genetics for lameness) the sole ulcer risk doubled. Similar associations were observed between the digital dermatitis genetic index and actual incidence of digital dermatitis. Effectiveness of the Lameness Advantage and digital dermatitis indexes will increase if farm lesion records improve and foot-trimming records include all lesions and are recorded on-farm software.

The additive genetic variance of foot lesions could be utilised to select for increased resistance to these lesions; novel traits such as DCT and sole lesion recovery may also be useful traits for this purpose.

References:

Barden, M., A. Anagnostopoulos, B.E. Griffiths, C. Bedford, M. Winters, B. Li, M. Coffey, A. Psifidi, G. Banos, and G. Oikonomou. 2022. Association between a genetic index for lameness resistance and the incidence of claw horn lesions in Holstein cows. Vet. Rec. 1–12. doi:10.1002/vetr.1632.

Genetics and Breeding

K42

What every farm advisor should know about dairy cattle breeding

Francisco Peñagaricano.

University of Wisconsin-Madison, USA.

Genetic selection is a very powerful tool for achieving lasting gains in dairy cattle performance. Contrary to improvements in nutrition, management or cow comfort, changes achieved through selection are incremental, cumulative, and permanent, which makes genetic improvement a very cost-effective strategy.

The basics of dairy sire selection: Dairy bulls are genetically evaluated for several traits, including different production, health, fertility, and type traits, and this genetic information is regularly compiled and published by each specific breed organization as sire summaries. There are at least ***three key concepts*** that appear in the sire summaries that should be carefully considered when making sire selection decisions. These relevant concepts are ***predicted transmitting ability*** (PTA; a measure of the genetic merit of the bull for a given trait), ***reliability*** (REL or %R; a measure of the degree of confidence in the PTA of the bull), and ***percentile rank*** (a measure of the rank of the bull within the evaluated population for the trait of interest).

Dairy sire selection for multiple traits: There are many traits, including production traits (such as milk yield and milk composition) and functional traits (such as fertility, health, longevity, and calving ability), that directly impact the profitability of any dairy production enterprise. The best method for selecting animals considering multiple traits is the use of an ***economic selection index***. This method combines multiple traits of interest into a single value, greatly facilitating the identification of the best animals. Individual traits are weighted based on relevant genetic information and their economic importance; these economic weights are based on current prices for both inputs (e.g., feed and veterinary costs) and outputs (e.g., milk prices) of a dairy production enterprise. These values are updated regularly to reflect current trends in the price of feed and milk.

K43

Economic trade-offs between productive life and genetic progress

Albert De Vries.

University of Florida, USA.

The rate of genetic improvement in dairy cattle has doubled in the last decade, in part due to the use of genomic testing which has greatly shortened generation intervals. As-

set replacement theory holds that an incumbent asset should be replaced earlier when the challenging asset is technically improved. The objective therefore was to explore how the increased rate of genetic improvement should generally affect cow replacement rates. A spreadsheet was built that determines the total cost per cow per year from the following 5 factors: cow depreciation cost, lack of maturity cost, aged cow cost, calf opportunity cost, and genetic opportunity cost. Data were based on generally available cost for the USA. Next, the average productive life was varied from 2 to 10 years and the total of these cost per cow calculated. The lowest total cost is an indication of the optimal productive life and hence the optimal replacement rate. After some sensitivity analyses, results showed that the optimal average productive life was between 3 and 4 years. The doubling of genetic progress increases optimal annual replacement rates by a few percent and therefore shortens productive life by a several months. However, the optimal productive life is still longer than the average dairy cow productive life observed in the USA which is approximately 2.6 years. The conclusion was that increased genetic progress shortens optimal productive life a little but is by itself not a strong enough factor to warrant the high replacement rates currently observed in the USA. Actual optimal replacement rates should be herd specific.

K44

Genomics: the latest revolution in dairy cattle

Francisco Peñagaricano.

University of Wisconsin-Madison, USA.

Genomic selection refers to selection decisions based on genomic-estimated breeding values. These genomic breeding values are calculated using genetic markers across the entire genome. This technology has revolutionized dairy cattle breeding worldwide because it allows breeders to make accurate selection decisions at a much earlier age, even when neither the animal nor its offspring have been assessed for the phenotypes of interest. Indeed, genomics has undoubtedly caused the most remarkable change in dairy cattle breeding since the introduction of artificial insemination.

Genomic selection has the potential to increase genetic gain considerably by reducing generation intervals and increasing selection intensity and selection accuracy. Progeny testing, the basis of dairy cattle breeding programs, is a very expensive and time-consuming process. At least 4.5 years are required for collecting semen of a potentially elite bull, rearing his offspring, and finally predicting his genetic merit based on his offspring's performance. If the bull is good enough to use in the entire population, then his first sons and daughters will be born when he is about 5.5 years of age. This long generation interval limits the rate of genetic progress. However, genomic testing allows breeders to identify superior bull calves within a few weeks of age, and hence, instead of waiting a minimum of 4.5 years, breeders can used genomic-tested young bulls before 1 year of age. This drastically reduces the generation interval. Similarly, genomic testing of heifer calves allows one to make accurate selection decisions at an early age, and superior females can eventually enter into in vitro fertilization programs, even before they reach sexual maturity. Moreover, for young bull calves and heifers, genomic testing provides more accurate PTA estimates than traditional parent averages, with average gains in reliability around 30%. Finally, greater selection intensity can be achieved using genomics because a large number of selection candidates can be screened in search of elite animals.

Overall, by shortening the generation interval and increasing the accuracy and intensity of selection, genomic selection in dairy cattle can at least double annual genetic gains. Note that the benefit of genomics is greatest for lowly heritable traits such as fertility, and traits that can be measured only late in life such as longevity. Indeed, genomics has doubled the annual rates of genetic gain for production traits but has increased from 3-fold to 4-fold for fitness traits, including female fertility, udder health, and productive life.

Finally, genomics has created opportunities to improve traits that are critically important, but too difficult or expensive to measure on the entire population. These relevant phenotypes can be measured only on a relatively small group of genotyped animals, and this reference population can then be used to predict genomic breeding values for the entire population, including young selection candidates. Examples of these important traits include feed efficiency and methane emission.

K45

Economic optimization of use of beef semen in dairy herds

Albert De Vries.

University of Florida, USA.

The use of beef semen in dairy herds in the USA has greatly increased in the last 5 years. This use coincides with the wide adoption of sexed semen which allows dairy farmers to obtain enough dairy heifer calves from targeted breedings within the dairy herd. However, various targeted breeding strategies exist, and it is not immediately clear which one is economically best and what the opportunity cost of non-optimal strategies are. Therefore, the objective was to explore how herd profitability is affected by user-defined beef and sexed semen breeding strategies, as well as finding the optimal strategy. A Markov chain herd budget calculator spreadsheet with daily steps was built that mimics the technical and financial performance of a closed herd consisting of dairy heifers and cows. Per parity, animals could be inseminated with beef, sexed, and conventional semen, depending on breeding number and genetic merit of the dam. Within breeding number, truncated normal distributions mimicked the variation in genetic merit. Hence, the genetic merit of dairy calves depended on the genetic merit of their dams. Surplus dairy calves, dairy bull calves, and crossbred dairy-beef calves were sold for fixed prices independent of genetic merit. Reproduction, milk production, feed intake, risk of culling, and associated prices were

also included such that a profit per milking cow per year was calculated. Four breeding strategies were simulated: conventional semen only, sexed + beef semen-by-age of the dam, sexed + beef semen-by-genetic merit of the dam, and the optimal breeding strategy. Surplus dairy calves were sold (using conventional semen) or not made (using the sexed + beef semen strategies). The optimal breeding strategy was sought with a non-linear optimizer within the herd budget calculator but was not necessarily found. The four strategies were evaluated at five levels of reproductive efficiency. Results showed that all sexed + beef semen strategies were more profitable than the conventional semen only strategy at all levels of reproductive efficiency. The advantage of sexed + beef semen strategies increased with greater reproductive efficiency. The user-defined semen + beef semen-by-genetic merit strategy was nearly as profitable as the optimal breeding strategy. The sexed + beef semen-by-age of the dam strategy was slightly less profitable. Genomic testing was profitable at greater reproductive efficiency but not for the sexed + beef-by-age strategy. In conclusion, the use of beef semen combined with sexed semen was more profitable than a conventional semen only strategy. Targeted breeding based on the genetic merit of the dam was slightly more profitable than when targeted breeding was based on the age and breeding number of the dam.

Teaching & Continuing Education

K47

Shortages of food animal veterinarians – a global threat

Ángel Abuelo Sebio.

Michigan State University, USA.

Many countries are experiencing shortages of food animal veterinary services in rural areas. This negatively impacts food safety and supply, public health, and animal welfare. Recruitment and long-term retention of food-animal veterinarians in rural areas are critical issues for the sustainability of the livestock industries located in regions with veterinary service shortfalls. However, these are multifaceted problems with no easy solutions. The issue of rural health workforce shortage, however, is not unique to veterinary medicine. There is a current worldwide trend demonstrating a reduction in the number of graduates from various health professions interested in pursuing a rural career. In human medicine, a 'rural pipeline into medical practice' to help to alleviate shortages of human doctors in rural areas was introduced. This pipeline is comprised by four stages: (1) structured contact between rural secondary schools and the medical profession, (2) selection of rural students into medical school programs, (3) rural experience during medical training, and (4) measures to address retention of the rural medical workforce. The impact of components of the rural pipeline on the choice of a rural medical career is strongly supported by the literature. This talk will compare the situation with similar problems of the human medicine profession and discuss what is being done and could be done to relieve these shortages through the adoption of the rural pipeline model in food animal veterinary medicine.

K49

Continue education in food animal medicine: does one size fit all?

Ángel Abuelo Sebio[1], Sabine Mann[2].

[1]Michigan State University, USA; [2]Cornell University College of Veterinary Medicine, USA.

It is now well established that the veterinary profession is facing a worldwide shortage of food-animal veterinarians in the public, private, industrial, and academic sectors. This negatively impacts food safety and supply, public health, and animal welfare. Recruitment and long-term retention of food-animal veterinarians in rural areas are critical issues for the sustainability of the livestock industries located in regions with veterinary service shortfalls. Providing access to continuing professional education (CE) is a major influencing factor for established rural professionals to remain practicing in these areas. CE has positive effects on participants' professional growth (improves confidence and competence of practitioners) and patient care. In addition, participation in CE

courses contributes to the development of a professional support network that helps to decrease burnout and compassion fatigue. However, most CE programs are not targeted specifically to graduates in their first years of practice despite new graduates choosing and needing different CE courses compared to senior veterinarians. Hence, it is essential to provide CE targeted to the areas that recent graduates in rural practice identify as their greatest needs. This presentation will showcase the development and authors' experience leading an online course on bovine medicine targeted at recent veterinary graduates in rural practice, emphasizing the differences in perceived relevance of CE topics between senior and early-career bovine veterinarians.

Nutrition & Metabolism Diseases

K51

Enegery balance and body condition, key drivers for a healthy herd

Adlai Schuler[1], Fernando Diaz Royon[2].

[1]Marshall Ridge Farms, USA; [2]Dellait, LLC., Brookings, USA.

Dairy cattle body condition and body condition scoring has long been a source of both study and debate among dairy professionals and lay people. While the scale for body condition differs depending on the country and even region, it is generally accepted that low values are equivalent to emaciation and high values are reflective of obesity. Despite a tremendous amount of literature demonstrating the effects of both overall body condition and body condition score change, widespread adoption of body condition as a documented tool for making management decisions by farmers remains relatively low.

Reproduction, production, animal health, and more recently, welfare have all been related to body condition or body condition score change. Several different reasons have been proposed for why the adoption of management strategies centered on body condition have been extremely limited. These include inconsistent training of staff on how to obtain BCS data, uncertainty on how to utilize the information when it is obtained, and a perceived lack of time by both professionals and dairymen. More recently, advancements in sensor and camera technology have helped to move subjective data measure into the objective category. Our aim with this presentation is to highlight how making body condition a key driver of decisions on the farm can improve herd health and subsequently profitability. This presentation will provide practical solutions for overcoming the challenges associated with body condition scoring in both a subjective and objective manner. It will also give examples demonstrating the benefits of tracking body condition on herd health outcomes over time regardless of the methodology used.

K53

Managing the transition period through protocols- A systems based approach

Adlai Schuler[1], Dr. Fernando Diaz-Royon[2].

[1]Marshall Ridge Farms, USA; [2]Dellait, LLC., Brookings, USA.

Producing a safe, wholesome, nutritious product at an economically viable price is the primary goal of dairy producers. Over the past century there have been significant technological and scientific advancements that have resulted in increased efficiency; increases production; decreases in environmental costs per unit of food; and decreases in the cost of production, in an unprecedented manner. While dramatic increases in productivity per cow as well as improved labor and feed efficiency ratios are the primary drivers of the reduced cost of produc-

tion, the individual cow is still the heart of the dairy economic model. No matter the size, farm level profitability is the direct result of the cow being influenced by four primary factors: 1) Genetics 2) Nutrition 3) People 4) Environment. When all four of these factors are aligned, a healthy cow results in a healthy herd, resulting in profitable farms.

To date, the dairy industry has largely relied on lagging metrics as indicators of performance. Percentage of heifers returned to the lactating herd, 21-day pregnancy risk, and 30- and 60-day cull rates, are only three of many of examples of common lagging metrics used to evaluate performance. Arguably, no singular portion of a cow's lifecycle has been as extensively documented as the transition period. The goal of this presentation is to describe the clinical aspects of managing herd health throughout the transition period. We will do this by focusing on well-defined and common disease conditions, managing those conditions extraordinarily well, and consistently utilizing the information to look ahead and see where the dairy is headed. We propose that by maintaining the cow as the center of system and implementing leading metrics (focused on the four primary factors above during the transition period) cow productivity and farm profitability can be reliably predicted.

Infectious Diseases

K55

Tools for the diagnosis and control of bovine paratuberculosis

Joseba M. Garrido.

NEIKER-Instituto Vasco de Investigación y Desarrollo Agrario, Derio, Spain.

Paratuberculosis (PTB) is an infectious enteropathy with worldwide distribution that mainly affects domestic and wild ruminants and is caused by *Mycobacterium avium* subsp. p*aratuberculosis* (Map) and triggers a regional chronic enteritis. Map infection significantly reduces the cost-effectiveness of cattle farms due to reduced milk production and early replacement of infected animals. Furthermore, Map has been associated with Crohn's disease, so it is considered by some authors as a zoonotic pathogen. The herd-level prevalence of MAP infection is over 50% in most countries with a developed substantial dairy industry. However, it should be considered that this percentage is limited by the small number of large epidemiological studies carried out in the different countries and by the difficulty in the laboratory detection of animals in subclinical stages.

Combining pathological lesions with clinical signs, two forms of infection, latent and patent, can be distinguished. Latent forms are those present in infected animals with focal lesions, very low bacterial load, low antibody titres and absence of clinical signs. On the other hand, patent forms are those presented by animals with multifocal and diffuse lesions, associated with a higher bacterial load and the presence of more or less evident clinical signs. Detection of infected animals before they present clinical signs of the disease is one of the challenges in laboratory diagnosis. So far, the combination of microbiological (culture and rt-PCR) and serological (ELISA) techniques with complementary sensitivity is the most effective alternative. However recent studies indicate that other techniques such as digital PCR could cover the shortcomings of current protocols.

Currently, the main disease-control strategy within dairy herds involves the combination of appropriate hygienic-sanitary measures and test and cull programs. Since test and cull programs are time-consuming, expensive, and eventually not as efficient as expected in part due to the lack of sensitivity of diagnostic tests, new approaches are needed. In this context, vaccination should be an alternative. It has been demonstrated its effect in the reduction of Map isolation in feces and tissues of infected animals and in the increasing of milk production and cow productive lifespan in infected farms. However, the possible interference of the vaccine with the tests used in the eradication control programs restrict its use. Another approach that is gaining relevance is the identification of genetic markers of resistance or susceptibility to the disease that can be considered in breeding programs.

In this talk all these aspects will be reviewed.

K56

What is new on IBR? Epidemiology and control at the farm level

María Guelbenzu.

Animal Health Ireland, Carrick-On-Shannon, Republic of Ireland.

Infectious bovine rhinotracheitis (IBR) is a highly infectious disease caused by the bovine herpesvirus type 1 (BoHV-1). BoHV-1, one of eight herpesviruses known to infect cattle, is an alphaherpesvirus and can also cause infectious pustular vulvovaginitis (IPV) and infectious pustular balanoposthitis (IBP). IBR has worldwide distribution and in addition to the impact on health and productivity also affects the trade of animals, semen and embryos.

In order to be able to apply successful strategies for the control of IBR at the farm level it is necessary to have a good understanding of the epidemiology of this disease.

BoHV-1 is mainly spread directly by close contact between animals. It can also be shed from the reproductive tract, including semen, resulting in venereal transmission. Aerosol transmission typically occurs over short distances but it may also occur over distances of up to 5m. The virus is moderately resistant to environmental factors so indirect transmission within or between herds can also occur indirectly through movement or sharing of contaminated facilities, equipment or personnel.

Clinical signs of BoHV-1 infection most commonly involve the upper respiratory tract and include nasal discharge, hyperaemia of the muzzle (red nose), conjunctivitis, fever and inappetence and on occasions, death. This may be accompanied by decreased milk yields and a range of negative reproductive outcomes depending on the stage of the reproductive cycle at which exposure occurs (failure to conceive, early embryonic death and abortion). However, it is also recognised that in herds with endemic infection the course of infection can be sub-clinical but nevertheless still be associated with a reduction in milk yield and negative reproductive outcomes.

Recovery following initial infection is associated with the development of immunity, but this does not eliminate the virus. Instead, the virus establishes lifelong latent infection in the trigeminal ganglion or pharyngeal tonsils. During this period the latent carrier is not shedding virus. However, at times of stress such as transport, calving, mixing stock etc, the virus may be reactivated and can begin to multiply and be re-excreted. This leads to new infection in other susceptible cattle, which in turn will also become latent carriers. These latently infected animals play a central role in maintaining IBR in infected herds, where they act as a reservoir of infection, and in spreading infection between herds.

Vaccines are used widely for a range of scenarios including to reduce the clinical impact of an outbreak, as part of an IBR control programme and to protect free herds against infection. Both live and inactivated marker vaccines are available which can reduce the clinical signs and the amount of virus shed following infection. When given intra-nasally, live vaccines can give rapid protection in the face of a clinical outbreak. When used as part of a control strategy, the percentage of infected cattle in a herd should decrease over a period of time as older, positive cattle are displaced by younger, uninfected stock. Animals vaccinated with gE-deleted marker vaccines can be discriminated from field-virus infected animals by a negative serological reaction for gE.

Understanding the relevant risk factors for (re)introduction of the virus is key information both for the design of effective control programmes and for individual farmers aiming to maintain their herds free of infection. These risk factors should be then translated into biosecurity measures that farms can apply. The main risk for introduction of infection is the purchase of latently infected animals. Biosecurity options to address this risk include maintaining a closed herd, buying known-negative stock and post-purchase isolation and testing. Other risks, including contacts at boundary fences, shows and sales, and movement of people and equipment, should also be considered.

In summary, control of IBR at the farm level is possible but, especially in endemic areas, this must be accompanied with the implementation of biosecurity measures to protect the farm from (re)introduction of the virus.

K57

Animal tuberculosis. Looking for the eradication

Joseba M. Garrido.

NEIKER-Instituto Vasco de Investigación y Desarrollo Agrario, Derio, Spain.

Animal tuberculosis (TB) is caused by infection with members of the *Mycobacterium tuberculosis* complex (MTC). *Mycobacterium bovis and Mycobacterium caprae* are the main etiological agents of animal TB a mycobacterial infectious disease with a worldwide distribution that affects cattle, other domestic hosts, wildlife and humans. The huge economic losses caused by bovine TB added to the impact of its zoonotic nature led to implement control strategies lasting for over a century in many countries. Although eradication of TB has been accomplished in some countries, the presence of *M. bovis* in herds continues to pose serious problems for animal and human health in developing countries. This discrepancy has been observed despite the similarity of the eradication programs used in the different countries. There are several reasons for the persistence of the disease in cattle, but it is usually attributed to the existence of wild reservoirs.

Different transmission pathways do exist. These include direct or indirect inhalation, oropharyngeal exposure and/or ingestion of the pathogen. Lesion distribution and progression seem to be shaped by the route of introduction of the bacterium. There is a general acceptance that the aerogenous transmission is the most frequent one in cattle and lesions are usually found in the respiratory system and associated lymph nodes (LN). Lesions can also reach these LN and other tissues or LN of the head region after oral exposure to the pathogen. However, ingestion of bacilli is usually associated with affected LN and tissues of the digestive system with or without visible lesions. Oral exposure to *M. bovis* could represent a

more relevant route of infection than previously thought. In the wildlife-livestock interface inter-species transmission is of an indirect nature, for instance through shared water or food. In these cases, infection will most likely enter the host by the oral route.

The intradermal test and the interferon-gamma release assay (IGRA) are the in vivo diagnostic methods used in test and cull-based eradication campaigns. These tests have been deemed of poor specificity because the confirmatory tests (pathological examination and culture) fail to demonstrate the presence of lesions and the involvement of *M. bovis* quite frequently. However, disagreements between confirmatory tests and in vivo methods are expected because their best sensitivity and specificity values are achieved at different immunopathological stages of the infection. In addition, cross-reactions with other mycobacteria present in the environment must be considered. Despite their limitations, these procedures have successfully eradicated the disease in many countries.

An alternative for TB eradication strategies in some scenarios could be vaccination. Probably, as has been observed with other mycobacteriosis as paratuberculosis, the first objective would be to control the disease through the reduction of mycobacteria excretion, which minimizes the risk of transmission. Several studies have been carried out in cattle and wildlife, principally using BCG but also with inactivated *M. bovis* vaccines, showing promising results.

The best option for TB control and subsequent eradication could be a combination of strategies including biosecurity measures, diagnostic follow-up of animals and vaccination, depending on the country, the situation and the host species involved in the epidemiology of this disease.

In this talk all these aspects will be reviewed.

K58

BVD. Actual situation in Europe

María Guelbenzu.

Animal Health Ireland, Carrick-On-Shannon, Republic of Ireland.

The concept of bovine viral diarrhoea (BVD) control has changed over the years. Initially, there was a focus to control the infection at herd level by prophylactic vaccination. Improved awareness of the economic impact that this endemic disease has on the cattle industry shifted the focus of control to a more systematic approach. Economic losses arise from infertility, reduced productivity and immunosuppression which, by increasing susceptibility to other pathogens, will result in increased veterinary and treatment costs as well an increased use of antibiotics on farm.

BVD is an excellent example of an infectious disease where a range of strategies have either succeeded or are currently advancing towards eradication. The main objective of this talk is to review the current status of the various control and eradication programmes for BVD across Europe and the strategies that these apply.

Two main approaches to BVD control and eradication exist and have been successfully applied by European countries. The first large scale systematic eradication programmes were initiated in the early 90s in Sweden, Denmark, Norway, Finland and the Shetland Islands. The approach taken is commonly referred to as the Scandinavian model and includes a strict non-vaccination policy. Herd-level tests are used to classify the herds into those likely to be non-infected and those with evidence of current or recent circulation of virus. Typically, these are based on antibody detection by antibody ELISA. Identification and removal of persistently infected (PI) animals from infected herds and continuous monitoring and certification of non-infected herds is also part of these programmes.

For other countries, where the seroprevalence at the animal and at herd level was considered to be too high to make the use of an initial antibody screen to classify herds suitable, the strategy focused on testing directly for PI animals. In Switzerland for example, the programme consisted of testing the whole cattle population for BVD antigen or RNA via ear notch testing in a short period of time, culling virus-positive animals, banning BVD vaccination and applying movement restrictions. This was followed by all newborn calves being sampled and tested with tissue sampling tags. In Ireland, ear notch tissue is collected from all new-born calves using modified official identity tags to test for BVD virus, supplemented by additional blood sampling in herds with positive results, including for testing of their dams. Culling of virus-positive animals and movement restrictions are also applied.

SOUND-control, a European Cooperation in Science and Technology (COST) Action, focuses on the topic of output-based surveillance for cattle diseases with either no or limited regulation under EU legislation. Limited regulation means that EU countries are not required to control the disease in their country. This COST Action provides an overview of national and regional control programmes for several cattle diseases. As highlighted by work done within SOUND control, in some countries there is a mixture of compulsory and voluntary BVD control plans depending on the region or cattle type e.g., mandatory for dairy and voluntary for non-dairy. Control plans are implemented at national or regional level and have different funding models.

A recent development in BVD control has been the inclusion of this disease as a Category C+D+E disease within the new Anima Health Law. This piece of legislation which has been in place since April 2021, includes, for the first time, an annex with requirements for programmes to be recognised at EU level and for member statues/zones to be declarer BVD-free. A number of countries/regions have had their BVD programmes approved or have had recognition of BVD-freedom.

In conclusion, a wide range of strategies for BVD control are currently applied around Europe, ranging from non-systematic control to EU-recognised freedom of disease.

Cattle Welfare

K59

Social housing of dairy calves: Why, when, and how?

Trevor De Vries.

Guelph University, Ariss, Canada.

A shift if mindset around dairy calf housing has occurred in recent years. For many years, individually housing of calves in individual pens or hutches was viewed as optimal, and nearly exclusively used in the dairy industry. Some of the cited advantages of individual housing include reduced pathogen spread from animal to animal, and increased opportunity for individual monitoring and management. While elements of those things may merit, we also know that individual calf feeding and management can be labour intense and limits social contact for calves, which may negatively affect calf behavioural development and welfare. A large body of literature now exists to demonstrate that social housing of calves, in pairs or groups, may have several short- and long-term benefits; these include: improve social skills and cognition, decreased stress, earlier and greater solid feed intake (particularly at weaning), and greater growth. There is empirical evidence that these benefits are greater the earlier in life calves are exposed to social housing. As result, more and more adoption of social housing of dairy calves is occurring throughout the industry. Despite these benefits, challenges may also occur with social housing, including potential for cross-sucking as well as increased health concerns. While potential for these challenges exist, they need not to with good nutrition, housing, and management.

K60

Managing automated milking herds to optimize health and welfare

Trevor De Vries.

Guelph University, Ariss, Canada.

The use of automated (robotic) milking systems (AMS) brings several opportunities for dairy producers with respect to cow health and welfare. There may also be situations where health and welfare challenges arise with adoption and use of AMS. Some of these challenges may result from cows not milking voluntarily, and thus achieving adequate milking frequencies at regular intervals. This is often the result of situations where voluntary milking behavior is impeded, specifically when cows cannot milk when they want to, or when cows do not want to go milk (often related to cows experiencing lameness). These situations are highly influenced by housing and management in AMS barns. Udder health may pose a challenge in AMS, however, data would suggest that it need not be. Further, we have opportunities to improve udder health and cow welfare at the end of lactation through proper dry off management in AMS. While AMS provide greater opportunity for managing nutrition at the cow level, there are situations where imbalances may occur, increasing the risk of metabolic disease including ketosis. Overall, to address these challenges no only are milking and feed management important in AMS herds, bedding and hygiene must be also be well managed to maintain good hoof health, cow hygiene, body condition, and cow comfort. Finally, in addition to being able to preventatively manage many of these potential challenges, there are also many technologies and associated data in AMS that provide increased opportunities to monitor, manage, and improve cow health.

K61

Pain assessment and management in cows and calves – Part I

Xavier Manteca

Universidad Autónoma de Barcelona, Spain.

Animal welfare is an essential element of modern animal production. First and foremost, animal welfare is grounded on ethical concerns that derive from the fact that animals are sentient beings, i.e. able to suffer and experience emotions.

Societal concern over the welfare of farm animals has increased recently and a growing number of citizens in many countries now demand that farm animals are reared, transported and slaughtered as humanely as possible. For example, according to a survey done in 2015 and involving more than 27.000 citizens from the 28 Member States of the European Union, 94 % of them think that it is important to protect the welfare of farm animals. Interestingly, this percentage ranged from 86 and 99 %, showing that even in the EU countries that are supposedly less concerned about the welfare of animals, a clear majority of citizens believe that it should be protected.

Improving animal welfare may have additional benefits. As many welfare problems have a detrimental effect on production, improving the welfare of farm animals very often has positive effects on performance. Also, improving animal welfare is one of the strategies that may contribute to reduce the use of antimicrobials in fam animals.

It is widely accepted that animal welfare encompasses not only the physical health of the animals (i.e. the absence of diseases and injuries) but also their behaviour and emotions. Pain is not only a consequence of several diseases and injuries, but also an aversive emotional experience that often interferes with the expression of normal behaviour. As such, pain is a major welfare issue in farm animals in general, including cows and calves. Therefore, pain prevention and management are key aspects of animal welfare improvement strategies.

This paper will be divided into two sections. In the first section, the principles of pain assessment in animals as well as the economic consequences of pain will be reviewed. The second section will address the major causes of pain in cows and calves. In addition, the general principles underlying some of the strategies to prevent such causes will be discussed.

Pain includes a sensory and an emotional component, the latter being particularly important from an animal welfare standpoint. As emotions are not easily measured in animals, pain assessment is difficult. Indeed, the gold standard of pain assessment in humans is self-reporting, which is not possible in animals. Although there are several physiological indicators that can be used to assess pain or inflammation in animals, including for example plasma concentration of cortisol and acute phase proteins, these are mainly useful in an experimental setting and are not feasible in field conditions. Indeed, assessment of pain in animals in field conditions is mainly based on the observation of behavioural changes. Some behavioural changes will appear regardless of the cause of pain and these include a reduction in feed intake and rumination; licking, rubbing or scratching painful areas; grinding teeth; altered social interactions, and changes in posture to avoid moving or causing contact to a painful body area. Scores based on facial expressions were originally developed to assess pain in laboratory animals and have more recently been developed for some farm species. These scores have been shown to be valid and reliable tools to assess pain and have the advantage of requiring minimal training.

There is growing evidence that pain in farm animals has negative consequences on production efficiency and economic profit. For example, work done in dairy cows has shown that the administration of an anti-inflammatory drug with analgesic properties to cows with mastitis in addition to the usual antibiotic therapy reduces subsequent culling rate. Although the mechanisms underlying this effect are not properly understood, it has been suggested that it may be due to the negative effects of pain on fertility. Similar beneficial effects of NSAIDs in calves with respiratory problems have been found. Studies carried out in several species suggest that preventing pain caused by parturition has positive effects on both the dam and the offspring.

K62

Pain assessment and management in cows and calves – Part II

Xavier Manteca.

Universidad Autónoma de Barcelona, Spain.

Pain in farm animals can be caused by diseases and injuries, husbandry practices, and parturition. Mastitis and foot problems leading to lameness are among the main painful conditions in dairy cows. Hyperalgesia (i.e. an increased sensitivity to pain) has been described in farm animals because of painful conditions such as lameness and mastitis.

Research has shown that all clinical mastitis, including grade I mastitis (i.e. those that result in changes in the aspect of milk only) are painful. For example, it has been shown that cows with mild and moderate mastitis have significantly larger hock-to-hock distances compared with healthy cows, suggesting that they modify their stance to reduce pressure on the udder. Moreover, increased restlessness during milking, including a high frequency of kicking and stepping, has been observed for at least 3 days after mastitis detection.

Animals with foot conditions suffer long-lasting pain that may commence well before lameness is apparent. Although different locomotion scoring systems have been developed for routine use by farmers, it should be noted that lameness is usually underreported by producers. Signs such as head bobbing, arching of the spine and changes in stride length allow a rapid identification of lame individuals.

Management of pain caused by mastitis and foot conditions includes both the appropriate use of analgesia and anaesthesia as well as changes in management and husbandry that reduce the risk of mastitis and lameness. The link between animal welfare and animal health (including the absence of conditions such as mastitis and lameness) includes several aspects. As health is an important part of welfare, medical conditions must be considered as welfare problems. Additionally, many welfare problems that are not directly related to physical health have an important effect on the risk of animals developing medical conditions such as mastitis and lameness. This is partly due to the fact that chronic stress (which is a welfare problem) may cause immunodepression. Also, many welfare issues related to housing and behaviour (including for example thermal and physical discomfort, negative social interactions between animals and poor human-animal relationship, among others) may have a direct effect on the risk of animals developing lameness and mastitis.

Some of the main painful husbandry practices in cows and dairy calves are tail docking, disbudding and dehorning. The alleged benefits of these practices should be weighed against their negative effects on animal. Tail docking provides a good example to illustrate this principle. For example, dairy cows are oftentimes tail docked based on the assumption that tail docking reduces the risk of mastitis; however, there is no scientific evidence at all that supports this assumption.

Disbudding and dehorning are always painful, regardless of the method used and the age of the animals. Moreover, there is now evidence that pain caused by disbudding may result in negative cognitive bias in calves, i.e. calves will judge a neutral stimulus as being negative. Cognitive bias is considered to be a useful indicator of the general emotional state of the animals, and the effects of disbudding on cognitive bias illustrate the far-reaching consequences that this practice -if not done with appropriate analgesia and anaesthesia- may have on the welfare of the animals. Also, the experience of pain very early in life may have long-lasting consequences in pain sensitivity and there is now evidence that animals that suffer pain shortly after birth may remain more sensitive to subsequent pain for a long period.

It is well accepted that dystocia results in pain in both the dam and the offspring. Whether normal parturition is also painful is less clear. However, even after normal parturition, plasma levels of haptoglobin remain higher than normal for at least 15 days in heifers and 4 days in cows. Haptoglobin is an acute phase protein that increases because of tissue damage and inflammation.

Feedlot

K65

Bovine Respiratory Disease in Feedlot Production – Past, Present, and Future

Calvin Booker[1], Ryan Rademacher[2].

[1]Feedlot Health Management Services, Ltd., Okotoks, Canada; [2]Feedlot Health Management Services by TELUS Agriculture.

The ***objective*** of this presentation is to inform industry stakeholders about the evolution of bovine respiratory disease (BRD) prevention and control strategies in feedlot production.

The ***materials and methods*** utilized to achieve this objective include rigorous research and development, large scale field trials performed in commercial feedlots, and an extensive database and analysis of animal health outcomes. Additionally, the collection of experts at Feedlot Health are at the leading edge of new technologies and strategies for feedlot production and health management and thus have insights into the future of BRD.

The ***results*** of these data and experiences show us how BRD management has evolved and continues to evolve over time. In the early years of feedlot production, the tools available to manage BRD were limited. Not only has our knowledge of the disease process and risk factors improved, but so have the technologies (e.g. vaccines and antimicrobials) available to prevent and control disease. The industry has moved from treating all cattle entering feedlots relatively similarly, to differentiating sub-populations based on their specific risk factors and managing those populations accordingly. Into the future, new technologies will continue to be developed and properly evaluated to identify those that provide more precise and accurate disease prediction down to the individual animal level. In conjunction with continually improving understanding of disease processes, the advancements in disease prediction and detection technologies will allow for evaluation of differential management strategies tailored to the individual animal rather than the group average.

In ***conclusion,*** BRD management has evolved with better ability to identify risk factors and predict outcomes, as well as improved tools to prevent and control disease. In the future, new technologies will allow for differential management down to the individual animal level. Specifically tailored protocols will continue to improve animal health, animal welfare, judicious use of antimicrobials, and overall sustainability of cattle producers and the industry.

K66

The use of technology for cattle health management in the feedlot

Calvin Booker[1], Eric Behlke[2].

[1]Feedlot Health Management Services, Ltd., Okotoks, Canada; [2]Feedlot Health Management Services by TELUS Agriculture.

The ***objective*** of this presentation is to inform industry stakeholders about the current status and plans for future evaluations of several technological advances that have been developed, and are being tested for use in managing cattle health in feedlots.

The ***materials and methods*** utilized to achieve this objective included the evaluation of the following technologies in the large-scale commercial feedlot environment: Remote Early Disease Identification System; Whisper Veterinary Stethoscope; QScout Blood Leukocyte Differential; and Infrared Thermography. Following real-time monitoring and(or) data collection with each of these technologies, chute-site data collection software was used to track morbidity and mortality outcomes. The morbidity and mortality outcomes were then used to retrospectively identify the diagnostic value(s) of the data generated by each respective technology.

The ***results*** from these evaluations indicate that each of these technologies provide additional information that may have the potential to be used to make differential management decisions, but the cost effectiveness of this differential management has yet to be elucidated and will require continued evaluations of these technologies.

The collective ***conclusions*** from these evaluations are that feedlot veterinarians, owners, and managers continue to embrace and adopt technologies to optimize their operations and increase their return on investments. Like in many industries, a growing number of innovative technologies promise to enhance the efficiency and sustainability of production, but each technology must be scrutinized and assigned a value proposition prior to full-scale deployment.

Diagnostic Imaging

K67

The Evolution of Bovine Reproductive Ultrasound and the Role of Veterinarians

Jill Colloton.

Bovine Services, Edgar, USA.

Objectives: The objective of this presentation is to review the parallel evolution of reproductive management of cattle and the use of ultrasound as a diagnostic tool since 1995, to consider possible future developments, and to encourage veterinarians to be the primary providers of ultrasound diagnostic services.

Materials and methods: Historical documents and personal experience in practice were compiled to present a timeline of the evolution of management and diagnostic methods for bovine reproduction. Current research was reviewed to understand possible future developments.

Client interviews were utilized to understand the producer's perspective in choosing a provider for diagnosis and consultation regarding the reproductive health programs on their farms.

Results: Parallel advances in the management and diagnosis of bovine reproductive health over the last 30 years have driven the need for skilled consultants and diagnosticians. Producers recognize that their veterinarians are uniquely suited to understand the current science and best provide these services. They also feel their veterinarian is more cost effective and reliable than hiring a technician or training a farm employee.

Conclusions: As the capabilities of ultrasound technology and our knowledge of bovine reproductive management evolve veterinarians are uniquely equipped to provide the best service for producers.

K68

The Doppler in field reproductive management: effective resource or pure fantasy?

Giovanni Gnemmi.

BOVINEVET INTERNACIONAL Bovine Ultrasound Services & Herd Management, Huesca, Spain.

Objectives: Is the Doppler a technique that we can use on a daily basis in dairy/beef farms? Is Doppler a technique that only has a reason to be used in research?

Can we use Doppler for the diagnosis of non-pregnancy in cattle? Are there differences between dairy and beef cattle? When is it convenient to perform this examination?

Can we use Doppler for the selection of recipients in embryo transfer?

Is it possible to use Doppler to define the time of ovulation?

Is it possible to use the Doppler to define the time of the oestrus cycle?

Is it difficult to learn to use Doppler? Is it difficult to set up the ultrasound scanner to use the Doppler? Is it convenient to buy an ultrasound scanner with Doppler?

Can I use Doppler to define the physio-pathology of the uterus?

Can I use the Doppler to make a diagnosis of embryonic distress and/or can I use this technique to define possible reasons for a possible therapeutic strategy?

These are some of the questions that the practitioners involved bovine reproduction regularly asks their self when deciding whether to engage in this technique. The purpose of this presentation is to give a simple and honest answer to these questions.

Materials & Methods: Personal experience and a critical analysis of the bibliography on the subject of Doppler in bovine reproduction are shared. The point of view of the customer, i.e. the buyer of the service, is shared. The state of the art of the technique in practice and research is defined, and an attempt is also made to understand the possible developments of the technique also in the light of artificial intelligence.

Results: The Doppler today, for the veterinarian involved in reproductive management in the field, has its own reason for use in the indirect definition of the level of progesterone produced by the corpus luteum: this certainly opens up the possibility of more precise ultrasound reproductive management.

Doppler assessment of the corpus luteum allows an accurate diagnosis of non-gestation: 5.0-10.0% false positives and approximately 0.5-1.0% false negatives.

In the selection of recipients for embryo transfer, the possible use of the Doppler technique is opening up. However, according to the writer, it will take some time before this technique can be applied on a scale. Ad of In the selection of recipients for embryo transfer, the possible use of the Doppler technique is opening up. However, according to the writer, it will take some time before this technique can be applied on a scale. To date, the accuracy of the technique is greatly affected by two factors: the quality of the Doppler used and the experience of the technician.

The other possible applications of the Doppler technique are still at an experimental stage and have not, however, encountered definitive scientific validation.

Conclusions: It is certain that Doppler will be incorporated into all ultrasound units, even ultra-portable ones, within the next five years. With regard to its possible actual use in the field, i.e. outside scientific research, it is currently difficult to see a concrete application outside the assessment of the degree of vascularisation of the corpus luteum. The use of artificial intelligence will probably make the technique usable on a scale, reducing the objective limitations, today represented by the set up of the ultrasound unit and above all by the experience of the technician.

For those who work in the reproductive management of beef cattle and where fixed-time insemination with re-synchronisation is used, this technique finds immediate application. It is worth remembering that while around 25 million

fixed-time inseminations are carried out in Mercosur today, in other continents such as Europe, artificial insemination, and especially fixed-time insemination of beef cattle, is still an embryonic technique, also due to a whole series of limitations and prejudices related to synchronisation. In dairy cattle, on the other hand, where fixed-time insemination is widely used, there remains the problem of defining a re-synchronisation programme, currently unavailable, that allows a weekly diagnosis of non-gestation.

K69

Use of ultrasound to improve reproductive management in dairy or beef cattle

Giovanni Gnemmi.

BOVINEVET INTERNACIONAL Bovine Ultrasound Services & Herd Management, Huesca, Spain.

Objectives: The purpose of this presentation is to demonstrate how the use of ultrasound allows us to define in which phase (luteal-follicular) of the oestrus cycle the cow is, but also in which part of each phase (meta-oestrus, di-oestrus; pro-oestrus, oestrus). The main purpose of this evaluation is to optimise the use of oestrus synchronization of the ovulation, pre-synchronisation and re-synchronisation. But also to be able to establish, on the basis of the follicular map and the ultrasound stratigraphy of the uterus, if and when the cow could enter oestrus.

Materials & Methods: A portable or ultra-portable ultrasound scanner, working in B-Mode, allows us to define with great precision whether we are in the luteal phase or the follicular phase of the oestral cycle. In the luteal phase the hormonal profile is progestin, so the myometrium and endometrium have the same thickness and echogenicity when analysed at a frequency of 5.0-10.0 MHz. The uterine lumen, if the cow does not have a chronic inflammatory process of the uterus, will be a virtual space.

In the follicular phase, the hormonal profile is oestrogenic: the myometrium is contracted and, due to the increase in acoustic density, will be echogenic. The vascular tonaca is thickened and the endometrium is oedematous, causing dilation of the blood vessels, which will appear as small anechogenic spaces in the endometrial structure. The endometrium is hypo-ecogenic and the uterine lumen shows increased fluid, which is anecogenic. The shape of the uterine lumen is star-shaped or Christmas tree-shaped.

Once the phase of the cycle is established, we move on to define the follicular map and the presence of the corpus luteum or corpora lutei, whose age will be established on the basis of the presence of one or more dominant follicles and their size. Once it has been established in which moment of the cycle the cow is, it is possible to give indications as to whether and when she will come into oestrus, but also to establish with great precision, whether it is possible and/or convenient to resort to oestrus synchronisation (PGF2a), or synchronization of the ovulation.

Results: The more thorough the technician's knowledge of the oestral cycle and the better the quality of the ultrasound unit in use and its set-up, the greater the accuracy of the method. Possible sources of error may arise from the presence of co-dominance and/or the presence of a persistent dominant follicle from a previous oestral cycle. This assessment is based on a pre-judgment: that if we visit dairy cows, they are two-wave growth, whereas when we work with beef cows and/or heifers (both dairy and beef), they are three-wave follicular growth.

Conclusions: This technique makes it possible to define with extreme precision the estrous window in which the cow is located and whether the cow is cyclical. This makes it possible to optimise all forms of hormone therapy. However, this technique opens up a great opportunity in herds where, for various reasons, it has been decided not to resort to the systematic use of pre-synchronisation or oestrus synchronisation. It is a technique that fits in perfectly with all oestrus detection systems and allows confirmation of the indication derived from electronic or manual oestrus detection systems. It is a very precise technique, but based on a single ultrasound evaluation, it is not free of errors, which may result from the initial pre-judgment in the definition of the follicular waves and not only.

New ultrasound scanners, even ultra-portable ones, already allow automatic measurement of follicular maps. In a few years' time, with the advent of artificial intelligence, it is very likely that the accuracy of this diagnostic method will improve, making it possible to reduce the natural margin of error that exists today.

K70

Thoracic ultrasonograpy in calves: an affordable diagnostic tool in farms

Sebastien Buczinski.

Faculté of veterinary medicine, Saint-Hyacinthe, Canada.

Thoracic ultrasonography is an emerging field of research for bronchopneumonia diagnosis and management in cattle. The technique can be fastly applied in farms with the same ultrasound unit that is commonly used for reproductive purpose. Thoracic ultrasonography helps to characterize the importance of lung lesions (mainly consolidation) that are negatively associated with health and production outcomes. This ancillary tool can be useful to assess calf lung health and to monitor implementation of mitigation strategies for respiratory disease prevention and treatment. Thoracic ultrasonography is an emerging field of research for bronchopneumonia diagnosis and management in cattle. The technique can be fastly applied in farms with the same ultrasound unit that is commonly used for reproductive purpose. Detection of lung consolidation with ultrasound is reliable between different operators with minimal training. It is important to perform a bilateral examination because unilateral lung lesions are common. The right cranial lung is of special interest in calves and its specific

landmark with the internal thoracic artery and vein. Thoracic ultrasonography helps to characterize the importance of lung lesions (mainly lung consolidation) that are negatively associated with health and production outcomes. Animals with lung lesions (using various thresholds and consideration for case definition) have been found at higher risk of dyingor being culled before the first calving. Other findings associated with lung consolidation are decreased average daily gain during the preweaning period and decreased hazard of first pregnancy. Finally, decreased milk production during the first lactation has also been reported. In feedlot, beef and veal calves, thoracic ultrasonography although less studied has also been associated with various negative outcomes.

This ancillary tool can be useful to assess calf lung health and to monitor implementation of mitigation strategies for respiratory disease prevention and treatment. Thoracic ultrasonography is a fast and affordable diagnostic test that can be used calf-side with no specific investment. This is an extra-tool in the bronchopneumonia diagnostic toolbox. However, this is not a magic tool and limitations should be known when trying to implement it in practice.

Sustainable Cattle Productive Systems

K71

Sustainability in action – how do we "meat" demand without "milking" the environment

Jude Capper.

Livestock Sustainability Consultancy, Harwell, Oxfordshire, UK.

Sustainable food production is one of the most often-discussed issues within agriculture, given concerns regarding climate change, resource use, animal health and welfare, antimicrobial resistance and the provision of affordable food. Although myriad definitions of sustainable food exist, the most widely-accepted comprises a balance between economic viability, environmental responsibility and social acceptability, yet the latter component has recently become disproportionately important, as consumers have increasing questions about how their food is produced.

Livestock productivity must continue to increase in line with future population growth, so that sufficient animal source foods can be produced to fulfil consumer requirements, while lessening the impact on the environment. For example, in the USA, the move towards large-scale intensive farming conferred a reduction in the greenhouse gas emissions (GHGe) per kg of milk by 63% between 1944 and 2007, with a further 19% reduction between 2007 and 2017. Similar effects were achieved in U.S. beef systems, with an 18% reduction in GHGe per kg between 1977 and 2007; and in both pork (35% reduction between 1959 and 2009) and egg production (63% reduction between 1960 and 2010). A clear differentiation should be drawn however, between improving productivity in all livestock systems with due regard for social, economic and resource use constraints, and imposing or prescribing practices or systems with regards for sustainability impacts or trade-offs. Given the billions of smallholder and subsistence farmers across the world who rely on livestock for myriad reasons, a wholescale global transition to intensive production systems is not the solution.

Animal health is one of the key determinants of productivity, with over 20% of global animal protein lost to disease. As healthy animals produce greater yields of milk or meat, or grow at a faster rate, improving animal health reduces both the economic costs and the environmental impacts of livestock production. Animal health is also a significant consideration for many consumers, who want to be reassured that that the milk, meat and eggs that they buy come from healthy livestock. Good animal health therefore promotes social acceptability, reduces the risk of public health issues and reduces the need for veterinary medicines – a significant positive effect given the threat of antimicrobial resistance to both animal and human health. The economic benefits accruing from improved animal health, also allow improved affordability of meat, milk and eggs to the consumer.

The extent of the environmental and economic improvements conferred by improved livestock health and the quantity of data in the literature varies considerably between species and diseases. For example, the economic costs of bovine respiratory disease complex and infectious bovine rhinotracheitis

are relatively well-defined, and multiple papers have quantified the reductions in GHGe conferred by improving mastitis incidence in dairy cattle; yet the economic impacts of many other diseases tend to be dated, with no quantification of associated resource use or GHGe. Considerable knowledge gaps therefore exist relating to interactions between productivity, livestock disease, economic cost and environmental impact. These gaps urgently need to be filled, both to help producers to understand the economic and environmental cost:benefit ratios of management practices or treatment decisions, and to allow downstream food industry stakeholders (e.g. processors, retailers and restaurants) to make informed decisions.

Improving livestock productivity through enhanced animal health provides a strategy to mitigate environmental impacts, increase economic viability and reassure consumers for whom health and welfare are key concerns. This triple-win approach aligns with One Health principles and provides a clear mechanism to enhance sustainability, however, the implementation of global animal health protocols and access to veterinary medicines remains an ongoing challenge.

K72

Feeding the world without devouring it

Frank M. Mitloehner.

University of California, Davis, USA.

By 2050, Earth will be home to nearly 10 billion people, a tripled human population during our lifetime. Only 1.8% of the Earth's surface is arable land that can be used for growing crops, a resource not likely to increase, which means the amount of cropland per person will decline by 20%. In the face of finite resources and a changing climate, we need sustainable solutions to the 2050 food challenge. Our current food system is often criticized for not addressing chronic undernutrition, micronutrient deficiencies and obesity. And agriculture does contribute to issues of environmental sustainability. Livestock production is often seen as particularly egregious, and some people say we can better meet the 2050 food challenge by limiting or eliminating animal-source foods from our diet. Critics of animal agriculture go as far as to claim that globally, livestock produces more greenhouse gases than the entire transportation sector. Less livestock production would reduce greenhouse gas emissions, provide more food for humans by decreasing feed needed for livestock, and free up rangeland and feedlots for crop production. In short - Eat less meat to save the environment. At first glance, this simple solution seems elegant and one that should be readily adopted. However, the truth is far more complicated.

K73

Cows of the future – challenges and opportunities for sustainable cattle systems

Jude Capper.

Livestock Sustainability Consultancy, Harwell, Oxfordshire, UK.

There is no "one-size-fits-all" sustainable cattle system – the degree to which different livestock systems are considered sustainable depends on the region, culture, market and metric of choice. Sustainable systems balance environmental responsibility, economic viability and social acceptability, yet of these three factors, the first has become disproportionately important. The widespread media coverage of climate change issues, augmented by the outcomes of the recent COP26 Conference and the publicity afforded to the primarily plant-based "Eat Lancet" diet, means that ruminant systems are often cited as being environmentally damaging. An urgent need therefore exists to improve the greenhouse gas emissions (GHGe) of all cattle operations to demonstrate, highlight and communicate our dedication to improving environmental sustainability.

Livestock industries in high-income regions have tended to reduce GHGe per kg of food produced through improvements in genetics, nutrition, health and management over the past century. Given that the USA-based dairy cow that holds the world record for 365-d milk production yielded 35,437 kg, it is clear that further genetic gains may still be available in dairy systems. The difficulty of processing and marketing larger beef carcasses means that increasing carcass weight may not be a sustainable strategy for beef production, however considerable opportunities exist through improving both age at slaughter and reproductive efficiency. If appropriate breeding goals are identified to ensure that cows and calves can make the best use of the resources available; pasture and feed are managed efficiently and with due regard for optimal production; and livestock health is made a priority; then resource use and GHGe may be reduced. Producing beef from dairy systems can also reduce GHGe, because a considerable proportion of the dam's environmental impacts can be allocated to milk production. These systems therefore may improve environmental responsibility, in addition to solving one of the major social acceptability issues of dairy production – the fate of dairy bull calves. Traditional suckler/cow-calf systems will therefore need to be proactive in communicating their role in producing food from grassland that is unsuitable for other food or fibre production, while sequestering carbon.

Technology use should be encouraged wherever possible, from basic husbandry practices (e.g. weighing cattle), to reproductive, growth and management technologies that are novel, not yet widely adopted or still under development (e.g. sexed semen, hormone implants, methane inhibitors). Crucially, this must be undertaken in combination with improved data collection, recording and benchmarking. Future consumers will demand information on a range of sustainability metrics (e.g. GHGe, biodiversity, medicines use, community support, etc), yet this will only be possible via credible data. At present there is no standard GHGe tool for use across the globe, and the tools available produce wildly differing results. With national, supply chain and product GHGe quantification becoming mandatory, a standard tool is required to ensure fair comparisons

and provide insights into the effects of on-farm practices.

One of the greatest sustainability issues that the cattle industry needs to overcome is the gap in knowledge and understanding between the producer and the consumer, which, when hitherto-underknown practices are exposed, may lead to accusations that farmers lack transparency or are cruel to animals. This is challenging in an era where television, internet and social media have overtaken traditional print media and literature as information sources and arguments against livestock production that appeal to aesthetic or ethical values are sometimes more successful than science-led information. Rather than trying to combat anthropomorphic or ethical claims with scientific facts, we need to combine the two, acknowledging that we share consumer desires for affordable healthy food, excellent animal health and welfare and reduced environmental impacts, and demonstrating a clear commitment to systems and management practices that promote these.

Ultimately, consumer trust is key to maintaining the social acceptability of cattle production. A sustainable future for cattle production will be independent of either economic viability or environmental responsibility if the market ceases to exist for milk and meat. However, if we improve livestock productivity, technology adoption and data recording in conjunction with improved consumer communication, we should be able to balance the three pillars of sustainability and ensure that milk and meat are still on the menu in years to come.

K74

Livestock and climate change – Facts and fiction

Frank M. Mitloehner.

University of California, Davis, USA.

Animal agriculture is often shouldered with a large part of the blame when it comes to climate change, and that's in part to the fact that we haven't been looking at all greenhouse gases correctly. While methane – the main greenhouse gas associated with animal agriculture – is a potent climate pollutant that we can and need to reduce, it warms our atmosphere differently than other gases because of its short lifespan. Methane persists in our atmosphere for about a dozen years before it's broken down via oxidation, and it's that atmospheric removal that is often neglected when trying to characterize methane's warming impact. Furthermore, if we can reduce methane emissions to the point where more is being broken down in the atmosphere than is being emitted, we'll see animal agriculture go from being blamed for climate change to being recognized as a major climate solution. By rethinking methane, we can see that animal agriculture's path to climate neutrality is within reach as scalable solutions offer the global community tools to fight global climate change.

Surgery

K76

Cesarean section in cattle

Sylvain Nichols.

Montreal University, Canada.

Introduction: Dystocia in dairy and beef cattle are fairly common (1.1-6.8% of all calvings). Vaginal manipulation can resolve the dystocia. However, in cases of fetal disproportion, cervical inertia, malformation or complicated malposition, a c-section will be necessary.

Preoperative treatments: Preoperative antibiotics, such as procaine penicillin, should be given. It is also appropriate to give a NSAID, such as meloxicam. The surgery site is clipped and prepared appropriately for surgery (washed and scrubbed). Most c-sections are done with the cow standing and restrained in a contention chute. Sedation is rarely needed.

For a standing procedure, the flank is anesthetized by paravertebral block (proximal or distal), inverted L or line block. The technique chosen is often based on surgeon experience.

Surgical approaches: Typically, the left paralumbar fossa is used to access the uterus. From this approach, the rumen acts as a barrier to keep the jejunum in the abdomen. Exteriorization is crucial with a dead calf. However, with a live calf that had minimal obstetrical manipulation, the uterus can be opened within the abdomen.

Ventral approaches are possible in cattle. They are usually performed on dead or emphysemateous calves. The ventral-midline and the right paramammary have been described in beef cattle. Those are more difficult to execute on dairy cattle because of the size of the udder and the massive vascular network. The para-mammary/inguinal approach can be used in dairy cattle. The ventral approach provides a more direct access to the uterus.

Surgical techniques: Hysterotomy is performed on the greater curvature. The calf is extracted gently to avoid tearing the uterus. With the uterus still exteriorized, the uterus is checked for another calf. The placenta, if detached, is removed. If it is still attached, scissors are used to remove the part that comes out of the hysterotomy. A double inverting pattern is the technique of choice for closure of the uterus. The patterns that can be used are continuous Utrech, Cushing or lembert. On the second layer, it is important to burry the knots to avoid adhesions. Absorbable suture material of USP 1 is appropriate. Some advocate the use of monofilament instead of multifilament to decrease the drag effect. If the latter is used, it is important to push the tissue over the suture rather than pull the suture through the tissue to avoid the dragging effect and tearing of the uterus.

Before being returned in the abdomen, the uterus is cleaned of blood clots and debris. If the surgery was contaminated and if possible, the abdomen should be thoroughly lavaged with sterile isotonic solution. Ideally, the lavage solution is evacuated, by massage or by suction prior to closing the abdomen.

Postoperative treatments

Antibiotics should be continued and readjusted according to the surgical findings (live vs dead calf, clean contaminated vs contaminated surgery) and any co-morbidities occurring (mastitis, metritis, etc). NSAID should be given as needed and according to the general status of the cow.

The postoperative period following a c-section performed to extract an emphysematous fetus is challenging. Intravenous fluids, broad spectrum antibiotics and oral transfaunation are often necessary for the first postoperative days.

Complications and Prognosis: The status of the calf has an impact on the severity of the postoperative complications. The most severe complication would be peritonitis. Retained fetal membrane is another complication. It needs to be treated more aggressively because the hysterotomy site can be an opening and cause secondary peritonitis. According to Lyons et al, the presence of retained membrane has a negative impact on the survival of cows 14 days after the procedure.

According to Lyons et al, exteriorizing the uterus and removing the abdominal blood clots during surgery has a positive impact on the survival of the cow. Also, c-section done because of feto-pelvic disproportion of a female calf has a positive impact on cow survival.

Tenhagen et al, showed that cows undergoing a c-section produce less milk at the beginning of lactation and are more likely to be open at 200 DIM. Therefore, they are more likely to be culled when compared to a control group.

K77

Surgical management of common intestinal conditions in adult cattle

André Desrochers.

Université de Montréal, Canada.

The objective of this presentation is to present some basic intestinal conditions that can be handled in field situation with emphasis on duodenum and jejunum.

Surgical approach: The most common surgical conditions reported in the literature are: intussusception, volvulus of the jejunum, incarceration and jejunal hemorrhage syndrome. Other less frequent conditions are: gut tie in steers, duodenal obstruction, trichobezoar, ileal impaction and sigmoid flexure volvulus of the duodenum. Since the last few years, we have more sigmoid flexure volvulus than any other intestinal surgical condition combined. Intestinal surgeries are performed usually through a right flank incision while the animal is standing. Preoperative analgesia is provided to the patient before invading the abdomen. Mild sedation might be necessary. Broad spectrum antibiotics must be given before the surgery. The flank is anesthetized by linear infiltration of lidocaine or with a paravertebral block.

In cattle, the mesentery is short and fatty rendering vessels ligation difficult. It is recommended to infiltrate the mesentery with lidocaine before resection to alleviate the pain from pulling on the mesentery. Only the affected portion of the intestine is exteriorized. End-to-end anastomosis is more commonly done than side-to-side. Doyen forceps, silicone or rubber tubing, and umbilical tape have been used to keep the resected section tight while suturing. The anastomosis is sutured with one layer of full thickness simple interrupted sutures with 2-0 absorbable suture on a swaged on needle. Simple continuous suture can be used but should be interrupted at one point to avoid a purse string effect (2X 180º). The mesentery should always be sutured to avoid incarceration. **Specific intestinal diseases.**

Intussusception is reported to be more common in young cattle and Brown Swiss. The most common site of intussusceptions is jejunum followed by colocolic. Effective reduction of the intussusception in surgery is rarely feasible therefore, surgical resection is indicated. The prognosis is fair.

Intestinal volvulus is rapidly fatal if not treated. Volvulus can involve either the ileal flange of the jejunum or the root of the mesentery. Surgery is an emergency. Exteriorization of a portion of the jejunum is necessary to reduce the volvulus. Resection of the jejunum will be performed if the ileal flange underwent irreversible vascular damage. The prognosis is 86% if the ileal flange is involved and 44% with a volvulus of the root of the mesentery.

Jejunal Hemorrhage Syndrome has a high mortality rate (60-100%). Although medical assistance is necessary in all animals, surgery is indicated only if there are signs of obstructions: scant feces, distended jejunum with or without intraluminal mass at rectal palpation and transabdominal ultrasound findings. Otherwise the animal is treated medically with 2-4 liters of mineral oil orally, IV fluids or blood transfusion and systemic antibiotics (β-lactam). The affected segment of jejunum is easily identified at surgery. There are 3 surgical options: aborad massage of the clot, enterotomy and resection anastomosis. Decision is based on the length of the clot and the integrity of the jejunal wall. Prompt laparotomy and manual massage had a higher survival rates in one study. Medium and long term survival rate was higher in cattle referred 24 to 48 hours after onset of signs. The recurrence rate although was high.

Duodenal obstruction should be suspected if there is a severe hypochloremic metabolic alkalosis with a small 'ping' behind the last right rib and absence of intestinal distension at the rectal palpation. If the cause cannot be determined, a temporary diagnosis of ileus is given and large volume of intravenous fluids is administered for 12 to 24 hours. Enterotomy can be performed on the cranial and descending duodenum to remove a foreign body or trichobezoar. However, any obstruction involving the sigmoid flexure of the duodenum is difficult because it cannot be exteriorized.

The volvulus of the sigmoid flexure of the duodenum is a surgical condition and diagnosis is confirmed during the laparotomy. The sigmoid flexure is severely distended with gas and a volvulus is palpated at its root close to the neck of the gall bladder on the visceral part of the liver. The volvulus is reduced and cranial duodenal content is milked through the flexure to ensure that it's functional. The prognosis is good if the sigmoid flexure is not necrotic.

K78

Distal Limb Fracture and Pin Casting

Sylvain Nichols.

Montreal University, Canada.

Limb fractures are frequent in cattle. They often involve the metacarpus and the metatarsus, but they can occur in all long bones of cattle.

A pin cast is a modified type II external fixator. The commonly used sidebars are replaced by casting material made of fiberglass. A pin cast can be used on fractures involving the metacarpus and metatarsus and the distal radius and tibia.

Surgical Preparation: The procedure is performed with the animal under general anesthesia or under sedation with regional anesthesia (brachial plexus bloc, epidural, or IV under tourniquet). The position varies according to surgeon preference and the equipment available. The surgery site is clipped, cleaned and prepared for surgery. Preoperative antibiotics and an NSAID is given prior to the surgery.

Pin Selection and Surgical Planning: Smooth and positive profile centrally threaded Steinmann pins are available from 2 mm to 6.4 mm. The threaded pins have a better holding power. However, they can be difficult to insert in older animal with thick cortices. The size of the pins should not exceed 20% of the diameter of the bone. A minimum of 2 pins are needed proximal to the fracture. They are inserted in the proximal or distal metaphysis and diaphysis. The middle of the bone needs to be avoided. The distance between the pins needs to be at least 6 times the size of the pins. To increase the distance between the pins in small fragment, the pins can be placed in a divergent plane.

Insertion Technique: A stab incision of the skin down to the bone is realized. The appropriate drill bit is used to create the pin hole. In older animals with thick cortices, the drilling needs to be done in steps to avoid overheating the bone. The drill bit is flushed with cool saline during drilling. If a threaded pin is used, the thread will be created with the appropriate tap. When the drill bit reaches the far cortex, it is found under the skin and another stab incision is created. When the pins are all in place, they are cut with a bolt cutter, leaving approximatly 3 cm out of the skin. A light bandage is placed over the pins. A bandage is then applied as is done for a standard cast application (stockinette, felt over bony prominences, etc.). Finally, the casting material is applied while traction and support are provided to the fracture. Most of the cast material is pushed through or wrapped around the pins to create a solid construct. For the last roll of casting material, the material is placed over the sharp edges of the pins to cover them completely. The hoof has to be included. It is important that the cast end is not too close to the last pin, to avoid creating a leaver that can stress the bone and cause a catastrophic fracture.

Postoperative Care and Follow-up: Systemic antibiotics and NSAIDs are used in the early postoperative period. The animal is confined to a stall. In calves, the cast is changed after 3 to 4 weeks. The pins are removed by rotating them out. The holes are cleaned and flushed. A second cast is then applied. In older animals, the first cast and pins are kept in place for 4 to 6 weeks if the animal is comfortable.

Complications and Prognosis: Pins and bone fractures at the pin/bone interface is a catastrophic complication. Pin loosening and ring sequestrum (osteitis) at the insertion site is a frequent complication that is secondary to overheating of the bone during drilling. It may cause discomfort to the animal. However, it usually resolves with removal of the pins. Delayed healing can be seen when pins are kept for a prolonged period of time. Other complications are associated with coaptation of the limb.

In a retrospective study by Lozier et al, looking specifically at fractures fixed with a pin cast in ruminant (cattle, goat and sheep), 79% of the cases survived to the removal of the coaptation. At long-term follow up, 85% of the cases were performing according to the owners' expectation despite the fact that 45% of the animal had some degree of lameness.

K79

Clinical management of septic arthritis

André Desrochers.

Université de Montréal, Canada.

Septic arthritis can be caused by a direct trauma to the articulation (primary), an adjacent infection to the articulation (secondary), a systemic infection (tertiary). Direct trauma is a common cause of septic arthritis in adult cattle. Calves with infected umbilical infection or adult with endocarditis are at risk for polyarthritis from a remote site. Septic arthritis in cattle is of bacterial origin. Bacteria isolated from the articulations will depend of the cause of infection. The most common in adult are: *Trueperella pyogenes, Escherichia coli*, and other environmental bacteria. In calves, a recent study found that *Streptococcu*s spp followed by *Mycoplasma bovis* were the most common bacteria isolated in septic joints.

Diagnosis: Adult cattle affected of septic arthritis are severely lame. During the physical examination, emphasis should be on investigating the origin of the septic arthritis with a very special focus on the umbilicus in calves. The most frequent joint involved are the carpus, tarsus, stifle and fetlock. Arthrocentesis can be easily done. Macroscopic examination of the fluid is often diagnostic (increased turbidity, decrease viscosity, fibrin). Bacterial isolation and identification are possible in only 50 % of the samples submitted. Radiographic examination helps the clinician to specify his diagnosis, and to establish a prognosis. Soft tissues are better evaluated with ultrasound examination. In our clinics, we've been using ultrasound to determine the presence and location of fibrin in the joint helping the clinician in his choice of treatment.

Treatment: Basic principles have to be followed:1- control the infection, 2-drain abnormal joint fluid 3-control inflammation and 4-restore joint function. In any cases, primary cause should be treated; calves with omphalophlebitis have to go under surgery rapidly before other joint infections occur. The choice of the appropriate antibiotics is based on the microoganism suspected, route of administration, cost of the treatment and withdrawal in meat and milk. *Trueperella pyogenes* being

the most common microorganism isolated in cattle, penicillin procainic is often the first choice antibiotic. In the presence of a severely contaminated wound, antibiotics against gram negative should be considered. In calves with septic joints, the antibiotic chosen should have an effect on *Mycoplasma* spp if no organism is isolated and the umbilicus is unlikely to be the cause specially if there is a history of *Mycoplama* spp on the farm or clinical signs associated to it (otitis, pneumonia, arthritis, mastitis). The duration of the treatment should be 2 to 3 weeks after the beginning of clinical improvement. Other route has been described: intra-articular injection, intra-venous under tourniquet and antibiotics incorporated in a slow release medium. Removal of infected tissue, debris and inflammatory mediators in the joint is essential for normal return to previous function. The goals of joint lavage are to remove debris and dilute the abnormal constituent in the joint. Joint lavage is performed in different way: tidal, through and through and arthroscopy. The size of the needle used are 16 G to 14G in calves and 14G to 5mm canula in adult. Arthrotomy is performed if the medical treatment failed or the joint is filled up with fibrin or pus and through and through lavage is impossible. Sites of arthrotomy are the same as the arthrocentesis. The incision should be long enough to allow adequate drainage and introduction of a forceps to remove fibrin. More than one incision per joint is necessary to access the entire cavity and improve the debridement. The incisions are covered with a bandage or stents and additional lavage are performed if necessary. Arthrodesis is the final solution when no treatments were efficient or because of the chronicity of the disease, joint function will never be restored. Articulations of the distal limb are easily arthrodesed (fetlock, proximal and distal interphalangeal joints). Severe carpal infection has also been treated with arthrodesis.

Prognosis: In cattle, prognosis is generally good for a return to previous function and productivity. It will depends of the time of presentation, radiographic evaluation (bone lysis and proliferation), and degree of extracapsular ankylosis. If more than 2 joints are infected, the prognosis is poor. Animal with chronic septic arthritis with bony lesions do not have a good prognosis for complete recovery and becoming a productive animal.

Biotechnology

K80

Simplified superstimulation programs for in vivo and in vitro embryo production in cattle

Gabriel Bo.

IRAC, Argentina.

Knowledge of follicular wave dynamics obtained using real-time ultrasonography and the development of the means by which follicular wave dynamics can be controlled have provided practical approaches for the *in vivo* and *in vitro* production and transfer of embryos in cattle. Two very important factors influencing variability in superstimulatory response are the intrinsic number of antral follicles in donors, and the stage of follicular development at the time of initiating FSH treatments. Response can be predicted by antral follicle counts done with ultrasonography, or the measurement of circulating concentrations of anti-Müllerian hormone (AMH). High antral follicle counts have been associated with more ovulations and a greater number of transferable embryos following superstimulation with FSH than low antral follicle counts. Furthermore, the elective control of follicular wave emergence and ovulation has had great impact on the application of on-farm embryo transfer, especially when large groups of donors need to be superstimulated at the same time. Although, estradiol and progestins have been used for many years, practitioners in countries where estradiol cannot be used have turned to alternative treatments, such as follicle ablation or the induction of ovulation by the administration of GnRH for the synchronization of follicle wave emergence. Initially, attempts to synchronize follicular wave emergence for superstimulation with GnRH were unsuccessful because of failure to induce ovulation consistently when administered at random stages of the estrous cycle, but subsequent field data were more promising. In these cases, GnRH was administered 1.5 to 3.0 days after the insertion of an intravaginal progestin device which may have increased the probability of an LH-responsive follicle at the time of treatment with GnRH. Indeed, we have reported on the strategic use of PGF2α, a progestin device and GnRH to induce ovulation prior to initiating FSH treatments. Basically, a persistent follicle was induced by treatment with PGF2α at the time of progestin device insertion; following administration of GnRH 7 days later, ovulation occurred in more than 95% of animals. Superstimulation initiated 36 hours after GnRH (with the P4-device remaining in place) resulted in a superovulatory response that did not differ from controls superstimulated between Days 8 and 12 of the estrous cycle. More recently, a study performed with Angus donors reported no difference in superovulatory response whether GnRH was administered 2 or 7 days after insertion of a P4-device with FSH treatments initiated 2 days later.

In vitro embryo production (IVP) also benefits from the synchronization of follicle wave emergence prior to oocyte recovery. As *Bos indicus* cattle have high antral follicle populations, large numbers of oocytes can be obtained by ovum pick-up (OPU) without superstimulation. However, synchronization of follicular wave emergence and superstimulation is necessary

to obtain high numbers of oocytes by OPU and blastocysts following *in vitro* fertilization (IVF) in *Bos taurus* donors. In Bos taurus breeds of cattle, especially those with low AFC, oocyte recovery and blastocyst production have been improved by superstimulatory treatments with FSH. There is also clear evidence that superstimulation increases oocyte and embryo developmental competence and thus, blastocyst production rates. Although FSH treatment protocols have involved twice daily treatments followed by a period of coasting to optimize follicle and oocyte development competence traditionally, more recent protocols with a single administration of Folltropin-V in 0.5% hyaluronan 48 or 72 hours before OPU have been very efficacious, making protocols user-friendly, while minimizing errors in compliance. FSH-treated beef and dairy cows and heifers had a greater percentage of medium-sized follicles (6 to 10 mm), a greater blastocyst production rate and more transferable embryos per OPU session. Finally, it has been shown that it is possible to obtain embryos in vivo and in vitro sequentially in the same donor. Although no significant differences in superovulatory response were found, initiation of FSH treatment 3 days after OPU resulted in a greater number of grade 1 embryos than when the protocol was initiated 2 days after OPU. All these protocols demonstrate that it is possible to obtain embryos in vivo and in vitro in commercial beef or dairy herds using efficacious protocols that are easily implemented by farm personnel.

K81

Factors affecting oocyte quality for embryo production in cattle

Pietro Baruselli Sampaio[1], Bruna Lima Chechin Catussi[1], Laís Ângelo De Abreu.

USP, Sao Paolo, Brasil.

The use of ovum pick up (OPU) associated with in vitro embryo production (IVEP) has a great potential to disseminate selected genetics, diminishing the interval of generations (use of young donors) and improving herds genetic gain. However, several factors appear to be critical to oocyte quality such as environmental factors, genetic background, age and lactation status of donor animals. The aim of this review is to highlight some critical areas that can help veterinary practitioners to enhance OPU efficiency and successfully implement IVP into their routine practice. The animal category is an important factor that affects oocyte quality. Our group used 120 Holstein donors of four animal categories, as follows: prepubertal heifers (n= 30), pubertal heifers (n= 30), lactating cows (n= 30) and nonlactating cows (n= 30). Donors were submitted to OPU without previous synchronization of the follicular wave. No difference was observed between experimental groups, regarding total number of aspirated follicles (P = 0.08). Despite a similar number of total recovered oocytes (P = 0.12), prepubertal heifers had an intermediate quantity of viable oocytes, and non-lactating cows produced more viable oocytes (P= 0.03), when compared to lactating cows. Still, prepubertal donors had lower cleavage rate (P< 0.0001) and lower blastocyst rate (P< 0.0001) compared to other categories. We studied the effect of donor pregnancy at the moment of OPU on oocyte quality and IVP in 3 Holstein categories: prepubertal heifers (8 to 10 month; n = 60), pubertal heifers (10 to 12 month; n = 60) and pregnant heifers (14 to 18 month; n=59). Pubertal heifers had a greater number of recovered oocytes as well as COCs cultured compared to other categories. In contrast, cleavage rate was similar between pubertal and pregnant heifers. Interestingly, pregnant heifers had a greater number of embryos produced per OPU and greater blastocyst rate when compared to other heifer categories. It has been reported that IVEP is more efficient in *Bos indicus* breeds than in *Bos taurus* breeds. The greater antral follicle population (AFP) found in *Bos indicus* cattle would appear to result in a greater number of suitable oocytes for *in vitro* culture. In this context, *Bos indicus* (Nelore and Gir) are reported to have greater number of visualized follicles and to produce greater number of total oocytes per OPU session, cultured COC and blastocyst rates than *Bos taurus* (Holstein). In dairy cows, the lactation period and interactions with insulin resistance may influence oocyte quality. Our group studied Holstein cows that were either at early or late days in milk (DIM) at the moment of OPU-IVP. Results showed that insulin resistance associated with late lactation period can disrupt oocyte quality, promoting lower efficiency of IVP. The number of blastocysts, as well as blastocyst rates, were greatly reduced in cows at later lactation. In addition, a number of apoptotic genes were upregulated in cows with greater days in milk. One factor related to the poor IVEP yields in *Bos taurus* cattle can be partly attributed to the heat stress. A previous seasonal experiment demonstrated that once the pool of ovarian oocytes is damaged by heat stress, two or three estrous cycles are required (after the end of heat stress) to restore the follicular pool and oocyte quality (Roth et al., 2001). However, our study (Torres-Júnior et al., 2008) showed a carry-over effect of heat stress on blastocyst production up to 105 days after the end of the heat stress. Factors that affect oocyte quality for embryo production have to be taken into account to increase the efficiency of ET in cattle.

K82

Factors affecting pregnancy rates in beef embryo recipients

Gabriel Bo.

IRAC, Argentina.

The main objective of implementing embryo transfer in beef operations is to accelerate the rate of genetic progress in the herd. Among the main factors that affect the use of these technologies are related to nutrition, management and estrus synchronization. Although prostaglandin F2α (PGF2α) has been used most commonly for synchronization of estrus, the requirement for estrus detection and the variability in the interval from treatment to estrus and ovulation has adversely affected its performance in embryo transfer programs. To avoid limitations associated with estrus detection, treatments that synchronize the time of ovulation, which were developed originally for fixed-time AI, have been utilized f/or FTET. These

treatments are generally divided into those that are GnRH-based and those that are estradiol-based. In either case, the recipient protocols include the insertion of a progesterone (P4) releasing device for 5 to 8 days, depending on the protocol. Recent studies with GnRH-based protocols have suggested that reducing the length of exposure of the P4-releasing device insertion to 5 days and increasing the interval from P4-device removal to GnRH and fixed-timed AI to 3 days may improve pregnancy per AI (P/AI) as compared to the traditional 7-day GnRH/P4 device protocol in beef cattle. Furthermore, it was suggested that a reduction in the length of the growth phase of the ovulatory follicle prior to ovulation, as occurs in some animals treated with the conventional 7-day protocols, alters the steroidogenic capacity of the dominant follicle prior to ovulation and the resulting CL, and decreases the ability of the uterus to support embryo development. Similar pregnancy rates per embryo transfer (P/ET) were obtained with in vitro-produced embryos to those of recipients synchronized with two PGF2α treatments 14 days apart and estrus detection and those synchronized using a modified 5-day Co-Synch+CIDR protocol (no GnRH at P4 device insertion, PGF2α at P4 removal on Day 5 and GnRH on Day 8). Based on these findings, we evaluated the effectiveness of an estradiol/P4 treatment protocol in which the exposure to P4 device was reduced to 6 days and proestrus was lengthened by the administration of GnRH 72 h after P4 device removal instead of ECP at device removal. The protocol for FTAI was named J-Synch. This treatment protocol has resulted in higher P/AI rates in beef heifers compared to the conventional protocol in which the P4-device is removed on Day 7 and ECP is given at that time. Furthermore, in a series of experiments that were conducted recently to evaluate the performance of the J-Synch protocol in embryo transfer programs the P/ET rate was greater in the J-Synch (49.4%) than in the conventional synchronization protocol (41.0%; P<0.05).

Although the previously described protocols have performed adequately for several years, recent attention has been directed to the effect of estrus expression and estradiol concentrations during growth of the preovulatory follicle on embryo growth and pregnancy. In recipients showing estrus, we have shown a significantly (P<0.05) greater P/ET (48.3% vs 30.1%) and lower pregnancy losses (25,6% vs 66.7%) than in recipients not showing estrus. Therefore, use of tail-paint or estrus detection patches in recipients would help identify animals showing estrus by simply running them through the chute at the appropriate time, without the necessity of labor intensive estrus observations. These modifications can be easily implemented in recipient synchronization programs and should result in overall higher pregnancy rates.

K83

Improving fertility in dairy herds using in vivo and in vitro produced embryos

Pietro Baruselli Sampaio, Guilherme Felipe Ferreira Dos Santos, Ana Carolina Dos Santos.

USP, Sao Paolo, Brasil.

Embryo transfer is a reproductive technology commonly used around the world to reproduce animals with high genetic merit. However, the application of embryo transfer (ET) technology can also improve the reproductive performance of dairy herds artificially inseminated. Environmental conditions are important factors affecting oocyte developmental competence and embryo production in both *in vivo* and *in vitro* production systems. Heat stress (HS) has a deleterious effect on fertility in dairy herds around the world, especially in tropical areas in which summers are hot and humid. Studies have shown that embryo transfer is an effective tool to increase fertility during heat stress because it bypasses the damage to the oocyte and early stages of embryonic development caused by hyperthermia. Therefore, a useful management tool to maintain high pregnancy rates throughout the year would be to produce embryos during the cooler months and use them for ET during the periods of heat stress. A retrospective analysis of data from a large commercial herd in Brazil was performed in Holstein cows submitted to ET or AI. Pregnancy per ET (P/ET) was higher along the year than P/AI, but the differences were more pronounced in the warmer months than in the cooler months of the year. Furthermore, the transfer of embryos to repeat breeder cows resulted in increased pregnancy rates compared to AI, supporting that the fertility problem repeat-breeder cows may be associated with oocyte quality and/or failure of early embryo development. The seasonal profile of *in vitro* embryo production (IVEP) has been reported in non-tropical and in tropical areas. In USA (Wisconsin), the *in vitro* blastocyst production was reduced during mid-to late-summer, preceded by increased production starting during mid-to late-spring. Winter and fall months were characterized by stable, high yields of blastocysts, showing an evident effect of heat stress on IVEP in an area where summer is milder. *In vivo* embryo production (SOV and uterine flushing) in Holstein heifers and lactating cows was also shown to be affected by HS in the tropics (Brazil). Data from 1,562 SOV procedures indicated that the fertilization rate, proportion of transferable and freezable embryos and the number of embryos produced per SOV attempt (4.4 ± 0.4 vs 2.8 ± 0.3) was reduced during the warmer season compared with the cooler season. In a tropical environment, the number of ova/embryos produced after superovulation (SOV), fertilization rate and percentages of transferable embryos and freezable embryos were reduced during the warmer season when compared with the cooler season. Similarly, we observed in the tropics that during the hottest season of the year, oocytes obtained from lactating Holstein cows and heifers had a lower *in vitro* developmental competence compared to the coolest season. Although HS had an overall detrimental impact on *in vivo* embryo production and quality, its negative effects were more pronounced in lactating cows than in heifer donors. For example, during the hot season the decline in the

number of fertilized ova and in the rates of fertilization, transferable embryos and freezable embryos were greater in donor lactating cows compared with donor heifers. In addition, lactating cows generate more body heat and suffer greater hyperthermia when exposed to similar environmental temperatures compared with growing heifers and non-lactating cows, which may be related to the high metabolic energy associated with milk production. Regarding the embryo production technique, studies suggest that IVEP technology can be a viable solution to increase embryo production within a short period of time. The use of OPU-IVEP may be a valuable alternative to produce a large number of embryos and pregnancies within a reduced time period. The IVEP can also be associated with the use of sexed semen to increase the production of calves of a specific sex, which would benefit dairy industries worldwide. In summary, although the embryo transfer technology has been used primarily to reproduce animals with high genetic merit, this technology can be used to resolve reproductive problems such as the reduced fertility found during heat stress.

Pharmacology & Therapeutics

K84

Antimicrobial susceptibility testing and its clinical applications in bovine practice

Keith Dedonder.

Latham BioPharm Group.

Objective: Antibiotic resistance is currently at the forefront of human and animal health and has been for many years. Antimicrobial susceptibility testing is commonly used in modern veterinary practice and so much so that many veterinarians submitting for this type of testing likely do so without thoughtful consideration in regard to the testing procedures or the interpretation of the testing results. However, in order to continue to be judicious with the use of antibiotics, it is essential that the clinician be familiar with the testing procedures and its interpretation in order to increase the chance of treatment success. The objective is threefold; 1) to re-familiarize the bovine practitioner with the most common methods of susceptibility testing performed in laboratories, 2) to educate on what goes into the determination of susceptible “S”, intermediate “I”, and resistant “R”, and 3) to exercise application of susceptibility testing to case management / treatment selection in the clinical setting.

Materials and Methods: The preferred methods of antimicrobial susceptibility testing and interpretive criteria are described in published standards documents to ensure that all laboratories are performing the testing procedures and interpretation in the same “standardized” fashion. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) is the functioning body for such standardization in Europe and the Clinical and Laboratory Standards Institute (CLSI) functions as such in North America. For the purposes of this presentation the CLSI methods and definitions will be used; however, the themes presented will be applicable to all clinicians regardless of geography.

Results: Unfortunately, there is not a perfect correlation between antimicrobial susceptibility testing and clinical case outcome. Testing that yields bacteria that are “susceptible” to the prescribed antibiotic may have a poor outcome and those with “resistant” infections may have a positive outcome. In both cases, the ultimate outcome of the treatment may have been influenced by factors beyond just those of the “bug-drug” relationship, such as immune status, environmental conditions, individual pharmacokinetic differences, etc.

Conclusions: A clinician that thoroughly understands the basic methods of antimicrobial susceptibility testing, its interpretation, and its limitations will make sound clinical decisions and more judiciously select and utilize antimicrobials in their practice.

K85

Defining antimicrobial efficacy through randomized clinical trials with negative controls

Keith Dedonder.

Latham BioPharm Group, USA.

Introduction: Bovine respiratory disease (BRD) is a multi-factorial disease described as a complex or syndrome involving an interaction of stressors, viruses, and bacteria. To this day, BRD remains a major disease in all types of beef and dairy production systems. Administration of an antibiotic is not only the mainstay therapeutic treatment option for acute BRD, but also as means to control the disease in high-risk populations. The judicious use of antibiotics in both human and animal health remains vital to ensure continued antibiotic efficacy.

Objective: The objective is to present a comprehensive review of all published randomized clinical trials investigating the effects of a drug against a negative control in treatment of BRD. The number needed to treat (NNT) statistic will be used to convey the results of this investigation. The NNT is an epidemiological measure used in communicating the effectiveness of an intervention. The NNT statistic is defined as the inverse of the absolute risk reduction and has a major advantage of being more straightforward to readers less versed in thinking of events such as clinical outcome in terms of probabilities (e.g., risk ratio, odds ratio). As such, NNT is much easier to interpret by the practicing clinician as it speaks in terms of number of treatments needed to apply across a population to make a difference in one patient.

Materials and methods: A systematic review of the scientific literature was performed using online resources, with included studies being limited to those published in English and originating from North America. Other criteria for inclusion were that the publications were required to be investigations into the treatment or control of naturally occurring BRD with an antibiotic (only) in a randomized, blinded, negative control field trial study design. Studies on the treatment of naturally occurring BRD must have involved animals that were not mass medicated with an antibiotic prior to or as part of the study. All studies involving positive controls or experimental challenge models were excluded.

Additionally, the Freedom of Information (FOI) New Animal Drug Approvals (NADA) summaries were searched on the United States Food and Drug Administration Center for Veterinary Medicine (US FDA CVM) website at the same time. Inclusions from the FOI summaries included efficacy trials (ET), dose response studies (DRS), dose selection studies (DSS), single location field studies (FS), and multi-location field studies (MLFS). To qualify for inclusion, dose selection studies must have used the current labeled dosages in the study.

As a final step, published literature resulting from online searches were screened to ensure that they were not duplicative of FOI data; any duplicative data was removed from the data set prior to analysis.

Results: The overwhelming majority of trials showed a positive effect on case outcome in the therapy and control of BRD. The median NNT in therapeutic trials involving negative controls was 2 (i.e., for every 2 animals treated for BRD in the overall population of these studies, 1 case was a treatment success). The median NNT for preventing 1 mortality due to BRD in the trials reviewed was 6 (i.e., for every 6 animals therapeutically treated 1 BRD death was prevented). In BRD control studies, the median number of animals that needed to be treated to prevent 1 acute case of BRD was 5.

Conclusions: The objective was to determine the effectiveness, using the NNT statistic, of different antibiotics in the therapy and control of BRD in randomized clinical trials utilizing a negative control. The data from this analysis should not be used to directly compare the NNT of one antibiotic to another due to differences in trial design, sample size discrepancies, risk classification differences of BRD between trials, resultant spontaneous recovery rates, and potential differences in case definition and success/failure outcome between trials. The presentation of the data here does make for a succinct way of defining reasonable expectations of efficacy in the treatment and control of BRD in a field setting for cattle at high risk of BRD. However, the clinician interpreting these findings needs to bear in mind the external validity of these studies when applying it to the type of cattle, environmental setting, and typical BRD pathogens encountered in their practice.

Epidemiology

K87

Epidemiology of bovine mastitis on the modern dairy farm - Introduction

Ynte Hein Schukken[1], Ruth Zadoks[2].

[1]Wageningen University - Royal GD, The Netherlands; [2]The University of Sydney, Australia.

Objectives: Bovine mastitis remains one of the most important diseases on the modern dairy farm. Not only is mastitis associated with pain and decreased welfare of cows, but also with decreased quality of milk, with increased use of antibiotics, unwanted early removal of cows and ultimately with a negative impact on the economics of the farm. In the last decade much has been learned about the epidemiology of bovine mastitis, therefore the objective of this overview is to summarize these findings and to develop directions for further improvement of udder health on modern dairy farms.

Subclinical mastitis: The distinction between clinical and subclinical mastitis is one hand important and on the other hand also a gradual difference. Cows with very high somatic cell counts clearly have abnormal milk that in some cases looks like normal milk at the time of milking but starts flaking when standing in a collection vessel for only a few minutes. Still, the distinction is of value as subclinical mastitis primarily leads to increased somatic cell counts, delivery of milk into the bulk tank and therefore decreased milk quality. Whereas clinical mastitis leads to observed disease in cows and often to immediate treatment and milk withhold in the affected animals.

In recent years the improved diagnostic tools, particularly in the field of molecular diagnostics, has resulted in a much more precise diagnosis of bacterial organisms that are associated with intramammary infections. A much better insight into infections with staphylococci, streptococci and also gram-negative micro-organisms has improved our understanding of these infectious processes.

Improved molecular diagnostics has also resulted on Important research with regard to the microbiome of the mammary gland. Current research would indicate that the mammary microbiome of health quarters is substantially different from quarters that are affected by either clinical or subclinical mastitis. Further research will be necessary to show whether the mammary microbiome plays a role in prevention of intramammary infections with major mastitis pathogens.

Clinical mastitis: Clinical mastitis has become increasingly important as subclinical mastitis may be controlled using the NMC 10-point plan. Increased on-farm hygiene, improved milking management and adequate treatment has on one hand reduced subclinical mastitis but at the same time put the spotlight on clinical mastitis.

In recent years much research has been done on the Immunology of the bovine mammary gland, including interventions on the immune system using diets, vaccinations or immune stimulations. These interventions have shown to be valuable in reducing the incidence of clinical mastitis, reducing the length of clinical signs and the risk of culling of affected animals.

Societal pressure has resulted in a reduction of the use of antibiotics for the treatment and prevention of intramammary infections. This reduction of antibiotic use has ultimately lead to further improvements in managing risk factors for intramammary infections on farms but also on the increased importance of immune modulation.

Environmental versus contagious: In recent years, our understanding of the main infection routes that lead to intramammary infections has substantially changed. The main reasons for this were the increased use of molecular diagnostics that led to precise typing of bacterial strains and as a result a paradigm shift on the classification of mastitis causing micro-organisms. The classic approach was to divide mastitis causing microorganisms into environmental and contagious organisms. With streptococci and staphylococci being contagious and gram-negative being environmental. However, increased application of molecular typing schemes have shown that such a distinction does not hold up and that all bacteria that are able to cause persistent intramammary infections may lead to contagious behaviour on dairy farms. This had resulted in a portfolio of risk-factors, where transmission has become an important but not a singular part in the dynamics of intramammary infections.

Conclusions: Important changes in the biology, diagnostics, management and treatment of bovine mastitis in the recent decade has resulted in a vastly different understanding of intramammary infections and the treatment and prevention of these infections.

K88

Molecular epidemiology of mastitis: evidence versus paradigm

Ruth Zadoks[1], Zadoks Ruth[1], Schukken Ynte[2].

[1]Sydney University, Australia; [2]Royal GD, Wageningen University and Utrecht University, The Netherlands.

The epidemiology of mastitis is generally described as contagious or environmental. Contagious pathogens have their reservoir in the mammary gland and are transmitted from animal to animal during milking. Environmental pathogens, by contrast, have their reservoir in the environment, and infections can occur at any time, even in non-lactating cattle. There are exceptions to this dichotomous classification. For example, insects may act as vectors, as shown for transmission of Staphylococcus aureus among heifers by flies, so contagious transmission can occur without involvement of the milking machine. Conversely, the infected mammary gland may serve as reservoir of infection, but with transmission via the environment, as shown for Klebsiella spp. in free-stall herds. Occasionally, we see iatrogenic point source transmission, as demonstrated for Pseudomonas-contaminated water or wipes, and for Serratia-contaminated teat dip.

The main weakness of the contagious versus environmental paradigm, however, is how we as veterinarians interpret and implement it. Many veterinary curricula, textbooks, extension reports, and peer-reviewed publications classify bacterial species as contagious or environmental, regardless of the evidence. As a profession, we should have a more nuanced understanding of the sources and transmission routes of mastitis pathogens, because molecular epidemiology studies provide ample evidence that many bacteria can be spread in multiple ways. When we open our minds to this possibility, the distinction between modes of transmission can often be made even without using molecular typing. The tools that we normally use for mastitis investigations, such as inspection of the parlour, housing and animals for hygiene and management risks, and evaluation of data regarding parity, lactation stage and affected quarters, can largely be relied on to formulate a diagnosis and interventions. The success of those interventions will then inform on the accuracy of our diagnosis and the need for further investigation.

In genomic epidemiology, entire genome sequences are generated and compared as done, e.g., for SARS-CoV-2. This level of complexity is not needed for mastitis investigations, which can often be conducted using relatively simple molecular biology tools including polymerase chain reaction (PCR), or the more time-consuming former gold standard method of pulsed-field gel electrophoresis (PFGE). PCR amplifies bacterial DNA, PFGE cuts it. Either way, a bar code-like banding pattern is generated, revealing whether bacterial isolates from different sources have the same or different DNA fingerprint, e.g., across quarters, cows, and herds, or between animals and the milking machine, bedding, faeces, drinking water, teat dips and wipes, etc. Most practitioners and clinics would not have access to this technology within their laboratory but the scientific work to generate the evidence has already been done. It is largely the willingness to implement available knowledge that is the main hurdle.

Although a social science study into communication and uptake of evidence would be very valuable, the main focus of this presentation will be on the molecular evidence itself. It shows that major mastitis pathogens such as Staphylococcus aureus, Streptococcus dysgalactiae, and Streptococcus uberis can all “behave” as contagious pathogens as well as environmental pathogens. The manifestation on a specific farm or even country will depend on the combination of pathogen strain and herd management. For example, S. uberis is almost exclusively environmental in New Zealand (different DNA fingerprints in different cows), whereas most clinical S. uberis mastitis in England is the result of contagious transmission (same DNA fingerprint in multiple cows). If we suggest to farms that milking time hygiene or teat dipping are not a priority when they have S. uberis mastitis in their herd, we may aggravate the problem. Even Streptococcus agalactiae, long described as an “obligate intramammary pathogen”, may have environmental sources, including people and bovine faeces. Several publications describe S. agalactiae problems that could only be controlled by approaching them as an environmental mastitis problem.

By presenting some of the examples described in this abstract, it is hoped that a growing number of cattle veterinarians will be willing to confront the contagious versus environmental paradigm with molecular epidemiology evidence and open their mind to the fact that many bacterial species are more sophisticated than our dichotomous thinking.

K89

Epidemiology of infectious disease on dairy farms - Introduction

Ynte Hein Schukken[1], Ruth Zadoks[2].

[1]Wageningen University - Royal GD, The Netherlands; [2]The University of Sydney, Australia.

Objectives: Infectious on dairy farms have gained in importance in recent years. Infectious diseases have resulted in subclinical and clinical disease in dairy cows, increased risk of zoonotic events whereby infections from cattle transmit to humans and also to increased societal concerns on welfare of calves and cows. In this presentation the recent experiences from the Netherlands will be shared. Lessons learned and important necessary developments in bovine health will be discussed.

Control of infectious diseases: In recent years control programs for infectious diseases have been developed for the Dutch dairy industry. This includes programs to reduce or eliminate infections with Leptospirosa hardjo, Mycobacterium avium subspp. paratuberculosis, Tuberculosis, Brucella abortus, Enzootic Bovine Leukosis virus (EBLv), Salmonella species, Bovine Virus Diarrhea Virus (BVDv), Infectious Bovine Rhinotracheitis virus (IBRv) and to some extend Blue Tongue Virus (BTv). On top of this, programs to diagnose and control list A diseases are present but have not been necessary in the last decade.

With the increased interest and concern about zoonotic pathogens, the control of Leptospirosa hardjo, Mycobacterium avium subspp. Paratuberculosis (MAP), Tuberculosis, Brucella abortus, and Salmonella species are important steps to decrease such concerns. The control of Enzootic Bovine Leukosis virus, Bovine Virus Diarrhea Virus and Infectious Bovine Rhinotracheitis are not indicated because of zoonotic concerns but because of animal health improvement in general or for trading reasons.

The development of these control programs is particularly dependent on the ability to control transmission between animals within a farm and transmission between farms.

Within farm transmission: Understanding within farm transmission has been particularly important for infectious diseases such as MAP, salmonellosis, BVD and IBR. Elimination of MAP from farms has been difficult, but long term control programs have certainly resulted in important progress as indicated by a reduced incidence, less severe shedding and disease and a delay in the time to shedding. Carefully designed diagnostic programs for BVD and IBR have turned out to be essential to eliminate within farm transmission and certification of farms as infection free. Salmonella control programs are based on long term control of the key risk factors and on bulk milk monitoring, subsequent individual animal diagnostics and culling of persistently infected animals. Once a farm has been

certified as infection free, prevention of between farm transmission becomes the focus of the national control program.

Between farm transmission: A number of the indicated infectious diseases are controlled based on National legislation and with the aid of National enforcement of diagnostic programs and subsequent control procedures. This includes to control programs for Tuberculosis, Brucella abortus, Enzootic Bovine Leukosis virus (EBLv) and to some extend Blue Tongue Virus (BTv). As a consequence all cattle farms in the country are included in monitoring and control and the risk of between farm transmission is minimal. In contrast, control programs for Leptospirosa hardjo, Mycobacterium avium subspp. paratuberculosis, Salmonella species, Bovine Virus Diarrhea Virus (BVDv) and Infectious Bovine Rhinotracheitis virus (IBRv) are implemented by the animal industries and without legislation or enforcement support by the government. Hence, the success of these programs fully depend on the ability of the industry partners to work together and decided on control programs that are implemented across different players in the industry. It has been remarkable that this appears to be successful. Important components of the control programs are cattle registration, documentation of animal movements and dedicated participation of farmers in the developed control programs.

However, the control of these infectious diseases in the dairy industry alone will not lead to elimination of the infectious agents from the country as beef cattle farms, calf raising farms and veal calf operations are also potential sources of these infectious organisms. Further cooperation between these industries will be essential for a further reduction in between farm transmission.

Conclusions: Control of infectious diseases on dairy farms has gained in importance in recent years. Concerns with regard to zoonotic diseases, animal welfare and reduced use of antimicrobials all result in an increased need for continuous improvement of animal health and reduction or elimination of infectious diseases on dairy farms.

K90

Beyond mastitis: molecular epidemiology insights into transmission and control of bacterial, parasitic and viral diseases of cattle

Ruth Zadoks[1], Ynte H. Schukken[2].

[1]Sydney University, Australia; [2]Royal GD, Wageningen University and Utrecht University, The Netherlands

Introduction: As sequencing of DNA and RNA has become increasingly accessible and affordable, molecular methods have added significantly to our understanding of the transmission and control of bacterial, parasitic and viral disease of cattle. They have provided insight into past movement of pathogens and their current distribution, and inform rational design of future eradication strategies, diagnostic tests, and vaccines. They also help us understand how the pathophysiology of disease and the transmission of organisms and, hence, control strategies may differ, or need to differ, between production systems, countries, or continents. Parasites are comparatively large infectious agents. They are eukaryotic, like cattle and humans, and have large genomes spread across multiple chromosomes, which means that sequencing of entire genomes is still very challenging. Bacteria are prokaryotes, with smaller and simpler genomes than parasites. Less than 15 years ago, sequencing of the first bovine *Streptococcus agalactiae* genome cost more than the annual salary of the postdoctoral researcher analysing it. Now, it can be done within days or weeks for less than 100 Euro. Viruses are much smaller yet, and sequencing of entire genomes is cheaper and faster than for bacteria, although it can be tricky for segmented genomes such as those of influenza D, which was first described in cattle in 2011.

Parasites: In parasites, sequencing of housekeeping genes is a useful tool for accurate species identification. This approach led to the realization that most rumen fluke in the Republic of Ireland and the United Kingdom did not belong to the species *Paraphistomum cervi*, as long assumed, but to the species *Calicopheron daubneyi.* The two species have different life cycles and intermediate host snails. Control of rumen fluke, which has emerged as a common cattle parasite across much of western Europe, requires knowledge of how to manage the environment to limit the snail population and cattle exposure to infectious cysts on pasture. Where the culprit was thought to be a freshwater snail, it is actually a mud snail, completely changing our options for environmental control. Other desirable tools for diagnostics and management of parasitic diseases would be molecular markers of anthelmintic resistance. Although initial results for, e.g., the liver fluke *Fasciola hepatica*, were promising, they could not be confirmed in other studies, countries, or continents.

Bacteria: Differences in pathogen characteristics between continents can be considerable, and evidence-based control strategies may differ between countries. In our contribution on molecular epidemiology of mastitis, the difference between New Zealand and the UK is used as an example, whereby *Streptococcus uberis* mastitis is almost exclusively environmental in New Zealand but commonly contagious in the UK, as it is in mainland Europe or the USA. Another striking geographical difference is observed for *Coxiella*, the causative agent of Q-fever in humans and coxiellosis in ruminants. In the USA, up to 95% of bulk tank milk may test positive for *Coxiella* but reports of Q-fever are unusual. In Europe, Q-fever is primarily associated with small ruminants. By contrast, cattle are considered the main source of Q-fever in Australia. The difference may be due, at least in part, to differences in *Coxiella* strains, which may also impact on the efficacy of diagnostic assays coated with antigens from different parts of the world.

Viruses: The most detailed molecular epidemiology information is available for viruses. Strain typing is essential to inform selection of vaccines for a high-impact pathogen like Food and Mouth Disease (FMD) because dominant serotypes may differ between countries or change over time. It may also inform on the role of animal movements and non-bovine host species as reservoirs, both for epidemic viral diseases like FMD and for endemic viral diseases like bovine viral diarrhoea virus disease (BVDV). Viral sequence analysis has shown that sheep, humans, or inanimate fomites may act as source of BVDV. Insight into occurrence and prevention of such excep-

tions will become increasingly important as BVDV programs progress, which they are likely to do now that it has been added to the OIE list of notifiable diseases.

Although it is impossible to provide a comprehensive overview of the molecular epidemiology of bovine infectious diseases, selected examples will be presented to illustrate their contribution to our ability to understand and manage the health of cattle populations.

Public Health, Food Security and Antimicrobial Resistance

K91

Vulnerability of rural wells and organic produce to multiple drug resistant (MDR) bacteria from dairy cattle manure

Edward Robert Atwill, Kenneth Tate, Thomas Harter.

University of California – Davis, USA.

Objectives: Assess the public health risk of microbial contamination of raw produce such as leafy green vegetables and rural groundwater wells used for drinking water from AMR-MDR enteric bacteria in dairy manure and slurry.

Material and methods: A. We collected feces or manure from live animals, pen soil, and manure storage ponds on California commercial dairies in the San Joaquin Valley to quantify the occurrence of AMR-MDR commensal and pathogenic bacteria. AMR-MDR determination included phenotypic methods (disk diffusion, MIC) and/or whole genome assessments of the resistome using the Comprehensive Antibiotic Resistance or ResFinder database.

B. In parallel, we determined if rural municipal wells either on the dairy or in proximity of a dairy exhibited MDR bacteria and whether field irrigation of forages with effluent from manure storage ponds created a subsurface plume of bacteria. Groundwater samples were obtained using domestic wells or by drilling monitoring wells on the dairy and in nearby rural communities. Bacteria were concentrated using filtration methods, followed by primary bacterial culture and biochemical characterization of isolates.

C. Lastly, a series of randomized controlled trials were conducted in experimental Romaine lettuce production fields whereby furrows were spiked with animal feces that contained specific concentrations of marked *E. coli*. These inoculated lettuce fields were then overhead irrigated and heads of lettuce hand harvested, rinsed, and the concentration of *E. coli* determined for in order to calculate the percentage of bacteria transferring from fecal deposits in the furrows onto the heads of lettuce.

Results: A. AMR-MDR commensal bacteria were most prevalent in young calves on California commercial dairies compared to other age classes and production stages. Over 50% of hutch calves were colonized by MDR *E. coli* within 6 weeks of age, while cull dairy cattle exhibited prevalences of 30-40% of MDR *Enterococcus*. Use of genomic tools to characterize the larger resistome compared to phenotypic AMR methods found that hutch calves were more likely to harbor MDR bacteria compared to other age classes of dairy cattle, but numerous resistance genes were also found throughout all age classes of dairy cattle.

B. AMR genes were widely dispersed in manure matrices throughout the dairy environment and cattle loafing areas, manure lagoon storage system, surface pen soil, and in dairy manure solids used for soil amendments for forage crops and in limited cases produce farming. For example, concentrations of AMR *E. coli* ranged from 6×10^4 cfu/100 ml of manure slurry

to 8×10^7 cfu/100 g of soil in cattle pens. Alfalfa field irrigation with dairy slurry water resulted in a multi-log rapid rise the concentration of *Enterococcus* spp. 4 to 5 meters below the surface in subsurface water. Monitoring wells on commercial dairies had 24% and 97% of groundwater samples with detectable concentrations of *E. coli* and *Enterococcus*, respectively, with concentration significantly associated with being located downgradient of corrals and manure-treated fields, depth to water table, and season. In contrast, water samples from the domestic wells on these dairies had a prevalence of 4% and 83% for *E. coli* and *Enterococcus*, respectively, but at very low mean concentrations (0.01, 1.8 cfu/100 ml, respectively). All of the tested isolates of *Enterococcus* (n=30) exhibited resistance to at least one antibiotic, and 87% exhibited resistance to 3 or more antibiotics, but these levels of AMR-MDR did not appear appreciably different than isolates from domestic wells in greater proximity to the dairies.

C. Lastly, the produce field trials found that 20 to 95% of heads of lettuce exhibited *E. coli* contamination from fecal material in the furrow, with distance and age of feces significantly associated with the level of *E. coli* contamination.

Conclusions: Given the ubiquitous AMR-MDR commensal bacteria in dairy cattle manure and surface pen soil, and the millions of liters of slurry in manure storage systems and tonnage of surface soil on a typical 1000-cow dairy in California, lack of appropriate good manure management practices can lead to inadvertent AMR-MDR bacterial contamination of surface and ground water sources and impact produce safety when stack-aged manure is used as a soil amendment prior to subsequent irrigation. A variety of good agricultural practices are available to mitigate these AMR risks from dairy manure, which will be discussed in my next presentation.

K92

Good agricultural practices to minimize environmental and waterborne contamination of antimicrobial resistant (AMR) bacteria from cattle manure

Edward Robert Atwill, Kenneth Tate, Thomas Harter.

University of California – Davis, USA.

Objectives: Develop and evaluate the efficacy of good agricultural practices (GAPs) operating at the landowner scale that reduce the risk of excessive environmental and waterborne contamination from AMR bacterial originating from dairy and beef cattle manure.

Material and methods: A series of experimental trials and longitudinal observational studies were conducted at research field stations or commercial livestock operations to determine the efficacy of various GAPs to reduce fluxes of manure-associated bacteria in surface runoff (overland flow) associated with precipitation or flood irrigation of dairy areas or grazed rangeland. For example, the effect of width of grassland buffer positioned between livestock grazing and source water supplies was evaluated for its effect on microbial reduction in rainfall-associated runoff. Other trials focused on GAPs such as aging manure prior to land application, rotation grazing for pasture rest periods, seeding of cattle loafing areas, and constructed wetlands in combinations with site characteristics such as soil bulk density and slope to reduce manure-associated bacteria in runoff.

Results: Many of the GAPs we evaluated take advantage of either distance between livestock and source water supplies, and/or time by aging manure prior to either land application, prior to the occurrence of natural rainfall, or prior to flood irrigation of grazing land.

With respect to grassland buffers located between grazing or loafing cattle and down-slope source water supplies, widths of buffer of just 1 to 2 meters were associated with 90 to 99.9% percent reduction in the total load of *E. coli* being transported in overland flow for well-maintained buffers. Compacted soils, high rates of precipitation, and higher land slopes can reduce this efficacy of grassland buffers. Buffers containing a drainage or erosion furrow dramatically reduce its efficacy of bacterial reduction.

With respect to flood irrigation applied to natural rangeland during summer in order to boost forage production in arid California, reducing the velocity and total amount of applied water in order to reduce tailwater volumes has an immediate benefit in reducing off-site fluxes of bacteria but may also reduce total forage production at the tail-end of the field. Routing irrigation tailwater through small constructed wetland can further reduce *E. coli* concentrations, especially for longer residence times in the wetland from lower tailwater velocities.

With respect to dairy cattle pens and loafing areas, eliminating cattle from the exposed site prior to the onset of the rainy season was associated with a 99% reduction in fecal coliforms discharging from the pen. Seeding the loafing pens with grass prior to the onset of rainfall further reduced bacterial runoff, especially for high percent grass cover. Aging manure solids prior to land application, and spreading manure weeks-to-months prior to the onset of the rainy season can help reduce the total flux of bacteria discharging of these manure-amended hillslope sites. Increasing the residence time of manure effluent in the storage lagoon can also reduce the concentration of *E. coli* in the final effluent prior to land application, which of course requires better management of runoff across the dairy enterprise and possibly the expensive option of increasing storage capacity of the existing manure ponds or adding additional finishing ponds to the manure management system. Lastly, there is a considerable amount of free technical guidance on the proper construction and management of dairy manure storage ponds, covering aspects such as adequate construction materials to reduce groundwater seepage and adequate freeboard to handle unexpected intense rainfall (perhaps more common with climate change).

Conclusions: Implementation of GAPs by livestock producers in proximity to critical water supplies will help reduce the risk of human exposure to livestock-derived AMR bacteria, thereby helping promote sustainable livestock production for years to come. The benefit-cost of alternative GAPs will no doubt be different for each livestock operation given their unique characteristics, but the key principle is to design and maintain a site-specific collection of GAPs that are designed to mitigate the anticipated flux of enteric bacteria and their AMR-MDR elements from livestock manure.

K93

Current situation of antibiotic resistance in human health and future perspectives

Rosa del Campo Moreno.

Hospital Ramón y Cajal, Madrid, Spain.

Objective: The aim of this paper is to review the current situation of antibiotic resistance in the hospital setting, from a clinical and epidemiological point of view.

Material and methods: A search will be performed in PubMed and Google Scholar for the last 5 years including both the most prevalent and the most infrequent resistance mechanisms. In addition to hospital publications, we will also include those associated with health care centers, especially long-stay nursing homes, and in relation to antibiotic consumption.

Results: Resistance to carbapenems, beta-lactams and colistin in Enterobacteriaceae is the main current problem in the hospital environment as it affects the microorganisms that most frequently cause infections. On the other hand, the increased incidence of *Clostridioides difficile* is also a considerable problem, together with the intra-hospital dissemination of high-risk clones with multi- or pan-resistance to all antibiotics. Antibiotic stewardship policies allow a more rational use of the latest generation antibiotics; however, we are increasingly aware that we need new strategies to fight against antibiotic multi-resistant microorganisms. At this point, phages seem to be the most promising alternative, in addition to ecological strategies that aim to replace resistant populations with sensitive ones with higher fitness.

Conclusions: Antibiotic resistance is a silent pandemic worldwide and one of the major health problems directly linked to increased human death, morbidity, and healthcare costs. The search for alternatives to antibiotics is necessary and must include a microbiological and ecological perspective to avoid future problems.

Parasitism

K94

What´s new in bovine neosporosis

Luís Miguel Ortega-Mora[1], Pilar Horcajo[2], Iván Pastor-Fernández[2], Esther Collantes-Fernández[2].

[1]UCM, Madrid, Spain; [2]SALUVET, Animal Health Department, Faculty of Veterinary Sciences-UCM, Madrid, Spain.

Bovine neosporosis is caused by the parasite *Neospora caninum* a worldwide concern due to its global distribution and leading role as an abortifacient. The impact of *N. caninum* abortions in just ten countries representing the major cattle industries of the world was estimated to be on average US$ 1 billion per year. In a recent systematic analysis *N. caninum*, opportunistic bacteria, BVDV and *Leptospira* spp. were the pathogens with the highest participation in transmissible abortions. The presence of *N. caninum* was analysed in 3,492 cases of bovine reproductive failure from 26 different studies, being involved in 28% of them (CI: 22.3%-33.7%). The percentage of *N. caninum* detection obtained by histopathology (42%), was greater than that obtained by PCR (26.5%), serology (18.5%) and immunohistochemistry (12.9%).

In cattle, the only transmission routes demonstrated are the postnatal infection by ingestion of oocysts shed by a canid, usually the domestic dog (horizontal transmission), and the transplacental passage from the dam to the foetus (vertical transmission). Two different forms of transplacental transmission have been described: the endogenous transplacental transmission (EnTT), due to parasite reactivation in persistently infected dams during gestation, considered the main transmission route in cattle, and the transplacental transmission (ExTT), that occurs when dams are infected during gestation by ingestion of oocysts and is less frequent. Both ExTT and EnTT may result in abortion, either epidemic (storm-like, associated to ExTT and with a highly negative economic impact) or sporadic/endemic (low level occurrences, associated to EnTT). In recent years, we have had the opportunity to study various epidemic outbreaks of N. caninum abortion in large dairy herds with a high economic impact. Remarkably, *N. caninum* can also cause abortion in other domestic ruminant species such as sheep, goats, and water buffaloes. Recent reports of dairy sheep flocks have described abortion rates up to 25% and a seroprevalence of 32%. Seropositive sheep were twice (OR =4.44) or three or more times (OR = 10.13) more likely to abort than seronegative sheep, and EnTT was the main route of transmission.

Several host and parasite factors can influence the dynamics of the infection in pregnant bovines, resulting in abortion or the birth of healthy calves. However, the mechanisms that determine abortion occurrence are not fully understood. On one hand, tissue damage and disruption of placental functions due to active parasite replication have been proposed as abortion triggers. On the other hand, an immune-mediated mechanism has been also proposed as a possible cause of abortion. In this regard, the clinical outcome of neosporosis is strongly associated with the period of gestation at which primary infection occurs. This is because the response gen-

erated against the parasites is highly dependent not only on the status of the dam's immune system, but also the immunological maturity of the foetus. In addition, disease severity is heavily determined by the parasite strain. So far, several *N. caninum* strains isolated from different hosts at different locations have been genotyped by whole-genomics analyses. These studies support the presence of a single, highly inbred genome that has been expanded worldwide by vertical transmission. Despite this, marked differences in virulence in pregnant cattle have been observed among different parasite isolates. In this sense, we have demonstrated that the Nc-Spain7 isolate prompts foetal death and displays high rates vertical of transmission, an increased tissue dissemination, higher parasite burdens, and more severe lesions in placental and foetal tissues. In contrast, infection with the Nc-Spain1H isolate does not induce foetal death or lesions in target tissues.

Currently, there are no effective vaccines or treatments against infection with *N. caninum*. Thus, control options are restricted to diagnosis, the implementation of biosecurity measures, and the application of management practices. Despite having a pivotal role to control or reduce the abortions induced by *N. caninum*, works focused on this have been anecdotal in the last years. This contrasts with the extensive efforts invested on the development of inactivated, sub-unit or live vaccines against bovine neosporosis, with little success to date.

Consequently, major efforts are still required to understand the mechanisms underlying *N. caninum*-abortion and develop efficient control measures to prevent it. Until them, adequate management practices have the potential to buffer the negative effects of neosporosis in ruminant production.

K95

Piroplasmosis of cattle

Alessandra Torina[1], Valeria Blanda[2].

[1]Istituto Zooprofilattico Sperimentale della Sicilia; [2]OIE Reference Laboratory for Babesiosis- Istituto Zooprofilattico Sperimentale della Sicilia.

Cattle piroplasmosis is a tick-borne protozoal disease of wild and domestic Bovidae caused by blood parasites of *Theileria* and *Babesia* genera (phylum Apicomplexa, order Piroplasmida). They are transmitted by ixodid ticks and their life cycles occur in both vertebrate and invertebrate hosts.

After the tick bite, *Theileria* sporozoites infect leukocytes and multiply inside them by merogony, mature schizonts develop into merozoites, which are released and invade erythrocytes, forming piroplasms. *Theileria* species can be gathered into schizont "transforming" or "non-transforming" species, according to their ability to immortalize infected leukocytes.

Transforming parasites include agents of severe diseases, such as *Theileria annulata* (responsible of tropical theileriosis, TT, or Mediterranean theileriosis) and *Theileria parva* (agent of East Coast fever, ECF), while the most important non-transforming species is *Theileria orientalis* (*T. orientalis/buffeli* group), which causes Oriental theileriosis (OT) or *Theileria*-associated bovine anaemia (TABA).

As concerning their distribution, *T. parva* occurs mainly in sub-Saharan Africa and *T. annulata* in Southern Europe, North Africa and Asia. *Theileria orientalis/buffeli* complex consists of eleven identified genotypes, of which Chitose and Ikeda genotypes are associated with severe disease. *T. orientalis* is globally widespread, but clinical OT cases have been reported in Australia, New Zealand, Japan, Korea, China and Vietnam.

Theileria taurotragi and *T. mutans* generally cause no disease or mild disease and *T. velifera* is non-pathogenic. These last three parasites occur mainly in Africa, with an overlapping distribution.

Theileria species are mainly transmitted by ticks of *Hyalomma* genus, even if other genera, such as *Haemaphysalis* and *Rhipicephalus,* can be involved.

Cattle theileriosis is characterized by leukocyte proliferation following pathogen infection. Other symptoms include fever, lymphadenopathy, and anemia. Not treated animals die within 3–4 weeks of infection. The pathology causes a significant reduction in animal fertility and economic losses to the livestock industry. Oriental theileriosis is characterized by haemolytic anaemia, jaundice, lethargy, tachycardia and late-term abortion in pregnant animals. *T. orientalis* infections can remain subclinical and may exacerbate under stress conditions.

Current control measures include acaricides for vector control, therapy with anti-theilerial drugs, largely dependent on the use of hydroxynaphthoquinone, namely buparvaquone, and vaccination. A complex immune response is elicited by theileriae parasites. Cell-mediated immunity is the most important protective response in *T. parva* and *T. annulata* infections and several molecules of the pathogen able to induce host immune responses are currently under investigation as candidate vaccine antigens.

Concerning bovine babesiosis, *Babesia* injected sporozoites infect directly red blood cells and multiply inside erythrocytes, resulting in red blood cell lysis.

Babesia bovis and *B. bigemina* are worldwide widespread species affecting cattle and they are mainly transmitted by ticks of *Rhipicephalus (Boophilus)* genus. *Babesia divergens*, the other prominent species affecting cattle, is relevant in some European regions and it is mainly transmitted by *Ixodes ricinus.*

B. bovis is generally the most pathogenic one, with infections characterized by high fever, ataxia, anorexia, general circulatory shock, and sometimes neurological signs. Anaemia and haemoglobinuria usually appear at a later stage of the infection. *B. bigemina* and *B. divergens* show similar clinical signs, mainly characterized by fever, haemoglobinuria and anaemia.

Infected hosts develop both humoral and cellular immunity against *Babesia*. Vaccines consisting of live, attenuated strains of *B. bovis*, *B. bigemina* or *B. divergens* are available in some parts of the world but their use is limited to younger calves, as they have still a nonspecific immunity. Older vaccinated animals should be treated with antibabesial drugs, mainly imidocarb, despite side effects occur.

As the success of the therapy towards cattle piroplasmosis depends on the timeliness of the treatment, an early diagno-

sis of clinically affected animals is essential to initiate control actions.

Diagnosis of acute cattle piroplasmosis is based on clinical signs and on examination of Giemsa-stained smears of peripherical blood or aspirated lymph nodes. The indirect fluorescent antibody test is the most widely used diagnostic test. Molecular biology provides useful tools for *Theileria* and *Babesia* species identification and genetic characterization. Several molecular methods targeting different markers have been developed, including polymerase chain reaction (PCR) and sequencing, real-time PCR, reverse line blot hybridization, loop-mediated isothermal amplification.

This presentation will focus on the main agents of cattle piroplasmosis, with an update on their global distribution, arthropod vectors, treatments, and the development of vaccines and diagnostic tools.

K96

Best-bet integrated control strategies for managing trypanosomosis and trypanocidal drug resistance in African village cattle populations

Peter-Henning Clausen.

Freie Universität Berlin, Germany.

Objectives: Trypanosomosis Rational Chemotherapy, TRYRAC, was a 5-year project (2012-2017) funded by the Global Programme on Agricultural Research for Development of the European Commission. Its overall objective was improving the livelihoods of resource-poor livestock producers in smallholder production systems through sustainable control of African Animal Trypanosomoses (AAT) where the major constraint is drug resistance. Development of trypanocide drug resistance depends on a multi-factorial process driven by (i) trypanocidal drug use practices, (ii) quality of trypanocidal drugs, (iii) ability to detect resistance and (iv) availability of strategies minimizing and controlling resistance at the livestock keeper level, particularly smallholders. The TRYRAC project addresses each of these factors through improving the capacity and capability of African laboratories and veterinary services to detect trypanocidal drug resistance, to conduct quality control of trypanocidal drugs and to promote and monitor the use of best-bet control strategies aiming at a rational drug use approach.

Materials and methods: Cross-sectional surveys were conducted to assess the trypanosome infection prevalence in randomly selected village cattle herds in the provinces of Kara and Savanes of northern Togo and in the Gurage zone of Ethiopia. Thereafter, drug sensitivity studies were carried out in hot spot areas (with a trypanosome prevalence > 10%). Based on these results, best-bet integrated strategies consisting of (i) rational use of quality tested trypanocidal drugs in symptomatic cattle, (ii) targeted insecticidal spraying of the lower body parts of cattle with a deltamethrin formulation and (iii) strategic use of anthelminthic drugs (albendazole) were implemented in risk group animals (calves < 2 years). Anthelmintic treatments took place at the beginning and end of the rain season.

Results: Single and multiple drug resistance against the therapeutic drug diminazene aceturate (DA) and the prophylactic drug isometamidium chloride (ISM) at the recommended dosages were detected with treatment failure rates ranging from 0 to 30% for DA and from 0 to 50% for ISM (Tchamdja et al. 2017). More than 40% of the tested trypanocidal drugs from Togo (Tchamdja et al. 2016) and 28 % from Ethiopia (Tekle et al. 2018), respectively, did not conform to FAO established quality standards. Rational use of trypanocidal drugs in symptomatic animals in Togo, combined with targeted insecticidal spraying and strategic deworming, resulted in much lower numbers of trypanocidal treatments, compared to control herds, whereby the animals remained in good health (Tchamdja et al. 2019).

Conclusions: This study demonstrates that the implementation of an integrated best-bet strategy leads to a reduced trypanosome prevalence under lowered trypanocidal use. In the absence of new trypanocidal drugs, rational use of the available drugs will be an appropriate strategy minimizing the risk of drug resistance development.

References:

Tchamdja, E.; Kulo, A. E.; Akoda, K.; Teko-Agbo, A.; Assoumy, A. M.; Niang, E. M. M.; Batawui, K.; Adomefa, K.; Bankolé, A. A.; Kombiagou, K.; Hoppenheit, A.; Clausen, P.-H.; Mattioli, R. C.; Peter, R.; Napier, G. B.; De Deken, R.; Marcotty, T.; Van Den Abbeele, J.; Delespaux, V. (2016): Drug quality analysis through high performance liquid chromatography of isometamidium chloride hydrochloride and diminazene diaceturate purchased from official and unofficial sources in Northern Togo. Preventive Veterinary Medicine; 126, 151–158.

Tchamdja, E.; Kulo, A. E.; Vitouley, H. S.; Batawui, K.; Bankolé, A. A.; Adomefa, K.; Cecchi, G.; Hoppenheit, A.; Clausen, P. H.; De Deken, R.; Van Den Abbeele, J.; Marcotty, T.; Delespaux, V. (2017): Cattle breeding, trypanosomosis prevalence and drug resistance in Northern Togo. Veterinary Parasitology; 236, 86–92.

Tchamdja, E.; Clausen, P.-H.; Kulo, A. E.; Batawui, K.; Bauer, B.; Den Abbeele, J. V.; Delespaux, V.; Hoppenheit, A. (2019): How rational drug use reduces trypanosome infections in cattle in chemo-resistance hot-spot villages of northern Togo. Acta tropica; 190, 159–165.

Tekle, T.; Terefe, G.; Cherenet, T.; Ashenafi, H.; Akoda, K. G.; Teko-Agbo, A.; Van Den Abbeele, J.; Gari, G.; Clausen, P.-H.; Hoppenheit, A.; Mattioli, R. C.; Peter, R.; Marcotty, T.; Cecchi, G.; Delespaux, V. (2018): Aberrant use and poor quality of trypanocides: a risk for drug resistance in south western Ethiopia. BMC Veterinary Research; 14(1), 4.

K97

Venereal diseases in beef cattle: an old problem that needs new approaches

Luís Miguel Ortega-Mora[1], Esther Collantes-Fernández[2].

[1]UCM, Madrid, Spain; [2]SALUVET, Animal Health, Department, Faculty of Veterinary Sciences-UCM, Madrid, Spain.

Bovine trichomonosis (BT) and bovine genital campylobacteriosis (BGC) are sexually transmitted diseases of cattle listed by the World Organization for Animal Health (OIE). Both venereal diseases are considered an important cause of early reproductive failure in beef cattle kept under extensive management conditions, placing important restrictions on the international trade of animals and animal products. BT is caused by the flagellated protozoan parasite *Tritrichomonas foetus* (Tf). The causative agent of BGC is *Campylobacter fetus* subsp. *venerealis* (Cfv), a microaerophilic, Gram-negative and motile bacterium with a characteristic spiral form. *Tritrichomonas foetus* and Cfv colonize the preputial cavity of bulls and the urogenital tract of females and are transmitted during coitus. Bulls act as asymptomatic carriers, whereas the disease in females causes embryonic death or early abortion. Economic losses arise from prolonged calving intervals, reduced calf drop caused by failure of or delay in conception, increased culling of infected animals and increased veterinary costs.

BT and BGC are still prevalent in some areas of Australia, North and South America and Africa. In Europe, the widespread use of artificial insemination (AI) and effective control programmes have greatly reduced the incidence of both diseases. However, recent studies in beef cattle in Spain found a prevalence of *T. foetus* infection of 12.7% in bulls (385/3016) and 20.7% in farms. In a different study, the prevalence of BCG was 7.7% (400/5182) and 12.2% for bulls and farms, respectively. These results highlight the need to diagnose these diseases in geographic areas and in management systems where risk factors for venereal diseases are present. In terms of diagnosis, bulls should be the target for diagnostic investigations, epidemiological studies, as well as control and prevention strategies. The laboratory diagnosis for BT is well-established with sampling methods, transport of the sample to the laboratory and detection by parasite growth in culture and confirmation by PCR perfectly validated. However, the laboratory diagnosis of Cfv by microbial culture, direct immunofluorescence test and PCR can be problematic due to different factors related to its growth and the differentiation between Cfv and *C. fetus fetus*. Bacteriologic culture with subsequent phenotypic identification remains the gold standard for the detection of Cfv. Nevertheless, isolation of Cfv from field samples is difficult, showing low sensitivity. Subsequently, the majority of Cfv detection is currently achieved by PCR assays, although the specificity of these techniques is, at present, under discussion.

Programs for the prevention and control of venereal diseases are partially successful, because risk factors (e.g. communal pastures, the lack of diagnostic tests before the breeding season and a the use of old bulls) cannot be avoided. Vaccination could be a feasible control measure to reduce the economic losses. Vaccines are only indicated for cows since they do not induce protection in bulls. In most cases, vaccines will not prevent infection, but they will reduce the time to clearance of both pathogens from the reproductive tract before foetal loss occurs, improving pregnancy rates by decreasing the duration of endometritis. A new vaccine capable to stimulate humoral and mucosal immunity against Tf and reducing the length of the parasite genital infection in cows under experimental challenge has been recently developed. Under natural infection situations, this effect was translated into shorted calving intervals and earlier calving date, improving the reproductive performance and reducing the financial losses that come with open cows and late-born calve. BGC is mainly controlled by diagnostic testing, reporting, and culling of infected bulls. Antibiotic treatment (streptomycin or oxytetracycline) can be successful in bulls under 3 years old, but it is often not effective in older bulls, thus culling is recommended. Unfortunately, and despite several commercial vaccines for BGC have been associated with some protection in cattle, they are not commercially available worlwide.

HIPRA

When our customers
ARE AT THE CENTER

HEALTH CENTRICITY

Working together to understand and contribute
to **raising healthy animals.**

Avda. la Selva, 135, 17170 Amer (Girona), Spain
Tel.: (+34) 972 43 06 60 · hipra@hipra.com · www.hipra.com

WORKSHOPS

W01 – W03	ECBHM WORKSHOP
W04	REPRODUCTION IN BEEF CATTLE WORKSHOP
W05 – W07	REPRODUCTION IN DAIRY CATTLE
W08	REPRODUCTION WORKSHOP
W09	GENETICS AND BREEDING WORKSHOP
W10	CONTINUING EDUCATION WORKSHOP
W11 – W12	UDDER HEALTH WORKSHOP (IN FARM)
W14	CATTLE WELFARE WORKSHOP
W15	DIAGNOSTIC IMAGING WORKSHOP
W16 – W18	NUTRITION AND BIOTECHNOLOGY WORKSHOP
W19 – W21	BIOTECHNOLOGY WORKSHOP
W22	EPIDEMIOLOGY WORKSHOP
W23	SURGERY WORKSHOP
W24 – W27	APPLICATION OF SYSTEMS THINKING DISCIPLINE IN BEEF CATTLE VETERINARY MEDICINE



MSD Animal Health

Smarter Solutions.
Smarter Prevention.
Smarter Connections.

#ScienceOfHealthierAnimals

Visit MSD Animal Health on Floor 3, Booth #15/16

and receive your personalized WBC 2022 ear tag



Join MSD Animal Health for a Symposium on

"Connected Cattle: The benefits of smart monitoring solutions in veterinary medicine"

Roma Room (Floor 2)
Wednesday 7th September - 15:00 - 16:30

msd-animal-health-ruminants.com

Copyright © 2022 Merck & Co., Inc., Rahway, NJ, USA and its affiliates. All rights reserved.
GL-NON-220600015

MSD Animal Health

ECBHM WORKSHOP

W01

Influence of water quality to the Somatic cell count in dairy herds

Beat Berchtold.

Tierärztliche Bestandesbetreuung, Switzerland.

Water is the number one component in cattle feeding. Calves as well as heifers and cows react sensitively to an elevated bacterial load in water. The clinical signs can vary from indigestion, high somatic cell count (SCC), clinical mastitis or lameness problems. If the taste of water is affected, decreased water intake has a direct effect on milk yield. The pathogenesis of the clinical signs associated with increased bacterial counts in the drinking water of dairy cows is not known so far. A possible explanation is the presence of bacterial toxins in the water. The physical and chemical qualities of the water as well as the buildup of the water pipe, as well as the flow rate can have an impact on the unknown pathogenesis and observed clinical signs in cattle. Clarification of the water quality is done by bacteriological analysis of E. coli, Enterococcus sp. and aerobic mesophilic germs. A acceptable water quality is negative for E. coli and Enterococcus sp.. The amount of aerobic mesophilic germs must be less than 300 colony forming units per ml water.

The presented case report describes a rise in SCC and clinical mastitis rate after installation of an automatic milking system in a Swiss dairy farm. Investigation on the farm revealed inadequate water quality as the main risk factor. Bacterial analyses of the water show an elevated count of aerobic mesophilic germs. The result for E. coli and Enterococcus sp. was negative. After improving the quality of the drinking water by physical ultraviolet treatment, bulk tank milk SCC decreased and cases of clinical mastitis almost disappeared.

In case of concerns about cattle health, the water quality on farms should be considered as a potential risk factor after exclusion of other causes. Poor water quality may also compromize cattle health in combination with other causes. The importance of water in cattle feeding justify the regularly check of its quality in the frame of herd health management.

W02

The comparison of two heat detection systems in a large dairy herd in West Wales

Lies Beekhuis.

Tivy Vets, UK.

Objective: In the United Kingdom most dairy herds rely on oestrus detection to artificially inseminate (AI) dairy cows. When a cow finishes her voluntary waiting period it is essential that oestrus is detected timely and accurately to ensure AI takes place on the correct day, at the right time. The objective of this study was to compare and analyse interservice intervals as a measure for accuracy of heat detection for two different heat detection systems that were used consecutive years in a dairy herd in West Wales.

Materials and methods: Reproductive data was collected from one large dairy herd in West Wales. All data derived from cows in milk. Breeds used in the analysis were; Holsteins, Jerseys and Crossbred (Holstein x Jersey) cows. Four consecutive breeding seasons spanning from November 2017 to October 2021 were analysed. In the analysed period two different heat detection methods were used. From September 2017 to April 2019 oestrus was detected using manually applied chalk on the tail head and visual oestrus detection [chalk system]. From October 2019 to October 2021 heat was detected with heat detection collars and additional visual detection [collar system].

Results: The herd size was around the 800 cows during the study period. Cows calved down in autumn and winter and were bred from November to April each year. Average yearly submission rates were higher with the chalk system then with the collar system. Average yearly pregnancy rates were higher with the collar system than with the chalk system. Further analyses of the interservice intervals indicated that the collar system was more accurate in detecting heat than the chalk system.

Conclusions: It is important to keep in mind that timely and accurate heat detection in a dairy herd depends not only on the used heat detection system but also on other factors like nutrition and housing. The analysed data showed that submission rates were higher when the chalk system was used compared to when the collar system was used. Nevertheless, with the collar system cows were more likely to conceive to a service than when the chalk system was used.

W03

Sustainable parasite management in French cattle; treat as little as reasonably achievable

Philippe Camuset.

Cabinet vétérinaire Yvetot, France.

Objectives: In 1981, ivermectin was released on the French veterinary market. The then recently understood type 2 ostertagiasis could be successfully managed and warble flies became eradicated. Those treatments were applied at the end of the grazing season. During the following years, long acting devices, or protocols involving macrocyclic lactones, prevented the strongyle infestation, thus optimizing growth during the first and second grazing seasons. However, the combination of those preventative and curative worming treatments didn't allow the development of immunity against strongyles. So, to avoid parasitism being an issue after the first grazing season, it was necessary to repeat treatments in the second grazing season, even in adult cows. As a result, resistant worm populations were developing and becoming a real concern and, since the beginning of the 2000's, ecotoxicity of macrocyclic

lactones was reported and this now leads to a real One Health concern. This paper describes how our practice has been involved in parasite management since the 90's, with regular communication with our farmers, and, since 1997, the regular assessment of serum pepsinogen levels at the end of the first grazing season to manage winter worming programmes aimed at digestive strongyles. The first parasitism audits were implemented in 2004 with a sustained deployment from 2008. Currently, about fifty herds are followed up each year.

Materials and methods: The essence of the audit is based on the assessment of the parasitic risk, mainly on pasture. The parasites present on the farm are examined and, if necessary, laboratory based-tests (serology, coproscopy) are prescribed in order to get a complete picture of the parasitism of the concerned herd. A strong understanding of the pastures and the animal group management leads us to assess the parasitic risk for gastro-intestinal strongyles (GIS), Dictyocaulus as far as possible, and wet area trematodes. For GIS, the number of generations is estimated, the parasitic impact occurs after the second or the third one for naïve animals. For older ones, the immunity is taken into account. For dictyocaulosis, the presence of healthy carriers, the pasture management are studied. For wet area trematodes, the contact with the snail intermediate hosts is the key point to manage. According to the potential zootechnic or clinical impact we have assessed, agronomic or medical advice is proposed to the farmer. In a quality approach, laboratory based tests are used to validate the vet prescription at the end of the grazing season or during the next winter.

Results: Each year, 40 to 50 herds are followed up according to the described method; some for up to 14 years. Our stated goal is to "Treat as little as reasonably achievable". This concept is nowadays completely shared by our farmers. As an example among the 80 herds that have been followed up in our practice, JF Chauveau contacted us in October 2010 as his herd had clinical signs and collapsed milk production due to paramphistomes. Some cows were shedding more than 2000 epg in their faeces. Emergency measures were carried on and a complete audit was set up in March 2011. Thanks to reasoned treatments, paramphistomes were eradicated by 2021 and the farmer deworms now only first grazing seasons calves turned out from April to June with a suitable formulation of moxidectine in spring in order to balance zootechnical performances, ecotoxicity and resistance development. Older animals are dewormed in a targeted approach with eprinomectin.

Conclusion: Our practice is one of the leaders in France for bovine and equine parasitology audits. In our clinic, we have developed a parasitology unit implementing more than 600 coproscopies each year for herbivores. We have trained many colleagues to use this method in France and the 2020 mandatory bovine sanitary visit which we prepared with the French department of Ecology was about sustainable management of parasitism addressing the topics of ecotoxicity and resistances. Nowadays, farmers are demanding this kind of delivery, because they are conscious of their responsibility towards civil society. By this approach, we are completely involved in the One Health concept and, in our modest level, in the fight against the global warming process. Sustainable livestock breeding is one of the virtuous way of increasing the ability of soil flora and fauna to trap atmospheric carbon.

REPRODUCTION IN BEEF CATTLE WORKSHOP

W04

The role of the veterinarian in reproductive management programs: Maximizing fertility to FTAI in beef cattle around the glove

Gabriel Bo.

Instituto de Reproducción Animal Córdoba (IRAC), Zona Rural General Paz, (5145) Córdoba, Argentina. Instituto de Ciencias Básicas, Medicina Veterinaria, Universidad Nacional de Villa María, Villa del Rosario, Córdoba, Argentina.

The objective of the incorporation of valuable genetics in beef herds using AI is to improve profitability. The development of hormonal treatments that control the time of ovulation has allowed the development of Fixed Time Artificial Insemination (FTAI) protocols. However, it must be considered that a significant number of breeding herds are still using natural services with bulls. In these herds, a significant percentage of cows are in postpartum anestrus at the beginning of the breeding season, which causes a delay in their conception. This workshop will present experiments using cyclicity induction and ovulation synchronization programs with the use of intravaginal progesterone releasing devices combined with estrogen salts or with GnRH, in order to improve reproductive performance of commercial beef herds. A precise and detailed knowledge of the hormones involved in the FTAI protocols is essential. Furthermore, it is necessary to know the conditions of the herds and their productive objectives to determine which protocols best suit in their production system. In addition, other issues must be considered. As Veterinarians we must take care of all these issues to ensure satisfactory results and to avoid unpleasant surprises. The quality of the semen to be used, the personnel available to work with the animals with reasonable standards of animal welfare, and the quality of the facilities available to work with a large number of animals in a short period of time are essential to ensure good results. Health is an issue that will not be the focus of this discussion, but it is undoubtedly fundamental. It is necessary to implement an annual control and disease prevention program. In addition, a strict control of the entry of new animals is essential to prevent other diseases. Finally, although good pregnancy rates can be obtained, we must be clear that beef producers do not sell pregnancy rates, but rather their result is evaluated in kilograms of beef per hectare produced. Therefore, our responsibility is to coordinate and program activities that include everything necessary for the implementation of reproductive programs to be as efficient as possible. These technologies can contribute to the improvement of reproductive efficiency and, consequently, improve the profitability of beef production systems.

REPRODUCTION IN DAIRY CATTLE

W05

Calving management – how to predict and when to assist?

John F Mee[1-2], Carola Fisher Tenhagen[3], Otto Szenci[4].

[1]; [2]Teagasc, Moorepark Research Centre, Republic of Ireland; [3]Freie Universität Berlin, Germany; [4]University of Veterinary Medicine Budapest, Hungary.

Objective: Changing the way we manage cows on the point of, and during, calving has the potential to reduce time spent monitoring preparturient cows and unobserved calving-associated dystocia and stillbirth and improve perinatal outcomes for both the dam and her calf. Recent advances in agri-tech have generated numerous novel devices designed to predict calving time more accurately than traditional methods. In addition, the question of when to assist at calving has been addressed in recent research and the results may change the way we approach this task in future. This workshop addresses these topics. The format is modelled on workshop presented at the European Society for Domestic Animal Reproduction (ESDAR) annual conference in Saint Petersburg in 2019. The objective of the workshop is to present and discuss the latest research results on a topic of everyday interest to cattle veterinarians and their clients internationally: calving management, specifically prediction of calving time and assistance at dystocia.

Materials and methods: This is a genuine, interactive, delegate-focused workshop. The workshop will be opened by a moderator giving an overview introductory presentation on the topic of interest – calving management. The format of the workshop will be a Gallery Walk. This involves: 3 workstations with flip charts; delegates randomly divided between workstations to form 3 groups led by a delegate rapporteur; groups spend 10-15 mins at first workstation discussing allocated topic; groups (but not rapporteur) then rotate clockwise between the 3 workstations spending 5-7 and 5-7 mins at their second and third workstations. Moderator and speakers float across groups to answer questions but do not directly influence groups. When rotations are complete each rapporteur presents the conclusions for their topic to the whole workshop using the flip charts' bullet points. Moderator and speakers discuss. This is followed by two short oral communications by domain expert speakers on '*Devices to predict calving time'* and '*When to assist at calving?'* The moderator then concludes the workshop by summarising the key take home messages.

Results: The results of the workshop are the ideas, opinions and messages generated by the delegates at the three workstations: devices to predict time of calving, when/how to examine/assist at calving and how to train farmer clients in calving assistance/when to call the vet, in combination with the three oral presentations. The content on the workstation flip charts will be photographed and emailed to interested delegates following the workshop.

Conclusions: This workshop will present blended conclusions on the titular topic developed as a combination between delegate-generated feedback and speaker oral presentations. These may generate new ideas for future interactive discussion in this or related fields of interest to veterinary practitioners.

W06

Giving the right advice: expert's recommendation for calving management

Carola Fischer-Tenhagen[1], John F. Mee[2].

[1]Department Experimental Toxicology and ZEBET, Unit Animal Husbandry, Aquaculture and Reference German Federal Institute for Risk Assessment, Germany. [2]Department of Animal and Bioscience Research, Teagasc, Moorepark Research Centre, Fermoy, County Cork, P61 P302, Ireland.

Optimal calving management enhances the chances that the cow will transition into lactation successfully and that her calf will start life vigorous and healthy. Much research on physiology and pathology of the calving process has been published over the years. The object of this presentation is to distill these research findings into practical information which veterinary practitioners can use to improve both their calving management skills and those of their client farmers. Critically, it is essential to understand the physiological time scale of the birth process. Restlessness and visibility of fetal parts at the vulva are important predictors of an imminent calving. Information on the correct positioning/posture of the calf in the birth canal and "which are the easy ones" to correct, including hygienic procedures, should be topics in practical demonstrations by the veterinarian in charge on the farm. A clear time line for intervention and which dystocia cases are "the hard ones for the vet" are important principles to be delivered by the expert.

W07

Importance of predicting the onset of calving to prevent stillbirth on dairy farms

Szenci Ottó[1], Mee John F.[2].

[1]University of Veterinary Medicine Budapest; [2]Department of Animal and Bioscience Research, Teagasc, Moorepark Research Centre, Fermoy, County.

The profitability of cattle breeding is greatly influenced by the rate at which calves are born alive and reared to adulthood. Despite the recent developments in animal breeding, perinatal mortality in Holstein-Friesian heifers and cows is still very high (3.5 to 8%) in some countries. If the onset of calving can be accurately detected as well as appropriate calving assistance provided, the prevalence of dystocia, stillbirth, vaginal laceration, retained foetal membranes, and consequent clinical metritis/endometritis can be significantly reduced.

The goal is to provide obstetrical assistance at an appro-

priate time after detecting the onset of the second stage of labour. Traditionally detection of stage two was dependent upon intermittent/continuous observation. However, several devices are now commercially available to detect the onset of calving. These include biosensors which monitor body temperature, tail elevation, behavioural signs or the opening of the vulva.

The latest research results in this field are presented and the associated literature is reviewed. The advantages and disadvantages of the different predictive methods will be discussed to select the most accurate method for the detection of the onset of calving on a dairy farm. These results and the associated review indicate that currently the vaginal device is the most accurate method of predicting the onset of calving in dairy cows.

REPRODUCTION WORKSHOP

W08

Applied epidemiology: how to interpret and use research data in practice, using examples in reproduction

Aurora Villarroel.

Athyr Vet, LLC, USA.

Objectives: Clinicians often assume that anything that is published in a scientific journal is scientific evidence. However, not all publications represent research of a question or hypothesis, and not all publications are correctly performed or analyzed. The objective of this workshop is to train clinicians to differentiate various types of published papers, and how to determine if the methodology, analyses and conclusions are correct, so they can decide whether it is pertinent information to change the way they practice veterinary medicine.

Materials and methods: The workshop will start with short introduction explaining the various types of published papers and how to determine which type a specific paper is. Following this introduction, there will be real examples of published research of each type for clarification. At least two research articles will then be dissected to evaluate methodology, statistical analysis and author conclusions.

Results: At the conclusion of this workshop attendees will know not to use the abstract of an article as evidence, and instead consider it just an indication of whether the article may be able to provide the information they are seeking. Readers will be able to evaluate the actual information provided by the research only after reading the main sections of the article: “Materials & Methods” and “Results”. Attendees will be able to understand the difference between statistical significance (given by the p-value of a statistical analysis) and biological significance (the perceived utility of the conclusions provided by the research article for a clinician in their practice).

Conclusions: When reading published research, clinicians should focus on the “Materials & Methods” section of an article to determine if the methodology is appropriate to evaluate the studied research question, and then focus on the “Results” section to decide their own conclusions of the research, and whether they can use this new information to help in the treatment of their patients. It is valid to have different conclusions from those of the published article. The clinicians may have different conditions as those described in the study (for example, research done in a desert may not apply to a farm in a tropical setting and vice versa), or they may decide that the procedures required to obtain the studied outcome are not viable in their clinical setting (for example, handling cows multiple times for hormone application may not be an option). Finally, clinicians may conclude that the methodology or analysis of a research article is invalid and, therefore, no real conclusions can be drawn from it.

GENETICS AND BREEDING WORKSHOP

W09

How do we use genetic data to improve farm profitability

Francisco Peñagaricano.

University of Wisconsin-Madison, USA.

Effective use of genomics: Sire selection. Dairy sire selection has dramatically changed with the implementation of genomic selection. Nowadays, dairy farmers have basically two main options when they make sire selection decisions: use proven, progeny-tested bulls or use young genomic-tested bulls, i.e., young bulls with no progeny that have been evaluated using only their own genomic data. Notably, the number of young genomic-tested bulls currently in the market far exceeds that of progeny-tested bulls. The key concept regarding young genomic-tested dairy bulls is that, on average, these young bulls have greater predicted genetic merit values than the proven bulls. Now, these higher genetic values are accompanied by lower reliability values. The question is how dairy farmers should proceed in this scenario: farmers should use young genomic-tested bulls because they have greater PTA values, or, instead, farmers should use proven bulls because they have more reliable PTA estimates. The best strategy is to use a group or team of young genomic-tested bulls. The advantage of using a group of young bulls is that reliability of the average genetic merit of the team is considerably greater than the reliability of each individual bull.

Effective use of genomics: Replacement heifer selection. The selection of replacement heifers in commercial dairy farms has been traditionally characterized by very low intensity of selection, because, in general, farmers retain nearly every heifer calf as a future herd replacement. However, recent improvements in herd management and the use of sexed semen have led to produce a considerable surplus of heifer calves. In this context, the selection of replacement heifers is feasible, and genomic testing allows the identification of superior or inferior heifer calves accurately and at an early age. The identification of genetically inferior heifer calves allows early culling of these animals, significantly reducing the cost of rearing replacements. Alternatively, these genetically inferior heifers can be inseminated with beef semen to produce high-value crossbred beef calves. On the other hand, the identification of superior heifers through genomics can be combined with the use of advanced reproductive technologies to rapidly propagate these animals and generate superior replacements. These high-genetic-merit heifers can be used as donors in either an in vitro fertilization program or an embryo transfer program. Instead, these superior heifers can be inseminated using sexed semen from top sires. It is worth noting that genotyping replacement heifers has extra benefits other than making proper selection and mating decisions, including parentage verification, controlling inbreeding, and avoiding the spread of genetic disorders through genomic-enhanced matings. Arguably, these benefits add value to genomic testing.

CONTINUING EDUCATION WORKSHOP

W10

Models of clinical education of bovine medicine

Ángel Abuelo Sebio[1], Carlos Risco[2].

[1]Michigan State University, USA; [2]University of Oklahoma, USA.

The veterinary medical profession has a long and laudable history of meeting evolving societal needs throughout the world. From the beginning of our profession, and continuing today, veterinarians have been at the forefront in providing care and disease prevention to livestock. An emerging societal need is the global increased demand for a wholesome and economical source or animal-based protein. A challenge that the veterinary medical profession faces to meet this need, is the global shortage of food animal veterinarians. Without veterinarians, farmers, public health, and the food supply are more vulnerable to potential disease outbreaks and other sources of economic loss that impacts food security. The shortage of food animal veterinarians is due to multifactorial problems such as to growing student debt, long hours, and fewer graduates wanting to live in rural communities. Another challenge is that beef and dairy production units have modernized with the application of new technologies and grown in scale and a reliance on intensive capital and population-based approaches to animal production. This change has resulted in different expectations from these producers for veterinary services. Although some veterinary curricula have expanded to include education in the application of these new technologies in herd hearth and preventive medicine, food animal clinical training continues primarily with a focus on individual animal medicine, diagnosis and treatment of disease. Arguably, these management changes in beef and dairy production to a more system based, have resulted in a growing shortage of veterinarian who have the expected training and skills to meet the needs of producers that operate in an intensified technology based animal-based food production system.

Materials and methods: The presentation will address 1) Historical Perspective of Veterinary Medical Education in Food Animal Medicine - what are the gaps that can be filled in our current training ? 2) Strategies to reduce food-animal veterinary service shortages 3)Development of continuing education programs for veterinarians focused on production medicine - does one size fit all? The afternoon session will consist of a roundtable by faculty from colleges of veterinary medicine on the pros and cons of models used in cattle clinical education. Practitioners in attendance will be encouraged to provide input and participate in the discussion.

Results and Conclusion: The goal of the presentation and roundtable is to consider options on best educational practices to enable food animal veterinarians to be more successful in their professional careers and personal lives. More specifically, an educational program that will give food animal veterinarians the tools and skills that will increase the quality and quantity of the services provided to beef and dairy cattle producers. A summary of the discussions of the roundtable will be provided to attendees of the conference.

UDDER HEALTH WORKSHOP (IN FARM)

W11

The importance of milking time assessments (observations and not physical measurements)

Ian Ohnstad.

The Dairy Group (UK).

There are International Standards on the procedures and methods for testing milking machines (ISO 6690:2007) and the basic operating parameters required (ISO 5707:2007). As these tests do not include actual milking, the results may lead to an incomplete evaluation and provide misleading results (International Dairy Federation, Bulletin 396/2005).

Objective: To highlight a number of key points that will allow competent personnel to assess the suitability of the milking system.

Materials and Methods: There are three broad areas to consider.

1. *Mechanical observations*

- Vacuum levels – checking the accuracy of the gauge set for the type of machine in operation (high or low level milklines). Too low a level can extend machine on time, increase liner slip and may decrease milk yields. Teat congestion and incomplete milking indicate too high a level.
- Vacuum stability – as important as working level. The vacuum at the receiver vessel should fluctuate no more than +/- 2.0kPa during milking, nor between the receiver vessel and milkline for more than 95% of a normal milking.
- Vacuum in the liner mouthpiece chamber (MPC) - to average at least 10kPa less than the average claw vacuum during peak milk flow. Teat barrel congestion and palpable mouthpiece rings are usually reduced when the MPC vacuum is less than 20 kPa. Higher MPC vacuum levels are observed with over-milking and larger bore liners. The presence of palpable mouthpiece rings on more than 20% of teats warrants investigation (increased mastitis new infection rates).
- Operation of the vacuum regulator. A simple test when air is admitted. Listen to see if the regulator closes off or the speed of a VFC increases.
- Fall off test – to assess if the machine can keep the vacuum level stable when a unit is removed or kicked off.
- Pulsation – listen closely to each pulsator for uniformity. Check liners are fitted correctly and in good condition. Correct liner movement can be assessed using the thumb test.
- Liner slippage – if more than 5% per milking then investigate and action.

2. *Operator observations*

- Attachment of the milking units – with minimum air admission and a smooth, efficient manner. Units must hang squarely on the udder with equal weight distribution between all four teats.
- Removal of the milking unit. Clusters must not be removed while under vacuum. Check for blocked air bleeds which also lead to slower milking, liner slippage and teats being bathed in milk during peak milk flow.
- Over-milking must always be avoided. With twice a day milking, units should be removed promptly when the flow rate drops to between 0.3 – 0.5 kg/min and nearer 0.6 – 0.8 kg/min with three times a day milking. If hand stripping identifies that >20% of quarters yield more than 100ml milk, then investigate.
- Cleanliness of the operator and facilities is essential.
- Demeanour of the operator. Dairy cows respond positively to a quiet, calm and consistent milking environment, including cow collection. If more than 5% of cows defecate something is wrong.
- Consistency of milking routine, with all cows receiving the same preparation intensity and duration, with standard time lags from first contact to attachment of the cluster.
- Milk Let down. Good preparation is essential for good milk let down and achieving less than 10% of bi-modal milk flow. A calm, well stimulated cow should produce around 50% of her production within 2.0 minutes of unit attachment.
- Teat disinfection. Teats should be disinfected as soon as practical after cluster removal, ensuring total teat coverage.

3. *Cow Observations*

- Cow behaviour. An effective milking requires calm quiet cows for optimum milking efficiency and milk quality.
- Teat Condition. Routine assessment of teat condition is part of any milking time assessment. The National Mastitis Council (nmconline.org) has recently updated Teat Condition Scoring.
- Cow cleanliness. Essential for milking efficiency and milk quality. Changes in management, environment and housing can be clearly identified with regular scoring.

Conclusions: Detailed observations at milking time can highlight the strengths and weaknesses of the overall milking process. Any comprehensive assessment needs to take account of the complex interaction between milking machine, operator and cow. Failure to understand the relationship may lead to inappropriate conclusions being drawn and incorrect recommendations.

W12

Milking time assessment – an useful tool in the armoury for a dairy veterinarian

Ronald Erskine, Ian Ohnstad.

Michigan State University, USA.

Background: Most dairy farms have their milking equipment evaluated and maintained on a routine basis. Although proper equipment function is necessary for milking performance, it does not guarantee it. Two management areas that can lead to poor milking efficiency are: 1) milking routines that don't achieve consistent milk letdown and 2) overmilking. Either one of these problems can leave cows 'high and dry' and expose teats to high vacuum levels. Improper function of pulsation, milking vacuum, or the interaction of vacuum with liners and milking cluster design can be also problematic.

Additionally, many dairy operations are increasingly relying on hired labor, especially foreign-born workers. However, many dairy managers have limited human resource knowledge and experience; this often leads to frustration with protocol drift and employees who have little training to understand 1) milking dynamics, 2) the operation of the equipment they use every day, and 3) troubleshooting problems with milking equipment.

Methods: This will be an interactive session in which we will discuss case studies and applied research regarding milking dynamics and the relation to milk quality, udder health, and herd profitability. This will be an opportunity for dairy veterinarians to better understand the impact of milking protocols on milking performance of the cows. What do the cows tell us about their milking experience? What tools and observations can we use to improve the cow's experience? What are the outcomes we should monitor to evaluate changes in protocols and management?

Objectives:

1) Evaluating milking machine performance.
2) Learning observational tools to use during milking evaluation.
3) Tracking outcomes of changes in milking protocols and machine operation.

CATTLE WELFARE WORKSHOP

W14

Animal welfare assessment at farm level and its implications for economic sustainability of dairy farms

Trevor De Vries[1], Xavier Manteca[2].

[1]Guelph University, Canada; [2]Universidad Autónoma de Barcelona, Spain.

Animal welfare has become an essential aspect of modern livestock production. Animal welfare assessment tools are needed to identify problem areas and monitor progress when improvement strategies are implemented. The objective of this workshop is to discuss the principles underlying welfare assessment and how welfare assessment protocols may be used to improve the economic sustainability of dairy farms.

Animal welfare may be assessed using indicators, i.e. variables that can be measured objectively. Because of the multidimensional nature of animal welfare, no indicator is enough by itself to assess the welfare of an animal or group of animals. Thus, a combination of several indicators should be used if welfare is to be evaluated.

Welfare indicators should meet the following requirements: First, they should be valid, that is, they should really measure animal welfare. The validity of an indicator may be assessed by expert opinion or, preferably, by investigations in which this indicator is compared with an independent measure of welfare. Second, welfare indicators should show a high intra- and inter-observer reliability. Third, indicators should be practical and ideally minimally or non-invasive for the animals.

Welfare indicators are divided into two groups: animal-based indicators and environment or resource-based indicators. Animal-based indicators are all those variables that are measured directly in animals, such as frequency, duration or intensity of a behaviour, incidence or prevalence of health conditions, or plasma concentration of hormones, as examples. Environment-based indicators include the size and design of facilities where animals are kept, the quantity and quality of food they receive, the temperature at which they are exposed, etc.; in short, environment-based indicators are variables that are not measured in animals, but in their environment.

The main difficulty of environment-based indicators is that a given environmental variable can have very different effects on animal welfare. This is due, first, to the fact that individuals of the same species may respond differently to a feature of the environment. A second reason why the effects of environmental variables are not always predictable is that a phenomenon of interaction between variables may occur, often as result of different management within similar environments.

Because the effect of environmental variables on welfare may not always be reliably foreseen, several researchers have suggested that, as far as possible, animal welfare should be evaluated with animal-based indicators that provide direct information on the state of animals. This does not mean, in any way, that environment-based indicators are not useful. There are some welfare problems that may be more easily measured with environment-based indicators. For example, it is

often easier to check that animals have free access to good quality water than to assess any possible state of dehydration. In this case, several environment-based indicators (number, location, operation and design of the water troughs, and water quality) would be more useful than an animal-based indicator (signs of dehydration). Furthermore, although animal-based indicators are especially useful for evaluating welfare state, environment-based indicators are needed to identify the cause of a possible problem and allow for continuous improvements.

Animal-based indicators can be grouped into four main categories: indicators related to the behaviour of animals; indicators related to the appearance of animals; physiological indicators, and indicators obtained from farm records, such as the prevalence and incidence of diseases.

To assess the overall welfare of an animal or group of animals, the protocol used must combine several indicators and include a description of the method to be used to measure each indicator. One of the main difficulties of all welfare assessment protocols lies in the aggregation of different measures to obtain an overall score.

Animal welfare assessment at the farm level largely influences economic sustainability by ensuring compliance with set standards of animal welfare, which then increases societal confidence in that sector and, thus, helps ensure market share. In addition, there is evidence that that compliance with animal welfare standards may also be associated with greater cow health, production, and longevity, which all contribute to greater farm profitability and economic sustainability.

DIAGNOSTIC IMAGING WORKSHOP

W15

Extragenital applications of Ultrasonography

Sebastien Buczinski[1], Jill Colloton[2], Giovanni Gnemmi[3].

[1]Faculté de Médecine Vétérinaire, Université de Montréal, Canada; [2]Bovine Services, USA; [3]BOVINEVET INTERNACIONAL Bovine Ultrasound Services & Herd Management, Huesca, Spain.

Objectives: Today, reproductive ultrasonography has a plantar diffusion. The diagnosis of gestation, of non-gestation, the diagnosis of foetal sex and the diagnosis of the physio-pathology of the ovary and uterus, is carried out daily everywhere. Extra-genital ultrasonography, although more slowly, is also becoming more widespread and is beginning to be a complementary examination that the buiatrician who also or only deals with bovine internal medicine relies on to confirm and sometimes define a clear diagnosis. In many cases, the buiitra can carry out this examination without the need for instrumentation other than that used for genital ultrasonography. The aim of this presentation is to show possible applications of the ultrasound technique with instruments and probes, normally used in bovine reproduction, but also how to perform an ultrasound examination of the abdomen using lower working frequencies, and therefore different probes, than those normally used in bovine reproduction.

Materials & Methods: The ultrasound examination of the lung, the teats and the umbilical region of the calf, have one point in common: often the clinical examination, even if well done, does not allow the type of lesion, its extension and therefore a prognosis to be clearly defined. This examination is performed with a 5.0-7.5 MHz linear probe and portable or ultra-portable instruments. The ultrasound examination of the abdomen when performed on calves can be done with a 5.0-7.5 MHz probe, however when working on calves already 90-120 kg and adult cattle, 2.0-3.5 MHz, convex or linear probes must necessarily be used. These probes can be mounted on portable, ultra-portable instruments or on trolley-mounted ultrasound units.

Results: Ultrasound examination of the lung is performed to confirm whether a calf has a respiratory form, to define its site and to establish the extent of the inflammatory process and thus the prognosis. However, this can be used routinely to monitor the nursery by analysing sentinel calves on a weekly basis. This monitoring makes it possible to establish the state of health of the nursery, which animals (of which age group), if any, have problems. It makes it possible to establish the efficiency of the staff also makes it possible to establish whether a therapy and/or vaccination programe is giving the expected result. The ultrasound examination of the teat is an examination that should be used every time we have milking disorders. The clinical examination, in particular the digito-digital palpation of the teat, often does not allow us to establish either the type or the extent of the damage, which may affect the papillary canal, Furstemberg's rosette, the teat cistern, the milk cistern or Furstemberg's ring. The ultrasound examination of the umbilical region makes it possible to confirm and/or make a clear clinical diagnosis of umbilical pathology. Umbil-

ical ultrasonography should always precede surgical therapy, allowing, among other things, the anaesthesia to be adjusted and the surgical strategy to be defined in order to obtain the best results from surgical therapy.

Abdominal ultrasonography can be used for the diagnosis of various diseases including peritonitis (hardware disease and abomasal ulcers), differentiation between mechanical and paralytic ileus, pyelonephritis, liver abscesses and caudal vena cava thrombosis among others. Abdominal ultrasonography with a low-frequency probe (with deep penetration) is particularly helpful to determine the extension of the lesions but also to target the best spot where abdominocentesis can be performed. In cows with colic, where the exact diagnosis is uncertain, abdominal ultrasound helps in distinguishing medical vs mechanical causes of the ileus, therefore improving the management of the case if early surgery should be performed.

Conclusions: Today, extra-genital ultrasound is within the reach of all those who wish to approach bovine internal medicine in a different way. It is a complementary examination and therefore an examination that must always follow the classic clinical examination and never precede it. Today this technique is also absolutely affordable from an economical point of view: for some examinations it is possible to use the same probe/instrument that is used in reproduction, while in other cases it is necessary to purchase a low-frequency probe (2.0-3.5 MHz), which can, however, be mounted on the same portable or ultra-portable ultrasound unit that is used in reproduction.

NUTRITION AND BIOTECHNOLOGY WORKSHOP

W16

Milk fatty acids: The science behind it

Débora Santschi[1], Daniel Warner[1], Rachel Gervais[2].

[1]Lactanet, Canada; [2]Université Laval, Canada.

Fat is the most variable component of milk. Its concentration and its fatty acid (FA) profile vary according to several factors including the animal (parity, lactation stage, breed), the environment (season, management system) and nutrition (forage and grain type and proportion, amount, and composition of dairy fat supplements). A typical dairy cow ration contains five major fatty acids but due to the rumen metabolism and lipogenesis from the mammary tissue, approximately 400 different FA can be found in milk, summing up to 92-95% of milk fat. Although only a dozen of these FA is present in significant proportions in milk, both major and minor milk FA can reveal information about the rumen metabolism and overall health of the dairy cow. Traditionally analyzed by gas chromatography, a laborious and costly technique, advances in technology over the last decade allow measuring major milk fatty acids routinely and rapidly by mid-infrared (MIR) spectroscopy. This technique offers the opportunity to monitor milk FA in bulk tank or individual cow samples alongside and, thus, at the same frequency as analyzing for major components (e.g., fat, protein, lactose, milk urea nitrogen, somatic cell counts). In six seconds, it is possible to obtain the complete composition of a milk sample, including the FA profile with the main groups of FA. These include *De novo* FA (short chain FA synthesized in the mammary gland from rumen precursors); Preformed FA (Long chain FA derived from dietary FA transferred into milk and FA mobilized from adipose tissue); and Mixed FA (Medium chain FA from mixed origin). Bulk tank milk samples from 3,395 dairy herds on milk recording in Quebec, Canada are currently routinely tested by MIR. Bulk tank milk samples (N = 1.85M) analyzed from April 2019 to June 2022 were used in this analysis to evaluate the use of FA as a monitoring tool. On a milk basis, each 0.1-percentage unit of increase in de novo FA increased milk fat content by 0.201 unit (r_{corr} = 0.78) and milk protein content by 0.117 unit (r_{corr} = 0.77); whereas each 0.1-unit increment in preformed FA increased milk fat by 0.099 unit (r_{corr} = 0.49) and had no impact on milk protein (r_{corr} = 0.05). Individual or more minor groups of FA can also be predicted from MIR, with variable levels of accuracy. In conclusion, routine monitoring of FA in individual cow milk or groups of cows shows promising applications for herd management in terms of better understanding rumen fermentation, body reserve dynamics and potential health issues, as well as applications for niche markets and characteristics of specialty milks.

W17

Milk fatty acids: Use and applications in Canadian dairy farms

Débora Santschi[1], Rachel Gervais[2], Daniel Warner[1].

[1]*Lactanet, Canada;* [2]*Université Laval, Canada.*

Milk fatty acids (FA) are related to feeding and farm management practices. They originate from either synthesis in the mammary gland (*de novo* FA), feed intake or body fat mobilization (preformed FA), or both sources (mixed FA). Rapid analysis of bulk tank FA profiles through mid-infrared (MIR) spectroscopy can present a valuable tool in dairy management. Bulk tank milk samples from 3,395 dairy herds on milk recording in Quebec, Canada are currently routinely tested by MIR. Bulk tank FTIR milk FA profiles from 3,202 dairy herds in Quebec, Canada, over 3 years (April 2019–June 2022; 1.85M samples) were assembled in a national database (Lactanet, Canada). Milk fat and protein were correlated with *de novo* FA from bulk tank milk (r_{corr} = 0.77–0.78, on milk basis) and to a lesser extent with preformed FA (r_{corr} = 0.49 and 0.05, respectively). Seasonal cycles showed decreased *de novo* and increased preformed FA during the summer months. When comparing August monthly averages vs the ones from the previous April, Jersey herds had a less important drop in *de novo* FA than Holstein herds (−0.7 and −1.3 g/100 g FA, respectively. Across seasons, Jersey herds had higher *de novo* (+1.5 g/100 g FA) but lower preformed FA (−2.9 g/100 g FA) and an overall greater milk fat content (+0.8%-units) as compared to Holstein herds. A snapshot analysis was conducted with herd averages for April 2019 for 2035 Holstein herds having high *de novo* (HDN; mean ± SD of 27.9 ± 0.73 g/100 g FA) and low *de novo* (LDN; 25.9 ± 1.14 g/100 g FA) levels. A mixed model approach was used in R with Gaussian distribution for continuous variables, and with binomial distribution and log-link function for variables with binary outcome, considering herd as the random effect in the model. No differences between HDN and LDN herds existed in herd size, days in milk, and whether the herd was declared conventional or organic, but HDN herds had higher odds (2.0; P ≤ 0.001) of having a positive Transition Cow Index™, and lower odds (0.80; P = 0.023) of having a somatic cell count of 200 000 cells/mL or more on test day. HDN herds had a 0.89 kg greater (P ≤ 0.001) milk yield per cow on test day. Finally, a graphical and interactive tool was developed with the aim to visualize milk FA profiles with respective benchmarks and historical data for each farm. A prototype was developed in R, validated on farm, and deployed in production via an IBM Cognos platform to generate interactive reports. The bulk tank FA reports include benchmarks that allow comparing herds among their peers. To facilitate interpretation of changes in the FA profile amidst the intrinsic variation of FA analyzed by MIR, an automated anomaly detection system based on a rule-based artificial intelligence approach has been implemented within a python package. Long- and short-term changes can be detected via the deviation and via the variation and gradient, respectively. A rule-based diagnostic system has been further developed to help with the interpretation of a detected anomaly in the FA profile by offering a potential solution for a triggered alert. In addition, a prototype for milk FA from individual cows has been developed that allows for a more granular FA profile representation by parity and lactation stage for all cows or groups of cows within a herd at test date.

W18

Practical use of DHI info about milk fatty acid profile: Using that info into the field

Ramiro Antonio Fouz Ramiro.

USC, Spain.

Objectives: Expose the potential of milk fatty acids analysis from milk recording samples as a tool in decision making on the farm.

Materials and methods: Of the more than four hundred fatty acids that can be found in milk, only twenty of them are of any interest due to their higher concentration. Its classification is given by the length of the chain of carbon atoms or by its degree of saturation, although the classification that provides the greatest practical application in farm management is the one based on where the different fatty acids are synthesized.

DeNovo: those that are synthesized in the mammary gland (≤C14:0), mainly from acetate.

Mixed: they are synthesized both in the mammary gland and from dietary fat (C16:0, C16:1).

Preformed: come from dietary fat and mobilization of body fat, when the cow loses weight (C15:0, C17:0, ≥C18:0).

Official milk recording is a photo that is taken monthly of the herd, which allows you to see its evolution over time, with the added advantage of being able to compare it with other herds, which use the same measurement and analysis system. With all its limitations inherent to its periodicity, it is a very useful tool for the farmer to have a systematic and reliable control of how his herd is working.

Since the beginning of this year, fatty acid analyzes is available in individual cow samples from milk recording samples.

Results: These analyzes have been put into production, incorporating them into the monthly milk recording reports to have a new diagnostic tool in dairy cattle herds. The farmer and the technicians who work on his farm can download both the cow-by-cow values and the calculated index as herd averages and by production groups.

Conclusions: It is not a new analytic, since it has been implemented for years, but the work of various researchers has given a boost to its use as a control tool in livestock, especially with regard to ruminal health and energetic balance.

Its correlation with methane is also valued, since it is important that the farmer becomes aware as a producer of greenhouse gases, especially when the regulations that limit emissions go faster than the farmer's own awareness, with lack of knowledge about control and reduction measures.

Our experience is based on Holstein cattle (98% of the cows that are milked), although the breed has a strong influence on the milk fatty acid profile when extrapolating results.

In milk recording samples, possible deviations in samples

taken poorly, non-representative, with excess/lack of fat, which affect the analysis of fatty acids, must be taken into account.

It is essential to have a good backup laboratory, in order to carry out the necessary calibrations, since the maintenance of the equipment influences the quality of the data.

The different variation factors that affect the production of fatty acids must be considered, such as the number of calvings, days in milk, seasonality, the number of milkings, etc. when interpreting the results.

BIOTECHNOLOGY WORKSHOP

W19

In vivo and in vitro production of embryos: competing or complementary techniques?

Gabriel Bo[1], Pietro Baruselli[2].

[1]IRAC, Argentina; [2]USP, Brazil.

Commercial bovine embryo transfer began in North America in the early 1970s, but soon extended to several countries around the world, including in South America. Although North America has consistently accounted for more than 50% of in vivo-derived (IVD) embryos, South America became the center for in vitro embryo production (IVP) beginning in 2002. Between 2002 and 2012, IVP increased more than 600% in Brazil, and in 2016 represented more than 57% of the world's IVP embryos. However, IVP has also increased rapidly in North America, and by 2017, numbers were similar between North and South America. It is noteworthy that on a world-wide basis, the number of IVP bovine embryos now exceeds the numbers of IVD embryos.

The objective of ovarian superstimulatory treatments in cattle is to stimulate the growth of the maximum number of antral follicles that will produce competent oocytes. For *in vitro* embryo production (IVP), the necessity of superstimulation with gonadotropins prior to ovum pick-up (OPU) is still under discussion, and the approach may differ depending on whether the donors are of *Bos taurus* or *Bos indicus* breeding.

Besides the requirements for FSH treatments, some other advantages of using in vitro embryo production (IVP) vs multiple ovulation and in vivo embryo transfer (MOET) are the following: 1) frequency of collections: every 2-3 weeks for IVP vs every 35-60 days for MOET, 2) IVP can be performed in pregnant (up to 3 months) and prepubertal donors (2 to 8 months), 3) a reduction in the semen required for embryo production (1-2 straws can fertilize up to 100 oocytes vs a minimum of 2 straws per donor superovulated).

Although IVP results are improving throughout the world, some of the disadvantages of IVP vs MOET are the following: 1) high investment is required for IVP, the cow is the incubator to produce in vivo embryos, 2) IVP needs high numbers to pay the fixed costs vs relatively low fixed costs are required for MOET, 3), there are still lower pregnancy rates with IVP than with MOET embryos (35-45% for IVP vs 50-60% for MOET embryos transferred fresh, 30-35% for IVP vs 45-50% for MOET cryopreserved embryos), 4) more embryo/fetal losses with IVP (5-20% for IPV vs 5-10% for MOET between 30 and 60 days, 5-20% for IVP vs 3-5% for MOET between 60 days and calving). The objective of this workshop is to objectively discuss the pros and cons of both technologies and to propose alternatives by which the two embryo production technologies can be complementary in some programs.

W20

In vivo and in vitro production of embryos: competing or complementary techniques?

Pietro Baruselli Sampaio[1], Gabriel Bo[2].

[1]USP; [2]IRAC, Argentina.

Commercial bovine embryo transfer began in North America in the early 1970s, but soon extended to several countries around the world, including in South America. Although North America has consistently accounted for more than 50% of in vivo-derived (IVD) embryos, South America became the center for in vitro embryo production (IVP) beginning in 2002. Between 2002 and 2012, IVP increased more than 600% in Brazil, and in 2016 represented more than 57% of the world's IVP embryos. However, IVP has also increased rapidly in North America, and by 2017, numbers were similar between North and South America. It is noteworthy that on a world-wide basis, the number of IVP bovine embryos now exceeds the numbers of IVD embryos.

The objective of ovarian superstimulatory treatments in cattle is to stimulate the growth of the maximum number of antral follicles that will produce competent oocytes. For *in vitro* embryo production (IVP), the necessity of superstimulation with gonadotropins prior to ovum pick-up (OPU) is still under discussion, and the approach may differ depending on whether the donors are of *Bos taurus* or *Bos indicus* breeding.

Besides the requirements for FSH treatments, some other advantages of using in vitro embryo production (IVP) vs multiple ovulation and in vivo embryo transfer (MOET) are the following: 1) frequency of collections: every 2-3 weeks for IVP vs every 35-60 days for MOET, 2) IVP can be performed in pregnant (up to 3 months) and prepubertal donors (2 to 8 months), 3) a reduction in the semen required for embryo production (1-2 straws can fertilize up to 100 oocytes vs a minimum of 2 straws per donor superovulated).

Although IVP results are improving throughout the world, some of the disadvantages of IVP vs MOET are the following: 1) high investment is required for IVP, the cow is the incubator to produce in vivo embryos, 2) IVP needs high numbers to pay the fixed costs vs relatively low fixed costs are required for MOET, 3), there are still lower pregnancy rates with IVP than with MOET embryos (35-45% for IVP vs 50-60% for MOET embryos transferred fresh, 30-35% for IVP vs 45-50% for MOET cryopreserved embryos), 4) more embryo/fetal losses with IVP (5-20% for IPV vs 5-10% for MOET between 30 and 60 days, 5-20% for IVP vs 3-5% for MOET between 60 days and calving). The objective of this workshop is to objectively discuss the pros and cons of both technologies and to propose alternatives by which the two embryo production technologies can be complementary in some programs.

ANIMAL HEALTH WORKSHOP: Managing dairy calf health: New insights and back to the basics.

W21

Managing dairy calf health: new insights into the basics of calf management

David Renaud[1], Chris Chase[2].

[1]University of Guelph, Canada; [2]South Dakota State University, USA.

The level of morbidity and mortality in the perinatal and preweaning period remain high in dairy calves. To reduce the impact of disease on dairy farms and calf raising facilities, a well thought out calf management program is critical; however, when building a program, it is important to consider economic considerations as well as time constraints to ensure uptake of recommendations. The prepartum period before the calf is born is the place to start as the nutrition provided to dry cow's influences colostrum quality and quantity as well as the size of the calf born. In addition, managing heat stress in dry cows can not only reduce transition disease but also improve survivability of calves. Following that, the perinatal period (birth to 24 hours of age) is likely the most essential period to mitigate disease. Ensuring that timely and appropriate calving interventions are provided will substantially reduce the level of mortality seen during this period. In addition, ensuring timely provision of high-quality colostrum soon after calving as well as an additional meal within 12 hours of birth will maximize the chance the not only excellent levels of passive immunity are reached but will ensure the proper development of the structure and microbial community of the gastrointestinal tract. Beyond the first 24 hours of life, how calves are managed in the first 60 days of life will have a great influence on their future productivity. Mitigating disease, such as diarrhea and respiratory disease, is also critical to maximize productivity and improve the welfare of calves. This can be achieved through transition milk feeding, free choice supplemental water, managing the environment, and implementing a strategic vaccination strategy. In addition, a high plane of milk nutrition ($\geq$ 8 L of milk per day) in combination with a step-down weaning program is critical to not only reduce disease but take advantage of the high level of efficient growth that can be achieved during this period. By going back to the basics with producers and focusing on delivering information that can be practically implemented substantial improvement in calf health and productivity can be seen.

EPIDEMIOLOGY WORKSHOP

W22

Workshop in strategies and guidelines for the selective treatment of clinical mastitis and at dry-off

Alfonso Lago.

DairyExperts, USA.

Clinical mastitis in dairy cattle has significant ramifications including adverse effects on cow health and welfare, financial losses to dairy farmers and public health concerns because of the extensive use of antibiotics for treatment. Therefore, there is a need to implement effective management strategies that also allow for judicious use of antibiotics. Not all clinical mastitis cases benefit from antibiotic therapy; some cases may benefit from different treatment strategies to optimize cure (e.g. short *vs.* long duration) while chronic or otherwise nonresponsive cases may be best managed through other means (e.g. dry off the quarter, segregate or cull the cow). The type of agent causing mastitis may be a major determinant of the treatment or management strategy selected. Thus, knowledge of the etiology of each case of clinical mastitis before making a treatment decision could assist in selecting cases that will be treated with antibiotics and to determine the appropriate duration of therapy. However, this requires rapid and accurate diagnosis of the etiology of mastitis.

Etiology based treatment decisions for the selective treatment of clinical mastitis represent a tremendous opportunity to reduce antibiotic use on commercial dairy farms by more than 50% for the treatment of mild and moderate clinical mastitis cases without sacrificing the efficacy of treatment or the long-term health and production potential of the cow. Consequently, dairy herds could incur considerable savings on treatment related costs (e.g. discarded milk, drugs and labor), especially if extended treatment durations are implemented. Additional benefits could include reduced risk of antibiotic residues in milk, and a reduction in the potential risk for development of antibiotic resistance in mastitis pathogens. Furthermore, differential treatment (adequate antibiotic selection or treatment duration depending on etiology) could improve treatment efficacy.

Selective dry cow therapy (SDCT) refers to the treatment with long-acting antimicrobials of only cows or quarters identified with or at risk of having an intramammary infection at dry-off, or at risk of acquiring one during the dry period. Conversely, blanket dry cow therapy (BDCT) is the treatment of every quarter of every cow at dry off. BDCT has been widely adopted in the last decades and led to an important success in the reduction of contagious mastitis. However, recent studies report a low prevalence of intramammary infections at dry-off in many herds. This, in addition to the recent introduction of rapid on-farm diagnostic tests, and the availability of teat sealants, may allow to develop successful SDCT strategies. Bulk tank SCC, as well as intramammary infection prevalence and etiology at dry off have been used to select herds benefiting from SDCT. Thereafter, the accurate identification of cows or quarters benefiting from antimicrobial treatment is the cornerstone for the implementation of SDCT. Strategies followed vary from use of cow records (SCC records, clinical mastitis history, etc), culture results, cow-side diagnostic test results (California Mastitis Test, milk leukocyte differential count, etc), or a combination of them.

The current epidemiology of mastitis in addition to the availability of new technologies make SDCT a logical step to reduce antibiotic use in dairy cows. The most recent clinical trials show that either culture or data based SDCT programs can be implemented successfully. Antibiotic use was reduced by more than half without any negative effects on health and productivity. Therefore, it represents an additional opportunity to improve antibiotic stewardship on farms.

At the workshop it will be discussed the efficacy of different selective treatment strategies of clinical mastitis and at dry-off, tools to identify the cows to treat, opportunities to optimize cure, and economics of different management approaches. Finally, practical guidelines for the implementation of selective treatment programs will be provided.

SURGERY WORKSHOP

W23

What is your diagnosis on lameness?

André Desrochers, Sylvain Nichols.

Université de Montréal, Canada.

It has been well known that the feet are at the origin of most lameness in cattle. Unless there is an obvious visual cause to explain the lameness and feet have been checked, the affected animal is often treated empirically with antibiotics and nonsteroidal anti-inflammatory drugs (NSAIDs). A basic knowledge of the most common conditions and their prognosis might preclude the unnecessary administration of drugs avoiding the risk of residue. Moreover, a more precise diagnosis will help the veterinarian and the owner to make the best medical decision. In this workshop, we will present and discuss 6 clinical cases. Emphasis will be on locating the origin of the lameness, use the appropriate diagnostic tools to give an accurate prognosis to the owner. To help the discussion during the workshop, we present in this abstract some basis of the physical examination for a thorough lameness exam.

LAMENESS EXAMINATION

There are 4 steps in the determination of the cause of lameness: locomotion or mobility scoring, hands on examination, establish a differential according to the location of the problem, usage of appropriate diagnostic tools.

Locomotion or mobility scoring

Locomotion scoring is an important objective in assessing lameness. It helps to orient the veterinarian toward the most likely cause and can be used to evaluate improvement after treatment. Cattle lameness is generally obvious by observing the cow's stance. Attention should be paid to the posture of the cow, including the back, shoulders, pelvis, and major limb joints. With the animal standing, the general stance is observed first and more specifically each limb and digit. Compare one region to the opposite side and determine if obvious swelling, wounds, shifting of weight, and foot posture such as toe touching or displacement of weight bearing on the medial or lateral claw are present. In long standing diseases with severe lameness, the heels are taller and the wall longer on the affected claw compared with that of the healthy claw. A dropped fetlock may be noticed on the sound limb because of excessive load on the flexor tendons and suspensory ligaments. In young animals, angular limb deformities secondary to uneven weight bearing occurs rapidly with chronic lameness. Differential diagnoses for non-weight bearing lameness include sole abscess, fracture, major joint luxation, critical weight bearing ligament or tendon injury, critical nerve injury,septic arthritis, septic tenosynovitis.

Examination of the affected limb

Unless an obvious lesion is apparent, we start by palpation of the limb from the digit working up the leg. The clinician should watch for a pain reaction and determine if swelling, deformation, crepitation, warmth, and wounds are present. A hoof tester is used to evaluate pain of the claw. Examination of long bones is performed by applying firm pressure in regions of minimal soft tissue covering (e.g. medial aspect of tibia and radius, greater trochanter of femur, greater tubercle of humerus, etc). Each joint should be palpated separately and complete flexion, extension, abduction and adduction of the limb performed. Isolation of the shoulder and elbow or of the stifle and tarsus are difficult when flexion or extension movements are performed because these joints are united by muscle tendon units.

Usage of appropriate diagnostic tools

Once the affected area is located, it is often necessary to use diagnostic tools to rule out diseases, help to establish appropriate treatment and prognosis. Nowadays many diagnostic tests are available but we will describe those that are readily available and affordable. Arthrocentesis and subsequent cytological and bacteriological analyses of the synovial fluid are the complementary tests of choice for the diagnosis and the management of any joint disease. It is simple and cost effective. A portable x-ray machine will allow you to examine everything from the feet up to the elbow and a portion of the stifle depending of the size of the animal. In adult cattle, a more powerful machine in a referral center is necessary to evaluate the hip and shoulder joint. Most common diseases that are evaluated with the xray are : fracture, septic arthritis, sequestrum and osteochondrosis. Soft tissues are better evaluated with ultrasound examination.

APPLICATION OF SYSTEMS THINKING DISCIPLINE IN BEEF CATTLE VETERINARY MEDICINE

W24

Application of System Thinking Discipline in Cattle Veterinary Medicine

Dale Grotelueschen[1], John Groves[2], Brian Vander Ley[3], William Prokop[4].

[1]Professor Emeritus, University of Nebraska; [2]Livestock Veterinary Services, USA; [3]University of Nebraska-Lincoln, USA; [4]Dairy Innovations, LLC, USA.

Objectives: Many veterinary practitioners and consultants play critical and indispensable roles in the management and decision-making processes of large, complex production structures. The discipline of systems thinking offers opportunity and methodology to understand the interrelated forces that impact complex systems over time in ways that help to identify innovative and high-leverage solutions. It is the objective of this workshop to provide an introduction to the key concepts and principal methodologies of systems thinking by sharing examples of its application in an array of professional activities associated with cattle veterinary medicine.

Materials and methods: The historical context of systems thinking and its suitability for application in cattle veterinary medicine will be presented. The faculty will present applications in professional activities related to beef practice/consultation, dairy practice/consultation, and academia.

Key Concepts to be highlighted in the examples include:

- The Iceberg Framework is a tool used in systems thinking that helps accurately define and deeply understand the problem being investigated.
- Mental Models are instinctive theories we have about how the world works. They are driven primarily by our past experiences and how we have learned to solve problems before. The Systems Thinking discipline provides the framework to break out of the models that hinder your ability to innovate.
- Causal loop diagrams consist of balancing loops, reinforcing loops, vicious cycles, virtuous cycles, and system delays provide the "language" of Systems Thinking. The relationship between language and thought processes can't be underestimated. Most western languages are linear in nature, which tends to drive linear thinking and linear problem solving. A fundamental principle of Systems Thinking is that parts of a system are related in non-linear ways and are interconnected in circular associations and feedback loops.
- Creative tension is a systems thinking principle and is contingent upon creating both personal and shared vision regarding the desired state or output of a complex adaptive system. Systems Thinking is also rooted in personal mastery that is based on a commitment to the truth about the reality of the current state or output of the system. The difference between the current state and the desired state produces disequilibrium that drives the creative tension in the system. Without a vision, there is no real motivation to change. Without a clear understanding of where we are, we have no basis for effective action. To reach equilibrium, complex adaptive systems must resolve this tension.
- System homeostasis refers to the principle that the complex systems we work with have developed and evolved over a long time period and are very adept at maintaining their baseline function. Complex systems produce results they are designed to produce, but those results may not be what we were hoping for. Often when we try to change or modify how a system works, the system will "push back" and return to homeostasis. Systems tend to push back by producing unintended consequences to our interventions. Understanding these unintended consequences is a big part of becoming an effective systems thinker. High leverage interventions made to a system produce the largest desirable change while producing the fewest unintended consequences.

Conclusions: One of the main outcomes of this workshop is to help give insight into fundamental principles of systems, in order to more directly show that systems are capable of driving their own behavior. Even when all players in a system behave in rational and scientifically based ways, the system still exerts forces of which most of us are unaware. Deep understanding of these forces lies in understanding the nature and structure of interrelationships between variables in the system, not just a deep understanding of the variables. Another outcome is to open a dialogue regarding a long-term vision to be shared among system stakeholders. Within the discipline of systems thinking, creative tension can be generated as a tool to move toward change. In order to have creative tension or pressure to change, we need to establish a shared vision that is based on the current reality of where we are at today and a clear idea of what we wish to achieve.

W25

Application of System Thinking Discipline in Cattle Veterinary Medicine

John Groves[1], Dale Grotelueschen[2], Brian Vander Ley[2], William Prokop[3].

[1]Livestock Veterinary Service, USA; [2]University of Nebraska- Lincoln, USA; [3]Dairy Innovations LLC, USA.

Objectives: Many veterinary practitioners and consultants play critical and indispensable roles in the management and decision-making processes of large, complex production structures. The discipline of systems thinking offers opportunity and methodology to understand the interrelated forces that impact complex systems over time in ways that help to identify innovative and high-leverage solutions. It is the objective of this workshop to provide an introduction to the key concepts and principal methodologies of systems thinking by sharing examples of its application in an array of professional activities associated with cattle veterinary medicine.

Materials and methods: The historical context of systems thinking and its suitability for application in cattle veterinary medicine will be presented. The faculty will present applications in professional activities related to beef practice/consultation, dairy practice/consultation, and academia.

Key Concepts to be highlighted in the examples include:

- The Iceberg Framework is a tool used in systems thinking that helps accurately define and deeply understand the problem being investigated.
- Mental Models are instinctive theories we have about how the world works. They are driven primarily by our past experiences and how we have learned to solve problems before. The Systems Thinking discipline provides the framework to break out of the models that hinder your ability to innovate.
- Causal loop diagrams consist of balancing loops, reinforcing loops, vicious cycles, virtuous cycles, and system delays provide the "language" of Systems Thinking. The relationship between language and thought processes can't be underestimated. Most western languages are linear in nature, which tends to drive linear thinking and linear problem solving. A fundamental principle of Systems Thinking is that parts of a system are related in non-linear ways and are interconnected in circular associations and feedback loops.
- Creative tension is a systems thinking principle and is contingent upon creating both personal and shared vision regarding the desired state or output of a complex adaptive system. Systems Thinking is also rooted in personal mastery that is based on a commitment to the truth about the reality of the current state or output of the system. The difference between the current state and the desired state produces disequilibrium that drives the creative tension in the system. Without a vision, there is no real motivation to change. Without a clear understanding of where we are, we have no basis for effective action. To reach equilibrium, complex adaptive systems must resolve this tension.
- System homeostasis refers to the principle that the complex systems we work with have developed and evolved over a long time period and are very adept at maintaining their baseline function. Complex systems produce results they are designed to produce, but those results may not be what we were hoping for. Often when we try to change or modify how a system works, the system will "push back" and return to homeostasis. Systems tend to push back by producing unintended consequences to our interventions. Understanding these unintended consequences is a big part of becoming an effective systems thinker. High leverage interventions made to a system produce the largest desirable change while producing the fewest unintended consequences.

Conclusions: One of the main outcomes of this workshop is to help give insight into fundamental principles of systems, in order to more directly show that systems are capable of driving their own behavior. Even when all players in a system behave in rational and scientifically based ways, the system still exerts forces of which most of us are unaware. Deep understanding of these forces lies in understanding the nature and structure of interrelationships between variables in the system, not just a deep understanding of the variables. Another outcome is to open a dialogue regarding a long-term vision to be shared among system stakeholders. Within the discipline of systems thinking, creative tension can be generated as a tool to move toward change. In order to have creative tension or pressure to change, we need to establish a shared vision that is based on the current reality of where we are at today and a clear idea of what we wish to achieve.

W26

Application of System Thinking Discipline in Cattle Veterinary Medicine

William Prokop, John Groves, Dale Grotelueschen, Brian Vander Ley.

Dairy Innovations LCC /Arm and Hammer Inc, USA.

Objectives: Many veterinary practitioners and consultants play critical and indispensable roles in the management and decision-making processes of large, complex production structures. The discipline of systems thinking offers opportunity and methodology to understand the interrelated forces that impact complex systems over time in ways that help to identify innovative and high-leverage solutions. It is the objective of this workshop to provide an introduction to the key concepts and principal methodologies of systems thinking by sharing examples of its application in an array of professional activities associated with cattle veterinary medicine.

Materials and methods: The historical context of systems thinking and its suitability for application in cattle veterinary medicine will be presented. The faculty will present applications in professional activities related to beef practice/consultation, dairy practice/consultation, and academia.

Key Concepts to be highlighted in the examples include:

- The Iceberg Framework is a tool used in systems thinking that helps accurately define and deeply understand the problem being investigated.
- Mental Models are instinctive theories we have about how the world works. They are driven primarily by our past experiences and how we have learned to solve problems before. The Systems Thinking discipline provides the framework to break out of the models that hinder your ability to innovate.
- Causal loop diagrams consist of balancing loops, reinforcing loops, vicious cycles, virtuous cycles, and system delays provide the "language" of Systems Thinking. The relationship between language and thought processes can't be underestimated. Most western languages are linear in nature, which tends to drive linear thinking and linear problem solving. A fundamental principle of Systems Thinking is that parts of a system are related in non-linear ways and are interconnected in circular associations and feedback loops.
- Creative tension is a systems thinking principle and

is contingent upon creating both personal and shared vision regarding the desired state or output of a complex adaptive system. Systems Thinking is also rooted in personal mastery that is based on a commitment to the truth about the reality of the current state or output of the system. The difference between the current state and the desired state produces disequilibrium that drives the creative tension in the system. Without a vision, there is no real motivation to change. Without a clear understanding of where we are, we have no basis for effective action. To reach equilibrium, complex adaptive systems must resolve this tension.

- System homeostasis refers to the principle that the complex systems we work with have developed and evolved over a long time period and are very adept at maintaining their baseline function. Complex systems produce results they are designed to produce, but those results may not be what we were hoping for. Often when we try to change or modify how a system works, the system will "push back" and return to homeostasis. Systems tend to push back by producing unintended consequences to our interventions. Understanding these unintended consequences is a big part of becoming an effective systems thinker. High leverage interventions made to a system produce the largest desirable change while producing the fewest unintended consequences.

Conclusions: One of the main outcomes of this workshop is to help give insight into fundamental principles of systems, in order to more directly show that systems are capable of driving their own behavior. Even when all players in a system behave in rational and scientifically based ways, the system still exerts forces of which most of us are unaware. Deep understanding of these forces lies in understanding the nature and structure of interrelationships between variables in the system, not just a deep understanding of the variables. Another outcome is to open a dialogue regarding a long-term vision to be shared among system stakeholders. Within the discipline of systems thinking, creative tension can be generated as a tool to move toward change. In order to have creative tension or pressure to change, we need to establish a shared vision that is based on the current reality of where we are at today and a clear idea of what we wish to achieve.

W27

Application of System Thinking Discipline in Cattle Veterinary Medicine

Brian Vander Ley[1], Dale Grotelueschen[2], John Groves[3], William Prokop[4].

[1]University of Nebraska-Lincoln Great Plains Veterinary Educational Center, USA; [2]University of Nebraska-Lincoln, USA; [3]Livestock Veterinary Service; [4]Dairy Innovations, LLC, USA.

Objectives: Many veterinary practitioners and consultants play critical and indispensable roles in the management and decision-making processes of large, complex production structures. The discipline of systems thinking offers opportunity and methodology to understand the interrelated forces that impact complex systems over time in ways that help to identify innovative and high-leverage solutions. It is the objective of this workshop to provide an introduction to the key concepts and principal methodologies of systems thinking by sharing examples of its application in an array of professional activities associated with cattle veterinary medicine.

Materials and methods: The historical context of systems thinking and its suitability for application in cattle veterinary medicine will be presented. The faculty will present applications in professional activities related to beef practice/consultation, dairy practice/consultation, and academia.

Key Concepts to be highlighted in the examples include:

- The Iceberg Framework is a tool used in systems thinking that helps accurately define and deeply understand the problem being investigated.
- Mental Models are instinctive theories we have about how the world works. They are driven primarily by our past experiences and how we have learned to solve problems before. The Systems Thinking discipline provides the framework to break out of the models that hinder your ability to innovate.
- Causal loop diagrams consist of balancing loops, reinforcing loops, vicious cycles, virtuous cycles, and system delays provide the "language" of Systems Thinking. The relationship between language and thought processes can't be underestimated. Most western languages are linear in nature, which tends to drive linear thinking and linear problem solving. A fundamental principle of Systems Thinking is that parts of a system are related in non-linear ways and are interconnected in circular associations and feedback loops.
- Creative tension is a systems thinking principle and is contingent upon creating both personal and shared vision regarding the desired state or output of a complex adaptive system. Systems Thinking is also rooted in personal mastery that is based on a commitment to the truth about the reality of the current state or output of the system. The difference between the current state and the desired state produces disequilibrium that drives the creative tension in the system. Without a vision, there is no real motivation to change. Without a clear understanding of where we are, we have no basis for effective action. To reach equilibrium, complex adaptive systems must resolve this tension.
- System homeostasis refers to the principle that the complex systems we work with have developed and evolved over a long time period and are very adept at maintaining their baseline function. Complex systems produce results they are designed to produce, but those results may not be what we were hoping for. Often when we try to change or modify how a system works, the system will "push back" and return to homeostasis. Systems tend to push back by producing unintended consequences to our interventions. Understanding these unintended consequences is a big part of becoming an effective systems thinker. High leverage interventions made to a system produce the

largest desirable change while producing the fewest unintended consequences.

Conclusions: One of the main outcomes of this workshop is to help give insight into fundamental principles of systems, in order to more directly show that systems are capable of driving their own behavior. Even when all players in a system behave in rational and scientifically based ways, the system still exerts forces of which most of us are unaware. Deep understanding of these forces lies in understanding the nature and structure of interrelationships between variables in the system, not just a deep understanding of the variables. Another outcome is to open a dialogue regarding a long-term vision to be shared among system stakeholders. Within the discipline of systems thinking, creative tension can be generated as a tool to move toward change. In order to have creative tension or pressure to change, we need to establish a shared vision that is based on the current reality of where we are at today and a clear idea of what we wish to achieve.



ORAL PRESENTATIONS

Animal Health **(AH)**

Buffaloes and Camelids **(BC)**

Biotechnology **(BT)**

Cattle Welfare **(CW)**

Diagnostic Imaging **(DI)**

Epidemiology **(EP)**

Feedlot **(FE)**

Genetics and Breeding **(GB)**

Herd Health Management, Smart Farming and Economics **(HH)**

Infectious Diseases **(ID)**

Internal Medicine and Toxicology **(IM)**

Immunology and Vaccinology **(IV)**

Lameness **(LA)**

Nutrition and Metabolic Diseases **(NU)**

Parasitism **(PA)**

Public Health, Food Safety and Antimicrobial Resistance **(PH)**

Pharmacology and Therapeutics **(PT)**

Reproduction in Beef Cattle **(RB)**

Reproduction in Dairy Cattle **(RD)**

Sustainable Cattle Productive Systems **(SP)**

Small Ruminants **(SR)**

Surgery **(SU)**

Teaching and Continuing Education **(TE)**

Udder Health and Mastitis **(UH)**





Draxxin PLUS
DRAXXIN® PLUS
Recuperación Rápida* y Eficaz frente a la Enfermedad Respiratoria Bovina (ERB), con un solo tratamiento
*Referencias:
1. Freedom of Information Summary. NADA-TBD – DRAXXIN KP (tulathromycin and ketoprofen injection). Food and Drug Administration. 2020.
2. Datos en archivo, informe de estudio nº A136R-US-16-508. Zoetis Inc.
3. Datos en archivo, informe de estudio nº A131C-US-17-551. Zoetis Inc.
4. Datos en archivo, informe de estudio nº A131L-XC-17-528. Zoetis Inc.
zoetis
Draxxin® Plus 100 mg/ml + 120 mg/ml solución inyectable para bovino. Composición: Tulatromicina 100 mg/ml; Ketoprofeno 120 mg/ml. Indicaciones de uso: Tratamiento de la enfermedad respiratoria bovina (ERB) asociado con pirexia producida por Mannheimia haemolytica, Pasteurella multocida, Histophilus somni y Mycoplasma bovis sensibles a tulatromicina. Contraindicaciones: No usar en casos de hipersensibilidad a las sustancias activas o a algún excipiente. No usar simultáneamente con otros macrólidos o lincosamidas. No administrar a animales que sufran lesiones gastrointestinales, diátesis hemorrágica, discrasia sanguínea o afecciones hepáticas, renales o cardíacas. Precauciones: Se produce resistencia cruzada con otros macrólidos. No administrar simultáneamente con antimicrobianos con un modo de acción similar como otros macrólidos o lincosamidas. Este medicamento no contiene ningún conservante antimicrobiano. Precauciones especiales para su uso en animales: El uso del medicamento veterinario debe basarse en la identificación y la prueba de susceptibilidad de los patógenos de destino. Si esto no es posible, la terapia debe basarse en la información epidemiológica y en el conocimiento de la susceptibilidad de las bacterias de destino a nivel de la granja, o a nivel local/regional. El uso del medicamento veterinario debe estar de acuerdo con las políticas antimicrobianas oficiales, nacionales y regionales. El uso del medicamento veterinario que se desvíe de las instrucciones dadas en el RCP podría aumentar la prevalencia de bacterias resistentes a la tulatromicina y podría disminuir la eficacia del tratamiento con otros macrólidos, lincosamidas y estreptograminas del grupo B debido a la potencial resistencia cruzada (resistencia MLSB). Dado que muchos AINES poseen el potencial de inducir ulceración gastrointestinal, especialmente en bovino de edad avanzada y en terneros jóvenes, debe evitarse el uso concomitante del medicamento veterinario con otros medicamentos antiinflamatorios (AINES) o medicamentos antiinflamatorios esteroideos (por ejemplo, corticoesteroides) dentro de las primeras 24 horas de tratamiento. Posteriormente se debe vigilar estrechamente el tratamiento simultáneo con AINES y antiinflamatorios esteroideos. El uso del medicamento veterinario (que contiene ketoprofeno) en animales de edad avanzada o en animales de menos de 6 semanas debe basarse en una evaluación de beneficio/riesgo del veterinario responsable. Evitar su uso en animales deshidratados, hipovolémicos o hipotensos que requieran rehidratación parenteral, ya que puede haber un riesgo potencial de toxicidad renal. Debe evitarse la inyección intraarterial e intravenosa. Precauciones específicas que debe tomar la persona que administre el medicamento veterinario a los animales: Este medicamento veterinario puede causar hipersensibilidad (alergia). Las personas con hipersensibilidad conocida a la tulatromicina, al ketoprofeno o a los fármacos antiinflamatorios no esteroideos (AINEs) deben evitar el contacto con el medicamento veterinario. En caso de derrame accidental sobre la piel, lávela inmediatamente con agua y jabón. Este medicamento veterinario puede causar efectos adversos después de la exposición dérmica y la autoinyección. Tenga cuidado de evitar el contacto con la piel y la autoinyección accidental. En caso de autoinyección accidental, consulte con un médico inmediatamente y muéstrele el prospecto o la etiqueta. Los AINEs, como el ketoprofeno, pueden afectar a la fertilidad y ser perjudiciales para el feto. Las mujeres embarazadas, las mujeres con intención de concebir y los hombres que planean tener hijos deben tener extrema precaución al manipular este medicamento veterinario. Este medicamento veterinario es irritante para los ojos. Evite el contacto con los ojos. En caso de exposición accidental a los ojos, lávelos inmediatamente con agua limpia. Si la irritación persiste, consulte a su médico y muéstrele el prospecto. Lávese las manos después de su uso. Los estudios de laboratorio efectuados con tulatromicina en ratas y conejos no han demostrado efectos teratogénicos, tóxicos para el feto o tóxicos para la madre. Los estudios con ketoprofeno en especies de laboratorio (ratas, ratones y conejos) no han producido ninguna evidencia de efectos teratogénicos, pero han sido observados efectos en la fertilidad, la toxicidad materna y la toxicidad para el embrión. Hay efectos adversos conocidos de clase de los AINES y otros inhibidores de la prostaglandina durante la gestación y/o el desarrollo embriofetal. No se ha establecido la seguridad de la combinación de tulatromicina y ketoprofeno en las especies de destino durante la gestación y la lactancia. Utilícese únicamente de acuerdo con la evaluación beneficio/riesgo efectuada por el veterinario responsable. Tiempos de espera: Carne: 50 días. Su uso no está autorizado en bovino cuya leche se utiliza para el consumo humano. No usar en animales gestantes cuya leche se utiliza para consumo humano en los dos meses anteriores a la fecha prevista para el parto. Conservación: No congelar. Periodo de validez después de abierto el envase primario: 56 días. Eliminación: Todo medicamento veterinario no utilizado o los residuos derivados del mismo deberán eliminarse de conformidad con las normativas locales. Nº registro: 3904 ESP. Titular: Zoetis Spain, S.L. Medicamento sujeto a prescripción veterinaria.

AH-01

An outbreak of *Neospora caninum* abortion in a dairy herd from the State of Georgia, United States

Pedro Melendez[1], Marcia Ilha[1], Moges Woldemeskel[1], Justin Graham[2], Michelle Coarsey[1], Debi Boughman[1], Lisa Whittington[1], Hemant Naikare[1].

[1]University of Georgia, TVDIL, Tifton, GA, United States; [2]Brooksco Dairy, Qutiman, GA, United States.

Neospora caninum is a protozoan parasite from the phylum Apicomplexa affecting several animals' species, including canines, cattle, sheep, goats, deer, raccoons and rodents. Neosporosis has emerged as a serious disease of cattle and dogs worldwide. In cattle produces abortion; consequently, the main economic effect of neosporosis in cattle is its negative impact on fertility and milk yield. The objective of this study case was to report an abortion storm in a dairy farm from Georgia, USA. The outbreak consisted of 92 abortions (5.45% of pregnant cows) that occurred in a period of 3 weeks (May 19 to June 05, 2019) in Holstein cows that were between 3 to 7 months of gestation. Two subset of samples (aborted fetuses' organs, placental tissues, aborted cows blood) were submitted to the Tifton Veterinary Diagnostic and Investigational Laboratory, University of Georgia (Tifton, GA, USA). An abortion panel was conducted in several of the samples. Major abortion-causing agents (e.g. BVD, IBR, Brucellosis, Leptospirosis) were negative by culture, serology and PCR; however, 2 out 3 submitted fresh aborted fetuses were positive to *Neospora caninum* by PCR and immunohistochemistry, and the 3 dams were positive serologically to the same pathogen. The entire herd was being fed a grass silage harvested from a pasture where previously feral pigs were hunted and carcasses were left behind. As a consequence of this action a large population of wild coyotes were attracted, which could have contaminated the pasture with potential *N. caninum*-infected feces. After the abortion outbreak was resolved it was recommended that the farmers not leave cadavers of hunted animals, which may serve as food for coyotes or other carnivores and predators that may potentially spread diseases to cattle.

Keywords: Neospora caninum, abortion, cattle, outbreak.

AH-02

Development of a novel multiplex immunoassay for enhanced disease surveillance in dairy cows using biochip array technology

S. Gildea[1], C. Richardson[1], R.I. McConnell[2], S.P. Fitzgerald[2].

[1]Randox Teoranta, Dungloe, Republic of Ireland; [2]Randox Laboratories Ltd., Crumlin, United Kingdom.

Objectives: Infectious disease represents one of the biggest problems facing dairy producers today. This is largely due to the high economic costs associated with disease outbreaks. A prompt and accurate diagnosis is important to minimise the risk of disease spread and also for determining appropriate treatment. Conventional Enzyme-Linked Immunosorbent Assays (ELISAs), limited to measuring only one analyte, are the diagnostic tools routinely employed for determining antibody status using bovine milk. Samples requiring analysis however, may be collected from cattle that have been exposed to several pathogens. As a result, the same sample may require testing using multiple ELISAs in order to obtain an accurate diagnosis and not overlook the potential occurrence of co-infection. This is labour intensive in a laboratory and costly for a milk producer. Biochip Array Technology (BAT), by using a multi-analytical approach, consolidates the testing process. This study reports the Bovine Pathogen Array (BPA), based on BAT, the first multiplex immunoassay capable of simultaneously detecting antibodies against some of the world's most economically important bovine pathogens; bovine viral diarrhea virus (BVDV), bovine herpesvirus 1 (BoHV-1), *Mycobacterium avium subspecies paratuberculosis* (MAP), Leptospira, *Neospora caninum* and *Fasciola* hepatica from a single milk sample.

Materials and Methods: Simultaneous chemiluminescent immunoassays, defining discrete test regions on the biochip surface, were employed and applied to the Evidence Investigator analyser. Sample classification was determined against a multi-analyte positive control, results are qualitative. Test accuracy was examined using 349 milk samples collected from various European dairy herds and results compared to those obtained using conventional ELISAs. With the exception of MAP, where only 24 positive milk samples were available, 25 positive and 25 negative samples for antibodies against each pathogen were included. For BoHV-1, antibodies against both glycoprotein B (gB) and glycoprotein E (gE) were measured. Repeatability and total precision were determined using multiple BPA reagent batches and antibody specificity against respective BPA panel antigens was also examined. For the World Organisation for Animal Health (OIE) listed diseases BVDV, BoHV-1 and MAP, limit of detection was established and results compared to current test methods. No milk sample pre-treatment was required prior to testing.

Results: All simultaneous immunoassays were target specific and presented repeatability and total precision <15%. The BPA demonstrated 100% agreement in sample classification for antibodies against BVDV, BoHV-1 gB, BoHV-1 gE, Leptospira, *Neospora caninum* and *Fasciola hepatica* and 98% agreement in sample classification for antibodies against MAP when compared to commercial ELISAs. The BPA demonstrated superior limit of detection test capabilities for antibody detection against BVDV and equivalent limit of detection performance for antibody detection against BoHV-1 gB, BoHV-gE and MAP compared to ELISA. Time to result for all six pathogens using BPA was similar to that obtained for only one pathogen using current available test methods.

Conclusion: The BPA simultaneously detects antibodies against six economically important infectious diseases and offers great potential as a diagnostic and surveillance tool at herd level. The inclusion of multiple antigens for selected pathogens increases disease screening capabilities and enables Differentiating Infected from Vaccinated Animals (DIVA) against BoHV-1 when used in conjunction with selected vaccines. The availability of the BPA on BAT also delivers significant advantage when screening large numbers of samples

during epidemiological studies thus, safeguarding livestock against disease spread and ensuring continued financial viability for food producers and the agri-food sector as a whole.

Keywords: Multiplex immunoassay, Biochip array, Bovine pathogen, Milk.

AH-03

New species-specific turbidimetric immunoassays for the quantification of bovine acute-phase proteins Haptoglobin and ITIH4

Matilde Piñeiro[1], José Angel Robles-Guirado[2], Laura Arroyo[2], Lourdes Soler[1], Natalia García[3], M. Angeles Alava[3], Fermín Lampreave[3], Yolanda Saco[2], Raquel Pato[2], Raquel Peña[2], Anna Bassols[2], Francesca Canalias[2].

[1]Acuvet Biotech, Zaragoza, Spain; [2]Universitat Autònoma de Barcelona, Bellaterra, Spain; [3]Universidad de Zaragoza, Zaragoza, Spain.

Objectives: Haptoglobin (Hp) and inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4) are two key acute phase proteins in cattle, used in the diagnosis and monitoring of infectious and inflammatory diseases. Hp is characterized by a low serum baseline concentration and a prominent increase (up to 100 fold)) following the inflammatory stimuli, whereas ITIH4 shows a less prominent but more protracted response. The most widely used method for Hp measurement is the Hp-Hb binding assay, however this method is not sensitive enough to detect Hp in normal bovine serum, and it is substantially affected by hemolysis. Bovine Hp and ITIH4 can be measured by immunochemical methods such as ELISA or turbidimetry. Immunoturbidimetry is a very convenient method, because results are obtained in a few minutes, and the assay can be automated using a clinical chemistry analyser. The aim of the study was to validate two new, species-specific, turbidimetric immunoassays for the quantification of bovine Hp and ITIH4

Materials and Methods: The assays were set up in a fully automated clinical chemistry analyzer Olympus AU400 and studies of accuracy, imprecision, limit of detection, prozone effect and interferences carried out. Limit of detection was established measuring a blank composed by 150 mM NaCl and 50g/L BSA for 30 times. Linearity and prozone effect were calculated by serial dilution of a serum sample with high Hp (1.7 mg/L) or ITIH4 (9 mg/mL) concentration. Hemoglobin, bilirubin and triglycerides were analyzed as potential interferents by determining Hp and ITIH4 concentration in a given serum sample in the presence of different concentration of these compounds. Within-run and between-day precision was calculated by measuring duplicates of a given sample for 20 days.

Results: The assay for Hp kept linearity under dilution in a measuring range up to 400mg/L. Limit of detection was 5 mg/L. No hook effect was observed with Hp concentration of 1.7 mg/mL. The within-run and between day CV were 4 and 5 %, respectively. No interference from hemoglobin (20 g/L), triglycerides (10 g/L) or bilirubin (150 mg/L) was detected.

ITIH4 assay showed linearity until 4 mg/mL. Limit of detection was stablished in 0,003 mg/mL. No hook effect was observed with ITIH4 concentration up to 9 mg/mL. Coefficients of variation were always below 4% for within-run and between day determinations. There was no interference by the presence of hemoglobin (20 g/L), triglycerides (10g/L) or bilirubin (150 mg/L).

Conclusions: These new turbidimetric immunoassays are rapid, robust and precise methods for the quantification of Hp and ITIH4 in cattle, and have enough sensitivity to measure baseline levels. Assays are not affected by hemolysis as can occur with other assays for Hp quantification Both methods can be easily set up in automated clinical chemistry analyzers, providing a suitable tool for routine clinical use.

Keywords: Haptoglobin, ITIH4, Acute phase proteins, Immunoturbidimetry, Laboratory diagnostics.

AH-04

A Review of the Causes of Mortality in Pre-Weaned Dairy Calves

Katrina Henderson[1], Megan Fergusson[2].

[1]SRUC Veterinary Services, Dumfries, United Kingdom; [2]SRUC Veterinary Services, Ayr, United Kingdom.

Pre-weaning calf mortality is an important determinant of dairy enterprise profitability and is regarded as an important indicator of animal welfare. A recent UK study of eleven herds by Johnson et al (2017)[1] found that, on average, 4.5 percent of calves died in the first two months of life (range 1.9 to 8.3 percent). While there is published evidence as to the range of pre-weaning mortality rates across different countries and farms, there is little known about the causes of mortality. This information would allow targeted control measures to be put in place to reduce losses.

A review was carried out of all diagnostic carcase and viscera submissions submitted to SRUC Vet Services between 2014 and 2018. This review sought to provide information on the major causes of mortality in pre-weaned calves in Scottish dairy herds.

A total of 614 submissions were analysed, and a definitive diagnosis was reached in 603. This highlights the value of a post-mortem examination in this age of calf.

A total of 1017 diagnoses were made, with infectious disease responsible for 69 percent. Nutritional problems accounted for a further 25 percent, and the final 6 percent represented individual calf issues such as congenital deformities or bovine neonatal pancytopaenia. When suitable samples were available, calves less than 7 days of age were screened to assess colostral antibody transfer. Where hypogammaglobulinaemia was detected it was considered to predispose calves to deaths from other causes.

The five most common causes of mortality were cryptosporidia, rumen drinking, rotavirus, salmonellosis due to *Salmonella* Dublin and colisepticaemia. Pneumonias made up approximately 34% of the diagnoses, with *Mycoplasma bovis* the most common cause of pneumonia related deaths. Enteric

pathogens resulting in diarrhoea made up 34% of the diagnoses, with cryptosporidia the most frequently diagnosed.

It was considered that a nutritional component had played a role in the death of 26 percent of calves, with rumen drinking accounting for the majority. Cases of suspected underfeeding were not included in this total as feeding volumes were not known for all calves. Rumen drinking occurs when the rumeno-reticular groove fails to divert milk from the oesophagus to the abomasum. Fermentation of milk deposited in the rumen results in acid accumulation, and predisposes to yeast colonisation. It is often a result of management practices such as bucket feeding, irregular feeding times, or feeding milk at the incorrect temperature or height. Neonatal diarrhoea and stressful events such as transport or mixing of calves can play a role. The feeding history of calves with rumen drinking was compared to that of control calves that had died of non-nutritional causes, where the feeding method was not considered to have impacted on the cause of death. Rumen drinkers were more likely to be fed from an open bucket with no teat (odds ratio 4.35, 95 percent confidence intervals 1.91-9.87); less likely to be fed from an automatic feeder (odds ratio 0.13, 95 percent confidence intervals 0.05-0.31); and more likely to be fed a low volume of milk (≤4 litres a day, odds ratio 4.5, 95 percent confidence intervals 1.01-20.11). Notably, only rumen drinkers had been fed waste milk.

These findings confirm much of the previous research on factors that predispose to rumen drinking, and illustrate that management changes can be made to reduce the risk. The data was obtained as a result of passive surveillance, so may not be fully representative of calf mortality on all dairy farms; but it nonetheless highlights the contribution postmortem examinations can make when building up a comprehensive picture of calf health. The fact that 26 percent of deaths had a significant nutritional component demonstrates the importance of feeding management in ensuring the health and welfare of dairy calves.

1. Johnson KF, Chancellor N, Burn CC, et al. Prospective cohort study to assess rates of contagious disease in preweaned UK dairy heifers: management practices, passive transfer of immunity and associated calf health. *Vet Rec Open* 2017;4:e000226. doi:10.1136/ vetreco-2017-000226

Acknowledgments: SRUC VS receives financial support from the Scottish Government for farm animal disease surveillance activities.

Keywords: Dairy calf mortality.

AH-05

Importance of a systematic approach to investigating emerging disorders of ruminants acquired during gestation

Peter Windsor.

Production Animal Welfare & health Services, Scarborough, NSW 2515, Australia.

Objectives: Describe a systematic investigative process to more accurately define those disorders acquired *in-utero* that lead to congenital deformities of farmed calves, lambs and kids. Abnormalities present at birth may well be occurring more frequently due to numerous reasons. In particular, the effects of increased climatic variability has increased the ranges of vector-borne teratogenic viruses, plus there is a consistent increase in the transport and use of the germplasm of elite animals that has restricted the gene pools of the most popular breeds. To manage this ever-present issue, a systematic investigative process is described, aimed at identifying causes of outbreaks and sporadic occurrences of deformities, defining these as attributable to: (1) infection; (2) nutritional deficiency or excess; (3) teratogenic toxin from a plant or other chemical source; or (4) inherited abnormality.

Materials and Methods: As many pathological processes may damage the developing embryo or foetus during gestation, a systematic investigative approach is provided, developed from extensive reviews of the veterinary literature and 40 years of investigative studies of disorders of ruminant neonates.

Results: Initially, the history and epidemiology of potential risk factors is examined. Are the animals located in areas at vulnerable periods where infectious agents, teratogenic plants or nutritional aberrations may occur? Secondly, breeding metrics are calculated, including the rates of successful mating's, pregnancies, neonatal animals delivered, and then marked and weaned. Identifying when losses occurred in the reproductive cycle enables a 'most likely' differential diagnostic list to direct more cost-effective investigations. Thirdly, if infectious agents are suspected, collection of a foetus, neonate and/or placenta and body fluids & tissues may enable demonstration of pathogen presence and confirmation of attributable lesions via laboratory examinations. Fourthly, where arbovirus infections occur, viral serological studies and interrogation of insect vector distribution maps and databases conducted. Finally, if a genetic disorder is suspected, examination of pedigree information and the database of inherited disorders 'Online Mendelian Inheritance in Animals' (OMIA; http://omia.angis.org.au/) is advised.

Understanding the causes of abnormalities occurring during development in pregnancy is important, particularly with evidence that risks of teratogens that alter embryos and foetuses is increasing. The emergence of *in-utero* infections due to Bluetongue, Schmallenberg and Akabane arboviruses in cattle and sheep and Zika virus in humans, reflects broadening insect distributions. Conversely, increasingly severe droughts may induce embryonic harm from deprivation of maternal nutrition and increased intoxication episodes. In Australian droughts, the plant *Dysphania glomerulifera* is associated with severe neonatal ovine and bovine biliary atresia following grazing by dams adjacent to depleted water reserves that exposes the foetus to the unique toxin biliatresone that inhibits post-hepatic biliary development. This recent discovery informs the pathogenesis of the disorder in humans, the most common cause of infantile liver transplantation. Further, with inbreeding common in bovine, ovine and caprine production systems, there is increasing risk of inherited congenital disorders, particularly where artificial reproduction enables large numbers of progeny to be descendents of small numbers of elite individuals. Finally, numerous ruminant abnormalities are

yet to be fully characterized, with research on their pathogenesis required.

Conclusion: Veterinarians and animal scientists have a key role in educating producers and the public on the inevitability of congenital malformations in livestock production. However, they need confidence and access to investigative skills to initiate and conduct the systematic studies required, that can provide evidence-based diagnostics and management solutions for a society that is increasingly concerned with improved welfare of livestock and frequently alarmed by the appearance of congenital disorders. These opportunistic investigations may assist our comparative understanding of the pathogenesis of congenital disorders and is of relevance to human welfare. With anthropogenic-induced climate variability increasing the risk and occurrence of deformities occurring *in-utero*, the systematic approach to defining these emerging disorders is encouraged.

Keywords: Congenital, deformities, arthropod-borne, genetic, disease.

AH-06

The effects of heat treatment of bovine colostrum on bacterial and somatic cell counts, immunoglobulins, growth factors, and the colostral whey proteome

Giulio Curone[1], Tawny Chandler[2], Jessica Cha[2], Paolo Moroni[2], Ruchika Bhawal[2], Sheng Zhang[2], Sabine Mann[2].

[1]Unversity of Milan, Lodi, Italy; [2]Cornell University, Ithaca, United States.

The objective of this study was to investigate the effects of heat treatment on colostral low abundant proteins, immunoglobulins (Ig), insulin and insulin-like growth factor I (IGF-I), as well as bacteria and somatic cells.

Study procedures were approved by the Cornell University Institutional Animal Care and Use Committee. Colostrum samples > 8 L and Brix % > 22.0 were harvested from cows on a commercial dairy in NY, USA, were split into 2 aliquots using single-use colostrum bags, and either cooled on ice immediately after harvest (raw; R; n=11) or heat treated in a commercial pasteurizer for 60 min at 60°C (heat; H; n=11). All samples were analyzed for Ig via radial immunodiffusion in colostrum, and for insulin and IGF-I concentrations in whey by radioimmunoassay. Colostral total bacterial counts (TBC) and somatic cell counts (SCC) were determined using standard plate culture techniques and flow cytometry, respectively. A subset of 5 pairs (n=10) was further analyzed by nanoLC-MS/MS technique after ultracentrifugation at 100,000 x *g* for 60 min at 4°C to enrich the low abundant protein fraction in whey. Data were analyzed using statistical analysis accounting for the paired nature of the data using either paired t-test (JMP v. 14.0.0, SAS Institute, Cary, NC) or free online software to analyze proteomics data (MetaboAnalyst v. 4.0) for fold-change ≥ 1.5 between pairs, and false discovery rate (FDR)-adjusted paired t-tests with $P < 0.05$.

The median (range) reduction of IgA concentrations was 8.5 (0-38.0) % due to heat treatment ($P = 0.02$), whereas IgG concentrations did not change due to treatment ($P = 0.36$). Insulin concentrations decreased by a median (range) of 22 (7-45) % ($P < 0.001$) and IGF-I by 10 (0-18) % ($P = 0.005$) in H vs. R, respectively. Heat treatment was associated with a mean ± SE decline in SCC of 207,000 ± 68,000 cells ($P = 0.01$), as well as a reduction in total bacterial count by 13,162 ± 3,472 cfu/mL ($P = 0.001$). Proteomics analysis identified 328 unique proteins in all 10 samples. Among the 25 proteins that decreased by at least 1.5 fold in H vs. R., 9 were identified as complement proteins. Among the 45 proteins with FDR-adjusted paired t-test $P < 0.05$ and that decreased in abundance, 11 were identified as complement proteins, and 6 involved in immune response or coagulation.

We conclude that heat treatment of colostrum is associated with a reduction in the concentration of bacterial and somatic cell counts, IgA, insulin and IGF-I. In addition, proteomics analysis of colostral whey identified a number of complement components and other proteins that decreased in abundance due to heat treatment. The role of colostral complement in the intestine or circulation of the newborn calf has received relatively little attention to date. The biological significance of the observed changes in colostral components for the health and immune function of the newborn calf will need to be assessed.

Keywords: Colostrum, heat treatment, proteome, insulin, IgA.

AH-07

Assessment of ruminal fluid pH evolution across the weaning period in Holstein calves under field conditions

Panagiota Kazana[1], Nektarios Siachos[1], Nikolaos Panousis[2], Georgios Bramis[1], Georgios Arsenos[1], Georgios Valergakis[1].

[1]Laboratory of Animal Husbandry, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece, Thessaloniki, Greece; [2]Clinic of Farm Animals, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece, Thessaloniki, Greece.

Objective: The aims of this study were twofold: i) to identify ruminal fluid pH (RF_{pH}) evolution patterns in the peri-weaning period, and ii) to assess the association of these patterns with management practices, performance and metabolic parameters in dairy calves.

Materials and methods: A total of 237 Holstein healthy calves from 8 commercial dairy farms were enrolled in the study. For each calf, date of birth, pre-weaning housing system (individual or group), feeding management (access to hay before or after 4th week of age; ample access to water or not), milk replacer (MR) daily feeding plan (low: <6 L; medium: 6 L; high: >6 L) and method of weaning (abrupt or gradual) were available. At -7d, 0d and +7d relative to weaning (0d), calves were clinically examined and bodyweight (BW) was estimated from heart girth measurement. At the same time-points, blood samples [for serum β-hydroxybutyrate (BHB) and urea

nitrogen (BUN) determination] and ruminal fluid samples (for on-site RF_{pH} measurement with a portable pH-meter) were collected, 1-2 h post-feeding. Average daily gain (ADG) was calculated for the pre- and the post-weaning week (ADG1 and ADG2). A hierarchical cluster analysis (HCA) was performed to identify clusters based on RF_{pH} evolution. The RF_{pH} at -7d, 0d and +7d were inserted in the HCA as continuous variables. The number of clusters obtained from the agglomeration schedule was then used in a two-step cluster analysis (TSCA) to establish the RF_{pH} patterns. Comparisons among RF_{pH} clusters were performed using chi-square test and ANOVA, for categorical and continuous variables, respectively. Moreover, multivariable univariate regressions were performed to assess the effect of the RF_{pH} cluster membership variable on BW at -7d, ADG1 and ADG2. Repeated measures mixed models were used to assess the effect of RF_{pH} cluster membership on BHB and BUN. ROC curves were performed to investigate possible BHB and BUN thresholds predicting RF_{pH} classification. All analyses were performed with IBM SPSS v.25.

Results: Calves were classified in 5 clusters (CL). CL1 (n=21) had constantly a RF_{pH} <6.0, fluctuating during the test period. CL1 was characterized by grouped housing pre-weaning (95%), limited access to water (95%), early access to hay (90%) and medium MR feeding plan (62%). CL2 (n=50) had constantly a relatively stable RF_{pH} <6.0. No management factors were associated with this cluster. CL3 (n=30) had a RF_{pH} <6.0 at -7d and 0d, which increased >6.0 at +7d. CL3 was characterized by individually housed calves pre-waning (77%) and a medium plan of MR feeding (87%). CL4 (n=46) had a RF_{pH} constantly >6.0, but with fluctuations during the study period; 72% of calves had early access to hay. CL5 (n=90) had a stable RF_{pH} constantly >6.0. CL5 was characterized by individually housed calves pre-weaning (74%) and a high plan of MR feeding (96%). Mean ADG (kg/d) for each cluster for the pre- (ADG1) and post-weaning period (ADG2) were: CL1 (0.52, 0.78), CL2 (0.47, 0.69), CL3 (0.62, 0.53), CL4 (0.87, 0.55) and CL5 (0.79, 0.40), respectively. Cluster membership had a significant effect only on ADG1 (P<0.05). Calves in CL1 and CL2 had a lower mean estimated ADG1 of 0.992 kg (P<0.001) and 0.607 kg (P<0.05) compared to calves in CL5 and CL4. Moreover, CL5 had a lower proportion (13.3%) of calves losing BW from -7d to 0d compared to CL2 (45.0%, P<0.05) and CL3 (30.0%, P<0.05). Calves in CL1 tended to have lower BW at -7d although they were numerically older than all others. BHB and BUN were significantly affected by cluster membership (P<0.05). BUN was also affected by time-point × cluster interaction (P<0.001). BHB ≥436.2 (μM/L) and BUN <7.85 (mg/dL) at -7d were identified from the ROC curves as significant thresholds for predicting classification of calves in either CL1 and CL2 or in CL4 and CL5.

Conclusions: During the weaning period, different patterns of RF_{pH} exist and several management factors were associated with them. Patterns of rumen pH >6.0 were associated with higher growth rates, higher BHB and lower BUN values, indicating proper rumen function (enhanced volatile fatty acid production and absorption and better nitrogen utilization efficiency).

Keywords: Calves, Weaning, ruminal fluid pH.

AH-08

Effects of a single transdermal Flunixin Meglumine treatment on health, milk yield, culling risk, and fertility of early postpartum Holstein Friesian dairy cows

Ruth Schmitt[1], Jantijn Swinkels[2], Carl-Christian Gelfert[3], Laura Pieper[4], Rudolf Staufenbiel[1].

[1]Ruminant and Swine Clinic, Free University of Berlin, Berlin, Germany; [2]MSD Animal Health, Boxmeer, Netherlands; [3]MSD Animal Health, Vienna, Austria; [4]4Department of Farm Animal Surgery, Vetsuisse Faculty, University of Zürich, Zürich, Switzerland.

Objectives: Inflammation around parturition, although initially physiological, can reach pathologic extent in some individuals, with detrimental impact on health and immunity. Early postpartum (p.p.) dairy cows are highly susceptible to different puerperal diseases associated with inflammation, which represents a considerable welfare and productivity problem in dairy herds. Treatment of early p.p. dairy cows with non-steroidal anti-inflammatory drugs (NSAID) might alleviate pain, reduce inflammation, and hence diminish the incidence of puerperal diseases. The objectives of this study were to assess the effects of a single transdermal application of Flunixin Meglumine at 24–36 h p.p. in Holstein Friesian (HF) dairy cows on subsequent health, milk yield and fertility.

Materials and Methods: A total number of 500 HF dairy cows (153 primiparous (PRIM) and 347 multiparous (MULT)) from 3 large commercial dairy farms in North Eastern Germany were included in the study. The farms had a history of comparatively high serum haptoglobin concentrations in postpartum cows. At 24–36h p.p. the first clinical examination and treatment took place. Cows with stillbirth, twin birth, dystocia, signs of milk fever, retained fetal membranes or high fever (>40°C), were excluded from the study. Posture, behavior, tail position, back arching, rumen fill and locomotion were scored, rectal body temperature was measured. Cows were randomly assigned to one of two groups (250 cows each): the treatment group received 3.33mg/kg b.w. Flunixin Meglumine (FM) (*Finadyne®Transdermal, MSD Animal Health*) by transdermal application; the control group (CON) received the same amount of placebo fluid. Cows were examined daily up to day 8 p.p. and on day 15. On day 8 and 15 p.p., their vaginal discharge was assessed using an intravaginal device (*Metricheck®*, *Simcro Limited, New Zealand*). Additional blood samples were drawn on day 4 and 6 p.p. for haptoglobin (Hp), albumin (Alb), total protein, betahydroxybutyrate (BHB), non-esterified fatty acids, calcium, and phosphate. Monthly milk yield, composition, somatic cell count (SCC), and fertility data were obtained from the herd management software. Statistical analysis was performed using a commercially available software (*IBM® SPSS®)*. Linear, ordinal, binary and generalized mixed logistic regression models were used to assess the effects of FM treatment on clinical and production parameters. Both combined and separate models were calculated for PRIM and MULT cows.

Results: Compared to CON cows, FM-treated PRIM cows had a lower risk for purulent vaginal discharge on day 8 (OR 1.52, *P*<0.04) and day 15 p.p. (OR 1.63, *P*<0.01). FM-treated PRIM cows also showed lower serum Hp (0.90±0.08 vs. 1.17±0.07g/l; *P*<0.01) and higher Alb concentrations

(35.5±0.31 vs. 34.8±0.31g/l; *P*=0.02) on day 6 p.p. and lower rectal body temperature throughout the study period (*P*=0.04). Compared to CON, FM-treated MULT cows showed slightly lower serum BHB concentrations on d 4 and d 6 p.p. (*P*<0.01). Regardless of parity, treated animals were significantly less likely to abduct their tail (14.3 % vs. 23.6 %) or show arched back (27.9 % vs. 39.7 %) on the day after treatment (*P*<0.05). Daily milk yield was higher in FM-treated PRIM cows (30.73 vs. 29.51±1.35 kg; *P*<0.001) and lower in FM-treated MULT cows compared to CON (36.90 vs. 37.78±1.06kg; *P*=0.020). Milk fat and protein content were higher in FM-treated PRIM cows and lower in FM-treated MULT cows compared to CON. No effects of the treatment were observed on rumen fill, locomotion score, milk urea, SCC, fertility (first service conception risk, 200 DIM days open) and 60 DIM culling risk.

Conclusions: In this study, treatment with flunixin meglumine at 24–36 h p.p. decreased metritis risk and systemic inflammation in PRIM cows. FM-treated PRIM cows had higher milk yield compared to CON animals. No effects of the treatment on reproductive performance or culling risk were found. Therefore, in dairy herds with higher prevalence of systemic inflammation in clinically healthy early p.p. cows, transdermal administration of FM at 24–36 h after calving can be justified only for PRIM cows. Future research is needed to confirm the findings and to assess effects of FM in cows experiencing dystocia and in clinically diseased fresh cows.

Keywords: Post partum, inflammation, flunixin meglumine, dairy cows.

AH-09

Oral treatment of ketosis with red wine in fresh Holstein cows reduces beta-hydroxybutyrate in blood and resolves ketosis better than treatment with propylene glycol

Aurora Villarroel[1], John Mata[2].

[1]Athyr Vet, LLC, Philomath, OR, United States; [2]Ome Naturals, LLC, Corvallis, OR, United States.

Objective: Treatment of fresh cow ketosis is an ongoing problem on dairy farms. Current treatment protocols lack efficacy, therefore new options are needed. The most effective treatments so far are all based on commercial alcohol products. The objective of this study was to evaluate the effectiveness of two natural alcohol products (red wine and ethanol) for the oral treatment of ketosis in fresh cows compared to two treatment protocols based on oral propylene glycol.

Materials and Methods: This was a clinical trial performed at a dairy farm in Oregon equipped with in-line milk component analysis (AfiLab®, Afimilk, Israel) and milk meters that automatically collected milking information on each cow 3 times per day, every day.

Holstein cows between 4 and 30 DIM were flagged by the system if they had 3 consecutive milkings with a fat:prot ratio>1.4 and then confirmed by testing a venous blood sample for BHBA (beta-hydroxybutyrate acid) with a cow-side device (Precision Xtra™, Abbott, US). Cows were assigned to one of four treatment groups, stratified by lactation group (1st lact and 2+ lact). Treatment groups were (A) 2 L of red wine the first day followed by 1 L/day for 2 days, (B) 300 mL/day of propylene glycol for 3 days, (C) 2 L of ethanol the first day, followed by 1 L/day for 2 days and (D) on-farm drench protocol that provided 300 ml of propylene glycol in 20 L of water with a proprietary blend of electrolytes. A follow-up blood sample was tested the day after the last treatment (day 4).

Cows were randomly assigned to each group, and a minimum of 6 cows with complete data were to be enrolled per group. Changes in BHBA, milk production and milk components were evaluated and compared between the four treatment groups.

Results: Cows treated with wine had significantly larger decrease in BHBA (-0.69±0.37 mmol/L) compared to cows treated only with propylene glycol (-0.30±0.53 mmol/L) or only with ethanol (-0.10±0.37 mmol/L), but no difference with the on-farm drench protocol (-0.56±0.86 mmol/L). These changes resulted in the following proportions of cured cows after 3 days, defined as BHBA≤1.0 mmol/L: wine 57%, propylene glycol 25%, ethanol 36% and drench protocol 33%.

Conclusion: Overall, the proportion of cured cows was low in all groups demonstrating the insidious nature of ketosis in fresh dairy cows and the need for more research to find effective treatments. Oral treatment with wine resulted in the largest proportion of cows cured compared to all other treatments, along with the largest decrease in BHBA in blood, especially compared to oral propylene glycol. Oral treatment with wine resulted in similar decrease in BHBA but more cures than a custom on-farm drenching protocol that required extensive labor and could result in death due to drowning. Additionally, it is noteworthy that wine would be a simple and viable alternative for the treatment of ketosis in organic dairy farms.

Keywords: Ketosis, propylene glycol, ethanol, organic, dairy.

AH-10

Prevalence of and risk factors associated with failure of transfer of passive immunity (FPT) in beef calves in Great Britain

Rachel Bragg[1], Alastair Macrae[1], Samantha Lycett[2], Elizabeth Burrough[1], Geraldine Russell[1], Alexander Corbishley[1].

[1]R(D)SVS, Edinburgh, United Kingdom; [2]Roslin Institute, Roslin, United Kingdom.

Objectives: Failure of transfer of passive immunity (FPT) of colostral antibodies is well documented in dairy calves, with prevalence estimates varying from 19.2% to 33.1% worldwide. However knowledge of the rate of FPT in beef calves remains limited, with very few published studies.

The objectives of this study were to 1) investigate the current prevalence of FPT in GB spring calving beef herds, 2) identify associated risk factors for the development of FPT in spring born suckled calves at the calf, cow and herd level.

Materials and methods: 1131 individual blood samples were collected from calves in the first week of life from 86 farms across Scotland and England in spring 2018 (mean 13.5 calves per farm). The following information was collected for each animal blood sampled: sex, breed, date of birth, date of sampling, ease of calving (4 point scale – 1:No assistance, 2:Easy assistance, 3: Hard pull/calving jack required, 4:Caesarean section), any assistance the calf required to feed colostrum (7 point scale – 0: Feeding not observed, 1: Observed suckling, 2: Lead to feed off dam, 3: Bottle fed dam's own colostrum, 4: Tube fed dam's own colostrum, 5: Bottle fed artificial colostrum, 6: Tube fed artificial colostrum), the weight of the calf at sampling (indirectly assessed using heart girth measurement), the parity of the dam (heifer or cow) and the body condition score (BCS) of the dam (scale 1.0-5.0). Calves were chosen as a convenience sample for the age range required, excluding calves born by Caesarean section. Calf blood samples were analysed for serum IgG by Radial Immuno-Diffusion (RID). Risk factor analysis was performed using generalized linear mixed modelling techniques, with the farm included as a random effect.

Results: 15% of calves sampled had a serum IgG concentration ([sIgG] under 10 g/l, indicating failure of passive transfer of immunity. 37% of calves sampled had a [sIgG] under 24 g/l, indicating poor passive transfer of immunity. On 25 farms, greater than 20% of the calves sampled had ([sIgG] under 10 g/l indicating complete FPT.

All three levels of assistance with colostrum feeding used in this study were significant predictors for calves having a [sIgG] <24 g/L (Lead to dam odds ratio (OR) = 1.85, Bottle/tube fed dam's colostrum OR = 2.35, Bottle/tube fed artificial colostrum OR = 3.78), whilst bottle/tube feeding either dam's or artificial colostrum were also significant for [sIgG] <10 g/L (OR 2.66 and 2.34 respectively). Calving assistance was significant and increased the likelihood of [sIgG] <10 g/L and <24 g/L (OR 1.66 and 1.91 respectively). Male calves had a higher risk for [sIgG] <10 g/L (OR 1.68) whereas being a twin or being born to a heifer were predictive of having a [sIgG] <24 g/L (OR 3.31 and 1.57 respectively).

Conclusion: Overall prevalence of FPT in spring born suckled beef calves was similar to dairy calves, with 15% of calves having serum IgG concentration ([sIgG] under 10 g/L in the first week of life. Significant risk factors for serum IgG <10 g/L were calving assistance (OR 1.66), feeding assistance (OR 2.34-2.66) and being male (OR 1.68). Significant risk factors for serum IgG <24 g/L included dam parity (OR 1.57), twins (OR 3.31), assistance at calving (OR 1.91) and assistance with feeding colostrum (OR 1.75-3.78). This study raises important questions with respect to colostrum management practices on beef farms and highlights the need for revised protocols, particularly with respect to colostrum assistance, to improve calf health and productivity.

Keywords: Beef, Calves, Calf health, FPT.

AH-11

The maternal influence on microbiota maturation in neonatal calves in beef and dairy systems

Matthew Barden[1], Peter Richards-Rios[1], Erika Ganda[2], Luca Lenzi[1], Richard Eccles[1], Georgios Oikonomou[1], Jo Oultram[1], Joseph Neary[1].

[1]University of Liverpool, Liverpool, United Kingdom; [2]Penn State College of Agricultural Sciences, Pennsylvania, United States.

Objectives: The gastrointestinal microbiota of neonatal calves changes rapidly in the first weeks of life. The dam is considered an important source of microbes for the calf [1–3]; consequently, the development of calf microbiota may vary with farming system due to differences in the duration of time the calf spends with the dam. The objectives of this study were to characterise the early maturation of oral and faecal microbiota in beef and dairy calves and compare these to the anatomical niches on their dams which were likely to contribute to the vertical transfer of microbes.

Materials and methods: Hereford beef cows (N= 5) and Holstein dairy cows (N = 5) were enrolled on two farms and sampled at three timepoints: 4-8 weeks pre-calving, within 12 hours of calving, and four-weeks after calving. Samples were collected from the mouth, teat-skin, milk, vagina and faeces of cows at all timepoints; only oral and faecal samples were collected from calves. Beef calves could freely suckle from their dam throughout the study. Dairy calves were removed from their dams within six hours of parturition and fed artificial milk replacer. DNA was extracted from each sample and the V4 hypervariable region of the 16S rRNA gene was sequenced. Amplicon sequence variants (ASVs) were identified and the phylogenetic relationship between these ASVs was explored.

Results: A total of 14,125 ASVs were identified and taxonomically assigned. In beef and dairy calves, the oral microbiota became more similar to the oral microbiota of adult cows over the first four weeks of life. There was little similarity between the faecal microbiota of calves and cows; at four-weeks of age the faecal microbiota of calves was most similar to the oral microbiota calves and adult cows. At four-weeks of age, ASVs were identified in the calf oral microbiota that were also present in all cow samples immediately after calving, with the exception of dairy cow faeces. Very few ASVs were present in the calf faecal microbiota at four-weeks of age that were present in cow samples at calving. These results were observed in both beef and dairy calves.

Conclusions: The oral microbiota of calves matured more quickly than the faecal microbiota and by four-weeks of age it was similar to the oral microbiota of adult cows. The microbiota of calf faeces changed over the first four-weeks of life but bared little resemblance to the faeces of adult cows. Any maternal influence on these changes presumably occurred immediately post-partum as there were few differences between the trends observed in beef and dairy calves, despite dairy calves having limited contact with their dam. Specific microbes were present in the microbiota of both the calf and dam at calving but after four weeks more of these were still present in the calf oral microbiota than the faecal microbiota. This trend was observed in both beef and dairy calves and may suggest that immediately after calving the dam shares more microbes

with the calf oral microbiota than the faecal microbiota. Microbes identified in cow colostrum were also present in calf faeces at calving, but none were still present in calf faeces by four-weeks of age suggesting colostrum did not have a persistent seeding effect on the faecal microbiota of neonatal calves. Overall there were no marked differences between the development of the oral and faecal microbiota in beef or dairy calves during the first four weeks of life. This suggests that continued contact with the dam has little influence on the early maturation of oral and faecal microbiota.

References:

1. Alipour MJ, Jalanka J, Pessa-Morikawa T, Kokkonen T, Satokari R, Hynönen U, et al. The composition of the perinatal intestinal microbiota in cattle. Sci Rep. 2018;8:10437. doi:10.1038/s41598-018-28733-y.
2. Klein-Jöbstl D, Quijada NM, Dzieciol M, Feldbacher B, Wagner M, Drillich M, et al. Microbiota of newborn calves and their mothers reveals possible transfer routes for newborn calves' gastrointestinal microbiota. PLoS One. 2019;14. doi:10.1371/journal.pone.0220554.
3. Yeoman CJ, Ishaq SL, Bichi E, Olivo SK, Lowe J, Aldridge BM. Biogeographical Differences in the Influence of Maternal Microbial Sources on the Early Successional Development of the Bovine Neonatal Gastrointestinal tract. Sci Rep. 2018;8:3197. doi:10.1038/s41598-018-21440-8.

Keywords: Beef calves, Dairy calves, Microbiota, Neonates, Gastrointestinal tract.

AH-12

Factors affecting the price of young calves sold to Québec auction markets to be raised as veal calves (2008-2019)

Buczinski Sébastien[1], Fecteau Gilles[1], Blouin Louis[2], Villettaz-Robichaud Marianne[1].

[1]Université de Montréal, Saint-Hyacinthe, Canada; [2]Producteurs bovins du Québec, Longueuil, Canada.

More than 80% of Canadian veal calves are produced in the province of Québec. Calves coming from dairy farms are generally sold through auction markets and then commingled in veal farms. Little information is available on key drivers of calves' prices when sold through auction markets.

Objective: The objective of this study was to determine the risk factors of not being sold or being sold at a very low price in 1 457 257 calves sold in Québec auction markets during the study period (from January 1st 2008 to December 31st 2019).

Materials and methods: The sale data were retrieved from the general database managed by Québec beef producers (Producteurs bovins du Québec) from the 6 auction markets in activity during the study period. Specific data analysed were: the distance traveled from then farm of origin to the auction market, the season, the year, the breed (Holstein, cross-beef or non-Holstein dairy), gender, and calves' weight. The dependent variable was defined as a calf sold <0.1 CAD/lb (0.22 CAD/kg) which represented 30 024 calves (2.06% of the dataset) during the study period. Multivariable logistic regression modelsmodels were developped using relevant interactions between independent variables as previously studied in beef feedlot calves.

Results: The final model included year, season, sex, weight (categorized in deciles), distance traveled (categorized in quintiles) as well as interactions between distance traveled and site, sex, season, and weight. Low weight calves (<40kg) had higher odds of being sold at low price per crude weight. Cross-bred and female calves had lower odds of being sold at a low price. The odds of being sold at low price were higher in winter. Counterintuitively, calves coming from longer distances (>110km from the auction market) had lower odds of being sold at a low price. However, these odds were higher than other traveled distance strata during fall and summer but lower during winter and spring.

Conclusions: This study gives interesting insight on specific risk factors associated with low purchasing price and would potentially be helpful for improving calf supply quality and prices. There is a complex relationship between distance travelled and calf quality (as assessed by purchasing prices). Preparation before transportation and condition of transport are therefore of critical importance and should be thoroughly investigated.

Keywords: Dairy, veal, calves, multivariable.

AH-13

Administration of intranasal bacterial therapeutics or subcutaneous tulathromycin induces long-term modulation of the nasopharyngeal microbiota in beef cattle

Trevor Alexander[1], Samat Amat[1], Edouard Timsit[2].

[1]Agriculture and Agri-Food Canada, Lethbridge, Canada; [2]CEVA Animal Health, Libourne, France.

Objectives: The emergence of antibiotic-resistant pathogens associated with bovine respiratory disease (BRD) presents a significant challenge to the beef industry, as antibiotic administration is commonly used to prevent and control BRD in commercial feedlot cattle. Alternatives to antibiotics such as intranasal bacterial therapeutics (BT) are therefore needed as part of new management strategies to reduce antibiotic use and BRD. This study aimed to evaluate the longitudinal effects of intranasal BT on the nasopharyngeal (NP) microbiota, abundance of BRD pathogens, and quantities of antibiotic resistance genes in post-weaned beef calves, in comparison to a common metaphylactic antibiotic.

Materials & Methods: Beef heifers (N = 20 per treatment) from an auction market received either i) an intranasal cocktail of six *Lactobacillus* strains (3×10^9 CFU per strain), ii) a single injection of tulathromycin, or iii) intranasal saline on day 0. Nasopharyngeal swabs were collected pre- (day -1) and post- (days 1, 2, 4, 7, 14, 28, and 42) administration, and were processed for the assessment of bacterial microbiota using

culturing (BRD pathogens only), 16S rRNA gene sequencing, and real-time PCR.

Results: Despite being transient colonizers, a single inoculation of intranasal BT induced longitudinal modulation of NP microbiota while showing no adverse effects on animal health and growth performance. The BT-mediated changes in NP microbiota included reduced microbial diversity and richness, and an increase in the cooperative and competitive bacterial relationships. Tulathromycin altered the NP microbiota structure and composition by reducing bacterial load, increasing the antibiotic resistance gene *msr*(E) abundance, and disrupting bacterial network interactions. While the relative abundance of genera associated with BRD pathogens did not differ between treatments, tulathromycin did reduce prevalence of the pathogen *Pasteurella multocida* on days 7 and 14 based on culture analysis.

Conclusions: The results indicated that it was possible to use intranasal BT to modulate the bovine respiratory microbiota up to 42 days after inoculation, including enhancement of bacterial interactions. Intranasal BT may therefore have potential in conferring microbiome-mediated resistance against respiratory pathogens in feedlot cattle.

Keywords: bacterial therapeutics, cattle, respiratory microbiota, tulathromycin, bovine respiratory disease.

AH-14

Prepartum magnesium butyrate supplementation improves the health and performance of dairy cows

Levente Kovács[1], Ferenc Pajor[1], Mikolt Bakony[2], Joan Edwards[3].

[1]Hungarian University of Agriculture and Life Sciences, Kaposvár, Hungary; [2]University of Veterinary Medicine, Budapest, Hungary; [3]Palital Feed Additives B.V., Velddriel, Netherlands.

Objectives: In the dry period, the size of the rumen papillae decrease by more than 50%. After provision of a high energy lactation ration, it takes 50-60 days for the rumen papillae to fully redevelop. As a consequence, during early lactation the ruminal absorption of volatile fatty acids (VFA) and nutrients is limited. Rumen papillae redevelopment is stimulated by VFA, with butyrate being the most effective due to its ability to increase cell proliferation and to inhibit cell apoptosis. Butyrate also increases epithelial blood flow, magnesium absorption and VFA transport in the rumen wall. Furthermore, relative to parturition, butyrate supplementation has been demonstrated to improve colostrum composition in pigs. We hypothesized that dietary magnesium butyrate supplementation during the peripartum period of dairy cows would increase colostrum quality and improve the health and performance of dairy cows.

Materials & Methods: Two hundred-and-nineteen healthy multiparous Holstein-Friesian cows were blocked by expected calving date and then randomly assigned to treatment and control groups. For the cows in the treatment group (n=108), magnesium butyrate (Rumen-Ready®, Palital Feed Additives B.V.) was supplemented to the pre-calving total mixed ration (TMR) during the three-week close-up period. This resulted in cows receiving 150 g of Rumen-Ready® per day until calving. Rumen-Ready® contains 70% magnesium butyrate encapsulated in a fat matrix, and the magnesium butyrate is completely released and solubilized within the rumen. The pre-calving TMR of the control group (n=111) had no supplementation. At partum, cows were monitored in terms of calving ease, colostrum quality and quantity. The daily milk yield of the cows up to 70 days postpartum was recorded. Body condition and lameness of the cows were scored weekly during the trial. Fertility-related parameters of the cows were assessed: days to first heat, services per conception and late embryonic/early fetal mortality rate. The health status of the cows was determined until 70 days postpartum by a trained veterinarian who was blinded to the treatment groups. Significant differences, relative to the control group, were declared at $P<0.05$.

Results: Magnesium butyrate supplementation was associated with a significant decrease in the calving assistance rate and incidence of retained placenta. Colostrum volume and the total yield of IgG, protein and lactose were significantly higher in the treatment group. Supplemented cows had significantly higher milk yield during the first three weeks of lactation, and a significantly higher body condition score 3-9 weeks after calving. In terms of reproductive performance, the number of services per conception, the late embryonic/early fetal mortality rate and the number of days to first heat were significantly decreased by magnesium butyrate supplementation.

Conclusion: Supplementation of magnesium butyrate, in the form of Rumen-Ready®, significantly improved the colostrum, calving ease, early lactation performance, body condition, health and fertility of dairy cows. These wide-ranging benefits are underpinned by the positive stimulation of the rumen during the close-up period, and the provision of a readily available source of magnesium.

Keywords: Lactation, dry period, rumen, fertility, colostrum.

AH-16

Identification of potential predictive biomarkers and molecular mechanisms contributing to BRD-associated mortality in post-weaned beef cattle

Matthew Scott[1], Amelia Woolums[1], Cyprianna Swiderski[2], Andy Perkins[3], Bindu Nanduri[4], David R. Smith[1], Brandi Karisch[5], William Epperson[1], John R. Blanton Jr.[5].

[1]Department of Pathobiology and Population Medicine, Mississippi State University, United States; [2]Department of Clinical Sciences, Mississippi State University, United States; [3]Department of Computer Science and Engineering, Mississippi State University, United States; [4]Department of Basic Sciences, Mississippi State University, United States; [5]Department of Animal and Dairy Sciences, Mississippi State University, United States.

Objectives: Bovine respiratory disease (BRD) is a multifactorial disease complex possibly exacerbated by inflammatory responses to pathogenic agents. Diagnosis is commonly based on non-specific clinical signs, which lack sensitivity.

Elucidating early genomic mechanisms involved in BRD could lead to improved diagnostics. Moreover, early identification of cattle at increased risk for death might support interventions that decrease mortality. Our objective was to determine whether whole blood transcriptomes of post-weaned beef cattle identify predictive biomarkers and biological processes associated with BRD mortality.

Materials and Methods: Blood was collected at arrival from six cattle that ultimately developed BRD within the following 14 days. Replicates were placed into two cohorts based on BRD-attributed mortality (n=3 ALIVE; n=3 DEAD). Blood RNA was sequenced into 80 million 150bp paired-end reads per sample via an Illumina HiSeq 3000. RNA-seq was performed with a HISAT2/Stringtie/edgeR pipeline, utilizing the ARS-UCD1.2 assembly for alignment. Differentially expressed genes (DEGs) identified between groups (FDR <0.10) were modeled in WebGestalt, Reactome, GLAD4U, and STRING to identify GO-biological process (GO-BP) terms, pathways, disease phenotypes, and predicted interactions.

Results: 69 DEGs were identified between cohorts. GO-BP terms and pathways indicated increased expression of type I interferon-associated and antiviral genes in DEAD (FDR<0.05). Disease phenotyping of DEGs increased in DEAD indicated antiviral mechanisms (FDR<0.05). STRING analysis of DEGs predicted co-expression of interferon-associated gene products (CI>0.900).

Conclusions: RNA-seq and downstream analysis of whole blood collected at arrival demonstrated significant increases in type I interferon-associated genes in animals that succumbed to BRD, indicating that pro-inflammatory mechanisms at arrival are associated with BRD outcomes.

Keywords: Bovine, blood, interferon, transcriptome, gene expression.

AH-17

Effect of live yeast supplementation on growth and health of pre-weaned dairy calves

Nektarios Siachos[1], Panagiota Kazana[1], Clothilde Villot[2], Eric Chevaux[2], Guillermo Jimenez[2], Georgios E. Valergakis[1], Georgios Arsenos[1].

[1]Laboratory of Animal Husbandry, Veterinary Faculty, Aristotle University of Thessaloniki, Thessaloniki, Greece; [2]Lallemand SAS, Blagnac, France.

Objective: High growth rates of calves during pre-weaning period are associated with better performance later in life. However, this period is also characterized by high morbidity and mortality. Hence, the objective here was to investigate the effect of an oral probiotic supplement containing the live yeast strain *Saccharomyces cerevisiae boulardii* CNCM I-1079, on performance and health of pre-weaned dairy calves.

Materials and methods: Forty-one Holstein calves born from late September to mid-November 2019, in a medium-size (390 milking cows) dairy farm in Greece were enrolled in the study. All calves received the colostrum from the dam within 2-4 h of birth and transitioned to milk replacer (MR) at day 3 of age. A blood sample was collected at 2-3 days of age to assess the transfer of passive immunity using a Brix refractometer. Calves were randomly assigned in two experimental groups, designated as Test (T) and Control (C), stratified by dam's parity, gender and serum Brix (%) value. Calves were individually housed in elevated stalls and were offered daily 7L of MR, divided in 2 equal meals, in buckets, at a dilution rate of 0.125kg /L, providing 4.6 Mcal metabolizable energy (ME) / kg of dry matter (DM) (21.5% crude protein and 18% fat). They had *ad-libitum* access to water and were offered pelleted starter (6.72 Mcal ME/kg DM) after week 1 and chopped alfalfa hay after week 6 of age. T calves received 5g of live yeast at a dosage of 2×10^9 cfu/day, diluted in the MR for the whole experiment. Weaning was done on week 9 of age (day 60) with a gradual reduction of MR volume the previous 2 weeks. Calves' bodyweight (BW) was measured with a portable livestock scale at birth and week 9 (weaning). Average daily gain (ADG) was calculated for the whole nursery period. Starter and hay refusals were weighted weekly. Feed efficiency (FE), as total ME intake divided by BW gain, was calculated. Respiratory and fecal scores (RS and FS respectively) were assessed weekly by the same evaluator. Univariate regressions were performed to assess the effect of treatment on ADG and FE. The effect of treatment on pelleted starter and hay intake were assessed with linear mixed models. Repeated measures logistic regressions were used to assess RS and FS, using binary variables (0: scores 0 and 1; 1: scores 2 and 3). Statistical analysis was performed using IBM SPSS v.25.

Results: The initial BW (mean ±SD) was 41.2 ±3.9 kg and 41.9 ±4.0 kg for T and C calves, respectively (P =0.797), while mean BW at weaning was 64.3 ±15.3 kg and 61.0 ±7.0 kg for T and C calves, respectively (P =0.103). One T calf and one C calf died from acute bloat and pneumonia, respectively, and their data regarding ADG and FE were excluded from the analysis. Three out of 21 C calves (vs. 0/20 T calves) were decided by the farmer to be weaned 2 weeks later than scheduled due to poor performance. High overall morbidity was recorded during the study. Ninety percent of T calves and 95% of C calves were at least once assigned a FS 2 or 3, while 80% of T calves and 67% of C calves were at least once assigned a RS 2 or 3. Week-to-week starter and hay intakes did not differ between groups. On average, total starter and hay consumptions were 23.0 kg (±10.2) and 1.9 kg (±0.7), respectively for C calves, and 25.7 kg (±8.8) and 1.7 kg (±0.7), respectively for T ones. T calves tended to have higher ADG than C ones (estimated marginal means: 0.361 kg/d vs. 0.299 kg/d, respectively; P =0.066). T calves had a numerically better overall FE of about 0.9 Mcal ME/kg BW gain compared to C ones. The FS was not affected by the treatment while the T calves had about 1.3 more respiratory afflictions than controls (P =0.048) during the experiment.

Conclusions: Pre-weaned calves fed MR supplemented with *Saccharomyces cerevisiae boulardii* CNCM I-1079 tended to grow with higher rates than control calves despite the higher respiratory afflictions compared to untreated ones. This was more evident during the second month of life, probably due to better nutrient digestibility and better feed efficiency utilization.

Keywords: Probiotic, milk replacer, calf, growth, health.

AH-18

Impacts of bronchopneumonia in dairy calves: results from a systematic review and meta-analysis

Buczinski Sébastien[1], Timsit Edouard[2], Achard Damien[2].

[1]Université de Montréal, Saint-Hyacinthe, Canada; [2]CEVA, Libourne, France.

Objective: Bronchopneumonia (BP), also known as enzootic calf pneumonia, is one of the most common disease in dairy calves. Surprisingly, impacts of BP on health and performance of dairy calves have not been recently reviewed and summarized. This information is nevertheless crucial to increase awareness of BP's significance and thus increase adoption of management practices known to reduce BP prevalence.

The objective of this work was to quantify impacts of BP occurring during the first 12 months of life in dairy calves on subsequent risk of mortality and average daily gain (ADG).

Materials and methods: A systematic review and meta-analysis of the scientific literature was performed following PRISMA guidelines. PubMed and CAB Abstract databases were screened for relevant studies that were published in English (until Feb 3rd, 2020). Only studies reporting naturally occurring BP cases from birth to 12 months of age in dairy calves in Europe and North America were considered eligible. Both observational studies and randomized controlled trials were included. Random effect meta-analysis models were used to quantify impact of BP on outcomes of interest.

Results: From an initial pool of 525 studies, 13 studies reported impact of BP on growth and 10 studies reported mortality effect. In 1 and 3 studies, respectively a numeric effect could not be included in the meta-analyses. Case definition for BP differed among studies. Odds of mortality were 2.80 higher (95% CI: 1.42-5.51; n = 9 studies) for calves with BP. Average daily gain was decreased by 67g/day (95% CI: 34-99g/day; n = 10 studies) in BP vs apparently healthy calves. Heterogeneities of the models were important but was improved when accounting for adjusted (multivariable) vs non-adjusted (raw) estimates and prevalence of disease in the study.

Conclusions: To the authors' best knowledge, this systematic review and meta-analysis summarized for the first time impacts of BP on mortality and ADG. Calves with BP had on average 2.8 more risk of dying and grown ≈ 70 g/day less than apparently healthy herd mate.

Keywords: Meta-analysis, bronchopneumonia, calves, dairy.

AH-19

Feeding pre-weaned calves with waste milk containing antibiotic residues is related to a higher incidence of diarrhea and alterations in the fecal microbiota

Martina Penati[1], Giulia Sala[1], Filippo Biscarini[1], Antonio Boccardo[1], Valerio Bronzo[1], Bianca Castiglioni[1], Paola Cremonesi[1], Paolo Moroni[2], Davide Pravettoni[1], Maria Filippa Addis[1].

[1]University of Milan, Lodi, Italy; [2]Cornell University, Ithaca, NY, United States.

Objectives: Waste milk (WM) is a major by-product of the dairy industry that includes low-quality colostrum, transitional milk, milk from cows administered veterinary drugs for the treatment of mastitis and/or other diseases, milk with high somatic cell counts (SCC), and milk that cannot be marketed. The large amount of discarded WM generates environmental pollution and represents the loss of a valuable resource that may serve as a good feed source for dairy calves because of its high nutrient content. However, using WM for feeding calves might expose new-born calves to infectious agents. Moreover, residual antimicrobials may have a negative influence on the animals' gut microbiota in terms of selection of antimicrobial-resistant microorganisms as well as in terms of interference with its proper maturation, by acting on specific taxonomic groups or by inducing long-term dysbiosis. The constant antibiotic pressure exerted on the calf microbiota by these residues may interfere with its physiological development by selectively inhibiting specific phylogenetic subgroups and increasing the selection and transfer of antibiotic resistance genes to the gut microbiota. Therefore, despite the apparent economic advantages for the farmer, WM might impair the correct physiological and immunological functionality of the calf gut and favor the selection of antibiotic resistance traits. We assessed the impact of WM on the correct development of the calf intestinal microbiome by analyzing the effect on intestinal health and on the fecal microbiota of calves fed with WM in the first weeks of life.

Materials and methods: WM was assessed for nutritional content and antimicrobial residue concentration by mass spectrometry. The trial, carried out over 8 weeks, included 12 Italian Friesian male calves. After 3 days of colostrum, 6 calves were fed Waste Milk (WM group) and 6 calves were fed Bulk Tank Milk (BM group) for 2 weeks. Then, for 6 weeks, all 12 calves received a weaning diet of milk substitute and starter feed. Every week for the first 2 weeks, and every 2 weeks for the remaining 6, we subjected all calves to clinical examination and collected rectal swabs to investigate the fecal microbiota composition by 16S rRNA gene analysis. Research protocols were approved by the University of Milan (protocol number 78_2018).

Results: The composition on WM and BM in terms of SCC, fat, protein, lactose, and microbial inhibitors content was as follows. WM: 450,000 cells/ml; 3.7%; 3.6%; 4.7%; present. BM: 284,000 cells/ml; 4.23%; 3.60%; 4.97%; absent. At the end of the trial, the two groups showed statistically significant differences in both clinical terms, including reduced weight gain and increased occurrence of diarrhea events in the WM group, and in terms of fecal microbiota composition. Almost all WM calves (5/6) developed diarrhea in the first 2 weeks (vs 1/6

BM calves). In the following 6 weeks, only 1 episode of diarrhea occurred in 1 WM calf. WM calves' body weight was significantly lower than BM calves along the trial. The 16S rRNA gene analysis indicated a sharp reduction in the fecal microbiota alpha-diversity of WM vs BM calves, most significant at Wk4 ($p < 0.02$), two weeks after exposure to WM. Beta-diversity of the fecal microbiota was significant between time-points ($p = 0.0069505$). As for the alpha-diversity results, significant differences were observed between WM and BM calves at Wk4 ($p < 0.05$). Based on the normalized relative OTU levels, WM and BM calves showed significant differences at all time-points. At the end of the trial, Bacteroidetes, Firmicutes, and Saccharibacteria decreased while Chlamydiae increased. Significant changes were also observed in 7 classes, 8 orders, 19 families, 47 genera. Among the most relevant findings was the general decrease of beneficial taxa, like Faecalibacterium, vs an increase in other taxa and potential pathogens, including Campylobacter, Pseudomonas, and Chlamydophila. Lactobacillus and Lachnoclostridium increased, but since the first was present in the milk substitute, its higher abundance in WM calves might indicate a lower microbiome resilience.

Conclusion: In conclusion, these results suggest that adding to the risk of increasing antibiotic resistance, feeding pre-weaned calves with WM is related to a higher incidence of calf diarrhea and relevant changes in the fecal microbiota composition.

Keywords: Calf, gut microbiome, milk, antibiotic residues, mastitis.

AH-20

Bovine besnoitiosis control plan: a two-year experience in an extensive herd from the dehesa agroecosystem

Gema Alvarez Garcia[1], David González Barrio[1], Carmen Arrobas-Domínguez[2], Elena Mejías-López[1], Carlos Diezma-Díaz[3], Luis Miguel Ortega-Mora[1], Ignacio Ferre[1].

[1]Complutense University of Madrid, Madrid, Spain; [2]Advacas Extremadura S.L., Badajoz, Spain; [3]SALUVET-INNOVA S.L., Madrid, Spain.

Bovine besnoitiosis, caused by the parasite *Besnoitia besnoiti*, is characterized by local and systemic clinical signs. Currently, male reproductive failure is the major concern as breeding bulls may develop sterility. The disease is widely spread in Southwest Europe and is endemic at least in Spain, Portugal, France and Italy. Moreover, new outbreaks have been recently reported in Central and Northern Europe. Nowadays bovine besnoitiosis is one of the most relevant cattle diseases in extensive husbandry systems. In Spain the disease is present in mountainous areas in Central and Northeast Spain and in the dehesa agroecosystems in Southwest Spain. The lack of drugs or vaccines hampers the control, and it is a re-emergent disease in Europe. In this context, biosecurity measures coupled to an accurate diagnosis play a key role in the control and should be adapted to the facilities and management possibilities of the farm.

Objective: The aim of this study was to implement and monitor the efficacy of a control plan for two years (2018 and 2019) in a herd infected with *B. besnoiti* with a low fertility rate. Management measures were applied based on an exhaustive diagnostic protocol.

Material & methods: The herd under study is in southwestern Spain. It is fenced and covers an area of 3,000 hectares including a hunting reserve of Eurasian wild board (*Sus scrofa*) and red deer (*Cervus elaphus*) with Mediterranean scrubland mainly composed by evergreen oak (*Quercus ilex*) and cork oak (*Quercus suber*) and 800 hectares of dehesa agroecosystem dedicated to extensive cattle farming. The census initially included 154 crossbreed cows and seven breeding bulls of the Charolais, and Limousine breeds and the farm has its own replacement. At that time the herd fertility rate was 36.7% and with a calving interval of 524 days.

Once *B. besnoiti* infection was confirmed in one male by clinical inspection and serological analysis the whole herd was serologically tested in March 2018. Initially a validated ELISA test was used, and all doubtful animals were retested by western blot as a confirmatory test. Replacement heifers were tested at one year of age. The whole herd was tested again following the above-mentioned serological protocol at the end of the study in October 2019.

Results: Initially the intra-herd seroprevalence was 34.97% and six out of the seven (85.7%) bulls were seropositive. Sperm quality was monitored in all bulls and a low concentration of sperm was observed in four of them as well as defects in sperm motility and morphology.

Based on the diagnosis results obtained several control measures were recommended:

i. Infected (seropositive) from non-infected (seronegative) cows were separated and progressive selective culling of seropositive breeding bulls and cows was carried out. These seropositive animals were fattened prior to the selective culling.
ii. Replacement cattle were tested at least twice.
iii. Artificial insemination was implemented in the seronegative group for one year. In the meantime, new breeding bulls were entered into the herd and serologically monitored. Nowadays, reproduction management is based again in natural mating with seronegative breeding bulls.

As a result of these management measures implemented for the control of bovine besnoitiosis all animals present in the herd were seronegative to *B. besnoiti* at the end of the study, the calving interval between 2018 and 2019 decreased up to 469 days and the fertility rate increased up to 76.7%.

Based on the results obtained it is possible to design and implement effective control strategies in dehesa agroecosystems since the regular health status monitoring of new entries was carried out and the extension of these farms favour to manage the herd into two separate groups to avoid parasite transmission by direct contact. Other biosecurity measures to maintain a closed herd recommended such as vector control and the absence of contact with wildlife were not possible in this herd. The inexistence of new seropositive animals could be due to the lower frequency of vector borne cases and the low relevance of this parasitic disease in wildlife (eg. wild ru-

minants) since they do not pose a significant risk for parasite transmission to cattle.

Conclusion: This study represents the first successful attempt to implement and monitor the effectiveness of a control program for bovine besnoitiosis with an improvement of reproductive parameters.

Keywords: Bovine besnoitiosis, extensive herd, control.

AH-21

Salmonella enterica isolated from dairy cattle in Uruguay presents a diversity of antibiotic resistance genes and mobile genetic elements

María Laura Casaux[1], Bruno D'Alessandro[2], Martín Fraga[1].

[1]*Plataforma de Investigación en Salud Animal. Instituto Nacional de Investigación Agropecuaria, Colonia, Uruguay;* [2]*Departamento de Desarrollo Biotecnológico, Instituto de Higiene, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay.*

Objectives: The objective of this work is to study the presence and distribution of antibiotic resistance genes, plasmids and integrons in Salmonella enterica of bovine origin from Uruguay.

Materials and methods: In total 75 isolates from the collection of Plataforma de Investigación en Salud Animal-INIA-Uruguay collection were included. Forty-two isolates were obtained between 2016 and 2020 from 33 outbreaks of neonatal diarrhea and/or mortality due to bovine salmonellosis in calves that presented or succumbed to the disease with bacteriological and histopathological diagnosis. The remaining 33 isolates were obtained between 2019 and 2020 from one dairy farm with a history of bovine salmonellosis. Isolate sources included calves and cows' feces, water and food for animal consumption.

The genome of this isolates' set was obtained using Illumina MiSeq in MicrobesNG (UK). The sequence reads were uploaded to *EnteroBase*. The isolates' serovar was determined using the *SISTR1+SEqSero2* scheme, and the sequence type (ST) using the *Achtman* scheme. The assembled genomes were then further analyzed using the tools *ResFiinder 4.1*, *PointFinder*, *PlasmidFinder 2.1* and *IntFinder 1.0* from the *Center for Genomic Epidemiology (CGE)* for identifying acquired resistance genes, chromosomal mutations, plasmids and integrons.

Results: In total, 31 isolates were typed as *S.* Typhimurium (STy, 41.3%), 24 as *S.* Newport (SNw; 32.0%), 11 as *S.* Anatum (SAn; 14.6%), 6 as *S.* Dublin (SDb; 8.0%), one *S.* Agona (SAg; 1.3%); one *S.* Montevideo (SMv; 1.3%) and one as IIIb61:i:z53 (1.3%).

For every serovar identified, only a single ST was present. Every STy isolate was classified as ST19, SNw isolates as ST45, ST64 for the SAn isolates and SDb isolates were typed as ST10. Less frequent isolates SAg, SMv and IIIb61:i:z53 were typed as ST13, ST138 and ST430 respectively.

There was a high diversity of antibiotic resistance genes in the isolates collection. Every isolate carries the *aac(6')-Iaa* gene that confers resistance to aminoglycosides. Within this group, *aph(3'')-Ib* and *aph(6)-Id* were also frequently present. Fifty-one isolates presented resistance genes for tetracyclines, *tet(A)* was detected in 47 isolates while four carried *tet(B)*. The *sul2* gene was present in 29 isolates conferring resistance to sulfonamides. Interestingly some genes like *aadA1*, *aadA2*, *aadA17*, *floR* and *cmlA1* that generally are present in genetic mobile elements were detected. The $_{bla}$TEM-1B gene related to penicillin was detected only in 7 isolates of SA and ST isolates. Plasmidic qnr gene was carried by 5 isolates while 37 isolates had the *parC_T57S* mutation both conferring resistance to quinolones. One SD isolated showed the mutation *acrB_R717Q* to confer resistance to azithromycin.

Considering the presence of antibiotic resistance in all the genomes, twenty-four were multi drug resistant (MDR). This characteristic was only detected in STy, SA and SAg serotypes. Among these, one presented antibiotic resistance genes for 4 groups of antibiotics and five for 6 groups of antibiotics.

All except five isolates carried at least one plasmid. The most frequent plasmids were IncFII(pHN7A8), IncFII(S), IncFIB(S), Col440I and IncI1. Forty-nine strains had 1 plasmid, 17 strains had 2 plasmids, and 4 strains had 3 plasmids. All STy carried plasmids and the most frequent combination of plasmids was Col440/ IncFIB(S).

Two class 1 integrons were detected in the collection. In705, carrying aadA1 gene, was detected in two STy isolates while In1363, carrying aac(3)-IId and intI1, was detected in one SAn isolate and in one STy. These 4 isolates were MDR and carried at least one plasmid.

Conclusions: The evaluation of the genome allowed us to detect serotypes involved in the outbreaks with STy as the most frequent. A wide distribution of MDR isolates was found in the farm's outbreaks and environment. There was a high number of isolates carrying genes that confer resistance to aminoglycosides, tetracyclines and sulfonamides, antibiotics that are widely used in veterinary practice in Uruguay. Most of the serotypes detected in the collection could have an impact on human health. This work represents one of the first studies assessing the MDR genes in *Salmonella* from dairy cattle in South America.

Keywords: Salmonellosis, dairy cattle, antimicrobial resistance, genome, South America.

AH-23

Assessing the utility of leukocyte differential cell counts for predicting morbidity, mortality and growth in a grain fed veal facility: A prospective single cohort study

Taika E. Von Konigslow, David L. Renaud, Todd F. Duffield, Charlotte B. Winder, David F. Kelton.

University of Guelph, Guelph, Canada.

Objectives: Automated leukocyte differential cell counts (DCC) that can be acquired and analyzed quickly on farm may be useful to incorporate into calf risk identification protocols

at the time of arrival at a veal or dairy beef operation. The objective of this study was to assess the utility of DCC taken at the time of arrival at a grain fed veal facility and 72 hours post arrival for determining morbidity risk, mortality risk and growth during the production cycle.

Materials and Methods: Data were collected at a veal research facility in Ontario, Canada between June and October of 2018. A total of 240 calves were enrolled upon arrival and a subset of 160 calves were revisited 72 hours post arrival. Leukocyte differential cell counts were evaluated using the QScout BLD test for leukocyte differential cell counts (Advanced Animal Diagnostic, Morrisville, NC). At the time of arrival, all calves were screened using a standardized health scoring protocol and blood samples were collected to evaluate serum total protein (TP) and DCC. A second blood sample was collected from the subset calves 72 hours post arrival to evaluate DCC. Cox proportional hazards models were constructed for both morbidity and mortality outcomes. Mixed linear regression models were constructed for the outcome of average daily gain. Models were constructed in Stata 15 (StataCorp LP, College Station, TX).

Results: Results from data collected at the time of arrival suggest that TP values greater than 5.1 g/dL reduce the hazard of mortality (HR = 0.29, P < 0.001) and a rectal temperature greater than 39.6°C was associated with an increased hazard of morbidity (HR = 1.48, P = 0.04). Calves that were dehydrated gained less (-0.09 kg/d, P= 0.03), however, an increased lymphocyte count was associated with calves having a higher level of growth (+0.05 kg/d, P = 0.02). Results from DCC collected 72 hours post arrival suggest that lymphocyte counts between 4.8 and 5.8 x 10^9 cells/L decrease the hazard of mortality (HR = 0.22, P = 0.03) and > 7.0 x 10^9 cells/L decrease the hazard of morbidity (HR = 0.56, P = 0.02), whereas, neutrophil counts > 6.0 x 10^9 cells/L increased the hazard of mortality (HR = 5.2, P = 0.02).

Conclusions: This study demonstrates that machine DCC at the time of arrival and 72 hours post-arrival has potential for use in calf risk identification protocols in veal and dairy beef facilities. Further work to determine the effects of time post transportation on blood leukocyte measurements in this population of calves should be performed to help determine the best time to measure blood leukocytes post arrival.

Keywords: Male dairy calf, risk factors, biomarker, leukocytes.

AH-24

Effect of over-conditioning on the hepatic global gene expression pattern of dairy cows at the end of pregnancy

Osvaldo Bogado Pascottini[1], Jenne De Koster[1], Filip Van Nieuwerburgh[2], Mario Van Poucke[3], Geert Ospomer[1].

[1]Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium., Merelbeke, Belgium; [2]Laboratory of Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Ghent University, 9000 Ghent, Belgium., Ghent, Belgium; [3]Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium., Merelbeke, Belgium.

Objectives: Lipolysis in the prepartum has been linked with liver dysfunction and systemic inflammation, which in turn are associated with an increased incidence of postpartum clinical disease. We hypothesize that over-conditioning is associated with excessive (basal) prepartum fat mobilization, leading to steatosis and modifications in the global gene expression pattern of the liver. Thus, the aim of this study was to evaluate the effect of over- and normal-conditioning on the differential hepatic transcriptomics profile of prepartum dairy cows.

Materials and methods: Ten non-lactating pregnant Holstein cows were euthanized two weeks before expected calving. Body condition score (BCS) and backfat thickness (BFT) were evaluated, and blood samples for non-esterified fatty acids (NEFA) were taken before euthanasia. After slaughtering, liver biopsy samples were collected and frozen for further triacylglycerol (TAG) concentration analysis and RNA sequencing.

Results: Five cows were classified as normal-conditioned (BCS = 2.5–3.5) and five as over-conditioned (BCS = 3.75–5). Regression models revealed that normal-conditioned cows presented lower BCS (3.17 ± 0.10; mean ± SEM), BFT (1.29 ± 0.29 cm), and serum NEFA (0.16 ± 0.04 mmol/L) in comparison to over-conditioned cows (4.35 ± 0.21, 3.14 ± 0.43, and 0.38 ± 0.07 for BCS, BFT, and NEFA, respectively; P <0.05). Hepatic TAG concentrations were not different between normal- and over-conditioned cows (4.63 ± 0.40 and 6.06 ± 0.44 mg/g of liver fresh weight, respectively; P > 0.05). Compared to over-conditioned, normal-conditioned cows presented four up-regulated (PLAC8, COL27A1, IDO-1, and MEP) and two down-regulated genes (IGFVP-1 and CSE).

Conclusions: As expected, prepartum over-conditioning (based on BCS) was associated with greater BFT and basal lipolysis than normal-conditioned cows. However, hepatic TAG concentrations were similar between prepartum over- and normal-conditioned cows. This was reflected in the low number of differentially expressed genes between these groups of animals. All the differentially expressed genes were associated with protein-coding. Normalized counts and fold changes of targeted genes in KEGG pathways should be evaluated to further explore the potential effects that over-conditioning may have in the hepatic global gene expression pattern.

Keywords: Transition period, metabolism, lipolysis, transcriptomics.

AH-25

Risk factors associated with total and pathogen-specific immunoglobulin G concentrations in western Canadian neonatal beef calves

Lisa Gamsjaeger[1], Deborah M. Haines[2], Ed Pajor[3], Michel Lévy[3], M. Claire Windeyer[3].

[1]University of Zurich, Vetsuisse Faculty of Veterinary Medicine, Department of Ruminant Medicine, Zurich, Switzerland; [2]Western

College of Veterinary Medicine, University of Saskatchewan, The Saskatoon Colostrum Co. Ltd, Saskatoon, Saskatchewan, Canada; [3]University of Calgary Faculty of Veterinary Medicine, Department of Production Animal Health, Calgary, Canada.

Inadequate transfer of immunoglobulins from dam to calf via colostrum is a challenge on cow-calf operations because it negatively affects pre-weaning calf health and growth. There is limited knowledge about risk factors for poor transfer of passive immunity (TPI) and the specific antibodies being transferred to beef calves via colostrum in the perinatal period under current production conditions in western Canada. Thus, the objective of this study was to determine factors influencing total and pathogen-specific immunoglobulin G (IgG) concentrations against selected neonatal calf diarrhea (NCD) and bovine respiratory disease (BRD) pathogens in the serum of newborn beef calves. A total of 420 serum samples were available from 1 - 7 day old beef calves born on 6 farms in Alberta, Canada. Samples were analyzed by radial immunodiffusion assay for total IgG concentration and by enzyme-linked immunosorbent assays for pathogen-specific IgG concentrations against *Escherichia coli (E. coli)*, bovine Rotavirus (BRoV), *Cryptosporidium parvum (C. parvum)*, Parainfluenza Virus Type 3 (PI-3), Bovine Respiratory Syncytial Virus (BRSV), and Bovine Herpesvirus-1 (BHV-1). Data collected about individual dam- and calf-level risk factors included dam parity, dam vaccination status, calving ease, birth month, calf sex, twin status, and route of colostrum consumption (i.e., nursed from dam, bottle-fed, or tube-fed). Multivariable multilevel linear and logistic regression models were built to evaluate risk factors associated with total and pathogen-specific IgG concentrations, failed transfer of passive immunity (FTPI; serum IgG < 10 g/L), and inadequate transfer of passive immunity (ITPI; serum IgG < 24 g/L). Farm was included as a random effect in all models to account for clustering at the herd level. Five percent (n = 20) of calves had FTPI and 18% (n = 75) of calves had ITPI. Calves born to heifers and those receiving colostrum intervention (i.e., fed colostrum or colostrum product by either bottle or tube) showed significantly lower total IgG concentrations (P < 0.0001). Calves that received colostrum intervention had higher odds of FTPI (Odds ratio (OR): 6.1, 95% CI: 2.0 - 18.9) and ITPI (OR: 4.8, 95% CI: 2.1-10.8). Pre-calving dam vaccination was associated (P < 0.0001) with increased IgG-concentrations against *E. coli,* BRoV, and BHV-1, whereas PI3 and BRSV-specific IgG concentrations were unaffected by dam vaccination status. Vaccine practices varied greatly within and between farms, and producer compliance with label instructions was inconsistent. This study highlights the opportunity for improvement of management protocols pertaining to dam vaccination and colostrum intervention on cow-calf operations.

Keywords: Antibodies, immunity, colostrum management, vaccination, beef cattle.

AH-26

Impact of total and pathogen-specific serum immunoglobulin G concentrations on the health and growth of western Canadian beef calves

Lisa Gamsjäger[1], Deborah M. Haines[2], Edmond A. Pajor[3], Michel Lévy[3], M. Claire Windeyer[3].

[1]University of Zurich, Vetsuisse Faculty of Veterinary Medicine, Department of Ruminant Medicine, Switzerland; [2]Western College of Veterinary Medicine, University of Saskatchewan, The Saskatoon Colostrum Co. Ltd, Saskatoon, Saskatchewan, Canada; [3]University of Calgary Faculty of Veterinary Medicine, Department of Production Animal Health, Canada.

Neonatal calf diarrhea (NCD) and bovine respiratory disease (BRD) are the most common calfhood diseases in beef calves worldwide. Vaccination of pregnant dams against specific NCD- and BRD-associated pathogens and good colostrum management have the potential to increase the transfer of those pathogen-specific antibodies to the calf via colostrum and thus protect against disease during early life. However, little is known about the associations of pathogen-specific antibody concentrations and the risk of disease and mortality, as well as the impact of such antibody concentrations on pre-weaning growth in beef calves raised under current production conditions in western Canada. The objectives of this study were to determine the impact of total and pathogen-specific immunoglobulin G (IgG) concentrations on the odds of pre-weaning treatments and mortality, and on average daily gain (ADG). A total of 420 serum samples from 1 - 7 day old beef calves born on 6 farms in Alberta, Canada, were collected. Samples were analyzed by radial immunodiffusion assay for total IgG concentration and by enzyme-linked immunosorbent assays for pathogen-specific IgG concentrations against *Escherichia coli (E. coli)*, bovine Rotavirus (BRoV), *Cryptosporidium parvum (C. parvum)*, Parainfluenza Virus Type 3 (PI-3), Bovine Respiratory Syncytial Virus (BRSV), and Bovine Herpesvirus-1 (BHV-1). Data regarding pre-weaning treatments, mortality, and birth and weaning weights were collected. Multivariable multilevel logistic and linear regression models were built to evaluate associations between total and pathogen-specific IgG concentrations and the odds of pre-weaning mortality and ADG, respectively. Farm was included as a random effect in all models to account for clustering at the herd level. The impact of IgG concentrations on the odds of pre-weaning treatment was established by univariable logistic regression analysis. Receiving colostrum intervention (i.e., being fed colostrum or colostrum product by either bottle or tube) significantly increased the odds of mortality (OR: 7.4, 95% CI: 1.7-31.6). Calves with FTPI and ITPI were more likely to die (OR: 18.5, 95% CI: 3.7-93.4 and OR: 10.1, 95% CI: 2.6-40.2, respectively), calves with FTPI were more likely to receive treatment (OR 7.9, 95% CI 2.7-23.7), and calves with ITPI had lower ADG (-0.09 kg, SE: 0.03, P < 0.002). Calves that were treated in the pre-weaning period showed lower E. coli and BRoV-specific IgG concentrations at birth (P < 0.0001), and BRoV-specific IgG concentrations were lower in calves that died in the pre-weaning period (OR: 0.97, 95% CI: 0.95-0.99). These results suggest a protective effect of E. coli and BRoV-specific antibodies and highlight the importance of

increasing their transfer from dam to calf. Interestingly, neither being treated pre-weaning nor any of the pathogen-specific IgG concentrations were associated with ADG. This study highlights the need to review and refine protocols with respect to colostrum management on cow-calf operations to decrease pre-weaning treatments, calf losses and improve pre-weaning growth.

Keywords: Antibodies, immunity, pre-weaning infectious disease, beef cattle, vaccination.

AH-27

Evaluating the Efficacy of Colostrum as a Therapy for diarrhea in Young Calves

Havelah Carter[1], Michael Steele[1], Joao Costa[2], Michael Nagorske[3], David Renaud[1].

[1]University of Guelph, Guelph, Canada; [2]University of Kentucky, Lexington, United States; [3]Saskatoon Colostrum Company Ltd., Saskatoon, Canada.

Objectives: Diarrhea is the primary cause of morbidity and mortality in dairy calves. Many cases of diarrhea in calves are treated with antimicrobials, increasing the risk of antimicrobial resistance, therefore, creating a need for alternative therapies. The objectives of this study were to evaluate the effects of feeding colostrum at the onset of diarrhea on growth and duration and severity of the disease in pre-weaned dairy calves.

Methodology: At a calf-raising facility in Ontario, Canada, calves were scored for fecal consistency twice daily on a scale of 0 to 3 and enrolled into the trial when they had two consecutive fecal scores of 2 (runny or spreads readily) or one fecal score of 3 (liquid consistency, splatters). Calves were then randomly allocated to receive one of three treatments: 1) Control (CON) (n = 35): eight feedings over 4 d of 2.5 L of milk replacer at a concentration of 130 g/L (26% crude protein and 17% fat), 2) Short term colostrum supplementation (STC) (n = 35): four feedings over the first 2 d of 2.5 L of a mixture of milk replacer at 65 g/L and colostrum replacer at 65 g/L (26% immunoglobulin G and 14.5% fat) followed by four feedings over 2 d of 2.5 L of milk replacer at a concentration of 130 g/L, or 3) Long term colostrum supplementation (LTC) (n = 38): eight feedings over 4 d of 2.5 L of a mixture of milk replacer at 65 g/L and colostrum replacer at 65 g/L. Serum immunoglobulin G (IgG) was determined at arrival to the facility. Body weight (BWE), days to enrollment since facility arrival (DTE) and severity of diarrhea (SDE) were recorded and a fecal sample was taken to determine the diarrhea causing pathogens (PT) present at enrollment. Daily health exams evaluating fecal consistency were performed for 28 consecutive days after enrollment and body weight was measured at d 0, 1, 2, 3, 4, 7, 14, 21, 28, 42 and 56 after enrollment.

Results: The median days for resolution of an abnormal fecal score was 3.5 d (range = 0.5-11.5), 2.5 d (range = 0.5-11), and 3 d (range = 0.5-7) in the CON, STC, and LTC groups, respectively.

Using a Cox proportional hazards model, it was found that calves in LTC group had faster resolution of diarrhea compared to calves in the CON group (Hazard Ratio (HR): 2.29; $P = 0.01$; 95% CI = 1.31 – 4.01). In addition, there was an association between both DTE and BWE and resolution of diarrhea, where calves who were at the facility longer prior to enrollment (HR: 2.29; $P = 0.01$; 95% CI = 1.56 – 5.45) and heavier at the onset of diarrhea (HR: 2.37; $P = 0.01$; 95% CI = 1.23 – 4.56), resolved diarrhea quicker. Moreover, calves with a fecal score of 3 at enrolment took longer to resolve their case of diarrhea (HR: 0.44; $P = 0.01$; 95% CI = 0.28 – 0.70). The number of types of diarrhea causing pathogen also had an impact, with cases where no or one pathogen was isolated was associated with a faster resolution (HR: 3.30; $P = 0.01$; 95% CI = 1.62 – 6.68). In addition to a reduced time to resolution, improved growth was observed in calves in the LTC group gaining 98 g/d more than the CON group over 56 d following enrollment (HR: 0.98; $P = 0.04$; 95% CI = 0.01 – 0.19).

Conclusion: Supplementation of colostrum at the onset of diarrhea was associated with a reduced duration of diarrhea and improved growth in young calves. This may provide life-long benefits for calves diagnosed with a gastrointestinal disease in the pre-weaning period. Due to the preliminary nature of this study, future research is necessary to determine the most practical and effective dose, duration, and timing of this therapy.

Keywords: Morbidity, treatment, pre-weaned.

AH-28

Association between acute phase proteins and clinical signs of respiratory disease in dairy calves

Rohish Kaura, Elisabeth Dorbek-Kolin, Marina Loch, Dagni-Alice Viidu, Toomas Orro, Kerli Mõtus.

Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Estonia, Tartu, Estonia.

Objectives: Bovine respiratory disease (BRD) is a leading cause of morbidity, mortality, and antimicrobial consumption of pre-weaned dairy calves. Assessment of clinical BRD symptoms in field conditions impedes making inferences on the presence of infection and inflammation and thus sets subjective criteria for antimicrobial treatment. The acute phase response (APR) is a part of host innate immunity and the first response to infections and tissue damage, which causes significant concentration revision in acute phase proteins (APPs), e.g., haptoglobin (Hp), serum amyloid A (SAA), and fibrinogen (Fib), in the blood. APPs have poor diagnostic specificity but are considered very sensitive in detecting clinical or subclinical inflammation or infection. So far, there is limited information available about which of the respiratory disease signs better predicts the infection and inflammation of the respiratory tract.

Therefore, this study aimed to identify the possible associations between different APPs with clinical signs of respiratory disease in dairy calves under field conditions and to assess their potential as a diagnostic tool complementing current BRD diagnosis.

Materials and Methods: Sixteen large Estonian commercial dairy farms with >400 dairy cows were included in this study. At each farm, five clinically healthy calves and five calves with clinical BRD signs in the age range of one to ten weeks were selected. The following clinical parameters were measured in each calf: rectal temperature (°C; 0 = < 39.0, 1 = 39.0–39.49, 2 = ≥39.5), respiratory rate (breaths/min; 0 = <45, 1 = ≥45), nasal discharge (0 = no, 1 = yes), cough (0 = no cough, 1 = yes), and demeanor (0 = normal, 1 = depressed). Blood samples from the jugular vein of each calf were collected in EDTA tubes for Fib measurement and in serum tubes for SAA and Hp measurements. From one farm, six healthy calves were included, making for an overall sample size of 161 calves for SAA and Hp estimation. Fib measurements were performed from blood samples from 11 farms, making for an overall sample size of 110 calves. Serum Hp concentration was determined using an ad hoc hemoglobin-binding assay. Serum SAA concentration was measured with a commercially available ELISA kit. Plasma Fib concentration was measured using the heat precipitation method. Linear mixed-effect regression analysis with farm as random intercept was used to study the associations between the recorded clinical signs and concentrations of APPs; separate models were built for the three outcome variables Hp, SAA, and Fib. The final models were produced by backward elimination of the variables from the original models. Age was controlled in the statistical models, except for Hp, to avoid a possible confounding effect. Pearson's correlation analysis was used to identify the relationships between APPs. The modelling and the diagnostics were performed using Stata/IC 14.2. Results were interpreted as significant if $p < 0.05$.

Results: Clinical signs that were observed in calves were diarrhea (62.1%), increased rectal temperature (≥39.5°C; 36.0%), increased respiratory rate (29.2%), nasal discharge (16.8%), cough (14.3%) and depressed demeanor (5.0%). Serum SAA concentration was positively associated with increased rectal temperature of ≥39.5°C. Hp levels was positively associated with increased rectal temperature of ≥39.5°C and presence of nasal discharge. In case of Fib concentration, positive associations were found with increased rectal temperature of ≥39.5°C, increased respiratory rate of ≥45 breaths/min, and calves exhibiting cough.

Fib showed a moderate positive correlation with Hp (correlation coefficient = 0.46) and SAA (correlation coefficient = 0.42) concentrations, whereas between Hp and SAA a weak positive correlation (correlation coefficient = 0.32) was found.

Conclusions: This study found association between serum APP concentrations and clinical signs of respiratory disease in dairy calves. Increased rectal temperature (≥39.5°C) best represented the inflammation during respiratory disease measured through elevated APPs. Calf-side tests for Fib measurement could serve as valuable diagnostic tool to make inferences about the presence of systemic inflammation and to assess underlying tissue damage, thus aiding in treatment decisions.

Acknowledgements: Authors are grateful to all the farms and their staff that participated in the study. This work was financed by the Estonian Research Council grant (PSG268).

Keywords: Acute phase proteins, respiratory disease, dairy calves.

AH-29

Comparison of total protein, antibody ELISA and electrophoresis as tests for failed transfer of passive immunity in calves in a Bayesian latent class analysis

Laurens Chantillon[1], Giel Schoenmakers[2], Jozefien Callens[3], Jade Bokma[1], Bart Pardon[1].

[1]Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium; [2]Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium; [3]Flemish Animal Health Service, Torhout, Belgium.

Objective: Failure of passive transfer (FPT), the insufficient transfer of passive maternal immunity to calves, is a leading risk factor for many calfhood diseases, mortality and growth retardation. The prevalence of FPT is generally high and highly variable between farms. Regular monitoring of the FPT status of the herd is an essential element of calf health management. Direct tests (determining immunoglobulins) and indirect tests (determining total protein as a proxy of immunoglobulins) are available, among which total protein and brix refractometry are most accessible and best validated. Radial immunodiffusion (RID) is often used as a gold standard test in North America, but likely no real gold standard tests for FPT exists. Capillary gel electrophoresis and antibody ELISA are offered in many European veterinary laboratories, but their diagnostic accuracy to diagnose FPT has hardly been evaluated. Therefore, the objective of this study was to determine the diagnostic accuracy of capillary electrophoresis and a commercial antibody ELISA for FPT diagnosis in a Bayesian latent class framework with total protein refractometry as comparative test.

Materials and method: In this diagnostic test study, 200 clinically healthy calves (dairy and beef) of less than one week old were sampled for blood serum by practitioners throughout Flanders (Belgium) with a maximum of five samples per farm. Subsequently, on each sample, total protein and the various serum protein fractions were determined by capillary electrophoresis (MiniCap Flex Piercing, Sebia, France), and serum immunoglobulin concentration was determined by ELISA (Bio-X Diagnostics ELISA kit for Bovine Immunoglobulin Assays, Rochefort, Belgium). Subsequently, Bayesian latent class models in the statistical freeware program Winbugs (version 1.4.3., MRC Biostatistics unit, Cambridge, United Kingdom) were used to determine the diagnostic accuracy of the three tests. For this study, an independent model was used in which both an uninformed and an informed model were run. In the first model, prior information on all parameters was set at uninformative, beta(1,1). The second model included informative priors on the prevalence FPT in Flanders (18%), and the third model included informative priors on the prevalence of FPT and the sensitivity (76%) and specificity (89%) of 51 g/L total protein as a cut-off for FPT. Priors for the sensitivity (76%) and specificity (89%) of TP 51 g/L were estimated from a study of Buczinski et al. with 95th percentile 60%. The priors were modeled using beta distribution parameters of the corresponding prior distributions (Epitools), resulting in beta(21.1824;7.3734), beta(8.6849;1.9498), and beta(2.3087;6.9619) for sensitivity, specificity, and prevalence, respectively. All models were run with 200.000 iterations.



Results: All models converged, and the prior and posterior densities of models 1 to 3 were very stable between models. Also, the deviance information criterion values of the three models differed minimally (<2). As the informative priors narrows the parameter uncertainty, model 3 is expected to be the most accurate. Electrophoresis showed the highest sensitivity and specificity with 98.1% (95% credibility interval (CI) = 90.0 – 99.9) and 99.3% (95% CI = 96.1 – 100), respectively. Total protein sensitivity and specificity were respectively 63.8% (95% CI = 53.2 – 73.6) and 97.0% (95% CI = 93.4 – 99.0). The sensitivity and specificity of the ELISA were 95.5% (95% CI = 88.1 – 99.0) and 74.5% (95% CI = 66.8 – 81.3), respectively.

Conclusion: Based on analysis of this dataset electrophoresis appears a promising, near gold standard test for FPT, which is readily available in many veterinary laboratories. Given the lower specificity of the evaluated antibody ELISA, we recommend evaluation of antibody ELISA in a similar framework given the large differences in diagnostic accuracy between tests that may exist.

Keywords: Maternal immunity, calf health, specificity, sensitivity.

AH-31

Effect of serum total protein concentration on early life health and growth of dairy calves

Victor Cortese[1], Mark A. Kirkpatrick[2], Thomas H. Short[3], Brian Voortman[4].

[1]Zoetis, Simpsonville, United States; [2]Zoetis, Kuna, United States; [3]Zoetis, Parsippany, United States; [4]Herd Health llc, Caldwell, United States.

Objective: To assess the effect of serum total protein (STP) concentration on the early life health and growth of dairy calves. Materials and Methods A total of 39,619 neonatal Holstein, Jersey, and crossbred calves from 15 dairy operations. Calves arrived at a single calf-raising facility at approximately 2 days old. Each calf was weighed at facility arrival and a blood sample was obtained the next day for determination of STP concentration by refractometry. All calves were managed in a standard manner, and health events were recorded for 120 days. A subset of 3,214 calves was weighed at 120 days old, and the average daily gain (ADG) was calculated. Linear mixed models were used to assess the effect of STP concentration on specific health events. Results STP concentration was associated with the incidences of death, diarrhea, pneumonia, and whether a calf received IV fluid therapy. In general, the incidence of adverse health events decreased as STP concentration increased to 6.0 g/dL, plateaued at STP concentrations between 6.0 and 8.5 g/dL, and increased at STP concentrations > 8.5 g/dL. Although STP concentration was not associated with ADG, the ADG for Holsteins increased as STP concentration increased to 8.5 g/dL and then decreased at STP concentrations > 8.5 g/dL. Conclusion Results suggested that, for neonatal dairy calves, an STP concentration between 6.0 and 8.5 g/dL was optimal for health and growth, and calves with an STP concentration < 5.0 or > 8.5 g/dL should be considered at high risk for adverse health events.

Keywords: colostrum, neonatal calf health, passive transfer.

AH-32

Eradication of Paratuberculosis from a Dairy Farm - Following the Administration of *Mycobacterium vacccae*

Daniel Elad[1], Inna Lysnyansky[1], Rama Falk[2], Shlomo E. Blum[1], Limor Weisbelith[1], Inna Mikula[1], Adin Shwimmer[2].

[1]Kimron Veterinary Institute, Bet Dagan, Israel; [2]National Service for Udder Health & Milk Quality, Caesarea, Israel.

Background: Paratuberculosis is a chronic enteric infection, primarily of ruminants, that causes significant economic losses, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Young animals are at the highest infection risk. To date, on-farm prevalence reduction is based on protecting these animals from contact with the microorganism by hygienic measures and by removing shedding animals, a long, expensive, and partially successful process.

Mycobacterium vaccae is has been reported to have immunomodulatory and immunotherapeutic capabilities, by activation of the cellular immune system, of primary importance in preventing paratuberculosis and its evolution to the clinical stage. We have previously demonstrated the safety of the oral administration of live *M. vaccae* to new-born calves.

Objective: Assessment of the impact of introducing *M. vaccae* on the prevalence of paratuberculosis in a dairy herd.

Materials and Methods: Since some of the heifers were found, in a previous experiment, to shed *M. vaccae* and thus expose eventual control animals to the microorganism, a case-control study was not possible. Consequently, a "before-after" experimental design was implemented. In this model, untreated animals that lived on the farm before the onset of the experiment served as negative controls. A dose of 10^{10} CFU live *M. vaccae* was administered, by gavage, to all heifers on the farm, within 24 hours of birth and 2 weeks later. The prevalence of paratuberculosis was assessed yearly by milk ELISA (mELISA). Fifty percent of cows aged 3 years, born within 3 years before the experiment's onset, and all the treated cows were tested, at the same age, by qPCR for MAP shedding. Management improvements that might have had a bearing on the prevalence of MAP were not implemented during the experiment's period. Paratuberculosis positivity was not considered a reason to remove cows from the farm.

Results: The rate of mELISA positive cows was reduced from 6% to 0% within 3 years and remained unchanged thereafter. 48.9% oh highly positive cows reverted to being mELISA negative. Interestingly these cows were not treated with *M. vaccae* but were likely to have been exposed to the microorganism from their environment. Due to the disrepair of the dairy's fencing, shedding heifers mingled with the adult animals, thus exposing them to *M. vaccae*. The rate of qPCR positive control cows was 2.46% whereas that of the treated cows was 1%. Four years after the onset of the experiment

(October 2019) 2 control shedding cows, still on the farm, became qPCR negative. The test cow continued shedding MAP and was removed from the herd in December 2020 due to infertility. One additional test cow turned mELISA positive in February 2021 but did not shed MAP when tested in June of the same year. The cow is still in the herd.

Conclusions: The results of this study, seemingly unprecedented, indicate, pending further confirmation, that the introduction of live *M. vaccae* reduces paratuberculosis prevalence to the verge of eradication conceivably resulting from its immunoprophylactic and immunotherapeutic activity, in an economic, safe, and straightforward manner, even without the implementation of paratuberculosis mitigating management measures.

Keywords: Paratuberculosis, Dairy Herd, Live Mycobacterium vaccae, Eradication.

AH-33

The effect of colostrum supplementation during the first 5 days of life on calf morbidity, enteric pathogens, weight gain and immunological response

Anna Catharina Berge[1], Pleun Penterman[2], Iris Kolkman[3], Geert Vertenten[2].

[1]Berge Veterinary Consulting, Vollezele, Belgium; [2]MSD Animal Health, Boxmeer, Netherlands; [3]A7 Noord Dierenartsen, Drachten, Netherlands.

Objectives: The objective of this study is to investigate the preweaning health, performance, immunity, and enteric pathogen shedding in calves supplemented with colostrum from dams vaccinated against rotavirus, coronavirus and *Escherichia coli* F5 during five days after birth compared to non-supplemented calves.

Material & methods: On a commercial dairy farm, healthy and viable new-born calves from vaccinated (Bovilis® Rotavec Corona) dams received at least 3 liters of colostrum from their own dam in the first 24h after birth and were randomly assigned to the colostrum supplementation treatment (treatment calves) or control treatment (control calves). The colostrum supplementation treatment consisted of the addition of a liter of previously frozen colostrum (from Bovilis® Rotavec Corona vaccinated cows) to the milk replacer on the second day of life and a half a liter of previously frozen colostrum to the milk replacer on day 3, 4 and 5 of life. Control calves similarly received once daily the same amount of supplement made from skimmed milk with similar protein and fat composition as the colostrum supplement. The daily milk intake and health parameters were monitored during the first week of life. Calves were weighed at birth, on day 28, and at weaning (approx. two months of age). Average daily gain (ADG) for the different periods was calculated. Faecal samples were taken at 7, 14, and 21 days of age. Additionally, on the first day of diarrhoeal disease diagnosis a faecal sample was collected. The presence of faecal pathogens was determined using a rapid ELISA kit (rotavirus, coronavirus, *Cryptosporidia*, *Clostridia*, *E. coli* F5). Faecal samples may also be evaluated for the microbiome composition when significant changes in the results will be identified. Serum samples were taken at 1, 7, 14, and 21 days of age to determine serum antibody levels to bovine coronavirus, rotavirus, and *E. coli* F5. Additionally, total serum IgG concentration was determined in the sample taken on day 1 to evaluate the transfer of passive immunity. Outcomes were analysed in multivariate statistical models controlling for calf age, passive transfer of immunity status, parity of dam and other potential confounders. In outcomes evaluating serial measures on calves, a repeated measures design was used.

Results: The study is ongoing. Preliminary results are presented from 19 treatment calves and 21 control calves. The passive transfer of immunity in all calves was adequate to good and similar for both the treatment and the control calves. Limited enteric and respiratory disease has not revealed any significant effect of treatment on preweaning health in the calves. The ADG in the preweaning period of the calves was above 1 kg/day and no significant difference in ADG between treatment and control calves in the first month, the second month, or the overall preweaning period was observed. The faecal samples collected on day 7, 14, and 21 of age were all negative for coronavirus and *E. coli* F5. There was a non-significant trend for more rotavirus positive samples in control calves on day 7 and 14 of age and more cryptosporidia positive samples in control calves on day 14 of age compared to treatment calf samples. Furthermore, faecal levels of clostridia on day 14 of age were non-significantly higher in control calves compared to treatment calves. The specific serum antibody levels against rotavirus, coronavirus, and *E. coli* F5 were not significantly different between treatment and control calves. It may be decided to do a faecal microbiome analysis if significant differences will be found between treatment and control group to see if prolonged colostrum administration may have a positive effect on the microbiome and be an explanation for the significant findings.

Conclusion: This study indicates a trend towards lower excretion of cryptosporidia and rotavirus in preweaning calves supplemented with colostrum from vaccinated dams.

Keywords: Prolonged colostrum feeding, calf health, immunity, enteric pathogens.

AH-34

Prevalence of tail injuries in German Dairy cows

Prisca V. Kremer-Rücker, Saskia Meier.

Hochschule Weihenstephan-Triesdorf University of Applied Sciences, Triesdorf, Germany.

Objectives: Tail injuries especially often occur in fattening bulls. Their prevalence increases, when beef cattle are housed on slatted floors. The origin of tail tip inflammation and necrosis is often reported as technopathy, irritation due to manure contact or itching followed by scratching, but also subacute rumen acidosis and laminitis seem to occur in association with tail tip injuries in fattening bulls. Several kinds of tail tip scor-

ing systems already exist, foremost for fattening cattle. These scoring systems include between 4 to 6 grades of severity, but do not differentiate between different kinds of tail alterations. In literature, prevalences measuring tail alterations in fattening bulls range from 5% up to 90%. In dairy cows, prevalences between 2.5 and 7.7 % have been reported.

Since tail alterations in dairy cows are not investigated very well, the aim of this study was to develop a scoring system, which covers different kinds of tail alterations detected in dairy cows and could be used to phenotype this trait as a first step towards animal health improvement. Secondly, histo-pathological and clinical evaluations will follow to be associated with the scoring group and grade of severity.

Material & Methods: Data collection started in December 2019 from a German 75 German Holstein (HOL) cows dairy herd. All cows were examined every two weeks during six months regarding any kind of tail alterations. The findings were described and photographed. Data analysis resulted in seven different kinds of tail alterations: 1. very tip of the tail, 2. ring-like, 3. scurf, 4. swelling, 5. thinning, 6. axis anomalies, and 7. verruca-like mass.

Hereinafter, prevalences for the observed tail alterations were calculated based on monthly data collection from five different dairy herds: 3 HOL herds, counting average herd sizes of 75, 300, and 1300, respectively; 2 German Fleckvieh (FV) herds, counting 60 cows, each. All cows were housed in free stall barns with conventional (HOL, FV) or automatic milking systems (FV).

In total, 4443 Dairy Cows' Tail Scores were recorded. Data preparation and analysis were performed using R version 4.1.2. Prevalences for tail alterations were calculated by dividing the number of observations within by the total number of observations of each kind of tail alteration and was given in percent. For calculating the total prevalence per breed and farm, the occurrence of at least one tail alteration counted as an observation, was divided by the total number of cows under investigation and given in percent.

Results: The overall prevalence for any kind of tail alteration was 88% in German Holstein and 99% in Fleckvieh cows; it varied between farms from 74% to 99%. Prevalences for HOL and FV regarding alterations of the very tip of the tail were 26% and 71%, ring-like alterations 24% and 30%, swelling 26% and 42%, scurf 55% and 60%, thinning combined with axis anomalies 16% and 21%, and verruca-like mass 10% and 21%, respectively. Number per tail ranged for ring-like alterations and thinning/axis anomalies from 1 to 5 and for verruca-like mass from 1 to 3.

Conclusion: During this study, high prevalences for different tail alterations in HOL and FV dairy cows were found out. The grouping of different alterations as described above can be useful to phenotype tail alterations in dairy cows. However, further investigations regarding pathogenesis, aetiology, and genetics of the observed alterations in dairy cows' tails are needed to understand their origin and impact on animal health and welfare.

Funding: This research was funded by the Tönnies Forschung, Rheda, Germany.

Keywords: tail injuries, tail necrosis, tail inflammation, tail alterations, dairy cows.

AH-35

Nutritional supplementation of a multi-strains yeast fraction improves health and increases beneficial gut microbiota of pre-weaned dairy calves

Clothilde Villot[1], Edyta Kowalczuk-Vasilev[2], Lysiane Dunière[1], Marine Gauthier[3], Eric Chevaux[1].

[1]Lallemand SAS, Blagnac, France; [2]University of Lublin, Lublin, Poland; [3]Lallemand BIO, Blagnac, France.

Objectives: High morbidity and mortality rates of calves during pre-weaning period will dramatically impact the sustainability of the farm. The reduction of the prophylactic treatments in calves production has contributed to the development of new microbial-based solutions to improve gut heath and consequently production. The objective was to determine the effect on health of a multi-strains yeast fractions product (MSYF) introduced to the calves' diet during the rearing period (5-65 day of life).

Materials and methods: Thirty Holstein calves born from late June to mid-November 2019, in a medium-size (500 milking cows) dairy farm in Poland were enrolled in the study. All calves received the colostrum from the dam within 2 h and 2.5 L of transition milk, 2 times a day until 5 days of age. At enrollment, 6 days-old calves, were individually housed and were offered daily 8 L of MR, divided in 2 equal meals, in buckets, at a dilution rate of 0.125kg /L, providing 4.6 Mcal metabolizable energy (ME) /kg of dry matter (DM) (21.9% crude protein and 18% fat). They had *ad-libitum* access to water and pelleted starter (3.55 Mcal ME/kg DM). Calves were randomly assigned in the two experimental groups, designated as supplemented (MSYF) and Control (C), stratified by dam's parity, gender and initial BW. Supplemented calves received 0.8 g of a MSYF /kg of MR, diluted in the MR meals for the whole experiment. Fecal consistency (scores) was scored daily by the research team during the morning feeding using a 1 (firm) to 4 (watery) scale (Wenge et al., 2014). Two fecal samples were collected at time = 10 days and time = 60 days of the trial for each calf. Incidence of health disorders was recorded daily for each animal. Every abnormal health condition such as fever or bronchitis as well as every treatment (antibiotic, electrolyte therapy) was recorded.

A linear mixed model was performed to assess the impact of the supplementation on the different parameters measured. Calf was used as random effect, sex, group, and sex*group interaction were used as fixed effects and initial BW as a covariate. Statistical analysis was performed using IBM SPSS v.25 and RStudio.

Results: No calves of MSYF group experienced respiratory diseases during the trial whereas 50% of the non-supplemented calves had respiratory problems (P = 0.034). In addition, the number of diarrhea cases was lower for MYSF compared to Control group (2.2 vs. 0.9/calf respectively, P = 0.007) as well as the duration of the diarrhea case (5.6 vs. 2.9 days/calf respectively, P = 0.005). Consequently, a lower fecal score was reported all along the trial for MYSF compared to Control calves (1.6 vs 1.9 respectively, P = 0.002). Along with those observations, a lower number of calves received a therapeutic treatment in MSYF group compared to Control group

(18.8 and 62.5% respectively, *P* = 0.034), and the average number of therapeutic treatment used for MSYF calves was lower compared to Control calves (0.3 vs 2.7/calf respectively, *P* = 0.005). Finally, less MSYF calves were supplemented with electrolytes compared to Control calves (37.5 vs. 81.3% respectively, P = 0.035). From a fecal microbiota standpoint, MYSF supplementation in neonate calves increased milk associated bacteria (*Bifidobacterium, Lactobacillus)* and beneficial mucinolytic *Akkermansia* (stimulation of mucine production, and immune system) which might have improved milk metabolism compared to Control and optimize the nutrients supply from this feed.

Conclusions: The overall health of pre-weaned calves was significantly improved when they received daily a multi-strains yeast fractions supplementation. Consequently, less therapeutic treatment was applied to the supplemented calves. This better health could be explained by an enrichment of beneficial gut microbiota of supplemented calves likely involved in a better valorization of the milk.

Keywords: health, prebiotic, yeast fraction, microbiota, calf.

AH-37

Relationship between calf housing design and bovine respiratory disease prevalence in pre-weaned Irish dairy calves

John Denis Donlon[1], Conor McAloon[1], John Mee[2].

[1]University College Dublin, School of Veterinary Medicine, University College Dublin, Belfield, Co. Dublin, Republic of Ireland; [2]Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark Fermoy Co.Cork, Republic of Ireland.

Objectives: Bovine respiratory disease (BRD) is a significant welfare and economic problem in housed dairy calves internationally. While the type of housing, the environment within and the management of that environment are significant factors influencing the prevalence of BRD, the relative importance of each of these risk factors is unclear. Hence, the objective of this study was to determine the interrelationships between housing type, housing environment, management factors and the prevalence of BRD.

Materials and Methods: Spring-calving dairy farms (n=64) throughout the Republic of Ireland were either randomly recruited (n= 39) or referred as BRD problem herds (n= 24) by Regional Veterinary Laboratories. Farms were visited in 2020 and in 2021;Each farm was visited twice: once one month prior to the calving season to allow for installation of temperature and relative humidity sensors in a calf pen, and a second time during the spring period when the calf house was occupied. At the second visit, housing type, environment, calf management and respiratory health were characterised. To do this, housing type was recorded and the dimensions measured, Temperature and relative humidity sensors were recovered, air samples were taken to quantify bacterial load, a survey of calf management practices was conducted, and pre-weaned calves were examined.

To determine the herd-level prevalence of BRD in the calf house, 20 randomly selected calves between 4 and 6 weeks old were assessed in two ways. Firstly, clinical signs of BRD were assessed using the Wisconsin Respiratory Scoring System. Secondly, a thoracic ultrasound (TU) was performed by one veterinarian using a Linear 5 MHz ultrasound probe (Easy Scan Go, IMV). Each calf was assigned a TU score between 0 and 5, with 0 indicating healthy lungs, 1 indicating diffuse comet tails, 2 indicating patchy consolidation, 3 indicating lobar consolidation, 4 indicating lobar pneumonia affecting two lobes and 5 indicating pneumonia affecting more than two lobes. A score 3 or greater was considered a case of TU-BRD.

An Analysis of Variance (ANOVA) model was used to investigate the association between housing type and the herd-level prevalence of BRD as assessed by TU. As the distribution of BRD prevalence within housing type was not normal, a Kruskal-Wallis rank sum test was conducted.

Results: In total, 1,258 calves were examined by TU. Across all calves, the mean prevalence TU-BRD was 10%. The minimum, maximum and median prevalence TU-BRD in the 5 predominant housing types was:

1. Duo pitch (n=28) (Median Prevalence = 5%, Min = 0%, Max = 45%)
2. Round-top houses with additional lean-to roof(s) (n=12) (Median Prevalence= 15%, Min =0%, Max = 80%)
3. Mono pitch (n=10) (Median Prevalence = 7.5%, Min = 0%, Maximum = 30%)
4. Round Top (n= 9) (Median Prevalence = 0%, Min = 0%, Max = 10%)
5. Lean to (n = 5) (Median Prevalence = 0%, Min = 0%, Max = 15%)

There was a difference in the mean prevalence of TU-BRD cases between the different housing types ($p < 0.05$). The epsilon squared was 0.153 which is considered a medium effect size. A post hoc analysis relieved differences between the mean prevalence of TU-BRD in round-top houses compared to round-top houses with lean-to roofs ($p < 0.05$), and between lean-to houses and round-top houses with additional lean-to roofs ($p < 0.05$). However, when these p values were adjusted using the Holm-Bonferroni method for multiple comparisons, these differences were no longer considered significant.

Conclusion: The relationship between predominant calf housing designs present on Irish dairy farms and BRD prevalence remain unclear. The wide variation in the prevalence of TU-BRD between farms could not be solely attributed to the design aspects of calf housing investigated above. Further analysis of the temperature, relative humidity and air quality data collected in this study may contribute to a better understanding of the interactions between calf housing and BRD.

Keywords: BRD, Housing, Envrionment, Thoracic Ultrasound.

AH-38

Effect of colostrum replacer fat content on thermogenesis, calf behavior, health and growth

Ainhoa Valldecabres[1], Michael Nagorske[2], Deborah Haines[2], Manuel Campos[2], Alfonso Lago[1].

[1]DairyExperts, Tulare, United States; [2]SCCL, Saskatoon, Canada.

Objective: The objective of this study was to determine the effect of colostrum replacer fat content on thermogenesis, calf behavior and growth.

Materials and methods: A total of 58 Holstein female calves were assigned to Control Colostrum Replacer (CCR; colostrum replacer at 20% IgG and 22% fat) or Defatted Colostrum Replacer (DCR; colostrum replacer at 20% IgG and 5.7% fat) manufactured by SCCL (SK, Canada). Calves were tube fed CCR or DCR providing 150 g of IgG at 1 h and 100 g of IgG at 12 h after birth. At 0 (before 1st feeding), 6, 12, 18, 24, 36, 48 and 60 h after birth, vital signs [heart rate (HR), respiratory rate (RR) and rectal temperature (RT)], shivering and posture (lying or standing) were assessed, and blood samples were collected. Temperature data loggers were inserted into the vagina and accelerometers were placed in the right hind leg to evaluate lying behavior from 1st colostrum replacer feeding to 60 h after at 2- and 1-min intervals, respectively. Body weight and height were determined at birth, 90±2 and 127±6 d after birth. Disease treatment records were collected up to the end of the study. Multivariable linear or logistic regression were used for continuous and categorical data, respectively.

Results: Loggers' temperature readings were higher for CCR than DCR calves (38.81±0.03 vs. 38.76±0.03 °C; *P* = 0.04). Serum IgG and total protein within 60 h after 1st feeding were lower for CCR calves [14.07±0.64 vs. 17.40±0.63 mg/mL (*P* < 0.001); 5.04±0.07 vs. 5.30±0.07 g/dL (*P* = 0.01)]. Calves fed CCR had higher body weight and average daily gain by 90 d of age [96.44±2.02 vs. 89.95 kg±2.06 (*P* = 0.03); 0.65±0.02 vs. 0.58±0.02 kg/d (*P* = 0.01)] and 127 d of age [139.75±3.27 vs. 129.14 kg±3.27 (*P* = 0.03); 0.81±0.02 vs. 0.71±0.02 kg/d (*P* = 0.001)]. Respiratory disease incidence was double for DCR calves (Relative risk = 2.00; 95% CI = 0.86 to 4.60; *P* = 0.09) (tendency). Thus, the incidence of respiratory disease was 41% for calves assigned to DCR and 21% for calves assigned to CCR. Furthermore, a total of 2 calves assigned to DCR vs. 1 calf assigned to CCR died during the study period.

Overall, no statistically significant differences were observed for vital signs (RT, HR and RR), observed (shivering and posture) and accelerometers behavior measures, height and diarrhea treatments. Average RT was 38.78 °C, HR was 150.03 beats per min, and RR was 47.67 respirations per minute. A total of 9 calves were observed shivering at 6 h, 3 at 12 h; 4 at 18 h, 1 at 24 and 60 h, and no calves were shivering at 36 and 48 h. Accelerometers' data showed that calves spent 50 h and 55 min or 86.37% of the time lying down (also calves were lying down 78.77% of the times when posture was observed) and had 42.52 standing bouts during the first 60 h after birth. However, when two influential observations defined as observations with studentized residuals above |4| SD were excluded, lying time was higher for DCR (52 h and 15 min) than for CCR calves (50 h and 39 min); *P* = 0.04. Finally, diarrhea incidence was 21% and calves averaged 97.79 cm in height at 90 d of age.

Conclusions: In conclusion, feeding a full fat colostrum derived colostrum replacer versus a partially defatted form had a positive effect on thermogenesis, led to higher body weight and body weight gain within 127 d of age, and may have had a positive effect on respiratory disease, despite lower serum total protein and IgG within 60 h after 1st feeding.

Keywords: Calves, Colostrum, Fat, Thermogenesis.

AH-39

Effect of dry needle acupuncture to reduce rectal temperature in dairy cows using a single needle on acupuncture point GV14 (Da-zhui)

Aurora Villarroel[1], Yoojean Song[2], Alice Nakasone[2], Dana Tsuchida[2].

[1]Athyr Vet, LLC, Philomath, OR, United States; [2]Oregon State University, Corvallis, OR, United States.

Objective: To compare the effect of a single commonly-used size hypodermic needle on acupoint GV14 (Governing Vessel 14) versus no needle for the reduction of naturally occurring fever in commercial Holstein dairy cows.

Materials and Methods: Animals eligible for inclusion in this study were lactating Holstein dairy cows with a rectal temperature greater than 103.0 °F that were not receiving any other treatment or cooling interventions. Sample size calculation to detect 1.0 °F difference (alpha= 5%, power= 90%) showed the need of a minimum of 10 cows, when using the same animals as their own controls.

Animals with rectal temperature ≥103.0°F (Temp0) were enrolled in the study and the control period of the study started. After 20 minutes, temperature was measured again (Temp1) and if fever persisted, an 18 G x 1.5 inch hypodermic needle was placed on acupoint GV14 for 20 (Temp2). Rectal temperature was measured again 20 minutes after needle removal (Temp3).

The difference between the average change in temperature within each cow during the control period (no needle, Temp1-Temp0), during the 20-minute trial period (GV14 needle, Temp2-Temp1) and 20 minutes post needle removal (Temp3-Temp0) was analyzed by 1-sided paired t-test with a level of significance of *P*<0.05, because acupuncture on GV14 was expected to reduce fever, never to increase temperature.

The question arose during the study whether longer duration of needle placement would have any effect on the drop in temperature. Therefore, a second set of cows was enrolled, where some retained the needle for 20 minutes and others for 40 minutes. Temperature at 60 minutes post placement was compared by a 2-sided t-test for independent means with a level of significance of *P<0.05*.

Results: In total, 11 Holstein dairy cows were enrolled in the original study. Mean temperature difference during the 20-minute control period was + 0.04 ± 0.23 °F. Mean temperature difference for the same cows during the first 20-min-

ute study period with GV14 acupuncture was - 0.37 ± 0.28 °F. Overall, acupuncture on GV14 decreased temperature by -0.41 °F more than no intervention (*P*<0.001).

In the subsequent, 22 Holstein dairy cows were enrolled; 11 cows had the needle placed in acupuncture point GV14 for 20 minutes and 11 cows had it placed for 40 minutes. Mean temperature difference 60 minutes after initial needle placement was - 0.55 ± 0.43 °F for cows with a 20-minute placement and - 0.08 ± 0.53 °F for the cows with a 40-minute placement (*P=0.045*).

Conclusion: Acupuncture with a commonly-used size hypodermic needle on acupoint GV14 is an effective method of reducing fever in dairy cows. The difference in temperature change between animals seems to be associated with the disease causing the fever (diagnosis) as well as the potential duration of clinical signs (acute versus chronic disease).

Acupuncture is a viable option for reduction of fever in animals where pharmaceuticals cannot be used due to withdrawal concerns, adverse reactions, or organic farming limitations. Additionally, acupuncture at GV14 can be a useful tool to help stabilize patients while further treatment options are being explored and discussed with the owner.

Keywords: Acupuncture, fever, cows, GV14.

BC-01

Study on efficacy of anthelmintic drugs in German alpaca farm

Luise Kultscher[1], Barbara Hinney[1], Ronald Schmäschke[2], Anja Joachim[1], Thomas Wittek[1].

[1]Veterinary University Vienna, Vienna, Austria; [2]University Leipzig, Leipzig, Germany.

Objectives: Endoparasites are considered a major health problem of South American camelids. Although prophylactic and therapeutic measures such as application of anthelmintics are commonly used, treatment efficacy is typically not assessed. In a previous study a number of alpaca owners expressed significant concerns regarding the efficacy of anthelmintic treatment; further imported animals from countries with higher percentages of anthelmintic resistances may also contribute to decreased efficacy of anthelmintic drugs. The present study aimed to evaluate the efficacy of anthelmintic treatment with different anthelmintic drugs in German alpaca herds.

Material and Methods: Overall, 617 samples from 538 clinically healthy alpacas >1 year from 27 farms (n=11-157 animals/herd) were examined. After initial coproscopic examination by flotation and strongylid egg quantification (McMaster technique), animals with at least 150 eggs per gram faeces were included in a faecal egg count reduction test (FECRT) using fenbendazole (FBZ; n=71 samples), moxidectin (MOX; n=71) or monepantel (MON; n=66) which are the most commonly used drugs in Germany.

Results: The most frequent parasites detected by flotation were *Eimeria* spp. (75.1%) followed by strongylids (55.0%), *Nematodirus* spp. (19.3%), cestodes (3.1%) and *Trichuris* (2.7%). Pre-treatment larval cultures (n=23 positive pooled farm samples) revealed *Haemonchus* (87.0% of the samples), *Cooperia* (43.5%), *Trichostrongylus* (21.7%), *Ostertagia* (13.0%), *Nematodirus* and *Oesophagostomum* (4.3% each). On average FBZ treatment reduced egg excretion by 45%, MOX by 91% and MON by 96%. On the farm level, 13/18 farms that used FBZ, 6/6 farms that used MOX and 2/5 farms that used MON had individual FECR values <90% (FBZ) or 95% (MOX, MON). *Haemonchus* and *Cooperia* were overrepresented on the farms with reduced treatment efficacy.

Conclusions: Gastrointestinal strongylids are common in German alpacas and especially FBZ was not sufficiently effective to reduce strongylid egg excretion. Although the FECRT could not unambiguously determine anthelmintic resistance in the present study, the finding that small ruminant strongylids, especially *Haemonchus*, are common in alpacas indicates that determination of effective anthelmintic doses for alpacas, monitoring of efficacy and adapted (targeted selective) treatment regimens must be implemented as part of sustainable deworming practices.

Keywords: Alpacas, endoparasites, antiparasitic drugs, drug efficay.

BC-02

Abortion in Alpaca and seroprevalence to *Leptospira* sp. in a herd in Treviso, Italy

Matteo Cornaggia[1], Luca Palazzolo[2], Letizia Ceglie[3], Laura Bellinati[3], Laura Viel[2], Ernesto Pascotto[4], Luca Zandonà[4], Giovanni Trombetta[5], Luca Bano[2], Alda Natale[3].

[1]Istituto Zooprofilattico Sperimentale delle Venezie, Padova - Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna, Brescia, Italy; [2]Veterinary Diagnostic Laboratory, Istituto Zooprofilattico Sperimentale delle Venezie, Treviso, Italy; [3]Veterinary Diagnostic Laboratory, Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy; [4]Animal Health Veterinary Service, Unità Locale Socio-sanitaria 2 "Marca Trevigiana", Treviso, Italy; [5]Private Veterinarian, Paese, Italy.

Objective: Leptospirosis is a zoonosis affecting all mammals sustained by *Leptospira* sp.

Leptospira is worldwide distributed due to its persistence in humid environments and, to many asymptomatic carriers, spreading the pathogen for longtime through urines.

Several studies conducted in South America on new world camelids revealed a widespread seroprevalence to *Leptospira* sp. rising from 6,5 to 89,6% with various serovars positivity and different antibody titers (Rosadio, 2015).

Alpacas seem to not develop any clinical signs of leptospirosis but only some reproductive disorders such as abortion, infertility, and stillbirth (Tibary, 2006).

Materials and methods: In July 2021, a 9-month-old alpaca's abortion belonged to an educational farm was delivered to the Diagnostic Service of IZSVe, Treviso, Italy for diagnostic purposes.

Placenta, liver, kidney and lung samples were processed as routine histological investigation.

Major abortion pathogens (Bovine Viral Diarrhea Virus; *Chlamydia* sp., *Coxiella burnetii*, *Neospora caninum*, Schmallenberg virus) were investigated through molecular methods.

Investigations into bacteria were carried out on *Campylobacter* sp., *Salmonella* sp., and *Listeria* sp., and the content of abomasum was cultivated in standard bacteriological media.

Leptospira sp. was suspected as a cause of abortion.

A real-time PCR (rPCR) was conducted on DNA extracted from lung, kidney, and spleen following the protocol previously described (Smythe, 2002).

Urines and blood sera of all animals in the herd (3 llamas; 25 alpacas) were collected monthly from the beginning of the outbreak (July, August and September).

Frozen sera collected on December 2020 (before the event of abortion) for other diagnostic purposes were also analyzed to compare the results obtained in July 2021.

Antibody titers were established by means of microagglutination test (MAT; OIE, 2021) while urine samples were analyzed in rPCR.

Results: The carcass was partially mummified and a diffuse hemorrhagic infiltration in organs and subcutis was recorded.

Histologically, a mild degree of autolysis was observed. Le-

sions were characterized by hemorrhagic infiltration in organs. A necrotic placentitis occurred.

Molecular and microbiological investigations gave negative results, except for rPCR against *Leptospira* sp. that resulted mild positive in the kidney of the aborted fetus. The weak Ct recorded in rPCR did not permit the sequencing of the amplicon.

The mother of the aborted fetus was tested for *Leptospira* sp. antibodies resulting negative before and after the outbreak.

The serological study conducted to detect specific antibodies against a panel of 9 pathogenic serovars (*Grippotyphosa; Copenagheni; Icterohaemorrhagiae; Pomona; Canicola, Tarassovi, Bratislava, Ballum, Hardjo*) revealed a seroprevalence of 21,4%, 10,7%, 53,6% and, 28,6% to one or more serovars at December, July, August and September, respectively. The antibody titers varied from 1:100 to 1:6400; males showed a higher seroprevalence (*p* 0,0219) and a higher median of 1:200 titer values.

The most frequent serovar detected was *Icterohaemorrhagiae*; followed by *Copenagheni*, *Grippotyphosa* and *Pomona.*

No symptoms were recorded at any times, nor was observed molecular positivity on urine.

Conclusions: Our work confirmed a probable pathogenic role of *Leptospira* sp. in new world camelids in Italy, as suggested by other authors in Europe (Rüfli, 2011).

Even if the rPCR on the aborted fetus gave a weak positivity and no bacterial DNA was detected in urine samples, serological investigations allowed us to confirm the *Leptospira* infection within the herd.

In absence of a clear seroconversion, it has been impossible to date the beginning of the outbreak. Due to the high persistent titers in the same subjects, probably the abortion represented its final event.

In a public health vision, to limit the zoonotic risk, a parenteral antimicrobial treatment with doxycycline for at least 2 weeks was encouraged by the Public Veterinary Service in combination with the official detention of the animals until a sink in the antibody titers.

Male alpacas showed higher seroprevalence and a higher median of antibody titers with respect to females, included the one who aborted. In cows, it is known that chronically infected subjects can remain seronegative, while very few studies were conducted in bulls.

Although urine collection in alpacas can be challenging, rPCR performed on this matrix is highly recommended to ensure the diagnosis. Authors intend to emphasize the complexity of the diagnosis of leptospirosis and the need to combine multiple diagnostic tools for a proper epidemiological investigation to create management protocols that mitigate the zoonotic risk.

Keywords: Alpaca leptospirosis, Alpaca abortion, Leptospira sp.

BC-03

Serum metabolomics assessment of etiological processes predisposing ketosis in water buffalo through the ^{1}H-NMR spectroscopy

Enrico Fiore[1], Anastasia Lisuzzo[1], Luca Laghi[2], Kevin Harvatine[3], Elisa Mazzotta[4], Maria Chiara Alterisio[5], Matteo Gianesella[1], Chenglin Zhu[6], Barbara Contiero[1], Vanessa Faillace[1], Paolo Ciaramella[5], Massimo Morgante[1], Jacopo Guccione[5].

[1]University of Padua, Legnaro, Italy; [2]University of Bologna, Cesena, Italy; [3]Pennsylvania State University, State College, United States; [4]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy; [5]University of Napoli, Napoli, Italy; [6]Southwest Minzu University, Chengdu, China.

Objectives: The transition period is critically important to health and profitability of dairy buffaloes. The metabolic adaptation to negative energy balance (NEB) induced by the considerable increment of energy and nutrient requirements is still one of the major concerns that may decrease the productivity and predispose to different disorders. An inadequate metabolic adaptation is characterized by elevated concentrations of β-hydroxybutyrate (BHB). Nevertheless, a specifically BHB threshold for dairy buffaloes is not established and dairy cows' reference are often used. The metabolic processes can be investigated using the metabolomics approach, which reflects the animals' health status. The aim of the current study was to use the metabolic approach, specifically with the ^{1}H-NMR, to assess the metabolomic profile of Mediterranean buffaloes (MBs) to investigate the metabolic changes associated with different levels of energy deficit.

Materials and methods: The current cross-sectional investigation received an institutional approval by the Ethical Animal Care and Use Committee of the University of Naples Federico II (n.PG/2017/0099607). Sixty-two Italian MBs were selected within the entire group of fresh buffaloes (< 50 days in milk) from a single high-yielding dairy farm. All the buffaloes received a complete clinical examination before sampling to exclude clinical ketosis or other pathological statuses. The blood samples were collected from jugular vein into tubes containing clot activator to obtain serum for biochemical and metabolomic analysis. According to serum BHB concentration, animals were divided into two groups: Group healthy (Group - H) consisting of 37 MBs with a level of BHB < 0.70 mmol/L and Group at risk of hyperketonemia (Group - RK) made by 25 MBs with a level BHB ≥ 0.70 mmol/L. The statistical differences for biochemical parameters and metabolite's concentration were performed by one-way ANOVA and Wilcoxon test according to data distribution. A post hoc pairwise comparison among metabolite concentrations was performed using Bonferroni correction. A $p\text{-value}<0.05$ was accepted, whereas a $0.05\leq p\text{-value}\leq 0.10$ was considered as trend to significance. A robust principal component analysis (rPCA), a partial least squares-discriminant analysis (PLS-DA) with variable importance in projects (VIP), and the metabolic pathways overrepresentation analysis (ORA) were generated to summarize the structure of the data and to highlight the metabolic pathways influenced by BHB concentration.

Results: Among biochemical parameters, only AST was significantly increased in Group - RK. A total of fifty-seven metabolites were identified in serum samples: 27 amino acids and derivates, 9 organic acids, 5 alcohols, 4 carbohydrates, 3 amine and derivates, 2 fatty acids, 2 ketone bodies, 1 sulfone, 1 vitamin, 1 imidazole, 1 nucleoside, and 1 guanidine. Six of the identified metabolites showed a statistically significance, specifically: glycerol, taurine, and creatinine showed a significant reduction in Group - RK, whereas acetone, acetate and 3-hydroxybutyrate showed a significant increase. In addition, six metabolites showed a trend toward significance: methanol, proline, and glycine were reduced in Group - RK, whereas formate, citrate, glutamate were increased. The rPCA analysis failed to cluster groups, while the PLS-DA showed two cluster principally related to acetate, 3-hydroxubutyrate, acetone, and glycerol (VIP > 1.5). The ORA analysis identified five metabolic pathways possibly responsible for changes in metabolome profile: glyoxylate and dicarboxylate metabolism; pyruvate metabolism; glycolysis / gluconeogenesis; glycerolipid metabolism and taurine and hypotaurine metabolism.

Conclusions: Metabolomic analysis through ^{1}H-NMR is a useful tool to achieve knowledge about metabolic profiling related to serum BHB modifications in dairy buffaloes. The metabolic state of our animals at risk of hyperketonemia suggests an initial mobilization of body resources, subclinical inflammation and potential oxidative stress status, changes in ruminal fermentations, influence on urea cycle and thyroid hormone synthesis. This study demonstrates that the metabolomic approach identified potential relationships with the development of subclinical ketosis even if the BHB concentration did not exceed the threshold value.

Keywords: Metabolomics; Negative energy balance; Mediterranean buffaloes; H-NMR; Ketosis.

BC-04

First detection of "*Candidatus* Mycoplasma haemolamae" in alpaca (*Vicugna pacos*) in Italy

Giulia Sala, Gabriele Ratti, Vincenzo Ferrulli, Donatella Scavone, Angelica Stranieri, Alessia Giordano, Antonio Boccardo, Davide Pravettoni, Stefania Lauzi.

University of Milan, Department of Veterinary Medicine and Animal Sciences, Lodi, Italy.

Objective: *Candidatus* Mycoplasma haemolamae is a wall-less hemotropic prokaryote that infects camelids. To the author's knowledge, there have been no published reports of *Candidatus* M. haemolamae infection in alpacas in Italy. This study describes a clinical case of *Candidatus* M. haemolamae infection in an alpaca cria from northern Italy and the prevalence in its herd.

Materials and methods: A 2 month-old alpaca cria was referred to the Clinic for Ruminants of the Veterinary Teaching Hospital of the University of Milan for weakness and lethargy. At birth, the alpaca cria was immediately rejected by their dam and fed with artificial bovine colostrum (Locatim®, Boehringer Ingelheim, Germany) and pasteurized whole cow's milk. At admission, the cria presented pale mucosae, tachycardia, and moderate dehydration. The hemogasanalysis underlined severe hypoglycaemia (1.1 mmol/L) and anaemia (haematocrit 17%, haemoglobin 5.6 g/dl). The complete blood count (CBC) confirmed mild, regenerative anaemia. The blood smear revealed numerous small basophilic coccoid structures attached to the surface of erythrocytes compatible with *Candidatus* M. haemolamae infection. In addition, parasitology examination of the faeces revealed severe coccidian infestation. To confirm the presence of *Candidatus* M. haemolamae, a portion of the 16S rRNA gene was amplified using a species-specific real-time PCR on blood samples collected at day of admission and at day of discharge. Threshold cycle (Ct) number was used as the measure of bacterial load (the lower the Ct level the greater the amount of target nucleic acid is present in the sample). Subsequently, the other animals of the entire herd from which the primary case was detected were tested by real-time PCR and by blood smear examination to investigate the presence of *Candidatus* M. haemolamae (n=20).

Results: In the blood sample collected at admission, real-time PCR revealed a high level of *Candidatus* M. haemolamae DNA (Ct 11.7). The cria was stabilized by administration of a 10% glucose solution, iron dextran (5 mg/kg, subcutaneously once), B vitamins (10 mg/kg, subcutaneously once), E vitamins, and selenium (0.05 mg/kg, subcutaneously once). Furthermore, the animal was treated with long acting oxytetracycline (20 mg/kg, subcutaneously, q72h for 3 treatments) for the *Candidatus* M. haemolamae infection. By the third day of hospitalization, the animal's clinical condition had improved. Eight days after hospitalization, the haematocrit (30.1%) and haemoglobin (11.7 g/dl) were also within the reference ranges, and the alpaca was discharged. The blood smear, performed on the day of discharge, did not show the presence of *Candidatus* M. haemolamae whereas real-time PCR was still positive, showing lower DNA levels (Ct 24.7) compared to the first blood sample. Anticoccidial therapy was set at discharge with sulfadimethoxine (110 mg/kg, orally, q 24 h for 10 days).

A 65% (13/20) *Candidatus* M. haemolamae real-time PCR positivity was reported in the other animals of the herd, with Ct values ranging from 16.4 to 32. A poor agreement between PCR result and smear examination was observed. The dam of the cria showed positive molecular results. An overall 66.7% (14/21) prevalence was observed in the herd, including the alpaca cria. No animal other than the cria had clinical manifestations correlated to the infection.

Conclusion: This study reports the first identification of *Candidatus* M. haemolamae in Italy. As shown in other studies, clinical infection was observed in a young animal, and further parasitic infestations (such as coccidiosis) were associated with *Candidatus* M. hemolamae infection. Treatment with oxytetracycline during *Candidatus* M. hemolamae infection was valid only for symptom remission, but the alpaca cria continued to be PCR positive after treatment, in accordance with previous observations on treatment in positive alpaca. Despite the very high prevalence of *Candidatus* M. haemolamae, most infected alpacas did not show clinical abnormalities. The absence of maternal colostrum intake suggests that the cria was not infected by colostrum. Further investigations are needed to assess the transmission dynamics of *Candidatus* M. haemolamae in Italian alpaca herds.

Keywords: Alpaca, Candidatus Mycoplasma haemolamae, Anaemia.

BC-05

Improving the embryo developmental competence and success of in vitro produced calves during hot season in Buffalo

Omaima Kandil[1], Ragab Dohreig[2], Ahmed Abdoon[3], Esraa Esmail[3], Hosam Tawfik[3], Ahmed Shaban[3], Emilie Corbin[4], Pascal Mermillod[4].

[1]National Research centre, Cairo, Egypt; [2]Haram Institute of Reproduction, Giza, Egypt; [3]National Research Centre, Cairo, Egypt; [4]INRA institute, Tours, France.

Heat stress is a major problem for animal breeding in tropical and equatorial area as well as in the Mediterranean countries. This translates into several pathologies and high rates of reproductive failure through early embryonic loss in buffalo. The present work was conducted to investigate:1) Effect of cold and hot season in the oocyte quality and in vitro embryo development competence of buffalo cultured in buffalo oviduct epithelial cell (BOEC).2) Effect of heat stress (41°C) & IGF during in vitro oocyte maturation in oocyte maturation rate and embryo developmental competence.3)Transfer of in vitro produced embryo during hot season to Egyptian buffalo. Egyptian buffalo's ovaries were collected during hot (June-August) and cold (December-February) season from abattoir. Oocytes were classified to Excellent (Ex), Good (G), Fair (F), Denuded (D). EX and G oocytes were matured in in-vitro maturation medium (IVM) (TCM-199+ 10% FCS + 10 µg/ml FSH+ 10 ng/ml EGF+ 50 µg/ml gentamicin (gn) for 22 h in 38.5C and 5% CO_2. The matured oocytes (1st pb) fertilized using Frozen – thawed buffalo semen, for 18 hours incubation. Zygotes cultured in SOFM + 10% FCS, 5 µg/ml insulin and 50 µg /ml gentamicin, with or without BOEC and incubated for 8 days. 2) Ex & G oocytes IVM in TCM-199 + 10% FCS+ 10 µg/ml FSH + 10 ng/ml IGF + 50 µg/ml, for 41°C /1hr- then 38.5°C vs. 38.5°C incubation for 22 hours. Then matured oocytes were fertilized and cultured as described before. Blastocyst were Fixed for cell counting to validate the quality of blastocyst. 3) Non-surgical embryo transfer during hot season in National Research Centre farm, Fresh Two in-vitro produced embryos (IVM,TCM+IGF, cultured in BOEC) were transferred to each buffaloes came in natural oestrus (buffalo number=5).Pregnancy diagnosis after 40 days from transfer. Results, Oocytes were collected from (349) buffalo ovaries giving an average of 2.4 oocytes/ovary (range 1.8 – 3.0 oocytes/ovary).There were highly significant (P<0.01) differences in the recovery rate of total oocytes between the two seasons, cold and hot season (3.02% ± 005 and 1.76% ± 0.05 respectively). Analysis of quality of buffalo oocytes revealed that, there were highly significant (P<0.01) differences in the mean % ± SE of excellent and good quality oocytes between the two seasons cold (38.63% ± 0.5, 51.35±0.50 resp.) and hot (11.29% ± 0.64, 21.37±0.74 resp.). While, fair and denuded oocytes increased with higher significant differences (P< 0.01) in summer (58.45% ± 0.86) when compared with the cold (9.86% ± 0.23). Maturation rate (85- 84 %), cleavage rate (75-71 %), blastocyst rate (33- 2%) were significantly higher (P<0.1, P<0.5) in cold temperature when compared with hot temperature season, in range 71-70%, 63-60 % and 22-17 respectively. In vitro culture of embryo using BOEC vs. without BOEC significantly increase the blastocyst rate either in cold (33 vs. 27 %) or hot temperature (22 vs. 17 %). The cell number of the blastocyst was significantly higher (P<0.1) in the cold temperature (mean= 106-90) when compared with hot temperature (80-60) and in vitro culturing in BOEC significantly increase (P<0.1) the blastocyst cell number either in cold or hot season. Effect of heat stress 41°C during in vitro oocyte maturation on in vitro embryo developmental competence in buffalo. Higher temperature 41°C for one hour during in vitro oocyte maturation in buffalo significantly (P<0.01) decreased the mean ± SD and rate of maturation (32±3.79, 51), cleavage (16.5±2.65, 26) and blastocyst (13.5±2.65, 22), when compared with in vitro oocyte maturation in 38.5°C (77.50±7.46, 87%, 42.67±2.52, 72% and 37.00±5.03, 41% respectively). Non- Surgical transfer of in vitro produced fresh buffalo embryos to five buffalos (day 6 of natural oestrus), pregnancy and calving of two calves (2/5, 40% Emy & Medo) with 42 kg body wright.

In conclusion: Heat stress either during hot season (summer) or through experimental rise temperature during in vitro maturation of buffalo oocytes significantly decrease the in vitro oocyte competence and blastocyst rate. Supplementation of IGF in in vitro maturation medium and co-culture of in vitro fertilized oocytes using BOEC decreased effect of heat stress and improved in vitro embryo production of buffalo. Success of calving of two calves (EMY& Medo) through transfer of IVP buffalo embryos matured in TCM+IGF during hot season (summer).

Keywords: Buffalo, season, BOEC, embryo, ET.

BC-06

Herd, buffalo, and quarter specific prevalence and risk factors of subclinical mastitis in water buffaloes of Bangladesh

Shuvo Singha[1], Gerrit Koop[2], Momena Khatun[3], Fabrizio Ceciliani[1], Sofia Boqvist[4], Marjolein Derks[5], Md. Ahasanul Hoque[6], Mohammed Kawser Hossain[7], Md. Mizanur Rahman[6], Ylva Persson[8].

[1]Department of Veterinary and Animal Sciences, Università degli Studi di Milano, Via dell'Univesrità 6, Lodi, 26900, Italy, Lodi, Italy; [2]Department of Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL, Utrecht, The Netherlands, Utrecht, Netherlands; [3]Veterinary Physiology, University of Bern, Bremgartenstrasse 109a, 3012 Bern, Switzerland, Bern, Switzerland; [4]Department of Biomedical Sciences and Veterinary Public Health, Division of Bacteriology and Food Safety, The Swedish University of Agricultural Sciences, BVF, Bakteriologi och livsmedelssäkerhet, 750 07 Uppsala, Sweden, Uppsala, Sweden; [5]Farm Technology Group, Wageningen University and Research, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands, Wageningen, Netherlands; [6]Department of Medicine and Surgery, Chattogram Veterinary and Animal Sciences University, Chattogram 4225, Bangladesh, Chattogram, Bangladesh; [7]Department of Surgery

and Theriogenology, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Sylhet-3100, Bangladesh, Sylhet, Bangladesh; [8]National Veterinary Institute, 751 89 Uppsala, Sweden, Uppsala, Sweden.

Objectives: Subclinical mastitis (SCM) in water buffaloes is a prevalent production disease responsible for reduced milk yield with compromised milk quality. The risk factors for SCM in water buffaloes are largely unknown since little information is available in this species. The study objectives, therefore, were to i) estimate the prevalence of SCM in water buffalo at quarter and buffalo-level; ii) identify the quarter the risk factors associated with SCM at quarter and buffalo-level, and iii) determine risk factors for high bulk milk somatic cell count (BMSCC) at the herd-level.

Material and Methods: This cross-sectional study was carried out between February 2020 and April 2021 and included individual lactating water buffaloes or buffalo herds belonging to17 buffalo concentrated sub-districts in Bangladesh (11 coastal and semi-coastal districts regions). The buffalo herds represented five different production systems: bathan (n = 51), semi-bathan (n = 106), households (n = 33), semi-intensive (n = 54), and intensive (n = 4). The native name "bathan" is used to define solely grazing-dependent free-ranging system in remote islands while "semi-bathan" implies to the partial stay at household when feed source is very limited in islands. Herd-level and buffalo-level data were collected using a structured questionnaire. Milk samples were collected on a herd basis (average 1-5 buffaloes/herd) from the bathan, semi-bathan, household, and semi-intensive production system and all individual buffaloes of the intensive production system. A total of 3,491 functional quarters of 880 lactating buffaloes of 248 herds were included in the study. A buffalo was considered positive for SCM if any functional quarter was tested positive in California mastitis test (CMT) score ≥2 (1 to 5 scale) and no signs of clinical mastitis (CM). The composite milk mixture (n = 242) collected in the morning was used for herd-level BMSCC using a portable DeLaval cell counter. Quarter and buffalo-level risk factors for SCM were identified using two sets of univariable and multivariable logistic generalized linear mixed-effects models, with random buffalo and herd effects. Population averaged beta estimates or cluster-specific estimates were used to calculate odds ratios (OR) for different risk factors depending on whether these effects happen at quarter or buffalo level. The linear regression model was used to link the herd-level data for the identification of the risk factors of BMSCC. The significant (P≤0.05) variables from multivariable regression models were considered as the risk factors of SCM at quarter or buffalo level and BMSCC at the herd-level.

Results: The prevalence of SCM was 27% (95% C.I. 25-28) at quarter-level and 50% (95% C.I. 47-54) at buffalo-level. Out of 17 analyzed variables two (e.g., rearing system and quarter position) were associated with quarter-level SCM, and three (e.g., teat symmetry, previous history of CM, and the number of milkers) were associated with buffalo-level SCM. For quarter level SCM, intensive buffalo rearing system (OR, 6.6, 95% C.I. 2.2-19.6; P < 0.001) had a greater risk than bathan system; and left quarters had a greater risk than right quarters (OR, 0.8, 95% C.I. 0.6-0.9; P = 0.006). There was a greater risk of asymmetric teat position (OR, 1.8, 95% C.I. 1.3-2.4; P <0.001) than symmetric teats; previous occurrence of CM in last 12 months (OR, 3.1, 95% C.I. 1.2-8.0; P = 0.02) than no occurrence; hand milking performed by single milker (OR, 1.5, 95% C.I. 1.2-2.1; P = 0.005) than multiple milkers for buffalo-level SCM. In the herds, the BMSCC were lower in swamp-type buffalo than river-type buffalo; when caretakers were more experienced; and milking performed in full hand with occasional stripping or knuckling than full hand only (all, P < 0.05). Intensive rearing systems (P = 0.08) had a higher BMSCC (435,000 cells/mL) than any other rearing systems.

Conclusions: The water buffaloes in Bangladesh have a high prevalence of SCM where risks are associated with the rearing system, teat symmetry, teat position, and milking performed by a single milkman. The fact that several manageable risk factors were identified suggests that effective buffalo udder health control strategies can be designed.

Keywords: Water buffalo, SCC, CMT, risk factor.

BC-07

Comparative Efficacy of Herbal, Oral and Injectable Anthelmintics against Toxocara vitulorum infestation in Nili-Ravi buffalo calves

Muhammad Avais, Mohammad Qasim.

Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore, Sheikh Abdul Qadir Jillani (Outfall) road Lahore, Pakistan.

Objectives: Parasitic infections are major constraint of large ruminant production and causes colossal economic loss to dairy industry. Among these *Toxocara vitulorum is* most important infecting calves in tropical and subtropical climates. It is responsible for retarded growth, low productivity and increased susceptibility of animals to other infections. Present study was accomplished to evaluate the comparative efficacy of herbal, oral and injectable anthelmintics against *Toxocara vitulorum* infestation in Nili-Ravi buffalo calves.

Material & Methods: For this 24 buffalo calves positive for *T. vitulorum* were randomly divided into 4 groups of 6 viz. A, B, C and D. Calves in group A were treated with Albendazole at 10 mg/ kg PO while the calves in group B were given Levamisole at 8 mg/kg PO. The animals in group C were treated with Doramectin at 0.2 mg/ kg SC whereas calves in group D were given *Chenopodium album* (herb) 2g/kg PO. All the treatments were given once. Eggs per gram (EPG) of calves in each group was determined at days 0 (pre-treatment) and then at day 4, 7, 14, and 21 (post-treatment). Efficacy of particular treatment was calculated on the basis of fecal egg count reduction test (FECRT).

Results: At day 4 (post-treatment), fecal egg reduction in groups A, B C and D was 59.67%, 48.79%, 39.82% and 1.82%, respectively. At day 7, fecal egg reduction in groups A, B C and D was 98.43%, 82.50%, 73.0% and 3.5%, respectively while at day 14 fecal egg reduction was 100.00 %, 98.25%, 95.95% and 6.7% in groups A, B, C and D, respectively. While

100.00% fecal egg reduction was recorded in group A, B and C on day 21 (post-treatment).

Conclusion: It was concluded that oral wormers (albendazole, levamisole) and injectable wormers (doramectin) are equally effective in treating *T. vitulorum* infestation in buffalo calves while Chenopodium herb is not effective to treat *T. vitulorum* in buffalo calves.

Keywords: Albendazole, doramectin, herb, buffalo, calves.

BT-01

Post ovulation plasma progesterone concentrations in beef cows treated with 2000 IU of eCG or synthetic eCG-like glycoprotein

Maria Gracia Moscoso[1], Lucas Cutaia[2], Mariano Aviles[1], Leonel Alisio[1], Ignacio Videla Dorna[2], Dolores Moreno[1].

[1]Universidad Católica de Córdoba, Córdoba, Argentina; [2]Syntex SA, Buenos Aires, Argentina.

Objetive: Equine chorionic gonadatrophin (eCG) has been used for more than 50 years in reproductive management several species. The use of natural eCG is currently under review due to the natural origin of the product and animal welfare issues associated with its production. Thus, we have developed a synthetic substitute. The objective of this study is to compare the levels of progesterone (P4) on days 4 and 7 after ovulation in superovulated cows using 2000 IU of eCG or synthetic eCG-like glycoprotein based on the fact that treatment with native eCG increases circulating progesterone concentrations in the subsequent luteal phase after treatment.

Materials and methods: Fourteen cyclic Angus/Hereford cows, 3 to 5 years of age were used. The trial was conducted in two periods in a crossover design (Periods 1 and 2). All cows were treated on Day 0 of treatment with an intravaginal device (DIB, 1 gram progesterone, Syntex, Argentina) plus 2 mg im of estradiol benzoate (Gonadiol, Syntex). On Day 4, cows were divided into two groups and received 2000 IU im of eCG (Novormon, Syntex; eCG group; n = 13) or 2000 IU im of synthetic eCG-like glycoprotein (Syntex; eCG-like group; n = 14). On Days 6.5 and 7, 500µg im of Cloprostenol sodium (Cyclase, Syntex) was administered. On Day 7, DIB devices were removed and on Day 8, 100µg im of Gonadorelin acetate (Gonasyn, Syntex) was administered. Twenty-seven days after the end of Period 1, cows were treated with the same protocol in Period 2, except that cows in the eCG group were treated with eCG-like and cows in the eCG-like group were treated with eCG.

The animals were scanned by transrectal ultrasonography (Chison 500; 7.5 MHz, Doppler) on Day 8 of treatment and all follicles > 8 mm diameter (pre-ovulatory) were recorded. Daily ultrasound examinations were continued until Day 11 to determine the number of ovulations, defined as the disappearance of follicles larger than 8 mm present in the previous examination. On day 14 and 17 of the treatment (4 and 7 days post ovulation) plasma samples were obtained, and were analyzed for the concentration of P4 using (Elecsys P4 III Roche assay in the Cobas 8000 e602 Roche). The average number of follicles > 8 mm on Day 8, the number of ovulations between Days 8 and 11 and the P4 concentrations were compared by ANOVA.

Results: No differences were found in number of follicles > 8 mm on Day 8 (eCG group - 14.5 ± 2.33 vs eCG-like group - 15.0 ± 1.94; P = 0.9) or in number of ovulations (eCG group - 13.7 ± 2.54 vs eCG-like group - 13.3 ± 1.79; P = 0.83; Published in ICAR, 2020; Bologna, Italy).

There was no effect of Period (P = 0.96) or Period*Group interaction (P = 0.17) for P4 levels. No differences were found in P4 levels on Day 14 (eCG group - 45.92 ± 8.67 vs eCG-like group - 49.66 ± 8.40; P=0.71) or in P4 levels on Day 17 (eCG group - 76.45 ± 16.29 vs eCG-like group 77.73 ± 18.54; P=0,89).

Conclusions: We conclude that the synthetic eCG-like glycoprotein has the same biological activity as native eCG, for having the same number of ovulations and levels of P4 post-ovulation than the native eCG. Finally, we conclude that the eCG-like substute produced by Syntex could be a very acceptable alternative for native eCG in breeding management of cattle.

Keywords: eCG, eCG-like, glycoprotein, SPO, FTAI.

BT-02

Predictive value of cumulus cells transcription for oocyte developmental potential

Álvaro Martínez-Moro[1], Ismael Lamas-Toranzo[1], Elena O´Callaghan[2], Anna Esteve-Codina[3], Pat Lonergan[2], Pablo Bermejo-Álvarez[1].

[1]Departamento de Reproducción Animal, INIA, Madrid, Spain; [2]School of Agriculture and Food Science, University College Dublin, Dublin, Republic of Ireland; [3]CNAG-CRG, Barcelona, Spain.

Objectives: Within the ovarian follicle, mammalian oocytes are enclosed by cumulus cells, which play crucial nourishing and signalling roles during folliculogenesis. These cells are lost during or shortly after ovulation in vivo and are removed following oocyte maturation in vitro (IVM). Thus, they constitute an interesting material on which to perform molecular analyses aimed to predict oocyte developmental potential. The objective of this study was to determine by RNAseq the transcriptional differences between cumulus cells from oocytes exhibiting different developmental potential.

Material & Methods: Cumulus-oocyte complexes were obtained from slaughtered cattle and individually matured *in vitro*. Following IVM, cumulus cells were removed by hyaluronidase treatment, pelleted, snap frozen in liquid nitrogen and stored at -80 ºC until analysis. Cumulus-free oocytes were fertilized and cultured *in vitro* individually. Cumulus cells were allocated into three groups according to the developmental potential of the oocyte: 1) oocytes developing to blastocysts following IVF (Bl+Cl+), 2) oocytes cleaving following IVF but arresting development prior to the blastocyst stage (Bl-Cl+), and 3) oocytes not cleaving following IVF (Bl-Cl-). RNAseq was performed in 4 (Bl-Cl-) or 5 samples (Bl+Cl+ and Bl-Cl+) per group, using the Illumina platform with >30 M reads/sample. Each sample contained cumulus cells from 10 cumulus-oocyte complexes (COCs). Differential expression was analysed by DESeq2 software.

Results: RNAseq analysis revealed 1609, 1466 and 1420 differentially expressed genes (DEGs) for the comparisons Bl+Cl+ vs. Bl-Cl+, Bl+Cl+ vs. Bl-Cl- and Bl-Cl+ vs. Bl-Cl-, respectively, using a raw p value <0.05. These DEGs were narrowed down to 77, 80 and 32 DEGs for the comparisons Bl+Cl+ vs. Bl-Cl+, Bl+Cl+ vs. Bl-Cl- and Bl-Cl+ vs. Bl-Cl-, respectively, when an adjusted p value <0.05 was used. From

these subsets of DEGs, 49, 50 and 18 DEGs, respectively, exhibited a fold change greater than 1.5. Focussing on DEGs in cumulus cells obtained from oocytes developing to blastocysts, 10 DEGs were common to both comparisons (10/49 from BI+CI+ vs. BI-CI+, 10/50 from BI+CI+ vs. BI-CI-). These DEGs correspond to 6 upregulated genes (HBE1, ITGA1, PAPPA, AKAP12, ITGA5 and SLC1A4), and 4 genes downregulated (GSTA1, PSMB8, FMOD and SFRP4) in BI+CI+ compared to the other groups.

Conclusions: mRNA abundance of specific genes in cumulus cells can be used to differentiate oocytes capable of developing to blastocysts from those arresting before that stage following in vitro embryo production.

Keywords: IVF, embryo transfer, gene expression, oocyte, cumulus cells.

BT-03

Effect of gamete origin (Bos taurus – Bos indicus) and sexing sperm on in vitro embryo production in a commercial system in Colombia

Lilian Bonilla León[1], Ramón Guillermo Gomez Domínguez[2], Martha Liliana Torres Londoño[2], Andrés Mejía Gallego[1], Ricaurte Lopera-Vasquez[1].

[1]Universidad Cooperativa de Colombia, Ibagué (Tolima), Colombia; [2]In Vitro Colombia, Bogotá D.C, Colombia.

Introduction: *In vitro* embryo production (IEP) is considered an important assisted reproduction technique for bovine animal breeding in elite farms including tropical countries as Colombia. Under tropical environment the use of *Bos indicus* breeds and their crossbreeds with *B. Taurus* has an important impact on IEP. Also, the use of sexed sperm on bovine breeding farms are important to reduce economic impact of males or females effect.

Objectives: The aim of this study is to know the effect of gamete origin (male -M and female -F gametes of *B. Taurus - Holstein Friesian* (H) and *B indicus - Nelore* (N) *and Gyr* (G) breeds) and sperm sexing (conventional -C and sexed -S) on Cleavage (-D3), blastocyst yield (-D7), and low embryo quality (discard on D7 -LEQ) in an IEP commercial system in Colombia.

Materials and methods: A retrospective study were done on 2019 database of *in vitro Colombia*® laboratory. A total of 1145 OPU sessions were included and after randomly sampling, 40 samples for gamete origin (GMxGF, GMxHF, HMxGF and HMxHF) and sperm sexing C and S for N and G were analysed through one way ANOVA of each parameter (Sigmaplot®12.0) ($p \leq 0.05$). D3, D7 and LEQ were calculated from in vitro fertilization on day 0.

Results: When gamete origin were analysed, in terms of D3 rates of GMxGF were significantly lower when compared with HMxGF and HMxHF (68.2±2.2 vs. 75.7±1.5 and 75.5±2.8) respectively (p=0.026), however, these differences disappear on D7 rates with a range 16.1±2.9 – 23.2±1.3 % for GMxHF and HMxGF respectively (p=0.259). Also, LEQ analysis on gamete origin, present significant differences with a higher HMxHF discard rates (5.6±1.1) than GMxGF (1.4±0.4) (p=0.02). On the other hand, when sexing sperm were analysed for *B Indicus,* CN (79.6±1.7) were equal to CG (72.6±3.6), and significantly higher than SN and SG on D3 rates vs. 69.8±2.6 and 68.2±2.2 respectively (p=0.013), patterns that maintains on D7 rates with a CN (30.5±2.6) similar to CG (25.8±2.3), but significantly higher to SN and SG (22.2±1.9 - 18.3±2.0 respectively) (p=0.001). In addition, C sperm present higher LEQ rates than S in N and G (6.2±1.2 - 4.6±1.0 vs. 2.0±0.6 - 1.4±0.4) for CN, CG vs. SN and SG respectively (p=0.001).

Conclusions: In terms of origin, *Bos taurus* male gametes has a positive incidence on first stages of embryo development (D3), however, these differences disappear at blastocyst stage (D7), confirming that embryo development has relative independence of IVF, and the importance of male effect on IEP. *Bos taurus* could have a negative impact on embryo quality represented in a higher LEQ rates of HxH group. Finally, S sperm show lower IEP (D3 and D7) for N, and C sperm increase a LEQ inside both breeds (N and G).

Keywords: In vitro Embryo production, sexed sperm, embryo quality, blastocyst.

BT-04

Pregnancy and foetal sex ratio following transfer of in vitro produced (IVP) embryos to lactating dairy cows

Alan Crowe[1], Jose Maria Sánchez[2], John Browne[1], Stephen G. Moore[1], Michael McDonald[3], Rafaela Rodrigues[4], Maria Fernanda Morales[4], Leandro Orsi De Freitas[4], Federico Randi[5], Patrick Lonergan[3], Stephen T. Butler[1].

[1]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Republic of Ireland; [2]Centro Nacional Instituto de Investigación y Tecnología Agraria y Alimentaria (CSIC-INIA), Madrid, Spain; [3]School of Agriculture and Food Science, University College Dublin,, Dublin, Republic of Ireland; [4]Vytelle LLC, Hermiston, OR, United States; [5]CEVA Santé Animale, Libourne, France.

Objectives: The aims of this study were to compare pregnancy rates and foetal sex ratio in lactating dairy cows bred by timed artificial insemination (TAI) or timed embryo transfer (TET) with fresh or frozen IVP embryos.

Materials and methods: IVP blastocysts were generated from oocytes collected by ovum pick-up from genetically elite Holstein-Friesian (n=29), Jersey (n=11) and Angus (n=21) donors and oocytes from slaughtered commercial crossbred beef heifers (n=119). Following in vitro maturation, fertilisation using conventional unsorted semen and culture, single Grade 1 blastocysts were transferred either fresh or following freezing and on-farm thawing into lactating, predominantly Holstein-Friesian, dairy cows which had been synchronised with a 10-d PRID-Ovsynch protocol.

On day 0, a 2-mL i.m. injection of GnRH analogue (Ovarelin, 100 µg of gonadorelin diacetate tetrahydrate; Ceva Sante Animale, Libourne, France) was administered, and a

progesterone-releasing intravaginal device (PRID Delta; 1.55 g Progesterone, Ceva) was inserted. On day 7, a 5-mL i.m. injection of PGF2α (Enzaprost, 25 mg of dinoprost trometamol; Ceva) was administered. On day 8, a second 5-mL i.m. of PGF2α was administered and the PRID was removed. On day 9.5 (32 h after PRID removal), a second i.m. injection of GnRH was administered. On day 10, 20% of the cows (n=240) were assigned to receive AI (16 h after the second GnRH), and the remaining 80% of the cows (n=952) were assigned to receive ET on day 17. Pregnancy rate was determined between Day 32-35 after synchronised ovulation using transrectal ultrasonography. Between Day 62-65 after synchronised ovulation, foetal sex was determined by trans-rectal ultrasonography in 436 of the cows that had previously been diagnosed pregnant on Day 32-35.

Pregnancy Data were analysed using generalised linear mixed models including service treatment (TAI vs. TET) as a fixed effect. In addition to examining foetal sex in recipient cows on Day 62 to 65, Day 7 (n=63) and Day 8 (n=40) IVP blastocysts produced over 3 repetitions of IVP using abattoir-derived ovaries as the source of oocytes were snap-frozen, and their sex was determined by extraction of DNA and amplification of a 241 base pair fragment of the amelogenin gene (AML-X) or both a 241 and a 178 base pair fragment for AML-X and Y, respectively.

Results: Mean (95% CI) pregnancy/service event at Day 32 was not different between AI (47.7%; 41.0, 54.5) and ET (fresh/frozen combined, 47.9%; 44.1, 51.8) and did not differ between dairy and beef embryos (Dairy: 50.1% vs Beef: 46.1%). Pregnancy rate was lesser (P = 0.003) on Day 32 following transfer of frozen embryos compared with fresh embryos (Dairy: 40.1% vs 61.1%; Beef: 41.2% vs 51.7%, respectively). Pregnancy loss between day 32 and 62 was greater (P=0.003) for ET (15.6%) compared with AI (4.7%), with greater losses observed for frozen beef (16.6%), fresh beef (17.7%) and frozen dairy (22.8%) compared with fresh dairy (6.7%).

The sex ratio (M:F) of foetuses derived from TET (n=328 pregnancies) was 60.8:39.2. In contrast, corresponding values for foetuses derived from TAI was 42.7:57.3 (n=108) (P=0.002). There was no difference in sex ratio across the different IVP-ET treatments (i.e., beef vs. dairy, fresh vs. frozen). Overall sex ratio (M:F) among in vitro blastocysts was 61.2:38.8 (M:F), and was not affected by day (Day 7: 61.2:38.8; Day 8: 65.4:34.6; p=0.685).

Conclusions: In conclusion, pregnancy/service event on Day 32-35 was similar for TAI and TET, although 9.6% of cows initially synchronized for ET were rejected. Of cows that were pregnant on day 32, pregnancy loss was greater for TET than for TAI. The bias towards male foetuses on Day 62-65 in recipient cows was mirrored by a similar sex bias in IVP blastocysts on Day 7 and 8, indicating similar survival of male and female embryos after transfer to recipients on Day 7.

Keywords: In vitro Embryo Production, Timed AI, Timed Embryo Transfer, Sex ratio, Pregnancy.

CW-01

Effects of postpartum analgesia on the behaviour of Holstein cows experiencing both assisted and unassisted parturition

Nicola Gladden[1], Kathryn Ellis[2], Jessica Martin[3], Dorothy McKeegan[4].

[1]Farm Animal Department University of Nottingham School of Veterinary Sciences Sutton Bonington, Loughborough, United Kingdom; [2]University of Glasgow School of Veterinary Medicine, Glasgow, United Kingdom; [3]Royal (Dick) School of Veterinary Studies and The Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom; [4]Institute of Biodiversity Animal Health & Comparative Medicine, University of Glasgow, Glasgow, United Kingdom.

Objectives: Assisted parturition in cattle is common and has the potential to be both painful and stressful. Although pain and stress are adverse welfare states, the welfare effects of assisted parturition have rarely been studied. Although non-steroidal anti-inflammatory drug (NSAID) analgesia is commonly provided following veterinary-assisted parturition, conflicting results from different studies mean that the potential welfare benefits of postpartum NSAID analgesia are unclear. Few studies compare cows experiencing both assisted and unassisted parturition, as well as those administered either an analgesic drug or a placebo in a 2x2 factorial design considered 'gold standard' for assessing the welfare effects of a chosen treatment. The objective of this study was to use such a robust design to investigate the effects of postpartum NSAID analgesia in both farmer-assisted and unassisted parturition, as well as the effect of farmer-assisted parturition on dairy cattle welfare in the first 48h postpartum.

Materials and methods: The study was performed on a 700-cow commercial UK dairy farm; the cows are housed all year round and calve in a year-round calving pattern. Thirty-seven cows experiencing farmer-assisted parturition and 35 cows experiencing unassisted parturition were recruited and randomly assigned to either a NSAID treatment group (35 cows) or a placebo group (37 cows). Cows in the treatment group were administered ketoprofen and cows in the placebo group received a saline placebo; both treatments were administered within 3h of parturition. Cow behaviour was monitored for 48h postpartum using continuous video recording. Behavioural analysis of video footage was performed using instantaneous focal sampling with sample intervals of every 20min every other hour. Observations were assigned to behaviours according to an ethogram containing 13 behavioural categories, and were used to construct a 48h time budget for each cow.

Results: The most common specific behaviour exhibited by cows across the whole 48h time period was standing (49% of time budget). Collectively, active behaviours occupied a slightly greater proportion of the time budget than lying behaviours (53.8% compared to 46.3%) and cows were most active in the first 12h postpartum (p <0.001). The most common lying posture was sternal recumbency with the head elevated (26.2%). Lying postures were affected by both treatment status and assistance status. Cows experiencing assisted parturition (irrespective of treatment status) spent more time in lateral recumbency overall than cows experiencing unassisted parturition (p = 0.008) and more time in lateral recumbency with the head rested (p = 0.049). Cows treated with ketoprofen (irrespective of assistance status) spent less time in lateral recumbency than cows treated with the placebo, both overall (p = 0.031) and with the head rested (p = 0.008). Additionally, when lying in sternal recumbency, cows treated with ketoprofen spent more time with the head rested than cows treated with a saline placebo (p = 0.009). Cows in the treatment group that had experienced assisted parturition showed a tendency to spend more time engaged in feeding directed behaviours than cows in the other three interaction groups (p = 0.079).

A diagnosis of postpartum disease was associated with an increased proportion of the time budget spent lying in sternal recumbency with the head elevated and more time lying in sternal recumbency overall (p = 0.023 and p = 0.013 respectively). Time spent engaged in lateral recumbency with the head rested was 2.5 times higher in primiparous animals than multiparous animals (p = 0.002) and as a result, the proportion of the time budget engaged in lateral recumbency overall was also higher in primiparous animals (p = 0.004).

Conclusions: Cows that were administered ketoprofen analgesia immediately postpartum exhibited lying postures suggestive of reduced pain and improved comfort, regardless of assistance status. There was no interaction between time and treatment status, suggesting that the beneficial effect of ketoprofen lasted for up to 48h postpartum. A single dose of ketoprofen in the immediate postpartum period therefore has the potential to improve the welfare of all cows after parturition irrespective of assistance status. As parturition is a necessary event for cattle that usually occurs annually, this finding has the potential to contribute to the improvement of welfare of large numbers of cattle and provides a further method for farmers and veterinary surgeons to optimise the management of cows in the immediate postpartum period.

Keywords: Welfare, Behaviour, Parturition, Analgesia.

CW-02

Characterization of Welfare in Dairy Cows Based on WelfareQuality Protocol in Portuguese Farms

Rui D'Orey Branco[1], Daniela Brandão[1], Catia Matos[1], Maria Ines Antunes[1], Isabel Santos[2], Manuel Pinheiro[3], George Stilwell[4].

[1]Lacticoop URCL, Aveiro, Portugal; [2]Agros URCL, Vila do Conde, Portugal; [3]Proleite Crl, Oliveira de Azeméis., Portugal; [4]Animal Behaviour and Welfare Laboratory, Centre of Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, Lisbon University, Lisbon, Portugal.

The current consumers are demanding a new approach from the dairy industry. Today we need to assure the consumer that animal welfare is respected. In Portugal, the three largest milk buyers worked together to apply the Welfare Quality® protocol and audit all the farms that sell to our companies. The objective of this study is to characterize the current status of animal welfare in Portugal. We audited 456 dairy farms across Portugal with a number of milking cows varying from 5 to 1232. The main breeds here Holstein-Frisian and the crosses with

Swedish Red and Montbéliarde. The audits were performed as described by the Welfare Quality® referential and consisted of the evaluation of the four main welfare principles: good feeding, good housing, good health, and appropriate behavior. These principles are divided into 12 criteria that allow the auditor to access animal welfare based on the animal and small input from the infrastructure. The combination of these inputs will create a numerical result. Farms results can vary from 0 to 100, being 0 the worst situation an animal can be, 50 a neutral situation of welfare, and 100 the best situation an animal can be in. However, results are presented only on the form of a) Not acceptable - 0 to 20; b) Acceptable - 20 to 55; c) Elevated - 55 to 80; d) Excellent >80. The final score is reached through a complex calculation. Data was collected from September 2019 to March 2020 using the digital platform WFQApp ®, this is a new tool to access the results from the protocol WelfareQuality ®. WFQApp allows direct data input reducing the human error and the desk time after the audit, it also allows the auditor to have access immediately to the results and the report to the farmer. Data was analyzed using the statics program R version 3.6.3.

The 512 Farms were divided in 4 groups based on the number of milking cows: very small <20 (n=75); small 21<50 (n=206); medium 51<250 (n=213); Large >250 (n=18). From these 512 farms, we were able to audit in 447 farms to date, the results of these farms were: Not approved-10, Acceptable-177, Elevated -260 and none was Excellent. There was no difference ($p>0.05$) nor a correlation between the size of the farm and the results. The main reason for the Not approved farms is related to a low value in all the principles, especially on good feeding. Regarding the remaining audited farms, the areas most found to need improvement were increases in size and/or a number of water throughs and reductions of skin lesions mainly through better bedding.

In conclusion, this is the first large scale characterization of the welfare status in dairy farms in Portugal. The results show that 41% of the audited farms are below what is considered neutral for animal welfare. Therefore, there is still a long path to improve animal welfare in dairy farms in Portugal. This path should include continued auditing and farmer's education.

Keywords: Dairy, Welfare, WelfareQuality, Cows.

CW-03

Opportunities for enhancing welfare in the Australian dairy industry

Lucy Collins, David Beggs, Peter Mansell.

The University of Melbourne, Melbourne, Australia.

Objectives: Public perceptions influence what we farm, and how we farm it.

Like many global dairy industries, Australia's dairy industry has been thrust into the media spotlight repeatedly, with increasing public concern about many common animal husbandry and management practices. While the industry continues to work towards phasing out or finding viable alternatives to many of these practices, Australian dairy farmers also need to look for novel ways to maintain socially sustainable farming operations. The aim of this study was to investigate opportunities for Australian dairy farmers to move beyond the minimum requirements outlined in the Australian Animal Welfare Standards and Guidelines for Cattle, and identify possible socially acceptable ways to enhance the welfare of dairy cattle across all five domains in the eyes of the public.

Materials & Methods: Using an online survey, social media users were encouraged to respond to open-ended questions pertaining to best possible welfare for cows, considering nutrition, environment, health and behaviour. Participants were also asked to describe what they considered to be the industry's major cattle welfare strengths, challenges, and opportunities. Open-ended question responses were coded for thematic saturation using NVIVO software.

Results: A total of 1378 valid responses were recorded. Many respondents (31.6%) reported having some involvement with the dairy industry and most (78.8%) consumed Australian dairy products. When asked to independently rank the factors they considered when making purchasing decisions (not important; somewhat important; very important) most (77.3%) considered a high standard of animal welfare as being very important. This, and safe food production, were the two factors most commonly reported as being very important amongst respondents. Almost all respondents (95.7%) believed dairy cattle to be sentient. Many (33.7%) reported as having ever signed a petition or attended a rally pertaining to animal welfare. Many respondents (40.9%) felt that they were very informed about dairy farming practices in Australia.

Common themes surrounding best possible welfare across the 5 domains included: access to pasture and sunshine; adequate space and shelter; plentiful and quality feed; social interaction; regular activity; low-stress stock handling practices on farm; positive interactions with humans; access to quality veterinary medicines and care (including pain relief); regular health monitoring and prophylactic treatments; appropriate genetic selection and responsible breeding; hygienic facilities; humane euthanasia; and access to enrichment resources (most frequently noted as scratching posts or cow brushes).

Perceived welfare strengths of the Australian dairy industry included: public trust in farmers to care for their livestock; relatively high standards of national animal welfare on a global scale; smaller scale family-owned farms; a strong community of resilient and collaborative farmers; industry resilience to natural disasters; progressive key industry priorities surrounding animal welfare (particularly targets around provision of pain relief at disbudding; use of polled genetics and sexed semen; the phasing out of induced calvings; management of heat stress; and creating alternate pathways for bobby calves).

Current welfare challenges identified by respondents included: negative public perceptions; lack of enrichment; the potential for compromise of cow health when selecting for production trait; bobby calf management practices; common health issues such as mastitis, eye cancers, prolonged recumbency and lameness; appropriate nutrition and feed availability; cow-calf separation; overuse of antibiotics; underuse of analgesics; short voluntary wait periods; access to appropriate shelter- poor stock handling techniques; painful husbandry procedures; biosecurity; environmental stewardship and sus-

tainability; changing climactic conditions; lack of skilled farm workers; working conditions on farm; farmer physical and mental health; and farm profitability.

Respondents indicated future welfare opportunities for the industry encompassed: improving community engagement; promoting consumption of Australian made or local dairy products; adoption of innovative technologies and automations (including health monitors and voluntary milking systems); continued industry commitment to policy improvements and mandates (particularly around provision of pain relief); processor commitment to ensuring compliance; benchmarking and third party audits; and ongoing research, development, and extension.

Conclusion: Respondents in this study have provided insights into areas for future research and development to enhance public discernment around cattle welfare on dairy farms in Australia. This study reaffirms the need for ongoing industry stakeholder engagement to provide assurances that enhanced welfare across all five domains will improve public acceptability of farming practices. This will ensure the Australian dairy industry retains a social license to operate into the future.

Keywords: dairy, welfare, social license, cow, public perception.

CW-04

Dehorning of calves: Common practice and attitude of 3,267 Austrian farmers

Simone Steiner[1], Johannes Lorenz Khol[2].

[1]Rinderzucht Austria /Cattle Breeders Austria, Vienna, Austria; [2]Clinic for Ruminants, Vetmeduni Vienna, Vienna, Austria.

Introduction: The disbudding of calves is a routine procedure in cattle husbandry. It is a painful intervention, regardless of the method used. The legislation regarding dehorning varies worldwide. In Austria, the legal guidelines were changed in accordance with scientific recommendations in 2017.

In calves younger than six weeks, cauterization is allowed to be done by a qualified person, while in older calves disbudding or dehorning must be performed by a veterinarian. The use of sedatives, local anesthetics and analgesia are mandatory, additionally, drugs for pain management have to be administered by a veterinarian.

Objective: The aim of the study was to evaluate how the new legally required methods were implemented, which management changes were necessary, as well as the farmers´ attitude towards the new regulations.

Material and Methods: Approximately 17,720 cattle breeders taking part in the national milk and beef herd performance testing program (LKV Austria) were included in the survey. An internet-based questionnaire was designed comprising six areas of interest: farm characteristics, producer perception of disbudding-related pain, economic impact and degree of satisfaction with the new situation. Furthermore, the opinion of the farmers about a possible shortage in veterinary care now and in the future was evaluated.

Results: A total of 3,267 questionnaires were returned over a 2 months period, resulting in a response rate of 18.4%. The average herd size of the farms was 37.7 cows, with Fleckvieh as the main breed. In almost 80% of the farms, all cows were hornless. Based on the results of the questionnaire, hot-iron cauterization seems to be the method of choice in Austria.

On approximately 52% of the farms only the young stock kept for replacement was dehorned, on 33% disbudding was performed on all calves respectively.

On 54% of the participating farms cauterization is done by a veterinarian, only on 44.9% disbudding is done by the farmers. Almost 30% of the farmers stated to believe that disbudding caused little or no pain, another 34.1% of the farmers considered disbudding to cause moderate pain and the remaining 36.3% found it to induce a lot of pain. Approximately 61% of the farmers think that pain management medication is worth the money. Sedation is generally considered more important than local anaesthesia. Only 56% of the farmers stated that disbudding is performed in the morning, furthermore only 6.7% of the surveyed farmers dehorned their calves within the third week of life. In most cases the reason for the delayed dehorning of calves is a shortage in available veterinary care.

To be more flexible, 45% of the farmers would like to apply pain management by themselves. The demand for trainings to achieve the necessary skills also was expressed in the questionnaire by many farmers.

Conclusion: Disbudding is still a routine procedure and performed on most of Austrian farms. Not all farmers see the legal chances as a benefit for animal welfare. To reduce costs and increase flexibility many farmers would prefer to apply pain management medication by themselves, which has to be critically valuated, as abuse and misuse of drugs may occur in this case.

Keywords: Dehorning, disbudding.

CW-05

Prediction of calving assistance using a commercially available tri-axial accelerometer

Nicola Gladden[1], Kathryn Ellis[2], Dorothy McKeegan[2].

[1]Farm Animal Department University of Nottingham School of Veterinary Sciences Sutton Bonington, Loughborough, United Kingdom; [2]University of Glasgow, Glasgow, United Kingdom.

Objectives: Farmers are recommended to closely monitor cows that may need calving assistance to enable intervention to be optimised; however, this can be difficult to achieve, and it is currently not possible to accurately predict when cows will give birth, or whether they are likely to need assistance. As such, some cows that experience a difficult calving may not receive timely assistance and, conversely, some cows may be assisted unnecessarily — both are situations that may have negative welfare outcomes. The objective of this study was to investigate whether activity data generated by a commer-

cially available tri-axial accelerometers (IceQube, IceRobotics, Scotland) is able to accurately detect cows that subsequently required calving assistance.

Materials and Methods: Eighty Holstein cows on a commercial dairy farm in Scotland were recruited to the study. IceQube accelerometers were fitted to one hindlimb of each cow one to two weeks prior to their expected calving date (all cows were artificially inseminated, and pregnancy confirmed ultrasonographically by a veterinary surgeon). Cows were housed in a group calving pen from the last three weeks of gestation until calving, after which cows were moved to an adjacent postpartum pen. The date and time of calving was recorded by the farmer and confirmed using video footage recorded as part of a wider study.

Activity data generated by the IceQube accelerometers comprises: the amount of time engaged in lying and non-lying behaviours (both presented as absolute time and as a proportion of the time budget), step count (number of steps taken in a defined time period), lying bouts (number of lying bouts in a defined time period; a lying bout is recorded when the cow transitions from standing to lying and back to standing), and Motion Index (a proprietary measure indicative of activity). Accelerometer-generated data were downloaded for 48 h pre-partum and used to construct time budgets that were analysed in 12 h time periods (0 to −12 h, −12 to −24 h, −24 to −36 h, and −36 to −48 h) using Linear Mixed Models and Generalised Linear Mixed Models (Poisson distribution) as appropriate; animal identification number was entered as a random effect into all models to account for repeated measures. Data were further analysed using Classification and Regression Tree (CART) analysis to determine a data threshold for prediction of assisted calving. Thresholds were validated using 5-fold cross-validation.

Results: Multiparous cows were moderately over-represented in the study population (72.5% compared to 28.8% primiparous cows), and most cows (71.3%) did not require assistance at calving. Overall, cows engaged in lying behaviours more than non-lying behaviours in the 48 h pre-partum (54.4% vs. 45.6% of the time budget).

Cows that were subsequently assisted during parturition tended to engage in more lying bouts (p = 0.064) and have a higher step count (p = 0.054) in the 48 h pre-partum compared to unassisted cows. Classification and Regression Tree analysis supported these results, finding that step count had the greatest association with subsequent assistance status. A step count of 883 was identified by CART analysis as being the optimal threshold for detecting cows that subsequently required calving assistance. Discriminant analysis indicated this threshold had an acceptable sensitivity (69.6%) but low specificity (31.6%), with a positive predictive value of 29.1% and a negative predictive value of 72.0%, for detecting subsequent calving assistance. Additionally, a threshold of 10.5 lying bouts was identified as the optimal threshold for detecting the last 12 h of gestation (sensitivity = 78.8%, specificity = 87.1%, balanced accuracy = 82.7%).

Conclusion: These findings show that IceQube generated data have the potential to be used to predict the timing of parturition and to identify cows more likely to need calving assistance; however, cows less likely to need calving assistance were more accurately detected. Our results could underpin future computational advances and provide a robust basis for future researchers to develop algorithms to identify data thresholds that may be used to alert farmers of higher risk cows, enabling them to direct resources towards ensuring the welfare of these animals.

Keywords: Calving, remote monitoring, accelerometer, prediction, assistance.

CW-06

Effect of two *vs.* three milk replacer feedings per day on behaviour and stress in Holstein calves

Patricia Carulla[1], Adrián Ramón-Moragues[2], Mª Ángeles Martínez[1], Eduardo De Mercado[3], Andrea López-Saturnino[3], Mª Luz Pérez Solana[3], Susana Astiz[3], Francisco Sebastián[4], Fernando Estellés[1], Arantxa Villagrá[5].

[1]Instituto de Tecnología Animal. Universitat Politècnica de València, Valencia, Spain; [2]Centro de Tecnología Animal-Instituto Valenciano de Investigaciones Agrarias, Segorbe, Spain; [3]Instituto Nacional de Investigaciones Agrarias, Madrid, Spain; [4]Centro de Recría Cowvet SL, Titaguas (Valencia), Spain; [5]Centro de Tecnología Animal-Instituto Valenciano de Investigaciones Agrarias, Segorbe (Castellón), Spain.

Objectives: During the early stages of life, calves are very susceptible to stress, so it is necessary to assure a maximal level of animal welfare and health during this period (Kaske et al., 2010). Feed management during this stage has a great impact on animal welfare (Hammon et al., 2020). In nature, calves suckle their mothers more than 6 times a day, but in production systems they are normally milk fed twice a day. Thus, the objective of this study was to compare the effect of feeding milk with different frequency per day on calves' behaviour and stress level.

Materials and methods: The study was carried out in a rearing farm located in Titaguas (Valencia, Eastern Spain) from November 18, 2020 to July 6, 2021. Twenty calves between 4 and 35 days of age were included, and randomly distributed into two treatments according to feeding program: a) group 2T, in which animals received 6 litres of milk replacer distributed in two feedings of 3 litres each and b) group 3T in which animals received 6 litres of milk replacer in three feedings of 2 litres each. The calves were provided with *ad libitum* starter and water from the day of entry and housed individually during 7±2 days after arrival and in pairs until weaning at seven weeks of life.

Blood samples were taken at two moments: 1) Pre-Weaning (1S), when one of the milk intakes had been removed (49.54±5.94d) and 2) Post-Weaning (2S), one week after weaning (66.74±8.2d). IgM, C reactive protein (CRP), serum amyloid-a (SAA) and haptoglobin (HP) were measured with commercial ELISA kits (Cloud-clone CORP. Houston, USA). Behavioural observations were performed by scan sampling every 5 minutes during 2 hours at 0, 2, 6 and 24 hours after the animals were housed in pairs and once per week until weaning.

Behavioural data and blood parameters were analysed us-

ing a cross tabulation and analysis of variance ANOVA (Statgraphics Centurion XVIII®) respectively, using the number of milks feeding as a fixed effect. For blood parameters, the age of the animals was also assessed (1S vs 2S).

Results: Results showed significant differences in all the studied behaviors except walking and inactive between 2T and 3T (Table 1). The 2T animals showed a higher percentage of non-nutritive oral activities (suckling element and suckling empty bottle), as well as aggressive behaviours. Furthermore, these animals showed a higher percentage of eating and rumination compared to the 3T animals. Finally, 3T animals presented a higher percentage of positive behaviors such as exploring, grooming, playing and social interactions. Related to blood stress parameters, the number of feedings did not have any significant effect, whereas the moment of sampling did for SAA (0.0375) and HP (0.0364): animals showed higher values at pre-weaning than at post-weaning. Therefore, the effect of reducing the number of milk intakes previous to the weaning should be considered a stressor agent.

Table 1. Effect of feeding management on the distribution of different behaviors (%).

Milk Feeding	Aggression	Drinking	Walking	Suckling element	Eating	Exploring	Grooming
2T	62.50	30.43	50	82.35	64.52	36.11	29.03
3T	37.50	69.57	50	17.65	35.48	63.89	70.97
P-value	0.047	0.0000	>0.05	0.0000	0.0000	0.0000	0.0000

Milk Feeding	Inactive	Playing	Suckling	Suckling empty bottle	Rumination	Self-grooming	Social
2T	49.55	42.11	33.77	56.67	57.14	59.46	45.95
3T	50.45	57.89	66.23	43.33	42.86	40.54	54.05
P-value	>0.05	0.0000	0.0000	0.0000	0.0081	0.0000	0.0020

Conclusions: Feeding calves three times per day could have a beneficial effect on animal welfare, as it improves positive behaviours and reduces hunger-related and agonistic ones. Nevertheless, this pattern did not present enough effect to alter blood stress parameters, although this could be due to the reduced sample size. Finally, according to the results obtained, gradual weaning (1S) is shown to be a stressful event for calves which has to be accurately handled.

Funded by CDTI (Centro para el Desarrollo Tecnológico Industrial); Ministerio de Ciencia e Innovación; proyecto CDTI-IDI-20200936.

Kaske, M., Wiedemann, S. and Kunz, H.J. 2010. Metabolic programming: Background and potential impact for dairy cattle. Vlaams Diergeneeskundig Tijdschrift, 79: 445-451

Hammon, H.M., Liermann, W., Frieten, D., Koch, C. 2020. Review: Importance of colostrum supply and milk feeding intensity on gastrointestinal and systemic development in calves. Animal 14: 133–143.

Keywords: Behaviour, calves, milk replacer, stress, welfare.

CW-07

Identifying cow temperament to reduce incidence of infectious disease and improve milk yield in early lactation

Nick Britten, Gareth Pearce.

University of Cambridge, Cambridge, United Kingdom.

Objectives: Animal temperament refers to consistent individual differences in behaviour between animals. These differences can be quantified using standardised behavioural testing or a combination of behavioural and physiological measures. Temperament quantified by behavioural testing is predictive of health and production in dairy cattle (1), however standardised behavioural testing is difficult for producers to implement. This study aimed to determine whether non-invasive physiological measures of temperament could feasibly replace standardised behavioural testing to predict health and production outcomes during early lactation in dairy cattle.

Materials and Methods: This study was conducted in 58 Holstein cattle between calving and 60 days of lactation managed in an automatic milking system at Cambridge University Farm, UK. The behaviour of each cow was recorded in a series of standardised tests (human approach, crush restraint, runway and novel arena/novel object (NANO) tests) previously described (2) at 21±4 days of lactation. Animals showing any clinical signs of illness or receiving veterinary treatment at the time of testing were excluded.

Before the NANO test, each animal was fitted with a heart rate monitor (H10, Polar Electro, Finland) around the thorax and left undisturbed for 5 minutes. Intervals between successive heartbeats (R-R intervals) were then recorded for 5 minutes. Immediately following this, temperature of both eyes was measured using an infra-red camera (T335, FLIR systems, UK). Each cow then underwent the NANO test, during which R-R intervals were again recorded, and eye temperature measurement was repeated immediately after the test.

Milk yield at 21 and 60 days were collected from the milking software. Medicines records were used to identify occurrence of clinical infectious disease (mastitis, metritis, interdigital necrobacillosis) during the first 60 days of lactation.

Principal Component Analysis (PCA) in R Studio 2021.09.02 extracted latent variables from behavioural test

data with an eigenvalue ≥ 4/√3 to identify temperament traits. Heart rate (HR) and heart rate variability parameters (root mean square of successive differences, low frequency power (LF), high frequency power (HF) and LF:HF ratio) were derived from R-R intervals. Analysis of variance was used to quantify differences in temperament between animals requiring treatment for an infection and healthy animals. Linear regression was used to quantify the relationship between temperament and milk yield.

Results: PCA identified activity, neophobia, boldness and sociability as distinct behavioural temperament traits. Eleven cases of infectious disease were detected during the first 60 days of lactation: 4 mastitis, 3 metritis and 4 interdigital necrobacillosis. Cows contracting infections in early lactation showed lower boldness (p≤0.01), lower sociability (p≤0.05) lower pre-test eye temperatures (p≤0.05) and higher pre-test heart rates (p≤0.01) than non-infected animals. Milk yield at 21 and 60 days of lactation was positively correlated with boldness (p≤0.01) and negatively correlated with heart rate before and during the NANO test (p≤0.01) and with LF:HF ratio before that test. Eye temperature was positively correlated with boldness (left eye p≤0.01; right eye p≤0.05).

Conclusions: The higher heart rates and LF:HF ratios and lower eye temperatures seen in cattle showing cases of infectious disease and lower milk yields indicated that these animals exhibited more physiological signs of stress. Cattle showing lower eye temperatures also exhibited lower levels of the boldness temperament trait. Previous studies have demonstrated that cattle showing lower boldness and sociability are more susceptible to infectious disease (1) and have lower milk yields (3). The current study extends this to indicate that these individuals can be identified using the physiological measures of heart rate parameters and eye temperature.

As these measures are likely to be more feasible for on-farm use than behavioural tests, they present potential practical methods of identifying individual cattle that are at greater risk of infection in early lactation. Targeting of management resources to reduce risk factors in these more susceptible animals should reduce incidence of infection thereby reducing antimicrobial usage and improving animal welfare.

1. Britten N, Pearce G. Animal - Science Proceedings. 12(1): 104.
2. Britten N, Pearce G. Animal - Science Proceedings. 12(1): 103.
3. Hedlund L, Løvlie H. Journal of Dairy Science. 2015 98(9): 5819–28.

Keywords: Dairy, Stress, Temperament, Disease, Thermography.

CW-08

Correlation between the Welfare Quality® protocol and productive performance in Portuguese dairy farms

João Cerqueira[1], George Stilwell[2], Rui D'Orey Branco[1].

[1]Faculdade de Medicina Veterinária, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal; [2]Animal Behaviour and Welfare Lab, CIISA-Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisboa, Portugal.

Objectives: The objective of this study was to evaluate the relationship of animal welfare of ten dairy farms in Portugal, with productive performance. We hypothesized that there are positive correlations between Welfare Quality® protocol (WQ) results and milk productive performance parameters, and a negative correlation between the WQ results and mean calving interval (CI).

Methods and materials: Ten farms were used in this study and were visited between January and September 2021. Farm size ranged from 35 to 789 milking cows at the time of the visit. All animals were kept on an intensive-based free-stall production system with several diverse bedding choices and were milked twice a day, with with the exception of one farm where cows were milked three times a day. All farms were assessed for animal welfare using the WQ protocol. The productive performance indicators considered were the CI, average daily milk yield (kg/cow/day), the number of animals reaching 305 days in milk (DIM), 305 DIM cumulative production (kg), and bulk tank somatic cells count (SCC) (cells x10^3/ml). Productive performance parameters were obtained and calculated using Microsoft® Excel for Mac version 16.56, and data was obtained from Bovinfor® and Lactinfo©. The descriptive statistical analysis was calculated using RStudio® Mac version 2021.09, and all the proposed correlations were assessed using Spearman's bivariate correlation with a significance level of 5% ($p<0.05$).

Results: From the farms considered in the study, 80% obtained the WQ "Enhanced" level, while 20% were classified as "Acceptable" as the final score. No farms were classified as "Excellent" or "Not Classified". Table 1 describes the WQ assessment correlations with milk productive performance. Our main results showed that average daily milk yield was positively correlated with the WQ Final Score (p= 0.002), positively correlated with the "Good Feeding" principle (p=0.012) and tended to be positively correlated with "Good Housing" principle (p=0.055). The number of animals reaching 305 DIM was positively correlated with the "Good Health" principle (p= 0,016) and with the "Expression of social behaviors" criteria (p=0.025), and tended to be correlated with the "Good Housing" principle (p=0.053). Finally, 305 DIM cumulative production was positively correlated with the WQ Final Score (p=0.024), with the "Good Health" principle (p=0.002), and with the "Good Housing" principle (p=0.030). There were no significant correlations between the WQ assessment results, the CI, and SCC.

Table 1. Correlation between the final and each Principle score in the WQ protocol, and average milk yield, number of animals reaching 305 DIM, and 305 DIM cumulative production.

	Average daily milk yield		Number of animals reaching 305 DIM		305 DIM cumulative production	
WFQ	**ρ**	**p-value**	**ρ**	**p-value**	**ρ**	**p-value**
Absence of prolonged hunger	0.73	**0.017**	0.53	0.112	0.57	0.084
Absence of prolonged thirst	0.70	**0.025**	0.32	0.372	0.52	0.124
Good Feeding	0.75	**0.012**	0.47	0.173	057	0.087
Comfort around resting	0.62	**0.055**	0.63	**0.053**	0.68	**0.030**
Thermal Comfort	N/A		N/A		N/A	
Ease of movement	N/A		N/A		N/A	
Good Housing	0.62	**0.055**	0.63	**0.053**	0.68	**0.030**
Absence of injuries	0.34	0.328	0.44	0.200	0.66	**0.037**
Absence of disease	0.29	0.421	0.49	0.151	0.3	**0.016**
Absence of pain induced by management procedures	0.40	0.245	0.68	**0.030**	0.56	0.091
Good Health	0.53	0.117	0.73	**0.016**	0.84	**0.002**
Expression of social behaviors	0.50	0.138	0.70	**0.025**	0.53	0.111
Expression of other behaviors	N/A		N/A		N/A	
Good human-animal relationship	0,03	0,934	-0,16	0,651	-0,24	0,510
Positive emotional state	0,62	**0,055**	0,12	0,738	0,32	0,372
Appropriate Behavior	0,30	0,405	0,055	0,881	0,07	0,854
Final Score	0,85	**0,002**	0,563	0,090	0,70	**0,024**

Conclusions: With these results we can accept the hypothesis that dairy farms with better Welfare Quality® scores will have superior productive performance, more specifically higher milk yield per cow per day, more complete lactations, and higher 305 DIM cumulative production.

Keywords: Animal welfare, Welfare Quality®, Dairy cow, Productive performance, Average daily milk yield.

CW-10

Automatic assessment of feeding, ruminating and locomotion behaviours in dairy cows naturally affected by diseases during peripartum period

Fadul Mahmoud[1], Luigi D'Andrea[2], Maher Alsaaod[1], Giuliano Borriello[3], Carlo Ferravante[2], Antonio Di Loria[2], Paolo Ciaramella[2], Adrian Steiner[1], Jacopo Guccione[2].

[1]Clinic for Ruminants, Department of Clinical Veterinary Medicine, Vetsuisse-Faculty, University of Bern, Bern, Switzerland; [2]Department of Veterinary Medicine and Animal Productions, University of Napoli "Federico II", Napoli, Italy; [3]Department of Veterinary Medicine and Animal Productions, University of Napoli "Federico II", Napoli, Switzerland.

Objectives: Changes in cow behaviour are one of the most important criteria to assess animal welfare and health. Several parameters can be used to build up an early disease warning system aiming to gain higher animal health standards, above all during the transition period when a negative energy balance may favour the onset of diseases. Considering the premises, the goal of the study was to assess and compare the feeding, ruminating and locomotion behaviour between cows naturally affected by diseases and healthy cows in the first week after calving.

Materials and methods: Forty-two, free-stall-housed, pluriparous Holstein x Friesian cows were enrolled from 14 days (d) before up to 1 week (wk) after calving. Based on the output of a 3-dimensional accelerometer placed either in a halter equipped with a nose-band sensor or a pedometer [RumiWatch®, ITIN+HOCH GmbH, Fütterungstechnik, Liestal, Switzerland], different feeding, rumination and locomotion behaviors were continuously recorded during this period. *Eating time, ruminating time, ruminating boluses, eating chews, ruminating chews, other activity time* (time spent not in eating, ruminating or drinking), *lying time, standing time, walking time, lie down, stand up, lying bouts, standing bouts, walking bouts* and *number of strides* were the parameters considered. Cows' health status was continuously monitored by means of general clinical examination (every other day) and weekly complete blood analysis [d -14 and d -7 pre-calving, day of calving (d 0)

as well as + d 7 post-calving]. Animals affected by ≥1 disease were considered sick.

RumiWatch® data were converted into 24-hours summaries, and days around calving (d -1, d 0 and d +1) were excluded from the analysis. The mean values of wk -2 (from d -14 to d -8), wk -1 (from d -7 to d -2) and wk +1 (from d +2 to d +7) relative to calving were calculated. Moreover, activities registered on the day, the disease was first clinically diagnosed (dd0), one and two days before disease diagnosis were also described (dd -1 and dd -2, respectively). Lastly, differences between dd0 vs. dd-1 (ΔD1), dd0 vs. wk -1 (ΔD2), and wk +1 vs. wk -1 (Δweeks) were assessed.

Results: At the end of the clinical monitoring phase, cows were divided in group S (n=24 sick cow; all of them diagnosed in wk +1) and group H (n=18 healthy cows). No intra-group difference was observed between wk -2 and -1, for both of them. In group S, eating and ruminating parameters were significantly decreased in wk +1 compared to wk -1, while no difference was detected in group H, for the same time period. In groups S and H, *standing* and *walking time* as well as *number of strides* were significantly increased in wk +1 compared to wk -1. *Lying time* was instead significantly decreased in wk +1 compared to wk -1, in both groups.

Regarding the inter-group difference in feeding and rumination behaviours, at wk +1 and dd0, *eating* and *ruminating time*, *eating chews* and *ruminating chews*, as well as *ruminating boluses*, were significantly lower in group S compared to group H, while *other activity time* was significantly higher. For ΔD2 and Δweeks, the difference between *eating* and *ruminating time*, *eating* and *ruminating chews* was significantly lower in group S compared to group H. Regarding the locomotion behaviours, at wk +1 and dd-2, the *lying time* in group S was significantly higher compared to group H, while the *standing time* was significantly lower. In addition, the *number of strides* was significantly lower in group S compared to group H, at wk +1.

Conclusions: The present study investigates for the first time behavioural changes over time of cows associated with spontaneous diseases. The combined use of Rumiwatch® noseband sensor and pedometer allows to detect the disease state mainly at the day of clinical diagnosis. The model considering the change over time of the variables *eat chews*, *ruminate chews* and *other activity time* achieved the highest accuracy in detecting cows with a health disorder in week +1. The results of the study show that novel precision dairy farming technologies may provide essential support for early disease detection, allowing to improve animals' health and well-being as well as the overall farm efficiency.

Keywords: Behaviour, accelerometer, cow, precision farming, peripartum.

CW-11

Effects of individual compared to pair housing on behaviour and activity of dairy calves

Sophie Mahendran, Claire Wathes, Richard Booth, Nicola Blackie.

Royal Veterinary College, London, United Kingdom.

Objectives: Behaviour is an indicator of calf welfare, and is affected by both the management system of the farm as well as the health of the animals. It has been shown that calves will exert effort in order to achieve social contact with another, preferring full body contact in comparison to head contact only (Holm et al., 2002). Socialisation by pair housing of calves is now recognised to effect consumption of solid feed (De Paula Vieira et al., 2010; Jensen et al., 2015; Whalin et al., 2018) such that [pair housed calves end up significantly heavier than individually housed calves at weaning (Pempek et al., 2016). However, the behavioural effects of housing type is still limited, so the aim of this study was to establish levels of different calf behaviours (cross sucking, lying times and bouts, novel object approach) between individually and pair housed calves.

Materials and methods: This study was conducted on a single commercial dairy farm in the South-West of England, milking 1800 Holstein and Jersey dairy cows in an all year round calving pattern. Calves were moved from a group calving pen at 12 hours of age into housing within a large shed, using a commercially available partition system (Calf-Tel, USA) that allowed either individual or pair housing of calves until 8 weeks of age. Calves were enrolled between March and May 2020, and were systematically allocated at birth into individual or pair housing groups. Calves were fitted with a tri-axis pedometer (IceQube, Ice Robotics, UK) applied to the hind limb of calves within 1 week of age using a velcro strap. These measured calf activity (via motion index), lying times and lying bouts. A novel object approach study was conducted, with placement of an umbrella into each calf pen during the sixth week of age. The time was measured from placement of the umbrella until it was touched by the nose of a calf, with non-approach given after 10 minutes. In pair pens, the time was stopped when just one of the calves made contact with the umbrella. In addition, a subset of calves had CCTV placed above their pens to enable video analysis on six non-consecutive days to assess cross sucking behaviour and lying proximity of the pair calves to each other. This footage was manually analysed for occurrence of cross sucking on another calf and sucking on inanimate objects.

Results: A total of 90 calves were enrolled, with 23 on individually housed calves and 67 on pair house calves. When assessing calf activity, the motion index was significantly associated with the housing group ($F_{1,83} = 440.3$, $p < 0.01$), with pair housed calves having a higher mean value of 4503.6 ± 117.5 compared to 4388.0 ± 179.2 in individually housed calves. The Motion Index was significantly associated with the month of enrolement ($F_{1,83} = 3.5$, $p = 0.019$), and with the occurrence of disease in a calf ($F_{1,83} = 3.0$, $p = 0.088$), with diseased calves having a lower motion index of 4137.2 ± 153.5 compared to non-disease calves 4678.5 ± 120.7. The Motion Index was not associated with the breed of the calf ($F_{1,83} = 2.4$, $p = 0.13$). The novel object approach was significantly affected by housing group ($p < 0.01$), with individually housed calves approach-

ing the novel object in a mean of 84.0 ± 9.4 seconds (SEM), and pair housed calves approaching in 121.2 ± 9.2 seconds (SEM). Individually housed calves carried out more sucking on inanimate objects than pair housed calves.

Conclusions: There were significant impacts on calf behaviour associated with the type of housing a calf was kept in. Pair housed calves were more active overall, with activity generally linked to positive welfare indicators. Individually housed calves were significantly quicker at approaching the novel object, suggesting that these isolated calves are more interested in changes to their environment, possibly due to boredom or loneliness. These findings would suggest that pair housing is beneficial for the behavioural welfare of calves.

Keywords: Calf, housing, behaviour, activity.

CW-12

Seasonal pattern in the incidence rate of preweaning calf mortality in a large-scale Hungarian dairy herd

Mikolt Bakony, Viktor Jurkovich, Jenő Reiczigel.

University of Veterinary Medicine, Budapest, Hungary.

Objectives: On large scale dairy farms in Hungary, as in most dairies worldwide, preweaning calves are housed outdoors in individual hutches. In summer, it is a usual sight that calves are inactive, cuddled up in the shaded area of the hutch and are panting at a very high rate. Beyond the apparent welfare concern, heat stress can negatively affect animal health and productivity. Heat stress in hutch-reared dairy calves, however, is an overlooked area in the dairy industry. We hypothesized that the thermal stress caused by high ambient temperature in the summer months negatively affects the survival rate in preweaning calves.

Material & Methods: The farm management data of Enyingi Agricultural Ltd. (Kiscséripuszta, Hungary, 47°02'12.5"N 18°21'30.1"E) from 1991 to 2015 were used in the analysis. The farm had an average animal population of 1500-1800 Holstein Friesian cows and their offspring in the studied period. The calves were housed in individual wooden hutches with slate roofs from birth until weaning (around 60 days of age). We collected meteorology data from the National Centers for Environmental Information (Asheville, NC, USA), using the data from the Hungarian Meteorological Service station nearest to the farm (Siófok, Hungary, 46°54'35.1"N 18°02'41.2"E). Weather data included daily mean, minimum and maximum of hourly dry bulb temperature measurements. In the first analysis, we calculated the average daily mortality rates for calendar months and applied the chi-squared test to compare the annual distribution of mortality in the two age groups (0 to 14 vs 15 to 60 days). Second, we determined the average mortality rates of the first age group (0-14 days) in periods of heat stress and thermoneutral periods and compared them by Fisher's exact test. For this purpose, the study period was divided into consecutive 3-day blocks, and those in which the mean temperature was at least 22 °C on each day were considered heat stress periods (risk periods). Blocks with a mean temperature between 5-18°C on each day served as reference. For comparison, we repeated the analysis with 4-day and 5-day periods and with temperature thresholds 23, 24, 25 and 26 °C.

Results: In the studied period (from 1991 to 2015), 46,899 calves were born on the farm, of which 2,155 died before 60 days of age. The average daily mortality rate of calves younger than two months was 9.64 per ten thousand. The average mortality rate was higher in summer and winter. The monthly distribution of calf deaths differed between the 0-14 and 15-60 day age groups. The mortality risk ratio of the age group 0 to 14 days compared to the rest (15 to 60 days) was above 2 throughout the year. It was highest in July (6.92), the hottest month in Hungary, and lowest in January (2.37). In the second analysis, the mortality risk in the 0-14 day age group was twice as high in periods with an average temperature above 22 °C than in periods of thermoneutrality (mean temperature between 5-18°C). With a daily mean temperature of 25°C or more (heatwaves), the risks were three times as high as in the reference period. Varying the length of the reference and risk periods did not substantially change the calculated measures of association.

Conclusions: Our results provide evidence against the common belief that dairy calves cope well with heat. Increased calf mortality in the hottest month of the year highlights that heat stress abatement in preweaning calves is just as important as protection against cold. Heat stress reduction is advised in outdoor calf rearing when the average daily temperature reaches 22 °C, which is characteristic of summer weather in a continental region.

Acknowledgements: Viktor Jurkovich was supported by the Ministry of Innovation and Technology and the National Research, Development, and Innovation Fund (grant No.: MEC_21_R_140844).

Keywords: Dairy calves, heat stress, mortality.

CW-13

How dairy farmers perceive Animal Welfare

Joachim Kleen[1], Giuliana Hellmann[2], Matthias Kussin[2].

[1]CowConsult, Großheide-Coldinne, Germany; [2]University of Applied Sciences Osnabrück, Osnabrück, Germany.

Objectives: Animal Welfare is increasingly debated in the broader public, and the dairy sector is more frequently debated for perceived and actual problems around dairy cow husbandry.

While the public debate is generally characterized by statements of conflicting pressure groups, little is known regarding the attitude and perception of dairy farmers. This study tried to collect and categorize the opinions of dairy farmers regarding the subject of animal protection and animal welfare. It was aimed at exploring their perception their attitude regarding the public debate around the topic and challenges on their respective farms.

Material and Methods: Using the method of a semi-structured interview, an interview guide with 15 open questions was developed. A total of 12 dairy farmers from various parts of Germany were interviewed by telephone. Farmers were selected by the technique of theoretical sampling, covering most farm types present in Germany. Three of the interviewed farmers were female, the age was between 25 and 58. The herdsize varied between 60 and 5000 cows. The interviews lasted about 30 mins, were recorded and transcribed. The transcripts were then analysed for the relevant categories and structured accordingly. The results in the different categories were then collected and compared.

Results: The farmers generally define „Animal Protection" close to the wording of the German Law on Animal Protection as legal base for their work. The farmers felt responsible to protect animals from pain and suffering and meet the physiological needs of the animals. All farmers judged their farms having a high standard of animal welfare; on all farms, however, practicable examples for possible improvement were named. Practices as e.g., dehorning or calf separation were seen critically by some farmers. Contrary to the legal term "Animal Protection", the term "Animal Welfare" was not uniformly understood by the farmers. This term was regularly associated as a terminology to be used in marketing and sales of dairy products.

Animal Health was perceived as highly important by all farmers. All rejected the idea to cut costs in therapy or prevention for economical reasons. Prevention was generally preferred over therapy and investments in preventive measures were seen as logical.

All interviewed farmers rejected a negative correlation between herd size and animal welfare. Some farmers stated that larger farms had more opportunity to structure their farms, allowing better trained people to spend more time with the animals; this could have positive effects on animal health and welfare. Problems with farm staff like fluctuation could, however, have a strong negative impact on the Animal Welfare.

Farmers stated that public debate around Animal Welfare issues did not influence their professional attitude and work, nevertheless affected them emotionally. The debate was perceived as being unfair and negative consequences for then economic situation of the sector are expected. While farmers do criticize the knowledge of the consumer public, they say they remain open for discussion and wishes.

In the opinion of the farmers interviewed, weak points of the dairy sector are dehorning, shortage of qualified staff, overburdening of farmers, little appreciation of male calves and the condition of cull cows.

Farmers see a huge potential in animal breeding: In their opinion, genomics offer potential to improve Animal Health and Welfare. Conversely, breeding is getting more complex and needs careful selection. Farmers generally disapprove of a genetic selection towards higher production only.

Discussion: The study offered insight into the ideas and perceptions of dairy farmers. All farmers interviewed had a high level of education which needs to be considered when interpreting the results. While "Animal Protection" appears as undisputed and of high importance to farmers, "Animal Welfare" is less understood and being considered as a more theoretical concept. The farmers generally assess the level of Animal Protection being high on their farms. This appears to derive from professional ethics and economic considerations. Social demands are deemed less relevant. Interviewed farmers presented themselves as considerate in all aspects of Animal Welfare and open to discussion on the pros and cons of modern dairy farming.

Keywords: Animal Welfare, Interview, Ethics.

CW-14

Correlation between Welfare Quality® protocol and antibiotic usage in portuguese dairy farms

João Cerqueira[1], George Stilwell[2], Rui D'Orey Branco[1].

[1]Faculdade de Medicina Veterinária, Universidade Lusófona de Humanidades e Tecnologias, Lisbon, Portugal; [2]Animal Behaviour and Welfare Lab, CIISA-Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal.

Objectives: The objective of this study was to evaluate the relationship between animal welfare in ten dairy farms in Portugal with the use of critically or non-critically important antibiotics. We hypothesized that higher Welfare Quality® protocol (WQ) results, promote a reduction in the use of critically important and non-critically important antibiotics.

Methods and materials: Ten farms were used in this study and were visited between January and September 2021. Farm size ranged from 35 to 789 milking cows at the time of the visit. All animals were kept on an intensive-based free-stall production system with several diverse bedding choices and were milked twice a day, with with the exception of one farm where cows were milked three times a day. All farms were assessed for animal welfare using the WQ® protocol. Antibiotic use was obtained from the farms' treatment records, and only data from the year prior to the WQ audit was considered. General use of antibiotics was calculated using the Fifth OIE Annual Report on Antimicrobial Agents Intended for Use in Animals (OIE, 2021) guidelines. For each farm, animal biomass was calculated, only considering the WQ protocol sample as eligible. For each milking cow, an average weight of 450kg was considered. As for dried-up cows and heifers allocated together, a correlation of 0.8 of a milking cow was applied. Treatment records from the year prior to the farm visit were transferred into a Microsoft® Excel for Mac version 16.56 sheet, and antimicrobial use was flagged and separated into critically important antibiotic, if the active substance was from groups "A- Avoid" or "B- Restrict" of the Antimicrobial Advice Expert Group categorization; or non-critically important antibiotic, if the active substance was from groups "C- Caution" or "D- Prudence" of the Antimicrobial Advice Expert Group categorization (EMA, 2019). Finally, the Fifth OIE Annual Report on Antimicrobial Agents Intended for Use in Animals (OIE, 2021) adapted formula was applied to both critically and non-critically important antibiotics and correspondent productive biomass:

Antibiotic usage (mg/Kg)=Antimicrobial agents reported (mg)/Productive biomass (Kg).

The data was stored in a Microsoft® Excel for Mac version 16.56 sheet, and the descriptive statistical analysis and proposed correlations were calculated using RStudio® Mac version 2021.09, using Spearman's bivariate correlation with a significance level of 5% (p <0.05).

Results: From the farms considered in the study, 80% obtained the "Enhanced" level, while 20% were classified as "Acceptable" as the final score. No farms were classified as "Excellent" or "Not Classified". Critically important antibiotics' use showed a tendency for a moderate negative correlation with the "Absence of disease" criteria (p= 0.071). The remaining correlations between critically importance antibiotics use and the WQ scores, showed no significance (p≥0,336). Also, there were no significant correlations between non-critically important antibiotics use and the WQ scores.

Conclusions: Critically important antibiotics tended to be used less in farms with higher "Good Health" principle score. Our results show that welfare assessment protocols may be a way of identifying farms that overuse antibiotics.

References:

EMA. (2019). *Categorisation of antibiotics in the European Union Answer*. https://www.ema.europa.eu/en/documents/report/categorisation-antibiotics-european-union-answer-request-european-commission-updating-scientific_en.pdf

OIE. (2021). *Fifth OIE Annual Report on Antimicrobial Agents Intended for Use in Animals - OIE - World Organisation for Animal Health*. https://www.oie.int/en/document/fifth-oie-annual-report-on-antimicrobial-agents-intended-for-use-in-animals/

Welfare Quality. (2009). Welfare Quality Assessment protocol for cattle. *Welfare Quality® Assessment Protocol for Cattle*, 1–142. http://www.welfarequalitynetwork.net/network/45848/7/0/40

Keywords: Animal welfare, Welfare Quality® , Dairy cow, Critically important antibiotics, Non-critically important antibiotics

CW-15

Adapting a New Zealand animal welfare assessment protocol for extensive beef systems in Namibia

Y. Baby Kaurivi, Richard Laven, Kevin Stafford, Tim Parkinson, Rebecca Hickson.

Massey University, School of Veterinary Science, Palmerston North, New Zealand.

Objectives: Although beef production differs significantly between New Zealand and Namibia in terms of cattle management systems, topography and climate, both countries have extensive beef systems with a focus on exporting beef to lucrative markets. Thus, beef from both countries is expected to conform to the high animal welfare standards demanded by these markets. This is increasingly likely to require a formal assessment. However, simply transferring protocols developed for industrialized intensive systems to extensive systems is not appropriate. Assessment systems need to be developed for the system they are testing. We have recently created a specific welfare assessment for extensive pasture-based beef cattle in New Zealand using measures from Welfare Quality and the UC Davis Cow-Calf protocol, with additional New Zealand-specific measures. The aim of this study was to test this protocol on semi-desert rangeland-based beef cow-calf production systems in Namibia and adapt it to those conditions.

Material and Methods: The protocol was tested on 55 Namibia beef properties (17 commercial farms, 20 semi-commercial farming villages and 18 communal farming villages) in autumn (March to April). Cows were assessed in the yards during a pregnancy test (total 2459 cows) and at grazing. Stockmanship was evaluated by observing cow handling alongside yard design and facilities. A questionnaire guided interview with the fam manager was used to assess the health and management of cattle. Follow-up visits in winter evaluated changes in animal health, welfare and cattle management.

Results: We identified several issues that were not covered by the New Zealand-developed protocol, including compulsory cattle hot iron branding, ticks, flies, predation, snake bites, plant poisoning, and recurrent drought. There were also significant differences across the Namibian beef properties in the feasibility of applying the protocol. For commercial farms, the quality of the yards and the handling facilities were similar to those seen in New Zealand. In contrast, the facilities on the semi-commercial and communal properties were of a lower standard. This was exacerbated by the persistent drought that was present during the study period, which meant that cattle on semi-commercial and communal farms often had a long-distance to go grazing or water so were unavailable for assessment outside of very early morning. Additionally, many of those farms had cattle at temporary grazing sites where facilities were even poorer with yards made of thorn bushes and tree stumps and no separate pens. This resulted in difficult and protracted cattle handling which was accompanied by shouting and hitting from cattle handlers. Other welfare issues identified on semi-commercial and communal farms were the presence of horns on most cattle, and multiple brands and brand wounds.

A key part of the assessment is setting targets for welfare. For some assessment measures, there is an effect of the system on the likely welfare impact. For example, in spring, most New Zealand beef cows have watery feces. This is not a welfare issue. In contrast, similar levels of diarrhea in Namibian cows in spring would indicate a welfare problem. However, as much as possible, assessment should be consistent across systems and countries, with similar targets for similar conditions. For a cow, the welfare impact of a broken tail does not vary across systems, so why should the target vary between systems? Nevertheless, if systems are extremely different then targets may have to be modified. This is particularly apparent when comparing body condition (as an indicator of nutrition) between New Zealand and Namibian beef cattle. The drought meant that more than 40% of cows on communal farms were classified as emaciated, levels which would result in prosecution on New Zealand farms. Setting body condition score targets for Namibian farms based on New Zealand targets is not going to be appropriate, but if the targets are made too easy then they are no longer useful for meeting the welfare

demands of overseas consumers.

Conclusion: This study is the first integral step towards developing welfare assessment and monitoring scheme for beef cattle in extensive semi-desert rangeland management such as those in Namibia, with the aim of developing an assessment which will be useful for both export purposes and improving the animal welfare standards on Namibian beef farms.

Keywords: Welfare assessment, beef cattle, New Zealand, Namibia.

CW-16

Heat stress in a temperate climate leads to adapted sensor based behavioral patterns of dairy cows

Peter Hut, Josje Scheurwater, Mirjam Nielen, Jan Van Den Broek, Miel Hostens.

Utrecht University, Utrecht, Netherlands.

Most research on heat stress has focused on (sub)tropical climates. The effects of higher ambient temperatures on the daily behavior of dairy cows in a maritime and temperate climate are less well studied. With this retrospective observational study, we address that gap by associating the daily time budgets of dairy cows in the Netherlands with daily temperature and temperature humidity index (THI) variables.

During a period of four years, cows on eight commercial dairy farms in the Netherlands were equipped with a neck and leg sensors to collect data from 4345 cow lactations regarding their daily time budget. The time spent eating, ruminating, lying, standing and walking was recorded. Individual cow data was divided into three datasets: 1) lactating cows from five farms with a conventional milking system (CMS) and pasture access, 2) lactating cows from three farms with an automatic milking system (AMS) without pasture access, and 3) dry cows from all eight farms.

Hourly environment temperature and relative humidity data from the nearest weather station of the Dutch National Weather Service (KNMI) was used for THI calculation for each farm. Based on heat stress thresholds from previous studies, daily mean temperatures were grouped into seven categories: 0 (< 0°C), 1 (0-12°C, reference category), 2 (12-16°C), 3 (16-20°C), 4 (20-24°C), 5 (24-28°C) and 6 (≥ 28°C); THI values were grouped as follows: 0 (THI < 30), 1 (THI 30- 56, reference category), 2 (THI 56-60), 3 (THI 60-64), 4 (THI 64-68), 5 (THI 68-72) and 6 (THI ≥ 72). To associate daily mean temperature and THI with sensor based behavioral parameters of dry cows and of lactating cows from AMS and CMS farms, generalized linear mixed models were used. In addition, associations between sensor data and other climate variables such as daily maximum and minimum temperature and THI were analyzed.

On the warmest days, eating time in the CMS group decreased by 92 min/day, in the AMS group by 87 min/day and in the dry group by 75 min/day compared to the reference category. Lying time in the CMS group decreased by 36 min/day, in the AMS group by 56 min/day, and in the dry group by 33 min/day. Adaptation to daily temperature and THI was already noticeable from a mean temperature of 12°C or a mean THI of 56, above, when dairy cows started spending less time lying and eating and spent more time standing. Further, rumination time showed a decrease, though only in dry cows and cows on AMS farms. With higher values for daily mean THI and temperature, walking time decreased as well. These patterns were very similar for temperature and THI variables.

These results show that dairy cows in temperate climates begin to adapt their behavior at a relatively low mean environmental temperature or THI. In the temperate maritime climate of the Netherlands, they indicate that daily mean temperature suffices to study the effects of behavioral adaptation to heat stress in dairy cows.

Keywords: Dairy cow, heat stress, sensor data, time budget.

CW-17

Sensor based continuous heart rate monitoring in calves to evaluate stress induced by different sampling techniques of the respiratory tract

Florian Debruyne[1], Glenn Van Steenkiste[1], Jade Bokma[1], Stan Jourquin[1], Thomas Lowie[1], Justine Clinquart[1], Daniel Berckmans[2], Bart Pardon[1].

[1]Department of Internal Medicine, Reproduction and Population Medicine, Ghent University, Ghent, Belgium; [2]M3-BIORES- Measure, Model & Manage Bioresponses, KU Leuven, Leuven, Belgium.

Objectives: In recent years samples of the respiratory tract are more frequently taken to rationalize antimicrobial use. It is currently unknown how stressful or painful these techniques are and whether differences exist between commonly used methods like deep nasopharyngeal swabbing (DNS), non-endoscopic bronchoalveolar lavage (nBAL) or transtracheal wash (TTW). Heart rate monitoring holds potential as a precision livestock farming application for evaluation of production efficiency and animal welfare (detection of stress, pain and (positive) excitement). Sensor-based heart rate monitoring is largely unexplored in calves. Therefore, the objective of this study was to compare the effects of DNS, nBAL and TTW on parameters derived from sensor-based continuous heart rate measurements in relation to resting heart rate and changes associated with feeding. Additionally, behavior, cortisol and substance P were determined.

Materials and Methods: A crossover study was conducted under experimental conditions, including 5 male Holstein-Friesian calves. Inclusion criteria were absence of pneumonia by thoracic ultrasonography and no failure of passive transfer. During the study the calves were individually housed in a straw-bedded pen, each with an individual top view camera. Each calf was equipped with a sensor (Movesense, Vantaa, Finland), which transmitted to a self-developed gateway (ESP-32) in the top view camera, attached to a chest strap. The sensor continuously collected heart rate measurements.

Data was transferred when a change in heart rate was noticed (inherent to the sensor software). Five sessions were organized, and calves were randomly assigned to one of the 5 test groups (negative control, only fixation for 2 minutes, DNS sampling, nBAL sampling, TTW sampling).

Pain scoring was done according to the pain face checklist by an examiner at -2h, +3h, +7h, +22h and +27h relative to the respiratory tract sampling. A permanent catheter was placed in the jugular vein and blood samples were collected at -1h, 0h, +1h, +5h and +24h relative to the sampling.

Heart rate data were filtered using a Savitzky-Golayfilter and different variables were calculated for analysis being, mean heart rate, area under the curve of the handling ($AUC_{handling}$), AUC of the handling/time (corrected for the length of the handling), the triangular index and the LF/HF ratio (Low frequency power (LF)/High frequency power (HF)) . Resting heart rate was defined as the mean heart rate outside feeding or handling procedures. By visual inspection the length of the 'event', being the positive deviation from resting heart rate, was determined. Similarly, the AUC of the event (AUC_{event}) and the AUC/time of the event were calculated. Analysis was done by linear mixed models in R. Additionally, behavior, cortisol and substance P were determined.

Results: Resting heart rate, was 98.39 beats per minute (bpm) (standard deviation (SD)=25.0; quantile (q) (0.02)=63.0; q(0.98)=158.1). The average of DNS, nBAL and TTW sampling was 124.6 bpm (SD=25.5; q(0.02)=87.6; q(0.98)=171.9), 115.6 bpm (SD=16.5; q(0.02)=81.2; q(0.98)=144.7), and 108.5 bpm (SD=19.7; q(0.02)=74.4; q(0,98)=167.6), respectively. Mean heart rate during the handling procedure was 111.7 bpm (SD=18.4; q(0.02)=81.8; q(0.98)=165.9), whereas this was 142.1 bpm (SD=26,9; q(0.02)=92.2; q(0.98)=191.4) during feeding. The LF/HF ratio during feeding was significantly (P<0.05) higher than all of the sampling techniques. LF/HF was significantly larger in DNS sampling compared to control, nBAL and fixation (P<0.05). The triangular index was significantly higher in feeding events compared to control and all sampling procedures (P<0.05), but no differences between sampling procedures could be shown. There were no significant differences between the sampling procedures in any of the other heart rate variables studied. Mean heart rate was significantly lower in the last two sessions compared to the first one, which is likely an age or habituation effect.

Conclusions: The presented sensor system provided continuous and reliable heart rate measurements in calves. In this study, LF/HF ratio was the most promising parameter for non-invasive stress-evaluation in calves. Calves appeared to cope reasonably well with respiratory sampling techniques when compared to feeding events. Based on the LF/HF ratio, DNS sampling would be more stressful compared to the other methods. Confirmation of current findings on more animals and further evaluation of stress and pain induction by sampling methods by behavioral monitoring and blood analysis needs to be done.

Keywords: Heart rate variability, LF/HF ratio, Deep nasopharyngeal swabbing, Non-endoscopic bronchoalveolar lavage.

CW-19

Qualitative Analysis of working employees' motivation and satisfaction on two large dairy farms

Joachim Lübbo Kleen[1], Anna Wasmuth[2].

[1]CowConsult, Coldinne, Germany; [2]University of Applied Sciences, Osnabrück, Germany.

Objectives: As dairy herds continue to grow in size globally, employees play an increasingly important role: Their motivation and satisfaction are crucial factors in animal welfare and production. The specific demands in terms of work flow, hours working and the proximity to animals seems to make it increasingly difficult for dairy farms to recruit personnel. A competition among farms for personnel is frequently observed. As there is little information on the role of employees in relation to animal welfare and health, information on factors relevant for the motivation and satisfaction of personnel were to be collected. It was to be determined whether factors creating satisfaction (Hygiene factors) and motivation (Motivators) were discernible. The role of the animals in work satisfaction and motivation was to be analysed.

Material & Methods: The dairy farms chosen for this study had a herd size of 1300 and 750 cows, respectively. 17 employees of the farms were interviewed using a semi structured interview. The interviews were transcribed, encoded and analysed for relevant information concerning the status quo of satisfaction and motivation. Categories of relevant information were formed and summarized.

Results: Various factors affected individual motivation and satisfaction of employees. As positive factors were identified intrinsic motivation to work within a team, as well as organization of work and holiday by the farm management. Working with animals was positive for most employees. Negative factors were failure of farm equipment, animal health problems and little recognition of achievements by the respective superior. There was little intrinsic motivation recognisable towards actively improving the animal welfare and health situation.

Conclusion: The 17 semi-structured interviews can serve as an example for the attitude of personnel working on large dairy farms, the results must however not be generalized. While animal-related factors incite a sense of responsibility and working with them created satisfaction, there was little motivation in employees to actively improve the situation if deficits were recognized. Such deficits were nevertheless identified as creating dissatisfaction among employees. Unspecific factors such as working environment, colleagues and organization of working hours were regularly mentioned as source of dissatisfaction. It was not possible to clearly differentiate between hygiene factors and motivators, respectively. The status quo was identified as being either a state of "pseudo-satisfaction" or "fixed dissatisfaction". Further research on the role of employees seems to be necessary. On the farms surveyed, the staff felt responsible for the animals but did not feel able to change deficits by themselves. This may be interpreted as a cognitive dissonance which may further deteriorate motivation of the staff. The method of qualitative analysis was appropriate to understand the situation of farm personnel.

Keywords: Motivation, Satisfaction, Interview, Communication, Consulting.

CW-20

Relation between hair-cortisol concentration and welfare assessment protocols in dairy cows

Frank Van Eerdenburg, Tessa Hof, Bente Doeve, Lars Ravesloot, Rebecca Nordquist, Franz-Josef Van Der Staay.

Fac Veterinary Medicine, Utrecht, Netherlands.

Objectives: Several protocols have been developed to assess farm animal welfare. However, the validity of these protocols is still subject to debate. The present study aimed to validate and compare eight welfare assessment protocols for dairy farms. Long term stress has a negative influence on animal welfare. Because the hair cortisol level is related to stress over a long period of time, a negative correlation between cortisol and the result of the welfare protocol scores was expected.

Material & Methods: On 58 dairy farms, spread over the Netherlands, the following assessment protocols were applied: Welfare Quality©; a modified version of Welfare Quality; KoeKompas (= Cow Compass); WelzijnsWijzer (=Welfare Indicator); a new Welfare Monitor; Continue Welzijns Monitor (Continuous Welfare Monitor); Cow Comfort Scoring System and the Welfare Index. On each farm, hair was collected from 10 cows to measure cortisol concentrations. Correlation coefficients were calculated between each of the welfare assessment protocol scores and mean hair cortisol concentrations.

Results: Only Koekompas (ρ = -0.23) and a simple welfare estimation by veterinarians (ρ = -0.28) had a weak, but significant, negative correlation with hair cortisol. Additionally, the modified Welfare Quality® protocol parameters housing (ρ = -0.30), the new Welfare Monitor parameter health (ρ = -0.33), and milk yield (ρ = -0.33) showed non-significant negative correlations with cortisol.

Conclusion: Because only five out of all the parameter scores from the welfare assessment protocols showed a negative correlation with cortisol, the protocols might not be reliable or, alternatively, hair cortisol levels may not be a valid indicator for stress in dairy cattle.

Keywords: Welfare assessment, cortisol, stress.

CW-21

Herd-level risk factors for calf and cow mortality in Estonian dairy herds

Kaari Reimus[1], Karin Alvåsen[2], Ulf Emanuelson[2], Kerli Mõtus[1].

[1]Estonian University of Life Sciences, Tartu, Estonia; [2]Swedish University of Agricultural Sciences, Uppsala, Sweden.

Objectives: On-farm mortality (unassisted death and euthanasia) is an unwanted loss of an animal which negatively affects farm economy. On-farm mortality rates reflect animal welfare status and high rates indicate deteriorated animal health. The objective of this study was to determine the associations between herd characteristics, animal housing conditions and management routines and within-herd calf and cow mortality rates in Estonian dairy herds.

Material and methods: The study population included all dairy farms enrolled in the Estonian voluntary production recording system with herd size of ≥20 cow-years in 2015-2017. A questionnaire was developed to collect data about management routines and housing conditions of calves and cows. The 338 farmers fulfilling the inclusion criteria were contacted by mail or telephone between October 2017 and March 2018. In total, 214 completed questionnaires were gathered. The within-herd mortality rates of calves (21-90 days) and cows (over 24 months) in years 2017-2018 were calculated and used as outcome variables. Negative binomial and linear regression models were applied for risk factor analysis in calf and cow datasets, respectively.

Results: The final datasets for calves and cows included 212 farms (usable response rate was 62.7%). The median within-herd mortality rate for calves aged 21-90 days was 0.15 per 100 calf-months (quartiles 0.00; 0.36). The median within-herd mortality rate for cattle over 24 months of age was 4.57 per 100 cow-years (quartiles 2.44; 6.86). Factors significantly associated with the increase of mortality of calves were larger herd size ($p = 0.005$), higher proportion of stillbirths and abortions ($p < 0.001$), prophylactic administration of vitamins to all calves ($p = 0.041$) and housing pre-weaned calves in single pens only compared to housing in both single and group pens ($p = 0.020$). Also, farmers that attended trainings more frequently had higher calf mortality rates ($p = 0.008$). Having calvings only in group pens or tie-stalls compared to multiple places was associated with higher calf mortality rates ($p = 0.020$). Higher cow mortality rates were present in farms where employees handled cows ($p < 0.001$). Also, farms located in the North-East part of Estonia had higher calf ($p = 0.020$) and cow ($p < 0.001$) mortality rates. Housing cows in freestall barns ($p = 0.008$), grazing cows ($p = 0.006$) and more frequent hoof trimming ($p = 0.002$) reduced within-herd cow mortality rates.

Conclusions: On-farm mortality rates in Estonian dairy herds are similar to what has been reported in other countries. Still, a high between-herd variability in on-farm mortality rates in calves and cows was confirmed. Study results suggest that housing conditions have impact on the health and welfare of dairy cattle. Providing more natural keeping conditions to cows (freestall housing, grazing), mitigating important herd health problems (lameness, reproduction health) and motivating farm personnel could be considered as key factors in achieving improvements in on-farm mortality rates.

Keywords: Dairy cattle, euthanasia, mortality, unassisted death, housing conditions.

CW-22

Evaluation of the association between health status and other risk factors and low sale price in Québec veal calf markets

Salvatore Ferraro, Marianne Villettaz-Robichaud, Anne-Sophie Perrault, Gilles Fecteau, Sébastien Buczinski.

Département de sciences cliniques, Faculté de médecine vétérinaire, Université de Montréal, Saint Hyacinthe, Canada.

Objectives: The first objective of this study was to evaluate health status of young calves sold in the two largest Québec auction markets. The second objective was to assess which individual characteristic had an impact on the calf's sale price.

Materials and methods: A cross-sectional study was conducted during in "month" of 2019 in two auction markets in Québec, Canada. Calves' health was assessed upon arrival using umbilical characteristics (presence of umbilical cord, dampness, swelling, and pain). Presence of sunken eye and positive skin tent test, nasal and ocular discharge, lameness, dropped ears was also evaluated. Evaluation of body score and hide cleanliness was recorded.

A study was performed to assess individual characteristics associated with a low sales price per kg. Calves sold with a price under 10th percentile of the day were considered as cases and calves sold more than 50th percentile were considered as controls.

Results: A total of 1 871 calves were enrolled in the study. Of these, 81.8% were males. The majority were Holstein (76.0%), 15.7% were Angus crossbreed, 4.3% were other beef crossbreeds, and 4.0% were non-Holstein dairy breed.

Umbilical cord was present in 39.7% of calves. Wet umbilicus was present in 4.4% of calves. Umbilical swelling was observed in 17.5% calves. A total of 5.7% of calves were painful during umbilical palpation. Sunken eyes and skin stent greater than 2 sec. were present in 14.0% and 22.7% of calves, respectively. Ocular discharge was present in 41.5%, and nasal discharge observed in 0.1% of calves. Lameness was present in 0.5% of calves. Body condition was considered low in 4.6% of calves. Dirty hide was observed in 14.1% of calves. Diarrhea was observed in 2.6% of calves. Dropped ears were noted in 0.9% of calves.

The odds ratios to be a case were significantly higher for non-Holstein dairy breeds versus Holstein (OR=12.5, 95% CI 5.5-33.3), female calves (OR=4.6, 95% CI 3.2- 6.9), calves having a poor body condition (OR=9.1, 95% CI 16.6-4.50), calves presenting sunken eye (OR=2.1, 95% CI 1.4-3.2) or having a skin stent greater than 2 sec. (OR=1.9, 95% CI 1.3-2.7) and calves presenting dropped ears (OR=3.57, 95% CI 1.77-11.1).

Conclusion: The most frequent abnormal clinical signs observed in this study are related to the umbilicus and hydration status. Breed, sex and some clinical signs appears to impact the sales price in calves sold at auction market.

Future studies could aim to better understand if these anomalies are present before leaving the farm of origin or are occurring during transportation from the farm to the auction market.

Keywords: Veal calf, auction market.

CW-23

Pain detection of cows with mastitis in the barn and at milking: a piece of cake?

Ginger Laure[1], Ledoux Dorothée[1], Bouchon Matthieu[2], Rautenbach Isabelle[1], Bagnard Charlotte[1], Lurier Thibaud[3], Foucras Gilles[4], Germon Pierre[5], Durand Denys[1], De Boyer Des Roches Alice[1].

[1]Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, Saint-Genès-Champanelle, France; [2]INRAE, Herbipôle, Saint-Genès-Champanelle, France; [3]Université de Lyon, INRAE, VetAgro Sup, UMR EPIA, Marcy l'Etoile, France; [4]Université de Toulouse, ENVT, INRAE, IHAP, Toulouse, France; [5]INRAE, UMR ISP, Université François Rabelais de Tours, Nouzilly, France.

Objectives: Pain is one of the most detrimental factors affecting dairy cows' welfare, and induces production losses. Mastitis is a good model for pain research in cattle, as it is a commonly occurring inflammatory painful disease. When induced by LPS infusion in the udder, mastitis has a limited duration, and non-steroidal anti-inflammatory drugs (NSAIDs) can easily modulate it. To date, there is a lack of knowledge on the behavioral reaction of mastitic cows in cubicles and at milking. This study aimed at refining behavioral indicators of pain in dairy cows with mastitis, according to time and whether cows were observed in the cubicle barn or at milking.

Materials & Methods: Twenty-seven cows received an intra-mammary infusion of 25 µg E. coli LPS in one healthy quarter. Thirteen cows received 3 mg / kg ketoprofen IM (Ketofen® 10%) in addition to LPS (LPS+NSAID cows), and 14 cows received placebo (20mL NaCl 0.9% IM) instead of NSAID (LPS cows). Evolution of the local immune response was assessed using somatic cell counts (SCC) and cytokines/chemokines quantification. Cows' response to the challenge was monitored at regular intervals from 24 hours before to 48 hours post-infusion (hpi) through direct clinical observations (cardiac, ruminal and respiratory frequencies), through indicators of inflammation (in milk: cytokines/chemokines, haptoglobin, serum Amyloid A (SAA)) and stress (cortisol in blood and in milk), and through direct behavioral observations in the barn (i.e. postures, activity, social behavior, ear positions, etc.) and at milking (i.e. steps, lifts, kicks).

Results: In LPS cows, infusion induced significant changes of endocrinal, inflammatory and clinical parameters. Blood cortisol peaked at (mean [95% IC]) 69.18 [48.98-97.72] ng/ml at 3 hpi and remained above 65 ng/ml at 7 hpi. Milk cortisol peaked at 1.69 [1-3.01] ng/ml at 7hpi. SCC peaked at 1.8 x 10^7 cells/ml at 7 hpi and remained above 8 x 10^6 cells/ml until 48 hpi. IL-6, IL-1b and CXCL8 peaked at 7 hpi (respectively: 1.9 [1.7-3.0] x 10^3 nmg/ml; 1.6 [0.7-2.3] x 10^2 ng/ml; 2.7 [1.7-7.0] x 10^3 ng/ml). SAA in milk significantly increased at 7 hpi, 24 hpi, 31 hpi and peaked at 7.4 [4.5-12.8] x 10^4 ng/ml) 48 hpi. More LPS cows stopped feeding/ruminating and pressed their tail between their legs at 3, 5, and 24 hpi than before ($P < 0.05$ in all cases). They also tend to be more numerous to

be apathetic, dropping their head and dropping their ears at 5 hpi (P = 0.07 in all cases). At milking, significantly more LPS cows were lift their hooves at forestripping at 7 hpi than before (P=0.04.

LPS+NSAID and LPS cows showed similar pattern of responses for milk cortisol, SCC, respiratory rate, mAA, haptoglobin, IL-6, IL-1b and CXCL8 (P > 0.05). Compared to LPS cows, LPS+NSAID cows had significantly lower plasmatic cortisol levels at 3 hpi (61.66 [24.57-144.54] ng/ml). At 7 hpi, compared to LPS cows, LPS+NSAIDs cows' rectal temperature decreased by 0.66 [(-0.85)-(-0.48)] °C, their rumen motility rate increased by 1.04 [0.34-1.75] contraction/min. At 31 hpi, compared to LPS cows, LPS+NSAID cows' heart rate increased by 8.44 [1.00-15.91] beats/min, and their rumen motility rate increased by 0.89 [0.19-1.60] contraction/min. Compared to LPS cows, a larger proportion of LPS+NSAID cows were feeding or ruminating (P = 0.02), and few of them had ears down (P = 0.04) at 5 hpi; they were more numerous to lie down at 24 hpi (P = 0.02).

Conclusion: This study confirms the benefits to use behavioral indicators to early detect pain associated with mastitis in dairy cows, particularly during milking. These behavioral indicators (feeding/ruminating, tail position, reactivity at forestripping) could be useful for early detection of mastitis and for decision-making regarding the initiation of pain-relief treatment during mastitis in dairy cows. This will contribute to animal welfare improvement.

Keywords: Dairy cow, Pain, Behaviour, Welfare, Detection.

CW-24

First approach to monitoring animal welfare with Precision Farming devices during fattening

Alberto Benito Díaz[1], Esperanza Domínguez Iralde[1], Marta Elena Alonso De La Varga[2], Marina Lozano Marquéz[2], Juan Manuel Lomillos Pérez[3], Juan José García García[1].

[1]Instituto Tecnológico Agrario de Castilla y León, Guijuelo (Salamanca), Spain; [2]Universidad de León, León, Spain; [3]Facultad de Veterinaria. Universidad CEU-Cardenal Herrera, Valencia, Spain.

Objectives: Consumers´ concerns about animal welfare are continuously increasing and are consider one of the main sustainability drivers for animal production systems today and in the future. This study focuses on the possibilities of monitoring some animal welfare parameters, such as ruminating, feeding and resting time, using precision farming (PF) technology. The final objective of this work is to help farmers to adapt their facilities and handling protocols to improve animal welfare based on the objective data obtained with the PF devices.

Materials and methods: Four one year old fattening crossbred animals, from a commercial beef cattle farm in Salamanca were monitored throughout the last 30 days of the fattening period. Two electronic devices for monitoring variables of interest were used. On one hand, electronic eartags, with three dimensional accelerometer that allows determining animal's activity patterns, were used. Specifically, time spent eating, ruminating, and resting were measured every 60 minutes. On the other hand, ruminal bolus with pH and temperature sensors that records data every 10 minutes were also used.

Two samples of hair were obtained from the suprascapular region of each animal following the methodology recommended in the revised literature in order to use hair cortisol concentration (HCC) value as indicator of chronic stress. This assessment technique made it possible to have an objective stress measures. Determination based on difference in the initial and the final HCC during fattening period was carried out using competitive ELISA (kit ELISA cortisol Neogen 402710) in the physical-chemical laboratory of the Estación Tecnológica de la Carne (ITACyL). All data has been statistically analyzed with RStudio software. Interactions among variables were assessed by Pearson's correlation coefficient. Analysis of variance (ANOVA) was used to evaluate the significance of deviation between animals.

Results: The result showed a mean cortisol increase of 0.83 ± 1.93 ng/g and a pH and temperature average of 6,68 ± 0,37 and 39.75 ± 0.098 ºC, respectively. Mean rest time was 22.08 ± 0.41 min/hour. The mean rumination time was 21.98 min/hour with a standard error of 2.92 min/hour. Mean intake time per hour was 5.92 ± 1.87 min/hour.

This study reported a significant association between some animal welfare parameters. Elevated cortisol levels were associated with high temperatures (0.96). Temperature variations could be due to increased fermentation activity. Long rest times were related to a decrease in hair cortisol concentration (-0.89). Therefore, it is essential that the dimensions and characteristics of farm facilities allow all animals to rest. Lower pH values were associated with increases in cortisol levels (-0.61). A balanced diet maintains a stable pH, this improves animal welfare. Hence, it's important to use feed made with suitable formulas that prevent digestive pathologies. Positive effects of rest times on pH (0.88) and negative correlation with temperature (-0.88) were reported. Intake times were inversely related to ruminal pH (-0.99).

A pH below 6 maintained for 1430 minutes/day corresponded with the highest cortisol level (6.8 ng/g). Also, the same animal presented the lowest pH (4.95). In contrast, the animal with the shortest time (370 min/day) with a pH below 6 showed one of the lowest levels of cortisol (2.32 ng/g).

Conclusions: Based on the presented results it can be stated that several monitorable parameters can be used to improve animal management during the fattening period, thus animal welfare is enhanced. Finally, studies on the effect of cortisol concentration, temperature, ruminal pH, rest time, intake time and rumination time on animal welfare are needed to evaluate current recommendations.

This work was financed by EAFRD funds through the European Association for Innovation in Agricultural Productivity and Sustainability (AEI-AGRI), within the framework of the National Rural Development Program 2014-2020.

Keywords: Welfare, precision farming, hair cortisol, ruminal pH.

CW-25

Fly repellency effect of deltamethrin improves stress and fatigue indicators and increases feed consumption of pre-weaned dairy calves, exposed to heat stress conditions

Konstantinos Arsenopoulos[1], Eleftherios Triantafillou[2], Athanasios Gelasakis[3], Elias Papadopoulos[1].

[1]Laboratory of Parasitology and Parasitic Diseases, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece; [2]"Vet Analysis" Private Veterinary Microbiological Laboratory, Larissa, Greece; [3]Laboratory of Anatomy and Physiology of Farm Animals, School of Animal Biosciences, Agricultural University of Athens, Athens, Greece.

Objectives: The objective of the study was to assess the fly-repellency effect of deltamethrin on pre-weaned dairy calves and to quantify its association with serum cortisol (SC) and creatine kinase (CK) concentrations as well as with feed consumption in pre-weaned dairy calves, under heat stress conditions.

Materials and Methods: Two intensively reared dairy cattle herds of Holstein breed located at Thessaloniki (Central Macedonia, Greece) were involved in the study between July and August 2020. Fifty calves per farm were assigned in two similar groups (n=25 per group) according to their age and gender; deltamethrin treated group (D group) and placebo treated group (C group). In all cases, 10 mL of deltamethrin (Deltanil® 10 mg/mL, Virbac Hellas, Greece) was applied once on pre-weaned dairy calves at the age of 15 days old. The enumeration of the fly burden was carried out by direct observation of the animals every 10 days at the ages of 25, 35, 45 and 55 days to assess the repellency effect of deltamethrin until weaning. Moreover, 10 fly traps (5 per group) with sticky surface were set in each farm, in predefined locations, of equal distances within the pens at the level of the calves. Blood samples, from each calf, were collected at the forementioned time points and were transported to the laboratory to be further assayed. The estimation of SC concentration was performed with electrochemiluminescence immunoassay method and CK concentration was estimated using spectrophotometry. Daily consumption of concentrates and roughages were estimated per calf and calculated by subtracting daily refusals from the offered amount. Temperature-humidity index (THI) was used to describe severity of heat stress the studied calves experienced. The differences of the meteorological data were estimated with the chi-square test. Inverse Gaussian regression models were used to estimate the random effect of the s^{th} calf and the fixed effects of deltamethrin treatment, the sampling occasion, and the farm on the SC and CK levels, flies' number, and daily consumption of concentrates and roughages. All statistical analyses were performed using SPSS (v23).

Results: THI ranged from 94 to 96 throughout the study, without statistically significant variations among sampling occasions, indicating that all pre-weaned calves experienced similar heat stress conditions. In group D, the number of flies landing on calves was reduced by 60.6 flies ($p<0.001$) in comparison to group C. In group D, SC and CK concentrations were reduced by 1.86 μg/dL ($p<0.001$) and 1101 nkat/L ($p<0.001$) when compared to group C. Regarding the feedstuff consumption, in group D calves the concentrates and roughages' consumption was increased by 137 g/day ($p<0.001$) and 25 g/day ($p<0.001$), respectively compared to group C.

Conclusions: Fly infestation challenges health and welfare of pre-weaned dairy calves, creating unfavorable living conditions. Heat stress further deteriorates these living conditions, leading to stressful conditions resulting in increased SC and CK and decreased feed consumption, therefore consisting possible stress and fatigue indicators. Deltamethrin treatment decreased the number of flies landed on pre-weaned dairy calves, improved SC and CK level and was associated with increased feed consumption under heat stress conditions, which characterize Greek summer months.

Keywords: Feed consumption, houseflies, pre-ruminants, fly-repellent; temperature-humidity index.

CW-26

Structural equation modeling to assess farm level welfare estimations

Tuomas Herva.

Atria Ltd., Oulu, Finland.

Objectives: Structural equation models (SEM) are widely used to estimate the magnitude of latent variables, which cannot be measured directly. It is widely used in psychology, social sciences and quality of life assessments in humans. There are many reports using SEM to estimate animal welfare related attitudes in people, but I was not able to find any papers applying SEM to evaluate animal welfare as itself.

I aimed to study animal welfare of Finnish bulls based on data from the Finnish national herd health scheme NASEVA and the suitability of SEM for development of NASEVA as a welfare measure.

Materials and methods: NASEVA is an industry-financed herd health tool for Finnish cattle farmers and veterinarians. It includes modified, partially Welfare Quality -based animal health and welfare estimations on annual farm visits performed by local veterinarians. There are altogether 2 indicators scoring housing, outdoor visits, nutrition, health, behavior and biosecurity from "good" (1) to "action needed" (3). All indicators scoring housing were used to calculate a housing index, which was assigned to be 1 if all indicators were 1 and 0 if all indicators were 3.

Atria is the biggest slaughterhouse in Finland. There are detailed animal level records available for every slaughtered animal. I selected all 253 farms slaughtering over 50 dairy breed bulls with available NASEVA data.

SEM was used to find the best predictors and indicators for welfare in finishing beef farms in Finland. Housing index, nutrition and health indicators in NASEVA and other farm descriptors were used to find significant causes for latent welfare variables, which in turn were determined by observed welfare indicators in slaughter records and on welfare visits. Predicted values were compared with observed values to estimate the model fit.

Scenarios describing relationships between significant predictive variables, found latent variables and significant welfare indicators were built to be able to explore the found associations in practice.

Results: It was possible to determine two separate significantly co-varying latent indicators describing animal welfare: 1) ”Risk for impaired welfare” was found to be indicated by the number of culled animals, the number of bruises in meat inspection, cleanliness of animals and the number of fatty carcasses as well as animal behavior, lameness and lesion scores on welfare visits; 2)“Growth ability” was found to be indicated by the number of fatty carcasses, estimated daily gain and carcass grade score at the slaughter. However, Indicator variables did not reveal a latent variable describing animal welfare determined as feelings.

Housing index and farm size were found to be significantly positively associated with ”Growth ability” and the number of bruises in meat inspection. Housing index was negatively associated with ”Risk for impaired welfare”, but positively associated with the number of culled animals and the number of dirty animals at the slaughter. Farm size was positively associated with ”Risk for impaired welfare” and ”Growth ability”.

”Growth ability” was doubled, and ”Risk for impaired welfare” halved when housing index was changing from the worst to the best. Housing index had a remarkable association with used welfare indicators as well: for example, the proportion of good classifications for behavior increased from 60% up to 100% when housing index was changing from the worst to the best.

Conclusions: SEM was found to be a suitable and practical tool to evaluate animal welfare and animal welfare estimation protocols on commercial farms. It gives a well structuralized picture of on-farm welfare.

NASEVA was found to be a valid tool for animal welfare estimation. It allowed a formation of two separate latent variables describing existing animal welfare on Finnish beef farms. ”Risk for impaired welfare” is associated with various health and welfare deteriorations, whereas ”Growth ability” is predicting high daily gain, carcass fat and grade scores. Adding qualitative behavioral assessment protocol in NASEVA could possibly enable the measurement of animal welfare determined by feelings.

Keywords: Welfare, bull, housing, growth, bruises.

CW-27

Effects of xylazine administration prior to laparoscopic abomasopexy as described by Janowitz on cortisol, substance P, and behavior in cattle

Tschoner-Utza Theresa, Zablotski Yury, Knubben-Schweizer Gabriela, Feist Melanie.

Clinic for Ruminants with Ambulatory and Herd Health Services at the Centre for Clinical Veterinary Medicine, Ludwig-Maximilians-University, Munich, Germany, Oberschleissheim, Germany.

Objectives: Left displacement of the abomasum (LDA) is a disease diagnosed in cattle all over the world, causing huge economic losses. Fixation of the abomasum by laparoscopic abomasopexy (LA) has first been descrribed in 1998 by Janowitz. Usually, cattle are not sedated due to the risk of them going down. Studies showed that concentrations of cortisol, and indicator for stress, fear, and pain in cattle, are reduced in animals treated with xylazine prior to being put into lateral recumbency with a tilt table, compared with untreated animals. The objective of this study was to evaluate cortisol and substance P concentrations in cattle undergoing LA after administration of xylazine, compared with a control group, and to describe behavioral observations and feeding and rumination time.

Material and Methods: LA was performed in 28 cows (aged 6.0 ± 2.0 years) with LDA, accodring to a standardized surgical protocol. Cows were randomly assigned to either CON (control group, n = 14) or XYL (xylazine treated group, n = 14). All cows were treated with 10 liters of 0.9% saline and 1 or 2 liters of 40% glucose on the day before surgery, benzyl penicillin procaine (20.000 IU/kg bodyweight intramuscularly) from the day before surgery for 4.89 ± 1.26 days, and ketoprofen (3 mg/kg bodyweight intravenously) on the day of the surgery and the following day. Cows of XYL received xylazine (2 mg/kg bodyweight intravenously) at the start of the surgery. Animals of CON received a placebo (0.9% sodium chloride solution intravenously). Blood samples for determination of plasma cortisol (PCC) and plasma substance P concentrations (PSPC) and blood gas samples were taken 3 hours before surgery (baseline, +00:00 hours), at 11:00 am (start of the surgery, +03:00) and at +03:15, +03:30, +03:45 (dorsal recumbency), +04:00 (cow standing again, end of surgery), +04:40, +05:00, +06:00, and +27:00. Behavior of the animals was assessed on the day of the surgery and the following day at 08:00 am, 01:00 pm, and 05:00 pm, and during the surgery. Feeding and rumination time was recorded for 24 hours following surgery. Parameters were studied using a multiple linear mixed effects model.

Results: LA could be performed without any animal going down due to the sedation. Mean PCC were lower in XYL than in CON at all times. In CON, PCC increased after the start of the surgery, with highest concentrations at +04:00. In XYL, PCC decreased after the administration of xylazine, until +03:45, when animals were put into dorsal recumbency. In XYL, PCC were significantly lower compared with CON at +03:30 ($p = 0.03$). Compared with +03:00, PCC were significantly higher at +03:45 ($p \leq 0.01$), +04:00 ($p \leq 0.01$), and +04:30 ($p \leq 0.01$) in CON, and significantly lower at +03:15 ($p = 0.02$) and +03:30 ($p \leq 0.01$), and significantly higher at +04:00 ($p \leq 0.01$) and +04:30 $p \leq 0.01$ in XYL. PSPC did not differ significantly between or within groups. Glucose levels were significantly lower in CON compared with XYL at +03:45 ($p = 0.03$) and +04:30 ($p = 0.03$). Mean pCO_2 was significantly higher in XYL compared with CON at +03:30 ($p = 0.05$). In XYL, mean pCO_2 was significantly higher at +03:15 ($p \leq 0.01$), +03:30 ($p \leq 0.01$), +03:45 ($p \leq 0.01$), and +04:30 ($p = 0.01$) compared with +03:00. Compared with +03:00, pO2 was significantly lower at +03:45 ($p < 0.01$) and +04:00 ($p < 0.01$) in CON, and at +03:45 ($p< 0.01$) and +04:30 ($p = 0.04$) in XYL. Behavior did not differ significantly between groups during and after the surgery. In XYL, animals had significantly

higher number of chews per bolus (p = 0.02) over a period of 24 hours after surgery.

Conclusions: The results of this study show that PCC increase during LA, and that administration of xylazine results in significantly lower PCC during the course of the surgery, representing less stress for the animals. Thus, in the opinion of the authors, xylazine should be given in a low dose prior to LA to decrease the stress level. The application of xylazine before LA improves the welfare of cattle during and after a surgery even if it is thought to be a minor invasive surgery like the LA.

Keywords: Dairy cow, left abomasal displacement, pain, surgery, stress.

CW-28

First approach to develop an animal welfare assessment model for extensive suckler beef cattle sector

Marta Elena Alonso De La Varga[1], Marina Lozano Marqués[1], Jose Ramiro González Montaña[1], Alberto Benito Díaz[2], Juan José García García[2], Ángel Javier Alonso Díez[1], Juan Manuel Lomillos[3].

[1]Facultad de Veterinaria. Universidad de León, León, Spain; [2]Estación Tecnológica de la Carne. ITACYL, Guijuelo-Salamanca, Spain; [3]Facultad de Veterinaria. CEU Cardenal Herrera, Alfara del Patriarca – Valencia, Spain.

Objectives: The increasing change in consumer preferences about the productive orientation both in animal and vegetable systems made it necessary a 180 degree change in the trends that prevailed until the beginning of the 21st century. There is an evolution from a system focused on productivity to a model that considers the one health concept with the environmental and animal welfare (AW) as important aspects of the sustainability of animal production.

In this scenario, it is necessary to protect and give additional value to productive models that meet consumers´ demands in terms of AW with additional environmental benefits. Our principal objective was to contribute to the sustainability of the extensive suckler beef cattle sector by developing a model to characterize AW of this type of farms in northwestern Spain.

Material and Methods: A model of AW characterization and assessment was developed based on the five freedoms used at present in the definition of AW of the Word Organization for Animal Health (OIE). Each one of them (freedom from hunger and thirst or adequate nutrition, freedom from discomfort or physical and thermal comfort, freedom from pain, injury or disease or health and absence of pain, freedom to express normal behavior or appropriate and natural behavior and freedom from fear and distress or absence of fear and anguish) have been evaluated using different parameters, based on the international bibliography and agreed with farmers. Some of those parameters were evaluated individually such as body condition score, ruminal fill score, locomotion scoring, hygiene scores, but others were considered at group level, the temperature and humidity index (THI) among them. All parameters were considered on a scale of 1 to 5, with 1 being the value indicative of a situation of very poor AW and 5 value of the best possible situation. Each freedom accounts to a maximum of 20 points.

On eighteen farms, 6 from Asturias and León, 6 from Extremadura and from 6 from Salamanca, with a total number of suckler cows ranging from 40 to 120 per farm, a representative number of adult cows in each farm were evaluated based on the MIL-STD-105D.

Results: Mean values of freedom 1 ranges from 17.6 ± 1.16 on Asturias and León farms to 18.4 ± 0.83 in Extremadura without significant differences in scores on the freedom 1 or adequate nutritional assessment. There were significant differences in freedoms 2, 3 and 4 ($F_{(2,15)}$= 22.63612 p= 0.000029, $F_{(2,15)}$= 49.27239 p= 0.000000, $F_{(2,15)}$= 25.86748 p= 0.000014, respectively). Freedom from discomfort (2) significant highest values were obtained in Salamanca (19.9 ± 0.31) and Extremadura (19.7 ± 0.19) possible due to the influence of mild climatological conditions compared with the Cornisa Cantábrica. Similar significant differences were registered in freedom 3 (health state and absence of pain), with lower values in Asturias and León farms (18.8 ± 0.25). In some of these farms adult cows spend 2 to 3 months in tied stalls during winter period and this could have a negative influence of parameters such as lameness score or mastitis incidence. The shorter duration of suckling period in Extremadura farms (3 months) could be responsible for the significant mean lower value of freedom 4 (16.3 ± 0.47) compared with León and Asturias (18.3 ± 0.87) and Salamanca (18.8 ± 0.54) farms.

Taking into account that mean total points were 91.1 ± 2.49 the AW of the studied suckling cows could be consider excellent. The extensive conditions of this production systems could have a negative influence on the fear from human expressed as higher approach distance but the moths expended on pasture or the possibility to feed their own calf could positively influence the suckler cows' welfare.

Conclusion: The model used in this first approach could be a useful tool to assess AW in cows keep under extensive production systems providing valuable information to consumers and this could help farmers to achieve a better market position for their product contributing thus to its sustainability.

This work was financed by EAFRD funds through the European Association for Innovation in Agricultural Productivity and Sustainability (AEI-AGRI), within the framework of the National Rural Development Program 2014-2020.

Keywords: Welfare, assessment, suckler, sustainability.

CW-29

Correlation between Welfare Quality® protocol and workplace satisfaction in Portuguese dairy farms

João Cerqueira[1], George Stilwell[2], Rui D'Orey Branco[1].

[1]Faculdade de Medicina Veterinária, Universidade Lusófona de Humanidades e Tecnologias, Lisbon, Portugal; [2]2Animal Behaviour and Welfare Lab, CIISA-Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal.

Objectives: The objective of this study was to evaluate the relationship of animal welfare of ten dairy farms in Portugal with workplace satisfaction. We hypothesized that there are positive correlations among the Welfare Quality® protocol (WQ) results, and workplace satisfaction.

Methods and materials: Ten farms were used in this study and were visited between January and September 2021. Farm size ranged from 35 to 789 milking cows at the time of the visit. All animals were kept on an intensive-based free-stall production system with several diverse bedding choices and were milked twice a day, with with the exception of one farm where cows were milked three times a day. All farms were assessed for animal welfare using the WQ® protocol. Workplace satisfaction was accessed using a questionnaire. For each farm, a questionnaire was delivered to the farm manager and to all of the working staff. The questionnaire is an adaptation of the one used by Phillip Durst et al. (2018) in *"Evaluation by employees of employee management on large US dairy farms"*. All data was organized in Microsoft® Excel for Mac version 16.56. The questionnaire was divided into 4 parts. Part 1 of the questionnaire consisted in a demographic and work conditions characterization with multiple selection, and short answer questions. Parts 2,3, and 4 were the parts considered in the Employee Satisfaction Index (ESI) calculation and referred to work environment, satisfaction, and relationship with manager. Part 2 consisted of a binary response ("Yes"- 5; "No"-1). Parts 3 and 4 answers were obtained using a 5-point Likert scale. In order to assess workplace satisfaction, ESI was calculated, using the guidelines proposed by Singh et al., (2014). All negative answers were aligned to provide a correct answer scale. Both managers and staff questionnaires were considered in the calculation of ESI. Afterwards, the applied formula was:

Employee Satisfaction Index (%) = (Total score received/ Maximum total score possible)×100

Results: From the farms considered in the study, 80% obtained the "Enhanced" level, while farms H and I were classified as "Acceptable" as the Final Score. No farms were classified as "Excellent" or "Not Classified". All ESI results are described in table 3, alongside the percentage of respondents for each farms. Among the correlations, the strong positive correlation with the "Comfort around resting" indicator (p= 0.069) and the "Good Housing" principle (p= 0.069), tended to be significant. Also, there was a strong negative significative correlation between ESI and the "Good human-animal relationship" criteria (p= 0.042). The remaining correlations between the WFQ and ESI showed no significance (p ≥0.150).

Conclusions: Our results reinforce the need to discuss further the impact of workplace satisfaction on animal welfare, specifically regarding the "Good human- animal relationship" criteria.

References:

Durst, P. T., Moore, S. J., Ritter, C., & Barkema, H. W. (2018). Evaluation by employees of employee management on large US dairy farms. *Journal of Dairy Science*, *101*(8), 7450–7462. https://doi.org/10.3168/jds.2018-14592

Singh, A., Pathak, S., Choudhury, J., Kumar, R., Banerjee, S., & Nehar, Y. (2014). *Review of HRM Volume 3 April 2014 Editorial Advisory Board*. *3*.

Welfare Quality. (2009). Welfare Quality Assessment protocol for cattle. *Welfare Quality® Assessment Protocol for Cattle*, 1–142. http://www.welfarequalitynetwork.net/network/45848/7/0/40.

Keywords: Animal welfare, Welfare Quality®, Dairy cow, Job satisfaction, Employee satisfaction index.

DI-01

Application of texture analysis of b-mode ultrasound images for the quantification and prediction of intramuscular fat in living beef cattle: a methodological study

Giorgia Fabbri[1], Matteo Gianesella[1], Luigi Gallo[2], Massimo Morgante[1], Michele Muraro[3], Matteo Boso[4], Enrico Fiore[1].

[1]Department of Animal Medicine, Productions and Health (MAPS), University of Padua, Padova, Italy; [2]Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Padova, Italy; [3]Veterinary Service of Consorzio Agrario del NORD-EST, Verona, Italy; [4]Veterinary Service of Società Agricola Vio, Eraclea, Italy.

Objectives: Intramuscular fat (IMF) plays a key role in determination of beef meat quality, because it contributes significantly to the aroma and tenderness of the meat. However, methods to determine fat % in muscle mass rely on visual inspection or on fat extraction from meat samples, and therefore require the animals' to be slaughtered. IMF determination in vivo could permit early meat quality estimation, and thus lead to changes in the animals' management to influence meat composition and better meet market requests. The aim of this methodological study was the elaboration of a formula capable of IMF% prediction from real-time ultrasound (RTU) images live beef cattle.

Material and methods: 26 Charolaise heifers were enrolled in the study and their longissimus dorsi (LD) muscle was investigated. Ultrasound images were scanned on the animals' right side between the 12th and 13th rib with a portable ultrasound scanner (MyLabOne™, Esaote S.p.a., Genoa, Italy) equipped with a multi-frequency convex probe (SC3421, Esaote S.p.a., Genoa, Italy; 2.5 – 6.6 MHz). All scans had 4.3 MHz frequency, 15 cm depth, and 100% gain. Texture analysis of the collected scans was performed by means of a free purpose-specific software (MaZda v4.6; Technical University of Lodz, Institute of Electronics, Poland).

One week after the *in vivo* examination, the animals were slaughtered and the whole cut of the 12th rib was collected. The cut was dissected into muscles, fat and bones. The sample of LD was analyzed with centesimal extraction: IMF% was determined by extraction with petrol ether (Randall) method.

Animals were divided in 3 groups depending on their mean lipid content percentage in 100g meat, and thresholds where chosen using IMF mean ± ½SD (Group 1 included animals with IMF below 4.24 g; Group 2 included between 4.25 g and 5.75 g; and, Group 3 included animals with IMF higher than 5.76 g).

Texture parameters were screened with a stepwise linear discriminant analysis using IMF measured by chemical extraction (IMFqa) as the dependent variable, and the results of the texture analysis as explanatory variables, to identify the best combinations of high-quality variables. The aim was a parsimonious model with as few parameters as possible, to enhance stability during validation.

The differentiation efficiency of IMFqa was tested by means of the receiver operating characteristic (ROC) curves. Bland-Altman analysis was performed to validate the method and to assess the agreement between IMFqa and IMFpred.

Results: Each scan generated approximately 300 texture parameters. Among these, 6 variables were identified as predictive by the stepwise analysis and were molded into a multiple regression equation.

IMF in the samples was then predicted by means of the formula (Predicted IMF, or IMFpred), and compared to the quantified IMF (IMFqa).

Among all samples, the mean IMFqa extracted from the meat was 5.08 ±1.47 g, while the mean IMFpred was 5.07±1.35 g.

A high linear correlation between IMFqa and IMFpred was found (r^2=0.85) and results from the ROC analysis showed an Area Under the Curve (AUC) of 92%, with a sensitivity of 80% and a specificity of 93.7%, while results from the Bland-Altman plot were ± 1.96 (±1.11SD).

Conclusions: In the present study, IMF% from beef cattle LD muscle was estimated successfully and with high accuracy, using RTU, one week prior slaughter.

Application of this technology on wide-scale breeding could lead to important economic impacts. A good accuracy in IMF% prediction could permit screening of the animals that are going to be slaughtered, and see whether their fattening is optimal for the market requirements, maximizing the profits.

This technology could also permit monitoring of fattening cycles, leading to rapid estimation *in vivo* of adequacy of the diets fed to the animals. Lastly, it could be used for genetic selection, bypassing lengthy genetic progeny testing and therefore saving large amounts of time.

Further studies to validate the method both on a wider sample and on different sex and breeds are encouraged, but such technology could be a powerful heard selection tool as well as assist farmers in fattening practices.

Ethical Standards: All animals were slaughtered according to EU regulations (Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing).

Keywords: Beef Cattle, Fat Prediction, Intramuscular Fat, Texture Analysis, Ultrasonography.

DI-02

Practical use of thoracic ultrasonography at feedlot arrival to identify high-risk BRD batches in suckler calves

Carolina Tejero[1], Laura Elvira[1], Javier Lillo[1], Lorenzo Fraile[2].

[1]MSD Animal Health, Salamanca, Spain; [2]University of Lleida, Lleida, Spain.

Objectives: Bovine Respiratory Disease (BRD) is the most important disease in veal industry. The assessment of lung health of suckler calves' batches at their arrival to the rearing facilities is key for decision making and implementation of prevention and metaphylactic protocols for BRD control. However, classification and identification of high-risk BRD batches (30% prevalence) continues to be a major challenge. BRD diagnosis based on clinical signs is inaccurate (61.8%

sensitivity and 62.8% specificity; White, 2009).

The use of thoracic ultrasound (US) scanning to evaluate lung health has demonstrated to be a high sensitivity and specificity tool (79,4% and 93,9%, respectively; Buczinski, 2015). A previous study in 811 veal calves using this technique at arrival (Tejero et al., 2019) showed an average of 21% moderate and 10% severe lung lesions. However, large variability was found among batches (P <0.05).

In high-risk BRD batches, metaphylactic treatment has the potential to reduce the number of animals infected with a susceptible bacterial pathogen, clinical or subclinical, reducing the disease challenge in the environment for non-infected animals and limiting disease spread (Nickel, 2010). Thoracic US scanning has the potential to identify high-risk BRD batches where metaphylactic treatment is justified. However, under field conditions, thoracic US scanning is time consuming. The main objective of this study was to evaluate if the selection of a representative sample of animals for thoracic US scanning provides a reasonable accuracy to identify high-risk BRD batches.

Materials and methods: The number of calves to be sampled per batch was calculated using free epidemiological software (https://epitools.ausvet.com.au). An estimated prevalence of 30% (high-risk BRD) using a confidence level of 95% and an accepted error of 10% for calves with lung lesions equal or higher than 3, using Adams and Buzsinski (2015) scoring system, was used.

To check the practical use of the sampling table created, a simulation of this sampling procedure was done in 12 batches where thoracic US was assessed in 100% of the animals. A randomization procedure (Excel, Microsoft Office) was used to select the different sampling groups, performing 10 random samples of each batch in order to measure the degree of success to predict whether the batch was a high-BRD risk one.

Results: The percentage of calves sampled ranged from 21% to 62% for a 300 or 50 suckler calves' batch respectively.

Our Database of 12 batches, where thoracic US was performed in a 100% of calves at arrival, was used for random selection of calves. Real batches included 12 to 51% of calves affected with score higher than 3 (moderate and severe cases). Most of the batches were high (>30% of calves with lung lesions = 42%) or medium (20-30% of calves with lung lesions = 50%) and only one (8%) was a low risk batch (<20% of lung lesions). Randomized selection of a percentage of calves following the sampling table was performed 10 times. The average results for these 10 randomizations were very similar to the real ones, and the estimated sensitivity was 96% for batches with at least a 30 % of prevalence of disease.

Conclusions: Thoracic US scanning of a randomized percentage of calves at arrival may be a useful technique to identify high-risk BRD batches where metaphylactic treatment is justified. This technique can be a practical tool for a more rational use of antibiotics in the veal industry.

Keywords: Feedlot, BRD, ultrasound, thoracic, calves.

DI-03

Applications of infrared thermography as a non-invasive technique early indicator of fever in the lambs

José Luis Blasco Castello[1], Jorge Gutierrez Gonzalez[2], Ángel Ruiz-Mantecon[3], Almudena Sanchez Gomez[4], Mercedes Arroyo Porriño[4], Silvia Aviles Diadosa[4], A Faundez Vara[4], Maria Elena Alonso De La Varga[5], Jose Manuel Lomillos Perez[5].

[1]Technical Specialist Feedlots, Zaragoza, Spain; [2]Technical Specialist Small Ruminants, Palencia, Spain; [3]Ganadería de Montaña Center (CSIC) de León, Finca Marzanas, Ctra. León-Grulleros, 24346 Grulleros (León), Spain; [4]COVAP, C/ Mayor 56, 14400 Pozoblanco (Córdoba), Spain; [5]Animal Production Dpt, Veterinary Faculty, León University, Campus de Vegazana, 24071 León, Spain.

Objetives: Ovine Respiratory Complex (ORC) is one of the main causes of morbidity and mortality in sheep feedlots with high prevalence and economic repercussions (González, 2018). Early ORC diagnosis is essential to minimize the effects of the disease, related with both the productive results and welfare of the animals.

Body temperature is an objective clinical data, although its increase is not always associated with disease, as can be also a response to the high environmental temperature or exercise. However, when it is associated with other more specific clinical signs, it is very useful for early diagnosis. Indeed, studies monitoring temperature using ruminal boluses in cattle, demonstrate how the thermal increase occurs 12-136 h before the caregiver detected other more specific clinical signs of pneumonia (Timsit et al., 2011).

On the other hand, thermography is a technology with increasing use in different fields of the veterinary medicine. Thermographic cameras detect infrared radiation emitted by the surface of that body, converting it into an electrical signal that is transformed into a color image. Indeed, Schaefer y col. (2007) already demonstrate it is useful to identify early disease in cattle. However, environmental temperature at the time of thermal image can affect rectal temperature (Church et al., 2014).

Then, the purpose of this study was to evaluate the use of an infrared thermographic camera taking into account environment temperature for the detection of animals with hyperthermia in an intensive lamb herd.

Material and methods: This study was carried out in a lamb fattening farm located in Córdoba (Spain). Infrared temperatures were measured in a total of 2,842 multiple source commingled Merino lambs and its crosses, 60 to 120 days old, and 18 to 30 Kg live body weight. Following previous Schaefer et al. (2007 and 2012) studies in cattle, we performed an orbital eye measurement, as in other anatomical parts the presence of hair or wool can interfere with results.

The measurements were made after the passage of the lambs through the handling or sorting sleeve and in the lazarette measuring in duplicate both rectal temperature with a conventional thermometer and eye thermal image with an infrared thermometer.

For infrared thermography the camera Testo model 870 was used, with a resolution of 160 x 120 pixels (with the possibility of increasing to 320 x 240 pixels), thermal sensitivity

<100 mK, 34º lens with fixed focus and integrated and calibrated digital camera. The image processing software was the IRSoft Software, version 4.5. While the thermometer used was the Kruuse clinical thermometer, model Veterinär-thermometer SC 12, also calibrated, with automatic stop system by sound signal and with a measurement accuracy of 0.1ºC.

At measurement time, the following data were additionally recorded: date and time, farm identification, barn, pen, etc. In addition, the inside and outside barn temperature was noted. Subsequently, we calculate a new parameter: the difference between rectal and ocular temperature (DROT).

The data were analyzed using Anova one-way for the comparison of means, as well as the correlation between the variables and the multiple linear regression using the statistical program IBM SPSS Statistics, version 25.

Results and discussion: We evaluate the effect of barn temperature on the ocular and rectal temperature. On the other hand, we evaluate its effect on different rectal temperature strata.

The correlations between the different variables were significant. Indeed, the combined use of variables, eye temperature and barn temperature, allowed us to establish a formula predicting the rectal temperature with an excellent correlation (R^2 = 0.9996).

Rectal tª = 1.099 * ocular tª - 0.038 * tª nav

Conclusions: The rectal temperature of lambs under intensive conditions is an objective clinical parameter very useful for ORC early diagnosis. Infrared thermography is a fast, automatic and non-invasive technique helpful for early identification of sick animals. And considering the barn temperature at lecture time improves its correlation with rectal temperature.

Keywords: Fever, thermography, lambs, environmental Tª, ORC.

DI-04

Comparison between thoracic ultrasonography and visual observation for the detection of BRD in veal calves

Carolina Tejero[1], Javier Lillo[1], Eudald Arnau[2], Laura Elvira[1].

[1]MSD Animal Health, Spain, Salamanca, Spain; [2]Practitioner Specialist in Feedlots, 2 Feedlot practitioner, Spain.

Objectives: High levels of mortality and morbidity due to bovine respiratory disease (BRD) in combination with animal welfare, reduction of antimicrobial use, and antimicrobial resistance are currently the most important challenges in the beef industry.

The detection of BRD in farms is based on respiratory clinical signs (cough, dyspnea, ear position, nasal discharge) and increased body temperature. However, the use of clinical signs to detect lung lesions associated with BRD has some limitations (Leruste et al., 2012; Sensitivity (Se) 61.8% and specificity (Sp) 62.8%; White, 2009). Recently, new tools have been developed to achieve a more accurate early diagnosis. Among them, Thoracic Ultrasonography (TUS) shows the highest accuracy to detect pneumonia at farm level (Se 79.4% and Sp 93.9%; Buczinski, 2015; Se 89% and Sp 95%; Berman, 2019).

The objective of this study was to compare Thoracic US with BRD clinical diagnosis performed by two senior veterinarians both at arrival to the rearing facilities and later along the first month on fattening.

Materials and methods: Two different batches of a commercial veal feedlot with 62 and 45 male calves were evaluated. The day of arrival, two experienced veterinarians (observer 1;O1 and 2;O2) classified the 107 calves as “BRD” or “Healthy” based on their clinical signs. Then, an experienced veterinarian performed thoracic US scanning (TUS) and classified the calves as having or not having lung lesions using a scoring system (Adams and Buczinski, 2015). The three veterinarians performed their scoring in a blind manner. All animals were scored again by the three veterinarians 7 and 21 days after their arrival. The different scoring results were recorded to evaluate BRD prevalence and agreement between the three scoring results. The TUS score was considered as the gold standard technique.

The comparison between the use of the diagnostic techniques (Se, Sp, positive predictive value – PPV- and negative predictive value-NPV-) and observers (concordance kappa index) was performed with free epidemiological software (https://http://www.winepi.net) using a confidence level of 95%.

Results: The prevalence of lung lesions detected by TUS at arrival was 35% for batch1 and 36% for batch2 respectively. Using clinical scoring, the prevalence of BRD was different between the O1 and O2 for batch1 (O1: 45%; O2: 29%) and batch2 (O1: 47%, O2: 38%). In the first review performed 7 days after arrival, BRD diagnosed by TUS increased to 75% and 62%, respectively for batch1 and 2. BRD prevalence diagnosed by the observers was also different in batch 1 (O1: 65%; O2: 72%) and in batch 2 (O1: 53%, O2: 47%). In the examination performed 21 days after arrival, BRD prevalence diagnosed by TUS was 67% and 49%, respectively for batch1 and 2. BRD prevalence diagnosed by the observers was (O1: 60%; O2: 52%) in batch1 and (O1: 20%, O2: 27%) in batch2.

Although the average Se and NPV were quite similar for both observers (Se 63.8% and 64.9% and NPV 60.1% and 65.3%, for O1 and 2 respectively), the Sp and PPV was higher for O2 (Sp 66.0% and 79.9% and PPV 69.4 and 79.65, for O1 and 2, respectively).

Finally, the concordance among both observers was moderated (kappa = 0.598, IC95% 0.29-0.71). Indeed, one of the observers consistently presented a better detection of BRD signs than the other. However, even for this observer, 28% of BRD cases detected by thoracic US were not identified as BRD using clinical diagnosis.

Conclusions: The present study suggests that Thoracic Ultrasonography can be used as an on-farm technique for BRD diagnosis. However, clinical diagnosis by experienced observers may be good enough to be used as a screening technique to identify BRD calves. The results also suggest that, with training, observers may improve their Sensitivity and Specificity for a better detection of treatment of BRD calves.

Keywords: Ultrasonography, feedlot, BRD, diagnosis, clinical signs.

DI-05

Electrical impedance tomography (EIT) to evaluate peak inspiratory and expiratory flow levels and distribution of ventilation in steers

Olivia Brabant[1], Andreas Waldmann[2], Andy Adler[3], Anthea Raisis[1], Ulrike Auer[4], Martina Mosing[1].

[1]College of Veterinary Medicine, Murdoch University, Perth, Australia; [2]Department of Pneumology and Critical Care Medicine, Witten/ Herdecke University Hospital,, Cologne, Germany; [3]Department of Systems and Computer Engineering, Carlton University, Ottawa, Canada; [4]Veterinary University Vienna,, Vienna, Austria.

Electrical impedance tomography (EIT) is a novel non-invasive imaging technique that allows a visual representation of the distribution of ventilation in real-time. The aim of this study was to determine if changes in lung status can be detected by changes in EIT variables in anaesthetised steers.

In this randomised cross-over study, seven anaesthetised spontaneously breathing steers lungs were exposed to atmospheric airway pressure (NoPres), or continuous positive airway pressure of 7 cmH2O (PosPres). Steers were positioned in dorsal recumbency. EIT electrodes mounted on a belt were placed around the thorax to measure impedance changes. Post induction, EIT recordings were performed at 30, 50, 70 and 90 minutes respectively. From each recording ten breaths were retrospectively analysed and pooled for the pixel with maximal inspiratory ($FlowPix_{insp}$) and expiratory flow ($FlowPix_{exp}$) within the EIT image and the centre of ventilation, illustrating shift of ventilation distribution along the gravitational axis, between right and left lung (CoV_{r-l}) and ventral to dorsal lung (CoV_{v-d}). Descriptive statistics were used to describe the difference between NoPres and PosPres and are reported as mean (95% confidence interval).

Lower $FlowPix_{insp}$ and $FlowPix_{exp}$ were measured during PosPres compared to NoPres; $FlowPix_{insp}$ [311 (282-340) vs 352 (315-388)] and $FlowPix_{exp}$ [268 (249-287) vs 313 (282-343)], respectively. The CoV_{r-l} was lower [54 % (53-55)] in PosPres than in NoPres [61 % (58-63)] while only a small difference of 3 % was seen for CoV_{v-d}.

EIT can be used to detect differences in lung status by flow and CoV measurements in anaesthetised cattle.

Ethical Animal Research: This study was appoved by Murdoch University ethics committee R2998/17

Keywords: Electrical impedance tomography, Ventilation, Cattle.

DI-06

Preliminary analysis in the flank temperature pattern in dairy cows during pregnancy

Michal Trela[1], Malgorzata Masko[2], Malgorzata Domino[1], Dominika Domanska[1], Tomasz Jasinski[1], Bartosz Pawlinski[1], Zdzislaw Gajewski[1].

[1]Department of Large Animal Diseases and Clinic, Institite of Veterinary Medicine, Warsaw University of Life Sciences WULS – SGGW, Warsaw, Poland; [2]Department of Animal Breeding, Institite of Animal Science, Warsaw University of Life Sciences WULS – SGGW, Warsaw, Poland.

Objectives: Recent years have seen a significant increase in interest in the use of thermal imaging in veterinary diagnostics, especially in a health herd management of livestock animals. Owing to the possibility of fast, non-invasive and automated temperature measurement on the surface of a cows' body, this technique shows great potential in predicting local changes in the tissue metabolism and local blood circulation. The abdominal area demonstrated physiological changes during late-gestation related to the increased demand for the growing uterus and more loaded abdominal wall muscles. However, there is still a need for further research to standardize measurement data in thermal imaging. Therefore, the aim of the study was evaluated if the changes in physiological activity during late pregnancy may result in abnormal thermal patterns of the flank of dairy cows.

Materials and methods: Thermographic images of the right flank were obtained from 54 Holstein-Friesian dairy cows from a commercial 550 head dairy farm. All cows were housed under the same environmental conditions. The pregnancy was confirmed with ultrasound examination on day 32 after insemination and one the day of imaging in 30 cows. Based on ultrasound, the cows were divided into the following groups: NP - nonpregnant (n=24); EP - early pregnancy (1st to 3th months; n=6), MP - mid pregnancy (4th to 6th months; n=6) and LP - late pregnancy (7th to 9th months; n=24: 7th Mo, n=6; 8th Mo, n=6, 9th Mo, n=6). Images were taken indoor, with ambient temperature ranging from 14.0 to 16.0°C, using infrared radiation camera (emissivity 0.99; FLIR Therma CAM E60). Temperature pattern of the flank area was evaluated using five regions of interest (ROI 1–5) representing: the area along the transverse processes of the spine (ROI 1), the cranial area of the right abdominal surface (ROI 2), the cranial area of the right abdominal surface (ROI 2), the middle area of the right abdominal surface (ROI 3), the caudal area of the right abdominal surface (ROI 4), and the stifle area (ROI 5). Each ROI was analyzed for the minimal (T_{min}), maximal (T_{max}), and mean (T_{mean}) temperatures. The results (mean±SD) were compared between NP, EP, MP, 7th Mo LP, 8th Mo LP, and 9th Mo LP groups.

Results: The T_{min} was higher ($p<0.0001$) in all five ROIs in LP (19.67°C ±1.11) than in NP (13.55°C ±3.34), EP (14.92°C ±1.69), and MP (14.88°C ±1.64). There were no differences ($p>0.05$) in T_{min} between NP, EP and MP as well as between 7th Mo (18.85°C ±1.40), 8th Mo (19.14°C ±1.50), and 9th Mo (19.68°C ±1.41) of within LP group. The T_{max} was comparable in all five ROIs, with no differences ($p>0.05$) between NP (26.51°C ±2.24), EP (27.39°C ±2.57), MP (24.22°C ±3.16), and LP (25.53°C ±2.29) groups, regardless of the month of preg-

nancy. The T_{mean} was higher ($p<0.01$) in ROI 1 and 5 in LP (ROI 1: 22.35°C ±0.95; ROI 5: 24.78°C ±1.09) than in NP (ROI 1: 17.54°C ±4.42; ROI 5: 19.27°C ±4.09), EP (ROI 1: 18.96°C ±1.75; ROI 5: 19.88°C ±1.87), and MP (ROI 1: 19.71°C ±0.08; ROI 5: 19.97°C ±1.28), with no similar differences ($p>0.05$) in ROI 2, ROI 3, and ROI 4.

Conclusions: Thermography is proposed as a promising tool in determining changes in the flank temperature pattern in dairy cows during pregnancy. An application of different regions of interest during the superficial temperature analysis allows to established the discreet changes in the mean temperatures during late pregnancy. The minimal temperature seems to be the most reliable indicator of the superficial temperature changes related to the months of pregnancy. After examining more numerous groups of cows, the external temperature of the flank may be a useful method for the presumption of pregnancy in late gestation.

Keywords: Thermography, cow, pregnancy diagnosis.

DI-07

Associations between TUS scores and mortality and productivity outcomes on Scottish dairy farms

Katharine Baxter-Smith[1], Jimmy More[2], Robert Hyde[3].

[1]MSD Animal Health, Milton Keynes, United Kingdom; [2]The Ben, Kirkcudbright, United Kingdom; [3]School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, United Kingdom.

Objectives: A previously published study (1) used thoracic ultrasound (TUS) to assess farmer accuracy of diagnosing Bovine Respiratory Disease (BRD) in cattle youngstock. We found that sensitivity of farmer diagnosis of BRD was low; demonstrating that animals with lung lesions were not being identified and treated for BRD. We also found that some farmers treated animals which did not have signs of BRD on TUS, suggesting unnecessary use of antimicrobials. This study aimed to follow the same calves assessed in our previous work, to determine the effects of lung consolidation, as diagnosed by TUS, on future mortality and reproductive outcomes.

Materials & Methods: TUS examinations were conducted between 2019-03-13 and 2019-11-26, by a single veterinarian over 7 dairy herds. All calves on the farm between 21 and 61 days of age during these time points were scanned and scored. This included male and female calves destined for dairy or beef rearing, which ensured a larger dataset. On scoring, they were given a TUS score on a 0-6 scale (2) as well as a calf health score based on the Wisconsin calf health scoring system which was converted to a binary "normal" or "abnormal" TUS score. Further descriptions and results of this part of the study have been published previously (1).

For the current study, all calves which had a TUS examination were included in the dataset for analysis of mortality. Female dairy calves retained on the study farms were followed through to calving, for analysis of reproductive outcomes using the farms' management system and data collection software. Mixed effects logistic regression models were used to analyse associations between TUS score and mortality status, conception within 3 serves, time to conception and the number of serves to conception. Data was only collected for animals that were retained on the farm for the purpose of entering the adult milking herd. This meant a proportion of animals were lost to follow-up as they were sold for beef to other farms not included in the study.

Results: The initial dataset consisted of 347 ultrasonographic examinations. Of these, 53 calves (15.3%) were classified as abnormal and 294 (84.7%) as normal. Of the 53 calves classified as abnormal, only 13 (24.5%) were treated by the farmer, however, of the 294 classified as normal, 22 (7.5%) were treated. Of the 347 animals, 18 animals died during the follow up period of weaning to conception (5.2%), and mixed effects regression model results suggested that there was a tendency for animals with an abnormal TUS score as calves to be more likely to die during this time (OR 2.33, 95% CI 0.9-6.1, P = 0.08).

Data for pregnancy status within 3 serves were available for 149 animals, with 16/22 (72.7%) of animals with abnormal TUS status becoming pregnant and 114/127 (89.8%) of animals with normal TUS status becoming pregnant. Mixed effects regression model results suggested that abnormal TUS score was associated with lower odds of becoming pregnant within 3 serves (OR 0.32, 95% CI 0.1-1.0, P=0.06). No associations were found between TUS score and time to conception or the number of serves to conception.

Conclusion: Follow up analysis of dairy cattle which had undergone TUS between 21-61 days of age suggest a tendency for increased mortality in animals with higher abnormal TUS scores, and a tendency for decreased pregnancy rates within the first 3 serves. This reflects similar work undertaken in other countries (3,4). To the author's knowledge, this is the first study in the United Kingdom to ascertain the prevalence of subclinical BRD in pre-weaned dairy calves, and to follow these calves to adulthood to further investigate the effects of BRD on future performance.

Alongside the immediate health, welfare, and productivity impacts of clinical and subclinical respiratory disease, it is likely that subclinical BRD during the preweaning period is associated with future reproductive and mortality outcomes. The prevention of pneumonia in calves has the potential to improve health and production outcomes. For future work, we will follow these animals to the end of their first lactation to gather data on 305-day lactation yields to analyse for associations with calfhood pneumonia.

Keywords: Thoracic ultrasound, calf, bovine respiratory disease.

DI-08

Dynamics of subclinical pneumonia in male dairy calves in relation to antimicrobial therapy and production outcomes

Stan Jourquin[1], Thomas Lowie[1], Florian Debruyne[1], Laurens Chantillon[1], Nick Vereecke[1], Randy Boone[2], Bart Pardon[1].

[1]Ghent University, Merelbeke, Belgium; [2]Dierenkliniek Venhei, Kasterlee, Belgium.

Objectives: Quick thoracic ultrasonography (qTUS) is increasingly used as an on-farm method to diagnose clinical and subclinical pneumonia in dairy calves. The primary objective of this study was to describe dynamics of lung consolidation in a purchase dependent production system for male dairy calves (veal) in relation to antimicrobial therapy and respiratory diagnostic tests. In addition, the association of cured and uncured pneumonia with average daily growth (ADG) and cold carcass weight (CCW) were studied, as well as the effects of arriving with lung consolidation on the probability of developing chronic unresponsive pneumonia and reduced performance.

Materials and methods: A prospective cohort study was conducted involving 295 male Holstein Friesian calves, aged 14-21 days during one production cycle in a commercial veal farm. Both clinical examination and qTUS were performed on all animals upon arrival, at week 2, 3, 4, 6, 8 and 12. Pneumonia was defined as the presence of a lung consolidations ≥1 cm. Pathogen detection was done using cultures, Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) (Brüker Daltonik GmbH, Bremen, Germany) and whole genome nanopore sequencing (WGS) on non-endoscopic bronchoalveolar lavage samples (nBAL).

Results: Of the calves, 17.6% (52/295) arrived with a lung consolidation ≥1 cm. At the first outbreak of respiratory disease this number had risen to 30.8%. Initial therapy with tulathromycin and subsequently doxycycline appeared ineffective, resulting in a rise in the prevalence of animals with pneumonia up to 43.8% in week 4. At the start of the first outbreak in week 2, the vast majority (91,2%) of the pneumonia cases (n = 91) were subclinical. At week 4 the outbreak became more clinical and therapy with amoxicillin resulted in a cure rate of 52.7%. Bacterial cultures and nanosequencing diagnostics on nBAL samples identified bovine respiratory syncytial virus (BRSV) and Mycoplasma bovis as the dominant agents in the first outbreak. The isolated M. bovis strain had mutations encoding for macrolide resistance. The second outbreak was characterized by a Pasteurella multocida superinfection and isolation of a different M. bovis strain. Evaluated over the complete observation period, 53.9% of the calves cured upon antimicrobial therapy. Of the 46.1% calves that were not cured (n = 111), 29.7% (33/111) had developed chronic pneumonia. Calves with uncured or chronic pneumonia had a significantly lower ADG (992g/day ± 174 and 930g/day ±146, respectively) compared to calves that did not developed pneumonia (ADG = 1103 g/day ±156; P < 0.001). In contrast, calves that did fully cure had a similar ADG as calves that never developed pneumonia (1045 g/day ±139 and 1103 g/day ±156, respectively; P = 0.09). The effect of uncured pneumonia was no longer significant for cold carcass weight. Calves with lung consolidation upon arrival had a significantly lower ADG (981 g/day ±159 vs 1045 g/day ±159; P = 0.01) and were more likely to develop chronic pneumonia (OR=4.2; 95% CI: 2.1 – 8.6, P < 0.001). Animals with chronic pneumonia, in turn, had a significantly lower cold carcass weight (10.3kg ± 4.4; CI: 1.2 – 18.9; P = 0.02).

Conclusion: The prevalence of pneumonia in veal calf facilities proves to be much higher than previously expected, mainly because of its subclinical nature. Animals with pneumonia have a reduced daily growth but appear to be able to catch up to healthy animals when cure is achieved before the age of 12 months. Chronic unresponsive pneumonia is frequently encountered and the main reason for production loss. Calves that already had lung consolidation upon arrival on the veal facility had a higher risk of developing chronic unresponsive pneumonia. Both qTUS and nBAL diagnostics offer potential for better control and prevention of bovine respiratory disease in dairy calves.

Keywords: Bovine respiratory disease, subclinical pneumonia, Mycoplasma bovis, bovine respiratory syncytial virus, economics.

DI-09

Use of quick thoracic ultrasonography (qTUS) to guide and evaluate antimicrobial therapy in three natural outbreaks of Mycoplasma bovis pneumonia

Stan Jourquin, Laurens Chantillon, Thomas Lowie, Jade Bokma, Bart Pardon.

Ghent University, Merelbeke, Belgium.

Objectives: Quick thoracic ultrasonography (qTUS) is an accessible on-farm method to reliable diagnose (subclinical) pneumonia in both dairy and beef calves, offering great potential for more rational antimicrobial use and better control of bovine respiratory disease. The objective of this study was to describe the application of qTUS to guide and evaluate antimicrobial therapy in three natural outbreaks of Mycoplasma bovis associated pneumonia in a single beef farm.

Materials and methods: A retrospective analysis was performed on ultrasonographical and diagnostic data collected from 3 different outbreaks of respiratory disease in the same closed commercial beef farm housing 1000 Belgian Blue beef cows. Calves at risk aged between 2 and 6 months and were housed in groups of 6 in fully separated pens. Pneumonia was defined as a lung consolidation ≥1cm while cure was defined by reaeration of these lesions to <1cm in depth. In each outbreak, qTUS was used as a tool to initiate, stop or generally evaluate antimicrobial therapy. In outbreak 1, ultrasound-guided full metaphylaxis was used, meaning that all animals at risk (n = 130) were metaphylactically treated (one injection), after which antimicrobial therapy was only prolonged in animals which still had lung consolidation. Antimicrobial treatment was stopped as soon as ultrasonographical reaeration of the lung occurred. Outbreak 2 (n = 94) was approached by individual treatment of animals with ultrasonographically confirmed pneumonia, without metaphylaxis. Finally, in the third outbreak (n = 61), an ultrasound-guided targeted metaphylaxis,

only using group treatments in pens were 3 or more animals had consolidations ≥1cm, was applied. Again, as was done in outbreak 1, antimicrobial treatment was discontinued when reaeration appeared. In each outbreak, animals older than 3 months were treated with florfenicol while animals less than 3 months were treated with oxytetracycline, both administered as long acting formulation every 48 hours. Identification of primary pathogens was done by means of whole genome nanopore sequencing (WGS) on non-endoscopic bronchoalveolar lavage samples (nBAL). Strain typing of M. bovis and detection of possible genomic antimicrobial resistance was done by long-read nanopore sequencing.

Results: At the time of intervention 43.3% (55/130), 50% (47/94) and 37.7% (23/61) of the calves from outbreaks 1, 2 and 3 had a lung consolidation ≥1cm, respectively. In the first outbreak, where full metaphylaxis was applied, cure rate after 14 days was 98.2% (54/55). no new cases of pneumonia were detected, resulting in a total proportion of healthy calves of 99.2% (129/130) after the outbreak. In outbreak 2, over the 21-day follow up period 24 new cases of pneumonia were detected, resulting in a total of 75.5% (71/94) pneumonia within the herd. Cure rates were 61.7% (29/47) and 79.2% (19/24) for the initial and new pneumonia cases, respectively (total cure= 67.6 % (48/71). Finally, in outbreak 3, incidence of pneumonia started at 37.7% (23/61). After a 14 day follow up, the combination of targeted metaphylaxis and individual treatment resulted in a total cure rate of 78.9% (30/38). Of the initial cases, 78.2% (18/23) was cured. 15 new cases were detected in this outbreak, of which 80% (12/15) was cured at the end of observation. Cure rate was significantly lower in outbreaks 2 and 3, compared to 1 (P<0.01). In outbreak 1, 2 and 3, M. bovis was detected by WGS and strains were classified as belonging to Belgian M. bovis genomic clusters IV, I and I, respectively. In the third outbreak the coronavirus was also detected. Evaluated over the outbreak, in outbreaks 1, 2 and 3, a number of animal daily dosages (ADD) per animal of 3.0, 8.0 and 5.3 ADD/animal were necessary to control the outbreak, respectively. For comparison a standard metaphylaxis with tulathromycin stands for 7 ADDs/animal.

Conclusion: The qTUS technique showed potential as a reliable and practically feasible method both for detection of pneumonia and therapy evaluation in larger herds. Large differences in cure rate between the outbreaks were observed which may be related to co-infection, timing of the initiation of antimicrobial therapy, strain difference or the use of metaphylaxis. Especially the latter is a point of interest given that both ultrasound-guided metaphylaxis (full or targeted) resulted in lower antimicrobial use compared to individual treatment.

Keywords: Bovine respiratory disease, Mycoplasma bovis, lung ultrasound, antimicriobial reduction.

DI-10

Lessons learned from 1 year of quick thoracic ultrasonography (qTUS) training for practitioners to improve pneumonia diagnosis in calves

Bart Pardon, Stan Jourquin, Thomas Lowie, Florian Debruyne, Laurens Chantillon, Mathilde L. Pas, Wouter Van Mol, Justine Clinquart, Jade Bokma.

Department of Internal Medicine, Reproduction and Population Medicine, Ghent University, Merelbeke, Belgium.

Objectives: Thoracic ultrasonography (TUS) has become the key on-farm tool for bovine respiratory disease management (BRD), in particular to rationalize antimicrobial use. Despite growing interest to implement TUS on farm, many practitioners hesitate. Uncertainty about image recognition and scanning technique, as well as concerns on time investment are the main reasons given. Quick thoracic ultrasonography (qTUS) stands for a standardized lung scanning technique making TUS of a large number of calves in limited time possible. The objective of this study was to describe diagnostic performance of novice operators after a qTUS training course.

Materials & Methods: All Flemish veterinarians were contacted to volunteer for a project aiming at bringing qTUS to practice as a diagnostic aid in BRD management. Participants needed to complete an online course consisting of 4 modules (Module 1: positioning of TUS among other methods for pneumonia diagnosis; Module 2: ultrasound image recognition including common pitfalls; Module 3: qTUS scanning technique and Module 4: qTUS applications in practice). The training included 4 series of exercises with feedback, two on recognition of images, and 2 on recognition of full qTUS scan image loops. In addition, practical training sessions with experienced qTUS operators were organized at the discretion of the participant to decide how many to follow. Candidates were encouraged to scan in their own practice as well. Monthly online contact moments were organized between the trainers and trainees. To finalize the exam a practical test was organized. Three test occasions (sessions) were organized. Each participant needed to scan 23 (session 1 and 2) or 18 (session 3) calves and communicated to a recorder whether a lung consolidation of ≥ 1 cm was present. Performing other aspects of clinical examination was not allowed. Session 1 and 2 were done in a commercial veal calf farm with animals aged 8 (group housing) and 6 weeks (individual housing), respectively. Session 3 was done in a dairy farm on calves aged between 2 weeks to 4 months (individually housed until week 5). Total scanning time and information on intensity of online learning and self-training was collected. Diagnostic accuracy of the participants was compared with an experienced TUS operator.

Results: In February 2021, 45 veterinarians started with the online training, of which 34 had taken the certification test by January 2022. The prevalence of calves with lung consolidations ≥ 1 cm ranged between 39.1% and 75.0% depending on which calves the participants scanned. Overall diagnostic accuracy (Acc) was 72.3% (standard deviation (SD)= 10.8%; range (R)= 52.2%-95.7%), mean sensitivity (Se) was 64.4% (SD= 26.6; R= 0-100) and mean specificity (Sp) was 70.6% (SD= 17.7; R= 25-100). Average Se (45.0%) was significantly lower in session 3 compared to session 1 (74.4%) and 2



(73.0%) (*P*<0.05). In contrast, average Sp (60.6%) was significantly lower in session 2, compared to session 1 (80.5%) and 3 (78.9%) (*P* <0.05. Participants that followed more than one practical training session had on average a 21% higher Se compared to persons attending a single training (*P*< 0.05). Also the number of calves scanned in the own practice before the test trended towards a higher Se (*P*= 0.08). Gender, years graduated, hours invested in the online course and speed of scanning did not affect Se. Sp was not influenced by any of the factors mentioned above. The required scanning time per calf (including capture and fixation of the animal) was 4.7 min. (SD= 1.1; R= 1.7-6.5 min.) on average. After the course 60% of the candidates declared to feel skilled enough to use qTUS in practice, but this was not significantly associated with diagnostic performance.

Conclusions: With the qTUS training program (online + live trainings) in most novice operators a reasonable Se and Sp could be reached. However, variation in Se and Sp is substantial and may be influenced by age of animals and housing/scanning conditions. Increasing the number of practical training sessions significantly improved Se. Adequate training and continued self-assessment are necessary to sustainably integrate qTUS in the highly variable conditions in daily practice.

Keywords: Thoracic ultasonography; bovine respiratory disease; diagnostics; education; practice.

EP-01

The epidemiological and economic assessment of bovine viral diarrhoea infections worldwide

Pinior Beate[1], Roch Franz-Ferdinand[1], Richter Veronika[1], Firth Clair L[1], Scharnböck Bettina[1], Marschik Tatiana[1], Baumgartner Walter[2], Obritzhauser Walter[1], Wagner Peter[3], Garcia Sebastien[4], Minviel Jean J[4], Käsbohrer Annemarie[1], Raboisson Didier[4].

[1]University of Veteriary Medicine Vienna, Unit of Veterinary Public Health and Epidemiology, Austria; [2]University of Veteriary Medicine Vienna, University Clinic for Ruminants, Austria; [3]Department of Veterinary Administration, Styrian Provincial Government, Austria; [4]University of Toulouse, Population Medicine & Economics of Animal Health, France.

Objectives: The aims of this study were:

i. to determine the geographical distribution of bovine viral diarrhoea virus (BVDV) infections and intervention measures worldwide by conducting literature searches and questionnaires;
ii. to predict the temporal global development of BVDV infections, both with and without intervention measures;
iii. to identify potential sources of heterogeneity in the study by conducting a weighted-stratified meta-analysis of pooled prevalences, including countries, age groups, production systems, vaccination status, clinical signs, mitigation programmes, diagnostic methods and sampling material;
iv. to assess the extent to which epidemiological factors (e.g., BVDV new infection risk and circulation duration) and mitigation measures (e.g., vaccination, testing and culling, purchasing new stock or contact with neighbouring cattle herds) may influence production losses due to BVDV infections in cattle herds and
v. to analyse whether the implementation of BVDV mitigation activities is economically justified in Austria (Styria) and worldwide.

Material & Methods: To identify global studies focusing on the prevalences, mitigation measures, production losses and economics of BVDV control activities, an extensive systematic literature search was performed. Different meta-regression models were developed to analyse the data from the literature. By pooling the data from eligible studies, weighted meta-analyses were conducted using a random effect model to estimate the pooled prevalences of BVDV infected cattle while considering between- (heterogeneity) and within-study (sampling) variance as a result of sampling error. Univariate and multivariate meta-regression analyses were used to identify factors such as diagnostic methods, age of animal, production systems, or intervention measures that may have had a significant impact on the level of reported BVDV prevalences. Additionally, a meta-regression model was developed to identify potential influencing epidemiological factors (e.g., BVDV new infection risk) and mitigation measures on the production losses. Furthermore, a review of different economic assessments of global BVDV mitigation measures was conducted and the voluntary and compulsory BVDV eradication programmes in the federal state of Styria, Austria, were economically assessed by performing a cost-benefit analysis that considered potential trade effects due to implemented mitigation measures. In this context, a Bayesian structural time series model was developed to analyse a hypothesised positive impact of the compulsory BVDV programme on the Styrian cattle export market.

Results: Our questionnaire and literature research collected data for 107 countries. The meta-analysis covered 325 studies in 73 countries that reported BVDV infections in cattle from 1961 to 2016. In total, 310,548 herds and 6.5 million animals were tested for antibodies (AB) or BVD virus. The worldwide pooled prevalences of persistently infected (PI) cattle were found to sbe low (≤0.8% Europe, North America, Australia), medium (>0.8% to 1.6% East Asia) or high (>1.6% West Asia). The PI and AB prevalences in Europe decreased over time, while BVDV prevalence increased in North America. The highest mean pooled prevalences of PI animals were identified in countries that had failed to implement any BVDV mitigation programmes (including vaccination). Additionally, the meta-regression analysis revealed that the implementation of vaccination and biosecurity measures were associated with 8%–12% and 28%–29% decrease in BVDV-associated production losses on average, respectively, when simulated herds were compared with or without such mitigation measures. This reduction was partially offset when livestock holders purchased new cattle or allowed direct contact with other cattle herds.

An economic net benefit (benefit:cost ratio, BCR = 1.18) of the Styrian mitigation programme was demonstrated during the voluntary programme phase (January 1998–July 2004). If investments in the compulsory programme (August 2004–December 2016) were taken into account, there was an overall net economic loss (BCR = 0.16). The time point necessary to reach payback of total mitigation costs would have been the year 2020. The average number of cows and bulls exported from Styria increased significantly by 45% on average and the producer price increased by 10% on average during the compulsory mitigation period compared to the voluntary period.

Conclusion: The epidemiological and mitigation factors influencing BVDV prevalences and associated production losses presented here, as well as the economic assessment of control measures, could help to guide farmers and decision-makers to choose suitable strategies for the control of BVDV.

Keywords: Meta-analysis, Animal Health Economics, BVDV.

EP-02

Overview of control programmes of non-regulated cattle diseases in the European Union

Jaka Jakob Hodnik[1], Jože Starič[1], Jožica Ježek[1], George John Gunn[2], Inge Santman-Berends[3].

[1]Veterinary faculty, University of Ljubljana, Ljubljana, Slovenia; [2]Scotland's rural college, Inverness, United Kingdom; [3]Royal GD, Deventer, Netherlands.

Objectives: As part of a COST (European Cooperation in Science and Technology) Action SOUND control (Standardizing Output-based surveillance to control Non-regulated Diseases of cattle in the EU) more than 100 veterinarians, epidemiologists, statisticians, stakeholders and others, from 31 countries collaborate to evaluate existing control programmes (CPs) for non- regulated cattle diseases throughout Europe and beyond and to look for possibilities to move towards output based comparison of the results of these CPs.

Materials and methods: The SOUND control project is divided in to 5 working groups. Working group 1 (WG1) was entrusted with the task of describing existing CPs for non-EU regulated diseases. To collect the information on control programmes we created standardized tables members had to fill with information on the diseases that aren't regulated by the EU but are controlled in their country. For this we aimed at diseases that are not part of the A or B list of the new European Animal Health Law as most of those diseases are regulated by the EU. To be able to qualify as CP, it was agreed that a plan of eradication, control or active surveillance should be in place. Passive surveillance was excluded. The following information requested for each infection that was controlled by at least one COST country: if there was a CPs in place, type of cattle the CP applies to, if participation is voluntary or compulsory, if the programme is regional or national, funding, if there are additional EU guarantees in place, the country status for the disease (i.e. free, endemic, sporadic, etc.), and the last occurrence of disease in the country.

Results: We have obtained data for 24 diseases. Most of the participating countries have a CP for Enzootic Bovine Leukosis (N=25), Bluetongue (N=24), Infectious Bovine Rhinotracheitis (N=23), Bovine Viral Diarrhoea (N=22), Paratuberculosis (N=16), Anthrax (N=15), Bovine Genital Campylobacteriosis (N=13) and Trichomonosis (N=11). While other diseases are controlled only by a few or just by a single country. The country with the most controlled diseases is Norway with 18 CPs and the countries that declared themselves free from most diseases are Finland and Denmark with freedom from 11 diseases. We made maps that illustrate the number of CPs and the presence of disease and/or CP in each country. The results are summarised in Deliverable 1.1 on the SOUND control webpage (https://sound-control.eu/).

Conclusions: The results show the diversity in the number and type of disease control programmes in European countries and provides invaluable information for epidemiologists, veterinarians and stakeholders in Europe. In the next step we will collect information on specifics of the cattle industry in each of the countries and a more detailed description of the CPs they have in place. This information will be combined in a handbook that will provide a thorough overview of the CPs in place for non-regulated cattle disease in Europe. This will help to get an overview of the level of heterogeneity between different CPs and guide the data and methodology needs when developing an output-based surveillance system.

Keywords: Cattle, non-regulated diseases, control programme,

EP-03

Nation-wide screening of antibodies to *Mycoplasma bovis* in Swedish dairy herds and herd characteristics associated with the infection

Emma Hurri[1], Anna Ohlson[2], Åsa Lundberg[2], Anna Aspán[3], Karl Pedersen[3], Madeleine Tråvén[4].

[1]Clinical Sciences, SLU/National Veterinary Institute, SVA, Uppsala, Sweden; [2]Växa Sverige/Section of Animal Health, Stockholm, Sweden; [3]National Veterinary Institute, SVA, Uppsala, Sweden; [4]Clinical Sciences, Swedish University of Agricultural Sciences, SLU, Uppsala, Sweden.

Background: *Mycoplasma (M.) bovis* is considered an emerging pathogen that causes severe disease in cattle in many countries and is poorly responsive to treatment. An increasing prevalence of the infection, suggested by preliminary data, may pose a threat to the Swedish favorable situation concerning antimicrobial resistance (AMR) in cattle herds.

In Sweden, *M. bovis* has caused severe symptoms with outbreaks of pneumonia mainly in fattening herds. Before this study, there were around 20 dairy herds and 30 fattening herds that had been diagnosed in other projects or in field work. In 2016 a national screening on bulk tank milk (BTM) was performed to determine the prevalence of *M. bovis*. The samples were analyzed with PCR and ten farms out of 3,473 were positive, 0.3 % (1). There are disadvantages with the use of PCR since the bacterium is shed intermittently and milk from infected cows might not be included in the BTM at the time of sampling. Therefore, analyzing antibodies (ab) could provide a more correct prevalence of *M. bovis* infection in Swedish dairy herds.

Objectives: The objectives of our study were to investigate the BTM prevalence of *M. bovis* in Swedish dairy herds by determining the presence of antibodies and *M. bovis* DNA and to study herd characteristics associated with a positive BTM sample.

Material & Methods: Samples of BTM delivered from all dairy herds in Sweden, 3,144 herds, were obtained from the dairy routine laboratory with the help from Växa Sverige. The samples were analyzed with ID screen® indirect ELISA (ID-vet, Grabels, France). High sensitivity and specificity for *M. bovis* antibodies in serum was recently shown for this newly developed ab ELISA (2). The samples were also analyzed with a real time PCR (PathoProof Mastitis Major 4, Thermo Fisher Scientific, Vantaa, Finland). Herd level data on health parameters were retrieved from the Dairy Herd Improvement database (Växa Sverige), for a period of 12 months prior to the BTM sampling. The association between the herd-level *M. bovis* antibody status (negative/positive) and each of the herd variables was first evaluated by chi-square test (χ^2) for categorical variables and Student's t-test for the continuous ones. All variables with $P \leq 0.20$ were further analysed in multivariable logistic or linear regression models, correcting for biologically plausible variables.

Results: BTM samples from 4.8 % of the herds were positive for antibodies to *M. bovis.* There were large regional differences with the proportion of ab positive herds per region ranging from 0 to 20 percent. All herds were negative on PCR

analysis. For the herd characteristics there was a higher risk of antibody positivity in larger herds and there were also significant associations between antibody positivity and having a mortality of more than 0% in older calves (age 2-6 months) as well as in young stock (age 6-15 months). Moreover, there was a tendency of having a higher incidence of cows with more than 120 days between calving and final insemination in antibody positive herds compared to negative ones (*P*=0.052).

Conclusions: This is, to our knowledge, the first time a nation-wide screening of BTM antibodies to *M. bovis* is presented using the ID screen® indirect ELISA. The results of this study indicate that *M. bovis* infections are more prevalent among Swedish dairy herds than previously recognized, and that ab ELISA may be an efficient way to detect infected herds. The association between infection status, as measured by BTM antibody ELISA, and young stock and late calf mortality suggests that *M. bovis* infection affects animal health and welfare in Swedish dairy herds.

References:

1. Landin et al, IDF mastitis conference, 2019. Prevalence of *Mycoplasma bovis* and *Streptococcus agalactiae* in Swedish dairy herds. https://www.researchgate.net/publication/333162023_Prevalence_of_Mycoplasma_bovis_and_Streptococcus_agalactiae_in_Swedish_dairy_herds
2. Andersson et al. BMC Veterinary Research (2019) 15:369. A European inter-laboratory trial to evaluate the performance of three serological methods for diagnosis of *Mycoplasma bovis* infection in cattle using latent class analysis.

Keywords: Elisa, prevalence, risk factors, M. bovis, calf mortality.

EP-04

Calf-level risk factors for neonatal diarrhoea in Belgian Blue beef calves

Maude Lebrun[1], Geert Hoflack[1], Cécile Demblon[2], Geert Vertenten[3], Bart Sustronck[1].

[1]MSD Animal Health, Brussels, Belgium; [2]Veterinary Practice Demblon, Hastière, Belgium; [3]MSD Animal Health, Boxmeer, Netherlands.

Objectives: Neonatal calf diarrhoea (NCD) is one of the most important causes of morbidity and mortality in calves. NCD is considered to be a multifactorial disease, caused by a complex interplay of pathogens, environmental factors, and risk factors at the calf-level. The aim of this study was to assess different factors that influence the risk of NCD in Belgian Blue calves. Moreover, the prevalence of failure of passive transfer of immunity in Belgian Blue calves was estimated.

Materials & methods: This prospective study was conducted on a Belgian Blue farm from November 2017 to October 2018. In total 221 calves were followed during their first month of life to record the occurrence of diarrhoea and different calf-level factors i.e., birth weight, sex, dam parity, season of birth, colostrum IgG concentration, volume of colostrum consumed, time of first colostrum feeding, use of colostrum replacers, tube feeding of colostrum, suckling versus artificial rearing, and transfer of passive immunity. Transfer of passive immunity was evaluated using serum radial immunodiffusion measurement of immunoglobulin G approximately 48 to 72 hours after birth of the calves. R (R Core Team, 2017) multilevel regression techniques were used to assess the association between calf-level risk factors and the occurrence of NCD in Belgian Blue calves.

Results: The prevalence of NCD was 15,38% ($CI_{95\%}$ [10,90 – 20,83%]). The classification of the calves according to their serum IgG concentration (Lombard *et al.*, 2019) was as follows: 54 calves (24,43%, $CI_{95\%}$ [18,92 – 30,65%]) were in the category < 10 g IgG/l, 101 calves (45,70%, $CI_{95\%}$ [39,00 – 52,52%]) were in the category ≥ 10 - < 18 g IgG/l, 52 calves (23,53%, $CI_{95\%}$ [18,10 – 29,68%]) were in the category ≥ 18 – < 25 g IgG/l and 14 calves (6,33%, $CI_{95\%}$ [3,51 – 10,40%]) had serum IgG concentrations of ≥ 25 g/l. Significant predictors of an increased odds of NCD were birth weight, use of colostrum replacers, time between birth and colostrum administration, season of birth and serum IgG concentration. For each kilogram increase in birth weight (mean: 54,13 ± 8,90kg) of the calves, the odds of NCD were reduced by 7% (OR 0,93; $CI_{95\%}$ [0,88 – 0,98]; p = 0,01). Calves that were fed colostrum replacer, exclusively or in combination with dam's colostrum, had three times higher odds of developing NCD compared to calves that only received colostrum from their own dams (OR 3,08; $CI_{95\%}$ [21,15 – 8,33]; p = 0,025). Administration of colostrum more than two hours after birth resulted in 7-fold increase of the NCD odds compared to administration of colostrum within two hours after birth (OR 7.07; $CI_{95\%}$ [2,29 – 22,00]; p < 0,001). Calves born in autumn and winter had nearly five times higher odds of developing NCD compared to calves born in the spring and summer (OR 4,76; $CI_{95\%}$ [1,78 – 16,67]; p = 0,004). When considering calves with a serum IgG concentration < 10 g/l as reference, the odds of NCD was reduced by 74% in calves with a serum IgG concentration between 10 and 18 g/l (OR 0,26; $CI_{95\%}$ [0,09 – 0,70]; p = 0,009) and by 79% in calves with a serum IgG concentration between 18 and 25 g/l (OR 0,21; $CI_{95\%}$ [0,05 – 0,75]; p = 0,022). Calves with a serum IgG concentration of more than 25 g/l had a numerically but not significantly reduced odds of NCD compared to calves with a serum IgG concentration of < 10 g/l, (OR 0,62; $CI_{95\%}$ [0,11 – 2,74]; p = 0,5), probably due to the small number of calves in this category.

Conclusions: According to new standards as proposed by Lombard *et al.* (2019), in about 25% of the calves, the transfer of passive immunity can be considered as an absolute failure (serum IgG < 10 g/l). In only 30% of the calves, the transfer of passive immunity can be classified as good to excellent (serum IgG ≥ 18 g/l). The work described here identified several calf-level factors as potential predictors of the risk of neonatal diarrhoea in Belgian Blue beef calves. Further investigation of those predictors is warranted and may be of benefit to producers and practitioners involved in the control of neonatal diarrhoea in Belgian Blue beef calves.

Keywords: Belgian Blue beef calves, neonatal calf diarroea, risk factors.

EP-05

Salmonella diagnostic testing and serotyping in southern Australian dairy herds

Damian O'Brien[1], Geert Vertenten[2].

[1]MSD Animal Health, Macquarie Park, Australia; [2]MSD Animal Health, Boxmeer, Netherlands.

Objectives: A Salmonella diagnostic programme was conducted between 2017 and 2019 in Southern Australian Dairy herds. The programme sought to identify Salmonella isolates in bovine faecal samples from suspect Salmonella cases and where a positive diagnosis of Salmonella was confirmed, to identify the Salmonella isolate present. Further, the programme sought to compare the isolates identified with those present in commercially available vaccines, to allow veterinarians to make an informed decision on Salmonella vaccination.

Method: During the 3 years of the programme, co-operating veterinarians submitted a total of 680 faecal samples from clinical cases where they believed that Salmonella species may have been contributing to disease in the cattle or on the property. Samples from mixed aged cattle, ranging from young calves to older cows, were submitted to a central veterinary diagnostic laboratory for culture using Salmonella enrichment media. Sensitivity testing was performed on any Salmonella isolates grown and isolates were sent to the Microbiological Diagnostic Unit of Melbourne University for serotyping. Serotypes identified and their serogroups were compared to the serotypes and serogroups that are represented in the commercially available Australian vaccines, those being *Salmonella typhimurium* (serogroup B), *S. dublin* (D), *S. bovismorbificans* (C2), *S. uganda* (E1) and *S. zanzibar* (E1).

Results: From 680 samples submitted, a total of 219 (32.2%) salmonella positive samples were identified, yielding 221 salmonella isolates. Two samples were positive for both *Salmonella typhimurium* and *Salmonella bovismorbificans.* All other positive samples were positive for only one salmonella serotype. *Salmonella typhimurium* was the most commonly isolated serotype (81 isolations, accounting for 36.7% of isolates), followed by *Salmonella bovismorbificans* (41, or 19.5% of isolates) and *Salmonella dublin* (28, or 12.7% of isolates). There were a further 15 samples that were identified as being from serogroup B, most likely *Salmonella typhimurium*, 5 from serogroup C, most likely *Salmonella bovismorbificans*, and 2 from serogroup D, most likely *Salmonella dublin*, that were not fully typed.

Salmonella isolates from 65 properties were in-vitro tested for antimicrobial sensitivity, 3 demonstrated resistance to a single antimicrobial and 3 demonstrated resistance to multiple antimicrobials.

The majority of samples analysed came from 3 geographical regions – North East Victoria, Victorian Western Districts and Gippsland – consistent with the main dairying regions in the State. Geographical mapping of isolates found that there was no consistent pattern in serotype occurrence and that geographical distribution of isolates was varied within the state.

Of the isolates that were fully serotyped, 87% (168/193) were found to have commonality to one of the serotypes in the Australian vaccines. When considering serotype and serogroup, it was found that 95% (209/221) of isolates shared either commonality of serotype or serogroup with the Salmonella strains in the vaccines. When considering the registered vaccine containing *Salmonella typhimurium* and *S dublin*, 49.4% of isolates were definitively identified as being one of these serotypes.

Conclusions: Salmonella culture and typing is an important diagnostic tool to be utilised in the management of salmonellosis in Victorian dairy herds. There are several multivalent vaccines available and they contain salmonella antigens from different serogroups. This study found that the serogroups and serotypes in the vaccines are very closely matched to the Salmonella isolates found in the field.

In this study a number of different Salmonella serotypes and serogroups were identified, but the majority shared commonality to the strains contained in the two available Australian salmonella vaccines. To fully maximise the benefits of a salmonella control programme incorporating vaccination, selection of the most appropriate vaccine or vaccines to use is a decision which should be done based on serotyping results and in consultation with the consulting veterinarian. Monitoring salmonella incidence and serotypes is important, even after a vaccination programme has commenced, to monitor the success of the programme and to check for any variation in the serotypes of salmonella causing disease over time.

Keywords: Salmonella, diagnosis, serotyping, dairy, Southern Australia.

EP-06

Interpretation of the results of Q fever ELISA tests in domestic ruminants: a user-friendly Shiny application based on latent class models in a Bayesian framework

Thibaut Lurier[1], Marie Laure Delignette-Muller[2], Florence Ayral[3], Elsa Jourdain[4], Elodie Rousset[5].

[1]Université de Lyon, INRAE, VetAgro Sup, UMR EPIA, Marcy l'étoile, France; [2]Université de Lyon, Université Lyon 1, VetAgro Sup, CNRS, UMR 5558, Laboratoire de Biométrie et Biologie Evolutive, Villeurbanne, France; [3]Université de Lyon, INRAE, VetAgro Sup, Usc 1233 UR RS2GP, Marcy l'étoile, France; [4]Université Clermont Auvergne, INRAE, VetAgro Sup, UMR EPIA, Saint-Genès-Champanelle, France; [5]French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Sophia Antipolis Laboratory, Animal Q fever Unit,, Sophia Antipolis, France.

Q fever is a worldwide zoonotic disease, due to the bacterium *Coxiella burnetii*, responsible for reproductive disorders, such as abortion in domestic ruminants. Although direct detection of *C. burnetii* by quantitative PCR is primarily recommended for the direct diagnosis of Q fever in abortive contexts and identification of bacterial shedding, serological approaches aiming at detecting antibodies specific for *C. burnetii* are also useful at both the individual and herd levels. At the individual level, ELISA tests may be used to identify animals that were

previously infected (with or without clinical signs and shedding) and may still be latently infected (carriers with or without shedding). At the herd level, ELISA tests may be used to reveal a past or recent exposure to *C. burnetii* within a considered farm, a key tool for a rapid screening (prevalence, current evolutive circulation).

Recent investigations about the diagnostic accuracy of the three ELISA tests currently commercialized for their use in domestic ruminants showed that these tests are moderately sensitive (sensitivities ranged between 40% and 94%) and that their specificities are inferior to 100% (specificities ranged between 95% and 99%). As a consequence, the diagnostic uncertainty should be considered to limit potential misinterpretations of the individual or herd serological status.

The objectives of this study were (1) to build a methodological framework allowing calculating predictive values of Q fever ELISA tests at both the individual and the herd levels and (2) to provide a user-friendly application that could be easily used by veterinarians to interpret the results of a serological sample plan applied to a ruminant herd.

We developed an advanced computing method based on latent class modeling, implemented using JAGS and R to calculate predictive values corresponding to the results obtained with any of the three commercialized ELISA tests, at both the individual and the herd levels. This method was integrated within an open source web application, using Shiny, to favor its accessibility to all the potential users of these ELISA tests (e.g., veterinarians, veterinary diagnostic laboratories, research laboratories). After completing the characteristics of the herd (species, herdsize, type of production) and the Q fever epidemiological context (if known), the users obtain the probability of true seropositivity of the tested herd and animals given the ELISA test results.

The operation of this application is illustrated on a true clinical case where a Charolais bull originating from an apparently 'free of infection' herd was tested positive for Q fever by one ELISA test at introduction. To confirm or infirm these test results, additional animals in the herd of origin were tested with the same ELISA, considering firstly five heifers raised with the bull and secondly all males and females more than two-year-old (N=149). Probabilities of true seropositivity of the bull and its originating herd were calculated thanks to the developed application. The five other animals initially tested animals that were raised with the seropositive bull were negative to the ELISA test, which corresponds, in regard to the test used and to the herd characteristics to a probability of the bull's true seropositivity estimated at 0.85 with a 95% credibility interval (CI) of [0.14; 0.98].

Among the animals older than two years present in the herd, 5 out of the 149 tested animals tested positive which corresponds for each positive animal to a probability of true seropositivity estimated at 0 with a 95% CI of [0 ; 0.65]. At the herd level, the probability that the proportion of seropositive animals was above 0 was estimated at 0 with a 95% CI of [0 ; 0.58]; and if the herd was truly seropositive, the proportion of truly seropositive animals in the herd was assessed to 0.06 with a 95% CI of [0.02; 0.12].

This application assists veterinarians in a proper interpretation of the results of Q fever ELISA tests according to the sampling size and to available epidemiological and herd information. Veterinarians can therefore easily take advantage of a complex statistical model in a Bayesian framework to support their daily work related to Q fever risk analysis in ruminants. In the absence of a perfect reference test, this application could also be useful for local and reference laboratories for the confirmatory diagnosis of an ELISA test result.

Keywords: Coxiella burnetii - serology - predictive values - seroprevalence -cattle.

FE-01

The usage of antibiotics and anti-inflammatories in calf-rearing units in Finland

Heli Simojoki[1], Heli Härtel[2], Tuomas Herva[3], Vesa Hihnala[4], Liisa Kaartinen[5], Erja Tuunainen[6], Outi Hälli[1], Atte Sandelin[1].

[1]University of Helsinki, Department of Production Animal Medicine, Saarentaus, Mäntsälä, Finland; [2]HKScan, Turku, Finland; [3]Atria Plc, Oulu, Finland; [4]Oy Snellman Ab, Pietarsaari, Finland; [5]Finnish Food Authority, Helsinki, Finland; [6]Animal Health ETT, Seinäjoki, Finland.

Objectives: The usage of antibiotics (AB) in meat production is of concern in many countries. In Finland, meat production is mainly based on bull calves originating from dairy farms. Bull calves are transported to the rearing farms at the age of 10–30 days. Respiratory diseases are common in calf-rearing units. Approximately half of the calves are grown in specialized calf-rearing units selling calves to finishing units at the age of 5–6 months. Rest of the units are integrated, finishing their calves on their own farm. Bulls are slaughtered at the age of 18 months.The aim of the study was to find out the amount and type of antibiotics and non-steroidal anti-inflammatories (NSAIDs) used in different types of calf-rearing units.

Materials and methods: A total of 73 randomly selected calf-rearing units from Finland participated in the study. Calves (n= 27 692) were transported to these units between January and October 2016 and followed up 180 days. All the medication data and reason for medication were collected from the farms. Bookkeeping was in electronic form in 44% of the farms and on the paper form in 56% of the farms. The mean age of the calf on arrival to the unit was 23 days (SD 9.1). After 5 months feeding, calves either stayed on the same farm (28 farms, n=3 745 calves) or from specialized calf-rearing units calves were sold to another farm for finishing (45 farms, n=23 946 calves). Four out of fifth calves (79%) were milk breed (n= 21 766) and 21% milk-meat crossbred (n= 5926). The majority of the calves (88.5%) were bull calves. Medications used for dehorning were excluded from the data.

Results: During 180 day-period, 35 390 AB courses were given to 27 692 calves, in average 1.3 AB courses per calf. In 90% of the cases, reason for treatment was respiratory disease. The most common AB treatment was parenteral oxytetracycline, followed by macrolide and benzylpenicillin; 65%, 23% and 6%, respectively. Total of 28 045 NSAID treatments were administered. Most commonly used NSAIDs were meloxicam (54%) and carprofen (42%). In treatment courses, NSAID was used together with AB in 66%, NSAID alone in 7% and AB only in 26% of the cases. In specialized calf-rearing units and in the units with integrated milk feeding and finishing 67% and 37% of the calves were medicated at least once, respectively. The percentage of all medications per number of raised calves by the unit varied in specialized calf-rearing between 4–280% (mean 81%) and in integrated units 0–141% (mean 42%).

Conclusions: The total numbers of AB courses were high. The type of AB was selected according to Finnish guidelines for treatment of respiratory diseases. NSAIDs were commonly used with ABs as recommended. AB treatment was more common in specialized calf-rearing units where infection pressure might be higher due to bigger farm sizes and greater amount of young susceptible animals in the farm.

Keywords: antibiotic, anti-inflammatories, calf, calf-rearing.

FE-02

The metaphylactic use of tildipirosin for the control of Bovine Respiratory Disease in pre-weaned high-risk calves housed in individual hutches

Pedro Rodriguez Fernandez[1], Vinicius Silva Machado[2], Maria Luiza Celestino[2], Rafael C. Neves[3], Michael A. Ballou[2], Todd Bilby[3].

[1]Merck Animal Health, NJ, United States; [2]Department of Veterinary Sciences, College of Agricultural Sciences and Natural Resources, Texas Tech University, Lubbock, TX, United States; [3]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Purdue University, West Lafayette, IN, United States.

Objectives: Bovine respiratory disease (BRD) is one of the major causes of morbidity and mortality during the pre-weaning period. It delays growth of pre-weaned heifers, which can lead to long term impact on fertility and productivity. In the modern dairy industry, many calves are reared in heifer raising facilities, where calves are acquired from different sources and are transported for long periods of time. Transportation is a stressful event and is a known risk factor for BRD. Therefore, the use of metaphylaxis can be indicated for transported calves. However, information on the effect of tildipirosin for the control of BRD on morbidity and mortality for high risk calves housed in individual hutches is scarce. Hence, the objective of this study was to evaluate the efficacy of two metaphylactic strategies using tildipirosin for calves transported within the first week of life.

Materials & methods: A total of 2,100 clinically healthy Jersey and Jersey-cross calves were enrolled in the study. Calves were transported from 12 different dairies located in Minnesota to a calf raising facility located in New Mexico, where they were housed in individual hutches until weaning (56 days of life). Three days after arrival, calves were randomly allocated into three treatment groups. Calves in META1 group received a single subcutaneous (SQ) injection of tildipirosin (Zuprevo™, Merck Animal Health) at enrollment at 4mg/kg. Calves in group META2 received one SQ injection of tildipirosin at enrollment (4mg/kg) and a subsequent SQ tildipirosin injection 17 days later. Calves in CON remained as untreated controls. Average age at enrollment was 7.8 days. BRD was diagnosed based on a scoring system that assesses six clinical signs (cough, eye discharge, abnormal respiration, nasal discharge, ear droop or head tilt, and rectal temperature ≥ 102.5°F). Body weight measurements were assessed at enrollment and at weaning to calculate the average daily gain. Mortality data was gathered from the farms' database. At enrollment and weaning, ultrasonography of the lungs was assessed for random subset of 200 calves per treatment. Blood was collected at enrollment, 10 and 27 days later, and at weaning, for a random subset of 100 calves per treatment

group to determine evidence of inflammation. Haptoglobin, serum-amyloid A, complete blood cell counts, and other biomarkers were analyzed using commercial kits. The data was analyzed using multivariate logistic regression, Cox Proportional Hazards models, repeated measures ANOVA, and multivariate linear regression models. The variables age in days at enrollment, body weight at enrollment, dam's parity, season, and rectal temperature at enrollment, and source of calf was included as a random effect.

Results: The BRD incidence was 11.37%, 10.8% and 9.39% for calves enrolled in the CON, META1 and META2, respectively (P = 0.44). Time to BRD diagnosis was not affected by metaphylaxis (P = 0.45). Lung lesions was found in 25.1%, 24.9%, and 24.9% of calves enrolled in CON, META1, and META2 groups, respectively (P = 0.99). Mortality tended to be greater for CON calves in comparison to META2 calves (1.55% vs 0.57%, P = 0.05), but did not differ between calves enrolled in CON and META1 groups (1.55% vs 1.17%, P = 0.48). Weight gain was not affected by metaphylaxis. The average daily gain for calves enrolled in CON, META1, and META2 was 516.7, 517.7 and 524.6 g, respectively (P = 0.25). Blood analysis revealed that some of the markers of inflammation assessed were lower for META2 calves compared to CON calves. At 27 days after enrollment, calves enrolled in the META 2 treatment group had decreased concentrations of haptoglobin and aspartate aminotransferase, and decreased neutrophil to lymphocyte ratio compared to CON calves (P < 0.05). Additionally, CON calves had increased concentration of globulins and lower albumin to globulin ratio than META2 calves at the end of the weaning period (P < 0.05).

Conclusions: In conclusion, metaphylaxis with tildipirosin did not decrease the incidence of BRD in pre-weaned calves that were transported within the first week of life. Weight gain was not influenced by metaphylaxis. However, metaphylaxis with two injections of tildipirosin at enrollment and 17 days later tended to reduce mortality and significantly decreased circulating biomarkers of systemic inflammation.

Keywords: High-risk calves, tildipirosin, metaphylactic treatment, BRD.

FE-03

Effect of implant treatments to suckling beef steers on growth performance for 200 days and on lifetime growth performance and carcass traits

R. M. Cleale[1], C. Trejo[1], M. Corbin[1], T. H. Short[1], T. H. Allen[2], B. H. Obendorf[2], M. R. Buyers[3].

[1]Zoetis, Parsippany, NJ, United States; [2]Obendorf Ranch, Jordon Valley, OR, United States; [3]Agri Beef, Moses Lake, WA, United States.

Objectives: The goals of the study were 1) to evaluate the impact of implant treatments administered to suckling beef steers on growth performance over 200 days following treatment, and 2) to determine if suckling calf implant treatments impacted growth performance during grower and feedlot phases when the same implant treatments were equally applied to all cattle, and to assess calfhood implant effects on carcass traits.

Materials and Methods: Growth by spring-born, primarily Angus beef steer calves (n = 261; initial BW = 63 ± 5.1 kg) implanted with Synovex® One Grass (SOG; 150 mg trenbolone acetate, 21 mg estradiol benzoate), Synovex® C (SYNC; 100 mg progesterone, 10 mg estradiol benzoate), or nothing (CON; negative control) was assessed over 200 d in a study with a randomized block design with two pasture management groups. Treatment groups were equally represented in each pasture management group, and individual calf was the experimental unit. Calves remained together with their dams throughout the suckling phase and did not have access to creep feed. After weaning calves were placed in pens in a drylot and fed long hay for 3 d, then were transitioned to a forage-based total mixed ration. At 200 d after suckling calf implant treatments were administered steers were assigned to two drylot pens, each containing proportionately the same number of calves from each treatment group as were enrolled into the study to begin an 85-d backgrounding phase; all cattle received Synovex® Choice at the start of this phase. Upon completion of the backgrounding phase steers entered a commercial feedlot and were fed an average of 193 d, then were harvested and carcass data collected; all steers received a Synovex® One Feedlot at initiation of the feedlot phase. Steer bodyweights were measured at the start and end of suckling, backgrounding, and finishing phases.

Results: Total 200-d BW gain for SYNC (178.7 kg) and SOG (183.4 kg) were greater (P < 0.01) than CON (166.4 kg), but not different from each other (P = 0.16). Similarly, 200-d average daily gain for SYNC (0.89 kg/d) and SOG (0.92 kg/d) were greater (P < 0.01) than CON (0.83 kg/d), but not different from each other (P = 0.16). Cumulative 285-d gain for SYNC (271.2 kg) and SOG (279.5 kg) were different from each other, and greater than CON (256.7 kg; P < 0.05). Total gain over the 193-d finishing period by CON (331.1 kg), SYNC (335.4 kg), and SOG (330.8 kg) were not different (P > 0.05). Thus, implant treatments given to suckling calves did not negatively impact feedlot performance of cattle. From initial implanting to harvest (485 d), SYNC steers gained 16.9 kg more than CON (P = 0.0530), and SOG steers gained 20.4 kg more than CON (P = 0.0202). Carcasses from SYNC weighed 11.5 kg more than CON (P = 0.0554), and SOG carcasses weighed 15.4 kg more than CON (P = 0.0107). Marbling score, ribeye area, backfat thickness, distributions to USDA Quality Grades, and distributions to USDA Yield Grades were unaffected by suckling calf implant treatments. There was no impact of treatments on morbidity or mortality, and there were no observed adverse drug effects attributable to experimental treatments.

Conclusion: Under conditions of this study, a single dose of SOG resulted in average daily gain over a 200-d grazing period of 0.92 kg/d, significantly greater than for steers that remained untreated (0.83 kg/d). Likewise, cattle treated with a single dose of SYNC gained significantly faster than non-treated controls (0.89 kg/d) over 200 d. Implants in suckling calves, with or without trenbolone acetate, increased BW gain, and did not affect performance during backgrounding or feedlot phases.

Keywords: Cattle, suckling, implant, growth rate, average daily gain.

FE-04

Risk factors for antibiotics use and spread of *Mycoplasma bovis* in veal calves feedlots

Marie-Anne Arcangioli[1], Thibaut Lurier[2], Adélie Colin[3], Anthéa Huleux[3], Paule-Azeline Guerineau[1], Karine Hauray[4], Florence Tardy[3].

[1]Université de Lyon, VetAgro Sup, Anses, UMR Mycoplasmoses animales, F-69280, Marcy l'Etoile, France; [2]Université Clermont Auvergne, INRAE, VetAgro Sup, UMR EPIA; Université de Lyon, INRAE, VetAgro Sup, Usc 1233 UR RS2GP, F-69280, Marcy l'Etoile, France; [3]Université de Lyon, Anses, VetAgro Sup, UMR Mycoplasmoses animales, F-69007 Lyon, France; [4]SNGTV, Cabinet le Clair matin, F-01000, Bourg en Bresse, France.

Bovine respiratory disease (BRD) is the most antimicrobial-consuming disease in veal calves production. The presence of *Mycoplasma (M.) bovis,* as one of BRD causative agents, in a feeding lot could contribute to a wider use of antimicrobials as *M. bovis* is known to be persistent and resistant to most antimicrobial families. Controlling some risk factors for introduction or spread of this particular infectious agent may help to also reduce antibiotic (AB) use.

Objectives: Two cross-sectional studies were set up to assess the effect of lot size and feeding systems on *M. bovis* infection spreading and on AB use. We also assess the effect of *M. bovis* spreading as a risk factor for increased AB use.

Material & methods: Twenty-six feedlots were monitored from the "all-in" entry of calves until 3 consecutive weeks without any collective antimicrobial treatment. The spread of *M. bovis* was estimated through seroconversion tested using the BioK302 ELISA kit from BioX Diagnostics, on 10 to 15 calves' sera randomly sampled in each feedlot, at the entry and at the end of the observation period. All oral and injectable AB used meanwhile were recorded. The feedlots were selected according to their feeding system, either individual bucket (n=7) or automatic milk feeders with shared nipples (n=19), and their size, less (n=9) or more (n=17) than 50 calves. For both seroconversion and AB use, statistical analyses were conducted using multivariable generalized linear model with fattening farms as random effect. In both models, we initially included the following variables: feeding system, lot size, age, weight of calves and seropositivity to *M. bovis* at introduction and first order interaction between each variable. Use of AB and seroconversion rate to *M. bovis* were respectively included in the models for seroconversion and use of AB.

Results: The lots were monitored for 42 to 81 days. *M. bovis* infection spread increased with lot size (odd ratio (OR) of 2.9 [1.4; 5.8] per two-fold increase in lot size). The proportion of seroconverted calves was lower in bucket-fed lots compared to automatic feeding lots with a shared nipple (OR = 0.03 [0.003; 0.41]). Analysis of the association with the presence of a seropositive calf at entry was inconclusive. AB use was enhanced in larger feedlots with an increase of 1.5 treatments per two-fold increase of lot-size. For same-sized lots, the use of bucket could decrease AB consumption by up to 1.03 (-2.18;0.14) treatments per calf compared to automatic feeding. Lastly, no association between seroconversion to *M. bovis* and AB use was evidenced.

Conclusion: *Bucket feeding in small size lots, i.e. with a maximum of 50 calves in a same room, contribute to limit seroconversion to M. bovis together with consumption of antibiotics.*

Keywords: Respiratory disease, Calves' lot, Antibiotics, Mycoplasma.

FE-05

Genotyping and antimicrobial resistance patterns of *Mannheimia haemolytica, Pasteurella multocida* and *Histophilus somni* isolated from the upper and lower respiratory tract of feedlot cattle

Edouard Timsit[1], Trevor Alexander[2].

[1]University of Calgary, Calgary, Canada; [2]Agriculture and Agrifood Canada, Lethbridge, Canada.

Background/Objectives: During the last 10 years, the prevalence of *Pasteurellaceae* resistant against drugs typically used for bovine respiratory disease (BRD) control has increased in feedlot cattle. Surprisingly, it is not clear whether this increase in the prevalence of multidrug resistant (MDR) bacteria is due to the spread of one or few multiple MDR clones among cattle during the feeding period (i.e. horizontal spread) or due to the recrudescence of MDR clones already present in the respiratory tract of cattle upon arrival at feedlots. Recently, we reported a high prevalence of MDR *M. haemolytica*, *P. multocida* and *H. somni* isolated from cattle with BP in 4 feedlots in Western Canada. Unfortunately, as we did not genotype these isolates, it was not possible to determine whether a few or a large number of MDR clones were present in these feedlots, supporting either a horizontal spread of MDR clones among cattle or a recrudescence from carriers. Therefore, the objective was to genotype *M. haemolytica*, *P. multocida* and *H. somni* isolates using pulsed field gel electrophoresis (PFGE).

Materials and methods: Newly-received beef-crossed feedlot calves (arrival body-weight ± SD = 282 ± 28 kg) with BRD (n = 210) and pen-matched controls (n = 107) were sampled by deep nasal swabs (DNS) and trans-tracheal aspiration (TTA) at 4 feedlots in Western Canada. *M. haemolytica, P. multocida* and *H. somni* were isolated from DNS and TTA samples and their AMR profiles were determined using broth dilution method. Isolates were then typed by PFGE and grouped into pulsotypes (≥90% similarity).

Results: In total, 195, 277 and 139 isolates of *M. haemolytica*, *P. multocida* and *H. somni*, respectively, were isolated from DNS and TTA samples. A high proportion of *M. haemolytica* (≥ 73%) and *P. multocida* (≥ 78%) isolated from DNS and TTA were resistant against oxytetracycline (OXY) and tulathromycin (TUL). Concerning *H. somni,* there were high levels of resistance against OXY (≥ 52%) and penicillin (PEN; ≥52%) in both DNS and TTA samples. None or few isolates were resistant to florfenicol (FEE), enrofloxacin (ENR) and ceftiofur (CEF). *M. haemolytica* isolates were distributed among 20 pulsotypes and 26 singlets. However, the majority of isolates (54%) belonged to a single pulsotype, which displayed resistance to TUL and OXY. This pulsotype was isolated from 29

different pens across all 4 feedlots. *P. multocida* isolates were distributed among 9 pulsotypes and 11 singlets with the majority of isolates (67%) belonging to one pulsotype that displayed resistance to TUL and OXY. This pulsotype was present in 33 pens across all 4 feedlots. *H. somni* isolates were distributed among 13 pulsotypes and 28 singlets that were either susceptible to all antimicrobials tested or resistant to PEN and OXY. No single dominant pulsotype was observed for *H. somni.*

Conclusions: The genotyping and antimicrobial susceptibility testing of *Pasteurellaceae* isolated from cattle recently placed at 4 feedlots showed that MDR clones of *M. haemolytica* and *P. multocida* can be shared among a large number of cattle within and between feedlots. As cattle were very likely from multiple origins, this finding suggests a horizontal transmission of these clones among cattle shortly after arrival at the feedlots.

Keywords: BRD, AMR, Shipping fever, Antibiotics, Macrolides.

FE-06

Comparison of body temperature measurement using the GUARDIAN® device in feedlots and its possible applications in the early diagnosis of bovine respiratory disease

Iñaki Espinosa[1], Rubén Prieto[1], Natividad Pérez-Villalobos[2].

[1]Tórax SL, Madrid, Spain; [2]Universidad Europea de Madrid, Villaviciosa de Odón, Spain.

Objective: The objective of the present study was to test the new GUARDIAN® radiofrequency body temperature measuring device for beef calves, to compare the temperature detected depending on whether the device is housed in the vagina or in the rumen and to assess possible applications in the early diagnosis of bovine respiratory disease (BRD).

Material & methods: A one-week pilot study was conducted to assess the performance of GUARDIAN®, an externally applied telemetric device for intra-vaginal or reticulo-ruminal use. It is a T-shaped cylindrical device, 9 cm long and 2.8 cm in diameter. This device takes measurements of body temperature in a serial way, once every hour, sending this information to mobile devices, including an alert system in case of fever detection and storage of the data in its own database. The device consists of a temperature sensor on an electronic board, with a timer for sending the data every hour, a battery that lasts longer than the feeding cycle and an antenna for transmitting data. All this is included in a waterproof and biocompatible wrap-around material. Given the long-lasting characteristics of the material, it is reusable and only requires an external antenna to receive the signals, housed in the pen.

The GUARDIAN® device was applied both vaginally and ruminally to five cross-bred beef calves at two different sites (three in the province of Avila and two in Toledo) to assess their functionality, possible undesirable effects (expulsion or interruption of rumination) and to evaluate applications in the early diagnosis of BRD. Calves from Avila were between 10 and 12 months old, while those from Toledo province were between 4 and 6 months old.

Results: All devices were functional regardless of the route of application, and no undesirable effects were detected (rumination was not interrupted in any of them and none of the devices were expelled).

All animals showed daily temperature peaks above 40°C when the devices were housed in the rumen, coinciding with the maximum of rumination activity. In contrast, daily temperature fluctuations emitted by vaginal-housed devices in healthy animals were minimal, coinciding with previous studies in healthy animals (SD=0.16 ºC). All the animals that showed temperatures above 40ºC by a vaginal GUARDIAN® were later explored and showed symptoms compatible with BRD and rectal temperature equal to or higher than 40ºC.

Conclusions: GUARDIAN® showed optimal performance without any undesirable effects and detected sick animals satisfactorily and early, especially those devices placed in the vagina.

Keywords: Radiofrecuency device, BRD.

FE-07

Highthroughput Antibody and Cellular Immune Response Profiling Against Respiratory Pathogens in Calves following a Preconditioning Protocol

Nathan Cebron[1], Vanbergue Elise[2], Blandine Gausseres[1], Julie Cournet[1], Guiadeur Marlène[2], Aurore Philibert[2], Clément Boudenne[3], Lucie Dutheil[3], Béatrice Mounaix[2], Gilles Foucras[3].

[1]IHAP, Toulouse, France; [2]Idele, Paris, France; [3]ENVT, Toulouse, France.

Objectives: Pre-conditionning of calves before entry in fattening units has been promoted as a good approach to reduce antibiotics treatment against bovine respiratory diseases (BRD). Enhancing immunity to respiratory pathogens by vaccination before the risk period may help reduce the prevalence of BRD. The purpose of our study is to evaluate antibody and cell-mediated response against the main repiratory agents in a case/control study in order to evaluate the effects of the pre-conditionning program on immunity to BRD.

Materials and methods: Two groups of calves (n=20) were allocated randomly to each treatment. Pre-conditionned (PREC) calves were weaned and grouped in loose housing approximately 50 days before entry in the fattening unit. They received a balanced diet and were vaccinated twice at 4 weeks interval with an inactivated vaccine against BRSV, BPI3 and *Mannheimia haemolytica.* During that time, control (CON) calves were kept with their dams on pasture without any change. After weaning, they were directly transported to the fattening unit where the vaccination program with the same vaccine was implemented. Blood samples were collected at recruitment in the protocol, and then at the date of entry, and 30 and 60 days after entry, to cover the period of highest BRD incidence in this breeding system. Antibody response against BRSV, BPI3 and *Mannheimia haemolytica* was assessed by ELISA (BioX diagnostics). Whole blood cell stimulation was

prepared with the same pathogens, to assess cellular reponses, and with lipoplysaccharide (LPS) and concanavaline A to measure the levels of innate and adaptive responses, respectively. Cytokine production was measured using a custom bovine cytokines Milliplex assay (MERCK-Millipore).

Results: At the time of inclusion, no difference between the groups was noticed. The average daily gain (ADG), zinc and glutathion-peroxydase plasma concentration were higher in PREC compared to CON (+ 440g /d; + 3pmol / L; + 118U / g Hb) at the time of entry in the fattening unit. Further, specific antibodies for BPI3, BRSV, and *Mannheimia haemolytica* were higher in PREC compared to CON, as was IFNg production in response to *Mannheimia haemolytica*. Immune traits were correlated with several parameters like trace minerals or weight gain despite the low number of evaluated animals and the possibility of confusing factors.

Conclusions: High-throughput profiling of the immune response in young bulls around the entry in fattening units highlight the benefit and limits of pre-conditionning, and opens up promising prospects for the management of BRD in cattle, and improvement of farming conditions.

Keywords: BRD, vaccine, preconditioning, young bulls.

FE-08

Activity, rumination, and performance of BRD treated calves compared to their own baseline activity and healthy cohorts

Jason S. Nickel[1], John P. Hutcheson[1], Pedro Rodriguez[2].

[1]Merck Animal Health, Kansas, United States; [2]Merck Animal Health, Ontario, Canada.

Objectives: The objective of this study was to determine rumination, activity, and performance of cattle treated for BRD with Florfenicol-Flunixin Meglumine combination when compared to their own baseline and healthy cohorts.

Materials & Methods: This experiment was designed as a prospective cohort study in 203 beef calves exposed to naturally-occurring bovine respiratory disease (BRD). Upon arrival, calves were processed with a standard feedlot receiving protocol. All calves were equipped with an ear monitoring tag (Allflex Livestock Intelligence) that captures biometric data. Calves were enrolled at the time of first BRD diagnosis. The case definition for BRD consisted of a clinical score of 1, 2 or 3 and rectal temperature > 104°F. All calves meeting the case definition (N=93) were treated with a fixed combination of Florfenicol–Flunixin Meglumine (Resflor Gold, Merck Animal Health, Madison, NJ, USA). Treated calves were allocated to a separate "sick pen" and followed for 46-days post-diagnosis. The remaining calves not diagnosed with BRD (N=110) were maintained in the original pens. Health, average daily gain (ADG), activity, and rumination parameters were collected on all calves. Data analyses were performed by generalized linear mixed models evaluating the calf as the experimental unit.

Results: No statistical differences were observed in the arrival body weight between calves ultimately categorized as healthy (463.8 lbs, 95% confidence interval [95%CI]; 362.5, 565.2) or sick (461.5 lbs, [95%CI]; 451, 472) (P>0.10). Compared to cattle diagnosed with BRD at least one time, calves never meeting the BRD case definition displayed a heavier final body weight (615.1 lbs [95%CI; 527.4, 702.8] vs 547.7 lbs [524.4, 571.1], respectively) and a greater ADG (3.3 lbs/day, [95%CI; 0.7, 5.9] vs 1.8 lbs/day, [95%CI; 1.4, 2.2], respectively) (P≤0.10). Rumination and activity were also greater (P≤0.10) among healthy calves throughout the study compared to calves treated at least once for BRD.

Additionally, calves treated more than once experienced ongoing reductions in ADG, rumination, and activity parameters (P≤0.10). In calves treated up to three times, the negative impact on ADG was observed as early as the timeframe between enrollment and first BRD treatment compared to calves treated only once or twice (P≤0.10).

The impact of the treatment with Florfenicol–Flunixin Meglumine (Resflor Gold, Merck Animal Health, Madison, NJ, USA) on rumination and activity pre and post administration was also assessed. Post-administration outcomes for both rumination and activity increased compared to pre-treatment levels among calves treated only one time for BRD (P≤0.10). No differences were observed between pre- and post-administration estimates for calves treated twice or three times (P>0.10). No adverse events were observed in this study.

Conclusions: This study showed further evidence that cattle diagnosed and treated for BRD display a negative performance compared to cattle that maintained optimal health. However, cattle that respond favorably to the initial BRD treatment outperform their cohorts that require ongoing therapy. Rumination and activity biometrics were not negatively impacted in this study regardless of the frequency of BRD treatments with Florfenicol–Flunixin Meglumine (Resflor Gold, Merck Animal Health, Madison, NJ, USA) suggesting that this product may not have a negative impact on the animal's gastrointestinal environment.

Keywords: Feedlot, calves, rumination, BRD, monitoring.

FE-09

Reduction in BRD antimicrobial treatments in a US feedlot-based multi-site study using conventional BRD control approach vs. targeted prediction technology

Jason S. Nickel[1], John P. Hutchenson[1], David G. Renter[2], David A. Amrine[1], Juan Pedro Campillo[3].

[1]Merck Animal Health, Kansas, United States; [2]Kansas State University, Kansas, United States; [3]Allflex Livestock Intelligence, Netanya, Israel.

Objective: The study objective was to determine if cattle health and performance comparing a targeted bovine respiratory disease (BRD) prediction technology (BRD_PT; Whisper® On-Arrival) was superior to a negative control (no metaphylaxis) yet no different than a positive control (conventional BRD metaphylaxis; 100% application).

Materials & Methods: Across 4 US study sites (Texas [2 sites; TX-1, TX-2], Oklahoma [OK], Nebraska[NE]), cattle were

procured from various livestock auction markets, processed with the same product regimen, and randomly allocated to one of four BRD control treatment groups: 1. Negative control (Saline), 2. Positive control (Tildipirosin [Zuprevo®] to 100% of the group), 3. BRD_PT-high (± Tildipirosin; more calves treated), and 4. BRD_PT-low (± Tildipirosin; less calves treated). Within either BRD_PT-managed group (i.e. groups 3 and 4), only calves identified to be at high risk for BRD by the technology were administered the BRD control drug; those at predicted lower risk were left without therapy. Calves were penned by treatment group. Three days after treatment administration, cattle were observed daily by pen riders blinded to treatment group assignment. The same BRD case definition and BRD treatment regimen was implemented across all 4 sites. Cattle were followed to either a short-term timepoint (TX-1, 50 days; NE, 60 days) or to closeout (TX-2, 230 days; OK, 240 days). Health and performance outcomes were collected at all respective timepoints. Carcass metrics were captured on those followed to closeout.

Results: Across all sites, BRD control antibiotic use was reduced by 11% to 43% between the two BRD_PT-managed treatment groups compared to the positive control where 100% of the cattle received antimicrobial therapy. The positive control and both BRD_PT-managed groups significantly (P≤0.05) improved numerous health and performance outcomes compared to the negative control. At one site (OK), the BRD_PT-high group displayed a significant improvement in hot carcass weight (P≤0.05) compared to the positive control. However, no further differences (P>0.05) were observed between either BRD_PT-managed group and the positive control at any of the 4 sites.

Conclusion: Across all 4 sites, the BRD_PT technology (Whisper® On-Arrival) maintained the benefits of a conventional BRD control program yet reduced BRD control antibiotic use by 11% to 43%. This technology has the potential to reduce antibiotic costs to the producer while supporting judicious antimicrobial use.

Keywords: Calves, BRD, prediction, technology, metaphylaxis.

FE-10

Environmental sampling for characterization of *Mannheimia haemolytica* shedding by feedlot cattle

William B. Crosby[1], Robert Valeris-Chacin[2], Cory Wolfe[2], Sheryl P. Gow[3], Amelia R. Woolums[4], Paul S. Morley[2].

[1]Missippi State University, College of Veterinary Medicine, Department of Pathobiology and Population Medicine, United States; [2]Texas A&M University, Veterinary Education, Research, and Outreach Program, United States; [3]University of Saskatoon, Western College of Veterinary Medicine, Canada; [4]Mississippi State University, College of Veterinary Medicine, Department of Pathobiology and Population Medicine, United States.

Objective: Control and prevention of Bovine Respiratory Disease (BRD) is a major reason for treatment of cattle with antimicrobial drugs. However, the prevalence of antimicrobial resistance (AMR) is increasing in *Mannheimia haemolytica* and other BRD pathogens which may limit treatment efficacy. Investigation of AMR impacts on BRD requires recovery and characterization of *M. haemolytica* by costly and time consuming sampling of individual cattle. Identifying group sampling methods that are comparable to individual sampling could facilitate surveillance and research. The objective of this study was to compare detection of *M. haemolytica* in cattle via individual sampling using culture and quantitative real-time PCR (qPCR) of nasopharyngeal swabs (NPS) with detection from group sampling methods - water bow swabs, ropes hung on pens, and pooled DNA from NPS.

Materials and Methods: Cattle housed in10 pens located at 3 commercial feedlots in Texas were sampled at 10-22 days on feed. Ten animals from each pen (n=100) were randomly selected and NPS were obtained using rayon swabs for bacterial culture and DNA extraction. Five 1-cm diameter polyester ropes were hung from each study pen for 24 hours. Water bowls from each pen were swabbed at 3 locations in the bowl: the bottom, the waterline, and the top. qPCR for the leukotoxin D gene of *M. haemolytica* was performed in triplicate using DNA from individual NPS (n=10 animals/pen), pools of NPS (n=3 pools/pen), ropes (n=3/pen), and water bowls (n=3/pen). Individual NPS (n=10 animals/pen), ropes (n=2/pen), and water bowls (n=3/pen) were cultured to identify *M. haemolytica*. Mean Ct for each sample type was compared by Kruskal-Wallis analysis of variance on ranks, with post-hoc Dunn test using Benjamini-Hochberg correction.

Results: *M. haemolytica* was only cultured from individual NPS, with animal within pen prevalence ranging from 0-50% of animals sampled (median=10 %). qPCR identified *M. haemolytica* in 41% of individual NPS (n=41), 60% of pooled NPS samples (n=18), 76% of rope samples (n=23), and 78 % of water bowls (n=14 - only 18 samples had sufficient DNA for testing). Overall, ope samples had a lower mean Ct than water bowls, NPS pools, and individual NPS (26.5 vs 30.1, 31.1, 34.9, respectively; p <0.05). However, when analyzing pens separately, for 7 pens, there was no difference in mean Ct for each of the methods (Kruskal-Wallis, p>0.05).

Conclusions: These results support the use of group sampling to characterize group prevalence of *M. haemolytica* via qPCR. Culture of *M. haemolytica* did not reliably identify the agent in group sampling methods.

Keywords: Real-time PCR, BRD, bacterial culture.

GB-01

Estimated breeding values of dairy sires regarding colostrum traits

Aikaterini Soufleri[1], Georgios Banos[2], Nikolaos Panousis[3], Georgios Arsenos[1], Georgios E. Valergakis[1].

[1]Laboratory of Animal Husbandry, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece, Thessaloniki, Greece; [2]Laboratory of Animal Husbandry, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece, Scotland's Rural College/Roslin Institute, Edinburgh, Scotland, UK, Thessaloniki, Greece; [3]Clinic of Farm Animals, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece, Thessaloniki, Greece.

Objectives: Colostrum administration provides newborn calves with important nutrients (fat, protein, lactose) and immunity (IgG). Significant heritability estimates have been reported on colostrum traits ranging from 0.15 to 0.27, suggesting the traits can be improved with selective breeding based on estimated breeding values (EBVs) of selection candidate. The objective of this study was to derive and examine EBVs of Holstein sires for colostrum traits.

Materials and Methods: The study examined daughter of 67 Holstein sires, raised in 6 commercial dairy herds in Northern Greece. Number of daughters (purebred Holsteins with full pedigree) for each sire ranged from 5 to 49; the total number of cows with records in the study was 699. These cows calved between February 2015 and September 2016. Cows were milked completely after calving and a colostrum sample was collected. Fat, protein and lactose content (%) were determined with Milkoscan. Colostrum total solids (TS) were assessed with a digital Brix refractometer and expressed in % Brix values. Sire EBVs for colostrum traits (TS, fat, protein and lactose content) were derived with univariate statistical analyses based on a mixed model. The model included the effects of farm, parity number, calendar season, age at calving, colostrum yield, time interval between calving and colostrum collection, dry period length, cow body condition score at calving and milk yield of previous lactation, and the random animal additive genetic effect. The ASREML software was used for all statistical analysis.

Results: Sire EBVs were normally distributed. The EBV for colostrum TS ranged from a -4.05 to +3.47 (average EBV reliability 0.42). The EBV difference between the 10th and the 90th percentiles was 3.20% (-1.50 and +1.70, respectively). The phenotypic difference in % Brix values between daughters of sires in the 10th and 90th percentiles was 5.6% (22.6 and 28.2, respectively)**.** The EBV for colostrum fat content ranged from a -2.60 to +1.29 (average EBV reliability 0.29). The EBV difference between the 10th and the 90th percentiles was 2.23% (-1.56 and +0.67, respectively). The phenotypic difference of fat content between daughters of sires in the 10th and 90th percentiles was 3.7% (4.2 and 7.9, respectively). The EBV for colostrum protein content ranged from a -2.76 to +2.04 (average EBV reliability 0.34). The EBV difference between the 10th and the 90th percentiles was 2.32% (-0.92 and +1.40, respectively). The phenotypic difference of protein content between daughters of sires in the 10th and 90th percentiles was 4.4% (15.7 and 20.1, respectively). The EBV for colostrum lactose content ranged from a -0.34 to +0.38 (average EBV reliability 0.24). The EBV difference between the 10th and the 90th percentiles was 0.33% (-0.21 and +0.12, respectively). The phenotypic difference of lactose content between daughters of sires in the 10th and 90th percentiles was 0.9% (1.7 and 2.6, respectively). Three bulls had positive EBVs and 5 had negative EBVs for all four traits studied. When considering TS, fat and protein content only, 13 bulls had positive EBVs and 19 had negative EBVs for all three traits. Mean EBVs for the 13 "positive" bulls were 1.05, 0.44 and 0.61, for TS, fat and protein content, respectively. Mean EBVs for the 19 "negative" bulls were -1.10, -0.66 and -0.67, for TS, fat and protein content, respectively.

Conclusions: Variability in sire EBVs regarding colostrum traits supports the concept of genetic selection leading to trait improvement. A synthetic colostrum quality index could be potentially developed comprising sire EBVs on individual traits. This index could be eventually included in an overall performance index that will drive future breeding programs.

Keywords: Breeding values, sire, cow, colostrum.

GB-02

The genetic architecture of susceptibility to claw horn disruption lesions in Holstein cows

Matthew Barden[1], Bingjie Li[2], Alkiviadis Anagnostopoulos[1], Cherry Bedford[3], Bethany Griffiths[1], Androniki Psifidi[4], Georgios Banos[2], Georgios Oikonomou[1].

[1]University of Liverpool, Liverpool, United Kingdom; [2]Scotland's Rural College, Edinburgh, United Kingdom; [3]University of Liverpool, Edinburgh, United Kingdom; [4]Royal Veterinary College, North Mymms, United Kingdom.

Objectives: Lameness is the most serious welfare problem facing the modern dairy industry. The lesions associated with the greatest impact on animal welfare, and those which incur the greatest financial costs to farms, are claw horn disruption lesions (CHDLs). The three most prevalent CHDLs are sole haemorrhage, sole ulcers and white line disease. In the United Kingdom, breeding strategies have not reduced the prevalence of lameness in the national herd and this is due, in part, to the paucity of detailed phenotypic data to inform genetic selection. Previous research from our group has identified the existence of genomic variation and regions that were associated with specific lameness lesions, suggesting value in pursuing this area of research [1]. The aims of this study were to determine the genetic variance and genomic regions which underly CHDL development.

Materials and methods: Over an eight-month period, 2,353 Holstein cows were enrolled on four dairy farms in the United Kingdom. Animals were assessed at four timepoints: prepartum (average: 56 days prepartum); freshly calved (average: 5 days postpartum), early lactation (average: 84 days postpartum) and late lactation (average:182 days postpartum). At each timepoint feet were inspected by a trained veterinarian and all lesions present were recorded and scored by severity.

Pedigrees were obtained for all cows with phenotypes, and 1,622 cows were genotyped with the Illumina 50K Bovine SNP chip. Lesion scores at the first three timepoints were combined to create an overall CHDL score, accounting for lesion severity and total number of feet inspections per cow (lesions from late lactation timepoint will be included in the final analyses). CHDL score was used as the phenotype for genomic analyses. A single-step GBLUP method was used to calculate heritability and the genetic correlations between CHDL score at different timepoints. Genome-wide association (GWA) analyses were applied to identify candidate genomic regions associated with CHDL score, using both single-marker and window-based association. Significance testing of SNP effects were adjusted using Bonferroni and False Discovery Rate approaches to control for multiple testing during GWA analyses.

Results: The average cow-level prevalence, across all timepoints and including all severity grades, was 43.3% for sole haemorrhage, 4.6% for sole ulcers and 35.8% for white line disease. Across all timepoints, the heritability for the combined CHDL score was 0.18 (SE: 0.03), using both pedigree-based and genomic approaches. The genetic correlation between CHDL score at different timepoints was high (>0.9). GWA analyses did not identify any SNP to be significant for CHDL score after the Bonferroni correction, but some SNPs exceeded a suggestive threshold after correcting for one false discovery per genome scan. When markers were grouped by 1Mb sliding windows, several candidate genomic regions were identified which explained larger proportions of genetic variance for CHDL score than other regions in the genome, approximately 3-4% of the total genetic variance. Estimated genomic breeding values for the combined CHDL score were calculated and showed good correlation with phenotypic values (0.67).

Conclusions: The genetic correlation between CHDL scores at each timepoint was high. This suggests an underlying genetic basis which was not dependent on environmental factors specific to the stage of lactation. As a combined phenotype, CHDL development is moderately heritable, and the estimated genomic breeding values indicate a reasonable correlation with the observed phenotype. Therefore, there is the potential to incorporate CHDL resistance into future breeding programmes. The presence of multiple candidate genomic regions suggests a polygenic architecture of CHDLs; further study is warranted in order to elucidate specific genes and molecular pathways involved.

References:

1. Sánchez-Molano E, Bay V, Smith RF, Oikonomou G, Banos G. Quantitative Trait Loci Mapping for Lameness Associated Phenotypes in Holstein–Friesian Dairy Cattle. Front Genet. 2019;10:926. doi:10.3389/fgene.2019.00926.

Keywords: Claw horn disruption lesions, GWAS, Heritability, Lameness, Welfare.

GB-03

Aging increases inflammatory response in dairy cattle

Gilles Foucras[1], Nathan Cebron[2], Sarah Walachowski[2], Aurélie Chaulot-Talmon[3], Charline Pontlevoy[3], Rodolphe Robcis[1], Alice De Boyer Des Roches[4], Dorothée Ledoux[4], Valérie Gelin[3], Christophe Richard[3], Hélène Kiefer[3], Hélène Jammes[3].

[1]ENVT, Toulouse, France; [2]IHAP, Toulouse, France; [3]BDR, Jouy-en-Josas, France; [4]UMRH, Saint- Genès-Champanelle, France.

Objectives: Old cows are reputed more sensitive to bacterial infections, developping more severe mastitis than youngest ones. However the biological basis of these observations are not known. Risk factors increase with aging, but inflammation and innate immunity are modified in the long term by previous exposure to microbes. Epigenetic changes of immune cells like monocytes could explain increased severity to infection. Monocytes are central in these mechanisms, and they undergo functional changes upon contact with pathogens or their products, and adapt their response to subsequent challenges. However, the molecular bases of long-term reprogramming are still poorly understood in cattle.

Materials and methods: Twenty-three cows were challenged through an intravenous bolus injection of LPS (0.5 µg/kg BW, ultrapure LPS, InVivogen) to induce a systemic inflammatory response. Fourteen of them were bovine somatic clones originating from the same cell line, in two groups of 5 and 15-years of age, respectively. A genetically-diverse group of 5 years-old cows (n=9) was also included in the study. All cows were raised and housed together as a single group since birth in an experimental farm. Cytokine production was measured sequentially (0, 3, 6, 12, and 24h) in plasma using a newly-developed custom bovine cytokines 15-plex Milliplex assay (MERCK-Millipore). Blood samples were collected twice at 0 and 24 hours after LPS injection. Monocytes were isolated and their genome-wide DNA methylation profile was determined by reduced representation bisulfite sequencing (RRBS) using a dedicated pipeline, in order to assess epigenetic marks according to the age, genetic background, and response to LPS.

Results: LPS exposure was associated with the production of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α). IL-6 and TNF-α production were higher in aged compared to young cows, and clinical signs were more severe in the former, indicating a stronger inflammatory response according to the age. Differentially-Methylated Cytosines (DMCs) targeting genomic regions important to monocyte identity and functions, independently of the genetic background, were identified. LPS stimulation causes hypomethylation in dairy cows whatever the age. Monocytes undergo epigenetic modifications after LPS challenge, indicating that previous exposure to Gram-negative bacteria, may modify the later capacity of the cells to respond to an infection. Comparison of young and old cattle led to identification of epigenetic marks related to aging.

Conclusions: Aged cows have a stronger inflammatory response that correlates with the presence of specific marks that develop during the course of life. Knowledge on epigenetic marks induced by aging may help define new breeding and prevention strategies.

Keywords: Inflammation, aging, epigenetic, dairy cows.

GB-04

Impact of host genetics on resistance of bovine monocyte-derived macrophages to *Mycobacterium avium* subsp. *paratuberculosis* infection

Maria Canive, Gerard Badia-Bringué, Marta Alonso-Hearn.

NEIKER, Derio, Spain.

The application of animal genetics in breeding programs is currently one of the important motors for efficient livestock production, not only to increase performance and productivity but also to ensure the resilience and health of livestock while maintaining or improving the longevity of animals. Genetic selection to enhance the resistance of dairy cattle to paratuberculosis (PTB) and other bovine diseases is being extensively explored. Resistance is defined as the ability of the host to prevent invasion or to clear the pathogen at the early stage of infection by mounting a protective immune response. However, the genetic loci influencing individual resistance to *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection and the primary molecular and cellular mechanisms underlying host resistance are still largely unknown. MAP spends most of its life cycle within macrophages which play a crucial role during all phases of infection. It is generally accepted that less Mycobacteria growth in macrophages *in vitro* implies immune restriction and hence less susceptibility, lower risk of infection or disease *in vivo*. Since macrophages functions are controlled by a limited number of genes under a controlled environment, the probability of identifying animals with a superior innate immune response against MAP using *ex vivo* macrophages models is much higher in comparison to field data. In the current study, we searched for genetic loci associated with resistance to MAP infection by evaluating the performance of MAP-infected monocyte-derived macrophages (MDMs) isolated from peripheral blood of 75 Holsteins cows and infected *ex vivo* with MAP. Bacterial load (log colony-forming unit, CFU) within MDMs was quantified at 2h and 7 days p.i. using a Bactec MGIT 960 instrument. In addition, the levels of some host biomarkers such as *Epiregulin* (*EREG*), *Complement C3 (C3)*, *galectin-9* (*LGALS9*), and *nitric oxide (NO)* were measured in the supernatant of the infected cells at 2 h p.i by ELISA. DNA from peripheral blood samples of the animals included in the study were genotyped with the EuroG MD bead Chip (44,779 single nucleotide-polymorphisms, SNPs). Linear mixed models were used to calculate the heritability (h^2) estimates and variance components for each phenotype; the amount of MAP within MDMs and biomarkers expression. After performing a genome-wide association study, the localization of associated SNPs, QTLs, and candidate genes was performed using the ARS-UCD1.2 reference *Bos Taurus* genome. The only phenotype that showed SNPs with a significant association ($P_{FDR} \leq 0.05$) was the bacterial load within MDMs at 2h (h^2= 0. 87) and 7 days (h^2= 0.83) p.i. The six identified SNPs were located on the *Bos taurus* chromosomes BTA2, BTA17, BTA18, and BTA21. Overlap was seen in two SNPs associated with the logCFUs at 2h and 7 d. p.i. All the identified SNPs had negative regression coefficients, and were, therefore, associated with a low bacterial load within MDMs. The following candidate genes were identified within a 100 Kb window of the significant SNPs: Oxysterol Binding Protein like 6 (OSBPL6), Serine Rich Nuclear Protein 3 (CSRNP3), and the Coiled-Coil Domain Containing 92 (CCDC92). OSBPL6 is an intracellular lipid receptor that contributes to the maintenance of cholesterol homeostasis by regulating cellular cholesterol trafficking and efflux. CSRNP3, also named TGF-Beta Induced Apoptosis Protein 2, is a transcriptional activator of apoptosis. The CCDC92 is an interferon-stimulated protein that plays a role in innate immunity and regulation of defense response. Estimated breeding values (EBVs) for the ratio of logCFUs at 7d/2h were calculated for each animal in the study population using the best linear unbiased prediction (GBLUP) model and the genomic predictions were validated in a population of animals with high (N= 8) and low (N= 8) EBVs. MDMs from these animals were infected *ex vivo* with MAP and the correlations between the logCFUs ratios within MDMs and EBVs were positive (Pearson R=0.77). Taken together, our results define a heritable and distinct immunogenetic profile in MAP-infected macrophages designed to limit bacterial load and inflammation early after infection. The identified SNPs could be used to develop genetic evaluations for immunocompetence in the Spanish breeding program which would allow producers to select cattle more resistant to MAP infection and likely to other intracellular pathogens as well; ultimately reducing the prevalence of diseases, preventing economic losses, increasing the length of cattle productive life, and improving food safety.

Keywords: Host resistance, breeding, macrophages, paratuberculosis, innate immune response.

GB-05

Inherited zinc deficiency-like syndrome in Holstein cattle due to a loss-of-function mutation of IL17RA-transmembrane protein

Sickinger Marlene[1], Häfliger Irene[2], Holsteg Mark[3], Raeder Leif M.[1], Henrich Manfred[1], Marquardt Siegfried[4], Drögemüller Cord[2], Lühken Gesine[1].

[1]Justus-Liebig-University Giessen, Giessen, Germany; [2]Vetsuisse University Bern, Bern, Switzerland; [3]Animal Health Service, Bad Sassendorf, Germany; [4]Veterinary Practice Dres Marquardt and Walter, Goch, Germany.

Objectives: Skin lesions and dermatoses in cattle are often associated with infections due to bacteria, fungi or environmental risk factors. Dermatoses with genetic etiology have also been described. Among these rare disorders, there are primary congenital disorders (e.g. epidermolysis bullosa) and dermatoses that are associated with inherited nutritional deficiencies, such as bovine hereditary zinc deficiency or zinc-deficiency-like syndrome. Our study presents cases with skin lesions observed on a Holstein farm in the Midwest of Germany that resemble zinc deficiency-like syndrome. The origin of all affected calves from a single sire and the same maternal grand sire let us to assume a potential genetic etiology.

Materials and methods: Close clinical and pathological examinations took place in two affected calves. Due to the suspected genetic background, genome sequencing of the

two affected Holstein calves followed by single-nucleotide variant and small-indel variant calling were performed. Available normal relatives of the two calves were genotyped by Sanger sequencing for identified potential causative protein-changing sequence variants.

Results: The two calves suffered from severe ulcerative dermatitis with hyperkeratosis, alopecia furunculosis and subcutaneous abscess formation. Blood analysis showed correspondent leukocytosis with neutrophilia whereas minerals, macro- and micronutrients were within the reference ranges. Genetic analyses delivered 4111 homozygous private variants including 23 protein-changing variants shared by both affected animals. Comparison with the 1000 Bull Genomes variant catalogue resulted in a single privately remaining protein-changing variant. This single-nucleotide deletion in exon 3 of *IL17RA* on bovine chromosome 5 was predicted to have a deleterious impact on the encoded protein due to a frameshift. Healthy mothers of affected calves as well as some other relatives were determined to be heterozygous for this mutation, confirming the assumed autosomal recessive inheritance.

Conclusions: A loss-of-function mutation of the IL17RA transmembrane protein that binds with low affinity to interleukin 17A could be identified as pathogenic variant for the psoriasis-like skin alterations observed in the two affected Holstein calves. In man, rare diseases associated with *IL17RA* include immunodeficiency 51 and chronic mucocutaneous candidiasis. The frequency of the recessive defect allele in the German (and global) Holstein population needs to be analyzed.

Keywords: Skin lesions, calves, genetics, immunodeficiency.

GB-06

Temperature-humidity effect on rumination time and activity in Holstein and crossbred Holstein x Jersey cows

Roberto Kappes, Deise Knob, Angelica Scheid, Bruna Mendes, Bruno Barreta, Laiz Perazzoli, André Thaler Neto.

Universidade do Estado de Santa Catarina, Lages, Brazil.

Objective: We aimed to compare temperature-humidity effect on rumination time and activity in different genetic groups of cows: Holstein, F1 Holstein x Jersey and R1 3/4Holstein x Jersey.

Material and methods: We made the study at the dairy cattle sector of Centro de Ciências Agroveterinárias of Universidade do Estado de Santa Catarina during the period between September 2018 and August 2019. There were 22 multiparous lactating cows, 7 Holstein, 5 crossbred F1 (½Holstein x Jersey) and 10 crossbred R1 (¾ Holstein x ¼ Jersey). Cows were mechanically milked twice a day, at 7:00 and 15:00. After milking the cows received concentrate in troughs separated by individual contention feeders. Posteriorly, the cows had free access to pasture with water ad libitum and available shadow area, staying in the paddock until the next milking. Before the study period, the cows were equipped with SCR by Allflex® electronic monitoring collars aiming adaptation and indexing in the software *HealthyCow24* – SCR.

To evaluate environmental interference over the cows' performance, we calculated the temperature-humidity index (THI) using environmental temperature and relative humidity through a Data Logger Akrom® model KR420, set to collect data every one hour.

For analysis of variance, the highest THI of the day data were used, and to evaluate THI effect on activity and rumination the mean was made every couple of hours. This mean matches the time given by the individual rumination and activity monitoring software. We used all the data for one year. Six THI classes were created: safe (<68), light (68≤ < 72), discomfort (72≤ <75), alert (75≤ <79), danger (79≤ <84) and emergency (≥84), but we didn't observe the last one.

Data were submitted to ANOVA with repeated measures over time, using the MIXED procedure of the SAS statistical package, and the covariance structure was defined based on the Akaike Information Criterion (AIC). Data were previously tested for residue normality by the Kolmogorov-Smirnov Test.

For the evaluations of the effect of THI on rumination time and activity at two-hour intervals, the statistical procedure used was similar to the one described above. Significant differences at the 5% and trend at 10% were considered.

Results: As shown in Table 1, activity unity was higher for R1 crossbreds and lower for Holstein, these not differing from crossbred F1 (0,0238). There was an increase in activity unity as THI increased (0,0001).

Table 1 – Mean values adjusted to the model ± standard deviation for different temperature-humidity indexes (THI) in different genetic groups for rumination time and activity unit.

	Activity				**Rumination**			
THI classes	**H**	**F1**	**R1**	**P value**	**H**	**F1**	**R1**	**P value**
Safe	52,5±2,1n	59,0±3,8ln	58,0±9,2mn	<0,0001	49,2±1,1ab	49,4±2,0ab	52,6±4,7a	0,7249
Light	71,1±2,1km	80,6±3,8lj	77,8±9,3hjkl		41,8±1,1cd	42,4±2,1cd	45,4±4,9bc	
Discomfort	86,1±2,1ghi	93,8±3,9dfgh	92,2±9,4efgi		31,6±1,2eg	31,9±2,2eg	34,0±5,1def	
Alert	101,9±2,1df	111,2±3,9bce	112,2±9,4cd		21,5±1,2h	23,7±2,2fh	22,2±5,1ghi	
Danger	119,4±2,3bc	133,8±4,2a	128,3±9,7ab		13,9±1,4i	13,5±2,6i	15,6±5,5hi	

*Different letters on the line represent significant difference (P <0.05).

We also observed a lower rumination time for Holstein (P=0,0002). While THI increases, rumination time decrease. When compared inside the THI class, there is no difference between genetic groups. There was no interaction between genetic groups and THI classes. (P=0,7249).

As shown in Table 1, Holstein cows showed lower activity unit in relation to R1 crosbreds, which did not differ from F1 crossbreds. We observed an interaction effect (<0,0001) between genetic group and THI classes for activity unity.

Conclusion: The highest THI negatively affects rumination time, increasing activity unity values. There is no interaction between genetic groups and THI on rumination time. It seems that no tested genetic group is best suited for high THI classes.

Keywords: Animal improvement, Heat stress.

GB-07

Conformation traits and Characteristics of Somatic Cells Counting in Holstein Cattle in Brazil - A Multivariate Approach

Angelica Scheid, Bruna Mendes, Rayllana Larsen, Luiz Schaitz, Mauricio Civiero, Marciel França, Adriana Hauser, Deise Knob, Roberto Kappes, Laiz Perazzoli, André Thaler Neto.

Universidade do Estado de Santa Catarina, Lages, Brazil.

Objectives: Identify the linear type traits which together affect the somatic cell count in Holstein cattle in Brazil.

Material and methods: We used recorded data from approximately 45,000 animals of the Holstein breed collected by the Dairy Control Service and type assessment by the Brazilian Association of Breeders of the Holstein Breed in the period from 2000 to 2010.

For somatic cell count (SCC) the average of observations from dairy controls was considered, and the data were converted into somatic cell scores (SCS). For the type characteristics (TYPE) the 21 characteristics were used.

Initially, genetic values for cows and bulls were estimated using the maximum likelihood method using the MTDFREML software. By the Stepwise method of the REG procedure of the SAS statistical package, the type characteristics that are individually related to the SCS were previously selected. With these characteristics, factor analysis was performed using the FACTOR Procedure, using the genetic values of all females and bulls with more than five female calves. The factors that together explained more than 70% of the accumulated variance were maintained. In addition, 20 bulls with a greater number of female calves were selected to submit the cluster analysis using the tocher method, which grouped similar bulls together.

Results: For cows' genetic values (Table 1) it is observed in the second factor that SCS presented opposite relation with legs side view and intermediate genetic values relation for anterior udder insertion, anterior teat placemen and median ligament. In the fourth factor the relationship between SCS and legs side view was positive, contrary to the average ligament and genetic relationship of intermediate values for the others.

Table 1 - Factor loads and percentage of variance explained by each factor referring to the genetic values of cows for SCS and TYPE.

Variables	Factor 1	Factor 2	Factor 3	Factor 4
SCS and TYPE				
SCS	0,24244	**-0,71914**	0,35003	**0,54411**
LSV	0,24445	**0,70645**	0,45605	**0,46085**
AUI	0,53970	0,09939	-0,62496	0,39049
ATP	0,71576	-0,04229	-0,19789	-0,25884
ML	0,60600	-0,03582	0,46633	**-0,44562**
VARIANCY%	25,79	20,58	19,55	18,53

Somatic Cell Score (SCS), Legs Side View (LSV), Anterior Udder Insertion (AUI), Anterior Teat Placement (ATP), Median Ligament (ML).

For the genetic values of bulls (Table 2), in factor 3, the ECS presented a contrary relationship to the legs side view and a relationship with intermediate genetic values for the other characteristics. In the fourth factor, this relationship was positive between them and with intermediate genetic values for the other characteristics.

Table - 2 Factor loads and percentage of the variance explained by each factor referring to the genetic values of bulls with more than five female calves for SCS and TYPE.

Variables	Factor 1	Factor 2	Factor 3	Factor 4
SCS and TYPE				
SCS	0,11943	0,30507	**-0.59546**	**0,73322**
LSV	0,29330	-0,01576	**0,75114**	**0,56175**
AUI	0,60973	-0,60710	-0.11042	0,08902
ATP	0,76077	0,02167	-0,20186	-0,29173
ML	0,42002	0,76633	0,17072	-0,20157
VARIANCY%	24,54	21,00	20,01	19,74

Somatic Cell Score (SCS), Legs Side View (LSV), Anterior Udder Insertion (AUI), Anterior Teat Placement (ATP), Median Ligament (ML).

Table 3 shows that most of the highly selected bulls have an intermediate value for SCS. However, there are bulls with high genetic value for SCS, which can impair the genetic gain in this trait. Among the improver bulls for SCS (negative genetic value) was not observed a profile for TYPE.

Table 3 - Groups of bulls separated by the variance of their genetic values for each trait resulting from the cluster analysis of the 20 bulls with the largest number of female calves for SCS and some trait characteristics.

SCS e TYPE					
	MEANS				
BULLS	SCS	LSV	AUI	ATO	ML
886; 391; 772; 452; 835; 478; 631; 501; 427; 1003; 250; 376; 160; 379;	0,016445	-0,09	0,476143	0,047143	0,422643
879; 798;	0,295323	-0,4875	0,4465	0,727	-0,2925
1005; 34	-0,35184	0,3265	-0,3465	0,4985	1,1645
987;	-0,65471	0,006	-1,261	-0,286	-0,743
692;	0,932983	-0,393	-0,144	0,52	0,957

SoAnterior Teat Placement (ATP), Median Ligament (ML).

Conclusions: Selecting for intermediate genetic values of anterior udder insertion, anterior teat placement and middle ligament tends to decrease the somatic cell score.

Keywords: Data bank, Genetic value.

GB-08

Dry matter intake, body condition score and, beta-hydroxybutyrate of Holstein and crossbred Holstein x Simmental cows during the transition period

Deise Knob[1], Roberto Kappes[1], Laiz Perazzoli[1], Bruna Mendes[1], Dileta Alessio[2], Wagner Bianchin[3], Armin Scholz[4], André Thaler Neto[1].

[1]Universidade do Estado de Santa Catarina, Lages, Brazil; [2]Centro Universitário Leonardo da Vinci, Indaial, Brazil; [3]Instituto Federal Catarinense, Santa Rosa do Sul, Brazil; [4]Ludwig Maximilians Universität München, Munich, Germany.

Objectives: We aimed at a comparison between purebred Holstein and crossbred Holstein x Simmental cows for dry matter intake (DMI), body condition score (BCS), body weight (BW) and β-hydroxybutyrate (BHB) during the transition period.

Materials and Methods: The research was carried out in a compost bedded pack barn confinement system in a commercial dairy farm in South Brazil. A total of 30 multiparous cows (18 Holstein and 12 crossbred F1 Holstein x Simmental cows) entered the study. Each cow entered the study 21 days before the expected calving day (prepartum) and stayed in the research group until day 21 after calving (postpartum).

Twice a day, the prepartum cows received a total mixed ration (TMR) based on maize silage and a commercial pre lactation concentrate. The postpartum cows received a TMR based on maize silage, ryegrass (fresh and silage), and concentrates. Cows were mechanically milked 3 times a day, and both genetic groups had an average daily milk yield of 29 kg. After each milking, the postpartum cows had access to the feed parlour.

The offered TMR and non-consumed feed of each cow were weighted to allow individual feed intake measurements. The TMR was offered *ad libitum* allowing 5-10% residuals. Weekly, body weights and BCS of cows were recorded. The BCS evaluation was based on a scale between 1 (extremely thin) and 5 (very fat). On the same day, blood was sampled for the immediately performed BHB measurement using an electronic handheld device (Precision Xtra meter, Abbott Diabetes Care). To obtain the daily rumination data, we used the data collected by the Heatime® (SCR/Allflex) system. The data were analyzed by the MIXED procedure of the SAS (SAS 2002) statistical package. The model was composed of the fixed effects genetic group, period (pre/post partum), and the interaction between them.

Results: Holstein and crossbred Holstein x Simmental cows have a similar DMI (Table 1). There is a difference between pre and postpartum DMI with a higher value after calving, which represents about 6-7 kg/day. Both genetic groups have similar BW, with an interaction between the genetic group and the transition period. Before calving, there was no difference for BW between both genetic groups, while the difference after calving reached 30 kg. The DMI % to body weight did not differ between Holstein and Holstein x Simmental crossbred cows. Even with similar DMI, crossbred cows have a better BCS during the transition period than the purebred Holsteins. This difference is highlighted in the postpartum period with 0.8 points advantage for the crossbred cows.

The BHB values do not differ between the genetic groups. It seems that the genetic group does not affect BHB since both genetic groups have similar milk yields and DMI. We just observed a difference for the period, with lower prepartum values.

Because of having similar DMI, there was no difference in rumination time between the genetic groups, as well as no interaction between genetic group and period.

Conclusions: Both genetic groups present a similar dry matter intake during the transition period, but the crossbred cows are more efficient by showing a better BCS before and after calving. The Holstein cows lose more BCS and body weight after calving than the crossbred cows.

Acknowledgments: To the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES)* – Finance Code 001.

Keywords: BHBA, BCS.

Table 1: Least Squares Mean ± mean squares error and P-value for Genetic Group (GG), transition period, and their interaction for the variables dry matter intake (DMI), body weight (BW), body condition score (BCS), rumination time (RT), and beta-hydroxybutyrate (BHB) for purebred Holstein (H) and F1 Holstein x Simmental (H x S) crossbred cows.

Variable	GG	Period		P-Value		
		Prepartum	Postpartum	GG	Period	GG*Per
DMI(Kg/day)	H	9.23±0.5	15.98±0.5	0.5224	<0.0001	0.0412
	H x S	9.32±0.6	16.44±0.6			
DMI % of BW	H	1.33±0.10	2.40±0.07	0.3623	<0.0001	0.5425
	H x S	1.16±0.14	2.36±0.10			
BCS	H	3.58±0.11	2.95±0.10	<0.0001	<0.0001	0.1287
	H x S	4.13±0.15	3.74±0.13			
BW(Kg)	H	744.7±17.5	632.3±17.1	0.5156	<0.0001	0.0379
	H x S	750.3±21.8	661.9±21.2			
BHB(mmol/l)	H	0.77±0.12	1.26±0.10	0.5226	0.0004	0.2610
	H x S	0.79±0.16	1.04±0.13			
RT(minutes/day)	H	478.7±14.5	552.0±14.1	0.4961	<0.0001	0.3003
	H x S	471.4±18.4	529.7±17.4			

GB-09

Milk yield and milk composition during the first three weeks of the postpartum period of Holstein and crossbred Holstein x Simmental cows

Deise Knob[1], Armin Scholz[2], Roberto Kappes[1], Laiz Perazzoli[1], Bruna Mendes[1], Wagner Bianchin[3], Dileta Alessio[4], André Thaler Neto[1].

[1]Universidade do Estado de Santa Catarina, Lages, Brazil; [2]Ludwig Maximilians Universität, Munich, Germany; [3]Instituto Federal Catarinense, Santa Rosa do Sul, Brazil; [4]Centro Universitário Leonardo da Vinci, Indaial, Brazil.

Objectives: We aimed at comparing purebred Holstein and crossbred Holstein x Simmental cows for milk yield (MY) and composition during the first three weeks after calving.

Materials and Methods: The research was carried out in a compost bedded pack barn confinement system on a commercial dairy farm in South Brazil. A total of 30 multiparous cows with 18 Holstein and 12 crossbred F1 Holstein x Simmental cows entered the study. All cows with 3 or more parities that calved within the experimental time entered the study. The cows had a dry off period of 60 days before the expected day of calving. Each cow entered the study 21 days before the expected calving day (prepartum) and stayed in the research group until day 21 after calving (postpartum). Both genetics groups had a postpartum dry matter intake of around 16 kg/day.

Cows were mechanically milked 3 times a day, and the individual MY was electronically recorded (DeLaval®). Individual milk samples were taken every 7 days in 40-mL bottles containing Bronopol® as a bactericidal preservative. Each sample consisted of an average mixture of the 3 daily milkings and was sent to the laboratory of milk analysis from the Universidade do Estado de Santa Catarina, UDESC/Lages, SC, Brazil. The samples were analyzed for milk composition by the infrared method with a DairySpec (Bentley®) equipment.

The data were analyzed by the MIXED procedure of the SAS (SAS 2002) statistical package. The statistical model included the fixed effects genetic group, week postpartum and the interaction between them.

Results: Both genetic groups produced about 21 liters/day at the calving day. Holstein cows and crossbred Holstein x Simmental cows yielded similar amounts of milk. After calving, the milk production increased quickly until reaching amounts of around 34 liters/day at the third week postpartum (Table 1). Genetic groups did not differ in the protein and lactose contents of the milk (Table 2). Yet for fat content, crossbred Holstein x Simmental cows tend to produce milk with higher percentages (P=0.0593) during the first 3 lactation weeks.

Table 1: Least squares means ± mean squares errors for milk yield of purebred Holstein (H) and F1 Holstein x Simmental crossbred (H x S) cows during the first three weeks after calving.

	Week	**H**	**H x S**
Milk Yield (kg)	1	31.9563±1.618	28.814±2.0454
	2	33.8914±1.5786	32.1865±1.9954
	3	34.4867±1.6364	33.4118±2.0336

Table 2: Least Squares Means (LSM) ± mean squares errors and P-value for Genetic Group (GG), week, and their interaction for the variables related to milk yield, energy corrected milk (ECM) and milk composition for purebred Holstein (H) and F1 Holstein x Simmental crossbred (H x S) cows.

Variable	GG	LSM	P-Value		
			GG	Week	GG*Week
Milk Yield (Kg)	H	30.51±1.43	0.4741	<0.0001	0.5006
	H x S	28.83±1.81			
ECM*	H	38.19±1.48	0.4123	0.4044	0.7712
	H x S	35.90±2.30			
Fat (%)	H	3.99±0.13	0.0593	0.0040	0.5161
	H x S	4.48±0.20			
Protein (%)	H	3.31±0.07	0.6316	0.0027	0.0807
	H x S	3.37±0.10			
Lactose (%)	H	4.57±0.03	0.8322	0.0009	0.3743
	H x S	4.58±0.06			
Fat + Protein (Kg)	H	2.54±0.09	0.5421	0.7828	0.8578
	H x S	2.42±0.15			

Conclusion: This study demonstrates that purebred Holstein and crossbred Holstein x Simmental present similar milk yields and milk composition during the first three weeks of the postpartum period.

Acknowledgments: This study was financed by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES)* – Finance Code 001.

Keywords: Transition period, Lactation.

GB-10

Genetic analysis of juvenile spastic paresis in Romagnola cattle

Joana G. P. Jacinto[1], Irene M. Häfliger[2], Anna Letko[2], Cord Drögemüller[2], Arcangelo Gentile[1].

[1]Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia (Bologna), Italy; [2]Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland.

Objectives: Bovine spastic paresis (BSP) is a juvenile-onset neuromuscular disorder that affects males and females occurring in various breeds of cattle. Clinically BSP is characterized by overextension of the gastrocnemius muscle causing a "straight hock" with an increase of the tibiotarsal angle of one or both hind limbs. Signs of BSP usually appear at the age of 3 to 8 months, although the disorder can be observed also earlier or later in life. BSP is usually associated to retarded growth, especially when animals experience difficulties in maintaining stance, spending considerable amount of time in recumbency and therefore are disabled to frequent access to food. Although the disease has been known for decades, it has not been possible so far to arrive to a definitive conclusion about the pathogenesis and etiology. Italian Romagnola cattle show a concerning prevalence for BSP that was estimated of 0.6% in 2002. As the occurrence of BSP is supposed to be genetically determined, we performed DNA-based molecular genetic analyses to unravel the underlying genetics causing this disorder in Romagnola cattle.

Materials and methods: We collected EDTA blood samples of 35 affected animals ranging from 1 to 21 month-old (median of 7 month-old). These 35 cases were clinically diagnosed with BSP severity grades ranging from 2 to 4 (median 3.5). A genome-wide association study (GWAS) was performed using high-density 777k SNP arrray genotyping data of the 35 BSP-affected and 32 controls. In addition, whole-genome sequencing (WGS) using the Illumina NovaSeq6000 was performed using DNA extracted of 6 BSP-affected Romagnola cattle. The obtained sequence reads were mapped to the ARS-UCD1.2 bovine genome assembly.

Results and Conclusions: Pedigree data of the collected BSP-affected Romagnola was not indicating a simple Mendelian inheritance. Preliminary GWAS results show no genome-wide significant association signal, although some regions with suggestive hits could be identified. No shared single-nucleotide variants (SNV) with predicted effect on the coding sequence could be detected on the six sequenced cases when compared with more than 500 control genomes of other unrelated breeds. These results indicate a more complex inheritance most likely due to regulatory mutations affecting several genes at different regions of the genome. Interestingly, human hyperekplexia shows similar clinical signs to BSP and it is supposed to be caused by mutations encoding glycerin proteins supporting our hypothesis of a possible genetic cause. Apart animal welfare issues and the economic impact in cattle production, BSP may therefore also constitute a model for comparative and translational medicine.

Keywords: "spastic paresis" "cattle" "Inherited diseases".

GB-11

Genetic parameters of sole lesion recovery in Holstein cows

Matthew Barden[1], Alkiviadis Anagnostopoulos[1], Bethany E. Griffiths[1], Bingjie Li[2], Cherry Bedford[1], Christopher Watson[1], Androniki Psifidi[3], Georgios Banos[2], Georgios Oikonomou[1].

[1]University of Liverpool, Neston, United Kingdom; [2]SRUC, Edinburgh, United Kingdom; [3]RVC, London, United Kingdom.

Objectives: Lameness in dairy cattle is primarily caused by foot lesions (Murray et al., 1996). Two of the most prevalent foot lesions are sole hemorrhage and sole ulcers (Cramer et al., 2008). It is thought that sole hemorrhage and sole ulcers represent different stages, or manifestations, of the same disease process (Lischer and Ossent, 2002); these lesions are collectively referred to as sole lesions. The objective of this

study was to estimate the genetic parameters relating to how well cows recover from such sole lesions.

Materials and Methods: A cohort of Holstein cattle were prospectively enrolled on four farms and assessed at four time points: pre-calving, immediately after calving, in early lactation, and in late lactation. Foot lesions were recorded at claw-level by veterinary surgeons at each time point, and used to define two binary traits: i) sole lesion recovery - whether sole lesions had improved between the early and late lactation time points, and ii) susceptibility to sole lesions - whether animals were affected with sole lesions during the study or remained unaffected at every assessment. Animals were genotyped and pedigree details extracted from the national database. Analysis was conducted in BLUPF90 software following a single-step approach; genetic parameters were estimated from threshold animal models using Gibbs sampling. The genomic estimated breeding values for both traits were calculated, and the correlation between breeding values was assessed in animals which had both phenotypes recorded.

Results: A total of 498 animals were used to estimate the genetic parameters of sole lesion recovery, 71% of animals had recovered between the early and late lactation assessments. The heritability of sole lesion recovery, on the liability scale, was 0.24 (95% highest density interval = 0.02 – 0.47). A total of 2,025 animals were used to estimate the genetic parameters of sole lesion susceptibility, 43% of animals recorded a sole lesion at least once during the study period. The heritability of sole lesion susceptibility, on the liability scale, was 0.23 (95% highest density interval = 0.14 - 0.32). The correlation between the genomic estimated breeding value for each trait was 0.06 (95% confidence interval = -0.03 - 0.15).

Conclusion: Our results indicate that recovery from sole lesions is a heritable trait, which suggests there is potential to breed cows which can recover from these lesions more quickly, although this finding should be corroborated in further studies. As sole lesion recovery appears to have a negligible genetic correlation with sole lesion susceptibility, genetic improvement would require selection on this recovery trait directly.

References:

Cramer, G., K.D. Lissemore, C.L. Guard, K.E. Leslie, and D.F. Kelton. 2008. Herd- and cow-level prevalence of foot lesions in ontario dairy cattle. J. Dairy Sci. 91:3888–3895. doi:10.3168/jds.2008-1135.

Lischer, C.J., and P. Ossent. 2002. Pathogenesis of sole lesions attributed to laminitis in cattle. Page in Proceedings of the 12th International Symposium of Lameness in Ruminants International Conference on Lameness in Ruminants, Orlando, FL, USA.

Murray, R.D., D.Y. Downham, M.J. Clarkson, W.B. Faull, J.W. Hughes, F.J. Manson, J.B. Merritt, W.B. Russell, J.E. Sutherst, and W.R. Ward. 1996. Epidemiology of lameness in dairy cattle: Description and analysis of foot lesions. Vet. Rec. 138:586–591. doi:10.1136/vr.138.24.586.

Keywords: sole lesion recovery, lameness, genetics, genomics.

GB-12

Novel insights into the genetics of schistosoma reflexum in Holstein cattle

Joana Jacinto[1], Irene Häfliger[2], Markus Freick[3], Jørgen Agerholm[4], Cord Drögemüller[2].

[1]Department of Veterinary Medical Sciences, University of Bologna, Ozzano Emilia, Italy; [2]Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland; [3]Faculty of Agriculture/Environment/ Chemistry, HTW Dresden-University of Applied Sciences, Dresden, Germany; [4]Department of Veterinary Clinical Sciences, University of Copenhagen, Højbakkegaard Allé, Taastrup, Denmark.

Objectives: Schistosoma reflexum (SR) is a lethal congenital syndrome in cattle characterized by U-shaped dorsal retroflexion of the spine and eventration of the viscera. A recessive mode of inheritance has been hypothesized but not yet proven. The aim of this study was to identify genetic causes of SR in a series of affected Holstein cattle by whole-genome sequencing (WGS).

Materials and Methods: Genomic DNA was extracted from ear cartilage of 10 SR affected Holstein calves, from EDTA blood of their dams and from semen of their sires (10 trios; 30 samples). Genomic DNA extracted from ear cartilage was available from additionally 9 SR affected Holstein calves. Short-read WGS was performed in all 39 animals, including the applied trio-approach for the 10 SR cases. The sequenced reads were mapped to the ARS-UCD1.2 reference genome and single-nucleotide and small indel variants were called. In order to identify private variants, the genotypes of the 19 SR cases were compared with a global cohort of 5347 cattle genomes of various breeds, including 1209 purebred Holstein. *In silico* tools were used to predict the biological consequences of the detected variants. Candidate variants were visually inspected. The term candidate was used to describe variants considering the affected gene function/associated phenotype, rarity and the predicted impact of the variant in the protein. In order to evaluate possible structural variants and chromosomal abnormalities the read depth along all chromosomes was calculated using a sliding window approach with a size of 10 kb and 200 kb. These coverage plots were obtained for all cases and available dams and sires.

Results: Assuming a recessive mode of inheritance, analysis of the WGS data reveled no single-nucleotide or small indel variants common to all cases. Assuming a dominant *de novo* event and therefore considering individually each case, it was possible to identify candidate causal protein-changing variants for 10 out of 19 SR-cases involving 10 genes. Particularly, by applying the trio-approach it was possible to identify *de novo* candidate variants for 3 SR-cases that were absent in both parents and in a global cohort of 5345 cattle control genomes. The identified variants affected *MLLT1* (p. Arg20Cys), *ACTL6A* (p.Met92fs), and *MAST3* (p.Pro1202fs) genes. Furthermore, in 7 SR-cases without sequenced parents it was possible to identify heterozygous, possible *de novo,* candidate variants that were absent in a global cohort of 5347 cattle control genomes. The identified variants affected the *ANO4* (p.Trp639Cys), *MYH1* (p.Thr663Ala), *DYNC1LI1* (p.Arg505Trp), *UBP1* (p.Arg388Gly), *SUGP1* (p. Arg326Cys), *SCAF8* (p.Val378fs) and *SYT12* (p.Ser238Leu)

genes. All these 10 coding variants were predicted to be deleterious. It could be speculated that the identified variants occurred either as a parental germline mutation or post-zygotically in the developing embryo. Furthermore, no evidence for larger structural variants or chromosomal abnormalities were detected by analyzing the obtained read depth and coverage along all chromosomes.

Conclusions: The previously hypothesized simple recessive inheritance for SR in Holstein cattle could not be confirmed. This study describes, for the first time, WGS findings for SR and provides evidence of unexpected heterogenic causality for SR by spontaneous *de novo* mutations affecting different genes. Herein, 10 protein-changing heterozygous variants are proposed as potential cause for SR located in candidate genes involved in embryonic and pre-weaning lethality thus giving a genetic diagnosis for 53% of the cases. So far, in cattle the efficiency of WGS for genetic diagnosis has not been investigated; however, the obtained results are considerable positive when compared with the efficiency of WGS-based genetic diagnostics in humans. Moreover, the unsolved genetic diagnosis for 9 cases might be explained 1) by limitations of the cattle genome annotation and/or 2) by limitations of the short-read WGS-approach. Sporadic lethal disorders such as SR affecting negatively dam's fertility and health, welfare and consequently economy in livestock are usually not diagnosed to the molecular level, mainly because of the lack of resources and diagnostic tools. Therefore, this study highlights that WGS-based precision diagnostics allows to better understand sporadic disorders and supports the value of surveillance of cattle breeding populations for harmful genetic disorders.

Keywords: Bovine, Dystocia, Congenital malformations, Precision medicine, Whole-genome sequencing.

GB-13

Analysis of meat yield control at the CENSYRA test station during the last 13 years

Andrés Domingo Montes, Gema Vara.

CENSYRA, Badajoz, Spain.

CENSYRA (Animal Selection and Reproduction Center) aims towards conservation, improvement and development of livestock breeds. CENSYRA collaborates with breeders' associations, which are recognized by the official authorities for the sole purpose of executing a breeding program for purebred breeding animals registered in the breeding book, through different reproduction centers and test stations.

Beef recording is a basic tool for herd management as well as for genetic evaluation and breeding. That is why the breeders´ associations incorporate meat yield control within their breeding programs. According ICAR (International Committee of Animal Recording) beef recording requires recording schemes that can accommodate beef production as implemented in practice and may be undertaken in breeding farms, finishing farms, individual test stations, progeny test stations or abattoirs.

The present research aims to analyze the meat yield data at the CENSYRA test station during the last 13 years, (2007-2019) taking three reference performance traits in two different beef cattle breeds, and watching its evolution through time.

The research is based on the results of 710 calves belonging to the Retinta (n=342) and Limousine (n=342) breeds. All animals entered the Test station had an age between 8 or 10 months, and a live weight between 350 and 425 kg. The test period duration was 112 days, with a previous phase to facilitate full adjustment to the station conditions (approximatly15 days).

The reference performance trait were:

- Chest girth circumference increase: Chest girth was recorded using a measuring tape the first day in Test station, and the 112 day.
- Average daily weigth gain is calculated as (FW-SW)*1000 / (AF - AS), where AS be the age at test start, expressed in days, AF be the age at test end, expressed in days, SW be the live weight at test start, expressed in kilograms, FW be the live weight at end of test, expressed in kilograms. Average daily weigth gain is expresed in grams per day.
- Feed efficiency: Efficiency of gain in beef production can be defined as the ratio of nutrient input to beef output. In this research, it is expressed as the kg of feed consumed per kg of live weight gain.

The results of the study were that the Limousine calves had better indexes of average daily weight gain and feed efficiency, but the Retinta calves presented major average chest girth circumference increases.

The Limousine breed average daily weight gain was 1407,17 gr. in the period from 2007 to 2019, while the average daily weight gain was 1573,89 gr. in the period 2012-2019 and 1709,38 gr. in 2019. This may mean that the evolution of Limousine breed yields is positive. The feed efficiency also improved throughout the study period. The average feed efficiency was 5,85 kg. in the period from 2007 to 2019, while the same trait was 4,96 in the period from 2012-2019, and 4,47 kg. in 2019.

The average chest girth circumference increase was 25,25 cm for Retinta breed calves, and 21,80 cm for Limousine breed calves. The Retinta breed average daily weight gain was 1307,68 gr. in the period from 2007 to 2019, and the average feed efficiency was 7,40 kg. in the same period. Unlike Limousine, the meat yields Retinta breed remain stable over the time. These data may be due to the fact that the Retinta breed breeding program is oriented towards maternal production, improving the characters of ease of delivery or weaning weight, and keeping meat yields constant over the time.

The final conclusion is that breeding programs can be useful for improving productive characters as in the beef cattle breeds.

Keywords: beef cattle, beef recording, test station, breeds, meat yield.

GB-14

Different Temperature-Humidity Indexes on Milk Yield and Composition; Somatic Cells Score and Stability to Alcohol Test in Grazing Holstein and Crossbred Holstein x Jersey Cows

Bruna Mendes, Roberto Kappes, Deise Knob, Angelica Scheid, Izabelly Telles, Laiz Perazzoli, André Thaler Neto.

Universidade do Estado de Santa Catarina, Lages, Brazil.

Objective: We aimed to compare genetic group and temperature-humidity index classes for milk yield, composition, somatic cells score and stability to the alcohol test.

Material and methods: We executed the study at the dairy cattle sector of Universidade do Estado de Santa Catarina (Lages, SC, Brazil) between September 2018 and August 2019. We used 22 multiparous lactating cows, 7 Holstein, 5 crossbred F1 (½Holstein x Jersey) and 10 crossbred R1 (¾ Holstein x ¼ Jersey). Cows were mechanically milked two times a day. After milking, cows received concentrate individually, after, they had access to grazing area. Cows had ad libitum access to water and available shadow area.

Weekly the individual daily milk yield was determined using Waikato® milk meters and milk samples were collected, obtaining a day compound sample. Part of this sample was transferred to a 40ml flask containing Bronopol® for analysis of milk composition by Fourier transform infrared spectrometry on DairySpec® (Bentley Instruments). Every two weeks we sent the samples to a laboratory of the Brazilian Milk Quality Network for somatic cells counting (SCC). SCC was transformed into somatic cells score (SCS). The milk samples were also analysed for stability to alcohol test, adding 2ml of milk and 2ml of alcohol in a Petri plate, considering the sample stable alcohol concentration previously to clots formation.

To evaluate environmental interference over the cows' performance, we calculated the temperature-humidity index (THI) using air temperature (AT) and relative air humidity (AH) through a Data Logger Akrom® model KR420, set to collect data every one hour. The THI was estimated as THI = (0,8 x AT + (AH/100) x (AT- 14,4) + 46,4).

The highest THI of the day were used to perform six THI classes: safe (<68), light (68≤ < 72), discomfort (72≤ <75), alert (75≤ <79), danger (79≤ <84) and emergency (≥84), the last one did not occur.

Data were submitted to ANOVA with repeated measures, using the MIXED procedure of the SAS statistical package, previously tested for residue normality. The model was composed by the variables genetic group, parity, days in milk, THI class and interactions between the variables.

Results: There was no difference in milk (P=0,5337) and energy corrected milk yield (P=0,2126) for Holstein, crossbred F1 and R1, respectively (Table 1), but crossbred cows showed higher fat and total solids content (<0,0001). Concerning protein contents, F1 crossbreds showed higher values, intermediate for R1 and lower for Holstein (<0,0001). Lactose contents were lower for F1 ones (0,0233).

We observed a reduction in milk yield and energy corrected milk (2,32 and 3,54kg/day, respectively) comparing THI classes safe and danger, as well as for milk components content.

F1 crossbred cows had higher SCS (0,002) in relation to the other genetic groups, not differing with THI classes increased levels (P=0,2627).

Lower milk stability to the alcohol test was observed in crossbred cows group (0,0001), as well as when THI grew (<0,0001).

Table 1: Mean values adjusted to the model ± standard deviation for different temperature-humidity indexes (THI) classes and different genetic groups for milk yield, energy corrected milk (ECM), fat, protein, lactose and total solids content, fat and protein yield, somatic cells score (SCS) and stability to the alcohol test.

	Genetic group			THI				
	H	F1	R1	Safe	Light	Discomfort	Alert	Danger
Milk (litre/day)	25,44±0,53	24,49±0,72	25,37±0,41	26,60±0,45a	24,76±0,51b	24,97±0,75ab	24,87±0,62b	24,28±0,91b
ECM	26,90±0,60	27,93±0,83	28,19±0,47	29,92±0,52a	27,30±0,59b	27,47±0,85b	27,32±0,71b	26,38±1,04b
Fat (%)	3,75±0,05b	4,21±0,07a	4,07±0,04a	4,14±0,04a	4,01±0,05ab	3,94±0,07b	4,00±0,06ab	3,95±0,09ab
Protein (%)	3,25±0,02c	3,55±0,03a	3,43±0,01b	3,47±0,02a	3,41±0,02b	3,45±0,03ab	3,42±0,02ab	3,30±0,04c
Lactose (%)	4,67±0,02a	4,57±0,02b	4,67±0,01a	4,69±0,01a	4,67±0,02ab	4,68±0,03ab	4,57±0,02c	4,59±0,03bc
Total solids (%)	12,47±0,06b	13,14±0,09a	12,99±0,05a	13,16±0,05a	12,89±0,06b	12,87±0,09bc	12,81±0,08bc	12,62±0,11c
Fat (kg/day)	0,95±0,02b	1,02±0,03ab	1,03±0,02a	1,09±0,02a	0,98±0,02b	0,98±0,03b	0,98±0,03b	0,95±0,04b
Protein (kg/day)	0,81±0,01	0,86±0,02	0,85±0,01	0,91±0,01a	0,83±0,01b	0,84±0,02b	0,83±0,02b	0,78±0,02b
SCS	3,01±0,31b	5,11±0,39a	2,93±0,21b	3,56±0,22	4,06±0,29	3,15±0,54	4,14±0,29	3,50±0,48
Alcohol	79,16±0,38a	75,74±0,53b	76,87±0,30b	78,60±0,32a	78,49±0,37ab	77,28±0,54bc	76,82±0,45c	75,11±0,68d

* Different lowercase letters on the line differ by Tukey's test for genetic grouping and uppercase letters for THI classes (P <0.05).

Conclusions: We concluded that crossbred cows have a productive performance similar to pure Holstein cows, with higher solid contents and lower stability to the alcohol test.

The higher temperature-humidity index values affect negatively milk yield and physicochemical quality.

Keywords: Milk quality, Heat stress.

GB-15

Relationship Between Longevity, Milk Yield and Somatic Cell Count in Holstein Cattle in Brazil - A Multivariate Approach

Angelica Scheid, Bruna Mendes, Rayllana Larsen, Luiz Schaitz, Mauricio Civiero, Marciel França, Adriana Hauser, Deise Knob, Roberto Kappes, Laiz Perazzoli, André Thaler Neto.

Universidade do Estado de Santa Catarina, Lages, Brazil.

Objective: To determine the joint relationship between milk yield, longevity and somatic cell count in Holstein cattle in Brazil.

Material and methods: Data from approximately 45,000 Holstein animals collected by the Brazilian Association of Breeders of the Holstein Breed Dairy Control Service from 2000 to 2010 were used.

As a longevity indicator, lifetime milk yield (LIFE_Y) was considered, and cows that were still alive at the time of the analysis were excluded from the data set because it is not known how long they will be alive. We estimated milk yield (MY) from individual milk control data. For somatic cell count (SCC) the average of observations from dairy controls was considered, being converted to somatic cell score (SCS). To estimate genetic values of cows and bulls, groups of contemporaries were created based on herd, month and year of calving for MY and SCS. In addition, animals that LIFE_Y, MY or SCS that were beyond the average were more or less two standard deviations. The genetic values for cows and bulls were estimated using the maximum likelihood method, using the MTDFREML software.

Based on the estimated genetic values, a factor analysis was performed using the FACTOR Procedure, using the genetic values of all females in the database, and of the bulls with records of more than five daughters, maintaining the factors that explained more together. 70% of the accumulated variance. In addition, 20 bulls with a greater number of female calves were selected for whom a cluster analysis was carried out using the *tocher* method, which grouped similar bulls together.

Results: In the factor analysis with genetic values of cows, two factors explained 70% of the accumulated variance (Table 1). This analysis demonstrates the importance of selecting a low genetic value for ECS, since it showed negative relationships with milk yield, without however showing a relationship with longevity.

Table 1 - Factor loads and percentage of variance explained by each factor referring to the genetic values of cows for LIFE_Y and TYPE.

Variables	Factor 1	Factor 2
SCS e LIFE_Y e MY		
SCS	**-0,73701**	0,07377
MY	**0,73859**	0,03426
LONG_P	0,02916	**0,99692**
VARIANCE%	36,92	33,39

* Somatic cells score (SCS), Lifetime milk yield (LIFE_Y) and Milk yield (MY).

In the factorial analysis for SCS and LIFE_Y and MY with genetic values of bulls, 2 factors were formed, which explained more than 70% of the variance (Table 2). In the first factor, SCS showed a contrary relationship with LIFE_Y and MY. In the second factor, LIIFE_Y presented a relationship contrary to milk production and a relationship with an intermediate value of SCS.

Table 2 - Factor loads and percentage of the variance explained by each factor referring to the genetic values of bulls with more than five daughters for LIFE_Y and TYPE.

Variables	Factor 1	Factor 2
SCS e LIFE_Y and MY		
SCS	**-0,72612**	0,01310
MY	**0,53537**	**0,73042**
LIFE_Y	**0,57067**	**-0,66856**
VARIANCY%	37,98	32,98

Table 3 - Groups of bulls separated by the variance of their genetic values for each trait resulting from the cluster analysis of the 20 bulls with the largest number of daughters for SCS, LIFE_Y, MY.

SCS, LIFE_Y and MY			
	ME		
BULLS	**SCS**	**LIFE_Y**	**MY**
1005; 452;886; 391; 1003; 771; 160; 427; 269; 501; 798; 250; 376; 987; 879; 379; 835;	0.028127	-0.17866	0.775123
692;	5.692925	-0.72717	-0.38632
282;	-0.06507	2.700032	2.168856
478;	0.000925	-0.05445	8.098696

Somatic cells score (SCS), Lifetime milk yield (LIFE_Y) and Milk yield (MY).

Through cluster analysis, it is observed that most of the selected bulls have an estimated intermediate genetic value for ECS. Bulls with higher genetic value for milk yield tend to have less genetic value for ECS and vice versa (Table 3). The analysis also shows that most of the bulls most used in Brazil in this period (2000-2010) were not selected for longevity.

Somatic cells score (SCS), Lifetime milk yield (LIFE_Y) and Milk yield (MY).

Conclusions: The selection for the lowest somatic cell score tends to increase, in the progeny, the genetic value for milk production and productive longevity.

Keywords: Data bank, Genetic value.

GB-16

Comparison of bull semen motility using a portable semen analysis device and computer-assisted sperm analyser

Kate Mitchell[1], David Beggs[2], Peter Mansell[2].

[1]University of Melbourne/Scottsdale Veterinary Services, Scottsdale, Tasmania, Australia; [2]University of Melbourne, Melbourne, Australia.

Objective: Bull breeding soundness evaluations (BBSEs) in Australia include a physical exam, scrotal circumference measurement, crush-side semen motility evaluation and often remote assessment of sperm morphology to provide a comprehensive assessment of a bulls' fertility and risk. The crush-side motility component of the examination typically involves the use of a microscope with a heated stage, requiring a large amount of space and power. The semen is assessed for progressive motility and an estimate is provided based on the forward movement of sperm observed by the practitioner. The crush-side assessment of sperm motility is relatively subjective and can be influenced by the experience of the practitioner, environmental temperature, concentration of the semen and the volume assessed. Previously developed for swine, the use of iPad-based sperm motility analysers has been developed to include most species of production animals including cattle.

The objective of this study was to compare the motility of fresh bull semen using a portable iPad-based semen motility analyser (iSperm) and a computer assisted sperm analyser (Androvision - Minitube).

Material & Methods: Semen was collected from three different proven stud bulls at a commercial bull collection facility, extended using Andromed (Minitube) extender and chilled. The ejaculates were transported to the semen laboratory the following day for assessment using the iSperm and Androvision system. The ejaculates were diluted and assessed simultaneously by the Androvision and iSperm for motility and concentration. The motility of the ejaculates was altered using additions of flash frozen dead sperm to allow for a greater scope of motilities to be assessed by the two devices. The motility, progressive motility and concentration were recorded. Analysis was repeated ten times to allow for later calculation of a coefficient of variation.

Results: A full analysis of the data is yet to be obtained however, the raw data indicates there is a repeatable correlation between the Androvision and iSperm when assessing progressive motility & concentration of fresh bull ejaculates.

Conclusion: Similar studies in other species have validated the use of a portable semen motility assessment device such as iSperm, allowing for assessment of semen in the field and removing assessor bias and environmental factors.

Keywords: bull, fertility, semen analysis.

HH-01

Controlled trial of the effect of negative dietary cation-anion difference on postpartum health and culling of dairy cows

Rita Couto Serrenho[1], Tony C. Bruinje[1], Emma Morrison[1], Trevor Devries[2], Todd Duffield[1], Stephen Leblanc[1].

[1]Univ of Guelph - OVC - Population Medicine Department, Guelph, Canada; [2]Univ of Guelph - Animal Biosciences, Guelph, Canada.

Objective: Negative Dietary Cation-Anion Difference (DCAD) diets fed during the prepartum period enhance calcium metabolism. Hypocalcemia influences neutrophil function and both are risk factors for clinical disease in the transition period of dairy cows.

The objective of this study was to assess the effects of a negative dietary cation-anion difference (DCAD) dry cow diet on postpartum health and culling.

Material and Methods: Cows from 4 commercial dairy farms in Ontario, Canada were enrolled in a randomized controlled trial from November 2017 to April 2019. Close-up pens (1 per farm) with cows 3 wk before expected calving were randomly assigned to a negative DCAD (TRT; -100 mEq/kg DM; target urine pH 6.0–6.5) or a control diet (CON; +95 mEq/kg DM with a placebo supplement). Each pen was fed TRT or CON for 3 months (one period) then switched to the other treatment for the next period, with 4 periods per farm. Body condition score (BCS) was measured at enrollment and urine pH was measured weekly until calving. Data from 1086 animals (TRT: n = 681; CON: n = 405) that received the assigned diet for > 2 wk were included. The incidence of milk fever (MF), retained placenta (RP), metritis, ketosis (blood BHB > 1.2 mmol/L, measured weekly in wk 1 and 2), clinical mastitis < 30 DIM (CM), displaced abomasum (DA), purulent vaginal discharge (PVD, assessed once at wk 5), ≥ 1 disease (DIS) or culling by 35 DIM were analyzed with logistic regression models with treatment, parity, BCS, and their interactions, accounting for pen-level randomization and clustering of animals within farm with random effects. There were no interactions of treatment with parity or BCS for any outcome. There were only 43 cases of MF, allowing only univariable analysis.

In a subset of 38 multiparous cows, blood was collected at -1wk, day 1 and 4 for assessment of: neutrophil phagocytosis (P) and oxidative burst (OB); ionized calcium (VetStat) and total calcium. Median fluorescence intensity for P and OB, and the shift of percentage of cells active for P and OB were measured with flow cytometry. Values represent stimulated cells minus unstimulated aliquot of the same sample.

Results: The incidence of MF was 5 ± 3% in CON and 1 ± 1% in TRT (*P* = 0.18). There were no treatment effects (CON vs TRT, LSM ± SE) on RP (7 ± 3%; 6 ± 2%; *P = 0.71*), metritis (11 vs 12%; SE = 4; *P = 0.83*), ketosis (21 vs 23%; SE = 4; *P = 0.59*, PVD (13 vs 12%; SE = 3; *P = 0.51*), or DIS (44 vs 41%; SE = 7; *P* = 0.41). Cows fed TRT had lesser incidence of CM (4 vs 2%; SE = 1, *P* = 0.09) and DA (3 vs 1%; SE = 1, *P* = 0.05). Culling < 35 DIM tended to be greater in CON (7 ± 2%) than TRT (5 ± 1%, *P* = 0.11).

There were no differences between treatment groups in the 4 neutrophil function outcomes (*P* > 0.25). Correlations of iCa or tCa with each of the PMN function outcomes were weak (r < |0.3|).

Conclusion: Contrary to our hypothesis, feeding a negative DCAD diet for 3 wk before calving did not improve neutrophil phagocytosis or oxidative burst activity. However, under commercial herd conditions, a negative DCAD fed 3 wk before parturition improved some but not all health outcomes assessed.

Keywords: Transition cow, nutrition, diseases, neutrophil function.

HH-02

An outbreak of acute respiratory disease in an adult daiy herd due to dust inhalation

Richard Sibley.

West Ridge Veterinary Practice, Witheridge, United Kingdom.

Objectives: This clinical study describes an outbreak of respiratory disease in an adult dairy herd comprising 850 milking cows. The affected animals responded poorly to treatment and so the focus was to identify the cause and prevent new cases.

Materials and Methods: 28 adult dairy cows were severely affected with respiratory disease over a period of six months. Thos that were clinically affected had a poor prognosis. Many others in the herd had intermittent mild signs of respiratory distress. Clinical signs included an afebrile pneumonia, with severe dyspnoea and respiratory distress in the worst affected cases. The response to treatment with antibiotics, non-steroidal anti-inflammatory drugs, and corticosteroids was poor. Most affected animals either died or had to be euthanized. Those that survived had poor productivity with chronic respiratory signs and were culled.

Four animals that had died soon after first clinical signs, or were sacrificed, were examined post mortem, with blood samples taken from five other affected animals to investigate the involvement of respiratory pathogens.

Results: Post mortem examinations and clinical pathology did not identify any involvement of bovine herpes virus, respiratory syncytial virus, mycoplasma bovis, or any other common respiratory pathogens that are considered potential causes of respiratory disease in adult cattle. The post mortem examinations and histopathology suggested an allergic or hypersensitivity reaction to an environmental exposure to an allergenic or irritable substance. Investigations at the farm discovered the use of a fine dust derived from Medium Density Fibre Board (MDF) as a bedding material, spread by a mechanical spreader each day in the cow housing. This dust was inevitably inhaled by some cattle present in the shed at the time of spreading.

Conclusions: The use of fine wood dust as a bedding material in a poorly ventilated environment can predispose to acute respiratory disease due to the inhalation of the dust and a hyersensitivity reaction in the respiratory tract. Recovered

cases become affected by chronic obstructive pulmonary disease and have markedly reduced productivity. The use of such bedding materials should be with extreme care.

Keywords: Pneumonia, respiratory disease, dyspnoea.

HH-03

The association of cough and other clinical signs with ultrasonographic lung consolidation: differences between dairy, veal and beef calves

Thomas Lowie[1], Katharina Van Leenen[2], Stan Jourquin[1], Mathilde Pas[1], Jade Bokma[1], Bart Pardon[1].

[1]Faculty of Veterinary Medicine - Ghent University, Merelbeke, Belgium; [2]Faculty of Veterinary Medicine - Utrecht University, Utrecht, Netherlands.

Background: Bovine respiratory disease (BRD) remains a leading cause of economic losses, hampered animal welfare and intensive antimicrobial use in the cattle sector. The latter contributes to public concerns and awareness about the development of antimicrobial resistance. To reduce antimicrobial use without risking economic losses or compromising animal welfare, it appears rational to limit antimicrobial treatment to calves with pneumonia, while withholding this treatment for just an upper respiratory tract infection at first instance. A true gamechanger in recent years is the use of thoracic ultrasonography (TUS) on farm, which was evidenced to be the most accurate diagnostic test for pneumonia in calves. Despite that rapid ultrasound techniques have been developed to better meet practitioners demands, continuously scanning all animals is practically and economically impossible. Therefore, the need for the development of early warning systems based on clinical signs, to timely detect animals with pneumonia is ever growing. However, a wide variety of definitions, based on clinical signs, to identify cattle with respiratory disease requiring antimicrobial treatment have been used in science and practice. Also, current scoring systems can be quite time consuming, whereas it is not known whether it is necessary to score all signs. Available scoring systems were developed and tested almost exclusively in pre-weaned dairy calves. Therefore, the question arises which clinical signs are associated with ultrasound confirmed pneumonia and whether the same clinical signs can be used postweaning or in other breeds or different production systems.

Objectives: To identify which clinical signs are associated with ultrasonographic lung consolidation (≥1 cm) in different breeds, production types (dairy, beef and dairy-mixed), and pre- and post- weaning. To identify how well individual clinical signs performed in the detection of ultrasound confirmed pneumonia compared to BRD clinical scoring cards in a new dataset.

Material and Methods: A cross-sectional study was conducted in the northern part of Belgium (Flanders) between 2016-2019. 956 untreated calves (70% Holstein-Friesian dairy and 30% Belgian Blue) from 84 herds were conveniently selected. At enrollment, all calves were clinically examined using 24 parameters, scored using the Wisconsin and Davis BRD clinical scoring card and subjected to TUS. Associations between clinical signs and pneumonia were determined using a generalized linear mixed model. Herd was added as random factor to take clustering of calves within a herd into account.

Results: Cough, both spontaneous and/or induced cough, was the only and best performing clinical sign, significantly associated with lung consolidation in all production types. Fever (≥39.4°C) was the second most promising factor, being significant in beef and veal calves, but not in dairy calves. At last, respiratory rate (≥43 bpm), eye and nasal discharge were only of significance in veal calves. However, across the complete dataset spontaneous cough (Acc (Accuracy): 65.1%), induced cough (Tracheal reflex Acc: 61.1%, Larynx reflex Acc: 58.1%), rectal temperature (Acc: 61%), breathing rate (Acc: 61.3%) and eye discharge (Acc: 59.1%) were significantly associated. Postweaning, none of the clinical signs studied were associated with pneumonia, with the exception of cough in dairy calves. Further, cough as a single clinical sign outperformed any combination of clinical signs, including the Wisconsin (Acc: 62.8%) and Davis (Acc: 61.5%) respiratory disease scoring systems, but sensitivity remained low.

Conclusion: Cough outperformed existing scoring systems based on multiple parameters, but still had too low accuracy to be useful for decision making regarding antimicrobial treatment. Based on the present study, cough is best regarded as a group level warning that the respiratory tract reacts on pathogens, environmental factors or their combination. Rather than using clinical signs, TUS remains absolutely necessary to responsibly use antimicrobials, avoiding treatment of animals with only an upper respiratory tract infection. Next to cough, rectal temperature and breathing rate are the most promising clinical signs for further exploring in continuous monitoring systems for pneumonia detection in calves. Given that the association of clinical signs with ultrasonographic pneumonia differed between production systems, breeds, and weaning status, validation of a scoring system in each breed, age category and housing system is recommended.

Keywords: Bovine respiratory disease, pneumonia, scorecards, predictive monitoring, thoracic ultrasound.

HH-04

Follow up of persistent pneumonia in a dairy cattle herd using quick thoracic ultrasound (qTUS) and non-endoscopic bronchoalveolar lavage (nBAL)

Laurens Chantillon, Stan Jourquin, Wouter Van Mol, Mathilde L. Pas, Jade Bokma, Bart Pardon.

Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium.

Objective: Every calf experiences respiratory tract infections. Generally, this will not severely affect the young animal unless the infection develops into a pneumonia. Pneumonia affects growth, production, mortality, welfare, antimicrobial use and fertility, making it one of the most important economic

diseases in cattle farming. Despite years of research and substantial efforts, still many herds face unsatisfactory results of preventative and therapeutic approaches.

The objective of this study was to describe an approach of individualized antimicrobial treatment using quick thoracic ultrasonography (qTUS) and diagnostics on non-endoscopic broncho-alveolar lavage (nBAL) samples in combination with targeted biosecurity measures in a large dairy herd with persistent pneumonia.

Materials and method: A 700 head dairy farm, with around 150 female calves aged under 4-5 months requested a herd visit for reasons of persistent cough and calf mortality since 2016. The herd relied on metaphylactic antimicrobial therapy with tulathromycin at first grouping for years. Vaccination against bovine parainfluenza type 3, bovine respiratory syncytial virus and bovine herpesvirus 1 was conducted. The planned approach consisted of a problem herd analysis investigation, followed by a series of follow-up visits. qTUS was performed with a linear 7.5 MHz probe, using 70% isopropyl alcohol solution as transducer agent. Calves with consolidations <1 cm were classified as healthy, between 1 cm and <3 cm were classified as mild pneumonia and consolidations ≥3 cm as severe pneumonia. Failure of passive transfer was assessed through total protein refractometry in calves less than one week old. Also, during the first and two other follow-up visits, five bronchoalveolar lavages (BAL) were taken from pneumonic animals. BAL fluid was cultured on blood agar and modified pleuropneumonia-like organism (PPLO) agar. Whole genome sequencing (WGS) with nanopore sequencing was performed on a pooled sample to identify all pathogens related to BRD. A *Mycoplasma bovis* PCR was done on a bulk tank milk sample.

Results: During the first visit, 56% of the calves were diagnosed with severe pneumonia and 10% with mild pneumonia. On aerobic culture *Trueperella pyogenes* was isolated and four PPLO agars tested positive for *Mycoplasma bovis*. WGS identified *M. bovis* and Bovine adenovirus. The genome of *M. bovis* was fully sequenced for known mutations previously associated with antimicrobial resistance and strain typing. Resistance against the macrolide tilmicosin was identified and the strain belonged to the Belgian genomic cluster V. Eight calves were evaluated for FPT and all had adequate antibody levels. Bulk tank milk PCR for *M. bovis* was negative.

Pneumonic animals were based on their age, either treated with oxytetracycline (age <6 weeks) or florfenicol (age ≥6 weeks). Treatments were carried out by the owner after thoroughly discussing the treatment plan after every visit with the veterinarian. To what considers biosecurity, individual housing pens and group pens were adjusted to avoid contact between pens. Calves were only grouped after a negative lung ultrasonography. Calves with unresponsive pneumonia were not commingled with healthy groups.

A total of 8 visits was needed, and at the end of the intervention 17% (22) and 18% (23) of the present calves remained with mild and severe pneumonia. A total of 71 new cases occurred in the follow-up period. Cure rate of the initial pneumonia cases was 15% while new cases cure rate was 57%. A year after the first follow-up, the average age at first calving, reduced from 24.7 months before the intervention to 23.5 months in the qTUS monitored group.

Conclusion: Severity of the pneumonia was greatly underestimated in this farm, partly because of the subclinical manifestation. The pathogen oriented approach, in this case based on the primary pathogen *Mycoplasma bovis*, consisted of both targeted biosecurity as tailored individual treatment based on susceptibility testing. The presented approach, using nBAL diagnostics to target treatment and prevention, and qTUS to personalize treatment and follow-up resulted in a significant decline in pneumonia prevalence in the calves. Cure rates were fair in animals, early detected by qTUS, whereas they remained disappointing in chronically affected animals. Hence, continuous qTUS follow-up remains necessary in *M. bovis* infected herds to assure early detection and effective treatment, given the predominance of subclinical pneumonia.

Keywords: Mycoplasma bovis, pathogen oriented approach, individualized treatment, biosecurity.

HH-05

Evaluation of umbilical involution in Holstein-Friesian calves after different treatments post natum

Theresa Maria Scheu[1], Dorothée Lang[2], Imke Cohrs[1], Christian Koch[1], Axel Wehrend[2].

[1]Educational and Research Centre for Animal Husbandry, Hofgut Neumuehle, Münchweiler an der Alsenz, Germany; [2]Clinic for Obstetrics, Gynecology and Andrology with Veterinary Ambulance, JLU Giessen, Giessen, Germany.

Objectives: An infection of the umbilical region, together with neonatal diarrhea and pneumonia, is one of the most common diseases in preweaning calves. The prevalence varies between 2% to 30% (Hathaway et al.,1993; Svensson et al., 2003). Disinfection of the umbilicus immediately after birth can decrease the risk of umbilical infections. Besides the approved and established disinfection solutions such as iodine, new products are being used hoping that they will lower infection rates. The aim of this study was to compare umbilical involution after the umbilicus treatment with iodine (J: Vet-Sept 10%; aniMedica GmbH, 48308 Senden-Bösensell, Germany) or Engemycin-Spray (E; active ingredient: Oxytetracyclinhydrochloride 25mg/ml; Intervet Productions S.r.l., Aprilia (LT), Italy) to an untreated group (U) of calves.

Materials and Methods: The study was done in 127 calves (n = 70 female; n = 57 male) at the Educational and Research Centre for Animal Husbandry, Hofgut Neumuehle, Münchweiler an der Alsenz, Germany. Animals were randomized to one of three groups by draw and were treated within one hour after birth. The assessment (including the degree of drying) and measurements of the umbilical structures (including circumference of the skin umbilicus and length of the umbilical cord) were done immediately after birth (<1h) and on day 1, 3 and 7 after birth. After day 7 the assessments were done once per week up to 10 weeks (n = 70 female; n = 33 male; n = 27 males were sold 14 days after birth). The calves were kept in single hutches for the first 14 days and in groups of 10 animals afterwards.

Results: The circumference of the skin umbilicus immediately after birth was unrelated to gender in all three groups: J: n = 43; 10.8 ± 2.0 cm; E: n = 42; 10.9 ± 2.1 cm; U: n = 42; 10.3 ± 2.0 cm. Subsequently there was a significant reduction in circumference in all three groups ($p < 0.0001$), but there was no difference between groups (J: n = 19; 8.8 ± 0.6 cm; E: n = 17; 9.9 ± 0.7 cm; U: n = 24; 9.1 ± 1.4 cm). The umbilical cord dried up during the first day after birth in 11 animals in group J, 7 in group E, and 8 in group U. By day 7 this number increased to 38 (88.4%), 32 (76.2%), and 39 (92.9%), respectively. The umbilical cord fell off after 3.0 ± 1.9 weeks (Group J), 3.3 ± 1.5 weeks (Group E), and 3.3 ± 1.7 weeks (Group U). None of the animals showed any sign of umbilical infection at any point in time.

Conclusions: This study did not show a difference between the two treatments compared to an untreated group. Based on this, it seems that other factors such as housing system or dryness of bedding have a higher impact on infection rates. The use of antimicrobial agents for treatment of the umbilicus immediately after birth also needs to be seen critically in the light of antibiotic minimization goals and therefore should be avoided.

Keywords: Umbilical infection, disinfection, Iodine, Engemycin-Spray.

HH-06

A high plane of nutrition is a protective factor against neonatal calf diarrhea on Bavarian dairy farms

Ingrid Lorenz[1], Huber Regina[2], Trefz Florian[3].

[1]Bavarian Animal Health Service, Poing, Germany; [2]Practice Mittermeier, Kraiburg, Germany; [3]Vetsuisse Faculty Bern, Bern, Switzerland.

Objectives: Calf mortality and morbidity is still unacceptably high worldwide. Neonatal calf diarrhea is the most important cause of calf losses in the first weeks of life. Therefore, risk factors for calf diarrhea as a herd health problem were investigated in a case-control study on Bavarian dairy farm.

Materials and methods: A total of 59 dairy farms were investigated by veterinarians of the Bavarian Animal Health Service due to problems with neonatal calf diarrhea (group P). The control group consisted of 18 farms out of the customer base of the Bavarian Animal Health Service that reported no veterinary treatments for calf diarrhea (group C). Management factors were assessed using a questionnaire during a face-to-face interview. Serum samples were collected from up to 10 healthy calves from 2 to 10 days of age for the examination of the quality of passive transfer using total protein analysis. Up to 10 colostrum samples were assessed for immunological and hygienic quality. Data were analysed using IBM SPSS Statistics 24.0.0.1. The association between the appearance of diarrhea on farm and the evaluated factors was analysed by univariable regression tests for binary or categorical variables, the Mann-Whitney U-test was used for continuous variables. Variables with a p-value ≤0.2 were entered into a multivariate regression model with calculation of odds ratios (OR) and associated 95% confidence intervals (95% CI) using a stepwise backward procedure with a Wald $P < 0.05$ as selection criterion and using presence of diarrhea problems on farm as a binary outcome variable. If two variables were closely correlated to each other ($r_S > 0.60$), only that variable was entered into the model which had the lowest P-value in the univariable analysis in order to minimize the effects of collinearity. The fit of the final logistic regression model was evaluated by means of the Hosmer-Lemeshow Goodness-of-Fit test.

Results: Herd size was the same (Median = 75 dairy cows) between groups. There was no difference in quality of passive transfer as assessed by serum total protein between problem herds and control herds. Also, no difference was found with regards to the immunological and hygienic quality of colostrum samples. Variables entered into the multivariate regression model were: Cleaning of calving pen after each calving (p=0.141); calf left longer than 3 hours with the mother (p=0.071); 3 litres of colostrum at first feeding (p=0.079); 3 litres or more at second feeding (p=0.012); ad-libitum feeding in first week of life (p=0.004); own feeding bucket for each calf in first week of life (p=0.032); calves housed near cows (p=0.042); administration of an iron containing preparation after birth (p=0.172).

Variables remaining in the final model were 3 litres or more at second feeding (OR: 0.209, 95% CI: 0.049 – 0.892, p=0.035); ad-libitum feeding during the first week of life (OR: 0.059, 95% CI: 0.006 – 0.599, p=0.017); and administration of an iron containing preparation after birth (OR: 10.935, 95% CI: 1.251 – 95.624, p=0.031). Hosmer-Lemeshow Goodness-of-Fit test: Chi-square: 0.721, df: 3, p=0.868.

Conclusions: Neonatal calf diarrhea is a multifactorial disease with numerous infectious and non-infectious factors determining if calves fall sick or stay healthy. In this study, two of the three variables in the final model gave evidence for a higher plain of nutrition being a protective factor against calf diarrhea on Bavarian dairy farms. In the past decades evidence is accumulating, that early life nutrition has an important impact on calf growth, development, health and well-being. Biologically normal feeding is recommended for the first three to four weeks of life, which means providing unrestricted amounts of milk or milk replacer as opposed to the traditionally practiced feeding of restricted amounts of milk, e.g. 10 or 15% of body weight. Ad-libitum or enhanced milk feeding results in elevated body growth and organ growth, e.g. of the small intestine, the mammary gland, thymus and endocrine pancreas. It either enhances or has no impairment on ruminal development postweaning. An adequate nutrient supply is important for the development of a stable intestinal microbiota and a successful intestinal immune response against pathogens. Therefore, results of this study are in line to previous findings and support the establishment of ad-libitum feeding programs in dairy calf rearing.

Keywords: Calf diarrhea, ad-libitum feeding.

HH-07

Motivations and barriers perceived by veterinarians when implementing herd health fertility management on UK dairy farms

James Brocket[1], Emma Fishbourne[2], Robert Smith[2], Helen Higgins[2].

[1]Craig Robinson Vets, Cumbria, United Kingdom; [2]Institute of Veterinary Science, University of Liverpool, United Kingdom.

Objectives: Vets can play a vital role in fertility herd health management. However, there is evidence to suggest that this is an area in which vets are not as involved as they could be, and sometimes not at all. By not proactively getting involved in fertility herd health management, vets are at risk of losing the role of consultant to other paraprofessionals. Furthermore, in a recent farmer satisfaction questionnaire, farmers rated proactivity as the number one rated attribute they look for in a vet (Jefferson-Loveday 2018). Why then as a profession are we not more involved in this area with farmers? We should be acting as the driving force for farm efficiency and profitability while keeping animal welfare at the forefront. The main aim of this study was to investigate the motivators and barriers that vets perceive when trying to implement herd health fertility programmes on UK dairy farms.

Methods: Face-to-face semi-structured interviews were conducted with 20, purposively sampled vets working in private practice in the north of England and southern Scotland, UK. All participants spent a minimum of 30% of their time working with dairy cattle and conducted routine fertility visits themselves. Interviews lasted between 20-60 minutes and were audio recorded and transcribed verbatim. The data was analysed inductively using qualitative NVivo software and followed the six-phase process of thematic analysis as described by Braun and Clarke (2006). Coding and theme generation was led by JB, with refinement through consultation with HMH, EF and RS.

Results: Gender was split 13 men and 7 women. Mean number of years qualified was 12.5 (range 3 to 31). There were 8 assistants and 12 senior partners/directors. Of the 20 participants, 10 were working in mixed practice, 10 were in farm-only practice and 4 had post-graduate cattle-related qualifications. The size of the veterinary practice they worked in varied from 3 to 28 farm vets, with a mean of 8.6.

The analysis generated four themes which together encapsulate the various barriers that the vets perceive to engaging with farmers in fertility health management, their approaches to engaging and their motivations for doing so. The first theme, “clinical baggage” explored past experiences that vets have had and how they shape, and in some cases seriously hinder, future attempts to engage with farmers. The second theme, “stuck in the comfort zone” highlighted, amongst other things, that if vets lack familiarity with data handling or have gaps in their knowledge this can contribute to a lack of confidence to engage on the subject of herd health fertility management. The third theme, “money matters” explores the financial motivations vets have for themselves but to a greater extent for the farmers they are engaging with. The fourth theme, “relationship with the farmer” included factors such as the importance of forging a positive relationship and trust with a farmer and how this is crucial for effective engagement. This was distinct from ‘friendly relationships’ that can impede vets attempts at making positive change.

Conclusions: Vets recognise the value of fertility herd health management, but are often unsuccessful in engaging with farmers for a variety of reasons. The themes identified in this study provide useful insight into the challenges they face and also provide key areas that could be targeted in future interventions to improve the uptake of fertility health management on dairy farms. Our suggests for future interventions will be presented at the conference.

Keywords: Engaging with farmers, Proactive, Preventative, Facilitating behaviour change.

HH-08

Effects of postpartum NSAID treatment on milking performance, reproductive performance and survival of cows and calves experiencing both assisted and unassisted parturition

Nicola Gladden[1], Dorothy McKeegan[2], Kathryn Ellis[2].

[1]Farm Animal Department University of Nottingham School of Veterinary Sciences Sutton Bonington, Loughborough, United Kingdom; [2]University of Glasgow, Glasgow, United Kingdom.

Objectives: Parturition assistance in cattle is common and has been shown to adversely affect reproductive performance, milk yield and culling risk. Effects of assisted birth on future productivity of calves have been less well studied although one study suggests that assisted birth may negatively affect future milk production. The underlying mechanisms for such adverse effects are not understood; however, multiple factors are likely to be involved, potentially including pain and inflammation. Although non-steroidal anti-inflammatory drug (NSAID) analgesia is commonly provided following veterinary-assisted parturition, it is uncertain whether any positive NSAID effects extend to production parameters. It is also difficult to draw conclusions from available data as few studies compare assisted and unassisted parturition, and both NSAID treatment and production outcomes differ between studies. Additionally, longer-term effects of administering NSAID treatment to calves at birth on their future production have not been reported. The objective of this study was to investigate the effects of both farmer-provided assistance and postpartum NSAID analgesia on subsequent production of cows as well as future production of calves. A factorial design allowed us to study not just the separate effects of assistance and NSAID treatment, but also the effects of interactions between assistance and treatment status.

Materials and methods: The study was performed on a commercial UK dairy farm; the cows are housed and calve all year round. Forty-seven cows and 47 calves experiencing farmer-assisted parturition and 42 cows and 44 calves experiencing unassisted parturition were recruited and randomly assigned to either an NSAID treatment group (45 cows and 46 calves) or a placebo group (44 cows and 45 calves). Ketopro-

fen or saline was administered within 3h of parturition to animals in the treatment or placebo groups respectively. Production data were obtained (with permission) from farm records for statistical analysis and animals were followed until either the end of their subsequent lactation (cows) or 1st lactation (calves), or until they left the herd.

Results:

Milking performance

Irrespective of assistance status, NSAID-treated cows had a mean 305-d mature equivalent yield (305ME) 664kg higher than placebo-treated cows (p = 0.011). Additionally, NSAID-treated unassisted cows had a tendency towards a mean 305ME approximately 1500kg higher than cows in the other three interaction groups (p = 0.073).

Reproductive performance

The median calving to conception interval of NSAID-treated cows tended to be 22d shorter than placebo-treated cows, irrespective of assistance status (p = 0.056). Additionally, NSAID-treated cows tended to be more likely to conceive overall (compared to placebo-treated cows) (p = 0.056) and a greater proportion of cows in the NSAID treatment group were inseminated to observed oestrus than in the placebo group (72% vs. 57%), although this was not statistically significant. Irrespective of treatment status, parturition assistance was associated with poorer reproductive performance with affected cows being less likely to conceive overall (OR 0.02; 95% CI 0.002-0.32; p = 0.005) and by 150 and 200 DIM (both p < 0.05).

First lactation reproductive performance of calves was negatively affected by assisted birth: assisted calves were bred more times before conceiving (p = 0.001) and had a longer calving to conception interval (p = 0.027) than unassisted calves.

Survival

Thirty-one cows (34.8%) left the herd before completing the subsequent lactation; assisted parturition was associated with an increased risk of leaving the herd early (OR 4.53; 95% CI 1.53 – 13.5; p = 0.004). Although treatment status did not affect the overall risk of leaving the herd early, median survival time of culled NSAID-treated assisted cows was 268DIM, compared to 169DIM for culled placebo-treated assisted cows, although this was not statistically significant.

Calves experiencing birth assistance were eight times more likely to not complete their first lactation than unassisted calves (OR 7.95; 95% CI 1.31-48.4; p = 0.024).

Conclusions: This study supports previously demonstrated adverse effects of assisted parturition on cows' reproductive performance and survival in the subsequent lactation. Only 305ME was positively affected by postpartum NSAID treatment, but this is an important finding due to the potential economic benefits provided by increasing milk yield. Results from this study also indicate that the effects of assisted birth may be long lasting and affect calves into their first lactation, although long lasting effects of ketoprofen treatment were not identified in calves.

Keywords: Parturition, NSAID, productivity, cow, calf.

HH-09

Characterisation of reticuloruminal contractions using a reticuloruminal accelerometer bolus, neck collar accelerometer and clinical examination (ultrasound and auscultation)

Giovanni Capuzzello, Lorenzo Viora, Elena Borelli, Nicholas Jonsson.

University of Glasgow - School of veterinary Medicine - Scottish Centre for Production Animal Health and Food Safety, Glasgow, United Kingdom.

Objectives: Physical activity and reticuloruminal contraction rate (RRCR) have been used as indicators of health status in cows. The three objectives of this study were to (1) determine whether a reticuloruminal tri-axial accelerometer bolus (smaXtec Classic Bolus) could provide observations that are consistent with RRCR as determined by clinical examination using auscultation and ultrasound; (2) describe the relationship between two commercial devices that indicate rumination rate (Afimilk Silent Herdsman neck-mounted tri-axis accelerometer collar; smaXtec Classic Bolus); (3) describe the diurnal pattern of RRCR using motility indices derived from boluses (smaXtec Classic Bolus).

Materials and methods: Six rumen-fistulated Jersey cows at the University of Glasgow were studied over two weeks in June 2021. Cows were fitted with collars (Afimilk Silent Herdsman neck-mounted tri-axis accelerometer collar) and boluses (smaXtec Classic Bolus). several weeks before the study. Cows were housed together in a single straw-bedded pen and fed *ad libitum* hay, replenished daily at 7.30-8.00 and 15.45-16.00. On Mon, Wed, Fri of the first week each cow was examined between 09.00-10.30h and at 16:30-18:00h. RRCR was determined over a 10 minutes period by ultrasound (US) with a convex 3.5 MHz probe placed on the ventral paramedian area of the abdomen, to the left of the caudal part of the xiphoid and simultaneously a second operator recorded reticuloruminal contractions by auscultation (AUSC) of the left paralumbar fossa. In the second week, no clinical examination was performed to avoid any interactions with the animals that might perturb the normal diurnal pattern of reticuloruminal contraction. Data were obtained from commercial web-platforms for both devices (collars: hourly summarised rumination, eating and activity indices; boluses: 10 minutes summarised temperature, rumination, activity indices) and boluses (smaXtec Classic Bolus) supplied inter-contraction interval (ICI) and contraction duration (CD) information summarised every 30-60s from their commercially protected algorithms, derived from raw accelerometer data. Time-series data were filtered to two datasets: 1) hourly bolus and collar data for the entire study period, 2) bolus data every ~30-60s during the 10-min periods of the clinical examinations. Distributions were checked. 10-min data were summarised to the hour for the main dataset. Pearson's correlation coefficients and Bland-Altmann plots were used to compare clinical examination (US and AUSC) with bolus contraction intervals and to assess the relationship between the rumination and activity indices from neck mounted collars and boluses. A cyclic Generalised Additive Model (GAM) with cow as fixed effect and smoothed time was used to describe the effects of hour of day (diurnal pattern).

Results: During the 10-min examination periods, mean

ICI (±SD) derived from US, AUSC and bolus were 40.1 ± 3.96s, 38.5 ± 3.33s, 40.4 ± 4.75s respectively. The Pearson correlation coefficients for estimated inter-contraction interval between US and AUSC with boluses, and between AUSC and US for the period of clinical observation were 0.55, 0.40 and 0.69 respectively (all $p<0.05$). Pairwise Bland-Altmann plots indicated similar performance of methods across all mean values, with small biases: Bolus-US = 0.27s, US-AUSC = 1.7s. The Pearson correlation coefficient for rumination index derived from neck mounted collars and indwelling boluses was 0.72, and for activity index it was 0.53 (both $p<0.001$). The GAM showed a strong diurnal pattern for all cows. The ICI was consistently high for all cows from 23:00 until 05:00h, falling then and increasing to a second peak which varied among cows between 10:00 and 14:00h, again falling after ~16:00h until ~20:00h. The effect of time and cow were highly significant ($p < 0.0001$), and the model explained 15% of the deviance. By examining the relationship between ICI and the CD, it was possible to identify two apparent types of contraction: one centred around a CD of ~10 s and an ICI of ~50s, the other at CD ~ 8.5s with ICI ~ 37s.

Conclusions: There was a robust relationship between clinical observation and the boluses for estimation of ICI, and between the bolus and collar for estimation of rumination index, indicating that the bolus provides useful clinical information about changes in rumination pattern. We are conducting further studies to assess the value of changes in ICI and CD for the early detection of disorders of the rumen and systemic disease states.

Keywords: Reticuloruminal contractions, diurnal pattern, ultrasound, 3-axis accelerometer, Precision Livestock Farming.

HH-10

Use of an automated monitoring system to assess rumination time and activity patterns in cows with metritis

André Pacheco De Carvalho[1], Brenda Barcelos[1], Denis Barbosa Alves Antonio[1], Jantijn Swinkels[2], Carlos Antônio De Carvalho Fernandes[3].

[1]MSD Animal Health, São Paulo, Brazil; [2]MSD Animal Health, Boxmeer, Netherlands; [3]University of José Rosario Vellano, Alfenas, Brazil.

Objectives: Postpartum uterine conditions such as metritis are common in dairy cows and represent a significant threat to animals' health and wellbeing, and frequently lead to a decreased reproductive performance in the subsequent breeding season. Changes in feed intake, rumination, and activity pattern in the periparturient period have been reported in cows that develop metritis in early postpartum period (Cocco et al., 2021). With an increased number of dairy farms equipped with automated monitoring systems, the use of health insights generated by such systems opens an opportunity for farmers and their vets to identify cows with higher risk of metritis.

The objectives of this study were to evaluate the efficiency of an automated monitoring system (SenseHub™ Dairy, Allflex® Livestock Intelligence™) to detect changes in rumination time and activity in dairy cows with post-partum metritis.

Materials & methods: The study was conducted throughout November 2019 to October 2020 with 493 Holstein cows (n=493) aged 2 to 6 years, on a commercial dairy farm, located in the Southern region of Minas Gerais state, Brazil. All animal procedures were following the recommendations of the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999). During the experimental period, cows were housed in a free stall. Cows were fed ad libitum a TMR based feed with corn silage as forage, and concentrates composed of corn and soybean meal, and added minerals and vitamins, which was balanced to meet or exceed the nutritional requirements of lactating dairy cows (NRC, 2001). Rumination time and activity pattern were monitored continuously in all cows using an automated monitoring system (SenseHub™ Dairy, Allflex® Livestock Intelligence™), from 30 days prepartum to 120 days postpartum. The cows were also monitored daily for signs of metritis. Cows between 5 and 15 days postpartum who exhibited signs of behavioral changes, depression, as well as the presence of abnormal uterine discharge and impaired uterine involution, were treated with antibiotics for three consecutive days (Conventional Treatment). After this period, treated cows in which no clinical resolution was observed, were treated with antibiotics for another 5 days (Extended treatment). Both treatment groups were compared to healthy cows with no signs of metritis (Healthy) from the same herd. The rumination time (minutes per day) and activity trend (provided by the system) were compared between groups using the SAS Glimix procedure (SAS Software, v9.4).

Results: Metritis was observed in 15.2% (75/493) of cows, out of which 66.7% (50/75) required extended antibiotic treatment for another 5 days. The average rumination time was 523.7 ± 1.3^{a}; 515.6 ± 3.1^{b} and 507.7 ± 2.7^{c} minutes (mean±std error) for the Healthy (n=418), Conventional Treatment (n=25) and Extended Treatment groups (n=50), respectively. The results revealed that cows affected by metritis had lower average rumination time ($P<0.022$) compared to the Healthy control group. The persistence of clinical signs and the need for extended treatment, which indicated a more severe process, were associated with a further reduced overall rumination time compared to the cows in which clinical cure was achieved after conventional treatment ($P=0.05$). The average activity trend generated by the system was 357.6 ± 0.9^{a}; $351.6\pm2,2^{b}$ and $332,0\pm1.6^{c}$ (mean±std error) for the Healthy, Conventional Treatment and Extended Treatment groups, respectively. The presence of metritis and severity of the condition (as indicated by the requirement for extended therapy) clearly affected the overall activity of the cows ($P=0.017$ and $P<0.0001$, respectively).

Conclusions: The results of the study confirmed that cows that go on to develop early postpartum metritis exhibit lower rumination time and decreased activity pattern than cows with no uterine conditions. Moreover, it also revealed that animals, in which the short antibiotic treatment did not lead to a clinical improvement, showed more pronounced deviation in the monitored variables.

These results open a significant opportunity to manage one of the biggest challenges in the postpartum period, identify in advance animals in high risk of metritis.

Keywords: Monitoring system, rumination, activity, metritis, pospartum.

HH-11

Effect of Formic Acid Treatment on Colostrum Quality and on Absorption and Function of Immunoglobulins: A Randomized Contolled Trial in Holstein Dairy Calves

Billy I. Smith, Sarah V. Cady, Helen W. Aceto.

University of Pennsylvania, School of Veterinary Medicine, New Bolton Center, Kennett Square, PA, United States.

Objectives: Good quality colostrum is characterized by high immunoglobulin levels and low pathogen load. Techniques to decrease pathogens can damage immunoglobulins, potentially reducing both absorption and function. Objectives were to determine the effect of formic acid treatment on colostral bacterial and immunoglobulin (IgG) levels before feeding, and serum immunoglobulin levels and neutralizing capabilities after feeding of either untreated or acidified colostrum.

Materials and Methods: Fifteen female Holstein calf pairs born < 12 h apart from different dams were randomly assigned to receive four liters of either untreated pooled (both dams) colostrum or the same pooled colostrum acidified to pH 4.0-4.5 with 10% formic acid. Colostrum characteristics including pH, bacterial load, immunoglobulin concentration and neutralizing capability were evaluated in both untreated and acidified samples. Blood samples were collected from each heifer pair on day 1 pre-colostrum followed by day 3 post-colostrum and then monthly for 6 months. Blood was analyzed for immunoglobulin G (IgG) concentrations and neutralizing capabilities against viral Infectious Bovine Rhinotracheitis (IBR), Bovine Viral Diarrhea Virus (BVDV) Type-1 and Type-2 and five serovars of bacterial *Leptospira*. Data were analyzed using STATA 17 (Stata Corp, College Station, TX, United States). A parametric t-test or non-parametric Kruskall Wallis test (which makes no assumptions about data distributions) were used to examine associations between feeding of untreated or acidified colostrum and outcome variables. Because many outcomes were below the detection limit of various assays, tobit regression was also used to censor those values and evaluate significance. A value of $P < 0.05$ was considered statistically significant.

Results: Birth weights among calf pairs were not significantly different. Compared to untreated colostrum (mean = 6.7, standard deviation [SD] 0.4, median 6.8, range 6.0-7.3) acidification significantly reduced colostrum pH (mean = 4.3, SD 0.3, median 4.3, range 4.0-4.5) $P < 0.001$. Total coliform count was also significantly reduced (untreated mean cfu/ml = 149, SD 444, range, median 1, range 0-1,700; acidified mean cfu/ml = 8, SD 31, median 0, range 0-120; $P = 0.02$). Colostrum IgG levels were not significantly different between untreated (mean 93.3 g/L, SD 39.7; median 92.80, range 36.7-164.4) and post-acidified colostrum (mean 101.9 g/L, SD 36.7; median 108.3, range 33.8-164.4; $P = 0.54$). There were no significant differences between untreated and acidified colostrum neutralizing titers for IBR, BVDV1, or BVDV2. Except for one calf in the untreated group, IgG levels on day 1 pre-colostrum were all less than 10 mg/dL. At 3 days of age, total protein levels in calves fed untreated colostrum (mean = 6.5 mg/dL, SD 0.4, range 5.4-7.0) and those receiving acidified colostrum (mean = 6.3 mg/dL, SD 0.7, range 5.2-7.6) were not significantly different, $P = 0.38$. Peak IgG levels occurred on day 3 (untreated mean = 26.1 g/L, SD 34.9, median 169.2, range 8.3-151.0; acidified mean = 30.2 g/L, SD 48.7, median 188.8, range 3.1-204.4; $P = 0.77$). Apparent efficiency of IgG absorption was not different between groups (untreated mean 24.3%, SD 11.4, median 25.3, range 8.6–51.3; acidified mean 22.6%, SD 21.7, median 21.6, range 4.1-58.9; $P = 0.65$). Thereafter, IgG levels slowly declined over the subsequent 6 months, but they were never significantly different between groups. Over the same period, maternal antibody titers against IBR, BVDV1, BVDV2, and *Leptospira canicola*, *L. hardjo*, and *L. pomona* were not different. Titers against *L. grippotyphosa* and *L. icterohaemorrhagiae* were unmeasurable in the majority of calves and the data were not analyzed.

Conclusions: Colostrum acidification significantly decreased bacterial load fed to newborn calves without affecting IgG concentration or virus neutralization. Nor did acid treatment impact serum IgG levels in calves or activity against common pathogens.

Keywords: Randomized Controlled Trial, Acidified Colostrum, Pathogen Load, Immunoactivity.

HH-12

Comparison of diagnostic testing strategies for failure of passive transfer (FPT) in Scottish dairy calves

Katie Denholm[1], Alexandra Haggerty[1], Kathryn Ellis[1], Colin Mason[2].

[1]University of Glasgow, Glasgow, Scotland, United Kingdom; [2]Scottish Agricultural College, Dumfries and Galloway, United Kingdom.

In dairy calves, failure of passive transfer (FPT) results in an increased risk of calfhood morbidity and mortality (Tyler *et al*. 1999; Pardon *et al*. 2015). Furthermore, calves with FPT have reduced liveweight gains following weaning (Furman-Fratczak *et al.* 2011) and reduced first milking lactation yields (Denise *et al.* 1989).

Background: Serum IgG levels of ≥10g/L are indicative of adequate passive transfer (Tyler *et al*. 1999; Virtala *et al.* 1999). The reference test for measuring IgG in serum is radial immunodiffusion (RID) which directly measures the IgG in a sample (Cuttance *et al.* 2017). In Scotland, this test is prohibitively expensive so indirect measures such as total protein (TP) tests are often substituted. Zinc sulphate turbidity (ZST) testing is another indirect measurement of IgG in serum (Hogan *et al.* 2015) and is commonly used in Scotland by clinicians and disease investigation centres to diagnose FPT in calves; despite a growing body of evidence to suggest its inferiority to other tests (Hogan *et al.* 2015), in particular its poor specificity. Brix refractometry has been used as an inexpensive and convenient calf side measure of FPT, which can also

be used to measure colostrum quality (Quigley *et al.* 2013).

Objectives:

- To explore the performance of various diagnostic testing strategies for FPT in Scottish dairy calves.
- To redefine optimal cutpoints for indirect testing strategies, including Brix refractometry, total protein (TP) and zinc sulphate turbidity (ZST).
- To challenge the *status quo* of indirect measures of FPT offered in commercial laboratories and disease investigation centres in Scotland.

Materials and methods: Serum samples were collected from 370 calves between 24 hours and 7 days old from 38 dairy farms in Lanarkshire, Stirlingshire and Dumfries and Galloway. Calves were excluded if they exhibited signs of ill health; in particular, dehydration. Samples were tested using ZST, Brix refractometry, TP and RID.

Receiver operating characteristic (ROC) curve analysis was used to determine the optimal cutpoints for correctly predicting FPT using concentrations of TP, percentage Brix and ZST. The optimal cut-point for each test was calculated using the Youden's index, where the sum of sensitivity plus specificity is maximised and equal weight is given to false positive and false negative results.

Results: False positive rates were high using indirect testing, particularly when ZST was used. FPT prevalence was 29.5%, 30.8% and 46.4% for TP, Brix and ZST respectively, compared with 14.1% for direct measurement of IgG concentration using RID. The results from this work confirm the poor test specificity of the ZST test and recommend a lowered cutpoint to improve test performance, in line with other published, international work. Existing cutpoints (from international literature) for indirect FPT tests were redefined using ROC (receiver operating curve) analysis and the accuracy of each test improved.

Conclusions: Despite over-estimation of the prevalence of FPT in this population of calves, it is still useful to monitor FPT using indirect tests due to their convenience and cost effectiveness; however, it is recommended that test cutpoints are lowered for all indirect measures, particularly ZST to improve performance.

It is recommended that clinicians take at minimum of 12 serum samples from a cohort of 1-7 day old calves at regular intervals to monitor the effects of any interventions to reduce the prevalence of FPT on a farm. Over-interpretation of indirect testing at one time point is a risk, especially if low numbers of calves are sampled.

Keywords: Calf, failure of passive transfer, diagnostics.

HH-13

A parallel evaluation of five cost-effective indirect methods for assessing failure of passive immune transfer in neonatel calves

Corbiere Fabien[1], Mugnier Amélie[1], Pecceu Katrien[2], Schelcher François[1].

[1]National Veterinary School of Toulouse, Toulouse, France; [2]MSD Animal Health, Beaucouzé, France.

Objectives: The aim of this study was to evaluate the diagnostic accuracy of five indirect methods that may be used in veterinary practices to assess the success of maternal immunoglobulins (Ig) transfer in neonatal calves. These methods include the assessment of STP by biochemistry analyzer in veterinary practice or by optical refractometry, digital Brix refractometry, calculated serum globulin concentration, and serum γ-glutamyl transferase (GGT) activity level.

Materials and methods: Blood samples were collected from 245 2 to 6-d-old calves. Serum IgG concentration were determined by radial immunodiffusion (RID). Serum total protein (STP_BA) and albumin concentrations and γ-glutamyl transferase activity (GGT) were determined by veterinary practitioners using their own biochemistry analyzer. Globulin concentration (GLOB) was calculated. Veterinary practitioners were also asked to assess STP using a digital Brix refractometer (%Brix) and an optical refractometer (g/L, STP_OP). Test sensitivity (Se) and specificity (Sp) were calculated using RID as the reference standard with failure of passive immune transfer (FTPI) defined as IgG < 10.0 g/L. Receiver operating characteristics curves were created and optimal cut-offs values for each of the five indirect measurement methods were selected based on the maximization of the Youden's *J* statistic.

Results: In this study, GLOB yielded the highest accuracy to identify FTPI (Se = 89.4%; Sp = 89.3% at 34 g/L). For %Brix, the optimal combination of Se and Sp was at 8.4% Brix (Se = 86.5% and Sp = 83.8%). The use of STP_OP was associated with the lowest diagnostic accuracy (Se = 69.7% and Sp = 81.6% at 52 g/L). For GGT the optimal cut-points were different for 2- to 3-d-old calves (Se = 87.5% and Sp = 87.8% at 393 IU/L) or 4- to 6-d-old calves (Se = 90.0% and Sp = 86.4% at 254 IU/L).

Conclusion: for on-farm use, digital %Brix refractometer can be a valuable and inexpensive tool to help producers and veterinary practitioners in their calf health management programs.

Keywords: Passive immunity transfer, immunoglogulin, refractometer, serum total protein, GGT.

HH-14

Association of combined serum macromineral profiles with clinical diseases in post-partum Holstein cows

Vangelis Tsiamadis[1], Georgios Banos[2], Nikolaos Panousis[3], Nektarios Siachos[1], Georgios Arsenos[1], Georgios E. Valergakis[1].

[1]Department of Animal Production, Faculty of Veterinary Medicine, Box 393, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece, Thessaloniki, Greece; [2]Scotland's Rural College and Roslin Institute, Edinburgh, Scotland, UK, and Department of Animal Production, Faculty of Veterinary Medicine, Box 393, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece, Thessaloniki, Greece; [3]Clinic of Farm Animals, Department of Clinics, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece, Thessaloniki, Greece.

Objectives: Post-partum serum concentration distortions of macrominerals (Ca, P, Mg, K) are negatively associated with health and productivity of dairy cows. The individual effects of each one has been studied separately from the others, so far. The objective of this research was to evaluate the association of combined macromineral concentrations during the first and second day in milk (DIM) on clinical diseases in early post-partum dairy cows.

Material and Methods: The research was conducted in compliance with international ethical standards. The study was conducted in 9 Holstein dairy herds; 938 cows were enrolled; parity 1, 2 and 3+ cows were 434, 232 and 272, respectively. Cows were blood-sampled on DIM 1 and 2 to assess serum concentrations of Ca (Ca1, Ca2), P (P1, P2), Mg (Mg1, Mg2) and K (K1, K2), and clinically examined on DIM 1, 2, 4 and 8. Body Condition Score (BCS) was evaluated at DIM 1 and 8. Calcium and Mg concentrations were determined with atomic absorption spectrophotometry, while those of P and K with a biochemical/electrolyte analyzer. The biochemical data set consisted of 7,504 macromineral records. The clinical data set consisted of 3,752 observations including retained fetal membranes (RFM), metritis, mastitis, displaced abomasum (DA) and clinical ketosis (KET). A new trait defined as at least one diagnosis on any of the aforementioned clinical diseases was created (CD_1-8). An Exploratory Principal Component Analysis (PCA) analysis with Direct Oblimin rotation was performed in order to test the null hypothesis that factors to-be-extracted were correlated. After rejecting it, a second PCA with Varimax rotation and Kaiser Normalization, which assumes that factors are independent and uncorrelated, was performed. In both analyses, macromineral concentrations were used as variables. Principal components (PC) were extracted using the elbow rule and used as continuous variables in the subsequent analyses. Afterwards, a univariate binary logistic regression (U-BLR) was performed to assess the effects of herd, parity, calving season, BCS and PC on the likelihood that cows have any of the aforementioned diseases diagnosed on DIM 1 to 8. The interaction between potential covariates was also examined. Predicted probabilities derived from U-BLR were used in subsequent receiver operating characteristic (ROC) analysis in order to evaluate the overall U-BLR model performance and to determine proper macromineral thresholds. All analyses were performed with SPSS ver. 25. Statistical significance was set at P≤0.05.

Results: ROC estimated thresholds (in mmol/L) were: Ca1≤2.1, Ca2≤2.03, P1≥1.9, P2≥4.6, Mg1≤1.01, Mg2≤0.95, K1≤4.5 and K2≤4.8. All estimated model Areas Under the Curve were >0.70 (0.64-0.78). Four components (namely first to forth) accounted for 68.3% of total macromineral variance. First component comprised mainly Ca1, Ca2 and Mg2; Ca and Mg concentrations above thresholds were associated with a decreased probability of metritis, DA and CD_1-8, by 77% (64-94%), 63% (41-96%) and 76% (65-89%), respectively. Second component included mainly Mg1 and Mg2; Mg concentrations above thresholds were associated with a decreased probability of metritis by 84% (72-99%). Third component comprised mainly Ca2, P2, K1 and K2; concentrations of Ca, K (above thresholds) and P (below threshold) were associated with a decreased probability of KET by 69% (50-96%). Finally, Forth component consisted mainly of P1, P2 and K1; concentrations of P (above threshold) and K (below threshold) were associated with an increased probability of mastitis by 50% (20-90%). Herd significantly affected all post-partum clinical diseases studied. Compared to parity 1, cows in parities 2 and 3+ were 2.3 (1.4-3.6) and 2.1 (1.3-3.4) times more likely to be diagnosed with RFM; compared to parity 1, cows in parity 3+ were also 3.9 (1.7-8.8) times more likely to be diagnosed with KET. Cows with 3.0≤BCS≤3.5 and ≥3.75 were 7.1 (1.7-30.9) and 9.9 (2.0-48.0) times more likely, respectively, to be diagnosed with KET than cows with BCS≤2.75. Cows with BCS≥3.75 were 3.7 (1.3-10.1) times more likely to be diagnosed with KET than cows with 3.0≤BCS≤3.5. In U-BLR analysis no interaction between potential covariates were identified.

Conclusion: When implementing preventive health management measures, serum concentrations of all four macrominerals must be considered simultaneously. Appropriate Ca, P, Mg and K serum profiles would greatly reduce morbidity of post-partum dairy cows.

Keywords: Macrominerals, Principal Components, Calcium, Magnesium, clinical diseases.

HH-15

Telemetric and Long Term Measurement of Reticuloruminal Motility - Precision and Accuracy of Derived Rumination Time

Johann Gasteiner, Christian Fasching, Michael Astl, Andreas Steinwidder.

Research Centre Raumberg-Gumpenstein, Irdning, Austria.

Objectives: Rumen motility is of basic importance for the physiological function of the digestive system and it is responsible for mixing, separating and transporting the ingesta. Reticuloruminal motility is described by specific contraction cycles. Reticular activity stands at the beginning of the primary contraction cycle. This is characterized by two reticular contractions and this is just found during resting and feeding. A third contraction is only seen during rumination, the so called rejection contraction (Kaske, 2015).

In order to evaluate the possibility of continuous measure-

ment of reticuloruminal activity, a trial was conducted at the Research Centre Raumberg-Gumpenstein. The objective of this study was to investigate, whether the extended contraction duration, due to the rejection contraction, is appropriate to record rumination time by continuously measuring reticular acceleration.

Materials and Methods: Reticular motility was measured continuously in four rumen fistulated cows over a period of 44 days by using a motility-monitoring bolus (MMB). On each day cows were visually observed for 120 minutes between 06.00 to 08.00 a.m. and rumination times were recorded. MMB were prototypes of a commercially available technology (smaXtec®). Boli were located in the reticulum as described by Gasteiner *et al.* (2015) and activity was recorded by an acceleration sensor. These data were used to determine the duration of reticular contractions (s), described as the pulse width (PW). The resulting rumination time recorded by the MMB was compared with visual observations by using the Pearson correlation coefficient (r), the linear regression coefficient of determination (R^2), and the Bland-Altmann plot for validation of precision. The Pearson correlation coefficient and the coefficient of determination from the MMB were categorized according to Hinkle *et al.* (2003). They were considered as precise if the correlation coefficient and coefficient of determination were high (> 0.70), and as accurate if the mean bias from the Bland-Altman plot included zero with the 95 % interval of agreement.

Results: PW was seen to be significantly higher during ruminating cycles ($P < 0.0001$), when compared to feeding or resting. When comparing these results with the outcome of visual observations, the r for rumination time was 0.97 ($P < 0.0001$) and the R^2 was 0.94 ($P < 0.0001$). The slope of regression was found to be 0.92 (± 0.03).The Bland-Altman plot was acceptable and did not show any bias. Mean differences ± standard deviation (MMB – observation) were 0.46 ± 4.09 min. The 95 % confidence interval encompassed 84 of 88 rumination time observations. Overall, the mean differences did, however, include zero within the 95 % interval of agreement, indicating no difference between the MMB and visual observation.

Conclusions: The rejection contraction during ruminating results in a longer reticular contraction time when compared to feeding or resting (12 s vs. 7 s). This enables us to record rumination time very precisely and accurately by using the described technique. When comparing our data with data from previous studies, the described MMB performed similarly to or better than other rumination monitoring systems. Thus the finding of the current study is important for implementation of the MMB on dairy farms because of the precise and automatic collection of physiologically relevant data gathered from the directly out of the reticulorumen. In addition to an exact definition of physiological functions the described technique has a great potential to detect reticuloruminal disorders and diseases at an early stage.

Literature:

Gasteiner, J.; Horn, M. und Steinwidder, A. (2015): Continuous measurement of reticuloruminal pH values in dairy cows during the transition period from barn to pasture feeding using an indwelling wireless data transmitting unit. *Journal of Animal Physiology and Animal Nutrition* **99** (2), 273-280.

Hinkle, D.E.; Wiersma, W. und Jurs, S.G. (2003): Applied statistics for the Behavioral Sciences. 2nd ed., *Houghton Mifflin*, Bosten.

Kaske, M. (2015): Vormagenmotorik und Ingestapassage. In Engelhardt *et al.* (Eds.): Physiologie der Haustiere, 5, *Enke Verlag*, Stuttgart, 361-372.

Keywords: Rumen motility, indwelling monitoring bolus, smart farming,

HH-16

Effects of Isoquinoline Alkaloids-based Product in Lactating Dairy Cows

Gilles Foucras[1], Nathan Cebron[2], Jérémy Poyedessus[1], Laura Leleu[1], Hervé Bezille[3], Santiago Bas[4], Cédric Audonnet[3].

[1]ENVT, Toulouse, France; [2]IHAP, Toulouse, France; [3]Feedéal SAS, Bourgbarré, France; [4]Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany.

Objectives: Improving animal health and welfare with low/no chemical input while achieving high milk yield is a difficult task in modern dairy production systems. To this end, many solutions have been tested with uncertain results. Plant-derived products have been favorably used in several species, but scarce data are available on their effects in dairy cattle. The purpose of this study was to evaluate the effects of a plant-based product derived from *Macleaya cordata* containing standardized concentrations of isoquinoline alkaloids (IQs) on milk production, but also udder and uterine health of lactating dairy cows.

Materials and methods: Primiparous (PRIM; n = 84) and multiparous (MULT; n = 602) cows from 18 commercials herds in France were evaluated. Cows were matched by lactation number, previous lactation milk yield and SCC (MULT) within farms, and were randomly allocated to receive two boluses containing IQs (IQS; n=343) or to serve as a control (CON; n=343). Boluses were designed to release an equivalent of 37.5 mg/d of IQs for 60 days, and 18.75 mg/d of IQs thereafter for three months following administration. Boluses were administered during the close up period, approximately 21 days before the expected date of calving. Data (milk yield, fat and protein, urea, SCC) from the first five official milk test-days were collected and analyzed retrospectively. In an independent set of five herds (n=52 cows in each group), mammary, uterine, hematological, and biochemical (NEFA, BHBA, haptoglobin) data were collected during the first three months of lactation. Statistical analyses were performed using R software (v3.5.1).

Results: Records show that raw and energy-corrected milk yields were higher (Two-way ANOVA, $p < 0.05$) in IQS cows, with a mean increase over than 1.5 kg in the set of 18 herds. No difference in milk fat, protein, and urea concentrations were noticed. Overall, mean SCC was lower (50×10^3 cells/mL; Two-way ANOVA, $p<0.05$), and the prevalence of cows with subclinical mastitis (SCC ≥ 250×10^3 cells/mL) was also lower ($p < 0.05$) in the IQS group on milk test-days during the first three months of lactation. The effect of IQs supple-

mentation is more pronounced in high-producing PRIM cows and improved milk yield in the two lowest quartiles. The number of clinical mastitis was lower in IQS cows ($p<0.05$). No difference for retained placenta or endometritis between IQS and CON were noticed in the first 45 days post-partum. No difference of NEFA, BHBA, or haptoglobin measured in the first week of lactation or fat:protein ratio thereafter could be detected, indicating that improvement cannot be solely explained by changes of the metabolic profile in early lactation.

Conclusions: These results show that administration of a plant-based product with standardized concentrations of IQs increased milk yield and improved udder health. IQs supplementation can be regarded as a strategy to improve productivity and to reduce SCC in dairy cows.

Keywords: Health, dairy cows, milk, plant-based product.

HH-17

Analysis of daily activities of herd managers and interrelations with the animal production and health situation on dairy farms in eastern Germany

Peter Hufe[1], Christina Felgentreu[1], Adriana Wöckel[2], Wolf Wippermann[2], Benno Waurich[1], Franz Schneider[1], Julia Wittich[1], Detlef May[1], Sven Dänicke[3], Hermann Swalve[4], Alexander Starke[5], Melanie Schären-Bannert[2].

[1]LVAT – Institute for Animal Breeding and Husbandry, Groß Kreutz, Germany; [2]"LVAT – Institute for Animal Breeding and Husbandry, Groß Kreutz", "Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, Leipzig University", Leipzig, Germany; [3]Institute for Animal Nutrition, Friedrich-Loeffler-Institute, Brunswick, Germany; [4]Institute of Agricultural and Nutritional Sciences, University of Halle, Halle-Wittenberg, Germany; [5]Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, Leipzig University, Leipzig, Germany.

Objectives: Different aspects of process controlling, such as establishment and assessment of control points and the analysis of workflows and daily activities of employees are standard in many industries to increase the quality and efficiency of production systems. Dairy production has evolved from traditional farming to modern and large-scaled production systems in many parts of the world. However, routines for process controlling are still in their infancy. Well-organized and efficient workflows allow the completion of tasks in an adequate manner and increase the quality of the result as well as the working conditions for employees. Animal production and health are measurable outcomes for the quality of herd management and animal husbandry. In a first approach, we therefore hypothesized an interrelation between daily activities of herd managers and the health status of the herd.

Materials and Methods: An analysis was conducted on 10 farms located in the state of Brandenburg with an average size of 592 German Holstein cows (229-1,221), an average milk production of 9,690 kg marketed milk per cow and year, and 32.9 % culling rate (excl. sales for breeding purposes). The daily workflows on the farm in different fields of activities (e.g. milking, feeding, reproduction, young stock rearing, herd management) were assessed in interviews. The person responsible for herd management was followed throughout a three-week period (mean: 13 d; 11-15, min-max) by a project employee. Activities were continuously documented on a minute-basis. An average of 954 entries per farm (537-1,326; min-max) and a total of 1,092 h (excl. breaks and tasks in other areas than dairy production) were recorded and assigned to 13 categories during data-processing (see results section). Herd data included key figures in following areas: production level (kg marketed milk per cow and year), milk quality (bulk tank and test-day data), stillbirth rates, culling (culling and mortality rates, period: 07/20-06/21). No statistical, only a descriptive analysis was performed.

Results: Of the documented 1,092 h the herd managers invested 4 % (1-9 %, min-max) in administration, office and management, 17 % (9-23 %) in communication, 11 % (3-19 %) in animal documentation, 17 % (11-28 %) in animal organization and movement, 9 % (4-16 %) in animal controls, 9 % (2-16 %) in animal treatment and health measures, 1 % (0-3 %) in feeding, 12 % (4-29 %) in accompanying of external service providers (e.g. veterinary, hoof trimmer, inseminator), 5 % (1-11 %) in assistance of employees, 5 % (0-30 %) in shift takeover from employees, 5 % (0-9 %) in process controlling, 2 % (1-5 %) in other activities and 3 % (0-11 %) of the time was not assigned/not recorded. The five farms where herd managers invested more than average time in animal and process controlling (animal health, feeding and other, ≥14 %) exhibited a higher production level (9,782 vs. 9,598 kg/cow/year), lower somatic cell counts (161,000 vs. 202,000 cells/ml), culling (30.4 vs. 35.3 %) and mortality (5.9 vs. 9.7 %-point of culling rate) rates and thereby higher lifetime productions of culled cows (36,698 vs. 30,400 kg), as well as lower stillbirth rates (6.5 vs. 8.0 %) and losses during the rearing period until 3 months of age (3.5 vs. 8.3 %). The forementioned herd managers invested more time in administration, organization and communication (37 vs. 28 %), but less time in tasks that should be delegated (e.g. moving animals, assistance and taking-over shifts of employees; 22 vs. 32 %) and treatment of animals (7 vs. 11 %). No difference between herd size was observed between the two groups [average of 624 (min: 229, max: 1,221) vs. 560 (min: 399, max: 783) cows].

Conclusions: 1. A large heterogeneity between farms in the activities of their herd managers exists, 2. In many farms original herd manager activities (management and controlling) are postponed by taking over tasks that should be delegated, 3. In most of the farms the controlling of the feeding process is largely disconnected from the person in charge for herd management, 4. Herd managers who spent more time in controlling achieve better results concerning the production level, health and longevity of their herd.

This project was funded by the European Innovation Partnership (EIP)-agri.

Keywords: Workflow analysis, process control, precision dairy farming, herd management, production diseases.

HH-18

The marginal cost of lameness in dairy herds: an integrated bioeconomic modelling approach

Robcis Rodolphe, Raboisson Didier, N'Diaye Youba, Lhermie Guillaume, Ferchiou Ahmed.

National Veterinary School, Toulouse, France.

Objectives: Lameness is a major concern in dairy cattle and foot disorders are responsible for 92% of total lameness cases. Different strategies are available for the farmers to manage lameness: short-term actions (curative trimming, footblocks, antibiotics, footbaths) and overall medium and long-term actions (hygiene, detection, preventive trimming). The objective of this study is to estimate the marginal cost of lameness and foot disorders, to define optimal bioeconomic strategy for managing lameness in dairy herds.

Materials and methods: A dynamic and stochastic simulation model (DairyHealthSim©) was used to simulate as precisely as possible the herd dynamics, reproduction management, the production and health events. The model offers a holistic representation of a dairy farm and simulates the interaction between production functions, diseases or damage function and veterinary or damage control functions according to farmer's practices. This model was previously used for studies on mastitis, reproduction and culling. A specific development (DHS-Lame) was done for lame and its different herd-level management strategies. Lame occurrences are simulated with a base risk for each aetiology of lameness (digital dermatitis – DD, interdigital dermatitis – ID, interdigital phlegmon – IP, sole ulcer – SU, white line disease – WLD), associated to a relative risk according to a farmer's practices. Lameness is simulated as lame state machine (lame score – LS – from 1 to 5) associated to a DD state machine (M1 to M4.1). The impacts are computed on milk production and heat expression. Once detected, lame cows are treated, and a recovery rate is applied depending on aetiology and treatment. A total of 880 scenarios were run including (i) housing (concrete vs. textured) (ii) hygiene (2 different scrapping frequencies), (iii) the existence of any preventive trimming or not, (iv) different thresholds of detected DD prevalence from which a collective footbath is applied to treat DD and (v) detection rate scenarios to represent farmer ability to detect lame. The economic evaluation is performed to calculate farmers risk adjusted income for each scenario and the marginal cost is calculated as an income loss per additional case for each lame aetiology and for additional lame cow (LS ≥3) Calibration was done thanks to peer-review literature review, grey literature or authors' experience if not available.

Results: The simulation results show a lameness prevalence varying from 27% to 83% depending the scenarios, showing a high capacity of the model to represent field. Digital dermatitis represented half of total lameness cases, followed by ID, SU, WLD and IP. Validity of the model was overall considered as very good.

Housing surface scenarios dramatically influence the prevalence of SU and WLD, while scrapping frequency and footbath threshold for application mainly determine the presence of DD. Results interestingly shows that preventive trimming allows a better reduction in lame prevalence than spending time for early-detection. Scrapping frequency was highly associated with DD occurrence, especially with a textured floor. The observed prevalence of DD reaches a plateau when the threshold of footbath application is over 30% (R2 = 0.94). Lameness prevalence variations also show a sensitive negative impact on total milk yield, with a logarithmic trend ($R^2 = 0.8$).

Economic results show that the milk losses represent the first component of lame costs, in agreement with literature. The marginal cost curve for the prevalence of lameness shows a downward logarithmic trend (lower marginal cost for higher prevalence): the marginal cost for lameness ranges from 193€ to 5€ while prevalence ranges from 25% to 60%, all aetiologies included. For DD, the marginal cost ranges from 248€ to 12€ with a prevalence range from 10 to 35%. For WLD and SU, the marginal cost ranges is from 281€ to 87€ and from 414€ to 22€ respectively for prevalence ranging from 2.5% to 5.5% and from 4% to 25% respectively.

Conclusions: The models allow to mimic with high precision the lame dynamics in the herd. The marginal cost of lame describes a downward logarithmic trend, meaning lower cost of one extra lame case for higher prevalence. The present tool can also be used as support for decision making and to identify the best strategy depending on the farm contexts.

Keywords: Lameness, economics, cow, management.

HH-19

Nutritional and health supplements in molasses blocks delivers increased rumen efficiency in smallholder large ruminant production in developing countries

Peter Windsor[1], Luisa Olmo[2], Nichola Calvani[2], Francesca Earp[2], Russell Bush[2].

[1]Production Animal Welfare & Health Services, Scarborough, NSW, Australia; [2]The University of Sydney, Camden, NSW, Australia.

Objectives: Summarize field studies examining the four different molasses-block supplements (consumed @ 100-150g/day) provided to large ruminants, for improved health and production outcomes, in a developing country context. Trials were conducted in Laos, where productivity is compromised by low growth rates, endemic adult and calf endoparasitism by *Fasciola gigantica* or *Toxocara vitulorum* respectively, with limited farmer knowledge and facilities for administration of therapeutics. Further, discuss a marketing feasibility study of this supplementation strategy and consider the environmental impacts of improving rumen efficiency through this technology.

Materials and methods: Field trials were implemented in villages in mainly the dry season in Laos from 2017-2019, studying the impacts of *ad-libitum* supplementation with high quality molasses blocks (20kg) (4 Seasons Pty Ltd, Brisbane, Australia), containing: non-medicated (NMMB); urea-medicated (UMB8; @ 8% urea); triclabendazole-medicated (Fasinex®, Novartis Australia, TMB5 @ 5g/kg, and TMB10 @ 10g/kg); fenbendazole-medicated (Panacur100®, Coopers Australia; FMB5; @ 5g/kg). Animal cohorts were enrolled into one of

three groups: (i) access to either UMB8, TMB5 or TMB10, or FMB5 medicated molasses blocks; (ii) access to NMMB; and where possible (iii) control groups without access to blocks. To serve as positive controls, additional cohorts were treated with oral anthelmintics. This inlcuded two FMB5 trials where oral pyrantel was administered at 2-3wks of age, plus use of oral triclabendazole in TBZ5 and TBZ10 trials. Weight data and faecal samples were obtained at weeks 1, 4, 8 and 12 for growth rates (average daily gain; ADG = grams/day) and faecal egg counts (FEC). Where data from cohorts not receiving blocks was unavailable, the results were compared with recently acquired baseline ADG data.

Results: Baseline measurements obtained from studies of the free grazing systems in Laos, confirmed low mean weights (182–204kg in cattle; 325–357kg in buffalo) and low ADG's (55–84g in cattle; 92–106g in buffalo), with animals likely to lose weight during the dry season due to feed deficiency. Trials with UMMB and UMB8 compared the weight gains of lactating cows (n = 46), calves (n = 25) and growing calves (n = 35) of famers (n = 20), with access to both blocks significantly increasing ADG in all 3 cohorts compared to baseline grazing-only data. The supplemented lactating cows (191g, 179g), calves (298g, 238g), and growing cattle (214g, 143g) all performed well respectively, but less than in a pen study conducted at a research station near Vientiane where UMMB-fed bulls achieved a superior ADG (320g). The initial triclabendazole trial (n=241) examined *Fasciola spp*. suppression over a 12 week period of access to TBM5 blocks, reducing FEC (~90%) and increasing ADG (201g) that was superior to the reduction in FEC (~19%), and ADG (124g) of those with NMMB access (*P*=001). A 2019 trial with TMB10 observed rapid elimination of the FEC following exposure to this block; these were replaced with NMMB for the remaining 10wks. In the fenbendazole trials, the FMB5 treatment produced rapid reduction in FEC relative to the control (*P*=0.062) and a high ADG (230g), exceeding that of control calves (170g) (*P*=0.002). Additional trials corroborated that FMB5 access was associated with higher predicted ADG (200g) compared to control calves (150g) (*P*=0.005). All farming families experiencing use of the blocks confirmed their animals were healthier with minimal parasites, they were easier to manage, plus they wished to purchase the blocks. Registration of molasses supplementation blocks in Laos has now occurred and sales of commercial blocks have commenced.

Conclusions: Provision of block supplements to large ruminants in Laos, especially lactating cows, enabled animals to maintain and even increase weight during the dry season when lactation occurs. Block supplementation also enabled efficient administration of endoparasite medication to large ruminants. Improved utilisation of low-quality forage and control of endemic parasitism, offers important opportunities for increasing large ruminant production efficiency. There is an emerging challenge to food security in providing more sustainable livestock systems in a climate emergency world. Significant improvements in cattle production efficiency means fewer animals required with shorter time periods necessary to achieve marketable levels of beef; one of several strategies that may help mitigate the impacts of beef production on greenhouse gas emissions from agriculture.

Keywords: Molasses-blocks, supplementation, anthemintics, cattle, buffalo

HH-20

Veterinary consulting on dairy farms with high rearing losses

Martina Hoedemaker, Johanna Sophie Dreyer, Anika Korte, Ina Gaude.

Clinic for Cattle, University of Veterinary Medicine Hannover, Hannover, Germany.

Objectives: Although the causes and risk factors for losses in youngstock are well known, apparently this area does not receive enough attention on many dairy farms. This might explain that calf mortality has not changed over the years quite often reaching values on farm level between 10% and 20%. It was the aim of the following study to analyse the rearing conditions on dairy farms with high rearing losses, to identify problem areas and provide extensive advice to the farmers. In addition, the success of veterinary consulting should be evaluated during a second farm visit and the reasons for non-implementation of measures should be recorded.

Material and Methods: Fifty-six dairy farms in Lower Saxony, Germany, participated in the study. Inclusion criteria were at least 30 dairy cows and rearing losses of ≥ 20%. Rearing losses were defined as number of calves recorded as live animals in the National Data Base which died within the first 6 month of live devided by the total number of calves recorded as live animals within a given time period. A questionaire was filled out together with the farmer followed by a farm inspection covering 10 different areas (calving management, newborn calf care, colostrum management, separation from dam, milk feeding, weaning, calf housing, heifer feeding, heifer housing, general monitoring of animals). The farmers received a thourough assessment and recommendations for improvements. Eight to 10 month after the first visit, the farms were visited again and analysed in the same way as during the first visit. In addition, the adoption of recommended measures was recorded. In case of non-implementation, the farmers were asked for reasons. Calf mortality was assessed during the intervall between the two farm visits and compared with the same time intervall in the preceding year to account for seasonal effects.

Results: Overall, 180 variables in the 10 different areas were analysed and evaluated whether or not good practices were fullfilled. The majority of farms (≥ 50%) performed only 42.8% of management measures accepted as good practice. Specific problem areas were newborn calf care, colostrum management, hygienic condition of calf housing and calving area as well as documentation and biosecurity. Overall, 250 different measures were recommended. Of those, 20.8% were not adopted at all; 40.8%, 30.0% and 8.4 % were adopted with a frequency of < 30 %, 30 to 80% and > 80%, respectively. Recommendations which were not adopted very well were hygienic measures in the calving and heifer areas and management measures considering calf health (vaccination, treatment, etc.). The acceptance was greater for measures dealing with the hygiene of milk feeding, calving management, newborn calf care, feeding of calves before weaning including colostrum management, and calf housing. On farm level, the mean frequency of adopted measures was 25.1%±10.4% (4.0% to 53.2%). The most frequent reasons for non-implementation were „not practicable" (19.9%), „to time consuming"

(13.7%) and „not promising“ (12.9%). Even though the selection criterium was ≥ 20% calf mortality at the time of random selection of the farms, calf mortality in the control time period was < 10% on 14 farms (26.4%), ≥ 10% to < 15% on 14 farms (26.4%), ≥ 15% to < 20% on 12 farms (22.6%) and ≥ 20% on 13 farms (24.5%), respectively. Overall, the calf mortality did not change, improve or got worse in 28.3%, 49.1% and 22.6% of the farms, respectively.

Conclusion: Dairy farms with high rearing losses do not follow good practices primarily in the areas of newborn calf care, colostrum management and hygiene of calf and calving cows areas. With about 25%, the adoption of recommended measures was fairly low. Also, the success of a one time consultation was only mediocre. Even though 49.1% of the farms could lower calf mortality, it increased on 22.6% of the farms during the study period. Obviously, rearing management represents a complex time and personell intensive part on dairy farms. The tasks often are difficult to predict leading to a high risk of calf mortality. A continuous expert advice appears to be necessary in order to achieve long-term and sustained success.

Keywords: dairy farms, calf mortality, veterinary consultation.

HH-21

Implementation of precalving vaccination programme against rotavirus, coronavirus and enterotoxigenic *E. coli* (F5) and effect on dairy calf survival

Dagni-Alice Viidu, Carmen Kuningas, Kerli Mõtus.

Estonian University of Life Sciences, Tartu, Estonia.

Introduction and objectives: Calves are born agammaglobulinemic and during the first weeks of life their resilience to infections depends on the extent of passive immunity acquired from colostrum. Diarrhea is one of the most common diseases and cause of death in neonatal calves whereas rotavirus is often reported as the most prevalent viral cause of diarrhea. Vaccinating cows and heifers before calving increases the level of pathogen-specific antibodies in the colostrum and enhances passive immunity potentially gained by the calf, however, the effect of precalving vaccination with trivalent vaccines on calf survival has not been thoroughly investigated. The current study aimed to investigate the implementation of the precalving vaccination programme against rotavirus, coronavirus and enterotoxigenic *Escherichia coli* (F5 antigen) in Estonian dairy farms and its effect on calf survival.

Materials and methods: The study included 13 dairy cow herds (herd size ranged from 242 to 2,457 cows) that applied precalving vaccination against bovine rotavirus, bovine coronavirus and enterotoxigenic *E. coli* (F5) (defined as PV). For each herd a questionnaire was filled out where the information about the vaccination procedures and calf feeding practices were asked. Farms were divided into correct and incorrect users (CU and ICU, respectively) of the PV based on the compliance with the product directions of use and colostrum feeding practices. To qualify as a CU the farm had to vaccinate all cows and heifers 3-12 weeks before expected calving and feed the calves whole milk from vaccinated fresh (up to four days in milk) cows at least during the first two weeks of calves´ life.

In order to analyse the effect of PV on calf survival the survival analysis was used comparing calf mortality rates in pre-vaccination (V-) and vaccination (V+) periods by using the registry data of Estonian Agricultural Registers and Information Board. The V+ period begun one month after the farm started vaccinating their animals and lasted for one year. The V- period was determined as exactly one year prior to V+ period. The start of the calf-level observation period started with birth or start of the observation period for calves that were born before the start of the observation period to account for left truncation. Calf-level observation period ended with either reaching 21 days of age or end of the observation period due to selling, slaughtering or death (euthanasia or unassisted death). Unassisted death and euthanasia (on-farm mortality) were considered as event of interest and observations that ended with selling, slaughtering or survival until the end of the study period were right censored. On a herd level, calf mortality rates were calculated as number of deaths per 100 calf-months for V- and V+ period. Farms were enrolled to the analysis only if they ear-tagged their calves during the first 4 days of life. The number of calf-based observations was 14,610 in the analysis.

Results: Six herds met the requirements of CU and seven farms were classified as ICU. Four out of the seven ICU farms did not vaccinate heifers and six fed the calves whole milk collected from vaccinated fresh cows for seven days or less.

In four out of the six CU farms, calf mortality rate decreased significantly during the V+ period compared to V- period. In one CU farm calf mortality rate was higher and in one CU farm it remained unchanged in V+ period compared to V- period. Average calf mortality rate over six CU farms was 17.08 per 100 calf-months during V- period (95% CI 15.85; 18.41) and 11.56 per 100 calf-months during V+ period (95% CI 10.64; 12.57). On average, calf mortality rate decreased among the CU farms during the V+ period compared to V- period (hazard ratio (HR) = 0.66, 95% CI 0.59; 0.74, p < 0.001) according to the mixed-effects Cox regression model (herd included as random effect).

Conclusions: The study revealed that vaccination protocols and practices of feeding calves colostrum from vaccinated cows vary in the farms. Also, we confirmed a positive field effect of PV on dairy calf survival in the herds that followed vaccination directions and fed calves colostrum from fresh cows at least during the first two weeks of life.

Keywords: Precalving vaccination, lactogenic immunity, calf scours, mortality.

HH-22

Cow behavior predicts and monitors calving diseases along the transition period

Doron Bar[1], Gilad Factor[1], Eran Friedman[1], Johan De Meulemeester[1], Monika Ptaszynska[2].

[1]Allflex Livestock intelligence, Netanya, Israel; [2]MSD Animal Health, Milton Keynes, United Kingdom.

Objectives: Calving diseases are a major problem in most dairy herds, negatively influencing cow welfare and dairy economics. Rumination behavior proved to be a good predictor of some calving diseases. New generation of these monitoring technologies are now able to describe the behavior of cows in a more detailed way (adding e.g. eating, resting, activity, etc.) The objective of this study was to characterize behavior of cows along the transition period by their health status, to improve the ability to characterize normal behavior of healthy cows and deviations from this behavior.

Material & Methods: We analyzed the behavior of a total of 1,553 cows calving in 8 farms in Israel, between February 2019 and November 2019. Farms were from various geographical locations, but similar in their feeding management (Total Mixed Ration [TMR]) and cow sheds (open, covered, dry compost barns). Calving events (i.e. twins, stillbirths, milk fever, retained placenta, metritis or endometritis, ketosis, and left displaced abomasum [LDA]) were recorded by the farmers and in addition, all cows were checked by a trained veterinarian between 6 to 12 days after calving. These data were stored in the Israeli cattle breeder association herd management software. The behavior of cows (the major activity in each minute) was obtained from a monitoring system (Sensehub Dairy, Allflex livestock intelligence) and summed to a total minutes per day. After initial descriptive statistics which is beyond the scope of this abstract, we present here the average effect of each major calving event in the last 10 days before calving and in the first 10 days of the lactation. Total minutes ruminating, eating, resting, or expressing high activity per day were dependent variables in mixed effect models (using the lme4 package in the R Foundation for Statistical Computing software) containing the day relative to calving day, calving event or diseases as fixed effects and cow nested in farm and parity as random effects.

Results: Last 10 days before calving: Cows with milk fever or retained placenta had a significant reduction ($P<0.01$) in the time spent ruminating days before the events happened. A significant reduction ($P<0.01$) in eating time and high activity time was observed in cows suffering later from stillbirth or ketosis, these cows had also a significant increase ($P<0.01$) in resting time in the last 10 days before calving.

First 10 days after calving: Cows with retained placenta, metritis or endometritis, ketosis, or with left displaced abomasum had a significant ($P<0.001$) decrease in rumination, eating, and high activity time. These cows had also a significant ($P<0.001$), substantial (~25%) increase in the time spent resting in the first 10 days after calving.

Conclusion: The finding of this study demonstrates the ability to identify cows developing one of the calving diseases early, thus possibly reducing their negative impact on cow welfare and farm profitability. Early identification of such cows before clinical signs are visible also faces the bovine practitioner with the challenge of how to best handle such cows. In addition, analyzing herd cow's behavior along the transition period, might provide insight into weak points and serve as an objective tool to monitor improvements.

Keywords: Dairy cattle, behavior, calving diseases, monitoring.

HH-23

Can an ATP bioluminescence meter be used as a cow-side tool for the evaluation of bacterial contamination of bovine colostrum?

Geert Hoflack[1], Pleun A.A. Penterman[2], Geert Vertenten[2], Bart Sustronck[1].

[1]MSD Animal Health, Brussels, Belgium; [2]MSD Animal Health, Boxmeer, Netherlands.

Objectives: Bovine neonates are born agammaglobulinaemic and rely on the absorption of immunoglobulins from colostrum shortly after birth. This absorption of immunoglobulins from the colostrum can, unfortunately, be hampered by a high bacterial load of the colostrum. Hence, strict hygienic procedures when collecting, storing and feeding colostrum are of paramount importance for a good transfer of passive immunity in bovine neonates. The adenosine triphosphate (ATP) bioluminescence meter has been advocated as a simple and useful tool for the evaluation of the degree of contamination of surfaces and liquids. The aim of the present study was to examine whether an ATP bioluminescence meter can be used as a cow-side tool to assess the degree of contamination of bovine colostrum and thus indirectly evaluate the hygiene applied during the collection, storage, and administration of colostrum on farms.

Materials & Methods: A total of 162 colostrum samples were collected from 88 different cattle farms in The Netherlands and Belgium. Samples of approximately 100 ml were collected immediately before the colostrum was fed to the calves. All samples were stored at -20°C until processing. The degree of bacterial contamination (total bacterial cell count) was determined using a Bactoscan™ FC+ (FOSS, Denmark) automatic bacterial count reader. The ATP bioluminescence, expressed as the number of relative light units (RLU) for each colostrum sample, was obtained using a SystemSURE Plus meter (Hygiena™, California, USA). On each occasion, the mean of three consecutive ATP bioluminescence measurements was calculated and used for analysis. The relationship between the total bacterial count of colostrum and the ATP bioluminescence relative light units of colostrum was investigated with a Spearman's rank correlation. Statistical analysis was performed in R (R Core Team 2017).

Results: Samples originated from HF dairy cows (n= 86) or from Belgian Blue beef cows (n= 76). The median total bacterial cell count of the colostrum samples was 7.000 CFU/ml and ranged from 4.000 to 1.000.000 CFU/ml. The median ATP

bioluminescence of the colostrum samples was 255,00 RLU (range 1,00 – 2486,67 RLU).

The total bacterial cell count of colostrum and the ATP bioluminescence of colostrum were negatively correlated ($\rho_{spearman}$= -0,27; $CI_{95\%}$ [-0,4 to -0,2]; p < 0,001).

Conclusion: The results of this study indicate that ATP bioluminescence measurements of colostrum cannot be used to evaluate the hygiene of the procedures applied during the collection, storage, and administration of bovine colostrum.

Keywords: Cattle, Colostrum, ATP bioluminescence, Total bacterial count.

HH-24

Dutch HF cow colostrum quality based on IgG concentration and total bacterial cell count

P.A.A. Penterman[1], G. Hoflack[2], G. Vertenten[1], F.H.J. Van Hagen[3], W.A. Schaap[4], Bart Sustronck[2].

[1]MSD Animal Health, Boxmeer, Netherlands; [2]MSD Animal Health, Brussels, Belgium; [3]Slingeland Dierenartsen, Varsseveld, Netherlands; [4]DGC Bekenland, Gelselaar, Netherlands.

Objectives: Because immunoglobulins cannot cross the epitheliochorial placenta of the cow during gestation, calves rely on the passive transfer of immunoglobulin G (IgG) through consumption of sufficient amounts of high quality colostrum within the first hours after birth. Besides by the concentration of IgG, colostrum quality is also determined by its bacterial contamination. To our knowledge, no recent data concerning the colostrum quality of Dutch Holstein Friesian (HF) cows are available. Therefore, the purpose of this study was to assess the colostrum quality from Dutch HF cows using different methods. The second aim of the study was to evaluate the influence of farm and veterinary practice on colostrum quality.

Materials & methods: Two large Dutch veterinary practices participated in this field study. 86 Dutch HF colostrum samples were collected at the time of first colostrum feeding on 19 different commercial dairy farms. For the quantification of the IgG's in colostrum, a commercial competitive ELISA-test kit (BIO K420, MonoScreen QuantELISA Immunoglobulin Easy, Bio-X Diagnostics S.A., Rochefort, Belgium) was used. Indirect evaluation of the IgG concentration of the colostrum samples was performed using a digital Brix refractometer (Milwaukee Refractometer MA871, Milwaukee Instruments Inc., NC, USA). On each occasion, the mean of three consecutive Brix refractometric measurements was calculated and used for analysis. The relationship between the IgG concentration and the Brix value of colostrum was investigated with a Pearson's correlation. The total bacterial cell count (TBCC) of the colostrum samples was determined using a bactoscan automatic bacterial count reader (Bactoscan™ FC+, FOSS, Denmark). The relationship between the IgG concentration and the TBCC of colostrum was evaluated using a Spearman's rank correlation. All statistical analyses were performed using R software (R Core Team, 2017).

Results: The number of colostrum samples collected per herd ranged from 2 to 5. The colostral IgG concentrations and the colostral Brix values followed a normal distribution with a mean of 53,19 ± 17,61 g IgG/l (range: 14,14 – 63,07 g IgG/l) and 21,10 ± 4,21 % (range: 9,33 – 29,27 %), respectively. The TBCC of the colostrum samples ranged from 4.000 to 1.000.000 CFU/ml with a median of 9.000 CFU/ml. Furthermore, 38,1% of the colostrum samples had an IgG concentration below 50 g/l and/or a TBCC exceeding 100.000 CFU/ml. A significant positive correlation was found between the IgG concentration and the Brix value of colostrum samples ($r_{pearson}$= 0,65; $CI_{95\%}$ [0,51 to 0,76]; p < 0,001). The colostral IgG concentration and the TBCC of the colostrum were negatively correlated ($\rho_{spearman}$= -0.30; $CI_{95\%}$ [-0,49 to -0,09]; p = 0,005). The mean IgG concentration (48,30 ± 18,71 g IgG/l) and the mean Brix value (19,90 ± 4,4 %) of the colostrum samples from practice A were both below the recommended threshold of 50 g IgG/l and 22 %, respectively. Compared to practice A, the colostrum samples from practice B had a significantly higher IgG concentration (58,68 ± 14,65 g IgG/l; p= 0,01) and Brix value (22,45 ± 3,59 %; p < 0,001). The non-parametric comparison of the TBCC of the colostrum samples revealed significant (p < 0,001) differences between the two practices (median practice A : 16.000 CFU/ml [range: 4.000 – 1000.000 CFU/ml] , median practice B : 6.000 CFU/ml [range: 4.000-1.000.000 CFU/ml]). The mean colostral IgG concentration was lower than the 50 g IgG/l threshold in 8 out of 19 farms. Only 10 out of 19 farms had a mean colostral Brix value of at least 22%. The median TBCC of the colostrum samples was higher than 100.000 CFU/ml in 3 out of 19 farms. Between the farms, obvious differences were observed. Certain farms (n=3) consistently presented poor-quality colostrum (IgG below 50 g/l and / or TBCC above 100.000 CFU/ml) results, whereas other farms (n=4) showed colostrum samples that all were of good-quality.

Conclusion: The results of this field study indicate that the colostrum quality of Dutch HF cows in general is rather poor, with nearly 40% of all samples not meeting the minimal requirements for good-quality colostrum. The differences observed between the veterinary practices could indicate that in some practices more attention is paid to the colostrum management compared to others. Farm level analysis, however, indicates that focused attention on certain farms may be necessary.

Keywords: Dutch dairy cattle, Colostrum quality, IgG ELISA, Brix refractometer, Bactoscan.

HH-25

Association between prepartum nonesterified fatty acids serum concentrations and postpartum diseases in dairy cows

Isabella Nicola[1], Hervé Chupin[1], Jean-Philippe Roy[1], Sébastien Buczinski[2], Véronique Fauteux[1], Nicole Picard-Hagen[3], Roger Cue[4], Jocelyn Dubuc[1].

[1]Université de Montréal, Saint Hyacinthe, Canada; [2]Université de Montréal, Saint-Hyacinthe, Canada; [3]Université de Toulouse, Toulouse, France; [4]McGill University, Ste-Anne-De-Bellevue, Canada.

Objectives: The objective of the present study was to evaluate the association between prepartum nonesterified fatty acids (NEFA) concentrations and development of postpartum diseases and culling occurring during the first 50 days in milk (DIM). A secondary objective was to identify optimal thresholds allowing identification of animals at greater risk of diseases or culling.

Materials and methods: We conducted a prospective cohort study over a 1-year period on 50 commercial Holstein dairy farms that were regular clients of the Bovine Ambulatory Clinic of the Faculté de Médecine Vétérinaire of the Université de Montréal (St-Hyacinthe, QC, Canada). Herds were selected by convenience based on being enrolled in a preventive veterinary medicine program involving herd health visits every 14 days, and on being willing to participate. At every herd visit, blood samples were taken from all cows within 35 days (±7) of subsequent predicted calving date. The samples were centrifuged (3,500 rpm x 10 minutes) and sera were stored at -20°C. After calving, the number of days between sampling and calving were calculated. Cows sampled between 1 and 14 days before calving were included in the study; their serum samples were submitted for quantification of NEFA concentrations. Data about diseases occurring during the first 50 DIM were collected. Disease definitions were standardized during the study and included: retained placenta (RP; ≥24 hours after calving); hyperketonemia (HK; BHBA ≥1.4 mmol/l) during the first 15 DIM; puerperal metritis (MET) during the first 20 DIM (fetid watery vaginal discharge with general signs of illness); displaced abomasum (DA) during the first 30 DIM; clinical mastitis (CM) in the first 30 DIM (milk aspect alteration with or without quarter swelling, with or without fever/anorexia); purulent vaginal discharge (PVD) at 35 ± 7 DIM inspected with a Metricheck device (threshold ≥ 3 based on McDougall et al., 2007), cytological endometritis (CYTO) measured at 35 ± 7 DIM with a leucocyte colorimetric test (threshold ≥ 1). Diseases were diagnosed by veterinary practitioners except for RP and mild or moderate case of CM. Descriptive statistics and multivariable mixed logistic regression models were computed. Diseases and culling were considered to be the dependent variables of each model. Independent variables included prepartum NEFA serum concentration, parity, and season of calving. Herd was also included in the models as random variable. At first, NEFA concentrations were offered to the models as a continuous variable. Subsequently, for variables with significant correlation with NEFA, NEFA concentrations were dichotomized in order to find the optimal thresholds using non-nested models (lowest AIC value).

Results: A total of 1,299 cows were included in the study. The incidence of diseases was 10% for RP, 20% for HK, 16% for MET, and 7% for DA, 13% for PVD, 40% for CYTO, 9% for CM, 3% for culling. Overall, 47% of cows had experienced at least one disease during the first 30 DIM. In the study, the NEFA concentration was associated with a greater risk of developing every individual disease for RP, HK, MET, DA and CM (P<0.05); with a greater risk of developing any of the aforementioned diseases in the first 30 DIM (P<0.05); and with a greater risk of being culled in the first 50 DIM (P<0.05). Based on the lowest AIC value from non-nested models, the optimal NEFA thresholds were ≥290 µEq/L for RP (OR 2.2, 95%CI: 1.5-3.1; P<0.01), ≥280 µEq/L for HK (OR 2, 95%CI 1.5-2.7; P<0.01), ≥300 µEq/L for MET (OR 4.0, 95%CI: 2.7-5.7; P<0.01) , ≥300 µEq/L for DA (OR 4.2, 95%CI: 2.9-6.1, P<0.01), ≥ 280 µEq/L for CM (OR 3.4, 95%CI: 2.2-5.3, P<0.01), ≥ 260 µEq/L for culling (OR 4.7, 95%CI: 2.2-10, P<0.01).

Conclusions*:* These study results confirm the association between prepartum NEFA serum concentrations and postpartum diseases (30 DIM) and culling (50 DIM). The optimal thresholds found in the present study were similar to those reported elsewhere. No association was found between NEFA and PVD or CYTO, in accordance with previous studies. We can conclude that prepartum NEFA thresholds of ≥280 can help to identify animals at greater risk of developing diseases in the first 30 DIM or being culled in the first 50 DIM.

Keywords: Nonesterified Fatty Acids, Post-partum diseases, Clinical mastitis.

HH-26

Relationship of nonesterified fatty acids with automated health-monitoring system variables and blood metabolic profile parameters in dairy cows

Dovile Malasauskiene[1], Mindaugas Televicius[1], Ramunas Antanaitis[1], Vida Juozaitiene[2].

[1]LUHS VA Large animal clinic, Kaunas, Lithuania; [2]LUHS VA Department of animal breeding, Kaunas, Lithuania.

Objectyves: The aim of this study was to assess the dependence of changes in nonesterified fatty acids (NEFA) on lactation number and to assess the relationship of NEFA with automated health-monitoring system biomarkers (AMS) variables and parameters of the blood metabolic profile in dairy cows.

Materials and methods: The study was conducted on 105 clinically healthy cows of Lithuanian Black and White breed. Cows were grouped by lactation number: 1 (n = 30), 2 (n = 60), 3 (n = 15) and NEFA level: NEFA <0.3 (n = 67) and NEFA ≥0.3 (n = 38) (James K. Drackley, 2000). All samples for NEFA were analyzed using an automated wet chemistry analyzer (Rx Daytona, Randox Laboratories Ltd, UK) with using reagents (Rx Daytona, Randox Laboratories Ltd, UK). Blood serum parameters, including NEFA, aspartate aminotransferase (AST), albumines (ALB), and γ-glutamyl transpeptidase (GGT) were examined using the Hitachi 705 analyser (Hitachi, Japan), and DiaSys reagents (Diagnostic Systems GmbH, Germany). Cortisol level was measured using the fluorescence enzyme immunoassay method by Tosh Corporation AIA-360 (USA). Milk yield (MY), progesterone and beta-hydroxybutyrate (BHB) were registred from AMS Herd Navigator (Lattecl/S. Hillerød. Denmark) in combination with a DeLaval milking robot (DeLaval Inc. Tumba. Sweden).

Results: The average NEFA value for all cows was 0.145 ± 0.013 (95% CI = 0.120-0.171). The study showed that (NEFA) tends to increase with increasing lactation (y = -0.019x + 0.1808; R^2 = 0.9998, P <0.001).The average NEFA value in primiparous cows (0.162 ± 0.034) was 12.04% lower compared with lactation 2 cows and 23.46% lower compared to lactation 3 (P <0.05). Analysis of the estimated indicators by

level of NEFA indicated statistically higher values of albumins (ALB) (41.61%, P <0.001), aspartateaminotransferase (AST) (26.37%, P = 0.05), γ-glutamyltransferase (GGT) (48.21%, P = 0.04) and a lower value of milk yield (MY) (20.72). %, P = 0.015) in cows of the NEFA group ≥0.3 compared to the group of NEFA <0.3.NEFA values were reliably correlated with serum ALB (r = 0.446, P = 0.001), AST (r = 0.292, P = 0.032), MY (r = 0.296, P = 0.05) and milk progesterone (r = 0.371, P = 0.006) and were negatively associated with GGT (r = - 0.298, P = 0.028).

Conclusions: Acording our study NEFA concentration has tendency to change on different lactation number. Highest NEFA concentration affect the concentration of some blood serum parameters. Our study showed that lower NEFA levels were associated with higher productivity and lower cortisol levels in blood of dairy cows.

Keywords: NEFA, dairy cows, health management.

HH-27

A description of colostrum quality on Scottish dairy farms and risk factors associated with poor colostrum quality

Alexandra Haggerty[1], Colin Mason[2], Kathryn Ellis[3], Katharine Denholm[3].

[1]The University of Glasgow/The Stewartry Veterinary Centre, Glasgow/ Castle Douglas, United Kingdom; [2]The Scotland's Rural Colleges, Dumfries, United Kingdom; [3]The University of Glasgow, Glasgow, United Kingdom.

Objectives: Poor colostrum quality is a major risk factor for failure of passive transfer (FPT) in neonatal dairy calves. FPT contributes to high incidences of calf morbidity and mortality as well as more long-term detrimental effects on productivity (DeNise *et al.*, 1989; Tyler *et al.*, 1999; Faber *et al.*, 2005). The two main measures of colostrum quality are immunoglobulin (IgG) concentration and bacterial contamination (total bacteria counts (TBC) and coliform counts (TCC)). Brix refractometry measures the total solids in colostrum by refraction of light and is highly correlated with IgG concentration (a threshold of 22% is used to indicate adequate quality) (Quigley *et al.*, 2013; Deelen *et al.*, 2014). US industry guidelines for bacterial contamination of colostrum state that TBC should not exceed 100,00CFU/ml and TCC should not exceed 10,000CFU/ml (McGuirk and Collins, 2004). The objectives of this study were to:

- measure IgG concentration and bacterial contamination of colostrum at point of feeding to neonatal dairy calves.
- establish risk factors for poor colostrum quality on Scottish dairy calves.

Materials and Methods: Thirty-eight farms in the Stirlingshire, Lanarkshire and Dumfries and Galloway regions of Scotland were enrolled between February and June 2019. A farmer questionnaire was completed by interview, detailing colostrum management practices. Two hundred and fifty-two colostrum samples were collected by trained farm staff at point of feeding. For each sample, Brix refractometry (Brix) was used to estimate IgG concentration and TBC and TCC were carried out using plate counts and Petrifilms™ respectively.

Brix, TBC and TCC were dichotomized to form categorical outcome variables either falling below, or exceeding industry thresholds and multi-level logistic regression models were constructed using farm as a random effect.

Results: Results for IgG concentration showed 111/252 samples, (44.05 %) were below 22% Brix and 77/252 (29.76 %) and 50/252 (19.84 %) exceeded the TBC and TCC thresholds respectively. When industry recommendations, in terms of Brix % and bacterial contamination, were considered only 99/252 (39.29%) met all the criteria.

Colostrum harvested from dams more than six hours after calving was half as likely to exceed the Brix threshold of 22% (reflective of adequate IgG concentration) (OR=0.47, 95%CI = -1.65–0.12, p = 0.09) compared with colostrum harvested less than six hours after calving. Furthermore, colostrum that was left in the collection bucket as opposed to being stored or fed immediately post-harvest was found to be 28.09 times (estimate = 3.34; 95%CI = 0.66–6.00) more likely to exceed TBC thresholds compared with colostrum that did not sit in a collection bucket post-harvest (p = 0.014).

Conclusion: A large proportion of colostrum samples (60.71%) failed to meet Brix thresholds, bacterial thresholds or both. Colostrum management strategies can be a risk for poor colostrum quality. Timing of colostrum harvest post calving was associated with a colostrum Brix <22%, and time spent in the bucket prior to feeding or storing was associated with a TBC exceeding 100,00CFU/ml. Poor colostrum quality could have significant impacts of calf health and welfare.

References:

Deelen, S. M. et al. (2014) 'Evaluation of a Brix refractometer to estimate serum immunoglobulin G concentration in neonatal dairy calves', Journal of Dairy Science. American Dairy Science Association, 97(6), pp. 3838–3844. doi: 10.3168/jds.2014-7939.

DeNise, S. K. et al. (1989) 'Effects of Passive Immunity on Subsequent Production in Dairy Heifers', Journal of Dairy Science. Elsevier, 72(2), pp. 552–554. doi: 10.3168/jds.S0022-0302(89)79140-2.

Faber, S. N. et al. (2005) 'Case Study: Effects Of Colostrum Ingestion on Lactational Performance1', The Professional Animal Scientist. Elsevier, 21(5), pp. 420–425. doi: 10.15232/S1080-7446(15)31240-7.

McGuirk, S. M. and Collins, M. (2004) 'Managing the production, storage, and delivery of colostrum', Veterinary Clinics of North America - Food Animal Practice, pp. 593–603. doi: 10.1016/j.cvfa.2004.06.005.

Quigley, J. D. et al. (2013) 'Evaluation of the Brix refractometer to estimate immunoglobulin G concentration in bovine colostrum', Journal of Dairy Science. Elsevier, 96(2), pp. 1148–1155. doi: 10.3168/jds.2012-5823.

Tyler, J. W. et al. (1999) 'Partitioning the mortality risk associated with inadequate passive transfer of colostral immunoglobulins in dairy calves.', Journal of veterinary internal medicine / American College of Veterinary Internal Medicine. John Wiley & Sons, Ltd (10.1111), 13(4), pp. 335–337. doi: 10.1111/j.1939-1676.1999.tb02191.x.

Keywords: Colostrum, Brix, Bacteria, Risk Factors.

HH-28

The carry-over effect of heat stress during late pregnancy on production and functional traits in the offspring generation

Kipp Cordula[1], Brügemann Kerstin[1], Zieger Peter[2], Mütze Katja[3], Möcklinghoff-Wicke Sibylle[4], König Sven[1].

[1]Institute of Animal Breeding and Genetics, University of Giessen, Giessen, Germany; [2]Diamond V, Cedar Rapids, United States; [3]Hessian Association for Performance and Quality Tests in Animal Breeding e. V., Alsfeld, Germany; [4]Innovation Team Milk Hessen, Friedrichsdorf, Germany.

Due to climate change, heat stress (HS) for dairy cows frequently arises. HS has tremendous unfavorable direct effects on dairy cow traits. Additionally, the negative long-term effect of in utero HS on birth weight, fertility and performance in the offspring generation under hot climate conditions have been observed. Regarding this background, the aim of this study was to analyze the carry-over effect of HS in terms of the average temperature-humidity-index (THI) during the last eight weeks of pregnancy on offspring performances.

In this regard, we considered a comprehensive dataset including Hessian Holstein dairy cows from calving years 2003 to 2013. THI was merged with fat content measured on first test day (fat%; N=171,150), non-return-rate after 56 days of first insemination (NRR56; N=94,981), calving to first insemination interval after first calving (CFI; N=134,885), productive life (PL; N=119,599) and lifetime productivity (LTP; N=119,634).

For THI 50–59 and THI ≥ 60 least squares means for fat% were substantially lower (4.21 % and 4.20 %, respectively) than for THI ≤ 39 and THI 40–49 (4.24 % and 4.23 %, respectively) ($P < 0.0001$). Moreover, offspring of mothers exposed to THI ≥ 50 had a lower ability to conceive than offspring of mothers exposed to THI ≤ 49 (80 % and 81 %, respectively) ($P < 0.0318$–0.0454). Calving to first insemination interval ($P < 0.0001$) and PL ($P < 0.0001$) in the offspring generation were also significantly negative affected (CFI: THI ≤ 39: 88 days, THI ≥ 60: 91 days, PL: THI ≤ 39: 915 days, THI ≥ 60: 889 days). Offspring of mothers, exposed to average THI ≥ 60, produced 18,137 kg milk during life. In comparison, offspring of mothers, who experienced THI-values ≤ 39 during late pregnancy, produced 18,422 kg ($P > 0.0375$). Epigenetic modifications triggered in the uterus can explain this long-term influence on the offspring.

Due to this detrimental effect across generations, an optimized heat management during dry period is recommended to create optimal conditions in uterus for the fetus and thus, set the base for best possible performance of the offspring in adulthood and improved longevity.

Keywords: In utero heat stress, dry period, fetus.

HH-29

Fresh cow's diseases prevalence and consequences in 42 Spanish Dairy Farms

Marcello Guadagnini[1], Noureddine Charfeddine[2].

[1]Elanco Animal Health, Sesto Fiorentino (FI), Italy; [2]Confederación de Asociaciones de Frisona Española (CONAFE), Valdemoro (Madrid), Spain.

Objectives: The transition period is the most important part of the lactation cycle for dairy farms profitability (Overton T. 2001). Most diseases occur during the first month of lactation (Leblanc S. et al., 2006) and can have a big impact on productivity. CONAFE, supported by Elanco Animal Health, started in 2015 a new project called "I-SA" on health traits monitoring with the aim of improving herd management and genetic selection. The scope of this data analysis is to increase the knowledge about fresh cow's diseases prevalence in Spanish dairy herds, understand their risk factors and impact on milk production.

Materials and Methods: 42 Spanish dairy herds across the country have been voluntary enrolled into a fresh cow's diseases data collection. Diseases occurrence have been recorded by the farmer and transferred to the local DHI association. Records include 18,043 lactations with 3 years calving dates from 2016 to 2018. Attempts to reach disease recording standardization have been made by providing disease definitions to farmers and suggesting to involve farm vets in disease diagnosis. Occurrence of the following diseases have been recorded throughout the first 68 days in milk (DIM), the highest 2nd test limit: Displaced Abomasum (DA), Clinical Mastitis (MAST), Retained Placenta(RP) and Metritis(METR). Other information proceed from the DHI database: herdcode, birthdate, calving date, parity, somatic cell count (SCC) and milk production at first and second test, BHB levels at first test (except for 3 farms), 305 days milk production. First tests between 5 and 25 DIM having BHB levels >0,1 mmol/liter have been considered as Ketosis (Viña C. et al., 2017, Renaud D. L. et al., 2019); first tests having >200 000 SCC/ml have been considered as Subclinical Mastitis (Ruegg P. 2017). Other variables were created: first calving age in categories, estimated cumulative production of the first 68 DIM (ICAR 2002). Descriptive statistics and multivariate models have been used to describe disease prevalence, determine risk factors and quantify concequeces on milk production. Statistically significant results were identified using a $p<0.05$.

Results: The dataset is composed by 33% first lactation animals (parity 1), 27% second lactation (parity 2) and 39% ≥3 lactations (parity 3+). Mean age at first calving was 25.3 months, with farm averages ranging between 23.4 and 28 months. Mean clinical diseases prevalence during the first 68 DIM was 3.11% for DA (0.76-7.73%) 4.58% for MAST (0-35.91%), 8.50% for RP (1.21-20.90%), 10.29% for METR (0.56-30.94%). Ketosis prevalence was 23%, with a farm prevalence range 4.55-50%. 31% of the farms (n=12) had a Ketosis prevalence greater than 25%. Subclinical mastitis at first test (5-35 DIM) was 21%. Statistically significant differences across parities were found for the risk of: DA (3+ vs. 2, Relative Risk (RR)=1.73 and 3+ vs. 1, RR=1.94), MAST (3+ vs. 2, RR=1.86 and 3+ vs. 1, RR=2.24), RP (3+ vs. 2,

RR=1.36; 3+ vs. 1, RR=1.61; 2 vs 1, RR=1.17), METR (3+ vs. 2, RR=1.23 and 2 vs. 1 RR=0.80), Ketosis (3+ vs. 2, RR=1.23; 3+ vs. 1, RR=2.18; 2 vs 1, RR=1.33) and Subcl. Mastitis (3+ vs. 2, RR=1.99 and 3+ vs. 1, RR=1.92). Age at first calving categories had a correlation with Ketosis risk (>27mo vs. <24mo, RR=1.80) and Subclinical Mastitis risk (>27mo vs. 24-27mo, RR=1.22 and >27mo vs. <24mo, RR=1.36).

Production losses at first test for ketotic cows vs. non-ketotic cows were 1.7 kg for lact=1 and 1.3 kg for lact>1 (p<0.0001). Production losses on 68 DIM cumulative production were 478 kg for DA, 179 kg for MAST, 163 kg for RP and 140 Kg for METR (p<0,0001). 305d milk losses were 725 kg for DA, 651 kg for MAST, 302 kg for RP and 173 Kg for METR (p<0.0001). Ketosis has not been included in the 305d milk model, as the proper way to analyze ketosis lactational impact on milk production is a testday model, which could not be built with currently available variables (Gröhn Y. et al. 1999).

Conclusions: Once again, transition cow's diseases importance have been demonstrated. Proper recording and analysis can increase our knowledge of the real prevalence at farm and country level, improve understandings of risk factors, estimate production losses and, finally, lead to a better transition management and genetic selection.

Keywords: Transition period, Dairy, Diseases, Spain.

HH-30

Comparison between general and analytical results account in three Galician dairy farms. A descriptive evaluation

Xosé Ramón Botana[1], Juan Cainzos[2].

[1]AIRA Sociedad Cooperativa Galega, Taboada, Spain; [2]Elanco Animal Health, Greenfield, IN, United States.

Objectives: With the aim of evaluate the economic result and the main areas of improvement, economic management programs have been implemented in Galician dairy farms. These economic evaluations are mainly based on the development of the general income statement (CRG) of the dairy farm activity based on General Accounting Plan 2007 (Real Decreto 1514/2007, PGC) procedures. However, development of an analytical income statement (CRA) allowing the differentiation of the economic results of the main economic activities present in dairy farms (Dairy – milk yield, Land - agricultural production (corn and grass silage), and Rearing - replacement heifers) was proposed. So, the objective of this study was to verify if this analytical accounting could provide useful information in the economic management of dairy farms compared with the income statement not split by sections.

Materials and methods: Three dairy farms (A, B, C) located at Lugo province (Spain) with different business models were selected. Farm A is a dairy farm with intensive management system, containing an average of 113 milking cows in production, 1,23 million of liters sold and 56 hectares of land. Heifers were reared by an external company. Farm B is a cooperative dairy farm with intensive management system, containing an average of 218 milking cows in production and 2 million of liters sold. They owns 149 hectares of land and heifers were reared into the farm. Farm C is a ATC* dairy farm (*Agrarian Transformation Company) with intensive management system, containing an average of 110 milking cows in production and 1,1 million of liters sold. They owns 60 hectares of land and heifers were reared by an external company.

For the analysis of their economic results, both the CRG and the CRA were performed in the three dairy farms during 2018, following the principles of the PGC. In the CRA, the income and expenses corresponding to each of the defined sections (dairy, land and rearing) were allocated, so that we obtain three independent income accounts. The necessary information is obtained from the following sources: Invoices of expenses and income of the companies, official accounting of farms, ReproGTV® (on-farm dairy management software) and Gescarro® (RMH® TMR software). All information is processed using the Excel 2016® program.

Results: The results of the general accounting for the three farms indicated that all of them obtained positive benefits during fiscal year 2018:

- Farm A: € 46.158 net profit, € 37,75 /1.000 liter of SCM* sold (*Solid Corrected Milk: 4.0% fat, 3.3% protein) and a net sales benefit of 7.7%.
- Farm B: € 95.147 net profit, € 47,60 /1.000 liter of SCM sold and a net sales benefit of 9.7%.
- Farm C: € 32.403 net profit, € 29,95 /1.000 liter of SCM sold and a net sales benefit of 6.9%.

However, the CRA analysis indicated that the result of the different sections evaluated was independent of the overall result, and also variable among the 3 dairy farms. Thus, in farm A and C the sections Dairy and Land have yielded benefits (€ 31.147 and € 2.283 for milk; € 24.457 and € 39.827 for Land, respectively), while the Rearing section has been deficient for both (€ -9.446 and € -9.707, respectively). In farm B the Land and rearing sections have yielded benefits (€ 135.202 and € 12.229, respectively), while the dairy section caused losses (€ -52.284).

Conclusions: Implementation of the CRG is an essential tool to evaluate the economic result of the farm in general, as well as to know the structure of expenses and incomes. This evaluation helped us to visualize the CRA benefits to identify areas of improvement that CRG can not identify, due to the fact that results of some sections can mask the results of others.

Keywords: Dairy,account,galician,economic,results.

HH-31

Use of a real-time location system to detect cows in distinct functional areas within a barn

J. M. Chapa[1], L. Lidauer[2], A. Berger[2], M. Ohlschuster[2], T. Potrusil[2], M. Sigler[2], W. Auer[2], M. Azizzadeh[3], M. Drillich[1], M. Iwersen[1].

[1]Clinical Unit for Herd Health Management in Ruminants, University Clinic for Ruminants, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna,

Austria; [2]Smartbow/Zoetis LLC, Weibern, Austria; [3]Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Objectives: Automated monitoring of animals by use of various sensor technologies is already used for many decades on dairy farms. Recent research and development of new sensor technologies and features aim to further improve animal health, welfare, and management procedures. Modern sensor technologies allow, among others, the tracking of animals in real-time in a barn. This can be used, for example, to estimate the time an animal spend at relevant 'functional areas' such as the feed bunk, cubicles, or alleys. The resulting data can then potentially be used as early indicators for disease, discomfort and to estimate the welfare status of an animal.

In this field study, we tested the real-time localization system (RTLS) of a commercially available system (SMARTBOW, Smartbow/Zoetis LLC, Weibern, Austria) to detect animals in predefined functional areas. The system consists of an ear-attached accelerometer that sends low-frequency signals to receivers, which transmit the data to a local farm server. Based on the incoming data of individual animals, the server software triangulates the location of an animal within the barn in real-time. The objective of this study was to determine the accuracy of the system to predict the location of the cow and the agreement between visual observations (VO) and RTLS observations for the total time spent by cows in relevant areas of the barn.

Material and methods: The study was conducted in May 2019 on a commercial dairy farm in Austria, housing approximately 35 Brown Swiss cows. The SMARTBOW (SB) tags were attached to the left ears of the animals. In advance of the study, functional areas of interest (i.e. the feed bunk, cubicles, and alleys) were predefined in the software of the sensor system. Cows were video recorded for three consecutive days using 9 digital cameras (DS-2CD2642FWD-IZS, Hikvision, Hangzhou, China). From these recordings, approximately 1 h was selected randomly each day for every cow (3 d × 35 cows). For each minute of an hour, animal position within a specific functional area was visually observed and labeled by use of specialized software for video analyses (Mangold Interact, Mangold International GmbH, Arnstorf, Germany). Data of the video observations (VO) served as gold standard in this study. A total of 6,030 pairs of location data, derived from VO and the SB system, were used for statistical analyses. Categorical data were used to estimate the agreement between the two methods. For each functional area of interest, the sensitivity (Se), specificity (Sp), and accuracy (Acc) were calculated. The total time spent (min/h) per cow in the specific areas was analyzed using Spearman correlations.

Results: Overall, a Cohen´s kappa of 0.78, indicating a 'substantial agreement', between VO and SB was obtained. Se and Sp were determined for locating the cows in the alley (74.0 and 91.2%), feed bunk (93.5 and 86.2%), and cubicle (90.5 and 83.3%), respectively, and overall accuracy of 87.6%. The correlation between VO and SB for the 'total time' an animal spent within an hour in alleys, at the feed bunk, and in cubicles was 'good' to 'strong' with correlation coefficients of 0.82, 0.98, and 0.92, respectively.

Conclusions: Overall, the real-time localization feature from SB was successful in predicting the position of an animal in a specific functional area. The estimated times, which an animal spent per hour in these specific areas were good. Future research should focus on, whether these times could be used as early indicators for disease, discomfort and to estimate the welfare status of an animal.

Keywords: Animal tracking, dairy cow, precision dairy farming, real-time location system, time budget.

HH-32

Application of Culling Form reveals patterns of dairy cow culling reasons

Kerli Mõtus, Tarmo Niine.

Estonian University of Life Sciences, Tartu, Estonia.

Objectives: Culling dairy cows often occur as a result of several, possibly related health issues however the registration forms for culling reasons are mostly not designed to capture the chain of the health problems that eventually lead to culling. The aim of this study was to develop and implement a Cow Culling Form (CCF) to reveal diseases or conditions resulting with culling, and their combinations. Also, farmers´ behaviour in marking culling reasons was analysed.

Materials and methods: The CCF developed to capture the culling reasons of cows slaughtered and dead on-farm was based on the Certificate of Death developed by McConnel and Garry (2017). It registered the general information of the cow, asked the respondent to list all diseases or conditions that could predispose or result in cow slaughter or death together with the time of onset and details about recovery. Also, it was asked to list all diseases the cow suffered in the ongoing lactation (date of onset, diagnosis, overall treatment protocol) and describe other problems or chronic diseases / conditions the cow had. Respondent was also asked to mark the culling reason that was inserted to the Estonian Livestock Performance Recording Ltd (ELPR) database for the culled cow.

In eight dairy farms, CCF was completed for every culled cow within the 12 months period. All completed CCFs were interpreted by the authors and were coded into underlying (disease or condition which initiated the possible chain of consecutive diseases or problems), intermediate, immediate (sequentially the last disease or disorder due to which the cow died or was sent for slaughter), and influential culling reasons (other diseases or problems that were outlined by the respondent as being related with culling but were not considered to be biologically related with other culling reasons). Based on the CCF data, culling code including three culling reasons (immediate, intermediate/influential and underlying) was created for each cow applying the extended coding system developed by McConnel and Garry (2017).

Results: The mean number of cows in eight study herds was 383 cows (range 126-564). The mean yearly cow culling rate of the study farms was 31.8% (range 23.0-40.7%) and the average on-farm mortality was 9.3% (range 3.7-18.5%).

Altogether, 686 and 250 CCFs of slaughtered and dead

cows were analysed in this study, respectively. Out of 686 slaughtered cows 14 (2.0%) were culled due to voluntary reasons, i.e. due to low milk yield only.

The number of unique three-reasons culling codes were 261 for slaughtered cows and 119 for cows that died on-farm. The most frequently identified underlying culling reasons in slaughtered cows were generic lameness (20% of slaughtered cows), impaired fertility (19.1%), abortion (9.8%), mastitis (8.5%), leg injury (5.8%), sole ulcer (4.2%) and metabolic disorders (3.8%). For the dead on-farm cows leg injury (14.8%), liver pathology (12.0%), milk fever (7.2%), generic lameness (7.2%), intestine inflammation (5.2%), mastitis (4.8%), dystocia (4.4%), sudden death (4.0%), displaced abomasum (3.6%), uterine torsion (3.2%), metabolic disorders (3.2%) and ketosis (2.8%) were identified most often as underlying culling reasons.

In general, the culling reason marked into the ELPR database more often concured with the underlying culling reason (72.6% and 63.6%) than with immediate reason (62.5% and 61.2%) identified from the CCFs of slaughtered and dead cows, respectively.

Conclusions: In general, herds that were included in the study had relatively high culling and mortality rates. Only negligible proportion of culls were voluntary reflecting deteriorated cow health and welfare also restricting the economic return of dairy farming.

Improved registration of culling reasons is needed to support informed herd-based decisions. Diagnosis tree, together with more broader disease categories, should be available in the recording systems to capture the relevant details related with death and slaughter. It is imperative to identify the triggering diseases or conditions to help to break the chain of sequential diseases that eventually cause culling. A tool which provides guidance to farmers through recording all important diseases and disorders, associated with culling, could be developed.

Acknowledgements: Authors are grateful to all farmers and veterinarians who were involved in this study. This work was supported by Estonian Research Council Grant (PSG268).

Literature: McConnel, C., Garry, F., 2017. Dairy cow mortality data management: the dairy certificate of death. Bov. Pract. 51, 64–72.

Keywords: Dairy cow, slaughter, death, causes, data registration.

HH-33

Association between rumination patterns detected by an ear-tag based accelerometer system and rumen physiology in dairy cows

Anne Simoni[1], Felix König[1], Karina Weimar[1], Andrew Hancock[2], Christian Wunderlich[3], Marcus Klawitter[3], Thomas Breuer[3], Marc Drillich[1], Michael Iwersen[1].

[1]Clinical Unit for Herd Health Management in Ruminants, Universitiy Clinic for Ruminants, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna, Austria; [2]Zoetis International, Dublin, Republic of Ireland; [3]Zoetis Deutschland GmbH, Berlin, Germany.

Objectives: Rumination is an essential part of the physiology of dairy cows. In this context, rumination activity is considered as a useful indicator for early detection of diseases and metabolic disorders.

The accelerometer-based sensor system SMARTBOW (SB, Smartbow/Zoetis LLC, Weibern, Austria) provides health alerts based on individual thresholds of rumination patterns in dairy cows. Detailed knowledge about the association between sensor-based rumination patterns and rumen physiology would help to interpret the clinical significance of rumination alerts. To the authors knowledge, no research has been carried out into the interaction between rumination alerts and changes of rumen fluid in dairy cows. These results could lead to a better interpretation of sensor-based rumination alerts.

Material & Methods: The study was conducted between April and October 2021 on a conventional dairy farm in the north of Germany, housing approximately 1900 Holstein-Friesian cows fitted with SB ear tags. According to our study 102 cows were matched in pairs based on the appearance of a rumination alert (ALRT) vs. no rumination alert (NALRT) and the lactation status. NALRT cows had to meet health criteria including a rectal temperature of < 39.5 C° and no signs of lameness (Sprecher >2). SB algorithms provided an 'acute rumination alert' and a 'long-time rumination alert' which were presented in Smartbow software and sent to a mobile device. A rumination alert was considered as valid if it persisted for at least 12 h, and the sample of rumen fluid was collected within the first 12 h of the alert.

Rumen fluid was taken twice using an oral stomach tube (SELEKT Rumen Fluid Collector, Nimrod Veterinary Products, Moreton-in-Marsh, UK). The first extraction (Ex1) was performed at the beginning of the alarm and the second extraction (Ex2) after the end of the alarm. The cows' status (ALRT vs NALRT) of each sample was blinded prior to examination by replacing the animals' identification number with a sample number randomly assigned by a second person.

The following parameters were examined in each sample: (1) rumen pH and redox potential by a portable electronic pH-meter (G1501 Serie, GHM Group Greisinger, Regenstauf, Germany; pH electrode GE 114-WD; redox electrode GR 175 BNC), (2) duration of methylene blue reduction time and sedimentation/flotation time, (3) microscopic evaluation of protozoa in a counting chamber (Fuchs-Rosenthal, Paul Marienfeld GmbH & Co.KG, Lauda-Königshofen, Germany).

To investigate potential differences in rumen physiology parameters between ALRT and NALRT, the rumen fluid parameters of the groups were compared at both extraction times (Ex1 and Ex2) by the Mann–Whitney U-test. For the detection of changes in rumen physiology within each group during the extraction period, rumen fluid parameters between Ex1 and Ex2 were compared for each group by related-samples Wilcoxon signed-rank test.

Results: The rumen parameters: pH, redox potential, methylene blue reduction time and sedimentation/flotation time differed significantly between ALRT cows and their NAL-

RT counterparts at Ex1 (*P < 0.01*). In contrast the number of protozoa showed significant differences between groups at Ex1 and Ex2 (*P < 0.01*). No differences were apparent for the other parameters at Ex2. The rumen fluid parameters of ALRT cows reached alignment with the values of their NALRT partners during the extraction period, except for the number of protozoa. Furthermore, ALRT animals differed in all rumen fluid parameters within the extraction period (*P < 0.01*) while those of NALRT cows remained constant.

Conclusions: The rumen fluid parameters were within the physiological ranges for ALRT and NALRT cows at both extraction times, but a higher variation was in ALRT cows at Ex1 was found. Higher variations in rumen fluid parameters of cows with rumination alerts could indicate a higher vulnerability to rumen health disorders. Considering the regeneration of protozoa to be a continuous process, protozoa were not able to fully replicate themselves until Ex2, which justifies the difference existing between ALRT and NALRT animals at Ex2. The collection of rumen fluid shows snapshots of rumen physiology in matched cows during and after rumination alert. Further research might focus on continuous measurement options for detecting rumen fluid parameters of cows at different health levels.

Keywords: Rumen fluid, rumination time, health alert, rumen disorders, accelerometer.

HH-34

Single and combined quality and quantity criteria of bovine colostrum and factors affecting them

Aikaterini Soufleri[1], Georgios Banos[2], Nikolaos Panousis[3], Georgios Arsenos[1], Georgios E. Valergakis[1].

[1]Laboratory of Animal Husbandry, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece, Thessaloniki, Greece; [2]2Laboratory of Animal Husbandry, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece/ Scotland's Rural College/Roslin Institute, Edinburgh, Scotland, UK, Thessaloniki, Greece; [3]Clinic of Farm Animals, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece, Thessaloniki, Greece.

Objectives: Colostrum administration ensures passive transfer of immunity to newborn calves. The common recommendation is to provide colostrum of adequate quality (IgG≥50g/L, Brix value ≥22%) and quantity (4kg), as soon as possible after calving. The objective of this study was to evaluate the effect of various management and cow factors on colostrum quality and quantity.

Materials and Methods: The study was conducted in 10 commercial dairy herds in Northern Greece, from February 2015 to September 2016. A total of 1,013 Holstein cows and their calves were included in the study. All cows were milked completely 232±195 minutes after calving, colostrum yield was recorded and a sample was collected. A Brix refractometer was used cowside to measure % total solids (TS); IgG concentration was calculated according to published equations. Body weight of calves was estimated using a heart girth tape. For each cow, records regarding calendar season and age at calving, milk yield of previous lactation, dry period length, time interval between calving and colostrum collection (TI) and body condition score (BCS) at calving were available. Target colostrum criteria were: a) colostrum TS content ≥22% (indicative of IgG content ≥50g/L), b) colostrum yield ≥4kg, c) 200g IgG in ≤4kg of colostrum and d) IgG quantity equal to 0.5% of calf body weight in ≤4kg of colostrum. Criteria (a) and (b) are individual colostrum traits while criteria (c) and (d) combine quality and quantity traits and represent practical recommendations regarding colostrum administration to newborn calves. Effects of calendar season, age at calving, milk yield of previous lactation, dry period length, TI and BCS on colostrum criteria were assessed with univariate general linear models. Farm was fitted as a random effect and all other factors as fixed ones in the models.

Results: Target values were not met by 18.3%, 25.4%, 23.5% and 19.8% of samples, for the 4 criteria, respectively. Farm (P<0.05), age at calving (P<0.05) and TI (P<0.05) had a significant effect on all criteria. Older cows produced colostrum of better quality and in higher quantities. When TI was longer, colostrum yield was higher but quality was lower. Adequate IgG quantity (≥200g and ≥0.5% of calf body weight) in a target colostrum quantity (≤4kg) was easier to achieve when TI was shorter. Autumn was the season with the highest quality (criterion a, P<0.05) but colostrum yield (criterion b, P<0.05) was low. Adequate IgG quantity (≥0.5% of calf body weight) in a target colostrum quantity (≤4kg) was more difficult to achieve during winter (criterion d, P<0.05). Previous lactation milk yield (P<0.05) and BCS at calving (P<0.05) had a positive significant effect only on colostrum yield (criterion b). Dry period length had a significant positive effect on colostrum yield (criterion b, P<0.05) but a negative one (P<0.05) on criterion c; adequate IgG quantity (≥200g) in a target colostrum quantity (≤4kg) was more difficult to achieve when the dry period length was >85 days.

Conclusions: Milking cows immediately after calving is a highly beneficial practice, resulting in colostrum of best quantity and quality combined, in most cases. Negative effects on colostrum quality associated with season, age at calving and dry period length were identified; however, they are inevitable under practical farm conditions. Recommended management practices (stock of frozen colostrum) can help overcoming them. The consistent effect of farm on all colostrum criteria necessitates the investigation of management factors associated with it.

Keywords: Dairy cow, colostrum quality.

HH-35

Factors affecting the skeletal muscle reserves during the transition period in Holstein cows

Nektarios Siachos[1], Nikolaos Panousis[2], Georgios Oikonomou[3], Georgios E. Valergakis[1].

[1]Laboratory of Animal Husbandry, Veterinary Faculty, Aristotle University of Thessaloniki, Thessaloniki, Greece; [2]Clinic of Farm Animals, Veterinary Faculty, Aristotle University of Thessaloniki, Thessaloniki, Greece; [3]Institute of Veterinary Science, Faculty of Health and Life Sciences, University of Liverpool, Neston, United Kingdom.

Objective: Association of dairy cow energy balance during the transition period with health status and performance has been thoroughly investigated. On the other hand, research on protein balance during the same period is limited. The objective of t*his study was* to assess factors affecting protein balance, expressed as skeletal muscle tissue reserves, during the transition period in Holstein cows.

Materials and methods: Two-hundred and thirty-eight multiparous cows in different parities (2: n=101; 3: n=72; 4+: n=65) from 6 Holstein dairy farms were included in this cohort study. Body condition score (BCS) and *longissimus dorsi* muscle thickness (LDT) of each cow was assessed at 7 time-points relative to calving: -45d; -21d; -8d; 0d; +8d; +21d and +28d, by the first author. Cows were scored for BCS on a 5-point scale with 0.25-unit increments; LDT was measured by ultrasonography, using a 5.0-7.5 MHz linear transducer. The probe was placed perpendicular to the vertebral column on the transverse process of the 4th lumbar vertebra, using ultrasound gel as a couplant. When a clear image was captured, LDT was measured at the site of the larger diameter of the muscle between the fasciae. Cows were classified as thin (BCS <3.00), normal (BCS 3.00–3.50) or fat (BCS >3.50) at their first assessment. The fixed effects of herd, time-point, parity, dry period duration, milk production of previous lactation, BCS_class, and the interactions of herd, BCS_class and parity with time-point on LDT measurements were assessed with repeated measures linear mixed models, accounting for the random variation of each cow's measurements. Factors with non-significant effects at the P>0.20 level, were excluded from the final model. The appropriate covariance structure was selected resulting in the lowest Akaike's information criterion value. Analysis was performed with IBM SPSS v.25.

Results: On a descending *F*-value order, time-point, BCS_class, herd, BCS_class × time-point interaction, herd × time-point interaction, parity and parity × time-point interaction had significant effects on LDT measurements. The effect of dry-period duration, although non-significant (P=0.144), remained in the final model as a covariate. On average, cows gained ca. 5% (P<0.001) muscle thickness from -45d (estimated marginal mean ±se: 34.0mm ±0.4) until -21d (estimated marginal mean ±se: 35.4mm ±0.4). Between -21d and -7d, LDT practically stabilized (estimated marginal mean ±se: 35.2mm ±0.4). Then, they lost ca. 26% (P<0.001) muscle thickness until +21d (estimated marginal mean ±se: 26.0mm ±0.4); LDT measurements appeared to stabilize again until +28d (estimated marginal mean ±se: 25.6mm ±0.4). Fat cows had significantly higher LDT measurements than normal and thin ones from -45d up to 0d and +7d, respectively, meaning that fat cows mobilized at the same time proportionally more muscle reserves than normal and thin ones. Moreover, significant differences were detected among herds in each time-point. Differences among parities were more obvious during the dry period.

Conclusions: Negative protein balance of transition Holstein dairy cows begins pre-partum and lasts until about 3 weeks post-partum. Mobilization of muscle reserves differed among parities, body condition scores and herds. Determination of specific herd factors affecting its variation is an essential step in developing appropriate management measures.

Keywords: Longissimus dorsi, transition, dairy cattle.

HH-36

Assessing the predictive capacity of a health monitoring system based on body condition score at the herd level

Ramiro Rearte[1], Nicolás Lorenti[2], Germán Domínguez[3], Rodolfo Luzbel De La Sota[1], Isabel Lacau - Mengido[4], Mauricio Javier Giuliodori[1].

[1]Instituto de Investigaciones en Reproducción Animal (INIRA), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata (FCV-UNLP), La Plata, 30, Argentina; [2]Actividad privada, Brandsen, Buenos Aires, 2, Argentina; [3]Actividad privada, Venado Tuerto, Santa Fé, 4, Argentina; [4]Comisión Nacional de Investigaciones Científicas y Tecnológicas (CONICET), CABA, 30, Argentina.

Objectives: The objectives of the current study were: 1- to define the herd threshold for cows with poor body condition based on its predictive capacity for disease risk at the herd level, and 2- to estimate the impact measures on disease rates due to body condition indicators in the transition period.

Material and methods: Data from two commercial grazing dairy herds (Herd A=5,034 and herd B=7,965 lactations) from Buenos Aires Province, Argentina were used to perform a longitudinal retrospective study during a 4-year period (January 2014-December 2017). Health, reproductive and body condition score (BCS) records were gathered. The BCS (5-point scale) was performed by farm personnel at calving (C-BCS), and at the end of the voluntary waiting period (40 and 50 days in milk [DIM] in herds A and B, respectively) by one of the authors. The difference between both measures of BCS was used to assess the change in body condition loss (∆BCS). All the cows not bred by 70 DIM were checked for anestrus (AN [yes/no]; defined as the absence of corpus luteum and a flaccid uterus). For herd monitoring, calving cohorts of 21-day were defined at each HERD (A or B) and PARITY (primiparous vs. multiparous cows) through the entire study period. The frequency of cows with CBCS<3 or ∆BC>-0.5 (BC%) at each cohort were calculated and used to define position measures (quartiles) through the whole study period, at each HERD and PARITY level. Quartiles were used, one at a time, as the threshold to dichotomize the cohorts (i.e.; over and above threshold) to predict the risk that a cohort has a frequency of AN over the median. The sensibility (SE), specificity (SP), area under the curve (AUC), and odds ratios (OR) for

each threshold were estimated, and the higher AUC was used as selection criterium to determine the herd level threshold at each HERD and PARITY level. Another visual approach was performed for herd monitoring by calculating the population attributable fraction (AF_P) of anestrus rate to body condition indicators at each cohort, for every HERD and PARITY level. The AFp was calculated from the adjusted risk ratio that was estimated by adjusting a stratified logistic model, for each HERD and PARITY level, which explain AN by main categorical predictor of body condition status (CBC<3 or ∆BC>-0.5; yes/no), and adjusted by calving season, calving year, and parity.

Results: According to the AUC, the herd threshold in primiparous cows was the 2nd quartile (BC%= 6%, [AUC=0.679; OR=4.545; SE=69.2%; SP=66.6%]) in Herd A. Providing that these cohorts having more than 6% of cows with poor BCS had 4 times higher odds for having anestrus rates over the herd median, the predictive capacity is moderate due to the estimated AUC, Se and Sp. Similarly, the 2nd quartile (BC%= 74%) in primiparous cows was defined as the herd threshold in Herd B [AUC=0.673; OR=1.485; Se=66%; Sp=68%]. Regarding multiparous cows, the herd threshold was set in the 3rd quartile (BC%= 20%, [AUC=0.574; OR=2.212; Se=81.5%; Sp=33.3%] in Herd A, and in the 1st quartile in Herd B (BC%= 78%, [AUC=0.611; OR=3.448; Se=37%; Sp=85%]).

The 21-day anestrus rate in primiparous and multiparous cows was higher in herd B than in herd A. In herd A, the median AFp in multiparous was 0.36 [Inter Quartile Range (IQR)=0.58], which means that around 36% of anestrus events would have been avoided if no cows would have poor BCS (assuming a causal relationship between BCS and anestrus). On the other hand, multiparous cows in Herd B, most of the time anestrus events were fully explained by the BCS (median AFp=0.98; IRQ=0.51). However, in those cohorts where anestrous incidence was high, the AF_p decreased in both herds.

Conclusions: We concluded that indicators of BCS are associated with the risk for anestrous at the herd, but their application as a monitoring tool has a limited predictive capacity. Therefore, other exploratory models such as those using impact measures (e.g.; AFp) could represent a more useful approach for monitoring performance during the transition period at the herd level.

Keywords: Body condition score, Anestrus, Herd monitoring, Dairy cows.

HH-37

Comparative case of Automated Health Monitoring and Health Scoring systems for dairy suckling calves

Eran Friedman[1], Doron Zahavi[1], Dan Navon[1], Elimelech Schreiber[1], Hagen Wile[1], Brandt R Kreuscher[2], Juan Pedro Campillo[1].

[1]Allflex Livestock Intelligence, Netanya, Israel; [2]Allflex Livestock Intelligence, Madison, Wisconsin, United States.

Objectives: Calf health control is one of the biggest challenges for dairy farms. Dairy producers have now access to new technologies to monitor the health of suckling calves, improving proper health management. The aim of the following trial was to compare the efficacy of an automated health monitoring system vs an intensive standardized health scoring system in a US dairy nursery.

Materials & Methods: For 9 months, a total of 586 female calves were monitored from 1-2 days of age until day 90, at weaning. The animals were reared in single hutches and were nipple fed. An ear monitoring tag (Allflex® Livestock Intelligence™) was applied to automatically monitor the health of each calf. The tag monitors the calf's behavior on a minute-by-minute basis and uses a proprietary algorithm to calculate an individual health index every hour. The health index values range from 40 to 100. The lower the value, the more severely sick the calf is. All monitored calves were also evaluated daily using a modified Wisconsin scoring system. Data on milk refusals, treatments, and mortalities was also captured. Rectal temperature was additionally measured for calves with one or more of the following: milk refusal, a total respiratory score equal or greater than 4, a fecal score of 2 or 3. Based on the collected health records, each Sickness Event evaluated with the modified Wisconsin scoring system was classified as mild, moderate, or severe. Mild cases were not considered for treatment. Farm and trial personnel were blinded to all data calculated by the automated monitoring system.

Results: From the standardized health scoring evaluation, 522 calves (89%) had at least one sickness event, and 265 calves (45%) had at least one moderate or severe sickness event. Average length of sickness events was 2.6 days. According to the automated monitoring system, average of the minimal daily health index (HI) was 97.51±0.02 for healthy days. Average of the minimal daily HI was 85.54±0.40, 82.03±0.52, and 78.76±1.65 for mild, moderate, and severe sickness events, respectively.

As the user can tune the desired sensitivity, the performance of the automated health monitoring system was evaluated using a HI score of 86 as a low sensitivity threshold for sickness (86-T), and 90 as a high sensitivity threshold (90-T). For 86-T, overall sensitivity and specificity were 63.6% and 96.9%, respectively. Sensitivity for mild, moderate, and severe cases was 56.7%, 73.3%, and 77.3%, respectively. For 90-T overall sensitivity and specificity were 75.7% and 92.4%, respectively. Sensitivity for mild, moderate, and severe cases was 71.1%, 82.2%, and 84.1%, respectively. Detection time (requiring continuous alert by the system) relative to treatment time by the farmer was 11 and 16 hours earlier for 86-T and 90-T, respectively. Severe cases detection time relative to treatment time by farmer was 27 and 37 hours earlier for 86-T and 90-T, respectively.

Conclusions: Our results indicate that the automated monitoring system (Sensehub Dairy, Allflex® Livestock Intelligence™) equals or betters an intensive health management system, while enabling to reduce the dependency on skilled labor. In addition, it provides the health index value that reflects the calf's sickness severity. Thus, providing another tool to improve health management and decision making.

Keywords: suckling calves, health index, monitoring, dairies.

HH-38

Changing the Course of Fresh Cows by Using Objective Monitoring

Travis Towns[1], Brandt Kreuscher[1], Juan Pedro Campillo[2].

[1]Allflex Livestock Intelligence, Madison, Wisconsin, United States; [2]Allflex Livestock Intelligence, Madrid, Spain.

Objectives: The implementation of an effective postpartum health control program is probably one of the main management goals of dairy farms. It is estimated that approximately 75% of disease in dairy cows typically happens in the first month after calving (LeBlanc et al., 2006). Conditions such as ketosis, metritis, endometritis, displaced abomasum, and retained placenta have a direct negative impact on reproductive performance and milk yield during the current lactation. Choice and efficacy of postpartum health screening methods depend on many factors including farm size, labor experience, and facilities. Visual observation is probably the most often used but is not always accurate or efficient. The aim of this study was to evaluate the impact of using insights from an automated monitoring system (Allflex® Livestock Intelligence™) on postpartum cow health by measuring milk yield performance at week 4 post-calving in a commercial dairy farm in the US.

Materials & Methods: In May 2019, a US Midwest dairy farm, milking approximately 5,500 cows implemented the insights generated by an automated monitoring system (Allflex® Livestock Intelligence™) to identify sick animals in the postpartum period (calving to 4 weeks). Every postpartum cow was wearing a collar device that monitors cow behavior on a continuous basis. Based on these data, the monitoring system calculates a rolling health index (HI), estimating the possible sickness status based on individual animal pattern changes. The HI range is from 0 to 100; the lower the value the more probability of animals being sick. This system provides the user with a health report listing animals with a Health Index below 86 to be checked for sickness. Prior to May 2019, the postpartum group was locked up daily for individual visual health observation; body temperature was checked only if animals were identified as sick by the farm staff. Animals were treated following the farm protocol. Cows assessed as healthy were moved to the general lactation pens before passing 4 weeks post-calving. After the system was installed, only animals included in the health report were evaluated in the fresh pen, reducing the time the pen was locked up. The system also identified cows for whom the movement to the general lactation pen was too early or otherwise problematic. No other relevant transition management changes took place except the implementation of an automated monitoring system. To assess the impact of this monitoring system on that farm, the average milk yield and the percentage of cows producing less than 60 lb (27 liters) at week 4 post-calving were evaluated. Only cows in the 2nd or higher lactation that calved during 2019 and with milk yield data available at week 4 were included in this analysis. Comparative results pre and post implementing of the system were analyzed.

Results: The results for 3,501 cows which calved between January and December were analyzed. Out of 1,707 cows calved between January and June, 183 (10%) did not reach 60lb (27L) at week 4 post-calving. Between July and December, after the automated monitoring implementation, 1,794 cows calved. Of these, only 29 (1%) failed to reach at least 60lb (27L) at week 4 post-calving.

Average milk yield at week 4 post-calving in cows (≥2 lactations) that calved between January and June was 101.4lb (45.9L). Cows with 2 lactations produced 103.8lb (47L), and cows with 3 or more lactations produced 99.5lb (45L). Average milk yield at week 4 post-calving (>2 lactations) in cows that calved between July and December was 105.6lb (48L). Cows with 2 lactations produced 103.5lb (47L) and cows with 3 or more lactations produced 107.2lb (48.6L).

Conclusion: The results of this study indicate benefits of implementing an automated monitoring system (Allflex® Livestock Intelligence™) for postpartum cow health on this particular farm. Objective health assessment compared to visual observation reduced labor skills impact and identified sicknesses earlier, in both the fresh and early lactation groups. This appeared to contribute to an improvement in daily milk yield by 4lb (2.1L) per cow and reduced the percentage of ≥2 lactation animals that did not reach 60lb (27L) at week 4 post-calving from 10% (calving from January through June) to 1% (calving July through December).

Keywords: Postpartum, health, monitoring, fresh cows, milk yield.

HH-39

Effect of twice *vs.* thrice milk replacer feeding/day on productive and health parameters in Holstein calves

Ángel Revilla-Ruiz[1], Eduardo De Mercado[2], Alejandro Pérez-Navarro[3], Patricia Carulla[4], Adrián Ramón-Moragues[5], Andrea López-Saturnino[6], M Luz Pérez Solana[2], Sonia Pérez-Garnelo[2], Fernando Estellés[7], Arantxa Villagrá[4], Francisco Sebastián[3], Susana Astiz[2].

[1]Medicine and Surgery Department, Veterinary Faculty, Complutense University of Madrid, Puerta de Hierro Avenue s/n, 28040 Madrid, Spain; [2]Animal Reproduction, INIA-CSIC, Madrid, Spain; [3]COWVET, valencia, Spain; [4]IVIA, valencia, Spain; [5]Facultad de Veterinaria, UCM, Madrid, Spain; [6]Facultad de Veterinaria , UCM, Madrid, Spain; [7]ETSIAMN, UPV, valencia, Spain.

Objectives: The management of calves and their health from the early stages is a fundamental aspect that can influence their entire productive life. Nutritional strategies during lactation are essential to ensure the proper growth of calves on the farm and their welfare. Historically, calves have been fed twice a day because producers milked cows twice a day. However, calves nurse their mothers more than 6 times per day. Therefore, the objective of this study was to compare two different nutritional management of dairy calves with an isocaloric diet administered twice or three times daily, evaluating its effect on productive and health parameters, up to 4-4.5m of age.

Material & methods: The study was carried out in a commercial rearing farm (Cowvet SL) in Valencia, Spain. We in-

cluded 288 Holstein calves between 5 and 38 days of age at entry, randomly distributed (block randomization controlling farm of origin and entry weight) into two feedings per day "2Fd" group or "3Fd" group, both groups of equal size (n = 144/group). The diet consisted of 6l per day of milk replacer without casein, formulated specifically for the farm (7% protein, 17% fat, 0.1% fiber; 1% calcium; 0.6% phosphorus and all recommended trace elements and vitamins) until weaning. The calves had *ad libitum* starter and water from the day of entry. The parameters studied were: Weight (kg), Ultrasonographic score respiratory disease (USRD; 0-5; according to Ollivett and Buczinski 2016), backfat thickness by ultrasound (BF; mm; Schröder and Staufenbiel, 2006) and calf health score (CHS; 0-21, according to Mahendran *et al.,* 2017) at three moments: 1) Farm entry (16.9±8.6 days old); 2) Weaning (53.8±3.3d) and 3) Grouping in growing batches (148.2±27.4d). The average daily weight gain (AWDG; kg/d) was calculated between moments.

We analyzed the effect between the 2Fd and 3Fd groups at each time point using non-parametric tests (non-normal distributed variables). Differences over time and interactions between factors were assessed with repeated measures ANOVA, including weight and age at entry as covariates (IBM SPSS® Statistics v. 25.0). Data are expressed as mean ± standard deviation.

Results: Age at entry was kept in the model of repeated measures (P<0.05). We found no significant differences between the treatments at any moment for Weight (Entry: 43.5±6.5 *vs*. 44.1±7.7; Weaning: 63.8±7.4 *vs.* 64.9±8.8; Grouping: 163.2±32.3 *vs*. 162.3±32.9 for 2Fd and 3Fd, respectively); for USRD (Entry: 2.03±0.2, *vs*. 2±0.2; Weaning: 1.95±0.5 *vs*. 1.8±0.6; Grouping: 2.1±0.25 *vs*. 2.1±0.25 for 2Fd and 3Fd, respectively); for Backfat (Entry: 3.2±0.5 *vs*. 3.1±0.4, Weaning: 4.2±0.3, *vs*. 4.2±0.3, Grouping: 5.4±0.2 *vs*. 5.5±0.3 for 2Fd and 3Fd, respectively), and AWDG (Entry-Weaning: 0.5±0.2 *vs*. 0.6±0.2, Weaning-Grouping: 0.8±0.2, *vs*. 0.8±0.2, and Entry-Grouping: 1±0.3 *vs*. 0.9±0.2 for 2Fd and 3Fd, respectively).

The health score (CHS) was significantly (P<0.05) higher in the 2Fd group calves (1.8 ± 0.51 *vs*.1.66 ± 0.57). This seems to indicate that feeding the calves three times per day could be associated with a better health condition of the calves until weaning, but we did not observe any effect of the treatment studied (two *vs*. three feedings) over time in any other variable assessed.

Conclusions: The nutritional regimen of three feedings/day could have a beneficial effect on lung health in rearing calves until weaning, but this improvement does not induce higher growth-rates, nor better health rates, long-term.

Funded by CDTI (Centro para el Desarrollo Tecnológico Industrial; Ministerio de Ciencia e, Innovación; proyecto CDTI-IDI-20200936.

References:

Ollivett, T.L., Buczinski, S. 2016. On-Farm Use of Ultrasonography for Bovine Respiratory Disease. Vet Clin North Am Food Anim Pract. 32(1): 19-35. doi: 10.1016/j.cvfa.2015.09.001.

Schröder, U.J., Staufenbiel, R. 2006. Invited review: Methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness. J Dairy Sci. 89(1): 1-14. doi: 10.3168/jds.S0022-0302(06)72064-1.

Mahendran, S.A., Booth, R., Beekhuis, L., Manning, A., Blackmore, T., Vanhoudt, A., Bell, N. 2017. Assessing the effects of weekly preweaning health scores on dairy calf mortality and productivity parameters: cohort study. Vet Rec. 181(8): 196. doi: 10.1136/vr.104197.

Keywords: Nutritional Managment, Rearing, Dairy, performance.

HH-40

Efficacy of the oral administration of specific immunoglobulins against e. coli, Rota- and Coronavirus for the prevention of neonatal calf diarrhea in dairy herds

Olivier Nussbaum[1], Richard Eicher[2], Josef J. Gross[3], Rupert M. Bruckmaier[3].

[1]Vet. Practice Abgottspon, Schwyz, Switzerland; [2]Clinic for Ruminants, Division Herd Health, Vetsuisse Faculty, University of Berne, Switzerland, Bern, Switzerland; [3]Veterinary Physiology, Vetsuisse Faculty, University of Berne, Switzerland, Bern, Switzerland.

Objective: The objective of the study was to evaluate the efficacy of a concentrate of specific immunoglobulins against *E. coli F5/K99*, Rotavirus and Coronavirus (Locatim®) given orally in addition to the dam`s colostrum to the calves for prevention of Neonatal Calf Enteritis (NCE).

Material & Methods: A total of 489 calves from 35 central Swiss herds without history of vaccination against NCE were included: 246 in the LOCATIM® group and 243 in the PLACEBO group. Within herds, calves were alternatively given either 60 ml of LOCATIM® or PLACEBO orally shortly before the first colostrum meal. LOCATIM® is a biological medicament manufactured from colostrum of cows hyper-immunized against *E. coli F5*, Rotavirus and Coronavirus, containing high levels of specific IgG to be given orally to the calf during the first hours of life. Every calf received the dam's colostrum according to the routine practice of the farm.

The farmer registered the clinical scoring of diarrhoea during the first 14 days of life. Diarrhoea score was defined according to following criteria: 0=no diarrhoea; 1=light diarrhoea without medical treatment; 2=severe diarrhoea requiring oral treatment; 3=more severe diarrhoea requiring parenteral rehydration or leading to death. Before treatment, a faeces sample was analysed for the presence of pathogens with a rapid immunochromatographic strip test (Speed® V-Diar 4).

A colostrum sample of the first milking was taken by the farmer. After at least three feedings, blood samples were taken from each calf by a veterinarian. Blood samples were analysed for total protein and IgG concentrations as well as for titers of specific antibodies against Rotavirus and *E. coli* F5. Colostrum samples were analysed for total protein and IgG-levels.

Statistical analyses: Univariable Chi-square analysis

was performed for diarrhea scores against all explanatory variables. For the multivariable logistic regression analysis, all explanatory variables with a univariable Chi-square p-value < 0.15 were included in the starting model. Non-significant variables were successively withdrawn with backwards elimination until only variables with a p-value < 0.05 were included.

The titers of the specific antibodies were logarithmically transformed and means were compared using the Student's T-test. As the transformed variables were still slightly skewed, an additional comparison of the median was performed with a non-parametric Kruskal-Wallis analysis.

Results: In 28 of 35 herds (80%), at least 1 calf had some kind of diarrhoea. A total of 138 calves (28.2%) suffered from diarrhoea during our study, 65 calves (13.3%) showed moderate to severe signs of diarrhoea (scores 2/3) requiring treatment. Four calves died in the first two weeks of life (mortality rate 0.82%). All 4 calves received the placebo.

The proportion of calves with diarrhoea was significantly lower in the LOCATIM group (21 calves = 8.5%) than in the PLACEBO group (44 calves =18.1%). The crude Odds Ratio (OR) was 0.42 (p = 0.002). In the final multivariable logistic regression model, the OR was even slightly lower (0.39, p=0.001).

There were no differences in serum total IgG or specific antibodies against *E. coli* F5 and Rotavirus within treatment groups and between sick or healthy calves.

Conclusion: Even in a region with good general management of dairy calves and good overall colostrum quality, the oral application of a concentrate of specific immunoglobulins against *E. coli F5*, Rotavirus and Coronavirus was effective to reduce the occurrence of NCE. The odds of getting NCE was statistically reduced (OR = 0.387) in the LOCATIM® group.

As the serum concentrations of IgG and specific antibodies measured 2-3 days after birth were not different between groups, the hypothesis concerning the mechanism of action for the clinical difference is that the protective effect was caused by antibodies present in the "kill zone" of the gut before the first contamination to support the local immunity in the very early phase.

Furthermore, the high level of the serum concentration of specific antibodies was possibly achieved earlier in the LOCATIM® group, leading to a more efficient protection against infection.

From a clinical point of view, the application of a sterile solution containing high levels of specific antibodies against *E. coli F5*, Rotavirus and Coronavirus given orally to the calf before the first colostrum meal, appears to be a valuable solution to reduce the risk of neonatal calf enteritis in addition to good management practices.

Keywords: Calves, Neonatal diarrhea, Prevention, Specific antibodies.

HH-41

Cost-benefit analysis of vaccination against Bovine Respiratory Disease

Bart Van Den Borne[1], Geert Vertenten[2], Robert Lavan[3], Henk Hogeveen[1].

[1]Wageningen University, Wageningen, Netherlands; [2]MSD Animal Health, Boxmeer, Netherlands; [3]Merck Animal Health, Madison, United States.

Objectives: Rearing young stock provides the future cows for a dairy farm. It entails, however, a large proportion of the cost price of milk (5-10 %). One of the diseases associated with rearing young stock is Bovine Respiratory Disease (BRD), which negatively impacts the health of the animal during its rearing phase as well as its performance during the first lactation. Vaccination is a possible management strategy against BRD. It reduces the number of cases in dairy herds but its cost-effectiveness has not been determined yet. The aim of this study was to assess the net economic benefit of vaccination against BRD.

Materials and methods: An existing calf level, bio-economic simulation model was adapted to first estimate the distribution of rearing costs under Dutch circumstances from two weeks of age until first calving and subsequently estimate the distribution of costs and revenues during the first lactation. The model simulates calf growth stochastically using a two phase growth function and incorporates the temporal uncertainties of BRD with the associated effects on morbidity and mortality until first calving. The model was extended with a Wilmink lactation curve and culling rules to simulate milk production and the associated effects of BRD throughout the first lactation until drying off. The model was adapted to compare the effect of two injections with a multivalent inactivated vaccine (Bovilis® Bovipast RSP, MSD Animal Health) at the age of 2 and 6 weeks with a default scenario without vaccination. An efficacy of 80% was assumed. All model input was based on scientific literature and, if not available, expert knowledge. Output of the model consisted of non-economic output such as BRD incidence, birth weight, first calving age, weight, milk production and culling incidence. Economic output included healthcare costs (vaccination costs, prevention costs and treatment costs), feed costs, barn costs, breeding costs, labour costs, depreciation costs, and milk and slaughter revenues. Total rearing costs and production revenues were estimated using 10,000 simulations. A sensitivity analysis was performed to investigate the effect of vaccine efficacy, BRD incidence risk, and other input parameters on the net economic benefit of vaccination.

Results: BRD incidence and death during the rearing phase and milk production of completed first lactations were 26.9%, 5.6% and 8117 kg on average, respectively, in the default scenario whereas they were 13.9%, 3.2%, and 8143 kg in the vaccination scenario. Reduced treatment costs (€4 vs €2 per animal) and reduced costs due to mortality (€41 vs €29) during the rearing phase only partially compensated the costs associated with vaccination (€25). However, increased rearing costs (€1727 vs €1735) were compensated by increased profits during the first lactation. Milk revenues (€2833 vs €2842) and revenues due to culling (€26 vs €28) were higher for the

vaccination scenario than for the default scenario. This resulted in an overall net economic benefit of €9 for each animal reaching its first dry-off. The model was sensitive to the BRD incidence risk and the efficacy of the vaccine. The net economic benefit became positive when the BRD incidence risk was around 18% assuming an 80% vaccine efficacy.

Conclusions: The estimates from this model are assumed to be conservative since only the effect of clinical BRD was simulated. Literature on production effects of subclinical BRD was scarce and was therefore not incorporated in the simulation model. Moreover, the vaccine efficacy of 80% was hypothetical given the lack of good data on this important input parameter. Nonetheless, this bio-economic simulation study showed that young stock vaccination against BRD is economically beneficial in dairy herds.

Keywords: Bovine Respiratory Disease, vaccination, cost benefit, dairy, Netherlands.

HH-42

Conducting herd health program in large commercial dairy herds – impact on herd health and farm economy

Kerli Mõtus, Ants-Hannes Viira, Kalmer Kalmus, Ants Kavak, Helis Luik-Lindsaar, Piret Kalmus.

Estonian University of Life Sciences, Tartu, Estonia.

Introduction: Veterinary herd health and production management (HHPM) programmes are defined as certain activities aimed to maintain the health and productive capacity of farm animals at the most efficient level while ensuring the farm's competitiveness and profitability. Although implemented in several countries the content and methods of these programmes vary. The health and economic impacts of implementing HHPM in large commercial dairy farms are not widely studied and previous studies have even shown controversial results. The aim of the present study was to analyse the health and economic consequeces of implementing dairy herd health programmes in large commercial dairy herds.

Material and methods: Five dairy herds with loose-housing cowsheds were enrolled in the study including roughly 100, 600, 600, 700 and 1700 dairy cows, respectively. Between April 2017 to March 2019 HHPM programme was implemented in these herds. In every quarter, four visits were performed by four herd health veterinarians covering the following topics: youngstock health and infectious diseases, udder health and milk quality, cow and heifer fertility, metabolic diseases and claw health. After each visit a summary report including analysis of the herd health data and the problem analysis together with recommendations for improvement was submitted to the farm managers. In all farms herd health and production-related data as well as farm treatment records were summarized on a monthly basis. Also, the following herd health related costs were analyzed routinely: cost for medicaments, value of discarded milk, heifer rearing costs related with delayed calving, lower milk yield associated with longer calving interval, costs for sperm and artificial isemination and extra charge or discounts for milk fat and protein content. Changes in herd health, production and economic performance over two years were analyzed to evaluate the impact of the implementation of herd health programmes.

Results and Discussion: During the study period, cattle health improved in most areas that were consulted by the veterinarians. Still, in some farms an increase of disease incidence and associated costs occurred. In some cases this could be explained by an improved disease detection and more complete registration of the disease data instigated by the consulting veterinarians. In general, the incidence of first month calf diarrhea reached below 20% by the end of the study in four herds but constant increasing trend of the diarrhea incidence occurred in one herd. Cost of antimicrobials used for the treatment of calfhood diseases decreased by 56% on average over four farms (could not be analysed in one farm due to lack of data). On average, the length of calving interval decreased from 412 to 396 days across five farms. The level of somatic cell count decreased in two herds but increased in three herds. In total, the number of clinical mastitis cases decreased from 54.1 to 41.6 cases per 100 cow/year. The linear trend of subclinically infected mastitis cows decreased in four, but increased in one farm. Proportion of cows with a diagnosis of hoof diseases decreased from 27% to 22% within two study years averaged over five farms.

The positive impact of the HHPM implementation was also illustrated by the change in the economic performance of the farms. While the weighted average of the herd health related costs for the study period was EUR 22 per tonne of milk produced, the costs decreased by an average of EUR 7-8 per tonne of milk produced in four farms by the end of the study. The main economic costs associated with livestock health were the cost of medicines (36%), discarded milk (34%) and milk price discounts due to lower content of milk fat and protein (15%).

Conclusions: In general, implementation of HHPM programmes improved animal health and productivity and, as a result, the economic performance of the farms.

Keywords: Herd health, economy, dairy herd, commercial farm, Estonia.

HH-43

Evaluation of Failure of Passive Transfer in neonatal dairy calves in the Netherlands

Pleun Penterman, Ruben Tolboom, Geert Vertenten.

MSD Animal Health, Boxmeer, Netherlands.

Objective: The absorption of maternal immunoglobulins in the small intestine during the first 24 hours after birth, termed passive transfer, helps to protect the calf against common endemic disease organisms until its own immature immune system becomes fully trained and functional. Colostrum management is the single most important management factor in determining calf health and survival. Although good progress has been made in the past years, there remains a consider-

able opportunity for many dairy farmers. A recent US study reported Failure of Passive Transfer (FPT) to affect 15.6% of calves tested, indicating a need for continued efforts to improve colostrum management. The objective of this study was to obtain prevalence data on FPT in dairy calves in The Netherlands and to identify risk factors for FPT at herd level.

Material and Methods: Neonatal dairy calves (n = 274) from several commercial farms in the Netherlands were enrolled in the study. Only calves aged between 2 and 7 days were included in the trial.

Serum samples were tested at Royal GD Deventer (The Netherlands) with a validated turbidimetric assay on a clinical chemical analyzer.

Different cut-off values for FPT and for evaluation of colostrum management were used to analyse test results. A cut-off value for FPT was defined as a serum gamma-globulin level lower than 10 g/L (Gay, 1983); a second cut-off value of 15g/L serum gamma-globulins was also used based on studies by Furman-Fratczak and colleagues (2011) and Windeyer and colleagues (2014) showing that dairy calves with serum IgG levels greater than or equal to 15 g/L experienced lower rates of respiratory disease. In addition, the revised individual and herd-based evaluation standards by Godden and colleagues, 2019 were used.These include ranking in 4 categories: excellent (≥ 25.0 g/L), good (18.0-24.9 g/L), fair (10.0-17.9 g/L), and poor (<10 g/L). These categories can be applied to individual calves and to the operation for herd-based evaluation based on the percentage of calves that should be represented in each category.

On each farm the following information was gathered to identify risk factors for FPT: number of lactating cows, youngstock raised on the dairy farm, number of animals younger than 1 year, number of animals between 1 and 2 years, average age at first calving, vaccination against respiratory disease, vaccination against scours, prevalence of scours, prevalence of respiratory disease and neonatal mortality.

Results: The average as well as the median of the serum gamma-globulin level in the sampled animals was 16 g/L ranging between 0 and 35 g/L. When using a cut-off value of 10g/L, 21% of the animals experienced FPT, while using a cut-off value of 15g/L would mean that 44% of the animals experienced FPT.

When using the four categories defined by Godden and colleagues, 11% of the samples were excellent, 33% good, 35% fair and 21% poor. These results are worse than the proposed distribution reflecting good colostrum management (>40% excellent, ~30% good, ~20% fair and <10% poor).

At the moment of submission of the abstract, the farm information was not collected yet. This will be presented in the final presentation during the World Buiatrics Congress. The aim is to identify risk factors for FPT on calf and herd level.

Conclusion: Depending on the used threshold 21 to 44 percent of neonatal calves in this study experience Failure of Passive Transfer. Although good progress has been made in the past years, there remains a considerable opportunity to improve the colostrum management practices on Dutch dairy farms.

Keywords: Failure of Passive Transfer, dairy calves, Netherlands, colostrum.

HH-44

Feeding calves with pasteurized colostrum and milk improves health in heifers and has a positive long-term effect on their productive performance

Ramon Armengol[1], Lorenzo Fraile[2].

[1]Universitat de Lleida, Lleida, Spain; [2]Agrotecnio Center, Lleida, Spain.

Objectives: The main objective of this study was to observe whether feeding female calves with pasteurized colostrum and cow's milk improved future reproductive performance, productive parameters and health over the course of the heifer-rearing process and the three first lactations.

Materials and methods: We carried out a study feeding calves non-pasteurized and pasteurized colostrum and cow's milk under the same environment and management conditions. Female calves born during 2013 and 2015 entered the study at 22 days of age, after having received pasteurized colostrum and milk (P group, n= 127) or non-pasteurized colostrum and milk (NP group, n= 134) during the first 21 days of life. All calves submitted to study showed a total serum protein ≥5.8 g/dL between day 2 and 5 of life. Calves were randomly grouped during the rearing period mixing animals from groups NP and P. During heifer rearing, reproduction parameters (number of artificial inseminations (AI) per pregnancy), production parameters (body weight (BW) and average daily gain (ADG)) and health parameters (Bovine respiratory disease (BRD) and diarrhea) were recorded. Productive (305-d milk yield, life milk yield and ADG), reproductive (AI per pregnancy and calving interval), and health parameters (milk somatic cell count (SCC/mL)) and age at culling were also recorded in a follow-up study for 6 years (2013 to 2019). Suitable statistical analyses were carried out to decipher the effects of the experimental group (P or NP) on productive performance throughout the study.

Results: A total of 261 female Holstein calves entered the study (134 in the NP group and 127 in the P group). Of these, 216 reached at least first parturition (NP=110 and P=106), 184 reached second parturition (NP=90 and P=94) and 139 reached third parturition (NP=71 and P=68). We did not observe significant differences in the probability of reaching the first, second or third parturition between the two groups.

Feeding on-farm pasteurized colostrum and milk during the first 21 days of life reduces morbidity of bovine respiratory disease during the first year of life and diarrhea during the first 180 days of life. In heifers that never reached first parturition (NP group=24 *vs.* P group=21), morbidity from both diarrhea (*P*=0.0492) and BRD (*P*=0.2797) was lower in the P than in NP group. Similar trends were seen in animals that reached first parturition and differences were significant for both diseases (BRD; *P*=0.02111 and diarrhea; *P*=0.0341). Moreover, it increases ADG from birth to first calving (P=670.5±5.6 *vs.* NP=616.2±6.2 g/day) and increases BW after first (P=531.7±3.8 *vs*. NP=492.6±3.8 Kg), second (602.2±4.6 *vs*. NP=575.1±4.6 Kg) and third lactation (P=645.9±5.3 *vs*. 626.4±5.3 Kg). Finally, it also significantly increases milk production at first lactation (P=10,147±160 *vs*. NP=9,574±209 kg). However, there were no differences in relation to reproduction performance and health of cows between animals be-

longing to the NP or P group.

Conclusion: Colostrum and milk pasteurization for dairy calves improves health and production parameters in heifers and cows, and has a positive long-term effect on cow's production parameters.

Keywords: Colostrum, milk, pasteurize, long-term effects, dairy.

HH-45

Is Brix refractometry an appropriate on-farm tool for measuring the IgG concentration in colostrum of Belgian Blue cows?

Geert Hoflack[1], Pleun A.A. Penterman[2], Geert Vertenten[2], Bart Sustronck[1].

[1]MSD Animal Health, Brussels, Belgium; [2]MSD Animal Health, Boxmeer, Netherlands.

Objectives: Transfer of passive immunity through the administration of colostrum is essential in bovine neonates because the epitheliochorial placenta of cows is impermeable for immunoglobulins (Ig). Adequate transfer of passive immunity can only be achieved when calves are fed sufficient amounts of good-quality colostrum shortly after birth. Colostrum quality is mainly determined by its concentration of immunoglobulins. However, the degree of bacterial contamination can have a negative impact on the intestinal absorption of these Ig's. The aim of the present study was to evaluate the quality of colostrum of Belgian Blue cows considering not only the IgG concentration but also the bacterial load of the colostrum. Additionally, the accuracy of indirect evaluation of the IgG concentration of colostrum from Belgian Blue cows by means of a digital Brix refractometer was assessed, as this is considered a handy cow-side on-farm tool.

Materials & Methods: For this field study, 23 Belgian bovine practices participated in the collection of colostrum from freshly calved Belgian Blue cows. On 69 commercial Belgian Blue farms, a total of 76 colostrum samples were collected. Sampling occurred immediately after caesarean section. Colostrum samples were stored at -20°C until further processing. IgG concentrations were measured using a commercial competitive ELISA-test kit (BIO K420, MonoScreen QuantELISA Immunoglobulin Easy, Bio-X Diagnostics S.A., Rochefort, Belgium). Indirect evaluation of the IgG concentration of the colostrum samples was performed using a digital Brix refractometer (Milwaukee Refractometer MA871, Milwaukee Instruments Inc., NC, USA). On each occasion, the mean of three consecutive Brix refractometric measurements was calculated and used for analysis. The relationship between the IgG concentration and the Brix value of the colostrum samples was investigated with a Pearson's correlation. Total bacterial cell count of the colostrum samples was determined using a bactoscan automatic bacterial count reader (Bactoscan™ FC+, FOSS, Denmark). Colostral IgG concentrations of < 50 g/l or a colostral Brix value of < 22% were considered as poor-quality colostrum. Furthermore, colostrum samples with a total bacterial cell count of > 100.000 CFU/ml were also classified as poor-quality colostrum. All statistical analyses were performed using R software (R Core Team, 2017).

Results: Colostrum from Belgian Blue cattle contained on average 81,24 ± 24,90 g IgG/l. The IgG concentration ranged from 24,90 to 150,00 g/l. The Brix value of the colostrum from Belgian Blue cattle had a mean of 25,27 ± 4,05 % (range: 16,77 – 36,40 %). A low non-significant negative correlation was found between the IgG concentration and the Brix value of colostrum samples from Belgian Blue cows ($r_{pearson}$ = -0,16; $CI_{95\%}$ [-0,38 to 0,07]; p = 0,172). The bactoscan values of the colostrum samples were highly skewed and were, therefore, analysed using non-parametric methods. The colostrum samples from Belgian Blue cattle had a median total bacterial cell count of 5.500 CFU/ml (range: 4.000 – 390.000 CFU/ml).. The IgG concentration was lower than the norm of 50 g IgG/l in 8,22 % of the colostrum samples. The total bacterial cell count was above the acceptable threshold of 100.000 CFU/ml in 5,48 % of the colostrum samples. Combining these two quality parameters (IgG concentration and bacterial count) 13,7 % of the colostrum samples were classified as poor-quality colostrum.

Conclusions: The colostrum quality of Belgian Blue cows can be classified as high since 92 % of the samples had an IgG concentration of at least 50 g IgG/l. Concerning bacteriological contamination, 95 % of the colostrum samples from Belgian Blue cattle were clean, defined as < 100.000 CFU/ml. When both quality criteria are taken together, more than 85 % of the colostrum samples from Belgian Blue cows fulfilled the requirements for high-quality colostrum, suitable for administration to new-born calves. In this study, no correlation could be found between the IgG concentration, as measured by competitive ELISA and the digital Brix refractometric value of colostrum from Belgian Blue cows. Hence, Brix refractometry seems inaccurate for the assessment of IgG concentration in Belgian Blue cow's colostrum.

Keywords: Belgian Blue cattle, colostrum quality, IgG concentration, Brix refractometry , Total Bacterial Count.

HH-46

Evaluation of metabolic health by pooled sample metabolic profiling on 305 Flemish dairy herds

Angelique Rijpert-Duvivier[1], Koen De Bleecker[2], Pieter Taelman[3], Herman Deschuytere[4], Johan Schaep[2], Jos Van Eecke[5], Julien Evrard[6], Robert J. Van Saun[7].

[1]Angelique Rijpert-Duvivier Senior Technical Consultant Ruminants Elanco Benelux, Utrecht, Netherlands; [2]Animal Health Care Flanders (DGZ), Torhout, Belgium; [3]Elanco Belgium, Antwerpen, Belgium; [4]Vetshops, Izegem, Belgium; [5]Hapco.Farm, Horebeke, Belgium; [6]Arsia, Ciney, Belgium; [7]Penn State University, University Park, PA 16802-3500, United States.

Objectives: Monitoring metabolic health of transition cows using pooled serum sample metabolic profiling (PMP) has been well described and reduces laboratory costs con-

siderably. PPM, including 21 biomarkers, was commercially introduced in Flanders (Belgium) by Animal Health Care Flanders (DGZ) in October 2020. Furthermore, DGZ developed an interpretation tool which presents results in a web diagram containing 8 metabolic key areas (Metabolic Scan). Study objectives were to evaluate metabolic profile results for close-up, fresh, and peak lactation cows from Flemish herds pooled and analyzed at the DGZ-laboratory between 12 October 2020 and 12 December 2021.

Materials and methods: A retrospective analysis was carried out. Beef cattle samples, incomplete and outlier results were excluded (50 profiles and 42 herds). Finally, 368 submissions from 305 different farms were analyzed and included 288, 265, 245 pooled sample metabolic profiles of following production groups: close-up (P1: 21-3 days prior to calving), fresh (P2: 3-21 days in milk (DIM)), and peak lactation (P3: 60-120 DIM) cows, respectively. Statistical analysis was performed using JMP computational software (version 15, SAS Institute Inc.). Evaluation included pool results and corresponding percentages of abnormal values per pool (methodology of Van Saun) for the following biomarkers: albumin, alpha, beta and gamma globulins, total protein (TP), beta hydroxybutyrate (BHB), calcium (Ca), chlorine (Cl), creatinine, phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), non-esterified-fatty-acids (NEFA), selenium (Se), urea, vitamin E, copper (Cu), zinc (Zn), gamma glutamyl transferase (GGT) and beta-carotene. Based on the biological significance for interpretation, individual biomarker results were assigned to metabolic key areas. Subsequently, results were converted into the percentage of animals at risk for these 8 key areas: Ketosis-negative energy balance (NEB-ketosis): BHB and NEFA; water intake: Cl and Na; liver function: GGT and urea; Cu and Zn; macro minerals: Ca, Mg and P; antioxidants: vitamin E, Se and beta-carotene; inflammation: alpha and gamma globulins, TP; dry matter intake (DMI): TP, albumin and urea. The key area results were categorized into 2 groups: CatA (0-20% at risk) and CatB (21-100% at risk).

Results: We found that 23%, 31% and 46% of the submissions contained 1, 2 or 3 production groups, respectively. The mean herd size was 187 (range 25 to 1955). Herd numbers located in Antwerp, Limburg, East Flanders, West Flanders, and Flemish Brabant (provinces) were 104, 21, 73, 94 and 13, respectively.

The following results concerning CatB are in order of P1, P2 and P3, respectively. In the NEB key area, 48, 58 and 42% were classified in CatB. The key area of water intake showed 39, 22 and 24% in CatB. In the key area liver function 47, 35 and 22% of the samples were found at CatB level. For the key area Cu and Zn 83, 82 and 78% were found in CatB. The macro mineral key area showed 18, 20 and 9% CatB results. For the antioxidant status, the percentages were 75, 55 and 26 respectively. In the inflammation key area 41, 46 and 22 % was found. Finally, the DMI key area showed 72, 54 and 22% in CatB for P1, P2 and P3 respectively. Significant correlation ($p < 0.05$) was found between key area percentages of all 3 production groups for NEB-ketosis, water intake, Cu and Zn, antioxidants, and DMI.

Conclusions: A considerable proportion of metabolic profiles showed signs of NEB-ketosis, most prominent in P2 and P1, but still proportional in P3 animals. Suboptimal water intake was seen most frequently in P1. For key areas, liver function, antioxidants and DMI, P1 was most often impacted, followed by P2, whereafter P3 seems to recover. Big deviations were found for Cu and Zn key area in all production groups. Further investigation is needed to see whether a low uptake, a poor homeostatic character of both elements or an erroneous reference value is responsible for this observation. The macro mineral key area showed the least deviation within all production groups. A state of inflammation was most often found in P2, followed by the P1 cows. Key area's NEB-ketosis, water intake, Cu and Zn, antioxidants and DMI showed correlation in percentages of animals at risk for all 3 production groups, indicating a need for further evaluation of farm management, feeding, cow comfort, animal welfare and preventative strategies.

Keywords: Metabolic profiling; pooled samples; transition.

HH-47

Effect of initial health status in Holstein calves on production and health parameters

Ángel Revilla-Ruiz[1], Eduardo De Mercado[2], Alejandro Pérez-Navarro[3], Patricia Carulla[4], Adrián Ramón-Moragues[4], Andrea López-Saturnino[5], M Luz Pérez Solana[2], Sonia Pérez-Garnelo[2], Fernando Estellés[6], Arantxa Villagrá[4], Francisco Sebastián[3], Susana Astiz[2].

[1]Medicine and Surgery Department, Veterinary Faculty, Complutense University of Madrid, Puerta de Hierro Avenue s/n, 28040 Madrid, Spain; [2]Animal Reproduction, INIA-CSIC, Madrid, Spain; [3]Cowvet SL, Valencia, Spain; [4]IVIA, Valencia, Spain; [5]Facultad de Veterinaria, UCM, Madrid, Spain; [6]ETSIAMN, UPV, Valencia, Spain.

Objectives: Health status can affect productivity and growth especially in the first growth periods of calves. The animals, at farm entry, are evaluated for their health status using a "calf health score" (HSc; Wisconsin Health-Score; Mahendran et al., 2017), which evaluates different health aspects simultaneously (rectal temperature, cough, nasal discharge, eye discharge, ear posture and fecal score), giving a higher score to those animals with the worst health status (0-21). The aim of this study was to evaluate how different productive and health parameters evolved in animals up to 4-4.5m of age with different health status at farm entry.

Material & methods: We randomly selected 235 calves from a commercial rearing-farm (Cowvet SL), Valencia, with an entry age of 5–38 days of age. All animals were evaluated by a modified version of the Wisconsin-Health-Score, and we categorized them into three study groups: HSc1 (HSc value=1; n=70), HSc2 (value=2; n=128) and HSc3 (value=3; n=37). We did not select calves with a value> 3 (only 5 individuals). Calves had *ad libitum* starter and water from the day of entry. The parameters studied were: weight (kg), ultrasonographic score respiratory disease (USRD; 0-5; according to Ollivett and Buczinski 2016), backfat thickness by ultrasound (BF; mm; Schröder and Staufenbiel, 2006) and HSc at three moments: 1) Farm-entry (17.7±8.6 days old);

2) Weaning (53.8±3.3d) and 3) Grouping in growing batches (148.2±27.4d). The average daily weight gain (AWDG; kg/d) was calculated between moments.

We analyzed the effect among groups at each time point using ANOVA and Tukey test for the means comparison (P<0.05; SAS® 9.0). Data are expressed as mean ± standard deviation.

Results: Health status at farm entry was associated with higher values of USRD (P<0.05; 1.96±0.2^{a}, 2±0.1^{a} and 2.11±0.3^{b}) and BF (P<0.05; 3.07±0.4^{a}, 3.25±0.5^{b} and 3.23±0.5^{ab}), indicating that this initial classification by health status is related to pulmonary status and physical condition of the animal. At Weaning, we observed a lower weight in animals with worse HSc at entry (P<0.05; 66.1±8.4^{a}, 63.1±7.8^{b} and 62.5±7.7^{b} for HSc1, HSc2 and HSc3 respectively) and again a lower BF in animals with a worse health status (P<0.05; 4.25±0.3^{a}, 4.14±0.25^{b} and 4.21±0.25^{ab} for HSc1, HSc2 and HSc3 respectively). However, at the moment of Grouping there were no significant differences in any of the variables. This could indicate that the initial health status slightly retards the growth rate until, at least, weaning, recovering later, with the AWDG reduced exclusively until weaning (0.634±0.21^{a}, 0.543±0.21^{b}, 0.551±0.17^{ab} for HSc1, HSc2 and HSc3 respectively), and a compensatory growth when the animals improved their health status. The Health Scoring showed how the animals reached similar health values, regardless of the HSc value at entry, showing the animals an average HSc of 1.7±0.5 at weaning and 2.1±0.25 at Grouping. Ultimately, all animals showed a similar, slightly increased health score, but no overcoming the value 6 (related to clinical condition). It is to highlight, the generally low HSc observed at this rearing farm.

Conclusions: The initial health status of the animals, even when very low (<4) slightly reduces the growth of rearing calves until weaning, but after this period the animals compensate the growth and achieve a similar state of health.

Funded by CDTI (Centro para el Desarrollo Tecnológico Industrial; Ministerio de Ciencia e, Innovación); proyecto CDTI-IDI-20200936.

References:

Ollivett, T.L., Buczinski, S. 2016. On-Farm Use of Ultrasonography for Bovine Respiratory Disease. Vet Clin North Am Food Anim Pract. 32(1): 19-35. doi: 10.1016/j.cvfa.2015.09.001.

Schröder, U.J., Staufenbiel, R. 2006. Invited review: Methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness. J Dairy Sci. 89(1): 1-14. doi: 10.3168/jds.S0022-0302(06)72064-1.

Mahendran, S.A., Booth, R., Beekhuis, L., Manning, A., Blackmore, T., Vanhoudt, A., Bell, N. 2017. Assessing the effects of weekly preweaning health scores on dairy calf mortality and productivity parameters: cohort study. Vet Rec. 181(8): 196. doi: 10.1136/vr.104197.

Keywords: Health Score, Dairy, rearing farm, performance.

HH-48

Effect of dairy heifer pre-breeding growth rate on first lactation milk yield in spring-calving, pasture-based herds

Ciara Hayes[1], Conor McAloon[2], Emmet Kelly[2], Catherine McAloon[2], Eoin G Ryan[2], John Mee[3], Luke O'Grady[2].

[1]Department of Agriculture, Food and the Marine, Cork, Republic of Ireland; [2]University College Dublin, Dublin, Republic of Ireland; [3]Teagasc, Animal Bioscience Research Department, Fermoy, Republic of Ireland.

Objectives: There is evidence that early life growth rate can have positive effects on future milk production, however very little is known about the impact in seasonal, pasture-based dairy systems. Therefore, the objective of this study was to investigate the association between pre-breeding growth rate and first lactation milk yield in spring-calving, pasture-based dairy herds.

Methods: Heifer calves (n=399) from a convenience sample of seven commercial, spring-calving dairy herds were weighed at birth and prior to breeding. Average daily gain (ADG) was calculated. Following first calving (22 to 27 months old), milk recording data were collected from the 265 cows that completed their first lactation using a minimum of three recordings/cow. Milk yields (305-day) were then standardised according to fat and protein content. A mixed linear regression model was used to investigate the relationship between ADG and milk yield.

Results: The final model included farm and month of calving within year as random effects. Heifer ADG was quadratically related to first lactation standardised milk yield. An ADG of 0.82kg/day was associated with maximum predicted yield. A heifer with an ADG of 0.82kg/day was predicted to yield 1120kg more than a heifer growing at 0.55kg/day, 218kg more than a heifer growing at 0.7kg/day and 103kg more than a heifer growing at 0.9kg/day. Age at first calving and predicted transmitting abilities for protein production and calving interval were also significant in the final model.

Conclusions: Pre-breeding ADG was quadratically related to first lactation milk yield, with an ADG of 0.82kg/day associated with maximum predicted yield.

Please see the reference to the published article and DOI below:

Hayes, C.J., McAloon, C.G., Kelly, E.T., Carty, C.I., Ryan, E.G., Mee, J.F. and O'Grady, L., 2021. The effect of dairy heifer pre-breeding growth rate on first lactation milk yield in spring-calving, pasture-based herds. Animal, 15(3), p.100169

DOI: https://doi.org/10.1016/j.animal.2020.100169

The article is available under the Creative Commons Attribution License (CCBY), available at https://creativecommons.org/licenses/by/4.0/

Keywords: Heifer, average daily gain, milk yield.

HH-49

Systematic evaluation of different fresh cow monitoring procedures

Felix König[1], Andy Hancock[2], Christian Wunderlich[3], Markus Klawitter[3], Thomas Breuer[3], Anne Simoni[1], Karina Weimar[1], Marc Drillich[1], Michael Iwersen[1].

[1]Clinical Unit for Herd Health Management in Ruminants, University Clinic for Ruminants, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria; [2]Zoetis International, Dublin, Republic of Ireland; [3]Zoetis Deutschland GmbH, Berlin, Germany.

Objectives: Intensive and well-structured monitoring of fresh cows is considered beneficial for the health and well-being of the cows and is an important factor for the success of the cows' further lactation. Due to the various metabolic and infectious diseases caused by a negative energy balance at the time of calving, fresh cow monitoring can become a very time-consuming task. However, currently it is necessary to lock up the animals for examinations and treatments, which restricts their natural behaviour and thus has a negative impact on the health and performance of the herd. However, performing this important but time-consuming examination during the fresh cow period without excessively affecting the animals' natural behaviour is often a challenge, especially when many animals need to be examined on larger farms. Automated monitoring by use of 'precision livestock farming' technologies is progressively applied on farms to identify animals at risk of disease at an early stage and to reduce routine examination times. Determining the exact times that cows are fixed in headlocks for examinations and treatments under practical conditions can be used to evaluate existing and develop new management strategies. This is particularly important for practitioners that want to develop time- and cost-efficient management strategies while minimising the impact on the dairy cow's time budget. PLF technologies could be a support here to reduce the fixation times of cows in headlocks and stuff working hours.

The aim of the study was to describe in detail the time required for routine examinations in the fresh cow area as well as the total duration each cow stayed in headlocks during these examinations.

Material and methods: This study was conducted from June 2021 to August 2021 on a commercial dairy farm in Germany, housing approx. 1,900 Holstein-Friesian dairy cows. Different methods of fresh cow monitoring procedures were compared against each other. These included, on the one hand, different routine examinations of fresh cows, on the other hand, three different workflows (systems) which differ in the order of examinations and treatments.

Prior to conducting the study, standard operating procedures (SOPs) were prepared in which the various examination and treatment steps, in particular the start and end times, were specified. For this purpose, the working times for conducting individual examination steps as well as the resulting fixation time of the animals were systematically recorded.

Sixteen digital observation cameras (network camera HYU-405, HYUNDAI Corporation, Korea) were installed in the fresh cow pen and recorded the fresh cow management procedures during the morning. Mangold-Interact (version 17.1.0.0, Mangold International, Arnstorf, Germany), a specialized software for visual evaluation of video footage, was used to analyze the time required for specific fresh cow management procedures. In total, 3973 examination steps and 1848 headlock times per cow were eligible for statical analyses. For comparison of the different systems as well as the examination steps, performed by the investigators, the Kruskal-Wallis test, respectively the analysis of variance (ANOVA) was used.

Results: Overall, the most frequently observed examinations were temperature measurement (n=2239, 56%), percussion-auscultation (n=329, 8.2%), succession-auscultation (n=325, 8.1%), and rumen fill estimation (n=217, 5.4%). The different examinations lasted on average between 1 and 115 seconds. Significant differences in the time needed to perform specific examinations were identified between the investigators.

The resulting fixation time differed significantly between the three different fresh cow management systems ($P < 0.05$). The animals were fixed in headlocks between 1 and 106 minutes.

Conclusion: Although significant differences in the examination times of different investigators were identified, these can be neglected from a practical point of view. Significant differences in the fixation time of the animals in the headlocks were determined based on the order in which the animals were examined and treated. The results of this study can be used in the future to develop optimal management strategies that consider cow needs, available labour and other economic factors. In this context, it could also be evaluated what contribution PLF technologies can make to reduce fixation times of cows in headlocks and stuff working hours.

Keywords: Dairy cow, health monitoring, transition period, accelerometer, fresh cow.

ID-01

The impact of bovine viral diarrhea virus infection on milk production of Dutch dairy herds

Xiaomei Yue[1], Wilma Steeneveld[2], Mariska Van Der Voort[1], Gerdien Van Schaik[3], Johannes C. M. Vernooij[2], Linda Van Duijn[3], Anouk M.b. Veldhuis[3], Henk Hogeveen[1].

[1]Wageningen University, Wageningen, Netherlands; [2]Utrecht university, Utrecht, Netherlands; [3]GD Animal Health, Deventer, Netherlands.

Objectives: Bovine viral diarrhea virus (BVDV) is endemic in many countries and can have a major impact on cattle health. BVDV can among others cause diarrhea, fever, pneumonia, growth retardation, immunosuppression, and reproductive disorders, thereby reducing milk production and consequently causing economic losses. Research on the effect of BVDV on milk production is relatively outdated and hardly investigates changes in milk production before and after a new BVDV infection in the herd. Moreover, it is unclear whether the impact of BVDV changes when an increased proportion of farms participates in a BVDV surveillance program. The objectives of this study are to determine the loss in milk production as a result of a new BVDV infection in dairy herds participating in the Dutch BVDV-free program between 2007 and 2017.

Material & Methods: Longitudinal herd-level BVDV surveillance data of 4,334 dairy herds participating in the BVDV-free program were combined with monthly test-day milk production data on herd level from 2007-2017. This combined dataset consisted of 3,126 herds, of which 2,486 herds were BVDV-free during the whole study period and were defined as free-herds. 640 herds lost their BVDV-free status at a certain moment during the study period and were defined as breakdown-herds. To estimate the impact of BVDV infection within breakdown-herd, milk production before and after the infection was compared. Since milk production fluctuates over time, the milk production data of the free-herds was used to correct for that. A linear mixed regression model was used to estimate changes in milk production before and after BVDV infection on an annual and quarterly basis. The model included the fixed variables BVD status, breakdown-herd or free herd, year, season, and a random herd-effect that takes the repeated measurements within the herd into account. The dependent variable was the average daily milk production on the test-day. In our analyses, we assumed that the risk period for milk production loss began with the BVDV infection. The moment of BVDV infection is difficult to determine accurately. Therefore, four scenarios were developed to reflect different possible moments of BVDV infection. In the default scenario, the breakdown date was assumed to be the start of BVDV infection, and the years/quarters after the breakdown date were included as a risk period for milk production loss. Three additional scenarios were studied in which it was assumed that the breakdown is caused by the birth of a persistently infected (PI) calf, being born 4, 6 or 9 months before the breakdown date. In these scenarios, the risk period for milk production loss started when the PI calf was born.

Results: Results for the default scenario showed that in the first year after the breakdown date, the average milk production loss per cow per day was 0.04 kg. In the first to fourth quarters of the first year after infection, the average milk production loss per cow per day were 0.13, 0.08, 0.01 and 0.07 kg, respectively. Overall, in the first year after BVDV infection of the default scenario, milk production losses ranged from 570 to 1,603 kg at a Dutch herd level, with an average of 1,086 kg per herd per year or 13 kg per cow per year. In the results of scenarios 1, 2 and 3, the negative effects of BVDV introduction were mainly in the second year after BVDV introduction, and the average milk production decreased by 0.05, 0.06, 0.05 kg/cow/day, respectively. Results show that the risk period for milk production loss due to BVDV introduction is mostly in the first two years after BVDV introduction, especially in the first quarter.

Conclusion: This study shows that the BVDV infection occurring in herds in a BVDV-free surveillance program has a limited but negative impact on milk production of dairy herds, mainly in the first year after infection. The main reason why a new introduction of BVDV caused such a small loss of milk production on dairy farms is probably due to the participation in the BVDV-free program. This BVD surveillance program enables participating farmers to identify and remove the PI animals very quickly after the infection, which may limit extensive transmission of the virus to the lactating herd and avoid large milk production losses.

Keywords: Bovine viral diarrhea, dairy, milk production.

ID-02

Case reports - *Mannheimia haemolytica* in dairy cows

Kirsten Stemme[1], Egon Thesing[1], Dirk Schäfer[2], Siegfried Marquard[3].

[1]MSD Animal Health, Unterschleißheim, Germany; [2]Praxis Schäfer, Dörmitz, Germany; [3]Tierarztpraxis Marquardt & Walter, Goch, Germany.

Objectives: *Mannheimia* (*M.*) *haemolytica* is one of the most important pathogens of respiratory disease in young cattle (calves and feedlot cattle). In recent years, however, cases of severe pneumonia caused by *M. haemolytica* in lactating dairy cows, have been reported. By means of two clinical cases in Germany, the wide range of clinical symptoms and diagnostic as well as treatment options are discussed to raise the awareness of this disease in dairy cows.

Material and Methods: The first case: an increased number of deaths occurred on a dairy farm with 500 lactating cows in Mecklenburg-Western Pomerania. When the veterinarian was consulted, five animals had already died, and more than 15 cows showed severe fever and an increased respiratory rate. Cows of all ages and in different stages of lactation were affected.

As a new silo had been opened preceding illness, Clostridia infection was suspected. Samples were taken from the affected animals for further diagnosis. One moribund cow was euthanized and sent to the state laboratory for postmortem examination.

The second case: the course of disease was more prolonged on a dairy farm with 110 lactating cows. One cow showed unspecific symptoms such as drop in milk in produc-

tion, anorexia and a slightly elevated body temperature. As metal particles were found by the metal detector of the feed mixer acute traumatic reticuloperitonitis was diagnosed. In the following days, 9 other cows fell ill with similar symptoms One of them developed severe disease (recumbency, bloody nasal discharge) and was euthanized for postmortem examination. On inquiry, the farmer said that three new heifers had entered the farm 12 days before.

Results: In both cases severe acute pneumonia was diagnosed on autopsy. On bacteriological testing, *M. haemolytica* was detected in lung tissue of both cows. *M. haemolytica* Serotype A1 was also found in other organs of the cow from case 1 and in nasal swabs and as well as in lung fluid taken via transtracheal aspiration (TTA) taken from diseased cows. Nasal swabs taken from diseased cows on farm 2 were also positive for *M. haemolytica*.

On farm two, paired serum samples were taken from all diseased cows and antibodies against BRSV, PI3-V, M*ycoplasma bovis* and *M. haemolytica* were measured. Only the titers of *M. haemolytica* antibodies showed an increase over time and, in six of ten animals, this increase could be valued as seroconversion.

Diseased animals were treated with antibiotics and nonsteroidal anti-inflammatory drugs (NSAID) after *M. haemolytica* was confirmed as infective agent and all cows responded well to this treatment. On farm 1, clinically healthy animals were vaccinated with a trivalent vaccine (Bovilis® Bovipast® RSP, MSD Animal Health). A few days after the first vaccination, the disease was already beginning to subside. After the second vaccination 4 weeks later, no further disease or death were observed in the dairy cows.

Conclusion: Both cases show that *M. haemolytica* can infect dairy cows and cause severe economic losses due to substantial drop in milk production or even sudden death of diseased animals.

Clinical symptoms may vary considerably and make it difficult to make the right diagnosis. To confirm an infection with *M. haemolytica*, either detection of bacteria in samples from the respiratory tract or proven seroconversion shown by an increase in antibodies can be used.

Vaccination can protect a herd, especially if new animals are entering the farm regularly.

Keywords: Mannheimia haemolytica, dairy cows, serology, vaccination.

ID-03

Different etiological agent associations detected in bovine respiratory disease (BRD) outbreaks in unweaned and fattening calves

Johan Manuel Calderón Bernal[1], Ana Fernández Ros[2], José Luis Arnal Bernal[2], Cristina Baselga Julian[2], Alfredo Benito Zuñiga[2], Ana Isabel Vela Alonso[3], José Francisco Fernández-Garyzábal[3], María Dolores Cid Vázquez[1].

[1]Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense, Madrid, Spain; [2]Exopol. Veterinary Diagnostic and Autogenous Vaccine Laboratory, Zaragoza, Spain; [3]Departamento de Sanidad Animal, Facultad de Veterinaria, Centro de Vigilancia Sanitaria Veterinaria (VISAVET), Universidad Complutense, Madrid, Spain.

Objective: Bovine Respiratory Disease (BRD) is a leading cause of economic loss, hampering animal welfare and intensive antimicrobial use in cattle operations. BRD is a multifactorial infectious disease that usually affects a group of animals and it is caused by a complex interaction between different viral and bacterial pathogens in single or mixed infections, the immune status of the host and environmental and management factors. The aim of this study was to investigate the frequency of detection of the main etiological agents involved in BRD and their association with BRD outbreaks.

Material and methods: One hundred fifty six (n= 156) outbreaks of BRD that were sent for diagnosis at the Laboratory of diagnosis EXOPOL between January 2020 and November 2021 were included in the study. Inclusion criteria were geographical localization of the outbreaks in Spain and complete test results for the detection of Parainfluenza 3 virus (PI-3), Bovine respiratory syncytial virus (BRSV), Bovine coronavirus (BCov), Bovine viral diarrhea virus (BVDV) and Bovine herpesvirus 1 (BoH-1v), and the bacteria *Mannheimia haemolytica (Mh)*, *Pasteurella multocida (Pm)*, *Histophilus somni (Hs)* and *Mycoplasma bovis (Mb)*. Diagnosis was performed in pools of 5 samples from different animals of the same outbreak by quantitative PCRs (qPCRs). Clinical specimens were bronchoalveolar lavages (n= 96), lung (n= 35), nasopharyngeal swabs (n= 8) and tracheal scrapes (n= 2) or mixtures of them (n= 15). Outbreaks were from unweaned (n=32), fattening (n= 107), replacement (n=8) and adult (n= 1) animals or unknown (n= 8).

A cluster analysis of categorical variables was performed using the hierarchical clustering method. The Boolean variables (presence/absence) included as active variables to define clusters were detection of PI-3, BRSV, BCov, BVDV, BoH-1v, *Mh, Pm, Hs* and *Mb*. Associations between cluster and categorical variables were analyzed using the chi-square test and significance level set at $P < 0.05$.

Results: The most frequently detected virus were BCov (39.7%), followed by PI3 (26.3%), BRSV (19.9%) and BVDV (17.7%) and the less frequent BoH-1v (3.2 %). The most frequently detected bacteria were *Pm* (85.9%), followed by *Mb* (77.6%), *Mh* (64.1%) and *Hs* (42.3%). Cluster analysis grouped outbreaks into two clusters. Cluster 1 included outbreaks were the detection of the PI3 (4.7 %), BRSV (4.7 %), BCov (24.5 %) and BVDV (6.6 %) viruses and *Mb* (72.6 %) was significantly lower and detection of *Hs* (51.9 %) was significantly higher than in the total of outbreaks. Opposite, cluster 2 included outbreaks were the detection of the PI3 (72.0%), BRSV (52.0 %), BCov (72.0 %) and BVDV (40.0 %) viruses and *Mb* (88.0 %) was significantly higher, and detection of *Hs* (22.0 %) was significantly lower than in the total of outbreaks. In cluster 1 and cluster 2, no statistically significant differences were observed in the frequencies of detection of *Pm* (84.0 and 90.0 %, respectively) and *Mh* (60.4 and 72.0%, respectively). It was found an association between both clusters and the productive stage of the animals ($P<0.05$). Thus, the frequency of outbreaks from fattening animals were significantly higher (OR

3.5; CI95% (1.5-7.8) in cluster 1 than cluster 2 (78.8% versus 59.2%, respectively), whereas the frequency of outbreaks from unweaned animals was significantly lower in cluster 1 than cluster 2 (14.1 versus 36.7%, respectively). There was no association between clusters and the geographic area of outbreaks.

Conclusion: These results suggest that in unweaned animals the respiratory viruses or *Mb* would have a greater clinical significance playing a role either as responsible for clinical processes or cooperating as predisposing agents for bacterial infections. On the contrary, in fattening animals, other predisposing factors such as stress associated with transport of animals or mixing of animals of different origins in feedlots would have a greater relevance. Consequently, the management and sanitary measures carried out for an efficient control of BRD in calves should be adapted to the different productive stages. In this regard, possible measures to consider could be the vaccination of unweaned animals against respiratory viruses or the incorporation of *Pm*, together with *Mh*, to the vaccines used in cattle which could help to substantially reduce the incidence of BRD.

Keywords: Bovine respiratory disease, outbreaks, cattle, BRD asociated infectious agents.

ID-04

Four years of mandatory BoHV1 (IBR) control programme in dairy herds in the Netherlands

Frederik Waldeck[1], Irene Bisschop[1], Jet Mars[1], Katrien Van Den Brink[1], Mattheus Hekman[1], Emma Strous[1], Bert De Roo[1], Paul Wever[1], Gerdien Van Schaik[2].

[1]Royal GD, Deventer, Netherlands; [2]Royal GD & Department of Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands.

Objectives: Bovine herpesvirus-1 (BoHV1), the causative agent of Infectious Bovine Rhinotracheitis (IBR) was first reported in the Netherlands in 1973. In 1997, a national eradication programme was initiated, with at the time an estimated nationwide herd prevalence of 84%. Only the use of gE-deleted markervaccines was permitted enabling testing for antibodies against wildtype BoHV1 with gE-ELISA.

In 1999, the national programme was abruptly suspended when a BVDV2-contaminated batch of IBR-vaccine resulted in severe clinical illness and death. After this disappointing end, the IBR-certification programme was continued voluntary for almost two decades. In 2015, when discussion on a new national programme for IBR-eradication started, 43% of dairy herds participated in IBR-certification routes (28% IBR-free, 15% IBR-unsuspected). The herd prevalence in dairy herds had decreased to 15.6% by that time.

In April 2018, a mandatory IBR-control programme only for dairy herds was introduced by the dairy industry and is carried out by Royal GD. The goal is to stimulate more herds to become IBR-free, to keep them IBR-free and mitigate the risk posed by infected herds through vaccination. Non-dairy cattle herds can voluntarily participate. The objective is to describe the progress of IBR control.

Materials & methods: Dairy herds are obliged to participate in one of the three major routes that can lead to a BoHV1-free herd status:

- IBR-free certification
- IBR-unsuspected certification
- IBR-vaccination certification

IBR-free certification starts with individual serum gE-antibodies screening of the herd, subsequent monitoring of the free status is performed by monthly bulk milk IBRgE testing.

IBR-unsuspected certification starts with a negative bulk milk gE-antibodies screening, subsequent monitoring of the unsuspected status is performed by monthly bulk milk IBRgE testing. After at least two years of IBR-unsuspected status, herds can qualify for an IBR-free status by individual serum gE-antibodies screening of cows older than 6 years.

IBR-vaccination certification is granted after the first whole herd vaccination with gE-deleted markervaccine (all cattle over 3 months of age) and is prolonged when the herd is vaccinated every six months thereafter. This route is mandatory for infected herds (positive bulk milk gE-antibodies screening).

In addition to the monthly bulk milk surveillance, other risk-based monitoring tools are applied. After purchase, cows from non-free herds are automatically noted in IBR-free and IBR-unsuspected herds and require mandatory testing for gE-antibodies. All cows that have aborted are mandatorily tested for gE-antibodies. In both cases, when cows test positive they need to be culled. Furthermore, when clinical signs of IBR are noticed in IBR-free or IBR-unsuspected herds it is mandatory to submit nasal swabs for PCR testing on IBR-virus.

The progress of the nationwide IBR-control programme is monitored by combining diagnostic test results with cattle movement data. Semi-annual analyses of the following key figures is performed: (1) the percentage of IBR-free, (2) IBR-unsuspected and (3) IBR-vaccinated herds, (4) incidence of new IBR-infections detected by bulk milk screening and (5) virus detection in nasal swabs.

Results: The percentage of dairy herds with a favourable IBR-situation almost doubled since the start in 2018. At the end of 2021, 55% of dairy herds were certified IBR-free, 27% IBR-unsuspected and 18% IBR-vaccinated. After four years of new IBR-regulation, the incidence of new IBR-infections detected by bulk milk screening (often subclinical outbreaks) decreased from 0.53% in 2018 to 0.26% in 2021. Annually, nasal swabs from around 200 herds are submitted for PCR testing and in 2018 BoHV1 was detected in 12.9% of herds. This figure dropped to 6.6% in 2021. Since the start of the new mandatory phase and up to the end of 2021, in total 109 IBR-free dairy herds had an outbreak (out of on average 15.304 dairy herds).

Amongst non-dairy herds, 22% is certified IBR-free on a voluntary basis. Even without mandatory control in these herds, the herd prevalence in non-dairy herds declined over the period 2016 to 2020.

Conclusion: Even though the Dutch IBR-programme is only mandatory for dairy herds, much progress is made in the

Dutch cattle sector. National implementation of IBR-eradication for all bovine herds by the government is discussed by the cattle industry and the Ministry of Agriculture. With regular monitoring, thorough and timely insight on the progress of IBR-eradication is obtained and evidence-based decisions can be made for further developments in the eradication programme.

Keywords: BoHV1; IBR; eradication; gE-antibodies; bulk milk.

ID-05

A synergy between influenza D virus and *Mycoplasma bovis* in bovine respiratory disease

Lion Adrien[1], Rancon Camille[1], Cebron Nathan[1], Secula Aurélie[1], Cassard Hervé[1], Salem Elias[1], Gaudino Maria[1], Bouleisteix Olivier[2], Zohari Siamak[3], Hagglund Sara[4], Delverdier Maxence[1], Baranowski Eric[1], Foucras Gilles[1], Guitton Edouard[2], Valarcher Jean-François[4], Ducatez Mariette[1], Meyer Gilles[1].

[1]Veterinary College of Toulouse, Toulouse- UMR1225 INRA-ENVT, France; [2]INRAE, UE 1277, Experimental Infectiology Platform (PFIE), INRA-Val de Loire research Centre, Nouzilly, France; [3]National Veterinary Institute Uppsala, Department of Virology, Immunobiology and Parasitology, Sweden; [4]Swedish University of Agricultural Sciences, Uppsala, Host Pathogen Interaction Group, Department of Clinical Sciences, Sweden.

Objectives: Since its discovery in 2011 in the United States, the novel influenza D virus (IDV) of the *Orthomyxoviridae* family was found spread among swine and ruminants on four continents so far, confirming a worldwide distribution. Cattle were suggested to be the main host. Experimental infections in naïve calves showed that IDV infects both the upper (URT) and lower (LRT) respiratory tracts, with a moderate pathogenicity and a high level of transmission (Salem et al. 2019). In addition, IDV detection during bovine respiratory disease (BRD) field outbreaks suggests that this virus can be considered at least as predisposing or co-factor of BRD (Mitra et al., 2016). To study the influence of IDV on other respiratory pathogens and to confirm its role as a co-factor of BRD, we performed experimental co-infections of calves with both IDV and *Mycoplasma bovis* (*M.bovis*). A frequent association between IDV and *M.bovis* was indeed found in 42% of animals with respiratory signs in veal calf units in France.

Material & Methods: Experimentation was performed under EEC guidelines (86/609/CEE) and official French ethical agreement. Twenty-nine calves (free of IDV and classical respiratory pathogens) were distributed into four separate pens. In three pens, 8 calves per group were intranasally nebulized at day 0 (D0) through a mask, with 10^7 $TCID_{50}$ per calf with a French IDV strain D/bovine/France/5920/2014, with 10^{10} colony forming unit per calf of the French *M.bovis* strain RM16, with both pathogens, respectively. Five non infected calves made up the fourth group. All calves were examined from 3 days before challenge (D-3) to the end of experimentation (D21) for clinical signs, gross and microscopic lesions (D6 for 3 infected calves per group and D21). IDV and *M.bovis* quantification was measured by qRT-PCR and qPCR in both URT (nasal swabs (NS) from D1 to D21) and LRT (bronchoalveolar lavages (BAL) at D-1, D2, D7, D14 and D21) and tissues (nasal turbinate, trachea and lung at D6 and D21). Gross lesions, histopathology, cell identification and counting from BALs and blood were investigated to assess pathology. The immune response was assessed for antibody seroconversion (IHA, ELISA) and cellular responses.

Results: Clinical examination confirmed that IDV induced a mild respiratory disease between D5 and D10 characterized by coughing, tachypnea, and dyspnea. Calves infected by *M. bovis* started showing moderate clinical signs later, from D7-D8 to D19. These calves mainly showed tachypnea, muco-purulent discharge, a more frequent and bad cough and sometimes labored breathing with abnormal lung sounds (wheezing). In the co-infected group, clinical signs were similar to those of the *M. bovis* group except that they occured earlier, starting fom D4, and were more severe. The mean clinical scores indicated significant differences between the co-infected groups and other groups from D6 to D8. The severity of the clinical manifestations, especially in the co-infected group, was correlated with severe gross and microscopic lesions in respiratory tissues (nasal turbinates, trachea and lungs), mainly characterized by loss of ciliature, necrosis of the respiratory epithelium, and mononuclear cells and neutrophils infiltrations in the lungs and BALs. No statistical differences were observed between groups for IDV replication in URT or LRT, except that IDV replication was longer in the monoinfected group. On the other hand the earlier replication of *M. bovis* in NS and respiratory tissues of the co-infected group correlated with the clinical differences observed between co-and mono *M.bovis* infected groups. After D10, only *M.bovis* was detected in NS, BALs and tissues in co-infected group while IDV was observed in NS, BALs and lungs in the IDV group until D21. Finally calves seroconverted against IDV as early as at D7 for IDV mono and co-infected groups while antibody response against *M.bovis* occured later. First results of cellular immune response examination indicate that IDV modulates the innate immunity against *M. bovis,* leading to more severe pathology.

Conclusion: Altogether, these results suggest a synergy between IDV and *M. bovis* respiratory infections in young calves with implications for the control of respiratory diseases. Indeed, IDV increases the severity of *M.bovis* infection leading to severe respiratory signs. First results of cellular immune response examination indicate that IDV modulate the immune response against *M. bovis* and confirm that IDV acts as an initiator pathogen for BRD.

Keywords: Bovine-Influenza D-Mycoplasma bovis-respiratory-BRD.

ID-06

Cross perceptions of farmers, veterinarians and physicians on Q fever: "one health" approach

Raphael Guatteo[1], Christophe Brard[2], Thibaut Cauderlier[3], Eric Collin[2], Renee De Cremoux[4], Kristel Gache[5], Brigitte Trezzani[3].

[1]Oniris & INRAE, Nantes, France; [2]SNGTV, Paris, France; [3]CEVA SANTE ANIMALE, Libourne, France; [4]IDELE, Toulouse, France; [5]GDS France, Paris, France.

Objectives: The objective of this study was to describe the knowledge and assess the perceptions, in a cross-sectional manner, of ruminant farmers, rural vet practitioners and physicians practising in rural areas. Under a "one-health" approach, the goal was to identify, if necessary, areas for improvement in terms of awareness of the disease and exchanges between these professions.

Materials and Methods: To this end, an opinion poll was conducted with the help of an independent polling institute (Via Voice). Hundred physicians and 100 veterinarians practising in areas < 20,000 inhabitants (rural areas) and 374 farmers geographically matched with both vets and physicians (respectively 134 sheep farmers, 100 goat farmers and 198 cattle farmers) were interviewed. All participants were randomly selected in the selected areas. The questionnaire was built around 3 main parts: (i) basic knowledge about Q fever in humans and ruminants, (ii) perception of the risk (including zoonotic risk) and (iii) knowledge about control measures both in humans and animals.

Results: Among farmers, 76% declared knowing the disease with difference depending on the species: 64% for cattle (57% for beef farmers and 76% for dairy farmers), 84% for sheep and 90% for goat farmers. For the rest of the survey, only slight differences were observed between goat, sheep and cattle farmers leading us to gather their results. The following table displays some (not exhaustive) important results. Abortion was the main clinical sign attributable to Q fever (45% for farmers and 70% for vets). Reproductive disorders were mentioned only by 7% of cattle farmers and 19% of vets. More than 29% of cattle farmers and 32% of small ruminants farmers and 21% of vets did not know about the clinical signs of Q fever highlighting the need to promote knowledge about the disease, particularly the negative impact on reproduction besides abortion. For physicians, the clinical signs the most frequently reported as leading to suspect Q fever was fever (67%) followed by joint pain (29%), pneumonia (24%) and fatigue syndrome (22%). Among all zoonotic diseases offered to the respondents, Q fever was ranked among the most at risk one as 2nd, 4th and 5th respectively for farmers, vets and physician in line with low perception for the zoonotic risk (despite recent and regular human outbreaks). However, awareness among farmers is higher among those who receive the public. For vets, the perception of farmers of the impact for their herd or their health was considered as non-sufficient in 62 and 80% respectively. Regarding the farmer's knowledge about prevention measures for their animals or their own health, vets considered it as non-sufficient in 77 and 82% respectively. Among barriers to tackle the disease, vets stipulated the lack of abortion reporting by farmer (65%), the funding's of ancillary test to confirm the disease (70%) and the choice of animals to be sampled (40%). Among farmers experiencing Q fever 53% did not implemented control measures. For those who implemented control measures vaccination was implemented in only 25% of the cases.

Table. Some examples of answers to the opinion survey.

Questions	Farmers (% respondants)	Vets (% respondants)	Physicians (% respondants)
I have been exposed to Q fever in my work	12%	49%	25%
I know this disease is zoonotic	65%	66%	83%
Introduction of Q fever is at risk for animal and/or human health	10%	30%	27%
Isolation of affected animals when Q fever is detected	55%	54%	35%
Vaccination considered as control action	31%	35%	14%

Conclusion: All together, these results support a very heterogeneous level of knowledge about the disease in any population. The perception of the risk, particularly the zoonotic one, also appears to be fairly low. The implementation of appropriate control measures needs to be improved, as does the dialogue between the breeder-veterinarian-doctor trio

Keywords: Q fever; Perception survey, Zoonosis, One health.

ID-07

Tracing the spread of bovine respiratory syncytial virus (BRSV) between herds

Sara Hägglund[1], Paul Johnson[2], Katarina Näslund[3], Sophia Lindberg[4], Ingrid Hyttsten[5], Anna-Karin Eng[6], Yongzhi Guo[4], Ewa Olszowy[4], Patricia Simões[4], Orton, Richard[2], Siamak Zohari[7], Daniel Haydon[2], Jean Francois Valarcher[4].

[1]Swedish university of agricultural sciences, Swedish university of agricultural sciences, Sweden; [2]University of Glasgow, Glasgow, United Kingdom; [3]Swedish university of agricultural sciences / National Veterinary Institute, Uppsala, Sweden; [4]Swedish university of agricultural sciences, Uppsala, Sweden; [5]Jämtlandsveterinärerna, Lit, Sweden; [6]ITVet Jemtland AB, Hammerdal, Sweden; [7]National Veterinary Institute, Uppsala, Sweden.

Objectives: Bovine respiratory syncytial virus (BRSV) causes respiratory disease in cattle and has been diagnosed in 12% to 83% of respiratory disease outbreaks in Europe [1,2]. In the north of Scandinavia, the control of BRSV relies on biosecurity rather than on vaccination despite that the epi-

demiological cycle and the modes of transmission of this virus are poorly understood. The objective of this study was to deepen this knowledge, at the national, regional and between-herd level, with the final goal to improve the control of BRSV.

Material and methods: Upsurges of outbreaks of respiratory disease occurred in 2016 and 2020, and whole BRSV-genome sequences were obtained from 54 cattle in 34 herds throughout Sweden. Partial genome sequences were additionally generated from the G-gene. In 2020, clusters of outbreaks were identified in the three counties: Dalarna, Uppland, and Jämtland, from which complete sequences were obtained from 5/5, 7/9 and 15/25 herds with diagnosed outbreaks. In three of the herds in Uppland, whole-genome data was additionally generated from BRSV collected in 2016. Phylogenetic analyses were performed to determine genetic relationships between different circulating viruses. Possible routes of introduction 3 to 4 weeks before the report of the outbreak were investigated, such as purchase or contact with animals from other holdings, visits, interactions with animal professionals, as well as contact with animal transport vehicles. Based on epidemiological and phylogenetic information, an attempt was made to identify how BRSV was introduced in some of the farms. One to two months after the last detected outbreak, bulk tank milk was collected from 30 herds in Jämtland, 23 of which without recent history of respiratory disease. This milk was analysed for BRSV-specific IgG1.

Results: Despite that BRSV is an RNA virus, few genomic changes were detected within counties. Nevertheless, there was a spatial clustering of virus genome sequences between counties, with rare introductions from one county into another. The viruses obtained in Uppland 2016 were more closely related to some of the viruses from the same region in 2020 than to viruses from other regions, indicating that the virus had remained in the area. Viruses collected in one herd in 2016 and 2020 had only 15 mutations difference, whereas viruses collected in different counties in 2020 differed with approximately ten times more changes. This data suggests that when a different lineage is detected in an area, it is the result of an introduction from another area rather than due to the rapid genetic evolution of strains.

BRSV-specific antibodies were detected in bulk tank milk from 18/30 herds in Jämtland after the wave of outbreaks in 2020. Based on sequencing data and epidemiological investigations, human, animal, material or transport contacts were suspected to be the origin of transmission of BRSV between some of the herds. Such contacts occurred 10-26 days before the first observed clinical signs.

Conclusions: The stability of the BRSV genome impedes the tracing of this virus. Nevertheless, the epidemiological information and preliminary phylogenetic data suggested that BRSV is introduced 2-3 weeks before outbreaks are reported, possibly sometimes by professionals or transports in contact with cattle on the recipient farms. The Swedish context is characterised by high biosecurity measures and scarce animal exchanges. These findings can be used as the basis to design and implement effective biosecurity recommendations to stop the spread of BRSV during an epidemic.

References:

1. Klem, T.B.; Rimstad, E.; Stokstad, M. Occurrence and phylogenetic analysis of bovine respiratory syncytial virus in outbreaks of respiratory disease in Norway. *BMC Vet Res* **2014**, *10*, 15, doi:10.1186/1746-6148-10-15.
2. O'Neill, R.; Mooney, J.; Connaghan, E.; Furphy, C.; Graham, D.A. Patterns of detection of respiratory viruses in nasal swabs from calves in Ireland: a retrospective study. *Vet Rec* **2014**, *175*, 351, doi:10.1136/vr.102574.

Keywords: Bovine respiratory syncytial virus, transmission, tracing, sequencing, epidemiological investigation.

ID-08

Prevalence, biosecurity and risk management of bovine coronavirus infections on dairy farms in Europe

Anna Catharina Berge[1], Geert Vertenten[2].

[1]Berge Veterinary Consulting, Vollezele, Belgium; [2]MSD Animal Health, Boxmeer, Netherlands.

Objectives: The objective of this cross-sectional field study is to obtain an estimate for the farm prevalence of Bovine Coronavirus (BCoV) in dairy production in Europe, and to characterize farm-level risk factors in management and biosecurity that are linked to BCoV infection in neonatal and weaned dairy calves.

Material & Methods: A convenience sample of 130 European Union (EU) dairy farms with at least 100 lactating cows each were enrolled in this study. The farm enrolment was based upon the country's relative magnitude of the national dairy production. Nasal and f aecal swabs were collected for BCoV detection, blood and bulk tank milk samples were collected for specific BCoV antibody detection. Depending on the farm size, 10-20 samples from neonatal calves under 3 weeks of age, 10-20 samples from the most recently weaned calves and 5-10 samples from fresh cows, and one bulk tank milk sample were collected. All samples were shipped and analysed at the same laboratory. BCoV presence was determined in nasal and faecal swabs using semi-quantitative Real Time PCR (RT-PCR). Bulk tank milk and serum samples were tested for the presence of BCoV antibodies using ELISA. On each farm an extensive questionnaire was performed to determine various husbandry (i.a. vaccination of mother cows against bovine coronavirus) and biosecurity management factors. The Biocheck survey (https://biocheck.ugent.be, Ugent, Belgium) was used to score the biosecurity system. Correlations between the results from testing of nasal and faecal samples, as well as blood and bulk tank milk samples were determined. Prevalence estimates for samples within farms and countries were calculated. Multivariable analysis was used with dichotomous outcomes (logistic models) or ordinal outcomes (cumulative logistic or linear models) to determine risks for the presence of BCoV and BCoV antibodies in neonatal calves, weaned calves, fresh cows, and bulk tank milk. Random effect models were used where appropriate.

Results: The study is ongoing. To date we have partial or complete results from 45 dairy farms in Belgium, Czech

Republic, Denmark, France, Italy, Netherlands, Portugal, and Sweden. Some preliminary results and data analysis are presented in this abstract, pending further enrolment. Antibody levels in bulk milk samples, measured as % inhibition, were on average 86 (++++) and all tested farms had animals with levels above 54 (+++). Mean antibody levels were 56 in preweaned calves, 39 in weaned calves and 62 in fresh cows. Approx. 50% of herds were using fresh cow vaccinations against bovine coronavirus during the dry period to boost colostral immunoglobulins, and there was a non-significant trend for increased antibody levels in neonatal calves and fresh cows in those herds. There was poor correlation (Pearson's correlation coefficient r=0,41) between bulk tank milk antibody levels and serum antibody levels. Presence of BCoV was confirmed in 17% of nasal and faecal samples from 27 herds. BCoV was found in nasal and/or faecal samples from 74% of herds. Fifty percent of herds had one or more animals that were BCoV positive on nasal swabs. Presence of BCoV was demonstrated in 23% of nasal samples from neonatal calves, 20% of samples from weaned calves, and 8% of samples from fresh cows. There was no significant difference in recovery of viral nucleic acid from nasal versus faecal swabs. There was poor correlation between animal antibody levels and virus shedding in animals (r=0,48). The biosecurity on 41 dairies was scored with overall score of 58%, external biosecurity score of 69% and internal biosecurity score of 41%. The biosecurity scores recorded were slightly above the world average score usually recorded for dairies that have used the Biocheck survey. The virus shedding in cattle tended to be higher in herds with overall higher biosecurity scores. However, antibody levels in cattle were non-significantly lower in herds with a higher biosecurity score.

Conclusion: The preliminary results from this study indicate that BCoV is commonly present in both the respiratory and enteric pathway in the dairy cattle population in the EU, with all herds being seropositive to the virus, and the virus present in numerous herds. The farm management and biosecurity measures associated with BCoV will be further investigated.

Keywords: Bovine coronavirus, prevalence, biosecurity, risk management, Europe.

ID-09

Pathogen-specific prevalence and pathogen associations during outbreaks of Bovine Respiratory Disease in calves in Flanders

Geert Hoflack[1], Maude Lebrun[1], Geert Vertenten[2], Bart Sustronck[1].

[1]MSD Animal Health, Brussels, Belgium; [2]MSD Animal Health, Boxmeer, Netherlands.

Objectives: Bovine respiratory disease (BRD) is a major health problem during calf rearing in many farms. The objective of this study was to obtain further insights into the importance of different pathogens involved and possible pathogen associations during outbreaks of BRD in calves in Flanders.

Materials & Methods: A cross-sectional study was performed from January 2019 until December 2021. The target population consisted of cattle herds from the northern part of Belgium (Flanders) with a current acute outbreak of BRD. Respiratory samples, consisting of either nasopharyngeal swabs, broncho-alveolar lavage fluid or lung tissue, were collected from affected calves in those herds and submitted for pathogen detection. Pathogen detection was performed using semi-quantitative real-time PCR test targeting seven bovine respiratory pathogens: bovine respiratory syncytial virus (BRSV), bovine parainfluenzavirus type 3 (PI3V), bovine coronavirus (BCoV), *Mannheimia haemolytica*, *Pasteurella multocida*, *Mycoplasma bovis*, and *Histophilus somni*. The results were analyzed using R software (R Core Team, 2017). Multivariable logistic regression models were constructed for each of the seven respiratory pathogens. As predictors, the PCR results of the other six pathogens besides the outcome pathogen were considered for the analysis. Additional predictors in the models were season of outbreak occurrence and sample type. Statistical significance was set at $p < 0.05$.

Results: In total 245 outbreaks of BRD were assessed. At least one pathogen was detected in 185 (75.5%) of those outbreaks. Single as well as multiple viral infections were detected in 31.8 and 43.7% of outbreaks, respectively. BRSV was the most frequently isolated virus (38 positive/245 outbreaks, 15.5%). In 73.7% of outbreaks where BRSV was detected, it was the only viral agent detected. BCoV was detected in 31 out of 245 outbreaks (12.7%) and was the only virus detected in 21 of those outbreaks (67.7%). PI3V was only detected in 3.7% of outbreaks and these were predominantly multiple viral infections (88.9%). *Pasteurella multocida*, *Mannheimia haemolytica*, *Mycoplasma bovis*, and *Histophilus somni* were detected in, respectively 58.8, 25.3, 21.6, and 20.8 % of the outbreaks.

In the present study, a PCR positive result for BRSV was associated with an increased detection rate of *Mycoplasma bovis* (OR 2.65, $CI_{95\%}$ 1.17-6.01) and PI3V (OR 9.63, $CI_{95\%}$ 2.14-52.5). *Mannheimia haemolytica* was associated with an increased detection rate of PI3V (OR 7.36, $CI_{95\%}$ 1.51-53.9), *Mycoplasma bovis* (OR 2.21, $CI_{95\%}$ 1.07-4.55), and *Pasteurella multocida* (OR 2.19, $CI_{95\%}$ 1.09-4.57). Detection of BCoV during an outbreak of BRD was associated with a higher risk for the detection of PI3V (OR 5.96, $CI_{95\%}$ 1.24-29.8). Besides the association of *Mycoplasma bovis* with *Mannheimia haemolytica* and BRSV, *Mycoplasma bovis* detection was also associated with a higher risk for the detection of *Pasteurella multocida* (OR 2.80, $CI_{95\%}$ 1.35-6.17), and *Histophilus somni* (OR 2.96, $CI_{95\%}$ 1.46-5.96). A seasonal effect was shown for BRSV (OR 4.85, $CI9_{5\%}$ 2.09-12.70) and *Mannheimia haemolytica* (OR 3.09, $CI_{95\%}$ 1.54-6.37) isolation, with a higher prevalence in winter and spring compared to summer and autumn. Sample type was only observed to be associated with the isolation rate of *Mannheimia haemolytica, with a* more frequent isolation from broncho-alveolar lavage fluid than deep nasopharyngeal swabs (OR 7.9, $CI_{95\%}$ 1.29-1.53).

Conclusion: The use of PCR as a diagnostic tool during BRD outbreaks is very valuable since in more than 75% of cases an etiological diagnosis could be established. BRSV and BCoV are the most frequently involved viral pathogens and acted predominantly as single viral agents. We demonstrated a seasonal influence on the occurrence of BRSV and

Mannheimia haemolytica with higher risk of disease in winter and spring. Finally, we found multiple interactions between pathogens responsible for BRD outbreaks in calves. This observation could be useful for the implementation of specific combined preventive measures at the farm level.

Keywords: Bovine respiratory disease, calves, diagnosis, PCR.

ID-11

Genomics-based epidemiology and antimicrobial susceptibility of *Mycoplasma bovis* isolates from veal, dairy and beef herds

Jade Bokma[1], Nick Vereecke[2], Linde Gille[3], Laura Van Driessche[1], Koen De Bleecker[4], Hans Nauwynck[2], Freddy Haesebrouck[5], Sebastiaan Theuns[2], Filip Boyen[5], Bart Pardon[1].

[1]Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium; [2]Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium; [3]Clinical Department of Production Animals, Faculty of Veterinary Medicine, University of Liege, Liege, Belgium; [4]DGZ (Animal Health Service-Flanders), Torhout, Belgium; [5]Department of Pathology, Bacteriology and Avian diseases, Faculty of Veterinary Medicine, Merelbeke, Belgium.

Objectives: *Mycoplasma bovis* associated pneumonia is feared around the world, in particular because of its poor response to antimicrobial therapy. A rise in antimicrobial resistance of *M. bovis* is reported worldwide over the last two decades. Whether different, more resistant strains exist in industries such as the veal industry (more intensive antimicrobial use) compared to conventional dairy and beef farms, is currently unknown. Therefore, the objectives of this study were to compare strains originating from the veal, dairy and beef industry both on their genetic relatedness and antimicrobial susceptibility.

Materials and methods: MIC-values were determined for macrolides, tetracyclines, florfenicol, gentamicin, enrofloxacin and tiamulin with microbroth dilution on 144 epidemiologically independent Belgian *M. bovis* isolates (31 dairy, 70 beef, 11 dairy-beef mixed and 32 veal farms), mostly obtained from the respiratory tract. For a selection of 100 of these isolates (29 dairy, 41 beef, and 30 veal), the whole genome was sequenced by MinION Nanopore sequencing. The reference strain *M. bovis* PG45 was used as quality control in all experiments. Antimicrobial susceptibility data were analyzed using the epidemiological cut-off estimated by the visual eye-ball method to distinguish between wild type (WT) and non-wild type (nWT). Single Nucleotide Polymorphism (SNP) analysis was performed to type *M. bovis* strains, but also to pinpoint specific genetic markers in targeted genes, which were shown to associate with the observed phenotypic susceptibility results. Binary logistic regression (0: WT; 1: nWT) was performed on different sectors to compare antimicrobial susceptibility between sectors. In addition, a phylogenetic tree was developed using CSI Phylogeny (Center for Genomic Epidemiology) for SNP calling on consensus sequences to compare strains between sectors.

Results: Highest MIC-values were observed for macrolides, where almost all strains showed acquired resistance against 16-membered macrolides (tilmicosin and tylosin), and about 50% against the 15-membered macrolide, gamithromycin. A limited number of isolates showed acquired resistance against gentamicin, florfenicol, enrofloxacin and tiamulin. Almost all strains belonged to the wild type population for the tetracyclines (oxytetracycline and doxycycline). A remarkable difference between sectors was observed for gamithromycin, showing that beef herds (59% nWT) had a three times higher odds (95%CI: 1.23-7.35) for gamithromycin resistant *M. bovis* than dairy herds (32% nWT)($P = 0.02$), whereas veal herds did not significantly differ from both sectors (47% nWT). The phylogenetic tree showed different clusters, although strains could not be associated with certain sectors. Specific genetic markers could be linked to acquired resistance for most strains.

Conclusions: This study shows that acquired resistance in Belgian *M. bovis* isolates is highest against macrolides, and minimal for tetracyclines. Secondly, no clear difference in acquired resistance (with the exception of gamithromycin) or strains between sectors were observed. This information could contribute to recommendations on antimicrobial therapy in case of *M. bovis* outbreaks and to further understanding of the epidemiology of this pathogen.

Keywords: Belgium, epidemiological cutoff, gamithromycin, tetracyclines.

ID-12

Intranasal Bacterial Therapeutics Reduce Colonization by the Respiratory Pathogen *Mannheimia haemolytica* in Dairy Calves

Edouard Timsit[1], Samat Amat[2], Trevor Alexander[3].

[1]University of Calgary, Calgary, Canada; [2]University of Alberta, Edmonton, Canada; [3]Agriculture and Agrifood Canada, Calgary, Canada.

Background/Objectives: Six *Lactobacillus* strains originating from the nasopharyngeal microbiota of cattle were previously characterized *in vitro* and identified as candidate bacterial therapeutics (BTs) for mitigating the bovine respiratory pathogen *Mannheimia haemolytica*. In the present study, these BT strains were evaluated for their potential to (i) reduce nasal colonization by *M. haemolytica,* (ii) modulate the nasal microbiota, and (iii) stimulate an immune response in calves experimentally challenged with *M. haemolytica.*

Materials and Methods: Twenty-four Holstein bull calves (1 to 3 weeks old) received either an intra-nasal BT cocktail containing 6 *Lactobacillus* strains (3 x 109 CFU per strain; BT + Mh group) 24 h prior to intranasal *M. haemolytica* challenge (3 x 108 CFU) or no BTs prior to challenge (Mh, control group). Nasal swab, blood, and transtracheal aspiration samples were collected over the course of 16 days after BT inoculation. Counts of *M. haemolytica* were determined by culturing, and

the nasal and tracheal microbiotas were evaluated using 16S rRNA gene sequencing. Serum cytokines (interleukin-6 [IL-6], IL-8, and IL-10) were quantified by enzyme-linked immunosorbent assay (ELISA).

Results: Administration of BT reduced nasal colonization by *M. haemolytica* (*P* = 0.02), modified the composition and diversity of the nasal microbiota, and altered interbacterial relationships among the 10 most relatively abundant genera. The BT + Mh calves also had a lower relative abundance of *Mannheimia* in the trachea (*P* <0.01) but similar cytokine levels as Mh calves.

Conclusion: This study demonstrated that intranasal BTs developed from the bovine nasopharyngeal *Lactobacillus* spp. were effective in reducing nasal colonization by *M. haemolytica* in dairy calves.

Keywords: Bovine respiratory disease, Lactobacillus spp., respiratory microbiota, dairy calves, probiotics.

ID-13

Prevalence of different pathogens of bovine respiratory disease (BRD) in feedlot cattle in Spain

José María San Miguel Ayanz[1], Michela Re[2], Francisco Javier García Peña[3], Alfredo Suarez-Inclán[1], Eugenia Peralta[1], Nacho Peón[1], Javier Blanco Murcia[2].

[1]Zoetis Spain S.L.U., Madrid, Spain; [2]Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, University Complutense, Madrid, Madrid, Spain; [3]GEMAS (Grupo de Estudio de Medicina para la conservación de Animales Salvajes), Madrid, Spain.

Objectives: Bovine respiratory disease (BRD) is a common endemic disease among Spanish feedlot cattle. BRD is the major cause of morbidity and mortality in feedlot cattle, resulting in significant economic losses due to treatment costs as well as reduced feed efficiency and animal product quality. Multiple factors such as the feedlot environment, co-infection with viruses, and stress related to transportation and mixing of cattle may contribute to the development of BRD. The disease complex is caused by one or more primary pathogens, including respiratory viruses and *Mycoplasma* spp., commonly complicated by a secondary bacterial infection, or by bacteria alone. Important bacteria associated with bronchopneumonia in feedlot cattle include *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*, *Trueperella pyogenes* and *Mycoplasma bovis.*

The studies on the prevalence of different pathogens in Spain are very few and there is no study that covers the prevalence of virus and bacteria and that focuses on the entire Spanish territory so far. The aim of the current study was to obtain real data on the prevalence of viral and bacterial pathogens in feedlot cattle in Spain in the two main production management systems, suckling calves and yearlings calves.

Materials and methods: The study was carried out in Spain in 2017. We collected samples from 58 feedlots to determine the prevalence of the major viral and bacterial pathogens. Bovine Herpesvirus type 1 (BoHV-1), Bovine Viral Diarrhea Virus (BVDV), Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza Virus type 3 (PI-3) and the bacterial pathogens *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Trueperella pyogenes* and *Mycoplasma bovis* are considered to be major primary and secondary pathogens in BRD.

The typical size of batches of cattle entering Spanish feedlots ranges between 80 and 120 animals. Serum samples were collected from at least 10 randomly selected animals at 30-40 days on feed (DOF), representing approximately 10% of the animals in a batch. Serum samples were collected from 58 different feedlots: 22 units feeding suckled calves and 36 units feeding yearling calves with an annual production of 270.000 calves, representing around 17 % of the total feedlot production in Spain. The feedlots were located in the main cattle feedlot regions of Spain: Catalonia, Andalusia, Castilla la Mancha, Castilla León and Galicia. A total of 723 serum samples were collected. Refrigerated serum samples were sent to a Spanish commercial laboratory for assay of viral and *M. bovis* antibodies using ELISA kits.

Deep nasopharyngeal swabs or trans-traqueal aspirations samples were collected for bacteriology from 21 feedlots from calves requiring BRD treatment during the feeding period. A total of 188 samples were analyzed in the laboratory. Upon arrival, the swabs were suspended and seeded onto a range of plates containing suitable culture media. The bacterial colonies were identified according to morphology, Gram staining, and biochemical and growth characteristics. Identification of *M. bovis*, *M. haemolytica* and *P. multocida* was confirmed by polymerase chain reaction test.

Results: The results obtained revealed the presence of *M. bovis*, *M. haemolytica* and *P. multocida* in BRD cases in 100 %, 53 % and 88 % of the feedlots respectively. The culture individual prevalence in the positive feedlots was 77 % for *M. bovis*, 42 % for *P. multocida*, 25 % for *M. haemolytica* and 0 % for *T. pyogenes* and *H. somni.*

A high seroprevalence for BoHV-1, BVDV, BRSV, PI-3 virus and *M. bovis* was found in both suckling and yearling calves feedlots. The seroprevalence for suckling calves feedlots was 100 %, 91 %, 82 %, 77 % and 91 % for BoHV-1, BVDV, BRSV, PI-3 virus and *M. bovis* respectively. The seroprevalence for yearling calves feedlots was 82 %, 94 %, 94 %, 97 % and 100 % for BoHV-1, BVDV, BRSV, PI-3 virus and *M. bovis* respectively. A high individual seroprevalence ranging from 30 to 70 % was also found at 30-40 DOF.

Conclusions: The prevalence of the major BRD pathogens in Spanish feedlots is high in both types of feedlot production units. The data indicate that contagion of the pathogen is rapid in Spanish feedlots. The results underscore the importance of selecting an antimicrobial which has proven efficacy against *Mycoplasma bovis* and the importance of design strong vaccination protocols and management practices to reduce the impact of viral pathogens.

Keywords: Bovine respiratory disease, prevalence, feedlot cattle.

ID-14

Prevalence of different pathogens of bovine respiratory disease (BRD) in dairy heifers in Spain

José María San Miguel Ayanz[1], Javier Blanco Murcia[2], Francisco Javier García Peña[3], Alfredo Suarez-Inclán[1], Eugenia Peralta[1], Nacho Peón[1], Michela Re[2].

[1]Zoetis Spain S.L.U., Madrid, Spain; [2]Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, University Complutense, Madrid, Madrid, Spain; [3]GEMAS (Grupo de Estudio de Medicina para la conservación de Animales Salvajes), Madrid, Spain.

Objectives: Bovine respiratory disease (BRD) is a common endemic disease among Spanish dairy farms, impacting mainly the young animals. BRD is the major cause of morbidity and mortality in dairy heifers, resulting in significant economic losses due to treatment costs as well as the appearance of chronic animals with a reduced productive life or with a delay in reaching the breeding time. Multiple factors such as the dairy farm environment, co-infection with viruses, and stress related to regrouping of heifers may contribute to the development of BRD. The disease complex is caused by one or more primary pathogens, including respiratory viruses and *Mycoplasma* spp., commonly complicated by a secondary bacterial infection, or by bacteria alone. Important bacteria associated with bronchopneumonia in dairy heifers include *Mannheimia haemolytica*, *Pasteurella multocida, Histophilus somni*, *Trueperella pyogenes* and *Mycoplasma bovis*. The studies on the prevalence of different pathogens in Spain are very few and there is no study that covers the prevalence of virus and bacteria and that focuses on the entire Spanish territory so far. The aim of the current study was to obtain real data on the prevalence of viral and bacterial pathogens in dairy heifers in Spain.

Materials and methods: The study was carried out in Spain in 2017. We collected samples from 50 dairy farms to determine the prevalence of the major viral and bacterial pathogens. Bovine Herpesvirus type 1 (BoHV-1), Bovine Viral Diarrhea Virus (BVDV), Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza Virus type 3 (PI-3) and the bacterial pathogens *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Trueperella pyogenes* and *Mycoplasma bovis* are considered to be major primary and secondary pathogens in bovine respiratory disease (BRD).

Serum samples were collected from at least 10 randomly selected animals at 30-40 days after a BRD outbreak. Serum samples were collected from 50 different dairy farms with an heifer census of 33.250 dairy heifers, representing around 7 % of the total Spanish dairy heifers. The dairy farms were located in the main dairy production regions of Spain: Galicia, Asturias, Cantabria, Catalonia, Castilla León, Navarra, Valencia, Castilla la Mancha and Andalusia. A total of 540 serum samples were collected. Refrigerated serum samples were sent to a Spanish commercial laboratory (Eurofins) for assay of viral and *M. bovis* antibodies using ELISA kits.

Deep nasopharyngeal swabs or trans-traqueal aspirations samples were collected for bacteriology from 37 dairy farms from dairy calves requiring BRD treatment during the weening period. A total of 316 samples were transported to the laboratory in transport media containing activated charcoal. Upon arrival, the swabs were suspended and seeded onto a range of plates containing suitable culture media. The bacterial colonies were identified according to morphology, Gram staining, and biochemical and growth characteristics. Identification of *M. bovis*, *M. haemolytica* and *P. multocida* was confirmed by polymerase chain reaction (PCR) test.

Results: The results obtained revealed the presence of *M. bovis*, *M. haemolytica*, *P. multocida* and *T. pyogenes* in BRD cases in 75 %, 11 %, 24 % and 16 % of the dairy farms respectively. The culture individual prevalence in the positive dairy farms was 41 % for *M. bovis*, 16 % for *P. multocida*, 21 % for *M. haemolytica,* 45 % for *T. pyogenes* and 0 % for *H.somni.*

A high seroprevalence for BoHV-1, BVDV, BRSV, PI-3 virus and *M. bovis* was found in dairy heifers. The seroprevalence for dairy farms was 21 %, 32 %, 41 %, 44 % and 30 % for BoHV-1, BVDV, BRSV, PI-3 virus and *M. bovis* respectively. A high individual seroprevalence ranging from 45 to 88 % was also found 30-40 days after the BRD outbreaks.

Conclusions: The prevalence of the major BRD pathogens in Spanish dairy farms is high. The data indicate that contagion of the pathogen is rapid in Spanish dairy farms. The results underscore the importance of selecting an antimicrobial which has proven efficacy against *Mycoplasma bovis* and the importance of design strong vaccination protocols and management practices to reduce the impact of viral pathogens in dairy heifers in Spain.

Keywords: Bovine respiratory disease, prevalence, dairy heifers.

ID-15

Use of quantitative serology as a new practical tool for veterinarians to follow up BVD status on vaccinated farms

Carlos Carbonell[1], Rita Aida Ambroggio[2], Rebeca Conde[2], Marcelino Álvarez[3], Laura Elvira[4].

[1]MSD Animal Health Spain, Salamanca, Spain; [2]Livestock Sanitary Group ADSG Mazaricos, Galicia, Spain; [3]University of Leon, León, Spain; [4]MSD Animal Health, Spain, Salamanca, Spain.

Objectives: The bovine viral diarrhea (BVD) virus causes significant economic losses in cattle farms. Indeed, various EU countries have done voluntary or mandatory eradication plans. Different schemes have three central elements: biosecurity, elimination of persistently infected animals and herd monitoring. In addition, many programs include systematic vaccination against BVD as an additional biosecurity measure (EU Thematic network BVDV, 2001; Moening et al., 2007).

For monitoring, the detection of antibodies continues to be the fastest, most practical and economical method to assess exposure to BVD (González et al., 2014). Therefore, some countries and regional programs, such as the ADSG in Galicia, base their control programs on ELISA tests that are carried out periodically on youngstock serum and bulk tank.

A possible concern regarding vaccination is the interference of the vaccine response with serological monitoring of

the herd. However, previous studies in a reduced number of farms have shown how the combination of sampling age (6-18 months old) and quantitative values of BVD p80 antibody ELISA, could be a useful tool to determine the BVD status of a herd, even if vaccinated with inactivated vaccines (Carbonell et al., 2019; Eze et al.,2019).

The main objective of this study was to analyze in many farms the practical use of quantitative BVD p80 antibody ELISA to differentiate between field virus circulation and BVD vaccination.

Materials and methods: Serum samples from 50 farms included in the Voluntary ADSG Control Program of Galicia were analyzed in 2019. The farms were classified based in their BVD status into three types: RC, recent circulation of BVD (5), BOV, vaccinated with an inactivated BVD vaccine (Bovilis® BVD) (40), LV, vaccinated with a live BVD vaccine (5). In the vaccinated farms (BOV and LV) no persistently infected animals had been detected in the previous two years.

All samples were analyzed in the Animal Health and Production Laboratory of Galicia by ELISA BVD antibody (IDEXX, BVDV p80 Ab Test). For this quantitative study, the results were expressed as Inhibition Percentage of the optical density (IP-OD). The ELISA results were categorized according to the manufacturer's instructions as: negative (>50), doubtful (40-50) or positive (<40). All positive samples (OD <40) were further divided over 4 groups (<10; 10-20; 20-30 ;30-40). Furthermore, the lower the PI, the higher the titer of antibodies present in the sample. For the statistical processing a descriptive analysis was performed using Excel 2016.

Results: A total of 976 serum samples were included in the analysis (101 RC, 780 BOV and 95 LV).

21.65, 70.5 and 23.66% of the samples were seronegative while 70.1, 8.9 and 48.4% were positive to ELISA with IP-OD <10 in RC, BOV and LV group, respectively. Moreover, the average PI value of positive samples was 6.5±4.8, 21.9±14.1 and 11.3±8.2 in RC, BOV and LV group, respectively.

A more detailed analysis by segmentation into four age categories (6-12m, 13-18m, 19-24m and > 25m) reveled two clear patterns: In the BOV group, the percentage of seronegative samples was even higher in animals 6-18 months old (82.9% in both); while the percentage of samples IP-OD <10 was very low (4.9%, 2.2%, 12.4% and 16.1%, respectively in all four age groups). However, in the RC and LV group the percentage of seronegative samples was much lower (40.0 -20.0% 6-12m and 31.3-20.8% 13-18m, respectively); while the percentage of positive ELISA IP-OD <10 was very high (60.0-60.0%, 86.7-50.0%; 50.0-56.7% and 73.2-38.2% for RC and LV per group, respectively).

Moreover, if we analyze the distribution of positive results based on the IP-OD result of ELISA in a boxplot, the first, second and third quartile of the BOV group (9.5,21.0,34.0) represents a different pattern to the RC group (4.0,5.0,7.0) and LV group (6.0,8.0,12.8); showing patterns that easily allows the differentiate the positives due to vaccination with the inactivated vaccine (BOV) vs virus circulation (RC). However, this serology is not useful to monitor LV farms as their patterns are difficult to differentiate to RC ones.

Conclusions: The use of quantitative IP-OD ELISA values, using IDEXX p80 antibodies ELISA test, seems to offer a useful tool to assess BVD status in farms vaccinated with inactivated vaccines. Moreover, this interpretation adds value to the technical advisor at farm level by improving their monitoring system.

Keywords: BVD, serology, quantitative, vaccine, monitoring.

ID-16

Pasteurella multocida, Mannheimia haemolytica and Bovine Corona Virus are the most frequently detected respiratory pathogens from bronchoalveolar lavages in Dutch dairy BRD calves

Henk Kuijk, Pleun Penterman, Geert Vertenten.

MSD Animal Health, Boxmeer, Netherlands.

Objectives: Bovine respiratory disease (BRD) is a common cause of morbidity and mortality in dairy calves, which has long-term consequences including decreased milk production, poor reproductive performance, and poor growth and longevity. Although management practices such as commingling and group housing increase BRD risk, viral and bacterial pathogens cause the lesions characteristic of BRD. Monitoring and testing for pathogens associated with BRD may facilitate the development of appropriate, targeted vaccination pro-grams.

From November 2019 until the end of December 2021, MSD Animal Health performed several diagnoses of respiratory pathogens by PCR on bronchoalveolar lavages (BALs) on Dutch dairy farms vaccinating against *Mannheimia haemolytica*, Bovine Respiratory Syncytial Virus (BRSV) and Parainfluenza 3 virus (PI3).

This manuscript gives an overview of the first results of the BALs performed on those Dutch dairy farms.

Materials & Methods: From several calves suffering from BRD on dairy farms vaccinating against BRD pathogens in the Netherlands, a BAL sample was taken as previously described (Van Driessche et al. 2016). Samples were transported at ambient temperature and analyzed at the Veterinary Laboratory Gelderland (Epe, The Netherlands) by PCR for *Pasteurella multocida*, *Mannheimia haemolytica*, Bovine Corona Virus, *Mycoplasma bovis*, *Histophilus somni*, BRSV and PI3.

Results: During the defined timeframe, 194 BALs from calves between 2 weeks and 6 months old were performed on 80 dairy farms from which 78,3%, 25,7%, 21,6% 13,4%, 12,3%, 10,3% and 1,5% of the samples were positive for respectively *Pasteurella multocida*, *Mannheimia haemolytica*, Bovine Corona Virus, *Mycoplasma bovis*, *Histophilus somni*, BRSV and PI3. This is in line with similar studies from Belgium (n=3234) (Griepbarometer DGZ, https://www.dgz.be/rundvee/gezondheidszorg/bioveiligheid-en-preventie/monitoring-en-vaccinatie/griepbarometer, accessed at Jan 12th 2022) showing the following prevalences for respectively *Pasteurella multocida*, *Mannheimia haemolytica*, Bovine Corona Virus, *Mycoplasma bovis*, *Histophilus somni*, BRSV and PI3 : 74,0%,

35,8%, 19,8%, 27,7%, 25,7%,21,1% and 5,5%. Both studies show that the most prevalent bacterial pathogens are *Pasteurella multocida* and *Mannheimia haemolytica*, while the most prevalent viral pathogen is clearly Bovine Corona Virus.

Finally, we compared the results of this PCR study with the seroprevalences of respiratory samples from BRD calves on non-vaccinating dairy farms (Kuijk et al., 2022). The most remarkable finding from this comparison was on PI3: only 1,5% of the BALs in this study (BRD calves on vaccinating farms) were positive for PI3 specific RNA where 66,6% of the animals in the serology study (BRD calves on non-vaccinating farms) were positive for PI3 antibodies. This demonstrates that many animals get in contact with PI3 field virus early in life or are still seropositive by the presence of maternal antibodies. But on the other hand, it is hard to detect PI3 BALs from BRD calves.

Conclusion: From this study, it can be concluded that *Pasteurella multocida, Mannheimia haemolytica* and Bovine Corona Virus are the most frequently detected respiratory pathogens from dairy calves suffering from Bovine Respiratory Disease.

Keywords: BRD, dairy calves, Pasteurella multocida, Mannheimia haemolytica, Bovine Corona Virus.

ID-17

Prevalence of respiratory pathogens on Danish cattle farms

Katja Mikkelsen[1], Liff Nielsen[1], Geert Vertenten[2].

[1]MSD Animal Health, Copenhagen, Denmark; [2]MSD Animal Health, Boxmeer, Netherlands.

Objectives: Bovine Respiratory Disease (BRD) is a costly and multifactorial disease of young and growing cattle. The factors that predispose to BRD include stress related to overstocking, moving or mixing cattle, poor ventilation or draughts, sudden climatic changes, mixing of various age groups, nutritional deficiencies, inadequate colostrum intake, and poor feed hygiene. These stress situations facilitate infection by primary viral pathogens which cause lung damage. The viral damage to the lungs may pave the way for various bacterial pathogens such as *Mannheimia haemolytica* (Mh)*, Pasteurella multocida* (PM) and *Mycoplasma bovis* (MB).

Vaccination is an important tool in managing BRD. Identification of major respiratory pathogens on farms with BRD problems can offer valuable insights helping establish an appropriate vaccination program.

In 2019 and 2020 MSD Animal Health used a testing approach BRD QuickScan to evaluate exposure to BRD pathogens present on several Danish farms. The BRD QuickScan involves detection of antibodies against Mh*,* Bovine Respiratory Syncytial Virus (BRSV), Bovine Corona Virus (BCV), Parainfluenza 3 virus (PI3V), MB and PM. The results of the BRD QuickScan and situation analysis was then used to provide the farms with a tailor-made advice including BRD vaccination and improvement of management factors predisposing to BRD.

This manuscript gives an overview of the prevalence of respiratory pathogens in Danish dairy farms and calf rearing operations with BRD problems.

Materials & Methods: The tested farms were selected based on the herd veterinarian defining them as having a BRD problem. On the selected farms, serum samples were taken from around 10 calves > 2,5 months old. The samples were subjected to the BRD QuickScan procedure in the Centre for Diagnostic Solutions (MSD Animal Health, Netherlands). An in-house ELISA test was used to measure Mh*,* PM*,* BCV and BRSV antibodies, whereas for PI3V and MB commercial ELISA kits were used (IDEXX and Bio-X respectively). The test for antibodies against BCV was only performed on samples from June 2020 onwards.

Results: In total, 51 BRD QuickScans were performed: 38 on samples from dairy farms (371 calves) and 13 from calf rearing operations (134 calves) meeting the same farm selection and calf-age criteria. At the farm level, 100% of the tested farms had samples positive for Mh and PI3V antibodies, whereas 94%, 73% and 40% were positive for PM*,* MB and BRSV respectively. All farms (100%) included in testing for BCV antibodies had positive samples.

At the calf level, no presence of antibodies against any of the pathogens tested was detected only in 1% of calves. Samples from 98% of calves were positive for antibodies against Mh*,* but only 3% of samples were positive for Mh antibodies only. Simultaneous presence of antibodies against Mh and viral BRD pathogens (BRSV and/or PI3V) was detected in 82% of samples. None of the samples had a mixed Mh - MB antibody presence.

Eighty-three percent of the samples were positive for antibodies against PI3V and/or BRSV. One percent were only positive for PI3V antibodies and none of the calves were positive for only BRSV antibodies. These findings confirm the importance of the viral pathogens in the BRD complex, mainly in combination with Mh.

Conclusion: All farms tested had calves positive for Mh and PI3V antibodies, and all farms tested for BCV antibodies had positive samples. At the calf level, the most frequently detected antibodies were those detected against Mh with a prevalence of 98% followed by PI3V with a prevalence of 82% of all calves tested with the BRD QuickScan. The results suggest that the pathogens circulating on Danish BRD problem farms were in most cases (82%) co-infections of bacteria and viruses, while Mh was involved in all cases.

The findings demonstrate that the BRD QuickScan can be a valuable tool supporting the vet and the farmer in the decision-making process around BRD control and prevention. For most pathogens circulating on farms with BRD problems, vaccines are commercially available and therefore an insight into the pathogens the animals are exposed to on a particular farm should help motivate the farmers to adopt prophylactic vaccination. Moreover, knowledge of the BRD pathogen landscape and specific farm risk factors should help the vets develop a tailor-made prophylactic vaccination programme for each farm.

Keywords: Bovine respiratory complex, pathogens, prevalence.

ID-18

Risk factors for BVDV introduction into Dutch dairy herds in a national control programme

Emma Strous, Irene Bisschop, Jet Mars, Linda Van Duijn, Frederik Waldeck, Paul Wever, Gerdien Van Schaik.

Royal GD, Deventer, Netherlands.

Objectives: After many years of voluntary control and decreasing prevalence, a national control programme for bovine viral diarrhoea virus (BVDV) is in place for Dutch dairy herds since 2018. Introduction of BVDV is minimized by regulations with respect to purchase of cattle from herds with a lower BVDV status. However, BVDV was introduced in several tens of herds in 2019-2021. The aim of the study was to determine risk factors for introduction of BVDV in the context of the national control programme.

Material & Methods: In a case-control design, herds with a confirmed BVDV introduction (cases) were compared with control herds that were located near the case herd, which remained free of BVDV. Both case and control herds were visited by a veterinarian and an extensive questionnaire was applied about the possible risk factors for BVDV introduction in the previous two years. In total, 149 cases and 148 controls were visited. Logistic regression analysis was carried out to determine the significant risk factors (P<0.05).

Results: The final multivariable model consisted of seven risk factors. Purchasing cattle from non-free herds (OR=1.25) and cattle from other herds that escaped and mingled with cattle from the own herd (OR=1.16) were risk factors related to direct animal contact. Risk factors for indirect external contact were: distance of less than 500m to beef cattle herds (OR=1.15), a permanent employee (OR=1.17) and the farmer working outside the farm in other cattle herds (OR=1.25). Risk factors that seemed more related to internal biosecurity were: housing of adult cattle and calves in the same barn (OR=1.22) and use of a group pen for calving (OR=1.16).

Conclusion: In conclusion, the risk factors for introduction of BVDV in free herds varied considerable between herds and had fairly low odds, indicating that there were many smaller biosecurity risks that need to be mitigated.

Keywords: BVDV, biosecurity, control programme.

ID-19

Voluntary control program against *Mycobacterium avium* subsp. *paratuberculosis* in cattle farms from the Livestock Health Defence Group Costa da Morte in Galicia (NW Spain)

Eduardo Yus[1], Francisco Javier Villaamil[2], Ignacio Arnaiz[3], Francisco Javier Diéguez[1].

[1]Facultad de Veterinaria, Lugo, Spain; [2]ADSG Coste da Morte, A Coruña, Spain; [3]Laboratorio de Sanidad y Producción Animal, A Coruña, Spain.

Objectives: Galicia is the main dairy cattle of the country, with 55% of the farms and 38% of the milk production. The mean herd size per farm is 43 cows. In 2004, a voluntary control program against *Mycobacterium avium* subsp. *paratuberculosis* (Map), causal agent of bovine paratuberculosis, was implemented in Galicia (NW Spain). 8,664 farms are currently integrated in the program, representing 26.2% of the herds (50.2% of the animals).

In the NW of Galicia, the Livestock Health Defence Group (LHDG) Costa da Morte, had 243 herds integrated in the voluntary control program (cattle and dairy farms) ranging from 539 to 5 animals (mean herd size of 68 cows).

The aim was to present the evolution of the program in LHDG Costa da Morte according to the laboratory results between 2014 and 2019.

Materials and methods: Blood samples are taken annually to animals older than 2 years for the 243 herds involved in the program, according to the following schedule:

- In Map infected herds, all cows over two years during the next years to elimination of fecal positive animals.
- 40% of animals over two years in herds not confirmed as infected.

The serums are analyzed for anti-Map antibodies with commercial ELISA and fecal samples of all ELISA-positive samples are analyzed by PCR or bacterial culture. Fecal positive animals must be sacrificed.

Additionally, all purchased animals were analyzed by ELISA. Positive ELISA or PCR animals must not be incorporated into farms.

Information of biosecurity and supervision of implementation of management measures to reduce fecal-oral contamination is also recorded in the program.

Results: The seropositive herds in LHDG studied (at least with one animal with anti-Map antibodies) varied between a maximum of 15.3% in 2015 and a minimum of 9.6% in 2018. These results are better than those obtained in all LHDG of Galicia with a maximum of 22% in 2015 and a minimum of 17.7% in 2017.

At animal level, the seropositivity varied between a maximum of 1.8% in 2014 (15.2% were positive-PCR/bacterial culture) and a minimum of 1% in 2018 (2.9% were positive-PCR/bacterial culture). In all LHDG of Galicia, it was observed a maximum of 2.8% in 2014 (20.8% were positive-PCR/bacterial culture) and minimum of 1.8% in 2017 (13.5% were positive-PCR/bacterial culture).

The percentage of seropositive in purchased animals did not exceed 2.8% any year, with a minimum of 0% in 2018 (never above 1.7% with a minimum of 0.7% in 2017 in all LHDG).

Only about 26% of the farms purchased cattle (33.7% in the whole region) and most farmers do not request information on the overall status of the origin farms.

Conclusions: Control programs are having an impact on the sanitary status. Collecting data is an important first step to identification of biosecurity shortcomings; however, apart from the control of purchased animals (mandatory), programs do not seem to have significant influence on the application of many other measures concerning biosecurity.

Acknowledgements: Animal Health and Production Lab-

oratory of Galicia, Xunta of Galicia

Keywords: Mycobacterium avium susp. paratuberculosis, cattle farms, control program, Spain.

ID-20

Comparison of different sampling sites and techniques for the detection of *Mycobacterium avium* subsp. *paratuberculosis* in environmental fecal samples in paratuberculosis positive cattle herds

Johannes Lorenz Khol[1], Martina Mattes[1], Michael Dünser[2], Waler Baumgartner[1].

[1]University Clinic for Ruminants, University of Veterinary Medicine Vienna, Vienna, Austria; [2]Austrian Agency for Health and Food Safety, Linz, Austria.

Objectives: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is causing paratuberculosis (Johne´s disease, JD) in cattle and is known to survive for an extended period of time in the environment. The objective of this study was, to evaluate in which areas within a barn MAP can be detected in positive cattle farms and to compare different sampling sites. Thereby, potential areas for MAP transmission, as well as the most promising places for the detection of MAP in positive cattle premises, should be identified.

Material and Methods: During the study, 14 Austrian dairy and beef operations were tested for the presence of MAP twice in a six months interval. In each farm at least one confirmed clinical case of JD was detected within a year prior to the study and the herd was therefore considered to be MAP positive.

On every farm, 7-10 paired environmental fecal samples from specific sites were taken. Sampling locations depended on the type of operation and included the calving area, alleyways, equipment, milking parlor and manure storage sites. Fecal samples were tested for MAP both by bacteriological culture on Herrold´s Egg Yolk Medium and real time PCR for IS*900* (Adiagene, Saint Brieuc, France), at the Austrian National Reference Laboratory for paratuberculosis.

Results: All farms enrolled in the study had at least one positive environmental fecal sample, confirming the classification as MAP positive. Fecal samples collected from the slurry pit, the alleyways in the feeding area as well as the manure channels (tie stall barns) proved to be most likely MAP positive. Altogether, 42.3% of the samples from the slurry pit were positive by culture and 51.9% by PCR, samples from the alleyways from the feeding area showed 44.4% and 30.0% positive results, and in the manure channel 87.5% of the collected samples were MAP positive by culture an 50.0% by PCR, respectively. The sensitivity of the samples could be increased significantly by collecting two samples from each site and reached 100% at the herd level, when several sample sites were combined within a farm.

Conclusions: The results of the present study indicate, that manure storage sites, as well as the highly frequented alleyways in a barn seem to be the most promising sites for the detection of MAP by environmental fecal samples. Based on these results, the use of environmental fecal samples seems to be a useful tool to assess the MAP herd level in cattle.

Keywords: Paratuberculosis, Johne´s disease, Mycobacterium avium subsp. paratuberculosis, environmental fecal sampling.

ID-21

Examples of and lessons learned from regional control programs for the abatement of *Mycobacterium avium* subsp. *paratuberculosis* in cattle

Johannes Lorenz Khol[1], Susanne Eisenberg[2], Irene Noll[3], Michael Zschöck[4], Tobias Eisenberg[4], Karsten Donat[5].

[1]University Clinic for Ruminants, University of Veterinary Medicine Vienna, Vienna, Austria; [2]Animal Disease Fund Lower Saxony, Hannover, Germany; [3]District Government of Giessen, Giessen, Germany; [4]Hessian State Laboratory (LHL), Dept. of Veterinary Medicine, Giessen, Germany; [5]Thuringian Animal Disease Fund, Institution by Law and 6Clinic for Obstetrics, Gynecology and Andrology with Veterinary Ambulance, Justus-Liebig-University Giessen, Jena, Germany.

Objectives: Paratuberculosis (Johne´s disease, JD) is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and may lead to substantial economic losses in infected cattle herds. Detection and control of the infection is challenging, therefore examples of regional control programs are presented and discussed.

Material, Methods and Results: In course of the program for the reduction of the MAP prevalence in Lower Saxony, Germany, dairy farms are obliged to test bulk milk samples for MAP antibodies, followed by testing of individual animals in seropositive farms. Subsequently, farmers can decide to join the accompanying MAP control program. Within the first year of the program 6,035 bulk tank samples were tested, 13% were MAP-positive and 670 farms joined the MAP control program.

Within the voluntary certification program in Hesse, Germany, the MAP herd status is evaluated using boot swab sampling (PCR and culture). In positive farms, animals are tested by individual milk or blood ELISA-serology. Until now, 100 farms participated in the program, of which 60 were MAP negative and 33 positive, respectively (no status assigned in 7 farms). The mean intra herd prevalence decreased from 7.56 % to 4.06% in participating farms.

The program for the abatement of MAP infections in cattle herds in Thuringia, Germany, is based on a yearly fecal examination of adult cattle within a herd. In 2017, fecal samples from 28,941 animals were tested of which 1.8% were MAP positive. Of the 136 participating farms, currently 64 are MAP negative and 72 positive, with 39 of the latter in the last step of the program before achieving a MAP-unsuspected status.

The biennial survey of the MAP herd status by boot swabs (PCR and culture) is the base of the MAP program in Tyrol, Austria. Positive farms may join the MAP control program to have their animals tested by individual fecal sampling. More

than 4,000 boot swab samples were tested in each run with 0.97% positive farms in 2016/17. In these farms 2,151 individual fecal samples were collected of which 2.3% were MAP positive.

Conclusions: The programs presented indicate, that a two-stage approach, with an evaluation of the MAP-herd level, followed by the testing of single animals, is generally well accepted by the stakeholders. Besides financial support, communication and cooperation of all participating parties seems to be crucial for the success of such programs. Furthermore, stigmatization has to be avoided and additional programs for positive farms should be available.

Keywords: Paratuberculosis, Johne´s disease, Mycobacterium avium subsp. paratuberculosis, regional control program.

ID-22

Wild cervids populations as Schmallenberg virus circulation sensors

Calixte Bayrou[1], Rosario Volpe[1], Christophe Lesenfants[1], Julien Paternostre[1], Damien Coupeau[2], Benoît Muylkens[2], Nassim Moula[1], Daniel Desmecht[1], Annick Linden[1].

[1]University of Liège, Liège, Belgium; [2]University of Namur, Namur, Belgium.

Background: The *Schmallenberg virus* (SBV) emerged in 2011 in Europe. The epicenter of this spreading was the region which straddled Germany, the Netherlands and Belgium. The dissemination of the virus is based on an arthropod/ruminant cycle. Midges belonging to the *Culicoides obsoletus complex* have been identified as the main vector population while the majority of tested ruminants supports the infection. After the remarkably fast spreading of the virus across Europe, questions raised about the virus implantation in the conquered lands.

Objectives: The objective of the study was to follow the circulation dynamic of SBV in Wallonia (Belgium) during six years after the emergence (2012-2017). We designed a seroprevalence follow-up of the wild deer populations to answer two main questions: (i) is SBV endemic in Wallonia and, if so, which kind of endemic profile characterizes its implantation? (ii) are wild deer a significant reservoir for the virus?

Material and Methods: The study is based on the annual sampling protocol of the Surveillance Network of Wildlife Diseases (SNWD) of the University of Liège (Belgium). The SNWD takes advantage of the hunting activities in Wallonia to collect, every year, during October, November and December, as many samples as possible to cover the largest number of communities. Blood is collected *post-mortem.* Sampled deer are classified as adults or juveniles (born the year of sampling) and belong to the two main deer species of Wallonia: roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*). All samples were analyzed using the commercial ELISA from IDvet: *ID Screen Schmallenberg virus Competition Multi-species*®. To assess the relative sensitivity (Se) and specificity (Sp) of the ELISA in this context, sero-neutralisation tests (SNT) were carried out on a subset of samples of years 2012, 2013 and 2014.

Results: The study led to the test of 2258 sera: 1140 from roe deer and 1138 from red deer. The geographical distribution of the samples is centered on the Belgian Ardenne. The subset of samples used for the SNT numbered 622 sera. The relative Se of the ELISA in this context was 70% and the relative Sp reached 93%. However, for the 2012 red deer cohort, an unexplained phenomenon dropped the relative Se to 30%. In consequence, the seroprevalence of this group was evaluated by SNT and not included in the statistical analysis.

The profile of the seroprevalence evolution over the six years is similar in the two species. Two years, 2012 and 2016, were characterized by a significantly higher level of circulation (roe deer: 2012 = 44±6% - 2016 = 47±7%; red deer: 2012 = 43±16% SNT-based evaluation – 2016 = 28±7%). Beside these two years, the seroprevalence was low in both species, especially in juveniles for which the seroprevalence did not exceed 6±5%, showing a very limited circulation of the SBV during 2013, 2014, 2015 and 2017. These low circulation years led to a a seroprevalence decrease in the whole population until a floor level close to 10% reached in both species in 2014.

Conclusion: Our study provides evidences that the SBV continues to circulate in Wallonia after 2011. Thus, Wallonia appears as an endemic area characterized by a global hypo-endemic state crossed by endemic pulsations. Such an endemic profile is classically explained by the Susceptible-Infectious-Recovered-Susceptible (SIRS) epidemiological model.

The intense 2016 circulation was observed and reported all across Europe. On the contrary, 2012 circulation is more specific to our study and is due to the specific topography of the Belgian Ardenne that locally slowed down the 2011 expansion of the virus.

The fact that floor level seroprevalence was already reached in 2014 for the two populations suggests that wild deer are not the main reservoir of the virus and have limited impact on the global circulation.

Keywords: Schmallenberg, Seroprevalence, Belgium, Deer, Epidemiology.

ID-23

Herd Environmental Sampling for detection of *Mycobacterium avium* subspecies *paratuberculosis* in Irish pasture-based dairy herds

Niamh Field[1], Conor McAloon[2], John Mee[1].

[1]Teagasc, Fermoy, Republic of Ireland; [2]University College Dublin, Belfield, Republic of Ireland.

Objective: The objective of this study was to determine the herd sensitivity (HSe) and herd specificity (HSp) of Herd Environmental Sampling (HES) for the detection of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in Irish

pasture-based dairy herds and to compare these metrics with those of whole-herd serology and confirmatory faecal PCR.

Materials and methods: In a three-year study (2019-2021), 122 commercial dairy herds were recruited from the Irish Johne's Control Programme (IJCP) https://animalhealthireland.ie/programmes/johnes-disease/irish-johnes-control-programme-ijcp/. Herds were visited once when cows were housed for the winter. Blood samples were taken from all animals over two years old. For the HES, six composite environmental samples were collected from areas of manure concentration such as slurry storage, adult cow housing, the collecting yard for the milking parlour and calving pens. Two herd-level tests for MAP were conducted on these samples: whole-herd serum ELISA (sELISA) with confirmatory faecal PCR (fPCR) of seropositive animals (sELISA + fPCR), and herd environmental fPCR. Blood samples were tested using IDEXX MAP ELISA kit and HES samples were tested by PCR using the Indical Bactotype MAP PCR kit. Individual faecal samples were tested with various different PCR kits. A herd was considered positive on sELISA + fPCR if at least one animal was positive on fPCR; and positive on HES if at least one composite environmental sample was positive on fPCR. The HSe and HSp of HES and sELISA + fPCR were estimated using a two-test, two-population Bayesian latent class model in R.

Results: Complete test results for both test methods were available for 97 herds. Fifteen and five herds were positive on sELISA + fPCR and HES, respectively, with seventeen herds identified as infected on either test; 80 herds were negative on both tests. The median herd level sensitivity (HSe) and specificity (HSp) and 95% credibility intervals for each test were as follows: sELISA + fPCR: HSe 0.57 (0.33-0.85), HSp 0.99 (0.98-1); HES: HSe 0.31 (0.16-0.5, HSp 0.99 (0.99-1).

Conclusions: In this study, the estimated HSe of HES was lower than the current standard method of testing herds for MAP in Ireland (whole herd serology with confirmatory faecal PCR). Further research is required to determine if the test methods used can be optimised to increase the sensitivity of HES in pasture-based dairy herds.

Keywords: Paratuberculosis, diagnostic, sensitivity, environmental, pasture-based.

ID-24

Comparison of different diagnostic strategies at the herd-level for *Mycobacterium avium* subspecies *paratuberculosis* in cattle

Francisco Jurado-Martos[1], Fernando Cardoso-Toset[1], Belén Huerta[2], Carmen Tarradas[2], Inmaculada Luque[3].

[1]1Dpto. de I+D+i, CICAP, 14400., Pozoblanco, Córdoba, Spain; [2]Dpto. Sanidad Animal, Facultad de Veterinaria, Universidad de Córdoba., Córdoba, Spain; [3]Dpto. Sanidad Animal, Facultad de Veterinaria, Universidad de Córdoba., Pozoblanco, Córdoba, Spain.

Objectives: Paratuberculosis (PTB) is a disease that causes significant production losses in cattle and its prevalence is expected to increase in the coming years due to the intensification of livestock systems. Knowing the status of PTB infection at the herd-level through accuracy and economical strategies would improve the control of the disease. Therefore, the objective of this work was to evaluate different diagnostic test to detect *Mycobacterium avium* subspecies *paratuberculosis* (MAP) at the herd-level from a cost-effective point of view.

Material & methods: A total of 26 dairy farms in southern Spain with an average of 107 milking cow were randomly selected for this study. The following samples were taken from each herd: blood serum samples, obtained by sterile BD Vacutainer ® from 20 randomly selected cows with at least one calf (n=520 samples). Also, a sample from the bulk milk tank (BMT, n=26) and accumulated feces (n=26) in the milking area (holding pen) from each dairy farm were obtained.

Both serum and the BTM samples were tested by enzyme-linked immunosorbent assay (ELISA) (IDEXX Laboratories, Westbrook, ME, USA), according to the manufacturer's instructions. The manure and BMT samples were analyzed by real-time polymerase chain reaction (qPCR). DNA extraction was performed following the instructions of the MagMAX™ Core nucleic acid purification kit, with a specific mechanical lysis module for MAP detection (Thermo Fisher scientific, Austin, Texas) and, in an automated manner, with the KingFisher™ mL equipment (Thermo Fisher scientific, Austin, Texas). The qPCR analysis was performed using the vetMAX™ MAP IS900-F57 kit (Thermo Fisher scientific, Austin, Texas).

The ability of each diagnostic test to detect MAP at the herd level was evaluated and compared with the other techniques. The agreement between the diagnostic tests was evaluated by Cohen's Kappa statistic (k) and interpreted as follows: k = 0.00 - 0.20, poor; k = 0.21 - 0.40, fair; k = 0.41 - 0.60, moderate; k = 0.61 - 0.80, good; and k = 0.81 - 1.00, excellent agreement (WinEpi software 2.0, Faculty of Veterinary, University of Zaragoza, Spain).

Results: In reference to individual serum samples, a total of 26 (5.0%) of the 520 serum samples showed MAP-specific antibodies. A herd was considered positive when at least one of the serum samples was seropositive, therefore, 14 herds (53.8%) were considered MAP positive by serology, most of them (64.3%) with only one animal seropositive. However, when analyzing the BMT samples by ELISA, 5 positive and 6 doubtful samples were detected, which were considered positive for herd classification, obtaining 11 seropositive herds (45.8%). When qPCR was used, the presence of MAP was detected in 11 (45.8%) fecal samples, but all BTM samples were negative with this technique.

Except for qPCR in BTM samples, the agreement between all other techniques was considered moderate (K=0.455-0.541). A total of 17 herds (65.4%) presented unanimity in the result of all the techniques studied (8 positive and 9 negative), while in 9 herds (34.6%) discrepancies were observed, standing out 3 herds (11, 5%) that were only detected by serum serology and 1 herd (3.8%) that was positive only to the BTM Elisa, which could be associated with the lack of specificity of the ELISA, described by other authors.

Conclusions: The diagnostic techniques analyzed for the classification of PTB positive and negative herds show moderate agreement. Therefore, we recommend the combination of indirect techniques (ELISA of individual animals or in BMT samples) and direct techniques (qPCR of manure) for the de-

tection of MAP at the herd-level as the most accurate strategy. However, the analysis of a sample of manure from the holding pen by qPCR turned out to be the most advantageous cost-effective strategy.

Keywords: Paratuberculosis, diagnosis, qPCR, ELISA.

ID-26

Mannheimia haemolytica serotypes detection by novel qPCR. Spanish cattle epidemiological situation

José Luis Arnal Bernal[1], María Dolores Cid Vázquez[2], Celia Sanz Tejero[1], Alfredo Ángel Benito Zúñiga[1], Oihane Alzuguren Ramos[1], Cristina Baselga Julián[1], Ana Belén Fernández Ros[1].

[1]Exopol, San Mateo de Gallego (Zaragoza), Spain; [2]Universidad Complutense Madrid, Madrid, Spain.

Introduction: *Mannheimia haemolytica* is described as a primary agent of bovine respiratory disease (BRD) which remarkably impacts in cattle cost. Most of healthy animals are natural carriers; nevertheless, this agent causes acute or chronic disease under certain circumstances such as environmental stress, shipping, weaning or infections by viruses or *Mycoplasma spp*.

Twelve different serotypes of *M. haemolytica* (A1,A2, A5-A9, A12-A14, A16 and A17) have been reported so far. Other serotypes, which were formerly included, are currently classified as *Mannheimia glucosida* (A11) or *Bibersteinia trehalosi* (T3,T4,T10 and T15). Previous publications reported serotype A1 and in a less extent A6 as the most frequently found in pneumonic lesions whereas serotype A2 is found mainly in the nasopharynx of healthy animals.

Different methods have been described for serotyping *M. haemolytica* until now, however, indirect hemagglutination test (IHA) has been the most common used. The lack of commercial antisera and cross reactions avoid the laboratories from offering this kind of diagnostic.

Objectives: This work aimed to develop a novel multiplex Real Time PCR (qPCR) which detects simultaneously main serotypes described in cattle (A1, A2 and A6) not only on the isolates but also directly on the clinical samples. Furthermore, this project researched the frequency of detection of the different serotypes of the *M. haemolytica* isolates obtained from diseased cattle in Spain.

Materials and methods: A novel multiplex qPCR targeting A1(FAM), A2(HEX) and A6(CY5) was developed and tested for inclusivity and exclusivity. A complete collection of reference strains including 12 isolates belonging to the respective 12 serotypes of *M. haemolytica* and 5 isolates from the four serotypes of *B. trehalosi* and *M. glucosida* (A11) were also tested.

Then, 59 strains isolated from lungs and broncheoalveolar lavages (BAL) of diseased animals belonging to different clinical cases were analyzed by qPCR and IHA. These isolates had been confirmed as *M. haemolytica* by Maldi Tof (Bruker) and commercial qPCR (EXOone Mannheimia haemolytica, Exopol). Cohen´s Kappa value was calculated (CI 95%) considering results from both techniques. Those strains with negative result for A1, A2 and A6 were considered as concordant if IHA resulted with a different serotype different from the three above mentioned.

Finally 26 clinical specimens sampled from diseased animals which resulted positive for *M. haemolytica* were directly analyzed by qPCR. This collection included nasal swabs(n=8), BAL(n=5) and lungs(n=14).

Results and discussion: Inclusivity and exclusivity test resulted as expected and reference strains were correctly identified. Isolates which were different from A1, A2 and A6 resulted negative but A17 which resulted positive for A2 qPCR test. This misidentification might not affect the study because A17 is not expected to be present in Spain.

Once analyzed the 59 isolates, 32 isolates (54.23%) resulted positive for A1, 12 isolates (20.33%) resulted positive for A2, 6 isolates (10.17%) resulted positive for A6 and 9 isolates (15.25%) resulted negative for A1, A2 and A6. Concordance resulted very high (kappa=0.95; CI 0.95, 0.87-1.0; SD=0.03). From those 9 isolates which resulted as non-typeable (nt) by qPCR two resulted positive to A16 by IHA and the rest remained as nt. These results agree those previously reported; A1, A2 and A6 represented the 85% of the cases and A1 was found as the most frequently detected in diseased animals.

Those clinical samples (n=26) which had resulted positive to *M. haemolytica* by qPCR (Cq range 18.9-32.6) obtained positive results for A1 (n=16, 61%), A2 (n=14; 53%) and A6 (n=5, 17.24%). In everysample but one was detected at least one of the studied serotypes. Coinfection was not frequently observed; just 3 samples resulted positive for two different serotypes.

The detection rates of serotypes A1 and A6 over the isolates were not significantly different from those when clinical samples were directly analyzed. Nonetheless, important differences were found in case of A2 ($p<0.05$). Several nasal swabs were directly analyzed by qPCR and could explain this fact.

Conclusions: This novel multiplex qPCR has proven to be an accurate technique to detect the most important serotypes of *M. haemolytica* in cattle. Moreover, this molecular tool overcomes the limitation of microbiological growth and serological proceedings. Epidemiological situation in Spain is not different from those previously reported in other countries, nevertheless, serotype A2 should not be neglected as it is frequently isolated from pneumonic lungs.

Keywords: Mannheimia, serotypes, qPCR, Spain.

ID-27

Topography of the respiratory tract bacterial microbiota in cattle

Edouard Timsit[1], Christopher McMullen[1], Trevor Alexander[2].

[1]University of Calgary, Calgary, Canada; [2]Agriculture and agrifood Canada, Lethbridge, Canada.

Background/Objectives: Bacterial bronchopneumonia (BP) is the leading cause of morbidity and mortality in cattle. While the bacterial composition of the bovine upper respiratory tract (URT) has not been studied in detail, the nasopharynx is generally accepted as the primary source of pathogenic bacteria that cause BP. However, it has recently been shown in humans that the oropharynx may act as the primary reservoir for pathogens that reach the lung. The objective was therefore to describe the bacterial microbiota present along the entire cattle respiratory tract to determine which URT niches may contribute the most to the composition of the lung microbiota.

Materials and Methods: Seventeen upper and lower respiratory tract locations were sampled from 15 healthy feedlot steer calves. Samples were collected using a combination of swabs, protected specimen brushes, and saline washes. DNA was extracted from each sample and the 16S rRNA gene (V3-V4) was sequenced. Community composition, alpha-diversity, and beta-diversity were compared among sampling locations.

Results: Microbiota composition differed across sampling locations, with physiologically and anatomically distinct locations showing different relative abundances of 1,137 observed sequence variants (SVs). An analysis of similarities showed that the lung was more similar to the nasopharynx (R-statistic = 0.091) than it was to the oropharynx (Rstatistic = 0.709) or any other URT sampling location. Five distinct metacommunities were identified across all samples after clustering at the genus level using Dirichlet multinomial mixtures. This included a metacommunity found primarily in the lung and nasopharynx that was dominated by *Mycoplasma*. Further clustering at the SV level showed a shared metacommunity between the lung and nasopharynx that was dominated by *Mycoplasma dispar*. Other metacommunities found in the nostrils, tonsils, and oral microbiotas were dominated by *Moraxella, Fusobacterium,* and *Streptococcus*, respectively.

Conclusions: The nasopharyngeal bacterial microbiota is most similar to the lung bacterial microbiota and therefore may serve as the primary source of bacteria to the lung. This finding confirms that the nasopharyngeal microbiota should be the focus of research as it relates to the role of the URT microbiota in BP. As well, this microbiota should be the main target for future interventions and pharmaceuticals aimed at controlling and preventing BP.

Keywords: Microbiome, bovine respiratory disease, natural cattle, lung.

ID-28

Metaphylaxis with tildipirosin did not alter the effectiveness of an experimental, monovalent vaccine of live, attenuated *Mannheimia haemolytica* administered intranasally to calves

Scott Nordstrom[1], Michael Bolton[1], Kevin Hill[1], Tim Parks[1], Jeff Knittel[1], Mani Vaidyanathan[1], Kelly Lechtenburg[2].

[1]Merck Animal Health, Madison, NJ, United States; [2]Midwest Veterinary Services, Inc., Oakland, NE, United States.

Objective: To determine the effect of tildipirosin administered subcutaneously (SC) on the efficacy of a live, attenuated, monovalent vaccine that contained *Mannheimia haemolytica (M. haemolytica)* administered intranasally (IN).

Materials and Methods: For this study, eighty-eight (88) healthy, single-source, Holstein or Holstein cross, male calves were approximately 14-weeks old at the time the study was initiated. Calves were seronegative for antibody to *M. haemolytica* leukotoxin; negative for persistent infection with Bovine Virus Diarrhea Virus; and, were not previously vaccinated. Calves had *ad libitum* access to fresh water and feed medicated with an ionophore. No other medications that could affect the vaccine or the virulent challenge organism were added to the water or the feed.

A completely randomized, 2 x 2 factorial, design was used; the individual calf was the experimental unit; and, 22 calves were enrolled per treatment group. Each calf was randomly assigned to one of four treatment groups:

1) an experimental intranasal (IN), monovalent, live, attenuated vaccine (VAX) that contained a proprietary seed stock of *M. haemolytica*;

2) VAX (IN) + tildipirosin (4 mg/kg; SC);

3) Placebo vaccine (PLBO) that contained the same medium and filler as in the experimental vaccine but without the bacterial antigen (IN);

4) PLBO (IN) + tildipirosin (4 mg/kg; SC).

After treatment was applied (Day 0), calves were housed in pens containing only individuals from a treatment group. The vaccine (VAX) was prepared using the same seed culture and titer of *M. haemolytica*, as commercially available in Merck Animal Health's licensed vaccines (Madison, NJ, USA).

Two calves in treatment group 2 and two calves in treatment group 4 died due to respiratory disease, unrelated to the experimental procedures, prior to the day of challenge (Day 70). The solution used for challenge was prepared with virulent *M. haemolytica* in Tryptic Soy Broth (TSB) (30 to 50 mL to deliver similar bacterial challenge); and, was administered intratracheally. After challenge on Day 70, calves remained in the same pen to the end of the study and were observed daily at approximately the same time each day. Clinical signs (respiratory scores, attitude scores, rectal temperature) of all calves were recorded. On Day 77, (7 days post-challenge) the calves were euthanized and lesions in the lungs were scored independently by two individuals. The average of those two scores was used for analyses. Samples (10 to 20 gm) of lung were submitted (within 48 hours of collection) for isolation of *M. haemolytica*. Personnel recording clinical scores, scoring lung lesions, or performing bacterial isolation procedures were blinded to the treatment group to which the animal was assigned.

The primary outcome variable was the Lung Lesion Score (LLS). Secondary outcome variables were clinical signs of respiratory disease (respiratory score, attitude score, rectal temperatures), and isolation of bacteria from samples of lung.

Results: There was no significant (P = 0.51) effect of tildipirosin on LLS whether calves were vaccinated with VAX or PLBO; however, VAX resulted in significantly (P = 0.046) lower LLS (VAX median = 1.48%) than did PLBO (median = 3.25%). Calves in all groups developed clinical signs; and, there was

no significant ($P > 0.05$) effect of any experimental treatment, on clinical signs. The percent of calves from which samples of lung yielded *M. haemolytica* was not significantly affected by VAX or PLBO.

Conclusions: Under the conditions of this study, VAX (IN) administered concurrently with tildipirosin (SC) was proven efficacious after an *M. haemolytica* challenge.

Keywords: Tildipirosin, intranasal vaccination, Mannheimia haemolytica, efficacy.

ID-29

Evaluation of risk factors of umbilical infection in newborn beef calves

Florent Perrot, Nicolas Herman.

National Veterinary School Toulouse, Toulouse, France.

Objectives: To describe and assess the potential risk factors associated with umbilical infection in beef farms located in the center of France, focusing on the passive immune transfer (PIT).

Materials and methods: Newborn calves from 22 French beef farms were followed up during a calving season (between November 2020 to March 2021). Each farm was visited twice a week. During the first visit (calves between 1 and 5 days of age), information on zootechnical practices and calving conditions was collected. During the first and the second visits (7 days after the first visit), housing conditions (cleanliness and humidity) and omphalitis were scored. Omphalitis scoring comprised thickening of umbilical stump (0 if < 2 cm and 1 if > 2 cm), presence or absence of purulent discharge (score 0 or 1), local pain (0 if no withdrawal when slight pressure applied, 1 if any) or ultrasound of internal structure. Each calf was also sampled during the first visit to assess the quality of passive immune transfer through serum total protein and Brix value optical refractometry.

Results: Nine hundred sixty-four calves were included in the study. The prevalence of omphalitis was 34% (326/964), first and second visits coupled. Univariable and multivariable statistical analysis revealed that ($P<0.05$): (i) male calves are more at risk of developing omphalitis than females (OR=2.6); (ii) the prevalence of umbilical infection is higher for calves weighing more than 50 kg and for calves with an umbilical cord length strictly less than 3 cm; (iii) a dirty calving pen increases the risk of omphalitis (OR=1.8); (iv) calves born from primiparous dams are more likely to develop an omphalitis than multiparous (OR=1.4). Furthermore, no statistical association was found between failure of passive immunity transfer (medium or bad) and omphalitis development (χ^2 Pearson > 0.05).

Conclusions: This study is the first to report the prevalence of omphalitis in beef cattle with a prevalence of 34% (326/ 964). Development of omphalitis was not associated with a failure of passive immunity transfer in this study. Risk factors related to calves were: sex, weight and umbilical cord length. Moreover, calving hygienic conditions are of prime importance to prevent omphalitis in the field.

Keywords: Beef calves, omphalitis, risk factors, passive immune transfer.

ID-30

Identification of BRD antibodies to install a tailor-made BRD Prevention Plan on Dutch dairy farms

Henk Kuijk, Hanny Swam, Pleun Penterman, Geert Vertenten.

MSD Animal Health, Boxmeer, Netherlands.

Objectives: Bovine Respiratory Disease (BRD) is a multifactorial disease of young cattle. The factors that predispose to BRD include stress related to stocking, moving or mixing cattle, poor ventilation or draughts, sudden climatic changes or extreme heat or cold, mixing various age groups, nutritional deficiencies, colostrum deficiency, and poor feed hygiene. These stresses lead to infection by primary viral pathogens which cause lung damage and may pave the way for various bacterial pathogens as *Mannheimia haemolytica (Mh)* and *Mycoplasma bovis*.

Vaccination is an important tool in managing BRD. Identification of major respiratory pathogens on a BRD problem farm may be essential to establish an appropriate vaccination program.

In 2019 MSD Animal Health started a BRD Prevention Plan including a serological screening, the BRD QuickScan, to identify which BRD pathogens are circulating on a farm. The BRD Prevention Plan is a decision tree guiding veterinary practitioners to manage BRD. Depending on the results of the BRD QuickScan, a tailor-made advice including BRD vaccination and improvement of BRD management factors is given.

This manuscript gives an overview of the BRD QuickScan results in 2020 and 2021 on Dutch BRD problem farms not vaccinating against BRD.

Materials & Methods: To run the BRD QuickScan, serum samples were taken from 5 calves (3-6 months old) on a BRD problem farm or BRD suspected farm. Those samples were analysed in the Centre for Diagnostic Solutions (MSD Animal Health, Netherlands) for antibodies against *Mh,* Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza 3 (PI-3) and *Mycoplasma bovis* by ELISA. An in-house test was used to measure *Mh* and BRSV antibodies, whereas for PI3 and *Mycoplasma bovis* a commercial kit was used from respectively IDEXX and Bio-X. The results of samples collected between October 2019 to December, 2021 are presented.

Results: During the defined timeframe, 325 BRD QuickScans were performed. In 1% of the BRD QuickScans, no antibodies against the selected BRD pathogens were identified. In those farms, a tailor-made approach was provided to improve the BRD status (www.rescalf.nl).

Eighty-three percent of the QuickScans were positive for antibodies against *Mh,* 5% of the QuickScans had only *Mh* antibodies, where in 78% of the QuickScans antibodies against *Mh* and viral BRD pathogens (BRSV and/or PI3) were present. On farms positive for *Mh,* vaccination with a vaccine providing

protection against *Mh* (Bovilis® Bovipast® RSP) was advised. Additionally, if on such farms BRD was already present in very young animals, supplementary vaccination of the pregnant dams was advised to improve the maternal antibody protection in those calves.

Eighty-nine percent of the QuickScans were positive for BRSV and/or PI3. Nine percent of the QuickScans were only positive for PI3 antibodies and none were only positive for BRSV antibodies. These findings confirm the importance of the viral pathogens in the BRD complex, mainly combined with *Mh*. On farms where high antibody titers against viral pathogens were detected, and BRD problems already were apparent in very young calves, an intranasal vaccination of the calves at the age of 1 week using a live BRD vaccine (Bovilis INtranasal RSP live) was recommended.). This could be followed by vaccination with a trivalent inactivated vaccine (Bovilis® Bovipast RSP) at a later age.

Twenty-two percent of the BRD QuickScans were positive for *Mycoplasma bovis*. On *Mycoplasma bovis* farms, a specific approach was recommended as no commercial vaccines are available. It was advised to create small groups of animals, avoid mixing of calves from several groups, and to vaccinate against other major BRD pathogens.

After the implementation of the BRD prevention plan including the BRD QuickScan, over 80% of the farms started vaccinating with an inactivated BRD vaccine (Bovilis® Bovipast® RSP), 9% started with a live intranasal BRD vaccine (Bovilis® INtranasal RSP® Live) and on 3% of the farms complementary examinations were performed. About 10% of the farms started the additional pregnant dam vaccination with Bovilis® Bovipast® RSP.

Conclusion: For most pathogens circulating on BRD farms vaccines are commercially available. Implementing a tailor-made BRD management program including vaccination is important to reduce BRD related losses. Practical tools as the BRD Prevention Plan and BRD QuickScan may be useful to reduce BRD. Based on serological findings farmers can easily be convinced (in dialogue with their veterinarians) to vaccinate against BRD.

Keywords: Bovine Respiratory Disease, QuickScan, serology, dairy, Netherlands.

ID-31

Elucidation of the efficacy of fecal microbiome transplantation (FMT) in healing calves with intractable diarrhea

Jahidul Islam[1], Masae Tanimizu[2], Yu Shimizu[2], Yoshiaki Goto[2], Natsuki Ohtani[2], Kentaro Sugiyama[2], Eriko Tatezaki[2], Masumi Sato[2], Eiji Makino[2], Toru Shimada[2], Chise Ueda[2], Ayumi Matsuo[1], Yoshihisa Suyama[1], Yoshifumi Sakai[1], Mutsumi Furukawa[1], Katsuki Usami[1], Hiroshi Yoneyama[1], Hisashi Aso[1], Hidekazu Tanaka[2], Tomonori Nochi[1].

[1]Tohoku University, Miyagi, Japan; [2]Chiba Prefectural Federated Agricultural Mutual Aid Association, Chiba, Japan.

Objectives: Fecal microbiome transplantation (FMT) has shown promising results that indicate the effective treatment of diarrhea in calves although the mechanisms by which FMT works has not yet been elucidated. Therefore, the aim of this study is to elucidate the efficacy of FMT in intractable diarrhea treatment and searching the potential bacterial taxa and metabolites responsible for FMT success.

Materials & Methods: Total 20 FMT trials, in which feces obtained from healthy donors were intrarectally transferred into recipient calves with diarrhea, were performed. Fecal samples were collected from donors on the day of FMT and from recipients before and on days 1 and 7 after the FMT. The samples were analyzed by high-throughput 16S rRNA gene sequencing, metabolomics via capillary electrophoresis time-of-flight mass spectrometry, and ELISA, respectively.

Results: Among the 20 FMT treatment, total 14 (70%) treatment were succeeded based on the clinical findings, diarrheal, metagenomics and metabolomics results. Considering the beta diversity, unweighted unifrac distance was found significantly different before FMT (D-success vs R0-success), but not in after FMT at day 7 (D-success vs R7-success) in successful FMT group. In unsuccessful FMT treatment, there was no significant difference observed between donor and recipient in before and after FMT. Thus, in unsuccessful FMT treatment group, calf exhibit impaired engraftment of the FMT bacterial community and failed to restore the commensal bacteria as well as metabolites, due to lack of optimal donor. On the other hand, genus *Selenomonas* confirmed donor–recipient compatibility in successful FMT treatments. A strong positive correlation between the microbiome and metabolome data, which is a prerequisite factor for FMT success, was confirmed by Procrustes analysis in successful FMT (r = 0.7439, P = 0.0001). A reduction in fecal amino acid concentration was observed in succeed treatment, which is strongly correlate with the remission of diarrhea. Additionally, weighted gene correlation network analysis confirmed the positively or negatively correlated pairs of bacterial taxa (family *Veillonellaceae*) and metabolomics features (i.e., amino acids and short-chain fatty acids) responsible for FMT success.

Conclusions: The findings obtained from the present study suggest that the FMT may directly or indirectly promote the cohabitation of certain bacterial taxa, which facilitate to recover recipient calves from intractable diarrhea in successful FMT treatment group.

Keywords: Diarrhea, microbiome, transplantation, metagenomics, metabolomics.

ID-32

Prevalence and antimicrobial susceptibility of BRD pathogens isolated from cattle with respiratory disease during over 10-years of supported testing program in Germany

Kirsten Stemme, Egon Thesing, Martina Resch.

MSD Animal Health, Unterschleißheim, Germany.

Objectives: Trans-tracheal aspiration (TTA) is a technique used for *in vivo* identification of mainly bacterial pathogens from cattle with bovine respiratory disease (BRD). MSD Animal Health in Germany has been supporting bacteriological analysis of samples from TTA for veterinarians and farmers since 2009.

The objective of this study was to investigate the dynamics in the prevalence of BRD associated bacteria (*Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, Trueperella pyogenes*) in the lower respiratory tract of calves with respiratory disease and their antimicrobial susceptibility over the past 13 years.

Materials & Methods: During the period of 2009 to 2021 samples were collected through TTA from calves with respiratory disease after a specific request by veterinarians to identify BRD related pathogens. The samples were collected from calves located on farms in all regions of Germany, dairy farms as well as fattening units. None of the sampled animals received an antimicrobial treatment prior to sampling. If possible, samples from 3 calves were pooled (e.g. animals housed in the same pen).

Isolation and identification of BRD-associated bacteria from the TTA samples were performed using bacterial culture, MaldiTof and subsequent determination of antimicrobial susceptibility by microdilution (samples collected since 2013 only). Isolates were evaluated using Clinical and Laboratory Standard Institute (CLSI)-approved methods. For *T. pyogenes* no antimicrobial testing was done as antimicrobial therapy is not considered viable and therefore no CLSI methods are available.

Results: During the period of supported testing, 1726 calves were sampled on 361 farms. As some samples were pooled, a total of 611 samples were submitted to laboratory testing. BRD-associated bacteria were isolated from 66.3 % of all samples with a yearly range between from 55.2 – 78,8 %.

P. multocida was the most prevalent bacterium (61.1 %; 33.3 – 73.9%) followed by *M. haemolytica* (16.6 %; 5.3- 33.3%). The isolation prevalence for *H. somni* was 7.3 % (0 – 15.6 %) and 15.0 % (6.9 – 26.3 %) for *T. pyogenes.* While there was no obvious trend, the prevalence of the four pathogens showed large variation from year to year.

None or only a few isolates of *P. multocida, M. haemolytica,* and *H. somni* were resistant to amoxycillin, ampicillin, ceftiofur, florfenicol, gentamycin, and trimethoprim sulfonamide. The percentage of *M. haemolytica* isolates that were susceptible to penicillin was 75 %, to enrofloxacin 97 %, to tetracycline 85 % and to tilmicosin 60 %, with only small variation during the years. Ninety five percent of *P. multocida* isolates were found to be susceptible to penicillin, 92% to enrofloxacin, 73% to tetracycline and 66% to tilmicosin. No isolate of *H. somni* was resistant to penicillin and enrofloxacin, while 96% of isolates were susceptible to tetracycline and 75% to tilmicosin.

Conclusion: *P. multocida* was the most prevalent bacterium isolated from the lower respiratory tract of calves with respiratory disease during the period of 2009 till 2021 in Germany. However, prevalence up to 30 % were found for *M. haemolytica* in certain years.

The results of antimicrobial susceptibility testing showed that *M. haemolytica*, *P. multocida,* and *H. somni* exhibited *in vitro* resistance to some important antimicrobial products that are frequently used to treat respiratory disease. On the other hand, it was also clear that several other antimicrobial treatments are available to which the isolated bacteria showed high susceptibility.

Keywords: Respiratory disease, trans-tracheal aspiration, BRD associated bacteria, prevalence, antimicrobial susceptibility.

ID-33

Mycoplasma bovis antibody testing in purchase protocol to reduce circulation between farms

Linde Gille[1], Callens Jozefien[2], Léonard Marie[3], Lipkens Zyncke[2], Ribbens Stefaan[2], Evrard Julien[3].

[1]University of Liège, Liège, Belgium; [2]Diergezondheidszorg Vlaanderen (DGZ), Torhout, Belgium; [3]Association Régionale de Santé et d'Identification Animales (ARSIA), Ciney, Belgium.

Background & Objectives: *Mycoplasma bovis*' importance as a causal pathogen of pneumonia, arthritis, mastitis, and other diseases has been well established in the last decades. In Belgium, a steady increase in *M. bovis*' presence has been noticed in bovine herds: in 2009, only 1.5% of dairy farms tested positive on culture of bulk tank milk (BTM) (1), while in 2011, already 11% of calves sold to the veal sector (corresponding with about 11% of herds) had antibodies at arrival (2), and in 2016, 32% of all dairy farms had either PCR or antibody positive BTM samples (3). This increase is worrying, given the enormous economic impact a *M. bovis*' introduction and circulation can have on a farm. The main cause of the increase is probably the purchase of carriers, given the enormous amount of cattle trade in Belgium: between 2005-2009, 40% of the cattle born in Belgian farms changed farms at least once (4).

As detecting carriers is not evident, given the intermittent excretion and existence of asymptomatic carriers, it is currently recommended to not purchase antibody positive animals to avoid *M. bovis* introduction in negative herds. To determine the risk of purchase of *M. bovis* antibody positive animals in Belgian herds, this study aimed to determine the number of animals testing antibody-positive at purchase.

Materials & methods: Throughout 2021, *M. bovis* antibodies were determined on every purchase protocol requested at 2 reference laboratories (ARSIA & DGZ), using a *M. bovis* antibody ELISA (BIO K432, Bio-X Diagnostics S.A., Belgium).

Results: In total, blood samples of 76285 animals were analyzed, of which 14.96 % (n= 11416) tested positive on M. bovis antibodies. One thousand two hundred twenty-seven animals were retested approx. 30 days after the first sample. Of these, 87,8 % kept the same result, 5,9 % seroconverted and 6,3 % seroreverted.

Conclusions: There is a non-negligible risk in introducing possible *M. bovis* carriers in seronegative herds trough purchase. There was 6,3 % seroreversion after 30 days, which could be due to antibodies truly dropping underneath the test

limit, given the poor persistence time of *M. bovis* antibodies (5), or could indicate the presence of false positives even with a test specificity of 97 %. For the seroconversion rate, the argument is different: probably, the 5.9 % conversion is only partially due to true seroconversion, but also due to the poorer sensitivity of the test used (Se 80.95%).

Given the possible presence of false negatives and the poor persistence of antibodies, it is quite possible that a part of the animals from *M. bovis* positive farms were missed in the first analysis. As such, it is advisable for *M. bovis* negative herds to test purchased animals twice, both at the beginning and the end of the 30-day quarantine, before releasing them in the herd. If either test is positive, the animal should be considered at-risk. Another option would be to test- or interpret testing on herd-level instead of animal level.

In conclusion, further research to identify *M. bovis* carriers, or development of farm-level testing procedures is sorely needed to stop the introduction of *M. bovis* into seronegative farms.

References:

1: Passchyn, P., S. Piepers, L. De Meulemeester, F. Boyen, F. Haesebrouck, and S. De Vliegher. 2012. Between-herd prevalence of Mycoplasma bovis in bulk milk in Flanders, Belgium. Research in Veterinary Science 92:219-220.

2: Pardon, B. 2012. Morbidity, mortality and drug use in white veal calves with emphasis on respiratory disease. PhD Thesis.

3: Gille, L., J. Callens, K. Supré, F. Boyen, F. Haesebrouck, L. Van Driessche, K. van Leenen, P. Deprez and B. Pardon. 2018. Use of a breeding bull and absence of a calving pen as risk factors for the presence of Mycoplasma bovis in dairy herds. Journal of Dairy Science, 101(9), 8284–8290.

4: Ensoy C., Faes, C., Welby, S., Van der Stede, Y. and Aerts, M. 2014. Exploring Cattle Movements in Belgium. Preventive Veterinary Medicine. 116(1-2), 89-101.

5: Petersen, M., Pedersen, J., Holm, D., Denwood, M. & Nielsen, L. (2018). A longitudinal observational study of the dynamics of Mycoplasma bovis antibodies in naturally exposed and diseased dairy cows. Journal of Dairy Science, 101(8), 7383-7396.

Keywords: Mycoplasma bovis, purchase, biosecurity, ELISA, surveillance.

ID-34

Validation and use of a new diagnostic protocol for Johne's disease control in New Zealand dairy herds

Andrew Bates[1], Rory O'Brien[2], Frank Griffin[3], Richard Laven[4], Simon Liggett[2].

[1]Vetlife Scientific Ltd, Temuka, New Zealand; [2]Disease Research Ltd, Dunedin, New Zealand; [3]Department of Microbiology and Immunology, University of Otago, New Zealand; [4]School of Veterinary Sciences, Massey University, New Zealand.

Objectives: This study reports the diagnostic accuracy and use of an initial screening test of four serum ELISAs followed by a confirmatory test of one quantitative fecal PCR (fPCR) for the diagnosis of paratuberculosis in mixed aged milking cows in New Zealand.

Materials and methods: Data from a cross-sectional study of 20 moderate prevalence herds was combined with existing data from low and high prevalence herds to form a combined dataset of 3,845 paired serum and fecal samples. Records of incidence of clinical cases of Johne's disease (JD) were used to classify herds into three prevalence categories. High prevalence herds (> 3% clinical JD per year for the last three years), moderate prevalence herds (< 1%) and low prevalence herds (zero incidence of clinical JD for at least the last five years). Positive ELISA data were declared if > 50 ELISA units and fPCR data at two cut-points (≥1x10^4 genomes/mL or >1x10^3 genomes/mL).

Fixed and mixed Bayesian latent class models were constructed at both fPCR cut-points, accounting for conditional independence, paired conditional dependence and all possible dependencies between tests using OpenBUGS. The aim was to identify *Mycobacterium paratuberculosis avium* (MAP) infected cows that met at least one of two criteria: shedding sufficient MAP in faeces to be detected by fPCR or mounting a detectable MAP antibody response.

The effect of using this new testing method as part of Johne's control programme in a large milking herd over a four-year period is also reported. A sub-set of this data was also used to look at the effect of infection status on milk production in sub-clinically infected cows (reported at Buiatrics Dublin, 2016).

Results: ***Model validation:*** The best fit to the data was obtained by modelling either pairwise dependencies between tests in a fixed model or including all dependencies in a mixed model at a faecal cut-off of ≥1x10^4 genomes/mL. Test performance differed with prevalence: for the random model, at a prevalence of 0.38 (95% predictive interval,(PI)=0.30-0.44), sensitivity was 0.54 (95%PI=0.47-0.62) and specificity 0.98 (95%PI=0.96-1.00). At a low prevalence (0.01 (95%PI=0.00-0.03), test sensitivity was 0.60 (95%PI=0.42-0.72) and specificity 1.00 (95%PI=1.00-1.00).

Models were robust to prior assumptions and this testing protocol had a positive predictive value of 0.96 (95%PI=0.87-1.00) in high prevalence herds and 1.00 (95%PI=0.99-1.00) in low prevalence herds. Correspondingly, the negative predictive value in high prevalence herds was 0.78 (95%PI=0.71-0.84) and 0.98 (95%PI=0.84-1.00) in low prevalence herd.

Reduction in prevalence of Johne's disease: We also report the successful reduction of infectious and infected animals in an endemically infected herd, where over a 4-year period, an annual test and cull policy using this approach reduced seroprevalence of positive cows from 26% to 2% and the proportion of clinical JD culls from 5% to 0.4%. Over this period, the seroprevalence in primiparous cows fell from 15% to 2.5%.

Effect of infection status on milk production: Previously, this testing method has been used to identify under-performing MAP infected animals with clinically normal, ELISA-positive animals producing 4% fewer kg milk solids (kgMS) per lactation and faecal positive cows producing up to 12%

fewer kgMS (World Buiatrics, Dublin 2016).

Conclusions: The results presented suggest that this is a useful tool in the control of JD on dairy farms, particularly in herds with higher levels of infection, where the sampling and testing cost per animal is defrayed across more detected animals.

Keywords: Johne's disease, Mycobacterium paratuberculosis avium, sensitivity, specificity, control.

ID-35

Tuberculosis diagnosis in bovine herd and associated cross reaction in the tuberculin skin test (PPD): a case report

Mónica Espada[1], Luis P. Figueras[1], Sofia Aventín[1], Aurora Ortín[2], Cristina Acín[2], Carmelo Ortega[2].

[1](1) Gabinete Técnico Veterinario,S.L., Zaragoza, Spain; [2](2) Animal Pathology Department. Veterinary faculty. University of Zaragoza (Spain), Zaragoza, Spain.

Abbreviations:

PPD - Purified protein derivative ((PPD) skin test (for tuberculosis))

T3 - Officially holdings free of tuberculosis (RD 1716/2000)

PCR - Polymerase Chain Reaction

Introduction: Bovine tuberculosis is a mandatory disease under Eradication according to the Spanish Tuberculosis Eradication Program. This program is based on the detection of positive animals using both, simple and comparative Tuberculin Skin tests (PPD also called Mantoux test in human medicine) and the elimination of the reactive animals.

The characteristics of the test (sensitivity, specificity and the predictive values) as well as the characteristics of the microorganism (different etiology complexes, species and subspecies) can produce, in some cases, cross reactions or interactions with other microorganisms that cause false positivity results in the tuberculosis program.

One of those cases is presented in this communication at the same time that it reflects, based on the origin of the problem, the importance of biosecurity as a basis for prevention.

Methodology and results (the case): The case took place in a cattle herd (Blond d´Aquitaine), which began its activity with 20 heifers aged between 11 and 16 months in 2016. Animals were introduced in the farm with a semi-intensive production system that included a covered ship and a park limited by a perimeter fencing. The farm was not in contact with other livestock, but in the area there were wild boars that are under control in the Wild Tuberculosis Surveillance Program (PATUBES) on which all the results were negative (0% prevalence).

The origin of the animals was a T3 farm (Tuberculosis free qualification level in the Spanish Tuberculosis Eradication Program). Previously to the movement of the animals, all of them were tested by a simple PPD tests which resulted negative in all of them.

In the reproductive control period, one of the animals was diagnosed with a congenital disorder that excluded it for the future. Therefore, the owner and the provider agreed to change it by one another heifer that arrived pregnant and brought a negative result to the simple PPD test, which carried to the farmer to introduce it directly into the flock.

After delivery, the new animal was affected by a enteritis process that was diagnosed as Bovine Paratuberculosis by staining Ziehl-Neelsen in feces samples and serology (Elisa test). After, the animal was isolated and slaughtered in the farm. PPD test was carried out in all the animals according to the Spanish Tuberculosis Eradication Program, on which 10 of the 20 animals resulted positive (differential values between the first and the second measures of 2.5-6 mm).

Results were contrasted by Compared PPD test which resulted in five positive animals that were sacrificed. These animals were sent to the slaughterhouse where samples were taken and sent to the Reference Center for Veterinary Health Surveillance (Visavet). The microbiological study of the samples of three animals showed a positive growth in specific Mycobacterium medium that resulted negative by PCR to both: Mycobacterium tuberculosis and Mycobacterium avium subspecies suis. It was concluded that positive reaction to the PPD test was not due to bovine tuberculosis.

At the same time, four animals of the farm were seropositive to Paratuberculosis (Elisa test) and one of them evolved in a clinical way and was sacrificed. After, a Paratuberculosis control strategy was applied in the farm at the same time that PPD tests continued with negative results in all animals.

Conclusions: The final conclusion is that there was a false positive reaction to the simple and compared PPD test that was not confirmed either by clinical or microbiology, which suggests any type of interference with some another Mycobacterium spp as it has been referred in another cases. While it is not possible to confirm it, we suspected that the interference in the test was caused by Mycobacterium avium subspecies Paratuberculosis since the outbreak occurred in the exploitation in that particular period.

Keywords: Tuberculosis bovine, Tuberculin skin test, Paratuberculosis.

ID-36

A sudden outbreak of paratuberculosis in a previously test-negative dairy herd; a case report

David Speksnijder, Betsie Krattley-Roodenburg.

University Farm Animal Clinic, Harmelen, Netherlands.

Objectives: Paratuberculosis (Johne's disease) in dairy cattle, caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is subject of an active control policy by the Dutch dairy industry, aiming to reduce the concentration of MAP in bulk milk delivered to the milk factories. In this abstract, we describe an outbreak of MAP infections in a previously test-negative dairy herd to show the complex epidemiology of paratuberculosis in dairy farming and the necessity

to take continuous precautionary measures, even on MAP test-negative farms.

Material and Methods: The high prevalence of paratuberculosis was detected in a dairy herd of ~180 adult cattle and ~150 young stock. Cows were housed in a freestall with cubicles and grazed during the summer months. Young stock were housed in a separate barn until ~18 months of age. The herd was not closed: in 2017 a total of 79 homebred young stock were temporarily raised on pastures of two other farms and between 2013 and 2018, another 53 animals were purchased from five different herds. Young calves were fed colostrum followed by milk replacer, grass silage harvested on the farm and concentrate.

Between 2008 and 2018, milk samples of all lactating cattle were tested biannually with the IDEXX Paratuberculosis Screening Ab Test (ELISA), using an elevated cut-off S/P ratio of 1.0 in order to increase the diagnostic specificity of the test. Until 2016, negative results were obtained only. However, in July 2018, three out of 132 tested lactating cows had a positive ELISA result. MAP infection was confirmed by positive faecal qPCR in two of these three cows, aged 6 and 7 years and homebred (index cases). As a follow up, cattle ≥ 12 months of age were tested by individual faecal qPCR in April / May 2019.

Results: In April/May 2019, 253 animals were individually tested by qPCR on rectally derived faecal samples. Twenty animals tested positive. All of these animals were born on the farm between January 2010 and July 2017, except one cow that was introduced from another herd at the age of 4 years. Neither the category of animal (homebred, temporarily raised in another herd, or introduced from another herd) nor age category (1, 2, 3 or ≥4 years of age) were significantly associated with the PCR result. However, a cluster of qPCR-positive homebred heifers born in 2017 became apparent: 7 of 12 tested heifers born in April and May 2017 in the case herd were qPCR-positive. These heifers had not been temporarily raised elsewhere. The dam of the oldest of these 12 heifers tested qPCR positive as well; 4 other dams which were still present on the farm tested qPCR negative. Also a heifer born in July 2017 tested positive whilst its mother tested negative on qPCR.

After informing about the management conditions around the time of birth of these 7 heifers the farmer indicated that the calving pen hygiene had been suboptimal and pooled colostrum and bulk milk had sometimes been fed to calves.

Conclusion: In this case herd, a cluster of MAP shedding cattle could be related to birth cohort. Potential transmission routes were a contaminated calving pen, transmission through feeding of colostrum and bulk milk from multiple cows to calves and calf-to-calf transmission. Our observations stress the importance of structurally taking preventive management measures to reduce the spread of MAP in test-negative herds as well.

The aim of the Dutch milk quality assurance program is to reduce the concentration of MAP in bulk milk and to provide assurance regarding milk quality in certified test-negative herds. For this purpose, herds do not necessarily need to be free of MAP infection. However, if farmers aim to eliminate MAP infection from their herds by test-and-cull, it is important to consider that faecal qPCR has a considerably higher diagnostic sensitivity to detect MAP shedding animals compared to the ELISA test and that a considerable proportion of infected cattle start shedding MAP before adulthood - as illustrated by the observations in this case herd.

Keywords: Paratuberculosis, Johne's disease, dairy.

ID-37

Detection of *Anaplasma phagocytophilum* in bovine abortions in Flanders

Hans Van Loo[1], Geert Opsomer[1], Jef Hooyberghs[2], Koen De Bleecker[3], Bart Pardon[1].

[1]Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium; [2]Federal Agency for the Safety of the Food Chain, Brussels, Belgium; [3]Animal Health Service Flanders, Torhout, Belgium.

Objectives: To monitor brucellosis in Belgium, farmers are obliged by the Federal Agency for the Safety of the Food Chain (FASFC) to submit each bovine abortion for analysis. Besides brucellosis monitoring, the abortion program concurrently screens the submitted samples for multiple infectious pathogens. Despite the extensive number of analyses included in this abortion program, about 60% of cases remain undiagnosed. One of the explanations could be the presence of unidentified abortifacients like *Anaplasma phagocytophilum*. The aim of this study was to evaluate bovine abortions in Flanders for the presence of *A. phagocytophilum* during the tick season (July-September), since anaplasmosis is a tick born disease.

Materials and methods: Between July and September 2012, the placenta and fetal spleen of 150 late term (> 7 months pregnant) bovine abortions were collected and analysed using an *A. phagocytophilum* PCR. All abortion cases were also tested for brucellosis by *Brucella* spp culture on the placenta and serology on maternal serum, for *Neospora caninum* by analysis for antibodies on maternal serum and for bovine viral diarrhea virus (BVDv) by antigen ELISA. The aborted fetus was examined for bacteria by aerobic, *Listeria* spp and yeast/fungal cultures on abomasal and lung tissue.

Results: Seven of 150 abortion cases (4.7%) were BVDv Ag positive on fetal spleen. In 12 of 150 cases (9.7%) yeasts or moulds were detected. Bacteria were found in 59 of 150 (39.3%) fetal abomasal samples and in 46 of 150 (30.7%) fetal lung samples. Seroprevalence of *Neospora caninum* in the aborted dams was 15.33% (23/150).

Of the placenta samples, 2.66% (4/150) was positive for *A. phagocytophilum*, while none of the fetal spleen samples was. No positive samples were found in the 7th month of gestation, while 1 positive sample originated from an abortion case that occurred in the 8th month of gestation and 3 samples were from abortions that happened in the 9th month of gestation.

Conclusions: The detection of *A. phagocytophilum* in late term bovine abortions suggests a potential role of this pathogen in (un)diagnosed cases of bovine abortion in Flanders. Placental tissue is probably the most preferred tissue to detect this pathogen in case of an abortion. Based on these findings, it can be interesting to include an *A. phagocytophilum* PCR in

the abortion monitoring program during the tick season.

Project financially supported by the FASFC and Sanitary Fund.

Keywords: Bovine, abortion, Anaplasma phagocytophilum.

ID-38

Biosecurity and biocontainment risks of Mycobacterium avium subspecies paratuberculosis entering and spreading in UK dairy herds

Richard Sibley[1], Peter Orpin[2].

[1]West Ridge Veterinary Practice, Witheridge, United Kingdom; [2]Myhealthyherd.com, Leicester, United Kingdom.

Objectives: The risks of Johnes disease entry and spread in over 3000 UK dairy herds using a risk assessment tool to measure, monitor and manage risks were studied to identify significant risks, with the objective of allowing farmers to manage those risks that predisposes their cattle to Johnes Disease.

Materials and Methods: A web based risk assessment tool was used by trained veterinary surgeons to assess, measure and monitor biosecurity and biocontainment risks on over 3000 UK dairy herds that engaged in some form of Johnes Disease management. The tool uses standard assessments and an algorithm to quantify risks and provide a summary and priority to identify and manage risks as part of the control plan.

Results: Biosecurity risks were defines as the risks of Mycobacterium Avium subspecies Paratruberculosis (MAP) entering the herd. 50% of herds were designated high risk of entry of MAP in to the herd, mostly due to purchasing cattle of unknown disease status and allowing cattle to drink from water courses that had passed through other livestock farms. Only 20% of dairy herds were designated as low biosecurity risk for the entry of Johnes disease.

Biocontainment risks were defines as those risks that predisposes to the spread of MAP within the herd. These risks are associated with prevalence of Johnes Disease within the herd and act as the multiplier of disease. 65% of herds were designated as high risk of disease spread, mostly due to the use of multiple calving areas and poor perinatal hygiene, and the use of pooled colostrum taken from high risk cows. Only 8% of dairy herds were designated as low risk of spread.

Of 2462 herds that were designated as having high risks of spread of MAP within their herds, 52% had high risks of entry of the disease, making these herds very high risk of a high prevalence of Johnes disease. 85% of these high risk herds were designated as infected by the attending veterinary surgeons.

Conclusions: Modern dairy farming systems tend to predispose to the entry and spread of MAP in dairy herds. Prevention and control of the disease will require significant changes in management and husbandry to prevent the entry and spread of the disease. The identification of specific risk factors allows rational management systems to be introduced to limit the spread of the disease in infected herds, and maintain freedom form disease in uninfected herds.

Keywords: Paratuberculosis, Johnes, biosecurity, biocontainment, risks.

ID-39

Mycobacterium Avium subspecies Paratuberculosis (MAP) Elisa tests on milk to predict future health and culling in a dairy herd

Richard Sibley.

West Ridge Veterinary Practice, Witheridge, United Kingdom.

Objectives: This study investigates the use of serial milk MAP elisa tests to predict health, productivity and emergency culling of adult dairy cows.

Material and methods: The study involved a large dairy herd of over 900 adult cows using milk MAP elisa tests on all milking cows every three months as part of a Johnes Control programme. Over 3000 adult cows that had been culled from the herd over a period of ten years, and which had milk elisa test results for their entire adult life were studied to determine if test results in the could be used to create a robust plan to manage test positive cows to predict and prevent health problems. Udder health, lifetime milk yield, fertility and emergency culling (as defined by being culled within 100 days of calving) were used to define health and productivity.

Results: Cows that had just one positive MAP elisa test in their lifetime had similar health and productivity to cows that had never had a positive test. Cows that had at least 2 consecutive positive tests had significantly higher somatic cell counts and higher emergency culling rates (34% of these cows were culled within 100 days of calving). Cows with at least four positive MAP elisa tests, with rising titres at each consecutive test had a very high emergency culling rate, such that 46% of these cows were culled within 100 days of calving for severe health issues. There was little difference in milk yields between positive and negative test cows.

Cows with repeated positive MAP test results have high risks of health problems that lead to emergency culling, with the consequent major economic losses incurred when culling lactating cattle with no salvage value.

Conclusion: Rising titres of MAP antibodies as measured by milk elisa tests indicate imminent severe health problems with poor prognosis.

Keywords: MAP, Elisa, Johnes, milk.

ID-40

Systematic review of herd-level test characteristics for *Mycobacterium avium* subspecies *paratuberculosis* in cattle

Niamh Field[1], John Mee[1], Conor McAloon[2].

[1]Teagasc, Fermoy, Republic of Ireland; [2]University College Dublin, Belfield, Republic of Ireland.

Objective: Conduct a systematic literature review to summarise the published estimates for herd sensitivity (HSe) and herd specificity (HSp) of diagnostic tests for *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in cattle.

Materials and Methods: A comprehensive literature search was performed in July 2020 to identify all published papers, including conference proceedings, published up to the date of the search, referring to herd sensitivity (HSe) and/or herd specificity (HSp) of a test method for MAP in cattle. All articles returned in the search went through a systematic four-stage screening process to identify relevant studies. Articles were included if they were written in English, available in full-text, had cattle as the species of interest (either dairy or beef) and contained an evaluation of the HSe and/or HSp of a diagnostic test method for MAP. Data extracted from each publication included sample population/s, method/s of analysis, reference tests, cut-off points, HSe and HSp. The relevant publications were classified based on the test method evaluated, and the results for each test method were summarised as a range of reported estimates.

Results: Forty-six publications with relevant results were eligible for inclusion in the final review, containing evaluations of whole-herd ELISA testing, bulk milk tank (BMT) ELISA, culture and PCR, pooled faecal testing and environmental sample testing. The ranges for HSe and HSp reported for each test method is summarised in Table 1.

Table 1. Summary of Johne's disease herd-level test characteristics.

Screening test	Herd sensitivity (%)	Herd specificity (%)
Whole-herd ELISA	40-100	21-96
Whole-herd ELISA + PCR	9-86	100[b]
BMT ELISA	8-97	53-100
BMT PCR/culture	0-85	100[b]
Pooled faecal testing	26-100	100[b]
Environmental sampling[a]	24-100	100[b]

[a] From studies evaluating a protocol using six composite samples
[b] Herd specificity can be assumed to be 100% due to direct detection of MAP bacteria.

The reported herd-level test characteristics for MAP demonstrate considerable differences in test accuracy. The wide ranges in reported estimates for each test are due to the variations in between-and within-herd prevalence, test protocols and cut-off points between studies. Whole-herd ELISA testing has potentially high HSe (40-100%) but potentially low HSp. This will result in many non-infected herds being classified as infected. The choice of seroprevalence cut-off point (ranged from >0% to 3% seroprevalence) for defining a herd as infected affects the estimates reported for whole-herd serology. The poor HSp of whole herd ELISA testing can be overcome by ancillary faecal testing of ELISA-positive animals, with an associated reduction in HSe. Bulk milk tank ELISA HSe estimates vary widely depending on the S/P ratio cut-off point used in the study. When manufacturer-recommended cut-offs are used, the HSe for BMT ELISA ranges from 8-30%. Environmental sampling and pooled faecal testing have relatively high HSe, 24-100% and 26-100%, respectively, and 100% HSp due to direct detection of MAP bacteria.

Conclusion: There are numerous options for herd-level testing for MAP in cattle. However, it is clear from this review that there are wide ranges in HSe and HSp estimates between studies. This makes it difficult to draw conclusions regarding the predicted performance of a test in a specific population with unknown disease prevalence. Decision-makers must balance the test characteristics with the resources available (funding, laboratory capacity) to identify the most suitable herd test method(s) in a population.

Keywords: Paratuberculosis, systematic, diagnostic, sensitivity, specificity.

ID-41

Control of paratuberculosis in small structured cattle farms

Johannes Lorenz Khol[1], Thorben Liening-Ewert[2], Laura Auböck[1], Paul Paul Ortner[2], Josef Kössler[2], Alexander Tichy[3], Michael Dünser[4], Christian Mader[5].

[1]University Clinic for Ruminants, University of Veterinary Medicine Vienna, Vienna, Austria; [2]Regional Veterinary Office Tyrol, Innsbruck, Austria; [3]Bioinformatics and Biostatistics Platform, University of Veterinary Medicine Vienna;, Vienna, Austria; [4]Austrian Agency for Health and Food Safety, Linz, Austria; [5]Veterinary Health Service Tyrol, Innsbruck, Austria.

Objectives: Since 2013, a voluntary survey and control program for MAP (*Mycobacterium avium subsp. paratuberculosis*), the cause of paratuberculosis (Johne´s disease) in cattle, is in place in the Austrian province of Tyrol. This alpine province is characterized by a small structured, traditional way of cattle farming. The aim of the present study was to evaluate data collected in the course of the MAP-program, related to the prevalence of MAP, as well as to the dynamics of the infection.

Material and methods: About 4,600 farms, representing approximately 70% of the Tyrolean dairy cattle, are participating in the MAP-program. In course of the program, MAP-positive farms are detected by boot swab sampling in a two years interval, followed by single animal testing and removing of positive animals. Additionally, basic hygienic measures to prevent further spread of the infection are advised in positive farms. During the program, structural date describing the farm characteristics and management practices were collected.

Results: In course of the program, the initial prevalence of 7.5% positive herds in 2013 decreased to 0.5% in 2019.

Detailed investigation of individual animal results revealed, that MAP-shedding decreased considerably after removal of single positive animals within a herd. Surprisingly, many cattle showed negative individual results and farms stayed MAP-negative in consecutive boot swab samplings thereafter, indicating possible passive shedding in some animals. Furthermore, these findings suggest, that fade out of the disease, after removal of MAP-shedding animals and the implementation of hygienic measures, may occur.

Analysis of risk factors showed, that the use of common alpine pasture seems not to be a significant contribution to the transmission of MAP. The same was found for sharing of equipment, which both seems to be in contrast to the current literature. On the other hand, rearing of calves with milk replacer, instead of whole milk, significantly decreased the chance to be MAP positive, which has been described before. Housing also seems to have an impact on the MAP-status of the farms, but the results are ambiguous and need further evaluation.

Conclusions: The data collected over a period of ten years indicate, that the use of common (alpine) pastures seems not to be a significant factor for the distribution of MAP. Furthermore, the dynamics of MAP infections in small structured cattle farms may contribute to the successful reduction of the MAP-herd prevalence by removing single positive animals and implementing basic hygienic measures.

Keywords: Paratuberculosis, Mycobacterium avium subsp. paratuberculosis, cattle, control, risk factors.

ID-42

A novel topical therapy for Foot-and-Mouth Disease improves animal welfare and reduces antimicrobial resistance risks

Peter Windsor[1], Sevidzem Lendzele[2], Syseng Khounsy[3], Jacques François Mavoungou[4], Francesca Earp[5], Isabel Macphillamy[5], Jim Young[5].

[1]Production Animal Welfare & Health Services, Scarborough, NSW, Australia; [2]Cattle Fattening & Milk Production Cooperative, Troua Belel, Cameroon; [3]Department of Livestock & Fisheries, Vientiane, Lao People's Democratic Republic; [4]Institute for Research in Tropical Ecology, Libreville, Gabon; [5]The University of Sydney, Camden, Australia.

Objective: Improving the therapy used by para-veterinarians and veterinarians in Foot-and-Mouth Disease (FMD) outbreaks, particularly in developing countries where FMD is endemic, is an important but largely ignored aspect of global FMD management. Whilst effective therapy is the focus of livestock owners, the priority for donors and policy makers is usually investments in vaccination programs that are frequently unsustainable. Treatment for FMD is typically use of inappropriate parenteral and/or topical antibiotics, for a viral infection, risking development of antimicrobial resistance (AMR) and food safety issues, plus increasing household and national socioeconomic losses from FMD. There is an urgent need for a therapeutic with clear animal welfare benefits, no AMR risks; one that encourages the reporting of clinical cases to authorities by farmers seeking an effective treatment for their valuable livestock.

Material and Methods: Opportunistic clinical studies were conducted during field FMD outbreaks in Laos and Cameroon in 2019, using a farmer applied 'spray-on' wound formulation, developed and registered in Australia for provision of analgesia, antisepsis and reduced healing times in animals undergoing routine husbandry procedures (Tri-Solfen®, Animal Ethics Pty Ltd, Australia). Study sites were villages with naturally occurring FMD outbreaks, occurring in April 2019 near Luang Prabang in Laos and November 2019 near Ngoundere in Cameroon. Animals treated included cattle and buffalo (n = 136) in Laos and cattle (n = 40) in Cameroon, all with clinical vesicular lesions of FMD. The therapy (Tri-Solfen®) was applied liberally (1-2mls per lesion) to all the oral and pedal vesicular lesions in the FMD-affected animals in Laos. In Cameroon, some pedal lesions were left untreated to compare healing rates and additional cohorts of FMD-affected animals were treated with a parenteral oxyteracycline antibiotic (n = 12; Moore Oxy®, Nigeria) or left untreated (n = 69), enabling comparisons of response to therapy of the pain relief (Tri-Solfen®) treated, antimicrobial treated and untreated animals. In addition to clinical observations during and following treatment, follow-up surveys involving questionnaires were conducted in both countries with the owners of treated animals approximately a week to 10 days following the clinical studies.

Results: There was a rapid response to treatment observed as a marked improvement in the demeanour of the treated animals and immediate unanimous approval of the efficacy of treatment by the livestock owners. The surveys confirmed that: oral lesions generally healed faster than feet lesions; that all topical pain-relief treated lesions generally healed within a week of treatment; in animals only treated with an antimicrobial agent (Cameroon) lesions healed after a week or more and longer for untreated animals; the appetite score was higher for pain-relief treated cases than with the antimicrobial treatment and untreated animals; pain during walking was relieved faster with topical pain relief medication compared to those treated with the antimicrobial agent and untreated counterparts; and in both countries, farmers reported a 100% appreciation for the pain relief product and were keen to have it available for general use. This product contains two topical anaesthetics (lignocaine and bupivacaine), adrenalin, and cetrimide in a gel matrix. As no antimicrobial agents are present, use of the product reduces AMR risk and with a pH of ~2.7 it is potentially viricidal and may reduce environmental transmission of FMDV if used prior to or during rupture of vesicles. Evidence from depletion studies suggests that with-holding periods of 4 days for meat and 3 days for milk will minimise any risk of tissue residues.

Conclusion: These clinical trials indicate that this novel pain relief therapeutic provided effective pain relief through blockage of nociception and ease of application and coverage encouraged the rapid repair of lesions. wounds. This may be an important intervention for improving animal welfare in FMD, potentially encouraging livestock farmers to report outbreaks as they seek supplies of a 'new medicine that works' as it provides readily visible amelioration of suffering. Sustainable FMD control requires improved surveillance and biosecurity practices, effective public awareness campaigns to encourage com-

pliance, plus in many instances, effective strategic vaccination programs. However, access to an efficacious and affordable therapy that contains no antimicrobial agents and is likely to be viricidal, is important for future FMD control programmes if it: encourages farmer reporting; decreases virus transmission; substantially improves animal welfare; and reduces AMR risk.

Keywords: FMD, therapy, welfare, AMR, cattle.

ID-43

Molecular typing of Mannheimia haemolytica isolates from UK cattle surveillance submissions

Colin Mason[1], Jane Errington[1], Geoff Foster[2], Jennifer Thacker[3], Oliver Grace[3], Katharine Baxter-Smith[4].

[1]SRUC Veterinary Services, St Marys Industrial Estate, Dumfries, United Kingdom; [2]SRUC Veterinary Services, Drummondhill, Inverness, United Kingdom; [3]SRUC Veterinary Services, Pentlands Science Park, Penicuik, United Kingdom; [4]MSD Animal Health, Milton Keynes, United Kingdom.

Objectives: *Mannheimia haemolytica* is an important and commonly recognised cause of respiratory disease in cattle and sheep worldwide. *M. haemolytica* serotypes A1, A2 and A6 are considered the most prevalent in cattle worldwide (Al-Ghamdi et al 2000) and are readily isolated from the nasopharynx of healthy cattle. Serotypes A1 and A6 have been reported as common isolates from pneumonic lung tissue (Klima et al 2014). *M. haemolytica* serotype A2 is considered primarily associated with disease in sheep (Fodor et al 1984). The aim of this study was to survey *M. haemolytica* isolates derived from pneumonia cases in the UK that were submitted for diagnostic investigation. Understanding the diversity of serotypes in the sample set is of use to inform future preventative strategies.

Materials and Methods: 100 *M. haemolytica* isolates derived from bovine clinical pathology and post mortem samples from pneumonia cases were tested using a multiplex PCR assay incorporating three serotype specific primer pairs for identification of *M. haemolytica* serotypes A1, A2 and A6 (Klima at al 2017). Primary bacterial cultures were made on Columbia sheep blood agar following 18-24 hours incubation at 37°C in capnophilic conditions, identification was confirmed using routine phenotypic tests and isolates were stored at -80°C.

Isolates were selected from cases which occurred between 2016 and 2018. Isolates were from nasopharyngeal swabs (17 isolates) or broncho-alveolar lavage fluid (2 samples) submitted from cases with a recorded clinical history of respiratory disease. The remainder were from post mortem samples (81 isolates) with a clinical history of respiratory disease and consistent gross pathology. Sample selection was not random or unbiased.

Results: 45% isolates were *M. haemolytica* serotype A1, 30% serotype A2, 18% serotype A6 and 7% un-typable using the techniques employed. Isolates were recovered from animals aged between 1 day and 8 years old and an equal proportion of male and female animals. 70% of isolates were derived from animals reared for beef production and 30% from animals reared for dairy production.

Conclusions: The finding that *M. haemolytica* serotype A1 is the most common serotype in this UK sample set is consistent with other studies looking at European isolates (Andrés-Lasheras 2019). *M. haemolytica* serotype A2 has been reported in North America as a commensal organism in cattle. 97% of the *M. haemolytica* serotype A2 isolates were isolated from pneumonic lung tissue. In 60% of the cases from which *M. haemolytica* serotype A2 was isolated, no other respiratory pathogens were detected. These findings do suggest a potential, causal role for *M. haemolytica* serotype A2 in bovine respiratory disease. In the UK there is only one vaccine which has been shown to protect or cross protect against *M. haemolytica* serotypes A1 and A6, but not A2.

References:

1. Andrés-Lasheras S, Zaheer R, Klima C, Sanderson H, Ortega Polo R, Milani M, Vertenten G and McAllister TA. (2019) Serotyping and antimicrobial resistance of Mannheimia haemolytica strains from European cattle with bovine respiratory disease. Research in Veterinary Science 124, 10-12.
2. Al-Ghamdi G.M., Ames T.R., Baker J.C., Walker R., Chase C.C., Frank G.H., Maheswaran S.K. (2000) Serotyping of Mannheimia (Pasteurella) haemolytica isolates from the upper Midwest United States. Journal of Veterinary Diagnostic Investigation 12, 576–578.
3. Fodor L., Varga J., Hajtos I., Szemeredi G. (1984) Serotypes of Pasteurella haemolytica isolated from sheep, goats and calves. Zoonoses Public Health 31, 466–469.
4. Klima C.L., Alexander T.W., Hendrick S., McAllister T.A. (2014) Characterization of Mannheimia haemolytica isolated from feedlot cattle that were healthy or treated for bovine respiratory disease. Canadian Journal of Veterinary Research 78, 38–45.
5. Klima C.L., Zaheer R., Briggs R.E., McAllister T.A., 2017 A multiplex PCR assay for molecular capsular serotyping of Mannheimia haemolytica serotypes 1, 2, and 6. Journal of Microbiological Methods 139, 155-160.

Acknowledgements: This study was funded by MSD animal health. SRUC Veterinary Services disease surveillance programme is funded by the Scottish Government.

Keywords: Mannheimia haemolytica, Cattle, Serotype.

ID-44

Leptospira hardjo re-introduction in a certified-free herd in the Netherlands

Katrien Van Den Brink, Marian Aalberts, Linda Van Wuijckhuise.

Royal GD, Deventer, Netherlands.

Objectives: *Leptospira* serovar hardjo (*L.* hardjo) is a zoonosis and cattle are the main carrier. The Netherlands is the only country in the world with a *L.* hardjo-free control program. In 1990 the estimated herd prevalence in dairy herds was 25%. Since 2005 only farms with a *L.* hardjo-free status are allowed to deliver milk to the dairy industry. The Dutch dairy population is free of *L.* hardjo, with very sporadic cases. The herd prevalence among non-dairy herds is 0,8%, based on national screening in 2013. Monitoring for re-introduction is necessary, as for many different reasons increasing numbers of cattle are purchased by Dutch farmers: in 2019 over 42,000 cattle were introduced from non-free herds as compared to on average around 6,000 in 2013. A considerable part of these cattle are imported.

This case report presents the results of a follow-up study after re-introduction of *L.* hardjo in a previously free herd.

Materials and methods: The *L.* hardjo-free control program is carried out by Royal GD. Herds are assigned the *L.* hardjo-free status after initial assessment consisting of testing the sera of all individual animals on the farm. Surveillance of *L.* hardjo-free herds is based on bulk milk samples every four months, serum-testing aborted cattle, monitoring cattle movement data and testing sera from cattle introduced from non-free herds. Bulk milk samples and sera are tested with an indirect *L.* hardjo antibody ELISA (Thermo Fisher Scientific).

In the event of a positive or inconclusive antibody test result, further testing is mandatory and the farm loses the *L.* hardjo-free status and receives the status 'unknown'. In case of an introduced animal with non-negative test results, the animal needs to be removed from the herd. Four weeks later, a confirmative bulk milk sample is taken, to test for spread of *L.* hardjo in the herd.

If control samples are *L.* hardjo antibody positive, individual testing of all the animals in the herd is mandatory to investigate spreading of *L.* hardjo. If spreading is confirmed, all animals in the herd are treated with an antibiotic and the herd status is changed to 'treated'.

To monitor for spread of *L.* hardjo in treated herds, a seronegative tracer group of animals > two years old are serum tested every six months.

Results: The 120 head dairy herd was *L.* hardjo-free since 1999. In the last control bulk milk sample of June 8th 2019, no antibodies for *L.* hardjo were detected.

On June 28th 2019, thirteen cows were imported from Luxembourg and introduced into the herd without a quarantine period. Mandatory testing of sera from these cows, taken on July 18th 2019, resulted in one inconclusive and twelve sero-negative test results. A second serum sample of the animal that tested inconclusive, was inconclusive as well. This animal was removed from the herd on August 15th 2019.

Four weeks later, a bulk milk sample tested positive, which was confirmed by another bulk milk sample. Subsequently, sera were taken from all cattle in the herd on October 4th 2019. Multiple animals had antibodies for *L.* hardjo, also animals that were not imported. After the confirmed spreading of *L.* hardjo, all cattle on the farm were treated with a single high dose of dihydrostreptomycin (25 mg/kg body weight, intramuscular).

The seropositive animals showed no clinical signs of *L.* hardjo infection. At the herd level, milk production was lower than expected with an increase in clinical mastitis cases.

Conclusions: In 2019, a *L.* hardjo infection was observed in a certified-free dairy herd in the Netherlands, after import of infected cattle from Luxembourg. As import of dairy cattle by Dutch farmers is increasing, import poses an increasing threat for the Dutch *L.* hardjo-free program.

Keywords: L. hardjo, control-program, bulk milk, import, re-introduction.

ID-45

Field Study of Control and Eradication of Endemic M.bovis in a Persistently Infected Dairy Herd using a Novel Testing Programme

Richard Sibley.

West Ridge Veterinary Practice, Witheridge, United Kingdom.

Objectives: A large dairy herd comprising 350 adult milking dairy cows and 700 youngstock and beef animals was enrolled in a novel management programme to attempt to eradicate M.bovis from the herd by managing biosecurity and biocontainment within the herd, concurrent with the statutory management of bovine tuberculosis by testing and culling.

The Gatcombe Herd had been persistently infected with tuberculosis for over 7 years, with the statutory single intradermal comparative cervical tuberculin test (SICCT) identifying over 100 reactors which were immediately culled in accordance with the statutory control regulations. Despite strict biosecurity during this time, the statutory controls failed to remove infection from the herd.

An assumption was made that infection was circulating in the herd, with cows shedding M.bovis without be identified by the SICCT, and thus transmitting the infection to susceptible cattle. The objective of the study was to prevent new infections from shedding cattle by management and husbandry.

Materials and methods: 192 cattle were identified and categorised in the herd using the staturoty Single Intradermal Comparative Cervical Tuberculin Test (SICCT) records to detect any animal that had ever had any form of reaction to the bovine tuberculin in its lifetime. Once categorised as high risk, these animals were repeatedly tested for the presence of M.bovis after each SICCT, which was performed every 60 days in accordance with the regulatory testing programme. 154 of these high risk animals were repeatedly tested for the presence of M.bovis in blood and faeces using viral phage testing and qPCR.

Results: 125 of the 154 high risk cows (81%) had at least one phage positive test, indicating that they were infected with M.bovis despite not being identified as reactors by the SICCT. 34 of these animals in the enhanced testing regime had at least one positive qPCR test on faeces, indicating shedding of the organism in their faeces.

Animals which were shown to be shedding were either culled or isolated to prevent transmission. High risk animals were prioritised for culling, but with economic performance

moderating culling decisions. All potential risks of transmission were identified and controlled by management and husbandry. Becasue of the unexpected high level of faecal shedding from some infected cows, risks of transmission were identified and managed to prevent further spread within the herd.

After three years of the control programme, the herd is now Officially TB Free and has had no SICCT reactors in the last 4 routine skin tests.

Conclusions: This study demonstrates the potential for controlling and eradicating M.bovis from persistently infected herds where endemic infection circulates within the herd and cannot be identified or controlled by the statutory control programmes that rely on the SICCT to identify infected animals.

The control programme allows the economic and meticulous removal of infection from the herd, and minimises the risk of re-introduction by minimising environmental contamination.

Keywords: Tuberculosis.

ID-46

Identification of potential microbial taxa involved in the efficacy of fecal microbiota transplantation (FMT) from healthy donors to recipients suffering from diarrhea in calves

Tomonori Nochi[1], Jahidul Islam[1], Masae Tanimizu[2], Yu Shimizu[2], Yoshiaki Goto[2], Natsuki Ohtani[2], Kentaro Sugiyama[2], Eriko Tatezaki[2], Masumi Sato[2], Eiji Makino[2], Toru Shimada[2], Chise Ueda[2], Ayumi Matsuo[1], Yoshihisa Suyama[1], Yoshifumi Sakai[1], Mutsumi Furukawa[1], Katsuki Usami[1], Hiroshi Yoneyama[1], Hisashi Aso[1], Hidekazu Tanaka[2].

[1]Tohoku University, Miyagi, Japan; [2]Chiba Prefectural Federated Agricultural Mutual Aid Association, Chiba, Japan.

Objectives: Calf diarrhea (CD) is a common disease and has an acute negative impact in the economy of the livestock industry. Antibiotics have been consistently used to treat the disease; however, fecal microbiome transplantation (FMT) has been attracting attention as an alternative therapy for CD. The objectives of this study were to characterize the fecal microbiota profile of healthy and diarrhea calves as well as potential donor selection for FMT using a machine learning approach.

Materials and method: Bacterial genomic DNA was extracted from feces of 156 calves, which were bred in different farms and classified as healthy (n=108) and diarrhea (n=48), and they were further compared with diarrhea calves used in an FMT study in which donors (n=20) and recipients (n=20) were enrolled. High throughput 16S rRNA amplicon sequencing for metagenomics was performed using a next-generation sequencer. In addition, a random forest (RF) prediction model was established, and a variable importance analysis was conducted with the aim of identifying microbial taxa those can be considered as potential predictors.

Results: The most abundant phylum in the faces of calves were *Firmicutes* and *Bacteriodetes*, which were found in healthy (51.17 % and 38.20 %) and diarrhea (46.03% and 31.91 %) groups, respectively. A principal coordinate analysis of the unweighted UniFrac distance matrix showed a significant difference between healthy and diarrhea calves. Specifically, in the RF model *Campylobacter*, *Actinobacillus*, and *Sporobacter* were identified based on the mean decrease in accuracy. *Campylobactor*, *Sporobacter*, and *Streptococcus* were identified as the most discriminating predictors based on the mean decrease in the gini criteria. Considering microbial abundance, *Sporobacter* was abundant in the overall healthy and donor groups for successful FMT. Along with RF, LEfSe analysis was subsequently performed among these groups, in which *Sporobacter* was found to be abundant in the healthy group. Taken together, these results suggest that *Sporobacter* may be a potential biomarker for the donors associated with FMT success.

Conclusion: FMT is an effective treatment option for the prevention of diarrhea in calves, specifically by identifying a beneficial microbial cluster. These findings have enormous significance for the livestock industry because FMT could eventually address the challenge of CD treatment as well as the use of excessive antibiotics.

Keywords: Diarrhea, fecal microbiome transplantation, calves, metagenomics.

ID-47

Farmer psychosocial factors associated with bovine viral diarrhoea control behaviours

Naomi Prosser[1], Martin Green[1], Eamonn Ferguson[2], Michael Tildesley[3], Edward Hill[3], Matt Keeling[3], Jasmeet Kaler[1].

[1]University of Nottingham, Sutton Bongington, Leicestershire, United Kingdom; [2]University of Nottingham, Nottingham, United Kingdom; [3]University of Warwick, Coventry, United Kingdom.

Objectives: Psychosocial factors are important for the uptake of health protective behaviours in human health, however many have not been investigated in the context of livestock disease control where the farmer is making decisions for their livestock. There are diverse approaches farmers can take to control bovine viral diarrhoea (BVD) in their herds, from BVD-specific vaccination, testing and culling to the more general prevention of the introduction of infectious diseases from other cattle (such as by having a closed herd, isolating new cattle and preventing contact with neighbouring cattle) and these may be influenced by psychosocial factors. This study investigated psychosocial and behaviour change factors in the context of BVD control methods used by cattle farmers.

Material and methods: A survey was completed by 475 UK cattle farmers in 2020. Multiple validated measures were used to investigate trust, psychological proximity (feeling close to another person), altruism and factors from a behaviour change framework (COM-B). The survey also investigated the methods of BVD control that the farmers used. Farmers were grouped by similar BVD control practices using latent class analysis, and a multinomial logistic regression model was used to investigate associations between the psychosocial and behaviour change factors and the BVD control classes.

Results: Farmers had the highest psychological proximity with (felt the closest to) their cows, followed by their vet, neighbouring farmers, the veterinary community, dairy farmers, beef farmers, the farming community, the National Farmers Union and the Government. Similarly, the farmers had the most trust in vets, followed by the NFU, other farmers and governmental organisations. The level of altruism among the farmers was similar to that of other people in general.

Farmers split into nine classes for how they controlled BVD from the latent class analysis. Similar classes were merged for further analysis resulting in five classes: 1) Does nothing (12%), 2) Vaccinates (25%), 3) Careful introducing new cattle (sources cattle from BVD-free herds and tests/isolates on arrival) (16%), 4) Vaccinates, careful introducing new cattle and prevents contact with neighbouring cattle (31%) and 5) Closed herd and prevents contact with neighbouring cattle (15%).

There were psychosocial differences between the farmers in each class that was using some BVD controls compared with the farmers who did nothing. Farmers who were vaccinating had higher psychological proximity with dairy farmers, lower psychological proximity with beef farmers and higher motivation to control disease (due to both automatic habits and emotions and reflective plans and goals). Farmers who were careful introducing new cattle had higher trust in other farmers. Farmers who were vaccinating, careful introducing new cattle and keeping them separate from neighbouring cattle had higher psychological proximity with their vet, lower trust in other farmers and had higher motivation to control disease. Finally, farmers who had a closed herd and were preventing contact with neighbouring cattle had lower trust in other farmers, higher psychological proximity to dairy farmers and lower psychological proximity to beef farmers, as well as being more likely to feel that they had enough knowledge and understanding of how and why to control infectious diseases and enough time and money to do so.

Conclusion: Various psychosocial factors were associated with the specific behaviours that farmers used to control BVD and understanding these will help veterinarians tailor their messages to encourage clients to take up disease control measures. Of particular relevance is that farmers with high psychological proximity with their vet were more likely to be proactively controlling BVD, which was more important here than the more extensively investigated trust. Therefore, it is important that vets work to ensure a close vet-farmer relationship to encourage proactive BVD control by farmers.

Keywords: Bovine viral diarrhoea, Infectious disease control, Farmer behaviour, Psychosocial factors.

ID-50

Prevalence of *Coxiella burnetii* in Central and Eastern European dairy herds

Attila Dobos[1], Miklos Gyuranecz[2], Zsuzsa Kreizinger[2], Aron Kovacs[2].

[1]Ceva, Budapest, Hungary; [2]Institute for Veterinary Medical Research, Centre for Agricultural Research, Budapest, Hungary.

Objectives: The aim of the present study was to assess the prevalence of *Coxiella burnetii* in different size of dairy herds in six Central and Eastern European countries based on enzyme-linked immunosorbet assay (ELISA) and real-time polymerase chain reaction (PCR) tests.

Material and methods: Bulk tank milk samples were collected from 370 dairy herds from six Central and Eastern European countries (Croatia, *n*= 13; Czech Republic, *n*= 138; Hungary, *n*= 126; Serbia, *n*= 24; Slovakia, *n*= 53; Slovenia, *n*= 16) between March and October, 2019. Samples were taken randomly from dairy herds of different size, but with focusing on larger dairies. Forty ml samples were taken from the bulk milk tanks and tested on indirect ELISA and real-time PCR targeting the IS1111.

Results: 1. The number of examined dairy herds varied according to the country of origin, but the overall *C. burnetii* infection status (percentage of positive herds/total number of herds with ELISA and PCR tests) ranged between 62.50-100.00% in the Central and Eastern European countries. Prevalence of *C. burnetii* differed according to the country of origin with Croatia showing 100.00%, the Czech Republic 98.55%, Hungary 97.61%, Serbia 70.83%, Slovakia 90.56% and Slovenia showing 62.50% average percentages of the positive herds. The analysis of the ELISA and PCR test results in association with herd sizes revealed, that herds of ≥250 animals showed significantly higher *C. burnetii* positivity (positive test results: 100%; Spearman's rank correlation, rho = 0.716, $p < 0.001$), than herds of <250 animals (positive test results: 73.03%). On the other hand, examining only the PCR test results, similar percentages of positive milk samples (40.63-44.94%) were detected among the herds of different sizes.

Conclusions: The present research assessed the prevalence of Q fever at dairy farms in Central and Eastern European countries, revealing increased seroprevalence in bulk tank milk samples compared to other European countries. Based on the analysis of the data, it is assumed that with growing numbers of animals in dairies and farm structures moving toward concentration, the risk of *C. burnetii* prevalence is increasing, underlining the importance of monitoring the herds' infection status and implementations of control measures

Keywords: Q-Fever,Central-Eastern Europe,Dairy cattle,Milk,Coxiella Burnetii.

ID-51

An Epidemic of Salmonellosis caused by Imported Rapeseed Meal Containing Salmonella at a Dairy Farm

Olli Ruoho.

Animal Health ETT, Seinäjoki, Finland.

Objectives: Finland has zero tolerance policy for salmonella. All Salmonella serotypes are considered to be a significant threat for public health. Zero tolerance means, that actions are always taken, if salmonella is detected from feed, animals or foodstuffs. The aim is to find the origin of infection or contamination and to prevent further spread. Within the

framework of the official Salmonella control program, lymph node and carcass swab samples are taken at slaughterhouses and raw milk is sampled at dairies. In addition, producers themselves take a large number of self-monitoring samples, e.g. in the context of animal trade. If salmonella is found on a cattle-, swine- or poultry farm, the farm is put under restrictions by the veterinary authorities and salmonella eradication must be started.

Materials and methods: A dairy farm in Western Finland was found to be positive for *Salmonella Infantis* in May 2019.

Heifers were to be sold from the farm and, hence, fecal samples were examined for Salmonella. The loose-housing dairy had 210 cows and 150 young animals. The farm was put under restrictions. This e.g. means, that animal movements to the farm an out of the farm are ceased. By special arrangements, the milk can be delivered to the dairy to be diverted to heat-treated products. The salmonella eradication was started immediately.

The principles of eradicating salmonella from the animals and the environment is to assure hygienic feed and water and to cut the contamination of feed with feces. The production facilities are cleaned and disinfected according to a separate plan. All animals were examined for salmonellosis by faecal samples. About 80 environmental swab samples were taken e.g. from feeding tables, water throughs, feeding equipment and feed storages. All samples were examined for Salmonella (ISO 6579:2017).

The animals were fed TMR (total mixed ration) with a TMR-wagon. TMR-feed itself and also each of its components were examined. The main component of the TMR-feed was pre-dried grass silage harvested from the own fields of the farm. In addition, the feed included rapeseed meal, minerals and farmed, ground barley and oats.

At first 25 % of the animals were salmonella positive. Some swab samples from feeding tables, feeding equipment and water throughs were positive but all feed was negative. The hygienic procedures were concentrated to the critical points found contaminated. Feeding tables and drinking throughs were disinfected twice a day and the wheels of the vehicles always before feeding. Feed handling and feeding equipment was cleaned and disinfected as well as possible.

Next month, however, 60 % of faecal samples were positive for salmonella. Hence, new samples from the environment and the feed were taken. Salmonella was detected in an environmental swab sample from the inner surface at the top of the rapeseed meal silo cover. This site had not been sampled before because a crane was needed for access. The rapeseed meal was imported from abroad. It was not heat-treated but tested on a sample of 1 bulk sample / 25,000 kg with negative result.

The silo was emptied, washed and disinfected. Rapeseed meal was excluded from animal nutrition and replaced by industrial heat-treated protein feed. The hygienic measures proceeded.

Results: The eradication procedure was monitored by faecal and environmental swab samples every second or fourth week. The animals got rid of salmonella in four months, as confirmed by two successive monthly faecal samples from the animals and one environmental swab sampling. Hence, the restrictions could be released.

Conclusions: The use of untreated rapeseed meal in animal feeding involves a risk of salmonella that cannot be completely eliminated by sampling. Contamination does not necessarily occur in feed samples alone but requires extensive swab and dust sampling of the feed environment throughout the whole feeding system. Feed storages and feeders may contain salmonella inoculants which, when mixed with feed, can cause infection in the herd.

Keywords: Salmonella, epidemic, rapeseed, dairy, farm.

ID-52

Facilitating the diagnosis of Q fever using FTA cards to store and ship bulk tank milk samples

Michaël Treilles[1], Pierre Charollais[1], Raphaël Guatteo[2], Carla Azevedo[3], Damien Achard[3], Juan Munoz-Bielsa[3], Philippe Gisbert[3].

[1]Laboratoire Qualyse, Champdeniers, France; [2]INRAE, Oniris, BIOEPAR, Nantes, France; [3]Ceva Santé Animale, Libourne, France.

Objectives: Coxiellosis, also named Q fever, is an infectious disease caused by an intra-cellular bacterium: *Coxiella burnetii* (**Cb**). In cattle, Q fever, when symptomatic, is mainly responsible for abortions, stillbirth, birth of weak calves, retained placenta and metritis/endometritis. But it is also a zoonotic disease, ruminants being the main reservoir of Cb.

Bulk tank milk (**BTM**) is an easy, inexpensive, and representative sample to detect Cb infections in dairy herds using RT-PCR. But one major limitation under field conditions is the need to deliver the BTM samples in adequate conditions (quickly, refrigerated and safely) to a qualified laboratory. In addition, sending non-inactivated biological material via regular post may be forbidden. A new innovative, easy, and accurate diagnostic tool (**QTest**) for Q fever was developed to overcome these constraints. Farmers or veterinarians simply place some drops of BTM on a WHATMAN FTA Elute Micro Card (**FTA card**) and let it dry before posting the card to the laboratory.

The objective of this study was to validate the reliability of this innovative technique.

Material and methods: This study had two complementary objectives, carried out in two steps.

The first step aimed at assessing the preservation of Cb DNA detection from BTM spotted on a FTA card over time under two different temperatures (20-22°C and 37°C) to mimic field conditions. A milk sample was artificially contaminated with Cb to reach a load of ~5x10^6 Genome Equivalent (GE)/mL which became the master sample. The master sample was then successively diluted at different dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}). Each diluted milk was then sampled several times on FTA cards and stored for up to 29 days either at 20-22°C or at 37°C. RT-PCR was performed for each dilution and each storage temperature on days 1, 4, 6, 8, 11, 15, 20 and 29.

The second step aimed at comparing, after ageing, the detection of Cb DNA by RT-PCR either when directly applied

on BTM or on BTM preserved on FTA cards. 70 BTM field samples previously tested positive for Cb by RT-PCR were stored as raw milk for 17 days before extraction or inoculated onto FTA card on day 15 and extracted on day 21. On day 21, a RT-PCR was performed for all the samples (raw milk and FTA card) and the results were compared between the two techniques for each sample.

Results: The first step showed that regardless of the duration of FTA card storage, all samples with a dilution below 10^{-3} (approximately 10^3 GE/mL) were detected to contain Cb DNA. Also, no significant loss of detectability was noted from d1 to d29, regardless of dilution or storage temperature. This means that the FTA card system ensures a stable preservation of Cb DNA in BTM samples stored at 20-22°C and 37 °C for at least 29 days.

For the second step, of the original 70 positive samples, we had 58 samples that were tested positive using one of both of the storage option. Of these 58 samples, 45 raw BTM samples tested positive and, of these, five tested negative when using FTA cards. In other words, there were 13 false negatives with older raw BTM samples while there were only 5 false negatives for older BTM on FTA cards. These five remaining were all with Ct value >35 indicating low quantities of Cb DNA. We can assume that the non-detection was likely due to the lack of reproducibility of the PCR technique for weak positive samples.

Importantly, for 13 samples, FTA cards produced positive PCR results while the equivalent raw BTM samples tested gave negative PCR results. This indicates that the detection rate was higher using FTA cards with aged BTM (91.4%) than with raw aged BTM (77.6%) samples.

Conclusion: Our study showed that the use of QTest makes BTM sampling, shipment, and storage very easy and cheap, while results do not seem to be impaired by the preservation/transportation method. Indeed, the stability of Cb DNA on an FTA card is maintained for at least 29 days at either 20-22°C or 37 °C. Therefore, this technique would facilitate an easier and more practical approach to diagnosis of Q fever at herd level and would be supportive of Q fever control strategies.

Keywords: Q fever, Coxiella burnetii, bulk tank milk, diagnosis, PCR.

ID-53

Towards the control of sheep associated malignant catarrhal fever (SA-MCF) in bison and buffalo in Wales, UK

Joseph Angell[1], Eleanor Bentley[2], James Stewart[2].

[1]Wern Vets CYF, Ruthin, United Kingdom; [2]The University of Liverpool, Liverpool, United Kingdom.

Introduction and background: Bison and buffalo are not commonly farmed in the UK, although they are considered an option for diversification due to the high value of the animal products. In the UK, a significant limiting factor for bison and buffalo production is the high susceptibility of these animals to sheep associated malignant catarrhal fever (SA-MCF) caused by ovine herpes virus-2 (OvHV-2) and strategies to limit this disease risk are essential to sustainable production.

Objectives: The aim of this project was to control SA-MCF in bison and buffalo in Wales, UK.

Objective 1: determine the exposure of farmed bison and buffalo to OvHV-2.

Objective 2: determine the presence/absence of OvHV-2 in species in contact with the bison/buffalo.

Objective 3: determine whether the bison/buffalo have been exposed to other infectious diseases that could potentially increase the risk of MCF following exposure to OvHV-2.

Objective 4: utilise a novel vector vaccine (Macavax) as part of a control programme.

Materials and Methods: Two farms were included in the project: 1) a mixed species farm with bison, cattle, sheep, poultry and deer; 2) a farm with buffalo and sheep.

Blood/tissue samples were obtained from a sample of the bison, cattle, sheep and deer from farm 1 and blood samples were obtained from a sample of the buffalo and sheep from farm 2.

qPCR was used to identify OvHV-2 on blood and tissue samples and commercial antibody tests were used to determine exposure to bovine viral diarrhoea virus (BVD), infectious bovine rhinotracheitis virus (IBR), *Mycobacterium avium* subspecies *paratuberculosis*, Neospora caninum and *Mycoplasma bovis*. Faeces were examined for the presence of gastrointestinal nematode eggs, *Fasciola hepatica* eggs and lungworm larvae.

Trace element analysis was carried out using commercially available tests.

Control strategies were deployed specific to the farms with a novel vector vaccine (Macavax) utilised on farm 1.

The farms were monitored for 18 months to determine the efficacy of the control strategies.

Results: Farm 1: no OvHv-2 was detected at the start of the project, however several deaths in the preceding years were confirmed as SA-MCF. The bison had evidence of previous exposure to IBR, *N. caninum* and *Mycoplasma bovis*, gastrointestinal nematodes and *Fasciola hepatica*. Copper, selenium and iodine deficiencies were also detected. The Macavax vaccine was administered twice to bison originating from the farm and once to new herd entrants that joined the herd after one year. No adverse events were observed. The health of the herd improved overall, however one death was recorded after 18 months with SA-MCF confirmed by postmortem and virus detection.

Farm 2: 4/19 (21.1%) of the buffalo had OvHV-2 detected by qPCR. 1/10 sheep also had OvHV-2 detected by qPCR. The buffalo had evidence of previous exposure to IBR and *Mycoplasma bovis*, with marginal trace element deficiencies. No deaths associated with MCF were observed prior to or during the study period.

Conclusions: Bison and buffalo can be farmed successfully in the UK, provided their health and welfare needs are met appropriately. Various commercial strategies already in place for cattle may be used in a similar way in these species with appropriate amendments where necessary. Where

possible the bison and buffalo should be kept as far away as possible from other species to reduce the risk of transmission of infectious diseases, especially OvHV-2. The novel vector vaccine (Macavax) was safe to use in bison, although further work as to the specific extent of its efficacy is needed.

Keywords: Bison, buffalo, malignant catarrhal fever, MCF, control.

ID-54

Detection rates of primary abortifacient pathogens isolated by conventional microbiology from dairy and suckler foetal submissions, 2020-2021

Ciara Hayes[1], John F Mee[2], Catherine McAloon[3], Bryan Markey[3], Miriam Casey[3], Elisabeth Innes[4], Cosme Sanchez[1].

[1]Department of Agriculture, Food and the Marine, Cork, Republic of Ireland; [2]Teagasc, Animal Bioscience Research Department, Fermoy, Republic of Ireland; [3]University College Dublin, Dublin, Republic of Ireland; [4]Moredun Research Institute, Penicuik, United Kingdom.

Objectives: The overall study objective was to establish the national prevalence of infectious abortifacients, including *Coxiella burnettii*, *Chlamydia abortus* and *Mycoplasma bovis*, in bovine foetal material submitted to the Irish Veterinary Laboratories Service (VLS) during the main (winter-spring) calving season 2020/2021. This abstract describes the sample set, available data and the results of routine foetal and placental cultures.

Materials and Methods: Sampling of bovine foetal material (abortions and stillbirths +/- placentae) was carried out between October 2020 and May 2021 at the six Regional Veterinary Laboratories (RVLs) of the VLS. This sample collection interval was chosen to span the period over which most abortions and stillbirths occur in Irish seasonal, grass-based dairy and suckler herds.

On voluntary submission of a bovine foetus to any of the six RVLs, relevant clinical history was taken from the herd owner/keeper, including details of the herd, the foetus/es and dam. Only foetuses with uninflated lungs were enrolled in the study. Straight crown-rump length (sCRL) was measured. The foetus and placenta, if available, were examined for gross abnormalities. A sample of foetal stomach content and a swab of placenta were collected and immediately plated on blood, *Brucella* and XLD agar. Blood and *Brucella* agar plates were incubated in 8% CO_2 at 37°C. XLD agar was incubated in aerobically at 37°C. The sample was also plated on Sabouraud's agar if requested by the investigating research officer. Plates were examined daily for seven days. Up to 25ml of additional foetal stomach content, a pooled sample of lung, liver and spleen and a sample of placenta (if available) were also collected and frozen pending further processing and testing. Lung, liver, midbrain and placenta were fixed in formalin. After five days, the fixed tissues were cut and placed in cassettes. These were then stored in wax blocks until further processing.

Data were extracted from the VLS Laboratory Information Management System and processed using Microsoft Excel and R Studio.

Results: The foetal carcasses originated from 855 individual herds with number of submissions per herd over the sample period ranging from 1 to 8. Herd size ranged from 1 to 750 with a median of 110.

In total, 1181 entire foetal carcasses were examined with (305) or without (876) placenta. The median, minimum and maximum sCRL of the foetuses was 75, 22 and 130cm, respectively. This implied median, minimum and maximum gestational ages of approximately 223 days, 108 days and full term, respectively, according to the formula:

DAY=8.4+0.087CROWN-RUMP+5.46CROWN-RUMP

Of the 1061 stomach content samples cultured, primary pathogens were detected in 281 (26.4%); *Trueperella pyogenes* (124), *Salmonella* Dublin (69), other *Salmonella* species (6*), Bacillus licheniformis* (31), *Listeria monocytogenes* (42) and *Aspergillus* species (9). All *Brucella abortus* cultures were negative.

Of the 186 placentae cultured, primary pathogens were detected in 55 (29.6%); *Salmonella* Dublin (22), *Bacillus licheniformis* (21), *Trueperella pyogenes* (12), *Listeria monocytogenes* (4) and *Aspergillus* species (2). Two primary pathogens were cultured from the same placental sample in six cases.

Various other bacterial and fungal species were isolated from cultures of placenta and foetal stomach content, thought to have a secondary role (i.e. opportunistic pathogens, capable of foetal or placental infection only under certain pre-existing conditions) in the pathogenesis of bovine abortion and stillbirth.

Conclusions: These results indicate that approximately 25-30% of bovine foetal mortality in this national cattle population could be attributed to primary (mainly bacterial) abortifacients detectable using routine culture methods. This raises the question as to the causes of the remaining cases, an international diagnostic challenge. To address this challenge, the sample set collected in this study will be used to quantify the role of other, less commonly tested for, primary pathogens such as *Neospora caninum*, *Coxiella burnettii, Chlamydia abortus* and *Mycoplasma bovis*.

Keywords: Bovine abortion, abortifacient pathogens.

ID-55

Prevalence of major enteric pathogens in Turkish dairy calves

Dogus Palavan Ozkan, Barlas Bozyegit, Suna Simsek.

MSD Animal Health, Istanbul, Turkey.

Objective: The aim of the study was to measure the prevalence of the most important causes of diarrhea: *E. coli* K99, Rotavirus, Coronavirus, *Cryptosporidium spp*. and *Clostridium perfringens* in dairy farms located in 5 geographic regions of Turkey.

Materials and Methods: The study has been performed

between February 2015 and November 2017 in 24 dairy farms located in Aegean, Mediterranean, Southeastern Anatolia, Central Anatolia and Marmara.

280 feces samples were collected from 1 to 45 days old calves with diarrhea. Most of them had still suckling reflex and watery to creamy feces. An on-farm diagnostic test was used to identify the 5 pathogens of interest in calves with diarrhea (Rainbow Test Bio-K306, Bio-X Diagnostics, Belgium).

Results: At least one pathogen was identified in 244 calves from 280 diarrheic calves (87.15%). In 75 of the 244 positive samples, more than 1 pathogen were identified (30.74%). In total 338 pathogens were identified in positive feces samples. The most prevalent pathogen was *Cryptosporidium* with 154 cases (45.6%), then, 87 cases (25.7%) of Rotavirus, 85 (25.1%) of *C. perfringens*, 10 (3.0%) of *E. coli*, 2 (0,6%) of Coronavirus. In 94 (61%) of the 154 samples positive for *Cryptosporidium* no other pathogen was identified. A *Cryptosporidium* positive mix-infected sample was mostly positive for Rotavirus as well (n=35, 23%). *Cryptosporidium* prevalence was highest in the Aegean region (84%), followed by Mediterranean (71,8%) and Southeastern Anatolia (70%).

Conclusions: In this study *Cryptosporidium* spp. and Rotavirus are the predominant agents causing neonatal diarrhea in the respective Turkish dairy herds. Effective control and prevention of *Cryptosporidium* spp. and Rotavirus should be the major focus points in the herds to reduce the prevalence of diarrhea. Vaccination of the pregnant cows with a neonatal calf diarrhea vaccine and proper colostrum administration may help to reduce the prevalence of rotavirus.

Keywords: Calf scour, enteric pathogens, Turkey, prevalence.

ID-56

Scottish BVDV spot tests: Why do we get single animals testing positive and what is their significance?

Helen Carty[1], George Caldow[2], Rachel O'Connor[3].

[1]SRUC Veterinary Services, Ayr, United Kingdom; [2]SRUC Veterinary Services, Edinburgh, United Kingdom; [3]SRUC, Ayr, United Kingdom.

Objectives: Young stock spot tests are used to determine if a herd has current evidence of exposure to bovine viral diarrhoea virus (BVDV). Serum samples from a representative proportion of young stock are tested for the presence of antibodies to BVDV. Within the Scottish eradication programme, typically five animals per management group are tested. If one animal in the group tests positive for antibodies to BVDV, the herd is deemed to be "not-negative" for BVDV. Movement restrictions are placed on herds that are "not-negative" for BVDV. This study aims to determine the reasons for and significance of single antibody positive results in Scotland over a one year testing period.

Material & Methods: 4728 young stock spot tests from Scottish herds were analysed for the presence of BVDV antibodies by Scotland's Rural Colleges (SRUC) Veterinary Services between 1st October 2017 and 30th September 2018. The spot tests were divided into one of three categories: (1) all animals tested negative for antibodies to BVDV; (2) more than 20% of the animals tested positive for antibodies to BVDV or (3) 20% or less of the animals tested positive for antibodies to BVDV. The data and notes from the spot tests in the third category were analysed to determine if there was a clear reason for low numbers (usually a single animal in a group of five) testing positive for antibodies to BVDV. Retest results, herd status for that year's calf crop and the subsequent calf crop were also assessed to determine the significance of these results.

Results: 4290 of the spot tests were in category 1, 209 were in category 2 and 229 were in category 3 (20% or less of the animals tested positive for antibodies to BVDV). After further data cleansing to remove inappropriate tests (those that were originally booked in as spot tests but in fact were tested for another reason), 216 spot tests in category 3 from 192 Scottish herds were used for this study. 40% of these spot tests were identified as having avoidable reasons i.e. use of vaccination, maternally derived antibody or incorrect calf tested. Following review of herd information, and in some cases retesting, 56% of the herds were assigned a "negative" status for the year and 44% a "not-negative" status. The subsequent calf crops from these herds were also tested, where applicable, and following analysis of those results 80% had a "negative" status, 17% a "not-negative" status and 3% had been declared as non-breeding herds. Animals persistently infected with BVDV were identified in three herds, despite apparent limited evidence of exposure to BVDV in the initial spot test.

Conclusion: 5% of spot tests carried out in the study period identified 20% or less of the tested animals as positive for antibodies to BVDV. 40% of these however had a clear, avoidable explanation. The results of this study can therefore be used to give clearer, evidence based guidance to minimise mistakes and misleading results with the spot test. The significance of these results on the herd status in the current and subsequent calf crops can also inform future policy on whether the cut-point for determining herd status should be changed.

Keywords: BVDV, eradication, testing, antibody.

ID-57

Bovine abortions in southern Belgium : 10 years of results

Delooz Laurent[1], Gregoire Fabien[1], Saulmont Marc[1], Saegerman Claude[2].

[1]ARSIA, Ciney, Belgium; [2]ULiege FMV UREAR, Liège, Belgium.

Since 10 years in Belgium, a national surveillance programme based on the compulsory reporting of abortions and subsequent analyses on their products reached several objectives including official surveillance of bovine brucellosis but also the monitoring of other bovine abortive diseases.

Some endemic, emerging or re-emerging pathogens could be identified, including *Brucella* spp., *Salmonella* spp., *Listeria monocytogenes, Campylobacte*r spp., *Coxiella burnetii, Leptospira* spp., Mycotic agents, Bovine viral diarrhoea virus,

Bovine herpesvirus 4, Bluetongue virus serotype 8, Schmallenberg virus, *Neospora caninum*.

In the context of the Belgian passive surveillance programme for bovine brucellosis, 46.967 bovine abortion's cases were collected from January 2010 to December 2019. They are originated from 6894 cattle farms distributed among the five Walloon provinces. In around 90% of cases, the fetus, the maternal serum and the placenta are collected and analyzed. These samples allowed a wide range of analyzes and an autopsy of the fetuses in the large majority of cases. In order to maximize the etiological diagnosis rate, a standard and systematic analysis protocol has been implemented.

To determine the cause of abortion, it is necessary to identify an abortive pathogen or congenital lesions incompatible with life. The etiologies of the abortions can be identified in approximately 30% of cases. In almost 20% of cases, an opportunistic pathogen is identified. This means that in 50% of the cases, no cause of infectious abortion could be identified. In order to improve the detection of abortive pathogens in herds with outbreaks of abortions without diagnosed etiology using conventional diagnostic methods (culture, PCR, etc.), the use of 16S RNA sequencing provides a definite advantage. Thanks to this new technology, several bacteria whose culture is complex and for which systematic PCR analysis would be too costly have been highlighted. Over 400 fetuses have been analyzed and bacteria such as *Ureaplasma diversum* have been diagnosed.

Standardizing the analysis protocol and improving it when no cause can be identified makes it possible to obtain a high rate of etiological diagnosis. The results obtained on more than forty thousand abortion's cases are an important source of information for the epidemiology and the surveillance of animal diseases in Belgium.

Keywords: Abortion, Cattle, Belgium, Diagnosis.

ID-58

Metagenomic sequencing of the respiratory virome in beef-suckler weanlings diagnosed with bovine respiratory disease

Kerrie Ní Dhufaigh[1], Matthew McCabe[1], Paul Cormican[2], Inmaculada Cuevas Gómez[2], Mark McGee[2], Tara McDaneld[3], Bernadette Earley[2].

[1]Teagasc, Animal Bioscience Building, Grange, Republic of Ireland; [2]Teagasc, Animal Bioscience Building, Dunsany, Republic of Ireland; [3]U.S Meat Animal Research Center, Clay center, NE, United States.

Objectives: Bovine respiratory disease (BRD) is a global cause of morbidity and mortality of cattle placed in feedlots, despite decades of development and application of vaccines and antimicrobials. The study objective was to compare untargeted viral metagenomic sequencing (on the Oxford Nanopore Technologies MinION and Illumina NovaSeq) and targeted species specific qPCR for characterisation of the upper nasal virome in clinically-diagnosed BRD and healthy beef-suckler weanlings on the day of BRD detection (day-BRD).

Materials and Methods: One hundred and fifty-three beef-sucker weanlings (209 days old [SD: 35.8] and 306 kg [SD: 26.3]) were purchased through auction marts, transported by road, and housed indoors for the duration of the study (Cuevas-Gómez *et al.,* 2020). Weanlings were vaccinated 24 h after arrival against *Clostridia* and against three known BRD-associated viruses (BoHV1, BRSV, BPI3) and one BRD-associated bacteria *M. haemolytica*. Sterile flocked swabs were inserted approximately 12 cm into the nasopharynx of each calf and gently rotated on the day of BRD diagnosis. Thirty animals with BRD and 30 matched healthy control animals were selected for virome sequencing. Viral nucleic acid (protected by nuclease-resistant capsids) was enriched by bead-beating and nuclease treatment. DNA and RNA was extracted and purified using the Qiagen MinElute Virus Spin kit. Double-stranded cDNA was generated then aliquoted and stored at -80°C until required. One aliquot of double-stranded cDNA was sent to a sequencing provider (CD genomics) where quality of DNA was assessed by Qubit fluorometry and agarose gel electrophoresis. Sequencing libraries were prepared using VAHTS Universal Plus DNA Library Prep Kit for Illumina (Vazyme). Sequencing was performed on an Illumina NovaSeq PE150 (generating ~20 M read pairs or 6 Gb data). A second aliquot of ds cDNA was used to generate barcoded nanopore sequencing libraries using the Rapid PCR Barcoding Kit (SQK-RPB004; Oxford Nanopore), which were pooled and sequenced on R9.4.1 flowcells on a MinION Mk1C. A positive control library and a negative PBS extraction control library were included in all sequence runs for both sequencing platforms. Data analysis and interpretation were performed via an in-house pipeline. A One Step TaqMan™ Fast Virus 1-Step Master Mix RT-PCR Kit was used to quantify BCoV and BRAV on day-BRD in unenriched samples. qPCR data were tested for normality using PROC UNIVARIATE of SAS (9.4), and analysed using the PROC MIXED procedure.

Results: BRD naturally developed within the BRD cohort as revealed by respiratory clinical scoring and thoracic ultrasonography (Cuevas-Gómez *et al.,* 2020). The two sequencing platforms had high concordance for detection of BRD-associated virus species. The common BRD agents (BRSV,BPI3, BoHV1) were detected at low levels on both platforms and we postulate this is a result of vaccination. Using qPCR to target two viruses (Cq cut-off point of ≤ 37), nasal swabs were positive for BCoV (mean healthy Cq = 22.24 ±6.97 *vs.* mean BRD Cq = 20.07 ±7.07) in all 30 health and all 30 sick animals. Nasal swabs from 22 healthy and 23 BRD animals were positive for BRAV (healthy Cq = 14.58 ±3.29 vs. BRD Cq = 10.5 ±3.24). There was no difference (P > 0.05) in mean Cq values between healthy *vs.* BRD for BCoV.

Conclusion: A diverse and complex virome was found in BRD and healthy beef-suckler weanlings on the day of BRD clinical detection. Both Illumina and Oxford Nanopore sequencing platforms are capable of characterising the metagenomes found in BRD animals, whilst the latter offers potential application to pen-side diagnostics due to its portability and the ability to identify viruses from nasal swabs within 6-24 hours. Two viruses were selected for RT qPCR based on high read abundance (BCoV and BRAV). However, statistical differences in virus quantity or virus prevalence were not observed between nasal swabs from healthy and BRD. This suggests that healthy animals were asymptomatic carriers of BCoV and BRAV.

Keywords: Bovine respiratory disease, nanopore sequencing, Illumina NovaSeq, viral metagenomics.

ID-59

Comparison of sampling and diagnostic techniques for recovery of *Mannheimia haemolytica* from feedlot cattle

William B. Crosby[1], Lee J. Pinnell[2], John T. Richeson[3], Cory Wolfe[2], Jake Castle[2], John Dustin Loy[4], Sheyl P. Gow[5], Keun Seok Seo[6], Sarah F. Capik[7], Amelia R. Woolums[1], Paul S. Morley[2].

[1]Missippi State University, College of Veterinary Medicine, Department of Pathobiology and Population Medicine, United States; [2]Texas A&M University, Veterinary Education, Research, and Outreach (VERO) Program, United States; [3]West Texas A&M University, Department of Agricultural Sciences, United States; [4]University of Nebraska-Lincoln, Nebraska Veterinary Diagnostic Center, United States; [5]Western College of Veterinary Medicine, Department of Large Animal Clinical Sciences, Canada; [6]Missippi State University, College of Veterinary Medicine, Department of Comparative Biological Sciences, United States; [7]Texas A&M AgriLife Research, Amarillo, United States.

Objective: Bovine respiratory disease (BRD) is caused by interactions among the host, environment, and pathogens. The current standard for antemortem pathogen identification in cattle with BRD is deep-guarded nasopharyngeal swabbing, which is challenging, costly, and waste generating. The study objective was to compare recovery of *Mannheimia haemolytica* via culture and real time-qPCR, and to characterize microbial community structure by 16S rRNA gene sequencing, using 75-cm deep-guarded nasopharyngeal swabs (DG), 41-cm unguarded proctology swabs (PS), or 15-cm unguarded nasal swabs (NS).

Materials and Methods: Samples were collected from beef steers and bulls (n=120, mean weight=262.2 ± 12.5 kg) 14 days after arrival at a feedlot after purchase from an auction market. One nostril was sampled with each swab type for bacterial culture, identification, and antimicrobial susceptibility testing by broth microdilution. The other nostril was sampled for DNA extraction for 16S rRNA gene sequencing and qPCR for the *M. haemolytica* leukotoxin D gene (lktD).

Results: There was high concordance among swab types for *M. haemolytica* culture and qPCR (complete concordance for 77% and 81% of animals across all 3 swab types for culture and qPCR, respectively). Microbial communities were highly similar among samples collected with different swabs types, and differences identified relative to treatment for BRD were also similar; however, sampling with NS was less effective in characterizing changes within less abundant phyla than sampling with DG or PS. Positive qPCR results for *M. haemolytica* were highly concordant across swab types (81% agreed completely), but samples collected by DG had higher C_t values (Kruskal-Wallis analysis of variance on ranks, $P < 0.05$; Dunn-test for pairwise comparison with Benjamini-Hochberg correction, $P < 0.05$) and lower frequency of positive compared to NS and PS (McNemar's Chi-square test, $P < 0.05$).

Conclusions: Though slight differences existed among results for different swab types within individual cattle, nasal swabs and proctology swabs yielded comparable results to deep-guarded nasopharyngeal swabs when identifying and characterizing *M. haemolytica* by culture and qPCR, and when characterizing the microbial community.

Keywords: Bovine respiratory disease, 16S rRNA gene sequencing, antimicrobial resistance, metagenomics, qPCR.

ID-60

Evaluation using latent class models of the diagnostic performances of three ELISA tests commercialized for the serological diagnosis of *Coxiella burnetii* infection in domestic ruminants

Thibaut Lurier[1], Elodie Rousset[2], Patrick Gasqui[3], Carole Sala[4], Clément Claustre[3], David Abrial[3], Philippe Dufour[2], Renée De Crémoux[5], Kristel Gache[6], Marie Laure Delignette-Muller[7], Florence Ayral[8], Elsa Jourdain[3].

[1]Université de Lyon, INRAE, VetAgro Sup, UMR EPIA, Marcy l'étoile, France; [2]ANSES, Sophia Antipolis Laboratory, Animal Q fever Unit, Sophia Antipolis, France; [3]Université Clermont Auvergne, INRAE, VetAgro Sup, UMR EPIA, Saint-Genès-Champanelle, France; [4]University of Lyon-ANSES Lyon, Epidemiology and Support to Surveillance Unit, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Lyon, France; [5]French Livestock Institute, Ruminant Health Management Joint Unit, Paris, France; [6]GDS France (National Animal Health Farmers' Organization), Paris, France; [7]Université de Lyon, Université Lyon 1, VetAgro Sup, CNRS, UMR 5558, Laboratoire de Biométrie et Biologie Evolutive, Villeurbanne, France; [8]Université de Lyon, INRAE, VetAgro Sup, Usc 1233 UR RS2GP, Marcy l'étoile, France.

Q fever is a worldwide zoonotic disease mainly responsible for reproductive disorders such as abortion in domestic ruminants. ELISA methods are the diagnostic tools recommended for the serological diagnosis of *Coxiella burnetii* infection in ruminants but their respective diagnostic performances are difficult to assess because of the absence of a gold standard.

Objectives: This study focused on three commercial ELISA tests with the following objectives (1) assess their sensitivity and specificity in sheep, goats and cattle, (2) assess the between- and within-herd seroprevalence distribution in these species, accounting for diagnostic errors, and (3) estimate optimal sample sizes considering sensitivity and specificity at herd level.

Materials and methods: We comparatively tested 1,413 cattle, 1,474 goat and 1,432 sheep serum samples collected in France. We analyzed the cross-classified test results with a hierarchical zero-inflated beta-binomial latent class model considering each herd as a population and conditional dependence as a fixed effect. Potential biases and coverage probabilities of the model were assessed by simulation.

Results: Conditional dependence for truly seropositive animals was high in all species for two of the three ELISA methods. Specificity estimates were high, ranging from 94.8% [92.1;97.8] to 99.2% [98.5;99.7], whereas sensitivity estimates

were generally low, ranging from 39.3 [30.7;47.0] to 90.5% [83.3;93.8]. Between- and within-herd seroprevalence estimates varied greatly among geographic areas and herds. Overall, goats showed higher within-herd seroprevalence levels than sheep and cattle. The optimal sample size maximizing both herd sensitivity and herd specificity varied from 3 to at least 20 animals depending on the test and ruminant species.

Conclusion: This study provides better interpretation of three widely used commercial ELISA tests and will make it possible to optimize their implementation in future studies.

Keywords: Q fever, Bayesian, diagnostic accuracy, herd sensitivity, conditional dependence.

ID-61

Sheep associated malignant catarrhal fever (SA-MCF): a series of cases on three farms in the UK

Joseph Angell[1], Eleanor Bentley[2], Hayley Crosby-Durrani[2], Rachel Hall[1], James Stewart[2].

[1]Wern Vets CYF, Ruthin, United Kingdom; [2]The University of Liverpool, Liverpool, United Kingdom.

Introduction and background: Malignant catarrhal fever (MCF) is caused by herpes viruses of the *Macavirus* genus and disease is observed worldwide. In the UK, the sheep associated form of the disease (SA-MCF) caused by ovine herpes virus-2 (OvHV-2) appears most commonly affecting many species including cattle. Clinical signs vary but are associated with a variably distributed systemic lymphoproliferative non-suppurative vasculitis and commonly include scleral congestion, keratitis and corneal oedema (which is often considered pathognomonic) together with pyrexia, depression and anorexia resulting in a loss of condition.

The aim of this project was to study naturally occurring clinical outbreaks in the UK in order to better inform farmers and veterinarians.

Objectives:

Objective 1: document the natural history of SA-MCF in naturally occurring outbreaks.

Objective 2: where possible identify sources of infection that could be addressed through specific and tailored on-farm advice.

Materials and Methods: Three farms were identified through a single veterinary practice in Wales, UK.

Clinical examinations of clinical cases were made, together with post-mortem examinations where possible. The presence of OvHV-2 was identified by qPCR on blood and tissue samples. A diagnosis of SA-MCF was made on the basis of clinical signs together with virus detection, and, where possible, histopathology carried out by a board-certified veterinary pathologist demonstrating a lymphoproliferative vasculitis.

On two farms (1 and 2) blood samples were obtained from asymptomatic in-contact cattle and subject to qPCR for OvHV-2 to determine the presence of asymptomatic cases.

On one farm (farm 2) blood and nasal swab samples were obtained from a random sample of asymptomatic sheep and also subject to qPCR for OvHV-2 to determine the likelihood of the sheep being the source of the infection.

Treatments of live clinical cases were empirical and included antibiotics and steroids or non-steroidal anti-inflammatory drugs (NSAIDs).

Results:

Farm 1

A group of 30 non-lactating dairy cattle were relocated to a field adjacent to a group of grazing store lambs. Commencing within 24 hours, and over a 12-day period, three animals demonstrated acute clinical signs with OvHV-2 detected in two. Two of the three animals affected died with one making a gradual recovery with virus still detectable one month later. Additionally, OvHV-2 was also detected in one asymptomatic animal from the same group.

Farm 2

A group of 14 store crossbred heifers were housed in an open sided shed directly opposite a group of 50 lambing Welsh mule ewes housed in a similar open sided shed, 5m away. A single heifer became unwell. Clinical signs included: reduced appetite, pyrexia, corneal oedema, nasal discharge, a generalised fine motor tremor, hindlimb ataxia and signs of colic. Despite treatment, she deteriorated rapidly resulting in generalised seizures, whereupon she was euthanised.

An immediate on-farm postmortem was carried out with a focal, extensive, ulcerative haemorrhagic and catarrhal rhinitis observed in addition to the pathology already observed. Varying degrees of lymphoproliferative/nonsuppurative vasculitis were observed in multiple organs.

OvHV-2 was detected in the clinical case, three of the in contact asymptomatic cattle, 7/10 blood samples from the sheep and 10/10 nasal swab samples from the sheep.

Farm 3

A single bulling Stabiliser heifer was observed to be unwell with no immediate contact with sheep. She developed clinical signs including: increased lacrimation, conjunctivitis, corneal oedema, cough, nasal discharge, ulcerated muzzle, pyrexia, and a hyperkeratotic dermatitis. She gradually deteriorated despite treatment and was euthanised 50 days later. An immediate on-farm postmortem revealed multiple pathologies throughout the carcass with a lymphoplasmacytic vasculitis observed in several organs.

Conclusions: A detailed clinical history, clinical examination, together with virus detection and postmortem examination are useful for diagnosing SA-MCF on cattle farms.

A clear link to a specific reservoir host may help clinicians implement appropriate mitigation of further infections by moving the cattle as far away from the reservoir host as soon as possible.

Recovery from MCF in cattle is possible, but rare, and may be related to the amount of virus challenge.

Subclinical infection in cattle is possible and may result in latently infected cattle.

Keywords: Malignant catarrhal fever, MCF, sheep, cattle, herpes virus.

ID-63

A parainfluenza 3 virus outbreak on a Dutch Veal Farm

Henk Kuijk[1], Herman Kleinjan[2], Ruben Tolboom[1], Geert Vertenten[1].

[1]MSD Animal Health, Boxmeer, Netherlands; [2]Dierenkliniek Den Ham, Den Ham, Netherlands.

Objectives: Bovine Respiratory Disease (BRD) is a multifactorial disease that can affect cattle of all ages but is predominantly seen in young cattle. Affected animals are highly infectious and shed large quantities of virus and bacteria through nasal discharge. The BRD pathogens are endemic in the Dutch cattle population. Calves may have serological antibodies in the absence of clinical disease. These antibodies may come from passive protection (e.g. colostrum) or as an active response to field infection or vaccination. A serological screening of healthy animals for several BRD antibodies, may give more clarity on the importance of these pathogens, as well as an indication to which level antibodies may have an influence on primovaccination. The latter may help to develop an adapted vaccination schedule against respiratory disease.

In this field study, antibody titers against the BRD pathogens Parainfluenza 3 virus (PI3), Bovine Respiratory Syncytial Virus (BRSV), *Mannheimia haemolytica* and *Mycoplasma bovis* were determined in healthy calves on a Dutch rosé veal farm without major BRD problems. No vaccinations against BRD pathogens were performed on farm. The objective of the study was to determine major infectious risk factors for BRD.

Materials and Methods: Eleven randomly selected calves were blood sampled every 4 weeks from arrival (February 19th, 2019) until week 24 after arrival (August 30th 2019). The eleven sampled calves were situated in a group of 220 animals. This group changed housing once in week 9 after arrival and was always separately housed from other groups on the farm. The group received 3 times an antibiotic treatment for 5 days starting from February 21st, March 4th and March 19th with respectively doxycycline, tilmicosin and doxycycline. All animals were monitored by the farmer and veterinarian as usual on this commercial veal farm.

All the collected samples were analysed at the Centre for Diagnostic Solutions (MSD Animal Health, Boxmeer, The Netherlands) by ELISA for antibodies against *Mannheimia haemolytica*, BRSV, PI3 and *Mycoplasma bovis*. An in-house test was used to measure *Mannheimia haemolytica* and BRSV antibodies, whereas for PI3 and *Mycoplasma bovis* a commerical kit was used from respectively IDEXX and Bio-X.

Results: Antibodies against all 4 pathogens were present in the selected samples. All the samples (each calf and each time point) were positive for *Mannheimia haemolytica* antibodies. The average titers for *Mannheimia haemolytica* increased over time to very high levels at week 20. Most of the samples (91%) were positive for PI3 antibodies. The average titers decreased gradually over time which may reflect the reduction of colostral antibodies over time. The majority of samples (97%) were positive for BRSV antibodies, however most of the BRSV titers (80%) were very low. The average titers also gradually decreased which clearly reflected the reduction of colostral antibodies over time. Only 1 animal was positive for *Mycoplasma bovis* antibodies at arrival. However, at a later stage, each animal was at least once positive for *Mycoplasma bovis* antibodies with variable titers. This could mean that the passive protection at arrival was very low and that animals get infected with *Mycoplasma bovis* over time gradually.

A surprising event at this well managed farm without major BRD problems was a BRD outbreak at week 21 after arrival. The animals were treated for 5 days with chlortetracycline. This outbreak was clearly reflected in the analyzed samples, as titers for PI3 antibodies increased exponentially at week 24 compared to week 20. This confirms PI3 infection as etiology for a BRD outbreak.

Although on this farm BRD complications and potential losses due to the PI3 outbreak could be avoided by antibiotic treatment, the serological analyses convinced the veterinary practitioner and farmer about the important role of PI3 in the BRD complex.

Conclusion: Serological analyses for BRD pathogens on veal farms are essential to understand the infectious pressure and design an appropriate BRD management program including vaccination. Vaccination against PI3 virus could have reduced the complications and potential losses of a BRD outbreak and improved the wellbeing of the animals on this farm.

Keywords: Bovine Respiratory Disease, Parainfluenza 3, veal, Netherlands.

ID-64

Effects of vaccination programs on Map faecal shedding and serological response in eight French dairy herds

Corbiere Fabien[1], Cournet Julie[2], Tasca Christian[1], Dubaux Emma[3], Foures Laurent[3], Foucras Gilles[2].

[1]UMR INRAa-ENVT IHAP, Toulouse, France; [2]UMR INRAe-ENVT IHAP, Toulouse, France; [3]GDS Meuse, Verdun, France.

Objectives: The main objective of this study was to evaluate the effects of Silirum® based vaccination programs on Map faecal shedding and serological status in French dairy herds infected with paratuberculosis. We also intended to evaluate the effect of age at vaccination on faecal shedding and serological status.

Materials and methods: The serological status (ELISA) and faecal shedding (qPCR) of 4- to 5-year-old vaccinated cows (n=237) were assessed every six months over a two-year period in 8 infected herds in the Meuse department, France. Within each herd, cows from the last non-vaccinated birth cohort (n= 249) were used as controls using the same sampling scheme. The probability of Map faecal shedding and the level of Map shed were modelled using mixed general linear regression models with herd and cow random effects, adjusting for age at sampling, days in milk within the lactation and vaccination status. The effect of age at vaccination was similarly investigated in the subset of vaccinated cows.

Results: In vaccinated cows the median age at vaccination was 5.7 months (interquartile range: 3.3-7.9 months). Only 36.3% of the vaccinated cows were positive on serum

ELISA, with cows vaccinated before the age of 6 months yielding significantly fewer positive results. Overall, 42.6% of vaccinated and 28.11% of non-vaccinated cows were positive on faecal qPCR, with strong differences between herds. However, only 5.2% of non-vaccinated and 6.7% of vaccinated cows shed more than 100 Map per gram of faeces. The probability of Map shedding (p=0.772) and the amount of Map shed (p=0.955) were not significantly different between seropositive and seronegative vaccinated cows and no effect of age at vaccination could be evidenced. Compared to non-vaccinated seropositive cows, vaccinated cows were at lower risk to shed Map in the faeces (Odd's Ratio = 0.40, 95 % confidence interval: 0.13 – 0.89, p=0.0234) and in lower amount (p<10-5). No difference was however evidenced between vaccinated cows and non-vaccinated seronegative ones, neither regarding the shedding probability (p=0.103) nor the shedding amount (p=0.410).

Conclusion: Based on these preliminary results, we conclude that the beneficial effects of vaccination on Map faecal shedding may be limited in the investigated herds. Moreover, the variability of serum ELISA response in vaccinated cows remains to be investigated.

Keywords: Paratuberculosis, vaccination, Map Shedding, serology.

ID-65

How the United Kingdom created a commercially driven National Johne's Management Plan with 95% dairy farmer participation in 10 years

Pete Orpin[1], Richard Sibley[2].

[1]Myhealthyherd, Hallaton, United Kingdom; [2]Westridge Veterinary Group, Witheridge, United Kingdom.

Objectives: In 2009 a National Johne's engagement plan was developed with the support of milk processors, laboratories, database providers, farming and veterinary groups. The initial objectives were to create an inclusive Johne's management plan which would be widely adopted by all dairy farmers in the UK. Farmers would have to be fully engaged for a minimum of 10 years to achieve effective control. To achieve this objective the Rules of the program would develop over time and by driven by industry and farmer demand. Creating common messages and managing peoples' beliefs, prior conceptions and increasing the importance of control were major focus points. The plan would be fully commercial with the farmer paying for advice and tests. Processors and vets would encourage participation.

By December 2020 it is anticipated that 95% of the UK Dairy Farmers will have completed a risk assessment, surveillance and have installed a JD control plan utilising an accredited vet.

Materials and Methods: The UK National Johne's Management Program evolved over a 10 year period. The initial first 5 years centred on synchronous education of farmers, vets and wider industry on the importance and benefits of control. Dairy UK (milk processor representative body) helped encourage milk processor to commit resources to help fund farmer education. A trained group of vets delivered extensive training across the UK (> 300 farmer meetings) and further farmer awareness was created through provision of training resources for private veterinarians to use in their own practices. The model was based on the RESET model (Rule, Education, Social Norms, Economics and Tools) with the greatest emphasis on Education, Economics and Social Norms in the early years.

Milk processors and retailers define the priorities for UK Dairy farmers as part of their contractual arrangements. A cohesive national plan (NJMP) for Johne's Disease (JD) control was developed in 2015 which with the objective of JD reduction in the UK. Processors encouraged their farmers to comply with the planning process.

The flexible framework for JD control was established allowing farmers to choose one of 6 potential control strategies using advice from an accredited JD vet. Less than 10% could adopt a strict biosecurity and monitor strategy. The most common strategies included improved farm management and testing. Firebreak vaccination is not commonly adopted due to complications with JD surveillance and Bovine TB testing.

Fig.1. Summary of chosen JD control strategies in 2019

Strategy	%
Biosecurity Protect and Monitor	9.3
Improved Farm Management	4.0
Improved Farm Management and Strategic Testing	50.0
Improved Farm Management with Test and Cull	29.0
Breed to Terminal Sire	7.4
Firebreak Vaccination	0.3

The success has been achieved through focusing on creating practical JD plans that match the need of the farmer and create commercial consultancy opportunities for the private vet.

The funding of the program has been minimal and has utilised stakeholder resources and commercial drivers for success. The farmer pays for all advice provided.

Results: 22 milk processors with an estimated 80% of GB milk volume engaged with NJMP. By October 2018, 6084 farmers were compliant with the NJMP. Declared strategies from 2923 farmers were improved farm management (IFM) and strategic testing (50%), IFM and test and cull (28%), biosecurity protect and monitor (10%), breed to terminal sire (7%) IFM alone (5%) and vaccination (0.3%). Data on strategies was not collated from 3161 farmers as two larger processors used their own software to collate participation.

Over 1100 vets have been accredited to deliver the JD plan through an online training portal provided by the British Cattle Veterinary Association.

In October 2019 the NJMP became a national farm assurance standard for all dairy farms supplying farm assured milk. All farms will have to undertake a risk assessment, have a compliant control plan and undertaken surveillance to track progress using the skills of an Accredited Johne's Veterinari-

an. 95% of UK dairy farmers will be compliant with the NJMP by 2020.

Conclusion: Milk processor and Retailer influence created drivers for engagement. The development of a practical, flexible and commercial approach to JD control, which appeals to all, is central to the success of the NJMP.

Keywords: Johne's, Paratuberculosis, MAP, Dairy.

ID-66

Effects of a CDK9 inhibitor RKP00156 on bovine papilloma

Tomas J. Acosta[1], Hiroshi Onogi[2].

[1]Obihiro University, Obihiro, Japan; [2]KinoPharma Inc., Tokyo, Japan.

Bovine papilloma virus (BPV) induces diseases of considerable veterinary importance in cattle and other ruminants. BPV infects the skin from wounds such as puncture scratches by bloodsucking insects. Papilloma grows as tumor at the site of infection, papilloma located on the teats and udder skin are often observed and it is associated with mastitis in dairy cows. Several genotypes of BPV was recently divided into three genera, Delta, Xi, and Epsilon. It is difficult to identify the virus type based on the gross characteristics of tumor located on skin. In most cases, animals are co-infected by multiple genotypes. An efficient treatment method for papilloma has not been established. Recently, a cyclin-dependent kinase 9 (CDK9) inhibitor RKP00156 that inhibit host enzymes used by the virus, was shown to be effective against a wide range of viruses in vitro and cold be used as an antiviral drug. The purpose of this study was to examine the effect of RKP00156 on papilloma and BPV expression in cattle.

Ten Holstein heifers (16 to 20 months), infected with papilloma virus were used in the present study. The left anterior teat of each animal was assigned to treatment group (T group) and the right anterior teat was used as control group (C group). RKP00156 (3%) ointment was topically applied to the teat (T group) and Vaseline ointment (vehicle) was applied to C group once a day for 2 months. Before each application, pictures of each teat was taken to evaluate the changes in the tumor size and the fate of tumors at fourth week (W4) and eight week (W8) after the start of treatment (W0), tumors samples were also collected for PCR analysis after DNA extraction. Genotype identification and virus expressions were evaluated on W4 and W8 in tissue samples. The changes in size and fate of papilloma was evaluated from the pictures, and the tumors were classified in three categories (improved; decreased in size or disappeared, no change; kept the same size, and worse; increased in size or appeared during the experiment). The differences in the size and fate of tumors between treatment and control groups were compared and analyzed by Fisher's exact test.

On W4, the improved rate of papilloma was higher and the worse rate was lower in T group than in C group ($P < 0.1$). Furthermore, on W8, the improved rate of T group further increased and the worse rate decreased. PCR analysis performed on W4 revealed that Delta and Xi types of BPV were expressed in the same number of teats in both groups, however, on W8, the number of teats expressing Delta and Xi decreased significantly compared with that of controls. These results indicate that administration of CDK 9 inhibitor RKP00156 ameliorated papilloma formation and reduced BPV expression, suggesting that RKP00156 has a good potential to be used as antiviral agent for papilloma treatment in cattle.

Keywords: Papillomatosis, bovine papilloma virus, CDK9 inhibitor.

ID-67

Seroprevalence of *mycoplasma bovis* in outbreaks of bovine respiratory disease from 2015 to 2019

Tim Potter[1], Carolyn Hogan[2].

[1]Westpoint Farm Vets, Horsham, United Kingdom; [2]Zoetis UK Ltd, Leatherhead, United Kingdom.

Objectives: In the UK Veterinary Investigation Diagnosis Analysis (VIDA) data there has been a steady increase in the percentage of diagnosable submissions being attributed to *Mycoplasma bovis*. In the period from January 2006 to December 2017 there were 1102 diagnoses of *M.bovis* associated with respiratory disease, mastitis and arthritis. Of these diagnoses 86.4% were associated with respiratory disease. As part of a UK wide scheme to support investigation of bovine respiratory diease outbreaks serological testing of affected groups was undertaken to gain a understanding of the role of *M.bovis* in the disease outbreaks and raise awareness of it as a respiratory pathogen.

Materials & Methods: As part of a subsidised surveillance scheme, blood samples were submitted for serological testing from outbreaks of bovine respiratory disease across the UK. The submitting veterinarian was able to choose from a screening panel of potential respiratory pathogens (Infectious Bovine Rhinotrachietis, Bovine Respiratory Syncitial Virus, Parainfluenza-3, Bovine Viral Diarrhoea Virus and *M.bovis)*. This abstract reports the findings from the samples screened for *M.bovis.* Blood samples were analysed for *M.bovis* antibodies using a commercial ELISA kit.

Results: Over the course of 5 years a total of 14451 samples were tested from across the UK. *Mycoplasma bovis* serology results are presented from samples submitted between 2015 and 2019 and summarised in table 1.

Year	Number of samples tested	Percentage of samples positive for *M.bovis*
2015	2460	50%
2016	3354	51%
2017	3351	48%
2018	2918	39%
2019	2368	41%

Table 1: Percentage of samples testing positive for *M.bovis* by year

Conclusion: The results highlight the importance of *M.bovis* as a potential respiratory pathogen on UK farms. It is important that farmers and veterinary surgeons are aware of the impact of the disease and are able to identify it's clinical presentations rapidly. The management of *M.bovis* on farm can present significant challenges. For farms that are unaffected the emphasis should be on preventing introduction through good biosecurity and stringent controls on purchasing stock. For herds where mycoplasma is already present steps should be taken to minimise spread and reduce the impact of the disease. The absence of accurate prevalence figures makes economic analysis difficult, although it is clear that the costs of mycoplasma disease include reduced production, drugs and labour for treatment, death and culling losses as well as the financial impacts of implementation of diagnostic and control measures. Because *M.bovis*-associated disease tends to be chronic, costs per case are typically high relative to other pathogens. In addition to the financial costs, *M.bovis* can contribute significantly to antimicrobial usage on farm.

Keywords: Mycoplasma, Pneumonia, BRD, Calves, Serology.

ID-68

Neurological signs in lambs as indication for BVDV circulation in a cattle young stock rearing herd

Betsie Krattley[1], Tine Van Werven[2], Mirjam Nielen[2], Frank Van Der Meer[3].

[1]ULP Harmelen, Harmelen, Netherlands; [2]Department Population Health Sciences, Farm Animal Health, Utrecht University, Utrecht, Netherlands; [3]Faculty of Veterinary Medicine, Ecosystem and Public Health, University of Calgary, Calgary, Canada.

Objectives: Twin lambs, born on a farm where young stock and sheep were raised together in the same barn, presented with neurological signs. The clinical signs resembled a border disease virus (BDV) infection without the previously described coat abnormalities. As BDV could not be ruled out, samples were sent for diagnostic testing and subsequent sequence comparison to identify potential infection with sheep or cattle pestiviruses.

Materials and methods: Both lambs were sampled and screened for bovine viral diarrhoea virus (BVDV) using an antigen ELISA. Also the young stock rearing herd, consisting of 2 adult cows, 26 heifers and 18 calves, were tested for both BVDV antibody and antigen using an ELISA. One PI calf was identified. The serum samples of both lambs and the PI calf were subsequently subjected to a 5'UTR RT-PCR. The PCR products were cloned in a pGEM-T easy vector and sequenced.

Results: The 5′UTR polymerase chain reaction (PCR) confirmed that both lambs and one calf were infected with a BVDV 1b strain. Phylogenetic analysis indicated that all BVDV (bovine and ovine derived) were type 1b. Additional analysis of the E2 and NS5b regions of the genome revealed a ~100% sequence identity for 5′UTR, E2, and NS5b.

Conclusion: Viral transmission from a PI calf to the sheep dam during pregnancy was considered the most likely route of infection. This case demonstrates that sheep can be infected with BVDV. Neurological signs and other health problems in sheep could indicate a BVDV infection in both sheep and cattle when reared in close contact.

Note: for a presentation supporting information is available:

1. A figure of the pylogenetic tree of the E1/E2 region of the type 1b BVDV viruses derived from both lambs and the calf with reference strains (GenBank reference).
2. A video of the 1-day old lambs with BVDV type 1b showing neurological signs.

Keywords: Bovine viral diarrhoea virus, BVDV, dairy cattle, sheep.

ID-69

Evaluating factors affecting recovery of *Mannheimia haemolytica* and *Pasteurella multocida*

Adriana Marcela Garzon, Alejandro Hoyos Jaramillo, Stephanie Hustad, Barbara A. Byrne, Heather M. Fritz, Richard Pereira.

University of California, Davis, Davis, California, United States.

Microbiological diagnosis is an important step in controlling and preventing bovine respiratory disease (BRD). Moreover, adequate transport storage type, elapsed time, and storage temperature before laboratory submission are critical for optimal results. The objective was to evaluate the effect of transport storage media, time, and storage temperature on *Mannheimia haemolytica* (MH) and *Pasteurella multocida* (PM) yield using an in-vitro model simulation. Semi-quantitative (quadrant method) and quantitative culture methods using colony forming units per ml (CFU/ml) were used to recover MH or PM using an in-vitro model with swabs. In both approaches, samples were grown in 5% sheep blood agar plates. In each trial, a sterile PBS solution was inoculated with MH or PM, achieving 0.5 McFarland using OD_{600} between 0.08-0.1. A total of 58 sterile cotton swabs were inoculated in a culture solution with MH or PM and placed in either: 1) sterile falcon tube (DRY); 2) Aimes culture media with charcoal (ACM); or 3) Cary-Blair transport Agar (CBA). Swabs were evaluated for recovery of MH or PM at three temperatures: 4, 23, and 36°C; and assessed at four-time points 0 (baseline), 8, 24, and 48 hrs. A multivariate mixed model was fitted to analyze the data using lme4 and lmerTest packages of R. When normality was not rejected, the dependent variable was the CFU/ml. The independent variables were storage media (DRY, ACM, and CBA), time points (8, 24, and 48 hrs), and the interaction between storage media and time points. Each swab was considered as an independent measure. When the normality was rejected, the non-parametric Dunn all pairs approach was used to compare CFU/ml between storage media, with one model created for each temperature and time point combination. The CFU/ml recovery of PM on samples stored at 4◦C was lower for ACM when compared to DRY at 8 hrs (P = 0.05) but higher



at 48 hrs ($P < 0.01$). For samples stored at 23◦C, ACM had a higher CFU/ml recovery than DRY at 24 hrs ($P < 0.01$), and at 48 hrs, ACM and CBA were higher than DRY ($P < 0.01$). At all-time points, samples stored at 36◦C had a higher CFU/ml recovery in ACM and CBA than DRY ($P = 0.02$). The CFU/ml recovery of MH on samples stored at 4◦C was higher for ACM and CBA than DRY at time points 24 ($P < 0.01$) and 48 ($P < 0.01$). Samples stored at 36◦C had a higher CFU/ml recovery for ACM and CBA than DRY at time point 24 ($P < 0.01$). These results support the value of ACM and CBA for the recovery of PM and MH isolates, especially if samples were not refrigerated properly. Also, the combination of longer elapsed time and higher temperatures can impair diagnostic accuracy.

Keywords: Mannheimia haemolytica, Pasteurella multocida, transport media, bovine respiratory disease.

IM-01

Association of barn air quality parameters and lung consolidation, airway inflammation and infection in group-housed calves

Katharina Van Leenen[1], Jonathan Jouret[2], Peter Demeyer[3], Laura Van Driessche[1], Lieze De Cremer[1], Christina Masmeijer[1], Filip Boyen[1], Piet Deprez[1], Bart Pardon[1].

[1]Ghent University, Merelbeke, Belgium; [2]Belgian Center for Occupational Hygiene, Zwijnaarde, Belgium; [3]Flanders Research Institute for Agriculture, Fisheries and Food, Merelbeke, Belgium.

Respiratory disease remains an important cause of morbidity and mortality in calves and is associated with substantial antimicrobial consumption. Reduction of antimicrobial use is demanded to fight increasing antimicrobial resistance. This reduction can be achieved by improved diagnostics, prudent antimicrobial use and disease prevention. Assuring a healthy environment with adequate air quality in calf barns is a preventive measure of major importance. However, which air quality parameters are exactly associated with (sub)clinical pneumonia or airway inflammation in calves is basically unknown.

A cross-sectional study was performed to explore the associations of air quality parameters with lung consolidation, pulmonary inflammation and infection in group-housed calves. Sixty beef and dairy herds were visited from January – April 2017 and a total of 428 indoor group-housed calves was sampled. To assess barn air quality the following measurements were performed at calf breathing level: continuous 24-hour measurements of ammonia concentration, relative humidity and temperature and punctual measurements of air velocity, ammonia and CO_2. Clinical examination, thoracic ultrasound and broncho-alveolar lavage for bacteriological and cytological analysis of broncho-alveolar lavage fluid (BALf) was performed. Data was analysed using multivariable mixed models and cluster analysis.

Barn temperatures showed the expected diurnal variation with an average of 14.2°C (standard deviation (SD) 4.4, range 5.5 – 23.9). Average relative humidity was 68.8% (SD 8.9, range 52.2 – 91.6). Ammonia concentrations were low with an average 24-hour concentration of 1.7 ppm (SD 0.9, range 0 – 4.0). Air velocity was variable, ranging from 0 – 2.0 m/s. This parameter was analysed as binary variable based on the optimal maximum air velocity cut-off value to detect a lung consolidation of ≥1 cm in depth, which was 0.8 m/s. Lung consolidations of ≥1 cm, ≥3 cm and ≥6 cm in depth were present in 41.1% (176/428), 27.1% (116/428) and 16.1% (69/428) of the calves, respectively. Lung consolidations of all depths were positively associated with mean or maximum temperature. Ammonia exposure, in hours ≥4 ppm, was associated with lung consolidation ≥1 cm (odds ratio (OR) = 1.73; confidence interval (CI) = 1.02 – 3.07; P = 0.04). The epithelial cell percentage in BALf was also positively associated with ammonia concentration (P = 0.01). The odds of lung consolidation ≥3 cm (OR = 7.7; CI = 1.35 – 41.7; P = 0.02) and ≥6 cm (OR = 25.0; CI = 2.5 – 33.3; P = 0.007) increased with air velocity >0.8 m/s.

Cluster analysis, based on average pen temperature and relative humidity, maximal air velocity, NH_3 and CO_2 concentration and bacterial air load, resulted in four different barn climate clusters. Cluster 1 was characterised by a warm and dry climate with ammonia accumulation (n = 19). Cluster 2 represented under-ventilated barns with accumulation of air pollutants (NH_3, CO_2) and high bacterial air load (n = 8). Cluster 3 was labelled as a presumably normal climate based on low concentrations of air pollutants and average temperature and relative humidity values, combined with low air velocity (n = 25). Cluster 4 was labelled as the draught cluster, characterised by high air velocity (n = 3). In cluster 4, the draught cluster, the prevalence of lung consolidations ≥1 cm was higher (81.8%; P < 0.001) compared to the presumably normal cluster (31.6%). Also, in the warm, dry and ammonia accumulation cluster the prevalence of these consolidations was higher (54.2%; P = 0.02) compared to the normal cluster. Furthermore, in the warm, dry and ammonia cluster the prevalence of consolidations ≥3 cm (38.1%; P = 0.04) and ≥6 cm (31.4%; P = 0.01) were higher compared to the presumably normal climate cluster (18.2% and 9.1%, respectively).

Average and maximum temperature, ammonia concentration and air velocity are associated with pneumonia in group-housed calves and therefore seem to be the most valuable parameters to measure when evaluating barn climate. Cluster analysis using different parameters can aid in identification of calf barns with sufficient air quality and further improve our understanding of calf barn climatic conditions.

Keywords: Temperature, ammonia, air velocity, thoracic ultrasonography, broncho-alveolar lavage fluid.

IM-02

Does Hemorrhagic Bowel Syndrome start with small mucosal erosions ?

Bert De Jonge[1], Bart Pardon[1], Filip Van Immerseel[1], Evy Goossens[1], Nick Vereecke[2], Mathilde Pas[1], Jozefien Callens[3], Koen Chiers[1].

[1]Ghent University, Merelbeke, Belgium; [2]PathoSense BV, Lier, Belgium; [3]Animal Health Service Flanders, Torhout, Belgium.

Objectives: To describe gross and histological lesions, as well as intestinal microbiome in cases of Hemorrhagic Bowel Syndrome (HBS).

Materials & Methods: In this descriptive study, 18 cows with HBS (7 necropsied immediately following euthanasia, 11 post mortem interval >12h) were grossly and histopathologically examined post-mortem. In addition, bacteriological examination and whole metagenome nanopore sequencing of intestinal samples of affected and unaffected intestine was performed.

Results: Hemorrhagic Bowel Syndrome was characterized by a small intestinal, intramucosal hematoma with dissection of the *lamina muscularis mucosa* (LMM), in all animals where histological determination was possible (10/18). In 57.1% (4/7) of the fresh cases, the small intestinal mucosa proximal to the hematoma, showed 9-14 multifocal solitary or clustered small mucosal lacerations or erosions measuring 4-45mm. In 73% (37/48) of these mucosal lesions, microscopic splitting of

the LMM comparable to the hematoma was present. Intestinal blood loss was more severe in animals with spontaneous death compared to euthanized animals (p = 0.038). Bacteriological cultivation and nanopore sequencing showed a polymicrobial population at the hematoma and unaffected intestine, with mostly mild presence of *Clostridium perfringens*. There was no infiltrative growth of fungi within the intestinal tissue.

Conclusions: HBS is characterized by an intramucosal hematoma which likely originates form small mucosal lesions through dissecting hemorrhage within the LMM. Fulminant intestinal blood loss is an important factor in the acute and lethal clinical course of this disease. Gross and microscopic pathology, as well as the culture and sequencing results, were not in support of involvement of bacterial or fungal agents in the pathogenesis of HBS.

Keywords: Jejunal hemorrhage syndrome, hematoma, intestinal, lamina muscularis mucosae, nanopore sequencing.

IM-03

Differentiating airway inflammation in calves by means of cytological profiles of bronchoalveolar lavage fluid

Justine Clinquart, Katharina Van Leenen, Jade Bokma, Bart Pardon.

Ghent University, Merelbeke, Belgium.

Objective: Bovine respiratory disease (BRD), the primary cause of antimicrobial use in youngstock, is an inflammatory disease caused by multiple infections interacting with host and environmental factors. To improve prevention of BRD a better understanding of airway inflammation is critical. Cytology of bronchoalveolar lavage fluid (BALf) offers many possibilities to characterize and diagnose different inflammatory airway diseases, both in humans and in animals. However, in calves to date no reference values for BALf cytology are available. This seriously hampers both research and practice, as identifying truly healthy (negative control) calves for respiratory diseases is difficult. Therefore, in this study the first aim was to apply unsupervised clustering in order to identify different airway inflammation profiles in BALf from calves. Second, based on identification of a healthy profile, reference values for differential cell counts in BALf as collected by non-endoscopic bronchoalveolar lavage were determined.

Material and Methods: A dataset with cytologic information on BALf was available. This dataset consisted of 339 group-housed calves aged between 1 and 6 months from 32 dairy farms and 30 beef farms. The differential counts in this dataset were evaluated by counting 400 nucleated cells on cytospin preparations. A k-means++ -algorithm, an unsupervised clustering method, was applied on the percentage of macrophages, neutrophils, lymphocytes, eosinophils, basophils and epithelial cells. The number of clusters was first obtained by validation with 'clValid' R package, evaluation of silhouette plots and biological relevance. Visualization of cluster centers was used to identify the different clusters. Subsequently, reference values for the normal cluster were calculated with 'Reference Value Advisor'. Lastly, a classification and regression decision tree was constructed to establish the cut off-values for cluster assigning of future observations.

Results: The ideal number of clusters was four, based on the different validation methods and visual evaluation. In three clusters one or two cell types were more prominent relative to the others clusters. As such, a neutrophilic (n=114), eosinophilic (n=9) and a lymphocytic-basophilic (n=31) cluster were identified. One cluster did not show any prominent change in cluster centers and was identified as normal (n=185). The mean and the calculated 95% interval reference values for the normal cluster were the following: 52.2% (22.7% – 84.3%) macrophages, 21.9% (1.4%-41.3%) neutrophils, 6.1% (0.3%-18.8%) lymphocytes, 0.2% (0.0%-1.5%) eosinophils, 0.0% (0.0%-0.0%) basophils, and 19.6% (1.0%-59.0%) epithelial cells. A classification and regression decision tree model used the percentage of neutrophils, basophils and eosinophils as main predictors of cluster type for future observations and provided an accuracy of 98.2%. The first cut off value used by this model was 42.0% neutrophils. If the percentage of neutrophils was lower than 42.0% a cut off value of 0.1% basophils and 1.9% eosinophils was employed. When neutrophil count was equal or above 42%, cut off values were 0.2% and 1.2%, respectively.

Conclusion: Unsupervised clustering provided a useful distinction of different inflammatory profiles. The main clusters were neutrophilic and normal clusters. Also, a smaller lymphocytic-basophilic and eosinophilic cluster were identified. The distinguishing of these four clusters, and identification of a presumably normal group, could contribute to novel insights in pathogenesis, diagnosis and prevention of various airway diseases in calves.

Keywords: Cytology, bronchoalveolar lavage, bovine respiratory disease.

IM-04

Association of ionized and total calcium in periparturient dairy cows

Imke Cohrs[1], Sophia Wächter[2], Lennart Golbeck[2], Walter Grünberg[3].

[1]Educational and Research Centre for Animal Husbandry, Hofgut Neumühle, Münchweiler an der Alsenz, Germany; [2]Clinic for Cattle, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany; [3]Clinic for Ruminants, University of Veterinary Medicine, Justus-Liebig-Universität-Giessen, Giessen, Germany.

Objectives: Hypocalcemia in cattle is commonly diagnosed based on serum total calcium (tCa) although only ionized calcium (iCa) is metabolically active. The objectives of this study were to study the association between tCa and iCa around calving and to identify factors most influential on the iCa:tCa-ratio.

Materials and methods: Lab results from 30 cows enrolled in an unrelated study investigating the effect of phosphorus-deprivation during the dry period were used. Cows were either fed a dry cow diet with adequate (0.30%) or low (0.16%) phosphorus content. Blood samples were obtained

daily from day -4 until calving, 0h, 6h, 12h, 24h and 2, 3, 4 and 7 days post-partum. Blood gas and blood biochemical analyses were conducted.

Results: Correlations between iCa and tCa during the first week of lactation ranged between r=0.55, *P*=0.002 and r=0.84, *P*<0.0001. The slope of the Deming regression lines varied considerably at the different sampling times. ROC analyses with a cut off value of 1.10 mmol/L for iCa conducted for each sampling time yielded a low area under the curve of 0.64 at calving that increased to 0.93 4 days post-partum. Parameters with strongest effect on the iCa:tCa ratio were plasma albumin (r^2=0.58, *P*<0.0001), pCO_2 (r^2=0.45, *P*=0.0003), standard-[HCO_3] (r^2=0.22, *P*=0.01), lactate (r^2=0.16, *P*=0.04) and NEFAs (r^2=0.15, *P*=0.05).

Conclusion: The association of tCa with iCa during the first week of lactation is weak and highly variable. Several blood parameters seem to affect the iCa:tCa ratio and thereby weaken the usefulness of tCa to predict iCa in particular during the first days of lactation.

Keywords: Ionized calcium, total calcium, periparturient dairy cow.

IM-05

Gain and loss of fat depots mass in dairy Holstein cows with different body condition during the transition period and early lactation

György Szura[1], Stephanie Schäfers[1], Dirk Von Soosten[2], Ulrich Meyer[2], Jeannette Klüß[2], Gerhard Breves[1], Sven Dänicke[2], Lena Ruda[1], Jürgen Rehage[1].

[1]University of Veterinary Medicine Hannover, Foundation, Hannover, Germany; [2]Friedrich-Loeffler-Institute, Braunschweig, Germany.

Objectives: Dairy cows suffer from negative energy balance (NEB) during the transition and early lactation period. NEB is an outcome of nutrient demand of late gestation, onset of lactation and inadequate feed intake. Body reserves, mainly from subcutaneous (SCAT) and abdominal adipose tissues (AAT), are mobilized to compensate NEB. Previous studies found differences in accumulation and mobilization of fat, as well as functional disparities between SCAT and AAT. The aim of this study was to compare the gain and loss of SCAT and AAT depot masses in Holstein dairy cows with initially lower and higher body condition (mean body condition score (BCS): 3.48 and 3.87, respectively) in the period from day (d)-42 to d70 relative to parturition.

Materials and methods: The study was performed at the Friedrich-Loeffler-Institute (FLI), Braunschweig, Germany. Estimated depot mass (eDM) of SCAT, AAT, retroperitoneal (RPAT), omental (OMAT) and mesenteric (MAT) adipose depot was determined by ultrasonography on d-42, 7, 28 and 70. Thirty-one pluriparous German Holstein cows were allocated into two experimental groups according to the eDM of SCAT on d-42 relative to parturition (low body condition (LBC) group: n=16, mean eDM 8.61 kg; high BC (HBC) group: n=15, mean eDM 15.6 kg). Average daily change (aDC; prepartum gain and postpartum loss) of adipose mass was calculated for the dry (DP; d-42 to d7), fresh cow (FCP; d7 to d28) and early lactation periods (ELP; d28 to d70). Additionally, dry matter intake (DMI) and lactation performance were recorded.

Results: Animals of the two experimental groups represented adequate and over-conditioned cows. The AAT depot had about 2 to 3 times higher mass than SCAT. Prepartum the AAT gain during the DP was greater compared to SCAT (0.33 kg/d vs. 0.14 kg/d). More fat mass was lost from AAT than SCAT depot after calving (0.23 kg/d vs. 0.14 kg/d). Cows of both groups had similar gains in AAT (0.33 kg/d) and SCAT (0.14 kg/d) masses during the DP. However, more adipose tissue was mobilized by HBC than LBC cows from both depots after calving (AAT: HBC vs. LBC: 0.30 vs. 0.17 kg/d; SCAT: HBC vs. LBC: 0.19 vs. 0.10 kg/d). Correlation analysis revealed that the higher was the gain of AAT mass (r^2: 0.36) during DP the higher was the loss in FCP. This was not the case for SCAT. In contrast to AAT, during FCP, a more negative EB was associated with a greater loss of SCAT mass (r^2: 0.18).

Conclusions: The results indicate that compared to SCAT the AAT depot is considerably bigger and is preferentially mobilized after parturition to compensate NEB. Correlation analysis revealed functional differences between AAT and SCAT depots. Greater knowledge of differences between AAT and SCAT depots could improve our understanding of excessive lipomobilization and its effects on health and performance of dairy cows during the transition period.

Keywords: Dairy cattle, adipose depot, ultrasonografy, transition period, fat mobilization.

IM-06

Do unmeasured strong ions predict mortality in sick goats?

Diego Gomez[1], Shannon Darby[2], Megan Palmisano[2], Dave Renaud[1].

[1]University of Guelph, Guelph, Canada; [2]University of Florida, Gainesville, United States.

Increased systemic concentrations of L-lactate and unmeasured strong ions (USI) are associated with an increased risk of mortality in humans and calves suffering from various diseases. The objective of this study was to investigate whether the concentration of L-lactate and USI, on admission to hospital, are associated with mortality in sick goats.

This study used a retrospective methodology. Medical records of 153 sick goats presented to a teaching hospital from January 2017 to September 2019 were reviewed and the following data were collected: Demographics, physical examination, venous blood gas, biochemistry analysis, and outcome. The USI concentration was calculated as: USI = SID_m — HCO_3^- — A^-; were SID_m is the strong ion difference calculated as SID_m (mmol/L) = (Na^+ + K^+) — (Cl^- + L-lactate$^-$); HCO_3^- is the plasma concentration of bicarbonate (mmol/L) and A- is the total negative charge of the plasma proteins (TP) (mmol/L) calculated as: A^- (mmol/L) = [TP x 0.343]/(1 + $10^{(pKa -}$

$^{pH)}$), where pKa (7.08) is the effective dissociation constant for bovine plasma weak acids.

Univariable logistic regression models showed that goats with USI > 2 had a 4.62 times greater odds (P = 0.01; 95% CI: 1.43 to 14.92) of dying than goats with normal levels (2 to -2 USI). No differences were found between those with an elevated lactate compared to those with normal lactate (OR: 0.82; P = 0.64; 95% CI: 0.36 to 1.88).

This study revealed that increased concentrations of USI, but not L-lactate, at admission were positively associated with mortality in goats.

Keywords: Unmeasured strong ions, lactate, goats, mortality.

IM-07

Measurements of acute phase proteins in pharyngeal swabs and serum from healthy dairy calves

Mette Bisgaard Petersen, Nynne Capion.

University of Copenhagen, Taastrup, Denmark.

Objectives: Early recognition and treatment of respiratory disease in calves can contribute to better animal welfare and possibly, reduce the antibiotic consumption. Detection of acute phase proteins (APP) in pharyngeal fluid could be the first step in making a non-invasive calf-side test for early detection of respiratory disease. The objective of this study was therefore to investigate if it was possible to detect the APPs serum amyloid A (SAA), haptoglobin (Hp) and lipopolysaccharide binding protein (lbp) in pharyngeal swabs from calves and if so, to compare the APP concentration in pharyngeal swabs with the serum APP concentration.

Materials and methods: Eighty-four healthy dairy calves aged 3-30 days housed in nine Danish dairy herds were included. All calves were assessed free from respiratory disease by a clinical examination and thoracic ultrasonography in the herd. After the clinical examination, serum samples were collected from the jugular vein and four long cotton swabs were placed in pharynx and moved around until saturated. The swabs were stored in cryotubes with 1 ml of phosphate buffered saline (PBS) for 24 hours, then removed and the cryotubes were stored at -80° Celsius until analysis. The swabs and serum were analysed for SAA, Hp and lbp at the Veterinary Diagnostic Laboratory at University of Copenhagen. The range and mean concentration of SAA, Hp and lbp in serum and swabs were compared.

Results: The SAA concentration in serum ranged from 32.93 - 180.94 ug/ml, with a mean of 118.28 mg/L and a standard deviation (SD) of 40.56 mg/L. There was a significant association between age and serum SAA, which decreased with age. The Hp concentration in serum ranged from 45.14 - 788.75 mg/L, with a mean of 100.60 mg/L and a SD of 100.74 mg/L and the lbp concentration in serum ranged from 7.25-56.32 ng/ml, with a mean of 20.53 ng/ml and a SD of 9.30 ng/ml. In the pharyngeal swabs the SAA concentration ranged from 0.00-8 ug/ml, with a mean of 2.18 ug/ml and a SD of 1.47 ug/ml. The Hp concentration in pharynx swabs ranged from 0.00-60.83 mg/L, with a mean of 20.13 mg/L and a SD of 13.61 mg/L. The lbp concentration in pharynx swabs ranged from 0.00-47.94 ng/ml, with a mean of 6.06 ng/ml and a SD of 11.85 ng/ml.

Conclusions: This is the first time that detection of the APPs SAA, Hp and lbp in pharyngeal swabs from dairy calves had been shown. From these results reference intervals for SAA, Hp and lbp in pharynx swabs from dairy calves housed in commercial farms can be established. This can be used further to compare with SAA, Hp and lbp in samples from diseased calves to establish the levels of APP in pharyngeal swabs of calves with respiratory disease.

Keywords: Acute phase proteins, calves, pharyngeal swab.

IM-08

Sepsis and other risk factors for mortality in critically ill calves

Mathilde Laetitia Pas, Filip Boyen, Jade Bokma, Bart Pardon.

Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium.

Objective: Critical illness in calves is a problem practitioners are frequently faced with, often resulting in mortality. It can be caused by several etiologies, with sepsis amongst them. Considering the strict antimicrobial legislation in food-producing animals, it's crucial to identify which of the critically ill calves are in need of (critically important) antibiotics. Knowledge on factors contributing to mortality in these calves can aid in better antimicrobial decision making. Therefore, the objective of this study was to identify risk factors for mortality in critically ill calves.

Material & methods: A retrospective cohort study was conducted on 230 calves presented with critical illness in our large animal clinic. Anamnestic information, as well as clinical, ultrasonographic and laboratory information was assembled. Critical clinical illness in combination with positive hemoculture (BD BACTEC™) were used as a proxy for the presence of sepsis. Multivariable logistic regression as well as classification and regression tree analysis were used to determine parameters significantly associated with mortality.

Results: In total 61.3% (141/230) of the calves died. Enteritis/diarrhea (51.7%, n=119) and pneumonia (45.7%, n=105) were most frequently detected, followed by sepsis (34.3%, n=79), neonatal respiratory distress syndrome (17.8%, n=41) and omphalitis (17.8%, n=41). Comorbidities (presence of multiple health conditions in the animal) were frequent (54.8%, n=126), but did not significantly increase the risks of mortality (*P*=0.79). The final multivariable regression model showed that abnormal behavior (OR=3.3; 95% CI, 1.1-10.0; *P*=0.03), male gender (OR=4.3; 95% CI, 1.4-13.2; *P*=0.01), pale mucosae (OR=10.4; 95% CI, 2.5-43.7; *P*=0.001), tachycardia (≥ 126 bpm) (OR=3.4; 95.0% CI, 1.1-10.8; *P*=0.04), acidosis (pH ≤7.18) (OR=5.3; 95% CI, 1.3 -21.9; *P*=0.021) and sepsis (OR=6.9; 95% CI, 1.9-25.0; *P*=0.003) were associated with

mortality. Regression tree analysis revealed behavior, chloride, glucose, potassium and lung ultrasonography to be useful in determining the odds for mortality in critically ill calves.

Conclusion: One-third of the critically ill calves in our study had sepsis, resulting in higher odds for mortality. The identified risk factors can be helpful for therapeutic decisions, taking farm economics, animal welfare and public health (e.g. antimicrobial resistance) into account.

Keywords: Cattle, Critically important antimicrobials, Hemoculture.

IM-09

Cardiovascular monitoring, laboratory findings and pain score in calves undergoing guided bilateral rectus sheath block for umbilical hernia repair: a randomized clinical trial

Maria Chiara Alterisio[1], Paolo Ciaramella[1], Fabiana Micieli[1], Giovanni Della Valle[1], Ludovica Chiavaccini[2], Giancarlo Vesce[1], Jacopo Guccione[1].

[1]University of Napoli Federico II, Veterinary Medicine and Animal Production, Napoli, Italy; [2]University of Florida Collage of veterinary Medicine Department of comparative diagnostic and population medicine, Gainesville, United States.

Objectives: Despite detection and relief of pain are gaining considerable attention in farm animals, only a few clinical studies assess the effect of the analgesia in cattle after abdominal surgery, so far. Cardiovascular findings, serum cortisol level (SCL) and blood gas analysis (BGA) outcomes, as well as the pain score (PS) assessment, are considered some of the most reliable methods of clinical monitoring to evaluated stress, fear, and pain in animals. Based on the previous statements, the current study aims to define the health and welfare of bovine calves undergoing herniorrhaphy under general field anesthesia using an ultrasound-guided rectus sheath block (RSB) by multiple clinical approaches based on a set of parameters.

Materials and methods: The study has been performed on fourteen calves randomly assigned to receive either bilateral ultrasound guided RSB with 0.3 mL/kg of bupivacaine 0.25% and 0.15 µg/kg of dexmedetomidine (Treated group, TG) or 0.3 mL/kg of 0.9% NaCl (Control group, CG). All animals were monitored by Holter recording to define the effects on the cardiac dynamic, as well as SCL, BGA, and UNESP-Botucatu pain scale to assess their health and welfare status. Holter monitoring has been continuously performed from -120 minutes (min) pre-surgery to +120 min post-surgery diving the mean results in interval 1 (Int1)=-120min pre-surgery to the beginning of induction time; Int2= beginning of induction to estubation time (EST-t); Int3=EST-t to +120min post-surgery; Int4=EST-t to +15min, Int5=EST-t to +30min, Int6=EST-t to +60min, Int7=EST-t to +120min. The SCL have been evaluated at -150min pre-surgery (baseline), at induction time, skin incision and EST-t, as well as at +30min, + 45min, + 60min, + 120min, + 360min after surgery. BGA at -120min pre-surgery, skin incision and EST-t, as well as at + 60min, + 120min. Finally, the PS was carried out at -150min pre-surgery and at +30min, + 45min, + 60min, + 120min, + 240min, + 360min post-surgery. Intra- and extra-groups differences have been calculated with appropriate parametric and non-parametric tests. *P*robabilities < 0.05 were considered statistically significant.

Results: No significant difference was observed regarding the heart rate between the two groups at any time-interval, while regarding the same parameter a significant intra-group difference has been observed for the TG (Int1=99.9±21.0 beat/min ±SD vs. Int2=92.7±22.0; $P<0.05$). A statistically significant difference was observed regarding the overall time both of respiratory arrhythmia (TG=4.08min vs. GC=25.21min; $P<0.0001$) and sinus tachycardia (TG=0.00min vs. GC=11.07min; $P<0.001$). A further significant difference was found regarding the SCL between the two groups at skin incision time (TG=0.45±0.08 ng/mL ±SD vs. CG=0.82±0.06; $P<0.01$), while none was observed regarding the BGA. Finally, calves receiving RSB exhibited significantly lower median PS ($P<0.05$) between the two groups at +45min (TG=1 vs.CG=4), +60min (TG=1 vs.CG=6), +120min (TG=0 vs.CG=1) and +240min (TG=0 vs.CG=1.5).

Conclusion: The current multiple clinical approaches based on a set of parameters assess the effects on the health and welfare of bovine calves undergoing herniorrhaphy under general field anesthesia after receiving an ultrasound guided RSB bupivacaine based, for the first time. The clinical procedures seem to reveal beneficial short-term (attested by HR and SCL) and long-term effects after surgery (attested by the PS). Although as compared to the traditional analgesic techniques, the use of this ultrasound based RSB seems to give greater beneficial effects on the health and welfare of calves affected by umbilical hernia, further studies are necessary to definitively confirm the effects and to enable this innovative analgesic procedure to become a milestone in the bovine field medicine.

Keywords: Holter calves, calves health, calves welfare, rectus sheath block, umbilical hernia.

IM-10

Association of gastrointestinal microbiota and anion gap acidosis in diarrheic calves

Lynna Li[1], David L. Renaud[1], Hanne M. Goetz[1], Jennifer Macnicol[2], Lisa Gamsjaeger[3], Diego E. Gomez[1].

[1]Ontario Veterinary College, Guelph, Canada; [2]University of Guelph, Guelph, Canada; [3]University of Zurich, Zurich, Switzerland.

Objectives: Diarrheic calves often develop anion gap acidosis associated with increased concentrations of unmeasured anions including D- and L-lactate. However, the mechanisms responsible for these acid-base disorders are not completely understood. The objective of this study was to investigate the fecal bacterial alterations of calves with diarrhea and to explore its relationship with changes in blood pH, bicar-

bonate, and the anion gap and the gastrointestinal microbiota of diarrheic calves.

Materials and Methods: Prospective case-control study. The fecal microbiota of healthy (n= 20) and diarrheic (n= 31) calves was assessed by sequencing of 16S ribosomal RNA gene amplicons. Blood gas analysis was completed using an i-Stat analyzer. Alpha diversity was assessed using the Chao-1, Inverse Simpson's and Shannon Even indices. The microbial membership and structure of the fecal microbiota was compared between groups using the Jaccard and Yue & Clayton indices, respectively. LefSe analysis was used to determine differentially abundant taxa in healthy and diarrheic calves.

Results: Diarrheic calves had a lower pH and bicarbonate concentration and a higher anion gap than healthy calves. In healthy calves, a higher richness, evenness, and diversity were observed compared to diarrheic calves. The Jaccard and Yue & Clayton indices showed that the community membership and structure, respectively, of healthy dairy calves was significantly different than that of diarrheic calves (AMOVA < 0.01), for both comparisons. LefSe analysis showed an enrichment in obligated anaerobes (*Phocaeicola*, *Bacteroides*, *Prevotella*, *Faecalibacterium*, *Butyricicoccus*, Ruminococcaceae and Lachnospiraceae) in healthy calves while in diarrheic calves there was an increase in facultative anaerobes (*Enterococcus*, *Ligilactobacillus*, *Lactobacilus*, *Gallibacterium Streptococcus*, and *Escherichia/Shigella*) (LDA score > 3 and P < 0.05). In diarrheic calves an increased relative abundance of lactate-producing bacteria including *Lactobacillus*, *Streptococcus*, *Veillonella*, *Ligilactobacillus* and *Olsenella* was detected compared to their healthy counterparts (LDA score > 3 and P < 0.05)**.**

Conclusions: Calf diarrhea was associated with a shift from obligated to facultative anaerobes and expansion of lactate-producing bacteria. Those changes were related to acidemia and increased concentration of unmeasured anions (L- and D-lactate), estimated using the anion gap. These results suggested that gastrointestinal microbiota could play an important role in the development of anion gap acidosis in diarrheic calves.

Keywords: Acidemia, D-lactate, acidosis, bicarbonate, unmeasured anions.

IM-11

Selective use of antibiotics in neonatal calf diarrhea and its influence on fecal microbiota

Giulia Sala, Valerio Bronzo, Antonio Boccardo, Vincenzo Ferrulli, Laura Filippone Pavesi, Maria Filippa Addis, Paola Cremonesi, Filippo Biscarini, Davide Pravettoni.

University of Milan, Department of Veterinary Medicine and Animal Sciences, Lodi, Italy.

Objective: The role of parenteral administration of antibiotics during neonatal calf diarrhea (NCD) is still an open issue. This study investigates the efficacy of antibiotic treatment and its influence on the gut microbiota of diarrheic calves.

Materials and methods: After approval of the Ministry of Health (approval number 14/2018), a total of 42 calves were selected from client-owned dairy calves with NCD, admitted to the Veterinary Teaching Hospital of the University of Milan from May 2018 to May 2019. We enrolled 1-to-10-day-old Holstein Friesian calves that did not present other concurrent neonatal diseases. Upon admission, the calves were clinically examined as described in Boccardo et al. 2017. The acid-base imbalance was assessed by venous blood-gas analysis. Serum total proteins (sTP) were measured in serum by a hand refractometer, and calves presenting a value < 55 g/L were considered as being affected by ITPI. Fecal cultures and antibiotic susceptibility tests were also performed. Conventional fluid therapy was performed on dehydration degree and acid-base imbalance. After infusion, calves were randomly assigned either to the group treated with antibiotics (Group A, n. = 21 calves treated with ampicillin 10 mg/kg IV q12h for 5 days), or those not treated with antibiotics (Group B, n. = 21). In both groups, ITPI calves received hyperimmune plasma containing a declared total protein quantity (PlasmaLife Calf®, Il Ceppo s.r.l., Italy). The amount of hyperimmune plasma was calculated using the formula:

Plasma amount (L) = (body weight (kg) × plasma volume [8.9%] × sTP GAP)/plasma sTP concentration

where sTP GAP = difference between pre–transfusion sTP and desired post–transfusion sTP (60 g/L); sTP concentration = concentration of sTP in the plasma as declared by the producer.

Calves were monitored daily with complete clinical examinations for 28 days. The clinical data were recorded using a Calf Health Scoring Chart (CHSC), and the probability of sepsis was calculated. Critically ill calves (sepsis score > 60%) were treated with antibiotics based on antibiotic susceptibility tests in both groups and this event was considered a failure of therapy. Each calf was weighed weekly, and a blood and fecal sample were collected. Blood serum samples obtained by centrifugation were analyzed to measure the concentration of sTP and IgG, while fecal samples were used for microbiota analysis.

The frequencies of mortality and treatment failure between the groups were compared with a $\chi 2$test. The difference in the average daily gain and days of diarrhea between groups was compared with a Mann – Whitney – Wilcoxon test. Statistical significance was considered for $p<0.05$. The fecal microbial diversity was assessed within samples (alpha diversity). All indices (alpha and beta diversity) were estimated from the complete operational taxonomic units (OTU) table at the OTU level, filtered for OTUs with more than 15 total counts distributed in at least two samples. In addition to the number of observed OTUs directly counted from the OTU table, within-sample microbial richness, diversity, and evenness were estimated using the following indices: Chao1 and ACE (abundance-based coverage estimator) for richness, and Shannon's, Simpson's, and Fisher's alpha for diversity.

Results: Eighteen calves (42.8%) presented ITPI (nine calves in group A, and nine in group B), and receiving the plasma transfusion (average 500 ml; min 300 ml; max 1000 ml). There was no statistical difference between groups A and B in terms of mortality rate (p=0.586), failure of treatment (p=0.449), average daily gain (p=0.556), and days with diar-

rhea (p=0.585), regardless of the presence or absence of ITPI.

The microbiota analysis showed that the microbiota of calves treated without antibiotics was re-established earlier than calves treated with antibiotics. Alpha diversity indexes were significantly higher in calves treated without antibiotics at the second week of convalescence than those treated with antibiotics (p=0.043). Furthermore, the antimicrobial treatment was associated with a decrease in the phylum Actinobacteria (p=0.045) and the family Lachnospiraceae (p=0.042) in the fecal microbiota of calves at the second week of study.

Conclusion: Our data suggest that antibiotic treatment should be omitted in the treatment of NCD both in calves with an adequate transfer of passive immunity and in ITPI calves treated with hyperimmune plasma. Furthermore, microbiota re-establishment seems to be significantly faster in calves treated without antibiotics.

Keywords: Neonatal Calf Diarrhea, Dairy Calves, antibiotic resistance, gut microbiota.

IM-12

Toxicity by Accumulation after 31 days of daily high dose administration of Meloxicam in an adult Boer Goat

Aurora Villarroel[1], John E. Mata[2].

[1]Athyr Vet, LLC, Philomath, OR, United States; [2]Ome Naturals, LLC, Corvallis, OR, United States.

Objective: The objective of this case report was to determine the cause for multiple unspecific and apparently unrelated clinical signs in a goat that was being treated for chronic laminitis on all four feet.

Materials and Methods: A 6-year-old intact non-gravid female Boer goat was presented for clinical signs of severe ventral edema, pleuritis, tachypnea, hypopnea, tremors, laminitis, weakness, inappetence and inability to stand. On anamnesis it was discovered that the goat had been treated daily with high doses (1.15 mg/kg) of meloxicam for 31 consecutive days. Observed clinical signs were consistent with meloxicam overdose in humans. Lacking any clinical reports in the literature on treatment options for this presentation, the decision was made to simply discontinue meloxicam and treat the most severe clinical signs (severe ventral edema, pain due to laminitis and inappetence) symptomatically with one session of acupuncture and supportive care.

Results: Given that the half-life of meloxicam in goats is approx. 11 h, complete clearance of the recommend standard dose (0.5 mg/kg (0.23 mg/lb) is estimated at >55 h. These pharmacokinetic properties, therefore, have the potential for toxic accumulation when this dose or a larger dose of meloxicam is administered every 24 h, especially during long-term therapy.

Ventral edema, pain due to laminitis and inappetence markedly improved within 24 hours. Ventral edema subsided completely within 48 hours of the single acupuncture session. All other clinical signs began improving on day 3 after meloxicam discontinuation and resolved completely within 19 days. All hooves presented complete detachment of the wall from the coronary band and required functional trimming over a period of 5 months until regaining full normal morphology.

Conclusion: To our knowledge, this is the first report of meloxicam accumulation toxicity in a ruminant. Based on this case and evidence in studied references, we propose to use lower dose or greater interval between doses (>24 h), and actively monitoring for toxicity signs when administering meloxicam other than at the recommended 0.5 mg/kg (0.23 mg/lb) a single time.

Keywords: Meloxicam, toxicity, toxic accumulation, goat.

IM-13

Particulate matter and endotoxin concentration in calf barns and their association with lung consolidation, pulmonary inflammation and infection

Katharina Van Leenen[1], Jonathan Jouret[2], Peter Demeyer[3], Pieter Vermeir[4], Laura Van Driessche[1], Lieze De Cremer[1], Christina Masmeijer[1], Filip Boyen[1], Piet Deprez[1], Eric Cox[1], Bert Devriendt[1], Bart Pardon[1].

[1]Ghent University, Merelbeke, Belgium; [2]Belgian Center for Occupational Hygiene, Zwijnaarde, Belgium; [3]Flanders Research Institute for Agriculture, Fisheries and Food, Merelbeke, Belgium; [4]Ghent University, Gent, Belgium.

Air quality is of paramount importance for human and animal health. Among the different air pollutants, particulate matter as a representation of dust of variable diameter has become a major health concern. Agriculture is an important source of organic dust, containing different particulate matter fractions and endotoxins, which have been associated with respiratory conditions in humans and horses. Dust concentration and constitution in calf barns is hardly documented and whether certain particulate matter fractions or endotoxins contribute to the bovine respiratory disease complex is currently unknown.

The objective of this study was to measure particulate matter fractions and endotoxin concentrations in calf housings and study their associations with respiratory inflammation and infection.

A cross-sectional study was performed and samples were collected on 23 dairy and 21 beef farms in Belgium, from January – April 2017. A total of 339 indoor group-housed calves were enrolled in the study. A Grimm aerosol spectrometer was used to sample PM_1, $PM_{2.5}$ and PM_{10} fractions during a 24-hour period. A separate PM_{10} sample was obtained in parallel, using an impaction sampler, for gravimetric PM_{10} and endotoxin analysis. Calves were clinically examined, thoracic ultrasonography and non-endoscopic broncho-alveolar lavage for cytology and bacteriology was performed.

Mean 24-hour PM_1 concentration was 16.8 ± 17.5 µg/m^3 (range (R) = 0.18 – 78.8), mean $PM_{2.5}$ 25.7 ± 25.5 µg/m^3 (R = 0.52 – 148.0) and mean PM_{10} 73.8 ± 55.4 µg/m^3 (R = 1.7 – 254.0). These values were above the 24-hour mean con-

centrations suggested in the WHO Air quality guidelines for human exposure, being 25 µg/m^3 ($PM_{2.5}$) and 50 µg/m^3 (PM_{10}), respectively (World Health Organization, 2005).

The mean EU/µg PM_{10} was 4.25 ± 5.8 (R = 0.02 – 30.3) and mean EU/m^3 200.2 ± 197.4 (R = 2.32 – 901.0). Lung consolidations with a depth of ≥1, ≥3 and ≥6 cm were present in 43.1% (146/339), 27.4% (93/339) and 15.3 % (52/339) of the calves, respectively. The odds of having lung consolidations of ≥1 (OR = 13.9; CI = 3.4 – 55.6) and ≥3 cm (OR = 7.0; CI = 1.8 – 27.0) in depth increased when endotoxin concentrations exceeded 8.5 EU/ µg PM_{10}, compared to when this cut-off was not exceeded. Exposure to small and fine particulate matter fractions ($PM_{2.5}$ and PM_1) was associated with increased odds of consolidations of ≥1 (OR = 3.4; CI = 1.6 – 2.7) and ≥6 cm (OR = 10.9; CI = 1.1 – 111.1), respectively. Total nucleated cell count of broncho-alveolar lavage fluid showed a positive association with the maximum PM_1 concentration and neutrophil percentage with the maximum PM_{10} concentration. Only Pasteurella multocida isolation was associated with average $PM_{2.5}$ concentration, for the other major respiratory pathogens no associations were found.

This study shows that particulate matter and endotoxin concentrations could play an important role in the bovine respiratory disease complex.

Reference: World Health Organization, W. (2005). WHO Air quality guidelines for particulate matter, ozone, nitrogen dioxide and sulfur dioxide: Global update 2005. 1–21. https://doi.org/10.1016/0004-6981(88)90109-6

Keywords: Particulate matter, endotoxin, lung consolidation, dust, pneumonia.

IM-14

One-year cross-sectional study of dermatological lesions in 433 dairy cattle in a veterinary teaching hospital

Eloi Guarnieri, Frédéric Sauvé, David Francoz.

Université de Montréal, St-Hyacinthe, Canada.

Little literature is available on the prevalence of bovine skin diseases. The objective of this study was to describe the dermatological lesions (DL) in dairy cattle admitted at the Faculty of Veterinary Medicine (FVM), Université de Montréal, from July 1, 2018, to June 30, 2019.

A study-dedicated dermatological lexicon was first developed by the authors. Over a year, all dairy cattle admitted at the FVM were included in the study. Dairy cattle readmitted or without integumentary examination within 48 hours of admission were excluded. DL involvingfeet and ear canals were also excluded. The morphological and location of the skin and the oral mucosal DL were recorded by a trained observer.

On the 610 cattle admitted at the FVM, 433 dairy cattle were included. Most of them were Holstein breed (90%) and females (98%). The mean age was 3.3 years old (+/- 2.8 years). Of these 433 cattle, skin and oral mucosal lesions were observed in 91% and 9% of the cases, respectively. Most cattle had at least one hock (55%), one carpus (47%) or one stifle (23%) affected. Crusts (55%), callus (54%) and alopecia (51%) were the most common skin DL. Erosions (43%) and ulcers (20%) were the most common oral mucosal lesions.

This cross-sectional study highlighted the high prevalence of the DL in this specific cattle population. Considering these results, further studies would be important to determine etiologies, economic and clinical impacts of these DL.

Keywords: Dermatology, Skin lesions, Bovine.

IM-15

Evaluation of the inter-rater agreement of the clinical signs of bovine respiratory disease in veal calves

Julie Berman, David Francoz, Abdelmonem Ahmed Abdallah, Simon Dufour, Sébastien Buczinski.

Université de Montréal, Saint-Hyacinthe, Canada.

Objectives: In veal calves, clinical signs of bovine respiratory disease (BRD) are nasal discharge, ocular discharge, dyspnea, drooping ears, cough, and temperature. Despite the existence of several clinical scoring systems, the variability of human recognition of these anomalies has never been reported. The objective of this study was therefore to assess the inter-rater agreement of BRD clinical signs in veal calves. We hypothesized that BRD clinical signs were not detected equally between veterinarians, technicians, and producers of the veal industry and that some clinical signs have a higher inter-rater agreement than others.

Methods: During 2017-2018, we prospectively recorded 524 videos of physical examination of random veal calves from 80 batches in Quebec, Canada. A researcher, not involved in the inter-rater assessment, classified each video as presence/absence of each of BRD clinical signs except rectal temperature. For each of the 5 clinical signs, 15 videos with and 15 videos without the clinical sign were randomly selected to limit kappa paradoxes. Those 30 videos were then presented in a random order to experimented rater with BRD in veal calves: 6 veterinarians, 6 technicians, and 6 producers. Raters assessed the clinical sign using a modified Wisconsin scoring system (0=absent, 1=slight, 2=moderate, 3=severe for nasal discharge, ocular discharge and drooping ears; and 0=absent, 1=moderate, 2=severe for dyspnea and elicited cough). We used the median percentage agreement (Pa), median Cohen's kappa (K), and Gwet's agreement coefficient 1 (AC1) to assess inter-rater agreement. Effect of scale combination was also tested to determine the optimal combination (4-scale 0/1/2/3 *vs* 3-scale 0/1,2,3 or 0/1/2 *vs* 2-scale 0,1/2,3 or 0/1,2). The differences of inter-rater agreement between veterinarians, technicians, and producers were estimated by a Wilcoxon rank-sum test (p-value < 0.05).

Results: The 2-scale combination (0,1/2,3 or 0/1,2) had a higher inter-agreement for all clinical signs. With this combination, elicited cough was the clinical sign with the highest inter-rater agreement (Pa = 0.93; K = 0.79; AC1 = 0.87) and dyspnea was the sign with the lowest inter-rater agreement

(Pa = 0.77; K = 0.20; AC1 = 0.74). According to Pa and AC1 values, the 2-scale inter-rater agreement of the 5 clinical signs was good (value > 0.6). According to K, only drooping ears and cough had a substantial 2-scale inter-rater agreement (K > 0.6). In general, the 2-scale inter-rater agreement was better between veterinarians than between technicians, and between producers except for the drooping ears where the agreement was better among producers ($p < 0.05$).

Conclusion: We concluded that with a 2-scale combination of severity the inter-rater agreement of BRD clinical signs was variable according to the sign but clinically acceptable in veal calves. BRD clinical signs were, however, not detected equally between veterinarians, technicians, and producers of the veal industry. Future research could determine if this discrepancy could be improved by standardized training.

Keywords: Inter-rater agreement, pneumonia, veal calves, clinical examination.

IM-16

Stage-of-lactation specific hematology reference intervals are needed for post-parturient Holstein dairy cows

Vangelis Tsiamadis[1], Nikolaos Panousis[2], Nektarios Siachos[1], Georgios Arsenos[1], Georgios E. Valergakis[1].

[1]Department of Animal Production, Faculty of Veterinary Medicine, Box 393, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece, Thessaloniki, Greece; [2]Clinic of Farm Animals, Department of Clinics, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece, Thessaloniki, Greece.

Objectives: Hematologic profile of Holstein cows has been investigated mostly during peak and mid-lactation and dry period. However, comparative studies for hematology of post-parturient Holstein dairy cows versus the other stages of lactation is scarce. Therefore, the objective of this study was to establish hematology reference intervals (RIs) from the same population of Holstein dairy cows during different production stages and estimate the percentage of cows correctly classified if they were used interchangeably.

Material and Methods: Initially, after clinical examination, 68 dry period (DP) and 87 peak-lactation [PL, between 30 and 120 days-in-milk (DIM)] healthy Holstein cows from 10 dairy herds were enrolled in the study, blood sampled once, and used to establish DP and PL RIs. Samples from each cow were collected into 3 sterile vacuum tubes: a) with anticoagulant (K3-EDTA, for hematology), b) with 3.2% Sodium Citrate (for fibrinogen), and c) without anticoagulant [for total protein (TP) concentration]. Complete Blood Count (CBC), fibrinogen and TP concentrations were determined using ADVIA 120 hematology analyzer, QBC VetAutoread, and ATAGO T2-NE desk refractometer respectively. Relevant RIs (DP and PL) were established with the Reference Value Advisor v.2.1. freeware. Subsequently, 1,021 Holstein cows from the same 10 herds were blood-sampled twice during the immediate post-partum period (on DIM2 and DIM8). Samples were collected and analyzed as previously described. These 1,021 cows were clinically examined daily from DIM1 to DIM8 and records from those that remained clinically healthy throughout this period (247 cows) were used to establish post-partum RIs (PP2 and PP8, respectively) with the aforementioned method. Reference intervals of PP2 and PP8 were fitted to DP and PL RIs, in order to estimate the percentage of cows correctly classified.

Results: Respective CBC 95% RIs (lower – upper limit) for DP, LP, PP2 and PP8 were: RBC: (5.15-7.63), (4.51-6.98), (5.37-8.01), (5.00-7.53) $x10^{12}$/L, HGB: (87.0-126.9), (71.5-113.5), (91.0-132.8), (83.1-121.0) g/L, HCT: (0.226-0.324), (0.192-0.305), (0.231-0.350), (0.215-0.315) L/L, MCV: (37.39-50.75), (35.03-47.86), (36-51), (35.7-49.7) fL, MCH: (14.34-19.38), (13.92-18.32), (13.84-19.30), (13.70-19.20) $x10^{12}$/L, MCHC: (361.9-416.5), (356.4-412.6), (352.4-417.6), (348.1-417.9) g/L, WBC: (5.63-13.11), (4.56-14.02), (4.16-15.30), (4.44-17.41) $x10^{9}$/L, neutrophils: (1.25-6.72), (1.40-7.60), (0.86-9.77), (0.85-9.77) $x10^{9}$/L, lymphocytes: (2.01-5.25), (0.89-5.64), (1.43-5.77), (1.61-5.66) $x10^{9}$/L, neutrophils:lymphocytes (N:L) ratio: (0.38-1.80), (0.44-1.96), (0.24-2.84), (0.24-2.84), monocytes: (0.02-1.05), (0.08-1.01), (0.08-1.93), (0.06-1.36) $x10^{9}$/L, eosinophils: (0.08-1.55), (0.03-0.95), (0.05-0.83), (0.04-0.61) $x10^{9}$/L, platelets: (62.5-532.4), (22.1-805.3), (48.2-604.8), (97.5-690.1) $x10^{9}$/L, TP: (53.7-88.4), (62.8-95.7), (56.3-84.0), (60.0-86.0) g/L, fibrinogen: (2.854-6.024), (2.294-7.236), (N/A), (2.702-7.884) mg/L. For PP2, percentage of cows correctly classified compared to DP and PL were: RBC, 96.2% and 71.2%; HGB, 95.8% and 61.4%; HCT, 91.6% and 79.3%; MCV, 97.5% and 91.9%; MCH, 98.7% and 85.8%; MCHC, 93.6% and 97.0%; WBC, 83.4% and 96.6%; neutrophils, 83.0% and 88.9%; lymphocytes, 88.9% and 99.0%; N:L ratio, 77.9% and 81.0%,; monocytes, 77.5% and 72.5%; eosinophils, 99.5% and 96.0%; platelets, 95.7% and 95.0%; TP, 97.0% and 84.3%. For PP8, percentage of cows correctly classified compared to DP and PL were: RBC, 99.0% and 92.3%; HGB, 96.7% and 88.8%; HCT, 97.0% and 96.0%; MCV, 98.6% and 94.7%; MCH, 96.6% and 91.8%; MCHC, 91.7% and 92.2%; WBC, 84.1% and 97.1%; neutrophils, 89.7% and 88.7%; lymphocytes, 93.7% and 97.0%; N:L ratio, 83.0% and 72.5%,; monocytes, 97.1% and 92.3%; eosinophils, 99.0% and 94.0%; platelets, 85.4% and 96.0%; TP, 99.0% and 97.6%; fibrinogen, 83.8% and 99.3%.

Conclusion: More than 10% of cows during the first week post-partum would be misclassified as non-healthy based on their hematology profile, if dry period and peak-lactation RIs were used. Reference intervals derived from cows in dry period or peak lactation are not suitable for fresh cows. Only RIs specially established for the first week post-partum should be used.

Keywords: Hematology reference intervals, Holstein dairy cows, post-partum period.

IM-17

Use of epidural long action steroids on calving paralysis treatment

Francisco Gálvez Sánchez[1], Carlos Coronel Araújo[2], Javier Blanco Murcia[2], Michela Re[2], Isabel Romero[1].

[1]Javier Blanco y Asociados Servicios Veterinarios, Los Molinos, Spain; [2]Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, University Complutense, Madrid, Spain.

Objectives: Calving paralysis is a syndrome that involve motor and/or sensitive disability and proprioception deficit of the hind limbs. This syndrome can present in different ways with a marked abduction associated to inability to adduce the hind limbs. Cows were diagnosed with one or more neurological syndromes: sciatic, tibial paresis, obturator or femoral, based on clinical findings consistent with damage to these peripheral nerves or their nerve roots. These cases of paralysis are more common in beef cattle since they are directly related to prolonged or dystocic calving, due to a maternal-fetal disproportion. The treatment that has been commonly established for this syndrome is based on systemic steroids and NSAIDs (nonsteroidal anti-inflammatory drugs), B-Complex vitamins and physiotherapy. This conventional treatment has shown relative success, being calving paralysis a syndrome that can hardly be reversed in beef cattle. The objective of this study is to provide a new tool for the calving paralysis treatment, through the application of methylprednisolone acetate epidural route. This innovative therapy is based on human medicine studies in which use this way to treat low back pain with very few systemic side effects. Applying epidural steroids, we get them to act directly by spreading from the epidural canal to peripheral nerves, thus decreasing the perineural inflammation that has occurred, encouraging a faster recovery.

Material and methods: This retrospective clinical study gathers the cases of 21 beef cows with calving paralysis. All patients underwent a clinical examination and a thorough medical and musculoskeletal clinical examination including flexor-withdrawal and sensitive test, that showed recumbency and sensory incapacity of their hind limbs, so they were treated with methylprednisolone acetate (0.5mg/kg) epidural route, being able to repeat the treatment at 10-15 days if it were necessary. All epidurals were performed between 0 and 24 hours postcalving. Along with the epidural, every cow received a dose of NSAIDs. This study shows the follow-up of all these cases, until the patient is properly able to stand up or, on the contrary, the death or sacrifice of the cow.

Results: The treatment based in the application of methylprednisolone acetate [Depo-Moderin®] (0.5mg/kg) epidural route was successful in 72% of the patients studied, getting up on their own on different days: 60% of the cows were able to stand up on the first 2 days, 27% of the cows on the 3rd or 4th day, and the remaining 13% from the 10th day.

Conclusions: In calving paralysis, the longer the cow remains in decubitus position, the worse the prognosis. So, with this results, the use of epidural long action steroids is proposed as a treatment to improve the prognosis and accelerate the recovery of patients. If the patient has a good body condition and is alert, epidural long action steroids combined with physiotherapy may be effective. Further studies are necessary, increasing the number of cases to be analysed, in order to ensure with certainty the effectiveness of this treatment.

Keywords: Calving paralysis, epidural, methylprednisolone.

IM-18

Bright blindness in a goat flock associated with fern (*Pteridium aquilinum*) intake

Maite Rodríguez Hernandorena, Asier Albite, Jorge Gimeno, Marina Ríos, Janira Magreñán, Paula Artigot, Paula Pacual, Jose Antonio García Del Jalón, Enrique Castells, Luis Miguel Ferrer, María Climent.

Universidad de Zaragoza, Zaragoza, Spain.

Objectives: In the month of August 2021, a clinical case of blindness in a goat flock was referred to the Ruminant Clinical Service of the University of Zaragoza by a practitioner working in the Basque Country (Spain). Affected animals belong to a pet flock of 12 Valais breed goats reared in an extensive production system.The goats were grazing in a 4-hectare field along with some animals of other species. According to the veterinarian, there was little food in the pasture, and ferns predominated, which the farmer reported that the goats ate greedily.

The practitioner referred that in June 2021, six goats were presented with nervous clinical signs. After neurological examination, he noticed almost complete blindness in all six animals.

Material and methods: A clinical examination of the affected animals was performed. They presented a low body condition (2/5), heart and breath rates were normal, as well as body temperature. In addition, the examination revealed mild blindness as the only neurological clinical sign. The farmer referred that goats presented a bright greenish light in the eyes at night.

One of the goats died and was referred to the Pathological Service of the Veterinary Science Faculty for the post-mortem exam. Cerebellum, encephalon and the eyes were taken for histopathology, while sterile swabs were collected for microbiology and Rt-PCR.

Results: A differential diagnosis was carried out based on the clinical signs and the history. Some disorders such as heavy metal or fern intoxication were taken into consideration. Likewise, listeriosis, vitamin A or B deficiency and individual causes such as retinal detachment, cataracts or an eye ulcer were included in the differential diagnosis.

The main suspicion was intoxication based on the chronic ingestion of fern (*Pteridium aquilinum*) referred to by the farmer. These plants contain high concentrations of ptaquiloside, a toxic component that causes stenosis in the retinal vessels damaging and vanishing the layer of rods and cones completely. This narrowness caused an increase in *tapetum lucidum* reflectance,which is called "bright blindness".

Microbiological study and Rt-PCR showed negative results

for *Listeria monocytogenes,* and thus the necropsy revealed it was not an inflammatory process but degenerative.

At the *post mortem* macroscopic examination, there were no major findings in the brain, and no signs of cerebrocortical necrosis were found under ultraviolet light. The following histopathological study only revealed representative damage in the eye: the layer of rods and cones had almost completely disappeared, and the consequent retinal degeneration reached the macula.

Conclusions: The ingestion of *Pteridium aquilinum* is associated with intoxication that can have an acute or chronic evolution with different clinical pictures. Acute poisoning, also called haemorrhagic fever, is more common in cattle than sheep and presents the characteristic clinical picture of a haemorrhagic syndrome. The continued intake of low doses of toxins (chronic intoxication) is associated with the appearance of bovine enzootic haematuria, gastrointestinal carcinomas, polioencephalomalacia (action of thiamine) and a particular form of the disease described in sheep known as "bright blindness". This clinical picture has not been described so far in goats.

Progressive bilateral blindness characterises this disorder due to a degeneration of the retinal neuroepithelium. The eyes glow abnormally in the gloom. There is no inflammation or opacity of the cornea. After the eye exam, the veins and arteries are narrower, and the main vessels are more separate than normal.

Keywords: Brigth Blindness, Fern, Goat.

IM-19

A Consensus on Calf Scour – Repositioning Vets at the Centre of the Solution

Matt Yarnall[1], Marie-Louise Ryan[2], Susana Astiz Blanco[3], Alice Nothhelfer[4], Sabrina Bertani[5], Henk Schooneveld[6], Bart Pardon[7], Thomas Vahlenkamp[8], Trine Fredslund Matthiesen[9], Barbara Wolfger[10], Ibrahim Haggag[11], Sioned Timothy[12], Manuel Cervino[13], Arnaud Bolon[14], Nicola Morandi[15], Monique Driesse[16], Jo Maris[17], Martina Bechter[18], Thomas Manske[19], Philipp Kukla[20], Mohamed Alnahrawy[21], Manfred Lang[22].

[1]Boehringer Ingelheim Vetmedica, Ingelheim, Germany; [2]Mulcair Veterinary Clinic, Cappamore, Republic of Ireland; [3]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain; [4]VetHelfer, Chasselay, France; [5]Ferraroni mangimi Ltd., Milan, Italy; [6]Schoonveldeweg, Koekange, Netherlands; [7]Gent University, Merelbeke, Belgium; [8]Institut für Virologie Zentrum für Infektionsmedizin Universität Leipzig, Leipzig, Germany; [9]Gråsten Kvægpraksis, Gråsten, Denmark; [10]Tierarztpraxiswolfger, St. Michael, Austria; [11]Al Safi Danone Dairy farm, Al-Kharj, Saudi Arabia; [12]Boehringer Ingelheim Animal Health UK, Bracknell, United Kingdom; [13]Boehringer Ingelheim Animal Health Spain, Madrid, Spain; [14]Boehringer Ingelheim France, Lyon, France; [15]Boehringer Ingelheim Italy, Milan, Italy; [16]Boehringer Ingelheim Animal Health Netherlands, Alkmaar, Netherlands; [17]Boehringer Ingelheim Animal Health Belgium, Brussels, Belgium; [18]Boehringer Ingelheim Germany, Ingelheim, Germany; [19]Boehringer Ingelheim Animal Health Nordics, Copenhagen, Denmark; [20]Boehringer Ingelheim Animal Health Austria, Vienna, Austria; [21]Boehringer Ingelheim Animal Health META Ltd, Dubai, United Arab Emirates; [22]Boehringer Ingelheim Switzerland, Basel, Switzerland.

Objectives: This abstract summarises the outputs of an expert group of calf health professionals, who met remotely to discuss this topic in October 2021. The objective was to provide an updated, qualitative assessment of the current challenges on farm with regards to calf scour, including diagnostic methods used and opportunities for improvement. The ultimate objective was to help vets to re-establish themselves as the go-to people for calf scour on farm.

Material and methods: A meeting was held on 6th October 2021, using the Zoom virtual meeting platform. Ten calf health experts from across Europe and the Middle East attended, along with 13 Boehringer Ingelheim technical managers with extensive calf health experience. Six of the experts were in the same room as their country BI technical managers. Participants engaged in a 2 hour workshop, covering 4 main areas, with interaction through the Miro whiteboard platform. Firstly, participants were asked about the rational and emotional challenges of veterinarians and farmers from calf scour. Secondly, participants considered the methods of detecting the causes of and contributors to diarrhoea, and methods of treatment. Thirdly, participants were asked to consider the strategies for dealing with these causes. And lastly, participants were asked to consider the opportunities for reducing the impact of calf diarrhoea.

Results: In considering the rational challenges of diarrhoea in calves for the vet and farmer, there is often poor understanding among farmers of the disease, including economic impact, and the need for prevention as well as treatment. Investigation of a disease outbreak often requires a much larger investigation to consider underlying contributing factors such as general health status, hygiene, nutrition and the transfer of passive immunity. Farmers find treatment difficult and time-consuming, therefore it needs to be straightforward, and involvement of the vet is required to help appreciate the “bigger picture”. Vaccination should have a good ROI and farmers must remember to continue vaccinating after the disease has gone.

When considering the emotional challenges of diarrhoea, the vet needs to be recognised as the expert and to feel confident in the interventions they recommend. The vet needs to feel empathy with the farmer, recognising the extra workload that disease brings to farmers, as well as sharing the decision “journey” in developing interventions. The farmer cares about their animals’ welfare, seeing healthy animals makes them feel good and they want to make sure their farm practices are the best they can be. They want more than just medicines and vaccines from their vet and need a good relationship if they are to bring the best to their farm. They want to be confident in the sustainability of their enterprise, but also have time to spend with their family now. They also want to give a good image to the public by producing good, high-quality food.

When considering the methods of identifying the causes of diarrhoea, a standard clinical examination, as well as an investigation of the overall farm environment and calf nutrition, are essential. Antigen tests (cow-side and laboratory) and other diagnostic opportunities may more specifically identify

an infectious cause. Farm audit and investigation of colostrum management are also of value. Farmers are often reluctant to support a full farm audit owing to the cost and time required, even though non-specific factors may be more significant in some outbreaks.

On-farm opportunities to reduce the impact of calf diarrhoea focus around creating a pride in producing a high-quality, high-welfare animal, and improving education and understanding of the disease (including economic impact) by the farmer, with the provision of formalised, specific treatment protocols and improved data recording. Ensuring good colostrum management is still considered an opportunity for improving calf health, and technological opportunities to improve the monitoring of calf health are considered valuable.

Conclusions: The diverse group of calf health experts identified that there is much potential for improvement on farm with regards to calf scour. This is despite the relatively widespread use of vaccines, however the vet has, in some instances, become disconnected from this discussion. The consensus of this Calf Expert Group was that vets are ideally positioned to provide a calf health solution, combining an understanding of and interventions in management, environment and immunity of neonatal calves.

Keywords: Calf scour, diarrhoea, consensus, vaccine, veterinary engagement.

IV-01

Macrophages regulate neutrophils' IL-1β release in the presence of *Mycobacterium avium* subsp. *paratuberculosis*

Iraia Ladero[1], Angela Holder[2], Jeannine Kolakowski[2], Heather Harris[2], Joseba Garrido[1], Natalia Elguezabal[1], Dirk Werling[2].

[1]NEIKER – Basque Institute for Agricultural Research and Development, Derio, Spain; [2]Royal Veterinary College (RVC), Hatfield, United Kingdom.

Objective: The main objective was to test the impact of IL-1β secretion and extracellular trap (ET) formation by neutrophils on inflammasome activation of macrophages in response to *Mycobacterium bovis* BCG (Mbv) and *Mycobacterium avium* subsp. *paratuberculosis* (Map).

Material & Methods: Blood was extracted from healthy cows (300 ml, n=5). Monocytes were isolated using anti-CD14-coupled microbeads (Miltenyi®) after peripheral blood mononuclear cell (PBMC) isolation with Lymphoprep®. $1x10^5$ monocytes were grown in 96 well plates supplemented with recombinant bovine M-CSF at 37°C and 5% CO2 for 7 days to generate monocyte derived macrophages (MDMs). Neutrophils were isolated after the Lymphoprep® isolation step, by hypotonic lysis to eliminate red blood cells and were seeded $2x10^5$cells/well in 96 well plates. Cultures of MDMs and neutrophils from the same cow were incubated separately and together. Cultures were stimulated with PMA, zymosan, Map K10-GFP and Mbv-GFP and incubated for 4 hours at 37°C and 5% CO2 in 96 well plates for fluorimetric estimation of ET and in 24 well plates containing round-coverslides for ET visualization by confocal microscopy after immunostaining against histones and neutrophil elastase. IL-1β levels were measured in culture supernatants with a specific bovine IL-1β ELISA kit (Invitrogen®).

Results: Fluorimetric assay results revealed that ET release was higher in MDM-neutrophil co-cultures (Map: 16%, Mbv: 18%) compared to neutrophils (Map: 12%, Mbv: 11%) and MDM cultures (Map: 5%, Mbv: 9%) after stimulation with mycobacteria.

MDM cultures showed more IL-1β release against Mbv (55-60pg/ml) than MDM-neutrophil co-cultures (20-24pg/ml) and neutrophil (21-22pg/ml) cultures. However, neutrophils released more IL-1β when challenged with Map (57-68pg/ml) than MDM- neutrophil co-cultures (13-19pg/ml) and MDM cultures (27-38pg/ml).

ET formation was seen in neutrophil cultures stimulated with Mbv or Map. MDMs with internalized Mbv and Map were seen in MDM cultures. In MDM-neutrophil co-cultures, Mbv and Map stimulation caused a loss of cell integrity leaving only a few MDMs with internalized mycobacteria.

Conclusions: Neutrophils have been implicated in the pathogenesis of bovine paratuberculosis (PTB) and tuberculosis (TB), but their role is not yet well defined and increasing knowledge in this field can aid in better vaccine designing. IL-1β has shown to be important for host resistance to human tuberculosis infection. Here we present data generated by using an *ex vivo* assay designed to study the interaction of bovine neutrophils with MDMs during mycobacterial infection.

ET release results suggest that this mechanism may not depend on the mycobacterial species. In contrast, the fact that IL-1β release was higher in neutrophil cultures in response to Map and higher in MDM cultures in response to Mbv suggests a different mode of action that can be species dependent. As far as we know, no one has described before the IL-1β production in response to Map by neutrophils. These findings support an important role of bovine neutrophils against Map triggering the Th17 response through IL-1β release and starting an effective response phagocytosing and immobilising bacteria with their NETs. As a consequence macrophages are attracted by IL-1β production and phagocytose neutrophils, being provided with the antimicrobial compounds that result from neutrophils' first contact with MAP.

Keywords: Mycobacterium avium subsp. paratuberculosis, Mycobacterium bovis, Neutrophils, Macrophages, IL-1β.

IV-02

Efficacy of a live *Trichophyton verrucosum* vaccine for control of bovine dermatophytosis in veal calves

Flaminia Valentini[1], Isabella Nicola[2], Edoardo Ramacciotti[3], Alberto Garofalo[1], Giulia Cagnotti[1], Antonio D'Angelo[1], Paola Gianella[1], Claudio Bellino[1].

[1]Department of Veterinary Sciences, Torino, Italy; [2]Université de Montréal, Saint-Hyacinthe, Canada; [3]Veterinary practitioner, Torino, Italy.

Objective: The aim of this study was to assess the safety and the efficacy of a single dose injection of a commercially available live *Trichophyton verrucosum* vaccine for control of ringworm in veal calves.

Materials e methods: A blinded case-control clinical trial was carried out on 709 veal calves of both sexes reared in multiple stalls in two farms of Piedmont region, in which the prevalence of dermatophytosis was about 30%. Animals of both herds were randomly divided into two groups: ***Exp)*** experimental group (n = 340) and *Ctr*) control group (n =369). In each group, calves with and without dermatological lesions were matched. The *Exp* group received a single dose of the commercially available live vaccine against *Trichophyton verrucosum* (TRICHOBEN, *Bioveta a.s*) inoculated intramuscularly in the neck 20 days after their arrival in farm (T0), while the control group did not receive anything. Calf were examined at T0 and at 5 subsequent experimental times: T1=35 d; T2= 55 d; T3= 75 d; T4= 80d; T5 = 95d. A thorough physical examination was performed in order to identify possible local or systemic side effects and a dermatological examination was performed in order to assessed the number of lesions of ringworm (typical annular, rick, grayish-white crust lesion) present on each calf. Data was expressed as mean ± standard deviation (SD). The Student *t* test and the *z* statistic were then used in order to assess differences between groups over experimental times. Statistical differences were set at $p < .05$. The Bonferroni *p* correction method was used for multiple comparisons. Statistical analysis was performed using R 3.4.3.

Results: No local or systemic side effects were observed in animals that received a single dose of the vaccine.

Overall, 217 out 709 calves (30.6%) showed lesions of ringworm at T0. The percentage of animals of the *Exp* group that showed dermatological lesions was 32,4% (n=110) at T0, 17,4% (n=59) at T1, 10,3% (n= 35) at T2, 1,2% (n=4) at T3, 0,6% (n= 2) at T4 and 0,3% (n=1) at T5. The percentage of animals of the *Ctr* group that showed dermatological lesions was 29,0% (n=107) at T0, 19.5% (n=72) at T1, 8,4% (n= 31) at T2, 3,2% (n=12) at T3, 1,3% (n= 5) at T4 and 0,5% (n=2) at T5. Overall, the number of calves that showed dermatological lesions decreased significantly in both herds and groups over all experiment intervals ($p < .0001$). No statistical differences were found between *Ctr* and *Exp* group over experimental times ($p < .05$). The average number of lesions recorded per animal in *Exp* group was 9,01 (± 9,9) at T0, 7,1 (± 10,1) at T1, 3,2 (± 2,9) at T2 and 3 (± 1,8) at T3. Two calves showed 2 lesions at T4 while one showed 3 lesions at T5. The average number of lesions per animal recorded in *Ctr* group was 9,2 (± 13,3) at T0, 9,1 (± 12,1) T1, 8,7 (± 14,0) at T2 and 5,5 (± 6,4) a T3 and 5,8 (± 4,3) at T4. Two calves showed 1 and 9 lesions respectively at T5. The average number of lesions did not significantly differ between *Ctr* and *Exp* group over the five experimental times ($p > .05$).

Conclusions: The results of the present study suggest that ringworm had a high prevalence among veal calves. Since *Trichophyton verrucosum* infection is influenced largely by immunological status, young animals are probably the most susceptible. As previously reported for different vaccine formulations, any local or systemic effect following vaccine administration was observed in the present study. When comparing the experimental and control groups, a single administration of a *T. verrucosum* live vaccine to veal calves with ringworm did not seem to hasten the resolution of lesions. However, the significantly decrease of infected animals in the non vaccinate group suggests that spontaneous recovery, with a low spread of the disease, is likely in younger veal calves. Further studies are needed to evaluated if a protocol with 2 vaccine injection could increase the efficacy of the commercial live vaccine in preventing/treating *Trichophyton verrucosum* ringworm in veal calves.

Keywords: Bovine dermatophytosis, Trichophyton verrucosum, live vaccine, veal calves.

IV-03

A step toward an effective vaccine against *Staphylococcus aureus* mastitis in a mouse model

Kamila Reis Santos[1], Fernando Nogueira Souza[2], Marcos Bryan Heinemann[1], Eduardo Milton Ramos Sanchez[3], Luiza Campos Reis[3], Hiro Goto[3], Adriano França Da Cunha[4], Mônica Maria Oliveira Pinho Cerqueira[4], Camila Freitas Batista[5], Alice Maria Melville Paiva Della Libera[1].

[1]Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, São Paulo, Brazil; [2]Universidade Federal da Paraíba, Paraíba, Brazil; [3]Instituto Medicina Tropical da Universidade de São Paulo, São Paulo, Brazil; [4]Escola de Veterinária da Universidade Federal de Minas Gerais, Minas Gerais, Brazil; [5]Universidade Cruzeiro do Sul, São Paulo, Brazil.

Vaccines for bovine *S. aureus* intramammary infections (IMIs) have been pursued for decades and approaches have focused mainly on opsonic antibody response aiming antibody-mediated bacterial clearance. However, immunoglobulins do not provide enough protection, and consequently the available mastitis vaccines only result in the reduction of the severity of clinical cases and increase the odds of cure of pre-existing cases of *S. aureus* IMIs. To be effective, the ideal *S. aureus* mastitis vaccine should either prevent infection or clear the bacteria from the mammary gland shortly after infection. It would seem that *S. aureus* mastitis vaccine that meets these criteria has not been developed yet. Thus, here we aimed to evaluate the expansion of a protective population of γδ T cells subpopulation ($V\gamma4^+$) in mice vaccinated with three *Staphylococcus aureus* recombinant proteins with a potential to prevent new intramammary infections in association or not with granulocyte-macrophage colony-stimulating factor (GM-CSF) DNA vaccine, which could boost antigen presentation. For the present study were used 18 mice (six mice per group) C57bl/6J lineage with six weeks of age. The animals were divided in three groups, each group with a different vaccine protocol. Firstly, we produced three *S. aureus* recombinant proteins ATP synthase subunit alpha (SAS); succinyl-diaminopimelate desuccinylase (SDD); and cysteinyl-tRNA synthetase (CTS), that was previously identified as potential candidates for preventing new IMIs in a previous study carried out by our research group using serum immunoproteomics. Beyond that, we also produce a DNA vaccine for granulocyte-macrophage stimulating factor (GM-CSF). The first group (G1) received the recombinant proteins and saponin as adjuvant at days 14, 28 and 42, and the second group (G1) received the same recombinant proteins and the GM-CSF at day 0. The third group (G3) received just the adjuvant saponin and serve as a unvaccinated control group. The spleen's γδ T cells subpopulation ($V\gamma4^+$) was determined by flow cytometry using a specific monoclonal antibody. We choose this population because the expansion of this lymphocyte population has been associated with protective against subsequent infections. The data were analyzed using the FlowJo software (Becton, Dickinson and Company, Oregon, United States). Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software, Inc., San Diego, USA). Firstly, the data were tested for normality of the distribution using the Shapiro-Wilk test. As the data showed Gaussian distribution, the data were subjected to a variance analysis followed by Tukey's test. The animals that received the three *Staphylococcus aureus* recombinant proteins with a potential to prevent new intramammary infections together with the GM-CSF DNA vaccine showed the highest percentage of this cell population .(4.40 ± 8.53; $P = 0.01$), followed by those that received just the recombinant proteins (2.94 ± 0.28; $P = 0.03$), which both are statistically different from the unvaccinated control group (2.65 ± 0.38). Thus, our immunogenicity findings indicated that these recombinant proteins identified in a previous serum immunoprotemic study have an important property associated with protective immunity against *S. aureus* infection.

Keywords: Mastitis, immune response, protective immunity, vaccine, Staphylococcus aureus.

IV-04

Assessment of in vivo immune and health status parameters in Holstein-Friesian calves fed milk replacer supplemented with microalgae displaying in vitro macrophage-stimulatory activity

Ana Rita Violante Pedro[1], Margarida Rosa Garcez Maia[2], Elisabete Gomes Martins[3], Carla Mendonça[4], Isabel Costa Ramos[5], Catarina Gonçalves[6], Tânia Lima[7], Cátia Mota[8], Joana Laranjeira[9], Ana Rita Cabrita[2], António Mira Da Fonseca[2], Manuel Vilanova[10], Alexandra Correia[10].

[1]ICBAS/i3S/LAQV/REQUIMTE-UP, Porto, Portugal; [2]LAQV/REQUIMTE/ICBAS-UP, Porto, Portugal; [3]ADM/EPIUnit-UP/EUVG, Coimbra, Portugal; [4]Centro de Estudos de Ciência Animal-Instituto de Ciências, Tecnologias e Agroambiente-UP, Porto, Portugal; [5]Cooperativa Agrícola de Vila do Conde CRL, Vila do Conde, Portugal; [6]International Iberian Nanotechnology Laboratory, Braga, Portugal; [7]i3S/IBMC-UP, Porto, Portugal; [8]ICBAS/LAQV/REQUIMTE-UP, Porto, Portugal; [9]Allmicroalgae, Pataias, Portugal; [10]ICBAS/i3S-UP, Porto, Portugal.

Objectives: Morbidity in newborn calves derives most commonly from gastrointestinal infections and respiratory syndromes. Although difficult to precise and extremely variable among regions, it has devastating effects on animal welfare and on long-term productivity and profitability. Most neonatal diseases derive from an imbalance in the pressure posed by pathogens in the surrounding environment and the host immune response to these pathogens. Maintaining good management practices is crucial to control morbidity rates; nevertheless, strategies that could enhance calves' immunity would be helpful in preventing disease, animal loss, suboptimal performance, and the overuse of antibiotics. Dietary supplements have been used to enhance calves' innate and acquired immunity. Yeast-derived beta-glucans trigger Dectin-1-mediated signalling, inducing epigenetic changes in innate immune cells, which result in a higher response to a subsequent stimulus (innate memory). Although scarcely studied, microalgae are promising alternatives as immune-modulators, due to their content of polyunsaturated fatty acids, organic minerals, beta-glucans, and antioxidants. This work addressed the effects of digestion products of microalgae on bovine innate immune cells and the use of microalgae as dietary supplements to newborn calves. We aimed at characterizing the *in vitro* response of macrophages to these dietary supplements and the zootechnical performance and immune parameters of newborn calves fed milk replacer supplemented with 1% *Chlorella vulgaris*.

Material and methods: *In vitro* assay: Peripheral blood monocyte-derived macrophages (MDM), from eight Holstein-Friesian calves, were stimulated with digestion products of *C. vulgaris*, *Nannochloropsis oceanica* and *Tetraselmis* sp. Monogastric (pre-ruminant) *in vitro* digestion of microalgae was performed according to previously described methodology. Two different dilutions of digestion products (1:10; 1:100) were used as representative of 2 and 0.2% supplementation, respectively, considering a daily intake of 8 L of milk replacer. Cytokine production (TNF-α, IL-6, IL-8, IL1β, IL-10), cytokine mRNA expression (TNFA, IL6, IL8, IL10, IL1 β, IL12A, IL12B and IL23A), and reactive-oxygen species (ROS) production were evaluated.

I*n vivo* assay: fourteen male Holstein-Friesian calves, 10 days old, were randomly allocated to control (milk replacer) and experimental (milk replacer with 1% *C. vulgaris*) groups. Prior to the beginning of the trial, animals were weighted, clinical evaluation was performed, and blood was collected for serum IgG and complete hemogram evaluation. Calves were fed 7 L of milk replacer (140 g/L) in two equal meals for 42 days. Starter feed, meadow hay and freshwater were provided *ad libitum*. Milk intake and refusals, faecal scores and health parameters were recorded. Animals were periodically weighed to evaluate average daily gain. Faeces were collected for pH, short-chain fatty acids and microbiome profiling. Blood was collected for hemogram and serum cytokine evaluation and to isolate peripheral blood mononuclear cells (PBMC) for assessment of proliferation and cytokine production in response to mitogens; peripheral blood monocytes were challenged with several toll-like receptor (TLR) and C-type Lectin-like receptor (CLR) agonists to evaluate cytokine expression, and MDM were used for phagocytosis assays.

Results: Digestion products of *C. vulgaris*, *N. oceanica*, and *Tetraselmis* sp., induced mRNA expression and production of all assessed cytokines, as well as of ROS by MDM. These results indicate that microalgae supplementation has a stimulatory effect on bovine macrophages. The *in vivo* supplementation of replacer milk with *C. vulgaris* had no negative impact on average feed intake, average daily gain, and health parameters. Serum cytokines and innate immune cells' response (cytokine mRNA and protein expression, phagocytosis) were similar between control and experimental groups. Also, no differences were found between groups regarding MDM phagocytosis capacity or PBMC proliferation. Assessment of faecal pH, short-chain fatty acids and microbiome profiling is currently ongoing.

Conclusions: Microalgae had an immunostimulatory effect on bovine macrophages. However, supplementation of calves' milk replacer with 1% *C. vulgaris* did not result in significant alterations in the evaluated parameters. Higher inclusion levels of *C. vulgaris* may be necessary to evidence putative immunomodulatory effects of *C. vulgaris* supplementation on immune and growth parameters in newborn calves.

Funding: This work received financial support from Fundação para a Ciência e Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES) through the project UIDB/50006/2020 and through the Project AlgaValor from Portugal 2020 (POCI-01-0247-FEDER-035234; LISBOA-01-0247-FEDER-035234; ALG-01-0247-FEDER-035234). AP was supported by phD grant PD/BDE/135540/2018 from FCT/MCTES, ADM Portugal S.A., and Cooperativa Agrícola de Vila do Conde. MM was supported by FCT through program DL57/2016–Norma transitória (SFRH/BPD/70716/2010).

Keywords: Bovine, microalgae, dietary supplementation, cytokines.

IV-06

Comparison between immune response observed in cattle vaccinated with a traditional toxoid vaccine or with the recombinant Hc-domain of botulinum neurotoxin type C and D

Luca Zandonà[1], Elena Tonon[1], Matteo Cornaggia[1], Adriana Di Castri[1], Ilenia Dirigo[1], Christelle Mazuet[2], Alessia Rizzardi[1], Joachim Frey[3], Luca Bano[1].

[1]Istituto Zooprofilattico Sperimentale delle Venezie, Diagnostic and Microbiology Laboratory, Treviso, Italy; [2]Institut Pasteur, Centre National de Référence des Bactéries anaérobies et du Botulisme, Paris, France; [3]Vetsuisse Faculty, University of Berne, Bern, Switzerland.

Objective: In the present study, we compared the immune response occurring in cows immunized with the recombinant heavy chain (Hc) domain of *Clostridium botulinum* neurotoxin (BoNT) type C and D (HcBoNT/C and HcBoNT/D), with that elicited by a commercial toxoid-based vaccine.

Materials and methods: Ten male calves, four-month-old, were vaccinated twice with a bivalent (C and D) traditional toxoid-based vaccine and ten male calves, hosted in the same stable, at the same age, were vaccinated with three doses of the Hc-BoNT recombinant vaccine. HcBoNT/C and HcBoNT/D were expressed in *Escherichia coli* and purified as previously described (1). Besides, five calves were injected three times with the only adjuvant, while other five were untreated and were included in the study as negative control. All in vivo experiments were approved by the ethical committee and authorized by the Italian Ministry of Health (n° 417/2017-PR).

Serum samples were collected before the first vaccination and three weeks after the second and the third vaccination. To evidence the antibody response, two ELISA tests were developed in-house using the immunogens as capture antigens. The neutralizing antibodies were investigated by means of a mouse protection assay (MPA) performed with BoNT type C, D and D/C (2). The neutralizing titer was quantified using two reference antitoxins.

Results: The group of cows vaccinated with the recombinant vaccine showed an average antibody titre of 1.031 ELISA units (EU) for type C and 1.14 EU for type D. In contrast, the average titre in animals vaccinated with the bivalent toxoid vaccine was lower, i.e. 0.461 EU for type C and 0.182 for type D.

The average antibody titers in untreated animals and animals inoculated with the adjuvant were 0.171 EU and 0.097 EU, respectively.

After two vaccinations, the mouse protection assay performed with BoNT type C revealed average neutralizing titers of 5.1 International Units (UI)/ml in bovines vaccinated with the recombinant vaccine or 0.625 UI/ml in those vaccinated with the toxoid vaccine. Despite the high humoral response evidenced by ELISA, the mouse protection assay performed with BoNT type D/C did not show any protection titre after two vaccinations and the antibody response recorded after the third vaccination was equal (1.25-2.5 UI/ml) to that obtained with the toxoid vaccine after two vaccinations. This result is probably due to the limited homology of the HcBoNT/DC with the HcBoNT/D (37%) and HcBoNT/C (77%) (3). The means of the neutralizing serum titers against BoNT/D were 2.06 UI for bovines vaccinated with the toxoid-based vaccine and 4.87 UI for bovines immunized with the recombinant vaccine.

Conclusions: A two-dose subcutaneous immunization with the recombinant vaccine stimulated a robust humoral response compared with control groups.

Our results demonstrate that the neutralizing antibodies titers against BoNT/C and BoNT/D stimulated by the recombinant vaccine were respectively 8 and 2 times higher than those of the classic toxoid vaccine included in the study. The recombinant vaccines encompass the immunogenic part of the protein that is biologically inactive, and does not contain formalin, hence it represents a safer tool for operators. The lack of protective antibodies against BoNT D/C in sera of bovines vaccinated with the recombinant peptides can be explained with the low structural homologies between the C-terminal-Hc of BoNT/DC and the C-terminal-Hc of BoNT/D (37%) and BoNT/C (77%). To prevent bovine botulism sustained by BoNT/DC, a new subunit vaccine set up with the C-terminal part of the heavy chain of the mosaic BoNT/DC should be tested.

References:

1. Stahl C., Unger L., Mazuet C., Popoff M., Straub R., Frey J. (2009) “Immune response of horses to vaccination with the recombinant Hc domain of botulinum neurotoxin types C and D”. *Vaccine* 27: 5661–5666.
2. Hatheway C.L., Dang C. (1994) “Immunogenicity of the neurotoxins of *Clostridium botulinum*”. In: Jankovic J., Hallett M. (eds) Therapy with botulinum toxin. Marcel Dekker, New York, pp 93–107
3. Nakamura K., Kohda T., Shibata Y., Tsukamoto K., Arimitsu H., Hayashi M., Mukamoto M., Sasakawa N., Kozaki S. (2012) “Unique biological activity of botulinum D/C mosaic neurotoxin in murine species”. *Infect Immun* 80:2886-2893.

Keywords: Botulism, recombinant vaccine, cattle.

IV-07

Reduction in BRD cases and antibiotics consumption using dry cow vaccination combined with early intranasal calf vaccination in a commercial Danish dairy farm

Jørgen Kragsig Olesen[1], Jonna Hjorth[2], Henrik Schmidt[2], Marina Solé[3], Martijn Seelie[3].

[1]Kvægdyrlægerne Midt, Bording, Denmark; [2]HIPRA Nordic ApS, VEJEN, Denmark; [3]HIPRA laboratorios, Amer, Spain.

Objectives: Bovine Respiratory Disease (BRD) has a considerable negative impact on production economics and on calf welfare, as well as a major impact on antibiotic consumption. The objective of this case study was to measure the changes in number of BRD treatments and the impact on antibiotic usage in the period 1-120 days of life when vaccinating dry cows with HIPRABOVIS® SOMNI/Lkt (*M. haemolytica* leucotoxoid & *H. somni* vaccine - HIPRA) in combination with

early intranasal calf vaccination with NASYM (live attenuated BRSV vaccine - HIPRA).

Materials and methods: A closed 285-cow dairy herd located in Denmark, with an average of 14 heifer calves born per month, experienced massive BRD problems among calves from the first days of life until 2 months of age.

Newborn calves are fed 3.8 L of good quality colostrum (Brix>22%) and are housed individually the first two days of life. After this they are housed in pairs. From approximately three weeks of age, they are housed 6 calves per pen.

Vaccination protocol: Dry cows are vaccinated subcutaneously with HIPRABOVIS® SOMNI/Lkt around dry-off and a 2nd dose 3-4 weeks later. NASYM is applied intranasally in calves during the first week of life.

Treatment data were gathered from April 2019 until December 2021.

A period of 12 months of vaccination is compared with a 12-month period prior to vaccination. An analysis of the first 120 days of life of all heifers born during the two periods has been performed. Bull calves are not included in the dataset as they are sold at the age of 2-5 weeks.

The number of BRD treatments and days under treatment pre- and post-vaccination was compared in order to estimate the impact of vaccination on the disease. Treatments are considered as a new case if there are 14 days or more between cases.

Results: Heifers born during the vaccination period had a significantly lower risk of experiencing one or more BRD treatments during their first 120 days of life compared to the heifers born during the 12-month period prior to the start of vaccination. The average number of BRD treatments per heifer was significantly reduced, from 1.04 before the start of vaccination to 0.36 in the vaccination period ($p<0.001$). A significant reduction was seen in the percentage of animals treated (from 73.5% to 30.8% $p<0.001$), and in the risk of being treated more than once (from 23.9% to 4.1% $p<0.001$).

If we consider the number of therapeutic days, there is a significant reduction from 8.62 before vaccination to 1.92 days of treatment per animal in the vaccination period, which means a reduction of 78%, with a corresponding decrease in antibiotic consumption.

The reduction in therapeutic days was significant throughout the entire study ($p<0.001$) but was highly significant from 1-60 days of life.

Conclusions: On a 285-cow dairy farm with known respiratory problems in calves, dry cow vaccination with HIPRABOVIS® SOMNI/Lkt and intranasal vaccination of the young calves with NASYM resulted in a reduction of average number of BRD treatments per animal from 1.04 to 0.36 ($p<0.001$). The number of therapeutic days was reduced significantly by 78% ($p<0.001$), with a corresponding decrease in antibiotic consumption. The reduction was noted in both the number of animals treated, and in the risk of being treated more than once. The reduction in therapeutic days was significant throughout the entire study ($p<0.001$) but was highly significant from 1-60 days of life.

Keywords: BRD, VACCINATION, Intranasal, passive immunity, cow vaccination.

IV-09

Single-shot vaccines against bovine respiratory syncytial virus (BRSV): comparative evaluation of long-term protection after immunisation in presence of BRSV-specific maternal antibodies

Jean François Valarcher[1], Sara Hägglund[1], Katarina Näslund[1], Luc Jouneau[2], Ester Malmström[3], Olivier Boulesteix[4], Anne Pinard[4], Dany Leguéré[4], Alain Deslis[4], David Gauthier[4], Catherine Dubuquoy[2], Vincent Pietralunga[2], Aude Rémot[5], Alexander Falk[6], Ganna Shevchenko[7], Sara Bergström Lind[7], Claudia Von Brömssen[8], Karin Vargmar[9], María Jose Rodriguez[10], Marga Garcia Duran[10], Isabelle Schwartz-Cornil[2], Geraldine Taylor[11], Sabine Riffault[2].

[1]Swedish University of Agricultural Sciences, Dept. of Clinical Sciences, Ruminant medicine, Host Pathogen Interaction Group, Uppsala, Sweden; [2]Université Paris-Saclay, UVSQ, INRAE, VIM, Jouy-en-Josas, France; [3]Swedish University of Agricultural Sciences, Dept. of Clinical Sciences, Ruminant medicine, Host Pathogen Interaction Group, Uppsala, France; [4]INRAE, PFIE, Nouzilly, France; [5]Université de Tours, INRAE, ISP, Jouy-en-Josas, France; [6]Uppsala University, Analytical Chemistry, Department of Chemistry-BMC, Uppsala, Sweden; [7]Uppsala University, Analytical Chemistry, Department of Chemistry-BMC, Uppsala, Sweden; [8]Swedish University of Agricultural Sciences, Dept. of Energy and Technology, Unit of Applied Statistics and Mathematics, Uppsala, Sweden; [9]Swedish University of Agricultural Sciences, Dept. of Biomedicine and Veterinary Public Health, Uppsala, Sweden; [10]Inmunología y Genética Aplicada, S.A. (INGENASA), Madrid, Spain; [11]The Pirbright Institute, Woking, United Kingdom.

Objectives: The objectives of this work were to compare the safety and the long-term clinical and virological protection induced by three single-shot vaccines against bovine respiratory syncytial virus (BRSV) in calves with BRSV-specific maternally derived antibodies (MDA).

Materials and methods: Four groups of six 3.5 to 8 week-old calves with BRSV-specific MDA were vaccinated either with i) 100 µg of the stabilised pre-fusion form of the BRSV F protein (PreF) in 2 ml ISA 61 VG adjuvant (MontanideTM, SEPPIC, France) intramuscularly (i.m) ii) 5 x 10^6 PFU of ΔSHr-BRSV intranasally (i.n) or iii) one dose of a commercial vaccine (CV) containing BRSV strain 375 ($10^{5.0}$-$10^{7.2}$ Cell Culture Infective Dose 50 %, Zoetis, France) i.n., or iv) were injected with a placebo consisting of 2 ml of ISA 61 VG i.m. Three months later, calves were challenged with BRSV Snook strain by aerosol and were monitored during 13 days post challenge. Clinical, immunological, virological and pathological investigations as well as mass spectrometry-based proteomic analyses were performed to assess the vaccine efficacy and safety.

Results: In line with previous results from this experimental model, all controls developed mild to severe clinical signs of respiratory disease. All vaccinated calves were clinically protected to some extent. The PreF vaccine tended to afford better clinical protection than either ΔSHrBRSV or the CV.

Virus RNA was detected by RT-qPCR, in all controls and in all ΔSHrBRSV- and CV-immunised calves, but not in 3/6 and 4/6 PreF-immunised calves, in nasal secretions and bronchoalveolar lavage (BAL), respectively. Based on the area under the curves of the nasal virus shedding, PreF induced a 10-fold better protection than ΔSHrBRSV and a 100-fold better pro-

tection than the CV.

At necropsy, although performed 13 days post challenge, lesions of interstitial pneumonia and emphysema were present in 6/6 controls and 3/6 CV-vaccinated calves, whereas none or only very limited lesions were observed in calves vaccinated with PreF or ΔSHrBRSV. Histopathological analysis revealed an acute to subacute bronchointerstitial pneumonia in controls, with an increased infiltration of neutrophils in the airways, compared to that in vaccinated calves. Based on BAL cytology and proteomic data with a focus on neutrophil proteins, calves immunised with PreF or ΔSHrBRSV were significantly better protected than those immunised with the CV. Three PreF-immunised calves had the highest number of eosinophils in a lung section, however no adverse clinical reactions were observed.

In contrast to ΔSHrBRSV and the CV, PreF induced BRSV-specific humoral responses pre challenge in most animals, such as BRSV-neutralising antibodies in 4/6 calves by D56 post vaccination (PV) (36 days pre challenge). On D84 PV (8 days pre-challenge), PreF-vaccinated calves had significantly higher titres of BRSV-specific serum IgG, and IgG1, than those vaccinated with ΔSHrBRSV or the CV. Additionally, BRSV-specific serum IgG2 levels tended to be higher in PreF-vaccinated calves than in calves immunized with ΔSHrBRSV or the CV on D84 PV. All three vaccines primed for rapid IgG responses following BRSV challenge and PreF primed for neutralising antibodies, but not IgA, in contrast to ΔSHrBRSV and the CV. BRSV-specific and IFNγ expressing T cells were detected by ELISpot in two CV-immunised calves 84 days PV and in several calves in all groups 13 days PI, but without any significant differences between calves from the different groups.

Conclusions: The i.m. PreF-based BRSV-vaccine induced slightly better clinical protection and a stronger virological protection than the two i.n. live-attenuated vaccines (ΔSHrBRSV and CV), in calves having BRSV-specific MDA at vaccination. Overall, ΔSHrBRSV performed better than the CV for pathological and virological protection. No safety problems were observed after vaccination and challenge. Finally, as a subunit vaccine, PreF allows the development of a DIVA (differentiation between infected and vaccinated animals) test that can facilitate monitoring the efficacy and the safety of the vaccine in the field. The vaccine can additionally be used in control programs.

Keywords: BRSV, vaccine, PreF, ΔSHrBRSV, long-term protection.

IV-10

Immunoglobulin G levels in beef cow colostrum in Finland

Tuomo Kähkönen[1], Heli Simojoki[1], Heidi Härtel[2], Tuire Tuukkanen[2], Maiju Pesonen[3].

[1]University of Helsinki, Helsinki, Finland; [2]HKScan Finland Oy, Turku, Finland; [3]Natural Resources Institute Finland, Ruukki, Finland.

Objectives: The aim of the study was to evaluate and determine the immunoglobulin G content of colostrum in Finnish beef cows by digital Brix refractometer and ELISA (enzyme-linked immunosorbent assay). Colostrum quality of beef cows has not previously been studied in Finland at the cow or farm level.

Material & Methods: The study was conducted on Finnish beef cow farms in the spring of 2021. Colostrum samples were collected from 19 farms. The farm collected calving and colostrum related data and took colostrum samples; in total 137 colostrum samples; 6 to 25 samples per farm. The colostrum samples were frozen in the farm and sent later to laboratory. The breeds of the cows were Hereford (n=37), Aberdeen angus (n=42) and other beef breed (n=43). Immunoglobulin G (IgG) levels were evaluated via total solids with a digital Brix meter (Atago PAL-S, Atago CO, Japan) and determined by ELISA (Bovine IgG ELISA kit, Bethyl Laboratories, Inc., Texas, USA). The measurement results were collected in an Excel (Microsoft Office 365) and the statistical analysis was performed with Stata software version 17.0 MP-Parallel Edition, (Texas, USA). The association of IgG content and variables was evaluated with a regression model. Variables included to the colostrum model were farm, parity and breed of the dam. The correlation between Brix measurement and IgG was tested with Pearson pairwise correlation test.

Results: Total solids Brix percentages of the beef cow's colostrum were median 24.5%, mean 24.7% and standard deviation (sd) 3.84%. Immunoglobulin G levels in the beef cow's colostrum were median 122.0 g / l, mean 128.7 g / l and sd 40.5 g / l. Dam's parity did not affect the IgG results of colostrum(p> 0.05). Breed and farm were highly associated to each other and both were associated with IgG when set separately into the final model. Aberdeen angus cows had higher IgG content in colostrum compared to Herefords or the group other breeds (coeff 23.8; p=0.006). Also the farm was associated to the IgG content of colostrum (Wald's test p=0.02). The lowest herd level mean IgG content was 89.3 g / l, and highest 173,0 g /l.

Conclusion: IgG levels in beef cow's colostrum were sufficient in all farms. IgG levels in beef cow colostrum differed between breeds and farms. Affordable Brix meters can be used as a tool to predict colostrum quality to ensure sufficient calf immunity in beef farms.

Keywords: Beef cow, Colostrum, IgG, Brix, ELISA.

IV-11

Evaluation of the influence of vaccination with a Type 1 and Type 2 BVDV vaccine on the milk yield of lactating cows

Lucy Metcalfe[1], Kathrin Sommer[2], Christian Guidarini[1], Matthew Yarnall[1].

[1]Boehringer Ingelheim, Ingelheim, Germany; [2]Boehringer Ingelheim, Hannover, Germany.

Objective: To determine whether there was an influence of vaccination with Bovela on milk yield in lactating dairy cows

under field conditions.

Materials and Methods: This field study was a randomized, blinded and negative controlled study according to the principles of Good Clinical Practice (GCP) in lactating cows. A total of 139 lactating cows in their high lactation phase, between 1 month after calving and 6 months of lactation, with different serological BVDV status were included in the study. Seven of the study animals were excluded before administration of IVP. Sixty-six (66) animals (group 2) were vaccinated once with a commercial titre of 10^5 $TCID_{50}$ / 2 ml vaccine. The same number of animals in the control group (group 1) received 2 ml of solvent (WPBS). The test and control items were administered intramuscularly (i.m.) in the same manner.

Daily milk yield, compared between both groups, was the primary parameter measured using an automatic milking system from one week before (day post vaccination (DPV) -7) vaccine administration until day 21, termination of the study. The experimental unit was the lactating cow. For each animal a baseline was calculated as the mean of the daily milk yields of DPV-7 – 0. The absolute changes from baseline and percent changes from the preceding seven days were calculated per animal for each day post vaccination (study days 1 – 21) and the mean daily milk yields were calculated per animal for each study week post vaccination. The period after vaccination was divided into 3 segments (DPV 1-7, DPV 8-14 and DPV 15-21).

Other parameters investigated were BVD-related clinical signs, rectal temperature, mastitis, injection site and BVDV-1 and BVDV-2 specific antibodies.

Results: The mean daily milk yield ranged between minimum of 26.43L on DPV 9 and a maximum of 29.07L on DPV 17 in group 1 and a minimum of 26.40L on DPV 7 and a maximum of 29.13L on DPV 17 in group 2.

No statistically significant difference were observed between group 1 and group 2 with regard to mean daily milk yield when comparing the three different periods (DPV 1-7, DPV 8-14 and DPV 15-21) after vaccination as well as between group 1 and group 2 with regard to changes from baseline when comparing the three periods after vaccination. No changes in mean milk yield of 10% or more was detected when comparing the daily mean milk yield of both groups to the milk yield of the seven preceding days.

The mean rectal temperature post vaccination stayed within the physiological range. There were no BVD-related clinical signs or reactions at the injection site reported. Antibody titres of the vaccinated group showed a statistically significant increase from study day (SD) 0 to 21 for both BVDV types. The antibody titre of the vaccinated group was also significantly higher than of the control group on SD21.

Conclusion: The primary parameter milk yield was evaluated between both study groups and revealed no statistically significant difference between vaccinated and control groups after vaccination. This demonstrates that there is no influence of vaccination with Bovela on milk yield. The vaccine is safe for use in lactating cows under field conditions.

Keywords: Vaccination, BVDV, bovine viral diarrhoea, milk yield.

IV-12

Impacts of meloxicam on IBR, BRSV, PI3, and CV titers and morbidity when administered concurrently with a modified-live respiratory vaccine in abruptly-weaned beef calves

Elizabeth R. Homerosky, Craig Dorin.

Veterinary Agri-Health Services, Ltd., Airdrie, Canada.

Objectives: The use of non-steroidal anti-inflammatories (NSAIDs) to mitigate pain and inflammation following stressful events or painful procedures has become common practice in the beef industry. However, a recent in-house study revealed that calves administered meloxicam on arrival at the feedlot were more likely to be treated for bovine respiratory disease (*P*=0.035). It remains unknown if the increased risk was due to impaired inflammatory response, decreased vaccination response, or a combination thereof. The objective of this study was to determine the effect of meloxicam on vaccination response and health by measuring titers for infectious bovine rhinotracheitis (IBR), bovine respiratory syncytial virus, parainfluenza 3, and coronavirus at three time points during the feeding period.

Materials and Methods: For this randomized controlled blinded clinical trial, 271 abruptly-weaned 650lb. steer calves were inducted into a single pen at an Alberta feedlot during October 2018. Upon induction, a venous blood sample was collected and calves received a 5-way modified-live respiratory vaccine, 8-way clostridial vaccine, growth implant, anthelmintic, and macrolide antibiotic. Calves randomly allocated to the control group (N=135) were administered saline, whereas calves allocated to the experimental group (N=136) received injectable meloxicam. Subsequent blood samples were collected at day 7 and 21 to measure early and peak immune response. Antibody titers were quantified using an ELISA test and results were interpreted by an immunologist. Calves were monitored daily for clinical signs of disease and treatments were recorded until an out-weight was measured at day 45.

Results: There were no differences in the direction and magnitude of antibody titers between treatment groups. Additionally, there were no differences in the proportion of calves that seroconverted to any of the viruses at day 7 and 21 between treatment groups except for IBR. At day 21 a greater proportion of calves in the meloxicam group seroconverted to IBR compared to the saline group (*P*=0.01). Morbidity was associated with treatment group as all four treated calves received meloxicam on arrival (*P*<0.05) and a single calf subsequently died. There were no differences in performance parameters at any handling events between treatment groups.

Conclusion: The association between meloxicam and increased morbidity is unclear, however decreased vaccination response is likely not a contributing factor. Stress associated with abrupt weaning methods, coupled with concurrent administration of vaccines and long-acting NSAIDs likely creates a complex immunological response. Further research is warranted to explore the relationship between NSAID administration and health outcomes.

Keywords: NSAIDs, immunity, bovine respiratory disease, feedlot, ELISA.

IV-13

Disease Protection and Immunogenicity of Two Commercial Intranasal Vaccines Evaluated with a BHV-1 Challenge of Weaned Beef Calves

Michael Bolton[1], Philip Griebel[2], Kevin Hill[3], Scott Nordstrom[3], Geert Vertenten[4].

[1]Veterinary Consultant, Michigan, MI, United States; [2]Intervac University of Saskatchewan, Saskatchewan, Canada; [3]Merck Animal Health, Madison, NJ, United States; [4]MSD Animal Health, Boxmeer, Netherlands.

Objective: This study compared both immunogenicity and disease protection between 2 commercial intranasal vaccines (Nasalgen IP® and Inforce 3®), when used in beef calves and challenged with BHV-1.

Materials and Methods: One hundred, 4 to 10 week-old calves at processing were randomly assigned to five intranasal treatment groups (n = 20/group): Group A - vaccine diluent at processing and weaning, Group B – NasIP (Nasalgen IP®) at processing and booster at weaning; Group C – vaccine diluent at processing and primary NasIP at weaning; Group D- Inf3 (Inforce 3®), at processing and booster at weaning; and Group E –vaccine diluent at processing and primary Inf3 at weaning. The analysis of BHV-1 serum neutralizing antibody titres confirmed IN vaccination was performed with maternal antibodies present. All calves were removed from dams at 5- 6 months of age and 14 healthy, BVDV negative calves from each group were shipped the same day from the ranch to the research station. The day after arrival, calves received the designated weaning vaccination. Three days later all calves were aerosol challenged with BHV-1 and monitored daily for clinical respiratory signs, body weight, and rectal temperature. Nasal secretions and serum samples were collected to quantify innate and acquired immune responses.

Results: Analyses of clinical responses following BHV-1 challenge revealed mean rectal temperatures among all vaccinated animals were significantly ($p \leq 0.0001$) lower than animals receiving diluent. Weight loss following BHV-1 infection was reduced compared to controls in all vaccination groups ($p < 0.0001$). Differences in temperature and weight were significant among vaccination groups but were numerically small.

Over the duration of the study all vaccinate groups shed less virus than the diluent control calves. Inf3 booster vaccination significantly ($p = 0.0008$) reduced virus shedding when comparing to primary Inf3 vaccination. NasIP booster group shed significantly ($p < 0.0007$) less virus beginning on day 4 post-infection. However, a significant reduction in virus shedding was not observed until day 5 post-infection with both Inf3 booster vaccination ($p = 0.0001$) and NasIP primary vaccination ($p = 0.03$). The primary Inf3 vaccination significantly reduced virus shedding only on day 6 ($p = 0.04$) and day 9 ($p = 0.001$) post-infection and this group did not significantly ($p = 0.197$) reduce the number of days virus was shed compared to the control group. In contrast, NasIP primary ($p = 0.017$), NasIP boost ($p = 0.0007$), and Inf3 boost ($p < 0.0001$) significantly reduced the number of days on which individual animals shed virus.

IFN alpha and gamma secretion was significantly ($p < 0.0001$) lower in all vaccinate groups compared to the control group. Both booster groups had significantly lower IFN alpha and IFN gamma secretion ($p < 0.0001$) compared to primary vaccination groups.

The NasIP booster group was the only group displaying a significant increase in BHV-1 serum IgG antibody titres three days after booster vaccination compared to controls ($p < 0.0001$) and all other vaccine groups ($p < 0.0003$). There were no significant differences in BHV-1-specific IgA antibody responses among treatment groups during the post challenge period ($p = 0.60$).

Conclusions: Differences in primary and boostered groups demonstrated that NasIP and Inf3 intranasal vaccination of young calves, when neutralizing maternal antibody was present in blood, induced BHV-1 specific immune memory that persisted for at least 4 months in the upper respiratory tract, evident primarily as significantly greater reductions in body temperature and IFN secretion post challenge. Primary vaccination with NasIP resulted in a more rapid onset of reduction in virus shedding compared to primary vaccination with Inf3 and primary NasIP vaccination resulted in a significant reduction in the number of days virus was shed but primary Inf3 vaccination did not. When comparing booster vaccination with NasIP and Inf3 there were small but significant differences in clinical disease but not virus shedding. The NasIP booster group was the only group to show a significant ($p = 0.0003$) increase in specific serum IgG three days after booster vaccination.

Keywords: Intranasal vaccines, BHV-1, protection, immunogenicity.

IV-14

Iron supplementation modulates the early immune response after intranasal vaccination of calves

Hans-Joachim Schuberth[1], Alina Kauke[1], Lennart Golbeck[2], Walter Grünberg[2], Imke Cohrs[3], Theresa Scheu[3], Esther Humann-Ziehank[4].

[1]University of Veterinary Medicine / Institute for Immunology, Hannover, Germany; [2]University of Veterinary Medicine / Clinic for Cattle, Hannover, Germany; [3]Educational and Research Centre for Animal Husbandry, Hofgut Neumuehle, Münchweiler an der Alsenz, Germany; [4]LABVETCON - Laboratory Veterinary Consulting, Burgdorf, Germany.

Objectives: Iron is involved in immune cell differentiation, metabolism and function. We addressed the question whether iron supplementation and the route of supplementation affects the response of calves towards an early intranasal vaccination with a live vaccine.

Materials and methods: A total of 29 healthy, purebred Holstein-Friesian calves were randomly assigned into one of three groups. Calves were born to pluriparous cows over the course of 11 months. Within 1h of birth calves received 4L of fresh colostrum from their respective dam (voluntary + rest drenched) and were treated with either a subcutaneous injection of 1000mg Fe3+ (INJ, n = 10), 1050 mg Fe3+ mixed into the colostrum (ORAL, n = 9) or with 0.9% NaCl as a sham treatment (CON, n = 10). Calves were then transferred to iglus

for 21 days and grouped thereafter. 10L of fresh milk from the own dam were fed for 5 days, feeding was then switched to 10L of milk replacer (1500 g/d, 15% DM, 65 mg FE/kg).

Calves were intranasally (i.n.) vaccinated at day 11 (between day 8 and 14 post natum) with a modified life vaccine against parainfluenza 3 and bovine respiratory syncytial virus (Bovalto Respi® intranasal). Heparinized blood was taken immediately before i.n. vaccination and 24 hours later. Major blood leukocyte subpopulations (granulocytes, lymphocytes, monocytes) were quantified flow cytometrically after hypotonic blood lysis. Mononuclear cells obtained after density gradient centrifugation were incubated with bovine-specific directly labelled monoclonal antibodies and cellular subpopulations (CD4+, CD8+, and gd T-cells, CD21+ B-cells, CD21-/MHC class II+ lymphoid cells, NK cells, classical, intermediate and non-classical monocytes) were determined flow cytometrically. Changes in leukocyte subpopulation numbers and ratios between cell types (24 h after vaccination versus before vaccination) were recorded as fold changes (FC). At day 30 (between day 28 and 32 post natum) all calves were vaccinated subcutaneously (s.c.) with an inactivated vaccine (Bovalto Respi® 3). Calves were categorized into those showing a swelling within 2 days at the injection site (SWELL+) and those with no side effect (SWELL-).

Results: Within the ORAL group, intranasal vaccination resulted in significant FCs > 1 of total leukocytes (FC: 1.10, p = 0.008), lymphocytes (FC: 1.35, p = 0.006), CD2+ T cells (FC = 1.6, p = 0.04), gd T cells (FC = 1.32, p = 0.012). The monocyte/lymphocyte ratio was significantly lower (FC = 0.79, p = 0.009) and the neutrophil/lymphocyte ratio dropped in tendency (FC = 0.87, p = 0.098). Within the INJ group, the numbers of CD4+ T cells dropped in tendency (FC = 0.85, p = 0.078) whereas the ratio between intermediate and non-classical monocytes displayed a significant rise (FC = 1.34, p = 0.0425). CON calves responded with a significant higher CD4/CD8 ratio (FC = 1.29, p = 0.033). The numbers of CD21-/MHC II+ lymphoid cells dropped in tendency (FC 0.86, p = 0.094), whereas numbers of intermediate (FC = 1.28, p = 0.078) and non-classical monocytes (FC = 1.38, p = 0.080) tended to rise. After s. c. vaccination on day 30, 1/9 ORAL calves, 4/10 INJ calves and 3/10 CON calves were SWELL+ (chi2, p > 0.1). SWELL- calves differed significantly (p < 0.02) from SWELL+ regarding their previous response after i.n. vaccination: SWELL- calves displayed positive FCs of lymphocytes (FC= 1.24 ± 0.08), CD4+ T-cells (FC = 1.61 ± 0.22) and CD8+ T cells (FC = 1.04 ± 0.12). FCs of SWELL+ calves were < 1 (lymphocytes: FC = 0.85 ± 0.07; CD4+ T cells: FC = 0.66 ± 0.09; CD8+ T cells: FC = 0.69 ± 0.06).

Conclusions: Intranasal vaccination generates signals resulting in an altered circulation behavior of immune cell subpopulations and/or their release from primary and secondary immune organs. Iron supplementation and the route of supplementation alters vaccination-induced changes of leukocyte subpopulation numbers in blood. A lack of side effects after s.c. vaccination in calves responding with raised numbers of circulating lymphocytes after i.n. vaccination, more frequently seen in orally iron supplemented calves, supports route-specific immune modulatory effects of iron supplementation.

Keywords: Iron supplementation, calves, vaccination, immune response.

IV-15

Comparing the effects on calf health after use of a commercially available *Mycoplasma bovis* vaccine in dairy herds in Scotland

Graeme Fowlie.

Meadows Veterinary Centre, Oldmeldrum, Inverurie, United Kingdom.

Objectives: *Mycoplasma bovis* is a bacterial pathogen of cattle with rising significance in the UK. With variable clinical signs such as pneumonia, mastitis and lameness causing substantial morbidity and mortality in all age groups and cattle systems coupled with difficulties in diagnosis and treatment *Mycoplasma bovis* control presents a real challenge to the cattle industry. Control methods of *M. bovis* infection include extended antibiotic courses metaphylactically, segregating and culling animals and pasteurising colostrum. Improvements in herd biosecurity has been recommended to prevent disease incursion into negative herds. Vaccination using autogenous vaccines has been used for some time in the UK with positive results in published data. A commercial 3-strain *M. bovis* vaccine available in the US, but not Europe, was acquired for this study. There is very limited published data on the efficacy of any commercially available *M. bovis* vaccine. The objectives of this study were to evaluate this commercial vaccine under UK field conditions in terms of safety, ease of incorporation into existing vaccine protocols (i.e. proof-of-concept), reducing mortality and reducing antimicrobial use in dairy calves up to 200 days old.

Materials and Methods: 4 dairies in North-East Scotland with evidence of mycoplasma herd infection and calf pneumonia were selected for vaccination, 4 other dairies were observed as controls. 902 calves born prior to vaccination (first time-period) and 680 calves born after vaccination was commenced (second time-period) were observed up to 200 days old for antibiotic use and mortality. Cows were vaccinated once at drying off (8 weeks pre-calving). Calves were vaccinated at 4 weeks of age. No changes to pre-existing farm vaccine protocols or routine herd management were made on any of the 8 farms during the study. A Kruskal-Wallis Rank Sum Analysis was performed on mortality data. Antibiotic usage was analysed using a Student's Independent T-Test two-sample assuming unequal variance. Vaccine safety and ease of incorporation into existing vaccine protocols was assessed with herd manager discussions.

Results: No adverse reactions were reported during the study in either calves or cows. All farms enrolled in the study successfully completed the vaccine protocols in cows and calves. All farms continued to use the vaccine after the conclusion of the study. There was a significant reduction in post-weaning mortality after vaccination in the second time period (P<0.02). There were 31 deaths in pre-vaccination born calves during the post weaning period (n=534), there were 2 deaths in the post-vaccination born calves during the post-weaning period (n=398). Calves born on non-vaccinating farms showed a slight increased mortality risk in the second time-period. There was a significant reduction in antibiotic usage after vaccination compared to control farms (p<0.05). Vaccinating farms purchased 70.2% less antibiotics after vaccination. Control farms (with qualifying data) purchased 33.9%

more during the second time-period.

Conclusions: This preliminary study demonstrated the safety and proof-of-concept of a commercially available *Mycoplasma bovis* vaccine in UK dairy herds. Due to study design and data gathering insufficiencies the significant reductions in post-weaning mortality and antimicrobial usage can only be broadly suggestive of an effect of the vaccine. Further work on assessing the effect of this vaccine in European cattle herds infected with *M. bovis* is warranted.

Keywords: Mycoplasma bovis, vaccine, dairy calves.

IV-16

Evaluation of health and lactation performance of dairy heifers supplemented with colostrum during the pre-weaning period

Jenna Stockler[1], Manuel Chamorro[1], Thomas Passler[1], Herris Maxwell[1], Manuel Campos[2], Tom Earleywine[3], Debbie Haines[4].

[1]Auburn University College of Veterinary Medicine, Auburn, Alabama, United States; [2]Saskatoon Colostrum Company Ltd., Saskatoon, Canada; [3]Land O' Lakes Animal Milk Products, Dane County, United States; [4]University of Saskatchewan College of Veterinary Medicine, Saskatoon, Canada.

Objectives: To determine if supplementing dairy heifers with 60 g of colostrum-Immunoglobulin G (IgG) in the daily milk-replacer ration during the first 75 days of life resulted in lower incidence of health events, greater first-lactation milk production, and greater survival to second lactation compared with age-matched dairy heifers that did not receive IgG supplementation.

Materials and Methods: A randomized controlled clinical trial was performed. Thirty Holstein heifers from a single dairy herd were separated from the dam immediately after birth and received 300 g of IgG from a colostrum-derived commercial colostrum replacer within 6 hours of birth. At 24 hours of age (Day 1), heifers were assigned to two different treatment groups. Group CR (n=15) received 150 g of a commercial colostrum replacer containing 30 g of IgG mixed with 304 g of a commercial milk replacer twice daily until weaning (day 75). Group MR (n=15) received 453 g of milk replacer with no colostrum replacer supplementation twice daily until weaning and acted as the control group. Blood samples were collected from all heifers at birth, at 24 hours of life to evaluate serum total protein (STP) and serum IgG levels. On-farm personnel blinded to treatment allocation monitored the calves daily and recorded morbidity and mortality events as well as treatments. Individual body weights were collected from all heifers monthly until calving. Milk production was recorded during first lactation and the corrected 305 Mature-Equivalent (ME) milk production was calculated for each heifer.

Results: During the pre-weaning period (Days 1 to 75), a greater proportion of diarrhea events and treatments were recorded in the MR group compared with the CR group ($P < 0.05$); however, respiratory disease, umbilical infection, joint infection and other morbidity events were similar between treatment groups ($P > 0.05$). Mortality was not observed among treatment groups during the pre-weaning period. The mean serum IgG and STP at 24 hours were similar between CR (27.6 g/L and 6.32 g/dL, respectively) and MR (26.42 g/L and 6.17 g/dL, respectively) calves ($P > 0.05$). Individual body weights were similar among treatment groups from birth to weaning and from weaning to calving ($P > 0.05$). The mean corrected 305-ME lactation was similar among heifers from the CR and MR groups ($P > 0.05$); however, survival to second lactation was greater in the CR group ($P < 0.05$). A greater number of heifers in the MR group were culled due to health reasons compared with heifers from the CR group (4 vs. 0, respectively).

Conclusions: The administration of 300 g of IgG through a colostrum-based commercial colostrum replacer to newborn calves results in serum IgG levels superior to what is considered excellent transfer of passive immunity (25 g/L) in dairy calves. Supplementation of dairy heifers with colostrum IgG throughout the pre-weaning period results in disease-sparing effects that extend to the start of the second lactation.

Keywords: Colostrum, IgG, Diarrhea, Survival, Lactation.

IV-17

Cellular and humoral immune response elicited in cattle through combined mucosal and systemic immunization with *Neospora caninum* membrane antigens

Alexandra Correia[1], Carla Mendonça[2], Margarida Duarte Araújo[3], Mariana Resende[1], Elena Pérez Antón[4], Luzia Teixeira[5], António Rocha[2], Manuel Vilanova[1].

[1]Instituto de Ciências Biomédicas Abel Salazar, University of Porto; i3S/IBMC, University of Porto, Porto, Portugal; [2]Instituto de Ciências Biomédicas Abel Salazar, University of Porto; Centro de Estudos de Ciência Animal-Instituto de Ciências, Tecnologias e Agroambiente, University of Porto, Porto, Portugal; [3]Instituto de Ciências Biomédicas Abel Salazar, University of Porto; LAQV/REQUIMTE, University of Porto, Porto, Portugal; [4]i3S/IBMC, University of Porto, Porto, Portugal; [5]Instituto de Ciências Biomédicas Abel Salazar, University of Porto; Unidade Multidisciplinar de Investigação Biomédica, University of Porto, Porto, Portugal.

Neospora caninum is an obligatory intracellular protozoan causative of abortion and stillbirths in cattle posing an estimated global economic burden exceeding one billion dollars *per year* to dairy and beef industries and farms. Vaccination is considered the most cost-effective approach to manage neosporosis, however no commercial vaccine is currently available to prevent this disease. We have developed an experimental intranasal vaccine against neosporosis, using *N. caninum* membrane antigens (NcMP) plus CpG adjuvant, that conferred IFN-gamma-dependent long-term protection to mice challenged with this protozoan.

Objectives: We aimed at improving the immunogenicity and mucoadhesiveness of this experimental vaccine in cattle by adding an additional adjuvant and administration route.

Material&Methods: Holstein calves were immunized by in-

tranasal administration of bovine-specifc CpG and NcMP, plus a carbomer-based adjuvant. To enhance the systemic immune response elicited by the reformulated mucosal immunization, we combined the intranasal administration with a subcutaneous boost of NcMP plus CpG. Cellular and humoral responses were evaluated in immunized and sham-immunized calves. Expression of genes encoding putative antimicrobial proteins was evaluated in bovine monocyte-derived macrophages infected with N. caninum and stimulated with IFN-gamma.

Results: The immunized calves presented elevated levels of parasite-specific IgG and IgA antibodies. Peripheral blood mononuclear cells collected from the immunized animals restimulated *ex vivo* with *N. caninum* antigens showed a marked proliferative response and elevated production of IFN-gamma. This host protective cytokine induced the *in vitro* upregulation of genes related with NO and ROS production, and autophagy in bovine monocyte-derived macrophages. These results show that the used immunization strategy induces parasite-specific humoral and cellular immunity in the bovine host. Additionally, it also stimulates the IL-12/IFN-gamma axis, a key protective mechanism against neosporosis.

Conclusion: Taken together, these results suggest that this optimized immunization approach may induce a protective response in cattle, a hypothesis that is currently being tested in calves.

Funded by FEDER through COMPETE 2020 and FCT - PTDC/CVT-CVT/31020/2017.

Keywords: Neospora caninum, mucosa, vaccination, IFN-gamma.

IV-18

Effectiveness of two intranasal vaccines for the control of Bovine Respiratory Disease (BRD) in newborn beef calves

Nicolas Masset[1], François Meurens[2], Maxime Marie[1], Pauline Lesage[1], Anne Lehébel[2], Nadine Brisseau[2], Sébastien Assié[2].

[1]INRAE, Oniris, BIOEPAR / SELAS EVA, Réseau Cristal, Nantes / Argentonnay, France; [2]INRAE, Oniris, BIOEPAR, Nantes, France.

Objectives: Bovine Respiratory Syncytial Virus (BRSV) is a major cause of Bovine Respiratory Disease (BRD) in newborn calves worldwide. Vaccination is widely used to prevent BRD and BRSV intranasal vaccines were developed to overcome interference with BRSV-specific maternally derived antibodies (MDA). Many experimental challenge trials have proven BRSV intranasal vaccine efficacy, but evidence of effectiveness under field conditions is still lacking, especially for newborn beef calves. The objective of this study was to compare the effectiveness in preventing BRD of a newly available commercial BRSV and BPI-3 intranasal vaccine (Bovalto Respi® Intranasal, Boehringer-Ingelheim) with that of the benchmarked one (Rispoval® RS + PI3 Intranasal, Zoetis) in newborn beef calves reared in a cow-calf farming system in France.

Materials and methods: A randomized non-inferiority multicenter trial was carried out to assess whether Bovalto Respi® Intranasal was at least as effective as Rispoval® RS + PI3 Intranasal, with a pre-stated margin of non-inferiority δ for the prevention of BRD in newborn beef cattle. Sample size was determined to demonstrate non-inferiority assuming α = 0.05, β = 0.20, δ = 0.05 and a prevalence of BRD in the active control vaccine group of 10%. Primary outcome was BRD cases during the in-housed risk period up to 3 months after vaccination. The statistical analysis of the primary outcome was carried out using a mixed logistic regression model. The variable in-housed risk period was kept in the model to adjust BRD occurrence to the variation of the duration of exposition to pathogens between calves. Least squares means were calculated from the model and used to calculate the difference in BRD prevalence between vaccine groups P_{BRD}(Bovalto Respi® Intranasal) - P_{BRD}(Rispoval® RS PI3 Intranasal) and its 95% confidence interval (CI). Secondary outcomes were compared between the two vaccine groups using the chi-squared test or Fisher test (mortality and lethality of calves, as well as antibiotic, non-steroidal and steroidal anti-inflammatory treatments administered during the in-housed risk period) and Student's t-test (time between the vaccine administration and the occurrence of BRD).

Results: A total of 935 Charolais calves from 39 farms were enrolled and randomized into 2 vaccine groups (Bovalto Respi® Intranasal n = 468; Rispoval® RS PI3 Intranasal n = 467). Age at vaccination, duration of in-housed risk period, parity of dams, sex ratio, and occurrence of diseases before vaccination do not significantly differ between the two experimental groups. There was no significant difference between the two vaccines regarding the occurrence of BRD during in-housed risk period. Using least squares means of model outcome, the difference of prevalence P_{BRD}(Bovalto Respi® Intranasal) - P_{BRD}(Rispoval® RS PI3 Intranasal) was estimated at -0.4%, 95% CI = [-1.6% ; 0.8%]. Moreover, no significant differences were observed between vaccines regarding mortality, lethality, duration between vaccination and the occurrence of BRD or treatments in the 2 groups.

Conclusions: Bovalto® Respi Intranasal is at least as effective as Rispoval® RS PI3 Intranasal for the prevention of BRD in newborn beef calves in a cow-calf system in field conditions.

Keywords: BRD, BRSV, Newborn Calf, Intranasal Vaccine.

IV-19

Systemic and local immune responses of beef calves vaccinated post transportation and at the time of a mild respiratory tract infection

Victor Cortese[1], Amelia Woolums[2], Brandi Karisch[2], Thomas Short[3], Merrilee Thoresen[2], Peres Badial[2].

[1]Zoetis, Simpsonville, United States; [2]Mississippi State University, Mississippi State, United States; [3]Zoetis, Parsippany, United States.

Objective: To assess the effect of transportation on immune responses to vaccination in calves.

Materials and Methods: Seventy-five weaned beef calves were randomly assigned to one of three groups (n=25). Group 1 was not transported (NTV) while groups 2 (TV) and 3 (TUV) were transported for 12 hours (day 0). Twelve hours later, NTV and TV were vaccinated intranasally with modified live bovine respiratory syncytial virus (BRSV), bovine herpesvirus −1 (BHV-1) and parainfluenza virus type 3(PI3V), and subcutaneously with modified live bovine viral diarrhea (BVDV) 1 and 2 with Mannheimia haemolytica (Mh) leukotoxoid vaccine. Nasal secretions and serum were collected pre and post vaccination for measurement of nasal interferon alpha, beta, and gamma and IgA to BHV-1 and BRSV, and serum neutralizing (SN) titers to BHV-1, BRSV, and BVDV 1 and 2.

Results: At vaccination some cattle had nasal discharge, fever, and coughing. Nasal swabs tested for common respiratory viruses pre-vaccination were negative. During the study, 5 cattle were treated for naturally occurring BRD. The BHV-1 and BVDV 1 and 2 SN titers were significantly higher in vaccinated than nonvaccinated calves on days 14 and 21. BVDV2 titers were significantly higher in TV than NTV. Response to vaccination was demonstrated in the systemic, but not nasal antibody responses.

Conclusions: This study demonstrates that cattle can mount a humoral response to vaccination in spite of transport and respiratory disease. Differences in serum and nasal responses further demonstrates the division between the local and systemic immune systems.

Keywords: Mucosal, immunity, vaccination, stress, shipping.

IV-21

Vaccination against Salmonellosis in veal farms in the Netherlands is an effective prevention method to reduce the use of antimicrobials

Niels Geurts[1], Henk Kuijk[2], Geert Vertenten[2].

[1]DAP Thewi, Tilburg, Netherlands; [2]MSD Animal Health, Boxmeer, Netherlands.

Objectives: Salmonellosis has been recognized as a disease in cattle all over the world for several decades. It has primarily been associated with S. enterica subsp. enterica serovar Dublin (S. Dublin) and S. enterica subsp. enterica serovar Typhimurium (S.Typhimurium). In view of the economic importance to the cattle industry and the potential to infect the human population, different vaccines have been developed. The objective of this study was to evaluate effect of vaccination with an inactivated Salmonellosis vaccine on mortality and reduction of antimicrobial use in veal farms in the Netherlands.

Materials & Methods: Forty-one animal groups in 6 Dutch veal farms with a history of both S. Dublin and S. Typhimurium were involved in the studies. Sixteen groups were vaccinated with an inactivated Salmonellosis vaccine (Bovivac® S, MSD Animal Health) according to the product data sheet and the other groups remained unvaccinated against Salmonellosis. The vaccinated groups were compared to the non-vaccinated groups over a rearing period of 25 to 26 weeks for mortality, daily antibiotic dose, daily antibiotic dose as defined by the SDa (Netherlands Veterinary Medicines Institute), the rearing cost per calf and the rearing cost per calf excluding vaccination.

Results: In total 42620 animals were included in the study: 17839 vaccinated and 24781 not vaccinated. The average animal group was 1115 and 991 animals for respectively the vaccinated and non-vaccinated groups. The average mortality was 2.54% in the vaccinated and 3.54% in the non-vaccinated group ($p<0.001$). The daily antibiotic dose (real and the one as defined by SDa) was significantly lower in vaccinated animals compared to non-vaccinated animals: 30.56 vs 40.20 daily dosages ($p= 0.001$) for the real daily antibiotic use and 13.06 vs 18.20 daily dosages ($p=0.009$) for the daily antibiotic dose as defined by the SDa. Finally, the average rearing costs were slightly higher in vaccinated (17.64 euro) compared to non-vaccinated animals (17.21 euro), but if the rearing cost excluding vaccination is considered, it is significantly ($p=0.012$) lower in the vaccinated group.

Conclusions: Vaccination against Salmonellosis with an inactivated vaccine is beneficial in Dutch veal conditions as there is less mortality and a strong reduction in antimicrobial use.

Keywords: Salmonellosis, vaccination, veal, antimicrobial reduction.

IV-22

The effect of local and systemic passive immunity acquired from maternal colostrum on clinical protection of beef calves against experimental challenge with BRSV.

Manuel Chamorro[1], David Martinez[1], Thomas Passler[1], Ricardo Stockler[1], Gage Raithel[1], Scott Silvis[1], Merrilee Thoresen[2], Paul Walz[1], Amelia Woolums[2].

[1]Auburn University College of Veterinary Medicine, Auburn, Alabama, United States; [2]Mississippi State University College of Veterinary Medicine, Starkville, Mississippi, United States.

Objectives: 1. To determine if vaccination of beef cows during the last trimester of gestation with two doses of an inactivated bovine respiratory syncytial virus (BRSV) vaccine resulted in greater transfer of local and systemic BRSV-specific passive immunity through maternal colostrum. 2. To determine the role of nasal BRSV-IgG-1 and IgA and serum neutralizing antibodies transferred from maternal colostrum on clinical protection of beef calves against experimental challenge with BRSV.

Materials & Methods: A randomized, controlled, clinical trial was performed. Forty, 3-month-old Black Angus-cross beef steers were assigned to 1 of 2 treatment groups. Group Vacc (n=20) nursed colostrum from cows vaccinated with 2 doses of an inactivated-BRSV vaccine before calving. Group NoVacc (n=20) nursed colostrum from unvaccinated cows. At 3 months of age, calves were challenged with BRSV by intranasal nebulization. Following challenge, respiratory signs were

scored. Nasal secretion and serum samples were collected before and after challenge for BRSV-specific nasal IgG1, IgA, and serum neutralizing antibody testing. Nasal secretion samples were collected after challenge for identification of BRSV by RT-PCR.

Results: Following BRSV challenge, mild respiratory scores were recorded in both groups (P > 0.05). The proportion of calves with fever (rectal temperature > 39.7 C) was greater in NoVacc calves. Nasal BRSV IgG-1 titers and serum neutralizing antibodies were greater in Vacc calves at 48 hours of life (P < 0.05); however, decayed similarly in both groups by 3 months of age (P > 0.05). Nasal BRSV IgA titers were non-existent following colostrum intake and before BRSV challenge and increased similarly (P > 0.05) in both groups after challenge. Calves in the NoVacc group had a higher probability of shedding BRSV in nasal secretions after challenge (P < 0.05). A greater proportion of NoVacc calves tested positive by BRSV RT-PCR after challenge (P < 0.05).

Conclusion: Vaccination of beef cows during the last trimester of gestation with two doses of an inactivated BRSV-vaccine was safe and resulted in greater transfer of local and systemic passive immunity to their calves. Moderate to low levels of BRSV serum neutralizing antibodies provide clinical protection against experimental challenge with BRSV. Nasal BRSV IgG-1 and IgA transferred from maternal colostrum do not play an important role on clinical protection of 3-month-old beef calves against experimental challenge with BRSV.

Keywords: Colostrum, IgG-1, IgA, neutralizing, BRSV.

IV-23

Evaluation of the cell-mediated immune response of dairy cattle vaccinated with an autogenous vaccine against mastitis sustained by *Staphylococcus aureus* and *Streptococcus agalactiae*

Matteo Cornaggia[1], Genovese Serena[2], Valentina Bertazzo[2], Katia Capello[2], Giulia Zarpellon[2], Tiziana Ferro[2], Lucillo Cestaro[3], Giulio Severi[4], Annalisa Stefani[2], Luca Bano[2].

[1]Istituto Zooprofilattico Sperimentale delle Venezie, Padova - Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna, Brescia, Italy; [2]Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy; [3]Veterinary Practitioner, San Donà di Piave, Italy; [4]Istituto Zooprofilattico Sperimentale dell'Umbria e Marche, Perugia, Italy.

Objective: Mastitis causes heavy economic losses in the dairy industry due to milk discharge, therapeutic costs, and culling rate of infected animals[4].

Staphylococcus aureus (SAU) and *Streptococcus agalactiae* (SAG) are important contagious bacteria agents of mastitis of cattle. In positive herds, their widespread is usually contained through the application of high hygienic standard measures, segregation, and blanket dry cow treatments that lead to a high consumption of antibiotics.

For this reason, authorities and consumers encourage the development of effective alternatives to the antimicrobial administration such as vaccines. Many vaccines against mastitis due to SAU have been tested with debated results, especially in field conditions[1,3].

The aim of our work was to evaluate the cell-mediated immune response elicited by a bivalent tailor-made vaccine produced with SAU and SAG as inactivated antigens, in field conditions.

Materials and methods: The herd was composed by 89 lactating Friesian cows hosted in the same experimental condition and served by an automatic milking system.

SAU and SAG were previously detected in the bulk tank milk. Bacteria were cloned and a tailor-made bivalent inactivated vaccine was prepared with a final concentration of $7.5x10^8$ UFC/ml.

Eighty-two subjects were vaccinated twice (V group) while 7 subjects served as negative controls (C) and received only the placebo composed of the adjuvants and vaccine medium without bacteria.

The peripheral whole blood and the individual milk samples were collected from 7 animals randomly selected within the V group and 7 from the C group at 0, 1, 2 and 3 months after the first immunization.

The cell mediated response was investigated by flow cytometry on the blood samples measuring the lymphocytes T CD4+ and CD8+ previously stimulated *in vitro* for 24h with heat-shocked cultures[2] (HSC) of SAU and SAG, and the lymphocyte activation pattern CD25+ was recorded. Concanavalin A (ConA) and PBS stimulation were used as a positive and negative control respectively.

Linear mixed models were adopted to evaluate the effects of groups (V and C), type of stimulation (SAG, SAU, ConA or PBS) and sampling time on parameters.

Results: Differences in cell response were significant (p=0.001) when blood was incubated in the wells with HSC, ConA and PBS. Statistical analysis revealed a significant difference in the increase expression of CD4+CD25+ against SAG (p= 0.0037) in the V group after the immunization. No differences were recorded for SAU vaccination.

Bacteriological evaluation of the individual milk resulted negative to SAG while 1 subject resulted positive to SAU in the V and C groups, respectively.

Conclusions: The study of the antigen-specific cellular subsets activated in response to SAG and SAU was aimed to achieve an *in vitro* test for cell-mediated response evaluation to study the effectiveness of vaccine against bovine mastitis.

The specific increase expression of $CD4^+CD25^+$ lymphocytes in the V group against SAG suggest that vaccination is able to stimulate properly the cells of the immune system and this observation is supported also by the nonproliferation in the C group which received only the adjuvant.

The poor results obtained with SAU are probably due to the expression of *S. aureus* exotoxins that act as superantigens and result in the overproduction of cytokines and activation of T cells that make the measurement inconclusive.

This preliminary study needs further confirmation in experimental conditions to better understand the role of the vaccines in the stimulation of the cell-mediated immune response for the prevention of mastitis in cattle.

References:

1. Bröker, B. M., Mrochen, D., Péton, V. (2016). The T cell response to *Staphylococcus aureus*. *Pathogens*.
2. Brown, A. F., Murphy, A. G., Lalor, S. J., Leech, J. M., O'Keeffe, K. M., Mac Aogáin, M., McLoughlin, R. M. (2015). Memory Th1 cells are protective in invasive *Staphylococcus aureus* infection. *PLoS Pathogens.*
3. Pereira, U. P., Oliveira, D. G. S., Mesquita, L. R., Costa, G. M., Pereira, L. J. (2011). Efficacy of *Staphylococcus aureus* vaccines for bovine mastitis: a systematic review. *Veterinary microbiology*.
4. Ruegg, P. L. (2017). A 100-Year Review: Mastitis detection, management, and prevention. *Journal of dairy science*.

Keywords: Dairy cows, flow cytometry, vaccine, mastitis.

IV-24

UK study: comparing antibody quantities in commercial calf scour pastes vs. the same volume of colostrum from cows vaccinated with a commercial calf scour vaccine

Kat Baxter-Smith[1], Robert Simpson[1], Geert Vertenten[2].

[1]MSD Animal Health, Milton Keynes, United Kingdom; [2]MSD Animal Health, Boxmeer, Netherlands.

Objectives: Neonatal calf diarrhoea (scour) is a common and costly disease on farm. There are many preventative approaches which can be undertaken to reduce the incidence and severity of this disease. It has been demonstrated previously that feeding colostrum with high levels of antibodies is protective against calf scour[1,2]. No published peer-reviewed evidence could be found on a literature search for the efficacy of scour pastes in preventing calf scour, however a recent survey completed by 479 UK cattle farmers revealed that they rated dam vaccination and scour pastes as equally efficacious scour prevention tools[3].

Little information is available regarding quantity of antibodies in commercial scour pastes, therefore the aim of this study was to measure antibody levels of rotavirus and coronavirus in commercially available calf scour pastes, and compare the results with the same volume of colostrum from cows vaccinated with a commercial calf scour vaccine (Rotavec® Corona, MSD Animal Health). This vaccine is licensed to reduce the incidence and severity of calf scour by raising the level of antibodies to rotavirus, coronavirus and E. coli K99 in the dam's colostrum.

Materials and Methods: Six different popular commercial scour paste brands were sourced from various agricultural trade stores. 2 tubes of each brand were purchased.

Samples of colostrum from 13 randomly selected heifers and cows which had been vaccinated during pregnancy with a commercial calf scour vaccine (Rotavec® Corona; as per SPC) were collected directly after calving and transported to the lab.

Rotavirus and coronavirus antibody titres in all samples were determined using a standard fluorescent antibody virus neutralisation test in all samples of colostrum and scour paste. The antibody titres reported were the reciprocal of the final dilution at which significant neutralisation of the virus by the sample was still seen.

Results: From the 12 scour pastes that were tested; there was an average virus neutralisation antibody titre of 10800 (min 80, max 81920) for coronavirus and 4341 (min 40, max 14482) for rotavirus.

The average virus neutralisation antibody titres of the same volume of colostrum from the vaccinated cows and heifers was 57383 (min 7241, max 81920) for coronavirus and 33846 (min 10240, max 81920) for rotavirus.

Conclusions: The 6 brands of calf scour pastes tested in this study contained on average markedly numerically lower levels of rotavirus and coronavirus antibodies compared to the same volume of colostrum from vaccinated cows. Although often listed as components of the tested scour pastes in this study, values for antibody quantities were not stated on the 'contents' or 'ingredients' labels. This study determined that on average commercial scour pastes do contain substantially lower levels of antibodies. The numerical value of antibody titres in the scour pastes varied considerably between brands.

Feeding a new-born calf 10% body weight (4L in a 40kg calf) of colostrum in the first hours of life is recommended to ensure a high level of passive immunity[4]. Using the results of this study based on average scour paste antibody levels, giving 4 Litres of scour vaccine-boosted colostrum would provide the calf with the antibody equivalent of 700-1000 scour pastes (paste size 30g).

Scour pastes are not licensed for the prevention of calf scour and evidence is lacking to support their use for this Therefore, this study illustrates that calf scour pastes are not a suitable substitute for good colostrum management and dam vaccination in preventing calf scour.

Farmers could be misinformed if they consider scour pastes and vaccination as equally efficacious at preventing calf scour; and more education from vets is needed.

Keywords: Calf scour, antibodies, scour paste, vaccine.

IV-25

Can an injectable trace element supplement increase the immune response of dairy calves?

Andrew Bates[1], Matt Wells[2], Richard Laven[3], Line Ferriman[4], Axel Heiser[5], Clare Fitzpatrick[6].

[1]Vetlife Scientific Ltd, Temuka, New Zealand; [2]Virbac Ltd, Hamilton, New Zealand; [3]School of Veterinary Sciences, Massey University, New Zealand; [4]Vetlife Ashburton, Ashburton, New Zealand; [5]AgResearch, Hopkirk Research institute, New Zealand; [6]Department of Microbiology and Immunology, Otago University, New Zealand.

Objectives: This study was carried out on a pastoral spring calving dairy farm in New Zealand with approval from Massey University Animal Ethics Committee. It was designed

to investigate the effect of TMS supplementation on the immune system in young calves before weaning.

Recent New Zealand (NZ) work suggests that neo-natal injection of a trace mineral supplement (TMS) containing copper, selenium, manganese, zinc and chromium can reduce morbidity and mortality on NZ dairy farms in the first 140 days of life. Overseas, evidence from older calves indicates this form of TMS leads to a heightened immune response. In light of the association between TMS and reductions in morbidity and mortality, this study was designed to investigate the effect of TMS supplementation under NZ pastoral conditions on the immune system in young calves before weaning.

Materials and methods*:* The first 40, Jersey-Friesian cross-bred heifer calves born after the mid-point of calving on a mid-Canterbury dairy farm (43.91° S, 171.75° E), were blood sampled within 24 hours of birth for serum total protein, Cu, Se and Zn. Thirty of these were selected, using stratified randomisation to form two equal groups (treatment and control) with the same distribution of serum total protein, Cu, Se, Zn and for the breed and age. From the remaining 10 calves, five were selected using stratified randomisation to form a sentinel group to verify no field exposure to *Salmonella sp*. occurred during the trial period.

All calves were housed in covered, open fronted bay-sheds with solid walls up to 1.5m in groups of 10-12 and 1.5m^2 allocated space per calf. Treatment and sentinel groups were randomly distributed amongst the housing groups. Calves were bedded on wood chips which were topped up weekly and all calves remained on the farm of origin for the period of study.

All calves received two injections of a killed vaccine containing *Salmonella typhimurium, S. bovis-morbificans*, *S. hindmarsh* and *S.Brandenburg* at two and six weeks of age. At the same time as vaccination, the treatment group received an injection of a TMS containing 40mg Zn, 10mg Mn, 5mg Se, 15mg Cu per ml. Sentinel animals received no injections. All animals were bled weekly from 2-9 weeks. Samples were analysed for neutrophil and monocyte phagocytic function, gamma interferon response, Salmonella sp. antibody titres and serum selenium, copper and zinc and differences examined using Bayesian statistics.

Results: At weeks 3 and 4 there was a wide difference between TMS and control calves in the percentage of white blood cells phagocytosing with the 95% predictive interval (PI) for the difference in the distributions excluding zero (+15% (95%PI=10.0-20.0) and +8% (95%PI=2.0-13.0) respectively. At weeks 3,4 and 5 phagocytosis per cell was also greater in the TMS calves (+12% (95%PI=2.1-21.6), +19% (95%PI=8.2-28.8) and +10% (95%PI=1.6-20.6) respectively). There was no statistical evidence of a difference in gamma interferon or antibody production between TMS and control claves although gamma interferon response was numerically greater in the TMS group.

Conclusions: This study adds to the evidence that TMS supplementation can increase some components of the innate immune systems in young calves. Neutrophil and monocyte function were increased, with a numerical increase in gamma interferon production. Although we found no conclusive evidence for an increase in antibody response in supplemented calves, both treatment groups showed an antibody response greater than the sentinel calves despite the presence of maternal immunity.

Keywords: Trace mineral supplement, mmunity, phagocytosis, calf.

IV-26

Field efficacy trials with a new intranasal BRD vaccine

Birgit Makoschey[1], Piet Nuijten[1], Björn Sander[2], Eva Zschiesche[2], Geert Vertenten[1].

[1]MSD Animal Health, Boxmeer, Netherlands; [2]MSD Animal Health, Schwabenheim an der Selz, Germany.

Objective: The objective of these studies was to determine the efficacy of a new intranasal BRD vaccine (Bovilis® INtranasal RSP™ Live) under field conditions. The vaccine contains live, attenuated BRSV and PI3 strains. During lab studies it was shown that it significantly reduced nasal shedding and clinical symptoms caused by BRSV and PI3 challenge infections as well as reduction of lung pathology and viral load in the lungs in a BRSV infection model.

Materials and methods: Two blinded, randomised, placebo-controlled field trials were conducted to investigate the efficacy. The first one was conducted in Portugal (3 farms) and the second one in Germany (2 farms) and France (6 farms). Calves (approx. 250 in total) aged 5 to 12 days received the vaccine or a placebo intranasally directly from the tip of a syringe. Groups were separated as much as possible during the first 2 weeks after vaccination as spreading of the live vaccine viruses is known to occur. Animals were monitored for signs of BRD like increased rectal temperature, coughing, increased breathing rate, nasal discharge, ocular discharge and depression. In case of BRD a bronchoalveolar lavage (BAL) was performed. Nasal swabs (NS) were taken weekly starting 2 weeks after vaccination. BAL and NS samples were tested by PCR for 8 different BRD pathogens, including BRSV, PI3, BCoV, BoHV-1, *Mycoplasma bovis, Histophilus somni, Pasteurella multocida and Mannheimia haemolytica*. Sera were collected on day 0, 14, 28 and 84 and tested in virus neutralisation (VN) assays for antibodies against BRSV and PI3. The efficacy of the vaccine was assessed based on differences between vaccinated and control calves in 1) clinical signs of BRD, 2) PCR results for BRSV and PI3 in BAL and NS samples, and 3) VN titers against BRSV and PI3.

Results: In the first study in Portugal, 124 calves were included, 62 in each group. In the vaccinated group 4 animals were excluded (N=58), whereas in the placebo group 17 animals were excluded (N=45), of which 15 animals were tested positive for the PI3 vaccine strain using a specific PCR. This proved the transmission of the PI3 vaccine strain from vaccinated to some of the placebo calves.

There was a notable difference in clinical signs between the two groups: more animals in the vaccinated group were without clinical scores than in the placebo group: 19% of the vaccinated calves had a total clinical score of 0 versus 11.1% in the placebo group, 55.2% of the vaccinated calves had BRD scores of 0 versus 37.8% in the placebo group, and

29.3% of calves in the vaccinated group had no BRD symptoms and a negative BRSV and PI3 PCR result versus only 15.6% in the placebo group. PCR analyses of many BAL and nasal swabs were positive for several other BRD pathogens like *Pasteurella, Mannheimia* and BCoV. As expected, serological responses were low on average and VN titers declined over time.

In the second study, a very low incidence of infection in France occurred but in Germany a BRSV field infection took place. Further detailed analysis of the different efficacy parameters for Germany showed: i) a significant difference (p=0.0406) for nasal swab samples positive for BRSV between the vaccinated group (61.5%) and in the placebo control group (85.7%), ii) that BRD clinical scores had the tendency to be higher in the placebo control group, iii) no clear differences in serological responses against BRSV or PI3, iv) a notable difference of BAL samples positive for BRSV: 30.8% in the vaccinated group was positive for BRSV in comparison to 42.9% in the placebo control group.

Conclusion: Field studies to determine the efficacy of vaccines against BRD pathogens are complex and influenced by animal health status, weather conditions, farm management and exposure to various pathogens including those that are not part of the vaccine. Indeed, in both studies most samples contained several other BRD pathogens, besides BRSV and PI3. Nevertheless, this new intranasal BRD vaccine (Bovilis® INtranasal RSP™ Live) induced significant protection against nasal shedding of BRSV during an outbreak. This is the first EU-licensed intranasal BRD vaccine for which efficacy has been proven in a field trial.

Keywords: BRD, intranasal vaccination, field efficacy, PI3, BRSV.

IV-27

Effect of periparturient intranasal vaccination on post parturient health parameters in Holstein cows

Victor Cortese[1], Pablo Pinedo[2], Juan Rodrigo Pedraza[3], Thomas Short[3], D. Manriquez[2], A. Velasquez-Munoz[2], G. Solano[2].

[1]Zoetis, Simpsonville, KY, United States; [2]Colorado State University, Boulder, United States; [3]Zoetis, Parsippany, United States.

Objective: The objective of this study was to evaluate the effect on health and reproductive performance of vaccinating Holstein cows were vaccinated with an intranasal modified live viral bovine respiratory syncytial virus (BRSV), modified live, temperature sensitive bovine herpesvirus −1 (BHV-1) and modified live, temperature sensitive parainfluenza virus type 3 vaccine (INV) during the peri-parturient period.

Materials and Methods: In a large commercial dairy, 4834 multiparous cows were vaccinated 18-24 days prior to expected calving date (n=1198), within twelve hours after parturition (n=1250) or at both time points (n=1141). A group was left as unvaccinated controls (n0 intranasal vaccination, n=1245). Cattle were blocked based on parity group and expected calving date and randomized to the experimental treatments (vaccination and control) within blocks. Health and reproductive outcomes were monitored and compared to matched, randomly assigned control cows.

Results: The greatest effects of vaccination were significant decreases in total cows removed from the herd throughout the lactation, decreased presentation of retained fetal membranes, and significantly lower incidences of pneumonia and mastitis. Overall, a greater impact was determined with two doses and, if one dose was administered, the results tended to favor administration at calving.

Conclusion: This study indicates that using this INV during the periparturient period can improved several postpartum health outcomes due to the up regulation of the immune system and supports earlier work indicating better immune responses when administered on the day of calving. While this vaccine can be used for immune modulation, more importantly this study suggests that future immune modulators may have a better outcome if administered to the local immune system.

Keywords: Postpartum health, postpartum immune suppression, calving stress, intranasal vaccination, immunomodulation.

IV-28

Benchmarking of the immune response induced by commercial intranasal vaccines against BRSV in dairy calves

Gilles Foucras[1], Lola Romanos[1], Nathan Cebron[2], Blandine Gausseres[2], Julie Cournet[2], Christian Tasca[2], Stéfanie Bernheim[3].

[1]ENVT, Toulouse, France; [2]IHAP, Toulouse, France; [3]Zoetis France, Malakoff, France.

Objectives: BRSV is a main respiratory agent in young calves. The intranasal vaccine Rispoval® Intranasal RS+PI3 (Zoetis) is available for more than ten years and has a long history of protective effects in bovine herds. Recently three new vaccines based on the same principle became available on the European market. However little is known about the local immune response provided by this way of immunization in cattle, and if there is any difference in the priming capacity of the viral strains used in these vaccines.

Materials and methods: Cross-bred dairy calves (n=40) were allocated randomly to one of the four commercial vaccines (10/group), with equal numbers of males and females. Calves had received 4L of fresh pooled colostrum (Brix index >22) at birth, and were housed in individual hutches. At the age of 8-17 days according to the label of the summary of product characteristics, calves were vaccinated intranasally with one of the four vaccines following the manufacturer's recommendations with the indicated material and modalities for application. Blood was collected before immunization and serum BRSV-specific antibodies were assessed (Monoscreen AbELISA BRSV, BioX Diagnostics), so that titres were not known at the time of vaccine administration. Mucosal lining was collected using nasal swabs (Copan) at 0, 7, and 14 days after vaccination. Virus load was quantified by RT-qPCR (Biosellal).

Local immune response was assessed by quantifying cytokine production with a Multiplex bovine cytokines assay (Milliplex, MERCK-Millipore) and by ELISA (Kingfisher Biotech).

Results: At the time of vaccination, all calves had BRSV-specific IgG1 antibodies with a mean value of 74% [43;105%] compared to the positive control of the ELISA kit. No group difference of the serum BRSV-specific antibodies of the calves before vaccination can be detected. BRSV was detected in 8/10 calves vaccinated with Rispoval® IN RS+PI3 at one of the two dates post-vaccination, but was more inconstantly detected in other vaccine groups. Cytokine concentrations were normalized to the total protein amount recovered by nasal swabing. Most of the 15 cytokines were detected in the mucosal lining and varied according to the date of sampling upon vaccination. Only trends were seen despite differences of BRSV strains, modalities of application, volume of vaccine and amount of virus administered in one or the two nostrils. Rispoval® IN RS+PI3 had a good capacity to induce Interferon gamma-induced protein 10 (IP-10 or CxCL10) production early at d7 in all calves tested.

Conclusions: With this design, no significant difference of the local immune response was noticed during a period of two weeks after vaccine application, despite some favorable trends and an homogeneous response induced by Rispoval® IN. Further data are needed to define further the difference of priming capacity among intranasal vaccines, and the consequences on the protection herewith afforded.

Keywords: BRSV, vaccine, intranasal, calves, immunity.

IV-29

Influence of vaccination on the seroconversion of 2 major respiratory pathogens in German beef rearing farms

Egon Thesing[1], Geert Vertenten[2].

[1]Intervet Deutschland GmbH, MSD Tiergesundheit, Unterschleißheim, Germany; [2]MSD Animal Health, Boxmeer, Netherlands.

Objectives: An important part of the German beef sector is the fattening of Simmental/crossbreed bulls. BRD (Bovine Respiratory Disease) is the main health problem on those farms. The causes are multifactorial, but the contribution of different pathogens is widely accepted. Bovine Respiratory Syncytial Virus (BRSV) and *Mannheimia haemolytica* (Mh) are 2 major pathogens playing a pivotal role in the BRD complex. Nowadays, the diagnostic of pathogens is mainly done by antigen detection on respiratory samples, rather than serological identification of antibodies. As serological studies during the rearing time in beef calves are not commonly available, a study was performed to obtain insights in the serodynamics of BRSV and Mh using paired blood samples including different BRD vaccination schemes.

Materials & Methods: The study was done on 2 beef rearing farms in different regions of Germany over 2 separate years (2017-2018) during the winter season. All calves were vaccinated with a live intranasal BRSV-Parainfluenza-3 vaccine (Rispoval® RS-Pi3, Zoetis) and received metaphylactic antimicrobial treatment at arrival on the rearing unit. The vaccination protocol was continued with either 2 administrations at 2 and 6 weeks after arrival with a monovalent attenuated vaccine (Rispoval® RS, Zoetis) (control group (cg)) or a multivalent, inactivated BRSV-Parainfluenza-3-Mh vaccine (Bovilis® Bovipast RSP (MSD Animal Health) trial group (tg)). Paired serum samples were taken from relevant subsets of animals in the cg and tg. The first sampling was performed just before the first vaccination and the second sampling 12 weeks later.

BRSV and Mh antibodies were measured in the Centre for Diagnostic Services (MSD Animal Health, Boxmeer, The Netherlands) by *in house* developed ELISAs and titers were expressed as log2.

Seroconversion for BRSV and Mh was defined as a titer change of 2 log2 steps or more.

A statistical evaluation was done for several parameters with significance level 0.05.

Results: In total 1.127 calves (farm A n=931, farm B n=196) were included in the study. Eventually, paired samples were taken from 196 calves corresponding to approx. 12 % (n=137) of the calves in cg and 5 % (n=59) of the calves in tg.

The baseline serum titers for BRSV and Mh ELISA at the time of the first sampling were not significantly different between groups (p=0.28) and there was no influence of age to the humoral response (p=0.44).

Most calves in the control group showed no humoral response in BRSV titer. Moreover, the titers dropped in a quarter of the calves. Only in 5 calves (4 %) the BRSV -titers increased 4 fold (>2 log2 steps) and consequently identified as a seroconversion. In contrast to this, 58 % of the animals in the trial group seroconverted. If one assumes that the infection pressure is equally high in both groups, one can establish a significant ($p<0.0001$) better induction of antibody formation after vaccination with the trivalent inactivated vaccine. Apart from the vaccination with the inactivated vaccine, additional field infections might play a role in this seroconversion. A good BRSV response to vaccination with the multivalent inactivated vaccine (Bovilis® Bovipast RSP) has already been described by Berge et al. (2021).

Concerning the Mh antibody induction, 42% of the cg calves had a seroconversion despite the absence of a Mh strain in the administered vaccine. This is indicative for a field infection in those animals. A clear humoral response for Mh under field conditions in young dairy calves is reported also by Jozan (2021). Almost twice the rate (81%) of animals showed serum conversion in the tg (17% > 2 log2 steps; 64% > 4 log2 steps) which is due to the combination of the vaccine effect and the field infection as demonstrated in the cg.

Conclusion: Serological screening of antibodies in beef calf rearing farms provides useful and practical insights into the occurrence of infections and the impact of vaccinations. Interpretation of this data supports the establishment of targeted prophylactic measures. *Mannheimia haemolytica* is a frequently circulating pathogen in beef calf rearing farms in which the use of a trivalent inactivated BRSV-Parainfluenza-3-*Mannheimia haemolytica* vaccine (Bovilis® Bovipast RSP) leads to a clear humoral response.

Keywords: Seroconversion, BRSV, Mannheimia haemolytica, vaccination, German beef farms.

IV-30

Impact of prepartum administration of an infectious calf diarrhea vaccine on nonspecific colostral immunoglobulin concentrations of dairy cows

Greg Chambers[1], William Kelton[2], Grant Smolenski[3], Emma Cuttance[1].

[1]VetEnt Research, Te Awamutu, New Zealand; [2]Te Huataki Waiora School of Health, The University of Waikato, Hamilton, New Zealand; [3]MS3 Solutions Ltd, Hamilton, New Zealand.

Objectives: Passive transfer of colostral immunoglobulins from the cow to the calf is essential for calf health. The objective of this study was to determine if prepartum administration of a vaccine stimulates increased concentrations of colostral immunoglobulins of dairy cows beyond what is explained by vaccine-specific immunoglobulins.

Materials & Methods: A prospective cohort study was conducted on a spring-calving commercial dairy farm that had applied a policy of only vaccinating cows with even ear tag numbers with a calf diarrhea vaccine, while cows with odd ear tag numbers were left unvaccinated. Cows in the vaccinated group (even ear tag numbers, n=204) received a sensitizer and booster vaccination with a vaccine against bovine rotavirus (serotypes G6 and G10), bovine coronavirus and *E. coli* having the K99 pili adherence factor. A sensitizer was given because the study vaccine was different to the vaccine previously used. Cows in the control group (odd ear tag numbers, n=194) received a 2 mL subcutaneous sterile saline solution. Both groups received two treatments at a three-week interval, completing the treatments approximately two weeks prior to the planned start of calving. During the calving period, technicians separated calves from cows immediately after parturition and prior to suckling, and cows were completely milked out within six hours of parturition. Using novel techniques, vaccine-specific, total, and nonvaccine-specific (total minus vaccine-specific) concentrations of immunoglobulin classes A, G1, G2a and M (IgA, IgG1, IgG2a and IgM respectively) were quantified by mass spectrometry for 20 colostrum samples from each treatment group.

Results: Technicians harvested colostrum from a total of 47 cows (n=24 control cows and n=23 vaccinated cows). Two cows were excluded due to low BCS (n=1) and a spurious colostrum volume (n=1), leaving 45 eligible samples. After matching, 20 samples from each treatment group were randomly selected for analysis. The mean colostrum Brix % was 24.65 and 26.20% for unvaccinated vaccinated cows respectively, but this difference was not significant at the 5% level. We found 2.2- to 3.0-fold higher concentrations of vaccine specific IgG1, IgG2a and total immunoglobulins in the colostrum of vaccinated cows compared to control cows, and no difference in the concentration of IgA. We also found a 1.6-fold increase in the concentration of IgM. IgG2a concentrations were lower but still within the detection range of our assay. While no significant differences were observed for the other immunoglobulin isotypes, we found a 1.5-fold increase in total IgM concentrations associated with vaccination.

After subtracting vaccine-specific from total immunoglobulin concentrations, we again found a 1.5-fold increase in total IgM concentrations associated with vaccination but no significant differences for the other immunoglobulin isotypes (Table 1). Furthermore, the difference in mean non-vaccine-specific colostral IgM concentration between vaccinated and control cows (2.72 mg/ml) was larger in magnitude than the difference in vaccine-specific IgM concentration (0.15 mg/ml). Though not statistically significant at the 5% level, the predicted difference in mean non-vaccine-specific IgG1 concentration between vaccinated and control cows was 10.78 (95% CI = -9.03 - 30.58) mg/ml, which was larger than what was explained by the difference in vaccine-specific IgG1 concentrations of 0.98 mg/ml. This increase in IgG1 concentrations drove the bulk of the 12.9 (95% CI = -10.26 - 36.07) mg/mL increase in overall non-vaccine-specific immunoglobulin concentrations.

Table 1. Predicted mean concentrations of non-vaccine-specific colostral immunoglobulins for control (n=20) and vaccinated (n=20) cows in a cohort study evaluating the effect of prepartum vaccination of pregnant dairy cows with a calf diarrhea vaccine on colostrum immunoglobulin concentrations.

	Mean (95% CI) concentration (mg/ml)		
Immunoglobulin class	**Control group**	**Vaccinated group**	**P-value**
IgG1	95.30 (81.30, 109.31)	106.08 (92.07, 120.08)	0.278
IgG2a	4.73 (3.96, 5.50)	4.71 (3.95, 5.48)	0.972
IgA[1]	9.87 (7.79, 12.50)	8.73 (6.88, 11.10)	0.462
IgM[1]	5.78 (4.74, 7.05)	8.76 (7.18, 10.67)	0.005
IgG	100.03 (85.57, 114.49)	110.79 (96.33, 125.24)	0.294
Total Ig	115.46 (99.08, 131.84)	128.37 (111.99, 144.75)	0.267

1. Values were log transformed and then transformed back to the original scale due to non-normality.

Conclusion: It is possible that the vaccine, in addition to managing infectious calf diarrhea, may also improve colostrum quality through increased non-vaccine-specific colostrum immunoglobulin concentrations. Further research is necessary to determine the mechanism for these preliminary findings and what impacts it may have on calf health outcomes.

Keywords: Vaccination, colostrum, immunoglobulin, dairy cow.

IV-31

Bovine Myeloid Antimicrobial Peptide-28 (BMAP-28) mRNA Expression by Bovine Cells and Effects of Synthetic BMAP-28 on Bovine Respiratory Disease Pathogens.

Santiago Cornejo Tonnelier[1], Cassandra Barber[1], Merrilee Thoresen[1], Daryll Vanover[2], Hannah Peck[2], Philip Santangelo[2], Amelia Woolums[1].

[1]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University., Mississippi State, MS, United States; [2]Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, Georgia, United States.

Objectives: *Mannheimia haemolytica* (MH) is the principal bacterial pathogen associated with bovine respiratory disease (BRD) in cattle. Bovine Herpes virus type 1 (BHV-1) can cause BRD by itself or it can be associated with MH or other opportunistic bacteria. Existing antimicrobials do not consistently prevent BRD due to MH, and do not have an effect against viruses; bovine antimicrobial peptides (AMP) have immune-stimulating and nonspecific antimicrobial effects that could improve BRD control. Messenger RNA (mRNA) treatment could be used to induce AMP expression in cattle, but efficacy must first be confirmed *in vitro*. Synthetic AMP can be generated to use as standards when characterizing mRNA-expressed AMP. We hypothesized that bovine cells can express synthetic mRNA coding for the AMP BMAP-28 and that synthetic BMAP-28 can inhibit the growth of MH and elicit antiviral effects against BHV-1 virus.

Materials & Methods: Madin-Darby bovine kidney cells were cultured and transfected with mRNA coding for BMAP-28 linked to the reporter nanoluciferase. After 4, 12, 24, and 72 hrs, relative light units (RLU) and protein concentration were measured. Results were expressed as RLU/µg of protein.

MH at 500 CFU/ml was incubated with synthetic BMAP-28 at 10 or 100 µg/ml for 0, 12, or 24 hrs, and quantitative culture was performed.

BHV-1 at 10^3 and 10^4 IU/ml were treated with synthetic BMAP-28 at 10 or 100 µg/ml and incubated at 37ºC for 2 hrs. A TCID50 assay on Madin-Darby bovine kidney cells was performed for each treatment. TCID50 units were calculated after 5 days post-infection (dpi).

Results: Bovine kidney cells expressed mRNA coding for BMAP-28 with peak expression occurring at 24hs in cell lysates and supernatants. Synthetic BMAP-28 at 10µg/ml inhibited MH growth at 12 and 24hs post-treatment. Synthetic BMAP-28 at 100 µg/ml elicited antiviral effects against BHV-1.

Conclusions: Treatment of bovine cells with synthetic mRNA induces BMAP-28 expression *in vitro*. BMAP-28 can inhibit MH growth and BHV-1 replication. These results provide support for further research to test the mRNA-expressed product against BRD pathogens *in vitro* and *in vivo*. mRNA treatment to induce AMP expression could lead to new BRD control strategies.

Keywords: BMAP-28, mRNA, BRD, Mannheimia haemolytica, BHV-1.

IV-32

Q fever: Vaccinate as much as needed and as little as possible

Jens Böttcher[1], Gregor Siegl[2], Stephanie Geischeder[1], Frank Dautzenberg[1], Elisabeth Deckinger[1], Michaela Alex[1], Vanessa Turowski[1], Britta Janowetz[1].

[1]Bavarian Animal Health Service, Poing, Germany; [2]Meltl GmbH & Co.KG, Grabenstätt, Germany.

Objective: *Coxiella (C.) burnetii* is a zoonotic pathogen. Endemic infection in dairy cow farms is frequently observed. A vaccine (Coxevac™, Ceva Santé Animal) is available to control infection in cattle. In this case report a controlled vaccination scheme was implemented in an infected dairy cow farm over a time period of 4½ years. Shedding of *C. burnetii* with milk and during calving was monitored. A long-term follow-up and characterization of chronically infected cows was possible as these were not immediately removed. In order to avoid side effects of vaccination no regular revaccination of all cows every 9 months was performed, however, long-term vaccination consisted of a primary vaccination of heifers and a single booster vaccination before 1st and 2nd breeding (after 1st calving), respectively. The immune response of seronegative heifers was assessed by phase-specific antibody ELISA and IFN-γ-Recall assay (IFN-γ-RA) before/after primary vaccination and revaccination.

Materials and Methods: In 2015 acute infection (abortion and stillbirths) was observed in a dairy cow farm (225 lactating cows, Simmental cattle). *C. burnetii* was identified as a possible cause. The farmer asked for coordinated intervention measures. Since October 2015 until January 2020 pathogen-shedding during calving was monitored by vaginal swabs. Additionally, individual milk samples were collected yearly. In spring 2016 primary vaccination of cattle (>12 months) was performed.

Vaginal swabs and milk samples were tested by quantitative PCR (Böttcher et al., 2013); milk and serum samples were tested for phase-specific antibodies and Li-heparin blood samples were tested by IFN-γ-RA (Böttcher et al., 2017).

A group of 15 heifers (2017) were tested before (t0), 4 months (t1), 16 months after primary vaccination (t2 i.e. at revaccination) and 1 month after revaccination (t3) for phase-specific-antibodies and IFN-γ-reactivity.

Results: Detection rates of *C. burnetii* in vaginal swabs were 9/59 in 2015 (Oct-Dec), 20/142 in 2016 (Jan-Jun), 19/145 in 2016 (Jul-Dec), 1/236 (2017), 6/253 (2018), 5/292 (2019) and 0/56 (Jan/Feb 2020). Two chronically infected cows (#129, #934) shed *C. burnetii* at calving in 2018. Four cows in the same calving pen with #129 tested positive ($<10^3$ C.b./swab), too. In 2019, #129 tested negative at calving, however, 5 cows in the same calving group tested positive ($<10^{3,5}$ C.b./swab). Detection rates of *C. burnetii* in milk were 7/191 (2015), 5/225 (2016/Mar), 13/211 (2016/Aug), 8/190 (2017), 0/161 (2018), 2/229 (2019) and 3/225 (2020).

Five and three chronically infected cows were present in the herd in 2018 (## 126, 129, 133, 214, 934) and 2019 (##129, 214, 934), respectively. Cow #129 shed *C. burnetii* at 2nd, 3rd and 4th, cows #126 and #934 only once at 2nd and 6th parturition, respectively. Vaginal swabs of #133 and #214 tested negative at parturition. Cows #126, #129 and #934 constantly shed large amounts in milk (mean $10^{3,4}$, $10^{4,1}$, $10^{3,4}$/ml, respectively) whereas #133 and #214 showed intermittent low-level shedding in milk (mean $10^{1,4}$, $10^{1,2}$/ml, respectively). Mean PhI-/PhII-titres (milk) of cows #126, #129, #133, #214, #934 were 2705/5561, 1324/6053, 566/95, 294/114, 109/2639, respectively. Cow #214 was the last one that established a

chronic infection (since Aug2016).

Primary vaccination and revaccination of heifers was analysed: PhI-titre (serum) was negative until t2, after revaccination (t3) a mean titre of 10000 was reached. PhII-titre increased to 10000 at t1, decreased to 2000 at t2 and increased to 100.000 at t3. IFN-γ-RA increased from <15% to 40% of the poke-weed-mitogen-reactivity at t1, decreased negative (<15%) at t2 and increased to 80% at t3.

Conclusions: To our knowledge this is the first report describing the long-term shedding of *C. burnetii* in a vaccinated herd. Herd vaccination decreased shedding of *C. burnetii* at calving about 9 months later. In other words, vaccination had no effect on the infected uterus. Vaccination had no effect on chronic shedders. Primary vaccination and only one revaccination protected cows from becoming chronic shedders. The strong PhII-titres and IFN-γ-reactivity after revaccination exceeding the level after primary vaccination suggests the induction of an immunological memory by primary vaccination.

Acknowledgements: This study was financially supported by the Free State of Bavaria and the Bavarian Joint Founding Scheme for the Control and Eradication of contagious Livestock Diseases.

References:

Böttcher, J. et al. (2013) Berl Münch Tierärztl Wochenschr 126, 427-435.

Böttcher, J. et al. (2017) J Vet Med Res 4(9):1106.

Keywords: Q fever, Coxiella, vaccination, chronic shedding.

IV-33

Duration of immunity after a natural infection with bovine respiratory syncytial virus

Sara Hägglund[1], Katarina Näslund[1], Hakan Enul[1], Cecilia Lefverman[1], Leonore Pascal[1], Anna Svensson[1], Menno Holzhauer[2], Karin Alvåsen[3], Catherine Dubuquoy[4], Isabelle Schwartz-Cornil[4], Sabine Riffault[4], Geraldine Taylor[5], María Jose Rodriguez[6], Marga Garcia Duran[6], Jean François Valarcher[1].

[1]Swedish University of Agricultural Sciences, Dept. of Clinical Sciences, Unit of Ruminant Medicine, Host Pathogen Interaction Group, Uppsala, Sweden; [2]GD Animal Health, Deventer, Netherlands; [3]Swedish University of Agricultural Sciences, Dept. of Clinical Sciences, Unit of Epidemiology, Uppsala, Sweden; [4]Université Paris-Saclay, UVSQ, INRAE, VIM, Jouy-en-Josas, France; [5]The Pirbright Institute,, Woking, United Kingdom; [6]Inmunología y Genética Aplicada, S.A. (INGENASA), Madrid, Spain.

Objectives: Bovine respiratory syncytial virus (BRSV) infections commonly occur as epizootic outbreaks in nearby cattle herds. In parallel with improved biosecurity, the maintenance of herd immunity could contribute to minimize virus circulation. With the overall goal to identify vaccine targets, the objective of this work was to monitor the duration of BRSV-specific humoral and cellular immunity in cattle.

Materials and methods: Local and systemic BRSV-specific immunity was monitored in cattle in one Swedish dairy herd following a BRSV-outbreak. The herd consisted of 534 cattle with an average age of 33 months and an average yearly milk production of 9971 kg ECM/cow. Cows were housed in a free stall with cubicles, weaned calves in group pens and unweaned calves in hutches outdoor. During the outbreak, two cows died in respiratory distress and there was a loss of delivered milk that had an estimated value of 2300 Euro. BRSV was detected by ELISA (seroconversion) and/or virus isolation and/ or RTqPCR in cows, heifers and calves.

Blood and milk (when applicable) was collected from 33 female cattle of the Swedish red and white breed every two months during 26 months after the outbreak (post outbreak, PO). The sampled animals were either born during or just after the outbreak (n=5), or were aged 2-3 months (n=6), 4-5 months (n=6), 7-11 months (n=8), or 23-30 months (n=8) at the time of the outbreak. The oldest animals were either at < 6 (n=4) or > 6.5 (n=4) months of gestation.

Nasal secretions and saliva were repeatedly collected from five animals. To verify the absence of BRSV re-circulation in the herd, blood was collected at the end of the study from 24 additional animals born >4 months PO. BRSV-specific antibodies of different isotypes were analysed by ELISAs, along with BRSV-neutralising antibodies (NAb) by a virus neutralisation assay. Furthermore, attempts were made to detect BRSV-specific T cell responses in peripheral blood, by lymphoproliferation and ELISpot assays.

Results: All cattle that were older than 2 months at the time of the outbreak had or developed BRSV-specific serum IgG and BRSV-F competing antibodies, which remained stable during at least 26 months. The four cows in > 6.5 months of gestation had the lowest levels of such antibodies and two cows did not secrete detectable BRSV-specific IgG in milk. The youngest cattle, which were possibly infected in the presence of maternally derived antibodies, became seronegative within 4-6 months PO (within 3-5 months of age) and remained so for at least 26 months, inferring that no re-infections occurred. All animals that were born >4 months PO were seronegative at the end of the study.

The BRSV-NAb responses and the BRSV-specific serum IgG2 followed a similar pattern as IgG, but with a greater individual variation. The highest NAb-titres were detected in some of the heifers that were 5-11 months old at the outbreak, but titres dropped within 4-6 months. Another such heifer, from which virus had been isolated, developed very poor NAb responses but high levels of BRSV-specific IgG2.

BRSV-specific IgA was detected in nasal secretions of all sampled cattle two months PO, but these responses were not stable over time. In contrast, BRSV-specific local IgG2 responses were more stable but varied between individuals, in agreement with the data on BRSV-specific IgG2 in serum. Overall, BRSV-specific IgG2 and IgA were poorly detectable in saliva. It was not possible to detect BRSV-specific T cell responses in peripheral blood two months PO.

Conclusions: In conclusion, it was demonstrated that BRSV-specific IgG lasts for at least two years in animals infected above 2 months of age but are not always detectable in milk. The BRSV-specific IgG2 antibody responses and the BRSV-NAb titers varied between individuals. In young animals, a drop of NAb titers was observed within 4 months, whereas

in adults, the NAb titers were low but stable. Cows that were near calving during an outbreak and all calves born later than two months before an outbreak, appear as the most critical candidates for vaccination in order to limit virus circulation in the field. More investigations are needed to confirm these data and to identify if previously infected animals are virologically protected by their antibodies upon reinfection.

Keywords: BRSV, immunity, duration, neutralising antibodies, respiratory disease.

IV-34

Virus detection by PCR following vaccination of naive calves with intranasal or injectable multivalent modified-live viral vaccines

Victor Cortese[1], Paul H. Walz[2], Benjamin W. Newcomer[2], Kay P. Riddell[2], Daniel W. Scruggs[3].

[1]Zoetis, Simpsonville, United States; [2]Auburn University, Auburn, United States; [3]Zoetis, Parsippany, United States.

Objective: To evaluate the duration and cycle times of PCR-positive results following administration of modified-live viral (MLV) vaccines to sero-negative beef calves.

Materials and Methods: Twenty beef calves were randomly assigned to either group 1 and vaccinated intranasally with a MLV vaccine containing bovine alpha herpesvirus 1 (BoHV-1), bovine respiratory syncytial virus (BRSV), and bovine parainfluenza virus 3 (BPIV-3), or to group 2 and vaccinated subcutaneously with a MLV vaccine containing bovine viral diarrhea virus 1 and 2 (BVDV-1, -2), BoHV-1, BRSV, and BPIV-3. Deep nasopharyngeal swabs (NPS) and transtracheal washes (TTW) were collected from all calves, and whole blood was collected from group 2 calves and tested by PCR.

Results: In group 1, the proportions of calves that tested PCR-positive to BVDV, BoHV-1, BRSV, and BPIV-3 on any sample at any time were 0%, 100%, 100%, and 10%, respectively. In group 1 calves, 100% of calves became PCR-positive for BoHV-1 by day 3 post-vaccination and 100% of calves became PCR-positive for BRSV by day 7 post-vaccination. In group 2, the proportions of calves that tested positive to BVDV, BoHV-1, BRSV, and BPIV-3 on any sample at any time were 50%, 40%, 10%, and 0%, respectively. All threshold cycle (Ct) values were >30 in group 2 calves, irrespective of virus; however, Ct values <25 were observed in group 1 calves from PCR-positive results for BoHV-1 and BRSV. All calves were PCR-negative for all viruses after day 28.

Conclusion: Following intranasal MLV viral vaccination, PCR results and Ct values for BRSV and BoHV-1 suggest that attempts to differentiate vaccine virus from natural infection is unreliable.

Keywords: Bovine virus vaccination, deep nasopharyngeal swabs, PCR.

IV-35

Efficacy of a vaccine to control Coxiellosis in goats 1 year after primo-vaccination: assessment of the duration of immunity

Philippe Gisbert[1], Tamas Szalai[2], Jean De Foucauld[1], Georges Orszagh[2].

[1]Ceva Santé Animale, Libourne, France; [2]Ceva Phylaxia, Budapest, Hungary.

Objectives: In small ruminants, Coxiellosis also named Q fever is responsible for abortion, stillbirth and weak born. In addition, Q fever is a zoonosis which can cause flu-like syndromes in humans but also abortions or cardio-vascular disorders. It is therefore a major public health concern. Control of Q fever is therefore of main interest.

In this challenge study, the efficacy of an inactivated vaccine against *Coxiella burnetii* - Nine Mile strain phase I (Coxevac®, Ceva Santé Animale) one year after primo vaccination on goats was evaluated on two main criteria:

- Reduction of abortion rate.
- Reduction of shedding in milk, faeces and vaginal mucus.

Material and methods: The study was conducted in compliance with the provisions of Directive 2010/63/EU relative of the protection of animals used for scientific purposes.

Forty 3 months old goats were vaccinated twice 3 weeks apart according to the product's label (Coxevac® 2 mL subcutaneously); forty goats of the same age were not vaccinated and were included as control. Eleven months later, goats were mated after oestrus synchronization. Finally, 14 pregnant goats in the vaccinated group and 7 from the non-vaccinated were selected.

At 75 +/- 7d of pregnancy (1 year and 27 days after the second injection of the vaccine), goats were challenged subcutaneously with a heterologous field strain of *Coxiella burnetii* (CbC1).

Abortion rate and number of live kids were assessed. Faeces and vaginal shedding were measured by qPCR from 14 days post challenge to 35 days post abortion/kidding. Milk shedding was measured by qPCR from the day of abortion/kidding to 35 days. For these three types of samples, both ratio of shedders animals and quantity of excreted *Coxiella burnetii* were measured.

Results: Five out of seven goats of the control group aborted (71.4%) while there were only three out of 14 in the vaccinated group (21.4%). The difference between the two groups was significant (p=0.0408).

The rate of non-viable or aborted kids was 71.4% and 11.1% in the control group and in the vaccinated group, respectively (p=0.0017).

From d56 post challenge to d35 post kidding/abortion, the proportion of shedders in faeces and vaginal mucus was significantly higher (p<0.003 and p<0.002, respectively) in the control group than in the vaccinated group. The mean level of excretion in faeces (measured in bacteria per g) was reduced by 4 log10 between the control and the vaccinated group. This reduction was 5 log10 for vaginal mucus (measured in bacteria per mL).

Regarding the shedding in milk, the excretion of *Coxiella burnetii* was significantly higher in the control group than in the vaccinated group ($p<0.0002$). The mean quantity of bacteria excreted per milliliter was reduced by 4 log10 in the vaccinated group.

Conclusion: This study showed that goat vaccination (Coxevac®, Ceva Santé Animale) was effective for one year in reducing the abortion rate due to *Coxiella burnetii* and excretion of the bacterium. This is of main interest to control the disease in the flocks and to reduce the zoonotic risk.

Keywords: Q fever, goats, vaccine, immunity.

IV-36

A field study evaluating humoral immune response in calves vaccinated with two multivalent respiratory vaccines

Anna Catharina Berge[1], Thibault Jozan[2], Camille Levesque[3], Geert Vertenten[4].

[1]Berge Veterinary Consulting BV, Vollezele, Belgium; [2]MSD Santé Animale, Beaucouzé, France; [3]LABOCEA, Javené, France; [4]MSD Animal Health, Boxmeer, Netherlands.

Objectives: Calf vaccination may be adversely affected by maternal antibodies. Two trivalent inactivated Bovine Respiratory Disease (BRD) vaccines that are commercialized in Europe (Bovilis® Bovipast RSP or Bovilis® Bovigrip, MSD Animal Health (BPAST) and Bovalto® Respi 3, Boehringer Ingelheim (BTO)), can be administered to calves from 2 weeks of age. BPAST has proven efficacy in calves with maternal antibodies, while BTO is only indicated for use in seronegative animals. In field conditions, calves rarely remain seronegative for BRSV due its high prevalence in cattle herds. The objective of this field study was to evaluate the seroneutralising (SN) antibody responses against bovine respiratory syncytial virus (BRSV) and specific humoral (IgG) ELISA response to BRSV, bovine parainfluenza 3 virus (BPI3V) and *Mannheimia haemolytica* (Mh) in young calves vaccinated with either BPAST or BTO and after booster vaccination 9 to 11 months later.

Materials & Methods: This field study was performed on one dairy farm in France, with 30 dairy calves that received at least 3L colostrum during the first 6h of life. Three study groups were created of 12 calves vaccinated with BPAST, 13 with BTO and 5 non vaccinated negative controls. Vaccines were administered at 15-30 days of age (T0), 1 month (m) later (T0+1m) and at 9-11 months of age (T10). Blood samples were taken at T0, T0+0.5m, T0+1m, T0+1.5m, T0+2m, T0+2.5m, T0+3m, T0+4m, T0+5m, T0+6m, T10, T10+0.5m and T10+1m. Serum antibodies in the individual calves after primo (first 2 injections) and booster (3rd injection) vaccinations were evaluated by calculating the areas under the curve (AUC) of the Log2 transformed BRSV SN titres and the optic density measures of the ELISA tests for BRSV, BPI3V and Mh. A seroconversion was defined as a four-fold or more increase in titres. Multivariate general linear models were used to evaluate the influence of the vaccination on the AUC of the serum measures within 6 months after the primo vaccination and 1 month after the booster vaccination.

Results: There was no significant difference in T0 titres between the groups. No negative control calves seroconverted, whereas respectively 15 and 75% of the BTO and BPAST vaccinated calves seroconverted for BRSV after primovaccination and respectively 0% and 75% of the BTO and BPAST vaccinated calves seroconverted to BRSV after booster vaccination. The BPAST vaccinated calves had significantly higher BRSV SN titres AUC following the primo vaccination and booster vaccination compared to the negative control calves and the BTO vaccinated calves. The BTO vaccinated calves did not have a significantly different BRSV SN titres AUC response after the primo or booster vaccinations compared to the negative control calves.

Although some animals experienced a natural infection with BPI3V, Mh or both, some differences in the antibody responses between the different groups could still be identified. BPAST and BTO vaccinated calves mounted a significantly higher AUC ELISA OD for both BPI3V and Mh compared to negative control calves after primovaccination with the highest AUC measured in the BPAST vaccinated calves. This in contrast with booster vaccination where only the BPAST vaccinated calves mounted a significantly higher AUC ELISA OD for both BPI3V and Mh compared to negative control calves.

Conclusion: This study indicates that despite containing antigens targeting the same BRD pathogens, immunogenicity of vaccines can be very different. Early vaccination of calves with multivalent adjuvanted inactivated BRD vaccines (e.g. Bovilis® Bovipast® RSP) can elicit a humoral response with a memory effect as indicated by the serological response after booster vaccination.

Keywords: BRD vaccines, commercial calves, BRSV, PI3, Mannheimia haemolytica.

IV-37

Concurrent vaccination for pneumonia in pre-weaned calves; a longitudinal study on the safety, and serological response elicited by delivery of two live intranasal vaccines

Anna Flynn[1], Catherine Mcaloon[2], Katie Surgrue[1], Ricki Fitzgerald[1], Cara Sheridan[3], Bosco Cowley[3], Emer Kennedy[1].

[1]Grassland Science Research Department, Animal & Grassland Research and Innovation Centre, Teagasc Moorepark, Cork, Republic of Ireland; [2]School of Veterinary Medicine, University College Dublin, Dublin, Republic of Ireland; [3]MSD Animal Health, Dublin, Republic of Ireland.

Objectives: Bovine respiratory disease complex (BRD) is an economically important disease with a high mortality rate. In Ireland, it remains the leading cause of death in calves between 1 and 12 months of age. Moreover, as antimicrobial resistance and animal welfare are areas of growing public concern, control of BRD through prophylactic vaccination will be increasingly important in decreasing calf rearer reliance on antibiotics. It has been suggested that vaccination of pre-weaned

calves from two weeks of age in the face of maternally-derived antibodies (MDA) can provide efficacious protection from the viruses most commonly associated with pneumonia.

Although numerous respiratory vaccines suitable for calves are available, achieving early protection is often challenging due to a limited vaccination time-window and labour demand. Reportedly, it is now common practice on farms to administer multiple intranasal vaccines together to pre-weaned calves. However, this practice is currently unlicensed and there is limited information on the effects of concurrent intranasal administration of these vaccines. The objective of this longitudinal study was to determine the safety of concurrent administration, from 3 weeks of age, of two currently available intranasal vaccines for the viruses Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza-3-virus (PI3V) and Bovine Herpes Virus 1 (BHV-1) and to examine the serological responses they elicited in the face of high levels of MDA.

Materials & Methods: Forty dairy (n=30) and dairy beef (n=10) calves, male (n=13) and female (n=27) were balanced in a randomised block design with 4 treatments; i) Live BHV-1 vaccine only (Bovilis® IBR Marker Live, MSD Animal Health) (BHV-1 only) ii) BRSV/PI3V vaccine only (Bovilis® INtranasal RSP Live, MSD Animal Health) (RSV/PI3V only) and iii) Concurrent vaccination with live BHV-1 and BRSV/PI3V vaccines (Bovilis® IBR Marker Live & Bovilis® INtranasal RSP® Live) (CV) and iv) non-vaccinated control group. Calves were vaccinated intranasally (IN) at an average age of 21.6 (±4.8) days with a 2 ml dose of each vaccine, 1 ml per nostril. Calves in the CV treatment received both vaccines separately, and consecutively. Control calves were given a placebo of the diluent used for both vaccines. All calves from vaccinated groups received booster vaccination against BHV-1 (Bovilis® IBR Marker Live, 2ml i.m) and RSV&PI3V (Bovilis® Bovipast® RSP, MSD Animal Health, 5ml, s.c.) at the age of 4 months. The serological response to vaccination was measured at 3 weeks post intranasal vaccination and following booster. The antibody response was measured with commercially available indirect ELISA test kits, and the results were compared between treatment groups.

In addition to serology, clinical health scores, including rectal temperature were taken before and after IN vaccination. Calves were weighed weekly and growth rates were compared between treatments. All data analysis was performed using SAS version 9.4 (SAS Institute Inc.) and significant associations in mixed models were confirmed at the $P<0.05$ level.

Results: There was no significant difference in rectal temperature before and after IN vaccine administration ($P= 0.219$). Concurrent vaccination had no adverse effect on weight gain or clinical score, with CV calves gaining on average 0.28 kg (±0.14) kg/week more than BHV-only and BRSV/PI3V only calves. Intranasal vaccination with BRSV/PI3V at three weeks of age resulted in an improved anamnestic response to the PI3V and BRSV antigens following subsequent administration of an inactivated vaccine against PI3V, BRSV and *M. haemolytica*. Therefore, intranasal vaccination at three weeks of age in the face of high levels of MDA acted to prime the immune response for a subsequent parenteral dose of inactivated BRSV/PI3V vaccine. BHV-1 antibodies increased in all treatment groups including the controls post-IN vaccination, which may indicate circulation of wild BHV-1 in the herd.

Conclusion: Although concurrent intranasal vaccination with a live vaccine against BHV-1 and a live vaccine against BRSV and PI3V is outside of the licensed use of the two products, in our study the concurrent use in 3-week old seropositive calves caused no adverse effect on weight gain or clinical parameters compared to calves vaccinated with the vaccines individually and non-vaccinated controls. Moreover, the enhanced immune response against BHV-1 and RSV following booster vaccination might suggest that the immune system had been primed. Any decision to use these vaccines concurrently needs to be made on a case-by-case basis by a veterinary professional.

Keywords: concurrent vaccination, intranasal, pneumonia, safety, serology.

LA-01

A prospective cohort study into the association between early-lactation mastitis and the development of sole ulcers in dairy cows

Christopher Watson[1], Matthew Barden[2], Bethany Griffiths[2], Alkiviadis Anagnostopoulos[1], Cherrill Bedford[1], Androniki Psifidi[3], Georgios Banos[4], Georgios Oikonomou[1].

[1]Department of Livestock Health and Welfare, Institute of Veterinary Science, University of Liverpool, Leahurst Campus, Chester High Road, CH64 7TE, UK, Neston, United Kingdom; [2]Department of Epidemiology and Population Health, Institute of Infection and Global Health, University of Liverpool. Leahurst Campus, Chester High Road, CH64 7TE, UK, Neston, United Kingdom; [3]Department of Clinical Science and Services, Royal Veterinary College, North Mymms, Hertfordshire, AL9 7TA, UK, North Mymms, United Kingdom; [4]Animal & Veterinary Sciences, SRUC, Roslin Institute Building, Easter Bush, Midlothian EH25 9RG, UK, Neston, United Kingdom.

Objectives: Sole ulcers (SU) are a major cause of lameness in dairy cattle and their incidence is highest in early to mid-lactation (1). SU take 8-12 weeks or more to develop, and it has been suggested many originate from an insult in the periparturient period (2). The risk for clinical mastitis is highest in the first 30 days in milk (DIM) (3) and we postulate a potential link between early-lactation mastitis incidence and later development of SU. A preliminary study that was carried out by our group involving 455 animals on 3 commercial dairy farms found that cows were significantly more likely to develop SU in early lactation if they had mastitis in the first month post calving (4). The aim of this prospective cohort study was to further investigate this association employing a much larger dataset and more intensive recording of lameness-causing foot lesions.

Materials and Methods: Data were collected from 2186 Holstein-Friesian cows and heifers raised in four commercial dairy farms in the UK. Data were collected from each animal on three occasions: - at drying off or 30-60 days before their expected first calving, in the first 10 days post calving and at 50-120 days post calving. On each occasion, feet were lifted and presence and severity of SU was recorded by a qualified veterinary surgeon. Mastitis episodes were recorded by trained farm staff. A series of univariable analyses were initially performed. Outcome of interest was the presence of a SU in early lactation. In order to account for potential confounding factors, a multivariable logistic regression model was also fitted to the data.

Results: 21.4% of cows that had mastitis in the first 30 days post calving developed SU in early lactation, whilst only 4.9% of cows that did not have mastitis in the first 30 DIM developed SU in early lactation (P = 0.0028). After correcting for parity group (primiparous versus multiparous), farm, and the presence or not of a SU at enrolment, cows that had mastitis in the first 30 days post calving were found to be 6.66 times more likely to have a SU in early lactation (CI 2.50-17.73, P <0.0001) comparing to cows that did not have mastitis in the first 30 days post calving. Multiparous cows were 1.64 times more likely (CI 0.95 -2.85, P = 0.078) to have SU in peak lactation than cows that were in their first lactation. Finally, cows were 13.3 times more likely (CI 6.36 – 28.02, P < 0.0001) to have an SU in peak lactation if they already had SU at enrolment comparing to cows that did not have SU at enrolment.

Conclusion: These initial results are consistent with those described previously by Griffiths et al. (2018). A strong association has been found between mastitis in the first 30 days and the presence of SU in peak lactation. The mechanism responsible for this association is not yet clear and warrants further investigation.

References:

1. Sanders AH, Shearer JK, De Vries A. Journal of Dairy Science. 2009;92(7):3165-74.
2. Shearer JK, van Amstel SR. Veterinary Clinics: Food Animal Practice. 2017;33(2):283-300.
3. Breen JE, Green MJ, Bradley AJ. Journal of Dairy Science. 2009;92(6):2551-61.
4. Griffiths B, Mahen P, Hall R, Kakatsidis N, Britten N, Robinson L, et al. Cattle Practice. 2018;26:81-.

Keywords: Dairy cattle, lameness, sole ulcer, mastitis.

LA-02

Metagenomic analyses of the bovine foot skin microbiome; associations with development of Bovine Digital Dermatitis.

Veysel Bay[1], Amy Gillespie[2], Erika Ganda[3], Nicholas Evans[2], Stuart Carter[2], Luca Lenzi[2], Anita Lucaci[2], Sam Haldenby[2], Matthew Barden[2], Bethany Griffiths[2], Enrique Sanchez-Molano[4], Rodrigo Bicalho[5], Georgios Banos[6], Alistair Darby[2], Georgios Oikonomou[2].

[1]Aegean Agricultural Research Institute, Izmir, Turkey; [2]University of Liverpool, Liverpool, United Kingdom; [3]Penn State University, Pennsylvania, United States; [4]The Roslin Institute, University of Edinburgh, United Kingdom; [5]Cornell University, Ithaca, United States; [6]Scotland's Rural College, Midlothian, United Kingdom.

Objectives: Previous studies have described the microbiome of the different lesion stages of Bovine Digital Dermatitis (BDD). The present study was designed to examine the healthy bovine foot-skin microbiome and determine differences in microbial populations associated with future development of BDD lesions.

Materials and Methods: Swabs were taken from the heel bulb region of the back-left foot of 259 Holstein-Friesian cows from three commercial UK dairy farms 3-4 weeks prior to calving. Feet were re-examined at one, four and 8-10 weeks post-calving to record any lesions that had developed and classify cows into four foot-health groups: those that remained healthy (HtHt), those that became infected (HtIn), those that were always infected (InIn) and those that were infected at enrolment but recovered (InHt).

16SrRNA gene amplicon sequencing was carried out for all samples using the Illumina® HiSeq 2500 platform, and taxonomic assignment of OTUs carried out using QIIME and the RDP classifier. Chao 1, Shannon and Simpson indices were calculated to describe alpha-diversity, and weighted and unweighted UniFrac distances were analysed using Principal Coordinates Analysis and used to describe beta-diversity for

foot health groups and farms. Robust response screening (JMP Pro 12, SAS Institute Inc., USA), was performed to evaluate differences in relative abundance of OTU assignments at genus level between samples from the HtHt and HtIn groups.

A subset of five samples from the HtHt and HtIn groups were used for shotgun metagenomic analysis to provide higher taxonomic resolution and detect differences in the functional profiles of the microbiome. Prepared libraries were sequenced using the Illumina® HiSeq 4000 platform and results analysed using LefSe to determine taxa most likely to explain differences between the two groups. HUMAnN2 was used to functionally annotate read data to show biological pathway abundance and completeness.

Results: Differences in sample richness and evenness were identified between foot-health groups. Alpha-diversity metrics overall suggested that HtHt samples had significantly greater microbial diversity than InIn or InHt samples, and a tendency to greater microbial diversity than HtIn samples.

Focussing on comparing HtHt to HtIn groups (where samples originated from morphologically healthy feet), PCoA showed clustering between HtHt and HtIn groups, indicating that overall the two groups share the same species. However, response screening showed at genus level that *Macrococcus* spp. and *Brachybacterium* spp. were more abundant in samples from the HtHt group, whilst *Succiniclasticum* spp., *Porphyromonas* spp., *Acholeplasma* spp., *Fastidiosipila* spp., *Peptoclostridium* spp. and *Prevotella* spp. were more abundant in samples from the HtIn group. *Treponema* spp. were present in the top 20 most prevalent genera for HtIn samples, but not for HtHt samples.

Shotgun metagenomic analysis showed some differences in taxa present in HtHt compared to HtIn samples. HtHt samples had increased relative abundance of many gram positive bacteria from the phylum Actinobacteria which would be expected to be part of the healthy foot skin flora. HtIn samples showed biologically relevant increases in wall-less bacteria from the *Tenericutes* phyla, which has previously been found to be more abundant in BDD lesions. Taxonomic assignment of reads was low and therefore the significance of these findings is uncertain; however, there may be some agreement with the 16S rRNA gene sequencing analysis in finding increased *Acholeplasma* spp. in HtIn samples and increased *Brachybacterium sp.* in HtHt samples.

Eight functional pathways were identified as significantly more abundant in the HtIn group. One indicated production of 4-deoxy-L-*threo*-hex-4enopyranuronate, which is a uronic acid resulting from the degradation of many polymers. These include plant polymers such as pectin and gellan, but also important components of connective tissue such as heparin, heparin sulfate, hyaluronan and chondroitin sulfate.

Conclusion: This is the first study to associate taxonomical differences in the healthy foot-skin microbiome with future development of BDD lesions. Our data suggest that loss of diversity may begin before the appearance of morphological lesions, and that *Macrococcus* spp and *Brachybacterium* spp. may be protective whilst *Succiniclasticum* spp., *Porphyromonas* spp., *Acholeplasma* spp., *Fastidiosipila* spp., *Peptoclostridium* spp. and *Prevotella* spp. may initiate dysbiosis that leads to lesion development. Upregulation of a functional pathway associated with polymer degradation may indicate the beginning of a pathogenic process in HtIn samples detectable prior to development of visible BDD lesions.

Keywords: Digital dermatitis, Microbiome, Metagenomics, Lesion development, Lameness.

LA-03

Initial validation of an intelligent video surveillance system for automatic detection of dairy cattle lameness

Alkiviadis Anagnostopoulos, Bethany Griffiths, Joseph Neary, Rob Smith, Georgios Oikonomou.

University of Liverpool, Leahurst Campus, Neston, United Kingdom.

Objectives: Lameness is a major welfare challenge facing the dairy industry worldwide. Monitoring of herd lameness prevalence, early detection and treatment of foot lesions are important aspects of lameness control. The objective of this study was to evaluate the performance of a commercially available video surveillance system for automatic detection of dairy cattle lameness (CattleEye Ltd.). Our aim was to investigate the validity of the mobility scores provided by the system by comparing them against those recorded by two experienced assessors. Additionally, we examined the system's ability to detect cows with at least one potentially painful foot lesion.

Materials and Methods: CattleEye utilizes an inexpensive 2D surveillance camera placed above the passageway exiting the milking parlour. Footage of cows exiting the milking parlour is sent directly to company servers where it is stored and processed. The final result of the analysis is a number between 0.0 and 100.0, indicating the degree of lameness. This variable is then transformed into 0-3 scale score (score 0 for 0-24, score 1 for 25-49, score 2 for 50-74 and score 3 for 75-100).From November 2020 to February 2021 three farms equipped with the CattleEye mobility scoring system were visited once a week by an experienced scorer (Assessor 1). During each visit, the entire milking herd was scored using the Agricultural and Horticultural Development Board (AHDB) 0-3 scale scoring method. Reports containing CattleEye scores were also stored but were not available to Assessor 1 before the end of the study. Assessor 1 also recorded lesions found during foot-trimming visits for two of the farms. Finally, a second experienced scorer (Assessor 2) recorded mobility scores on those two farms once during the study and within 48 hours of the last visit by Assessor 1. Percentage of agreement and Cohen's Kappa was calculated comparing the scores recorded by Assessor 1 and the CattleEye system in binary form (Lame/ Not Lame). The same analysis was performed for all possible pairs on the dataset containing scores from Assessor 1, Assessor 2 and the CattleEye system. Using the lesions recorded and their severity, cows were classified as having at least one potentially painful lesion or not. Lesions included in this analysis were: M2 stage of digital dermatitis, toe ulcer, sole ulcer and severe white line disease lesions. Confusion matrixes were calculated using the binary lesion score as reference and the binary form of Assessor 1 scores and the CattleEye scores as predictors.

Results: By the end of the study 6,040 mobility scores were collected by Assessor 1 that also had a corresponding CattleEye score, from which 1,102 also had corresponding scores from Assessor 2. Foot lesions from 84 cows were recorded.

Assessor 1/ CattleEye

Agreement between the binary scores of Assessor 1 and the CattleEye system for Farms 1, 2 and 3 were 82.6%, 84.1% and 88.9% respectively. The Cohen's Kappa for the same combinations were 0.44, 0.34 and 0.41 respectively.

Assessor 1/ Assessor 2/ CattleEye

For Farm 2, agreements between Assessor 1 and Assessor 2, Assessor 1 and CattleEye, and Assessor 2 and CattleEye were: 80%, 77.6% and 81.6% respectively. Cohen's kappa for those pairs were: 0.30, 0.26 and 0.30. Agreement among the same pairs but for Farm 3 were: 90.1%, 88% and 85% respectively. Cohen's Kappa for those pairs for Farm 3 were: 0.44, 0.40 and 0.32 respectively. Kappa values for agreement between Assessors 1 and 2 for farms 2 and 3 (0.30, 0.44 respectively) were very similar to those between Assessor 1 and CattleEye throughout the entire validation process (0.34, 0.41 for farms 2 and 3 respectively).

Assessor 1/ CattleEye/ Foot lesions

When using the lesion score as reference and Assessor 1 binary scores as classifier the confusion matrix produced a combination of 35% sensitivity and 88% specificity. Positive predictive value (PPV) was 38% and negative predictive value (NPV) was 87%. When using CattleEye scores as classifiers the confusion matrix produced a combination of 71% sensitivity and 81% specificity; PPV was 43% and NPV was 93%.

Conclusion: This study shows that the CattleEye system had a comparable performance to two experienced scorers when mobility score was used as a reference and outperformed the human scorer when lesion presence was used as the gold standard.

Keywords: Automatic lameness detection, dairy cattle.

LA-04

Sole Soft Tissue Thickness changes over the Periparturient Period and its Association with Sole Ulcers in Dairy Cattle

Bethany Griffiths[1], Alkiviadis Anagnostopoulos[1], Matthew Barden[1], Androniki Psifidi[2], Georgios Banos[3], Georgios Oikonomou[1].

[1]University of Liverpool, Liverpool, United Kingdom; [2]Royal Veterinary College, London, United Kingdom; [3]Scotland's Rural College, Midlothian, United Kingdom.

Objectives: Sole ulcers are a leading cause of lameness in dairy cattle. Despite their importance, we are still yet to fully elucidate their aetiopathogenesis. The sole soft tissues, which includes the digital cushion, has been theorised to play a role in the development of these lesions (Newsome et al., 2017). This abstract describes preliminary analyses of SSTT data collected longitudinally on a large number of dairy cows. The objective of this analysis was to describe periparturient changes SSTT measurements and its association with a number of explanatory variables.

Materials & Methods: Across four commercial UK dairy farms, 2,352 Holstein Friesian cows were prospectively enrolled prior to a calving date expected to occur between April and September 2019. Animals were assessed at four time points relative to their calving date; prior to calving (mean: -55 days, standard deviation (SD): 18), immediately post-calving (mean: +5 days, SD: 3), during early lactation (mean: +84 days, SD: 14) and again in late lactation (mean: +200 days, SD: 31). At each check the animals were mobility and body condition scored, all four feet were assessed for infectious and non-infectious foot lesions and these were graded for severity. An image of the digital cushion on the lateral claw of the hind left foot was taken using B mode ultrasonography for measurement at a later date.

In total 7,866 digital cushion images were analysed by a single assessor blinded to cow, farm and stage of lactation and using Image J software. Univariable analysis was undertaken. Simple linear regression models were constructed, from which least square means were calculated and Tukey HSD tests undertaken (R Core Team, 2020). The SSTT was the outcome variable.

Results: The SSTT nadir was shortly after calving, with SSTT then increasing throughout lactation (P <.0001). Parity was significant, with lower parity animals having significantly thinner SSTT compared to higher parity animals (P <.0001), this was especially apparent for primiparous compared to multiparous animals. There were significant differences in SSTT between farms (P<.0001), and a farm by stage interaction was noted (P<.0001). Two distinct patterns of SSTT change over the course of the lactation emerged. The first is as previously described, the SSTT starts relatively high, drops at calving and then increases in size again throughout lactation. This was evident on two of the farms. The other pattern featured a decline in SSTT from the dry period through to the nadir at early lactation, the SSTT then recovers in size by the late lactation visit. An interesting parity by time point of measurement by sole ulcer incidence interaction was also observed. Primiparous animals that developed a sole ulcer in early lactation exhibited a greater drop in SSTT thickness at calving compared to those primiparous animals which did not develop a sole ulcer. This change was echoed in the multiparous animals, but was not as sizeable. Initial analysis has also indicated that taller cows had thicker SSTTs, whilst cows with thinner sole horn had thicker sole soft tissues.

Conclusion: To the best of the author's knowledge this is the largest study of sole soft tissue thickness measurements. Initial analyses have highlighted that freshly calved animals and heifers have significantly thinner sole soft tissues and our project adds further evidence that fresh cow management and heifer management are important stages for managing the risk of lameness in the production cycle of dairy cows.

Acknowledgements: The authors thank BBSRC and AHDB for their support.

References:

Newsome RF, Green MJ, Bell NJ, Bollard NJ, Mason CS, Whay HR and Huxley JN 2017. A prospective cohort study of digital cushion and corium thickness. Part 1: Associations with body condition, lesion incidence, and proximity to calving.

Journal of Dairy Science 100, 4759–4771.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Keywords: Dairy, Lameness, Sole Ulcer, Digital Cushion.

LA-05

Use of intra-articular administration of isopropyl alcohol for facilitated ankylosis of the distal interphalangeal joint in cattle with septic arthritis

Katharine Simpson[1], Chad Baumwart[2], Robert Callan[1].

[1]Colorado State University, Fort Collins, United States; [2]Highland Veterinary Clinic, Arapahoe, Oklahoma, United States.

Objectives: To determine whether intra-articular (IA) injection of 70% isopropyl alcohol and hoof block application to the adjacent healthy claw will result in clinical ankylosis of the distal interphalangeal joint (DIPJ) in cattle with septic arthritis (SA), and whether lameness scores will be improved.

Materials and Methods: Four beef cows were diagnosed with SA of the DIPJ via clinical and radiographic exam (4), and/or culture of synovial fluid (2). Lameness scores were recorded. Six to 20 mLs of 70% isopropyl alcohol was administered into the affected DIPJ following regional intravenous perfusion of 2% lidocaine. A wooden hoof block was affixed to the adjacent healthy claw. Cattle were re-examined, lameness re-scored following hoof block removal, and clinical determination of ankylosis performed at follow-up visits at 2.5 – 7 (average 3.8) months later. Radiographs were repeated if economics permitted (2 cases). Long-term follow-up (> 1 year) was obtained by clinical and radiographic exam (1), by video (1), and/or via contacting the owner by telephone (all cases).

Results: All 4 cows had improved lameness scores and clinical ankylosis of the affected DIPJ. Long-term follow-up indicated that all cattle were pasture sound. Two cows had calved and all 4 were rebred. All owners expressed satisfaction with the outcome.

Conclusions: Clinical ankylosis and improved lameness scores were observed following an IA injection of 70% isopropyl alcohol into the DIPJ in cows with SA. All cows remained in the herd and were productive. Additional studies are warranted to determine efficacy of this procedure.

Keywords: Lameness, cattle, septic arthritis.

LA-06

Impact of hoof lesions and moment of occurrence in milk production in dairy cattle

Almudena Molinero Arguello[1], Nayara Magalhaes Goncalves[2], Juana C. C. Chagas[3], Phd Marcos Inácio Marcondes[4].

[1]UNIFORM-Agri BV, Pamplona, Spain; [2]UNIFORM-Agri BV, Belo Horizonte, Brazil; [3]Affiliation: Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences (SLU), Skogsmarksgränd, 90183, Umeå, Sweden; [4]Dairy Cattle Production and Nutrition, Universidade Federal de Viçosa, Brazil.

Objectives: Lameness is one of the main reasons for early culling in dairy cattle. Most lameness cases are caused by lesions in the hooves with dramatic consequences in cow's life. Thus, we aimed to describe the impact of lameness in milk production, depending on hoof lesion type and moment of occurrence.

Materials and methods: In this retrospective study, we used records of 5.565 cows from four farms located in the UK, which included data from 86,407 Milk Test records between September 2014 and October 2019. Lactations with less than five records were removed from the dataset. Lameness was considered as affecting MY if observed at least 21 d before the milk test day, and was scored as 1) horn lesions, and 2) infectious diseases of the hoof. Non-specified lameness lesions were removed from the databank. The effect of lameness on milk yield was studied based on the drop in MY when cow was lame. Firstly, we removed all lameness records from the data and fitted a wood's curve (WC) for each cow and lactation number. Secondly, we returned the lameness data to the databank and tested the effect of lameness in each WC parameter following the model: $MY = (1+ 1\times Lame) \times Wk^{2+ 2*Lame} \times e^{-(3+ 3*Lame) \times Wk}$, where 1, 2 and 3 were parameters determined for each cow, 1, 2 and 3 were the effect of lameness on MY, Lame is the binary occurrence of lameness, and Wk is the week of lactation. The lameness score (LS) was tested on all parameters. Lastly, a general WC was fit including in the general WC to estimate the MY loss throughout the lactation. Farm was included as random effect in all models. The model was run using PROC NLMIXED (SAS University edition) and parameters were considered different when $P < 0.05$.

Results: A total of 180 cows had, at some point of their life, a LS recorded. There were 87 cows with LS 1 and 93 with LS 2. Number of lactation when lameness occurred varied from 1st lactation to 10th lactation. Also, there was a big variability of occurrence among days in milk (from 4 to 443). The LS did not impact 1 ($P = 0.119$) but did affect 2 ($P = 0.029$) and 3 ($P = 0.046$). Additionally, lameness did not impact 1 parameter ($P = 0.397$), thus one equation was fit for each LS as follows: LS = 1) $MY = 34.064 \times Wk^{0.160-0.025\times Lame} \times e^{-(0.018-0.002\times Lame) \times Wk}$; and LS = 2) $MY = 34.064 \times Wk^{0.160-0.080\times Lame} \times e^{-(0.018-0.007\times Lame) \times Wk}$. Compared to her colleagues, a lame cow had a lower milk yield at lactation peak. Lame cows with an infectious disease had a peak production of 38,5 kg/d and 36 kg/d at peak for cows with hoof horn lesions, while healthy cows had on average 41,5 kg/d of milk production at peak. Milk loss was, on average, between 0,9 kg/day and 1,8 kg/day. Our analysis revealed that in some cases, infectious disease had no impact in milk loss (late lactating cows). On the other hand, cows with hoof horn

lesions had a milk yield loss from 0,25 kg/day to 4,5 kg/day. Week of lactation where lameness occurred had a strong impact in milk production. Highest loss was detected between weeks 12 and 19 of lactation, and percentage of total milk loss during this period was up to 4,7% for horn lesions and up to 12% for infection diseases of the hoof.

Conclusions: In conclusion, lameness impacted milk production and it is lower for infectious diseases than for hoof horn lesions. Additionally, the lameness impact is related with the time of lameness occurrence, being higher during weeks 12-13 of lactation.

Keywords: Lameness, dairy cattle, milk losses.

LA-07

A Disinfection Field Trial to Remove Bovine Digital Dermatitis Treponemes from Hoof Knives after Foot-Trimming

Amy Gillespie[1], Stuart Carter[1], Roger Blowey[2], Nicholas Evans[1].

[1]University of Liverpool, Institute of Infection and Global Health, United Kingdom; [2]Wood Veterinary Group, Gloucester, United Kingdom.

Objectives: Although lameness caused by bovine digital dermatitis (BDD) is widespread and much researched, the mode of transmission has not been clarified. Hoof knives become contaminated with BDD treponemes during foot-trimming(1), and these bacteria may survive for up to two hours under aerobic conditions on hoof knife blades(2).

This study tested a protocol for disinfecting hoof knives to mitigate the risk of transmitting BDD during foot-trimming. Three disinfectants were previously identified as candidates for hoof knife disinfection *in vitro* using a 20 second contact time(2). The current study tests these three disinfectants: 1:100 FAM30®, 2% Virkon® and 2% sodium hypochlorite- under field conditions during foot-trimming.

Materials and Methods: This study was approved by the University of Liverpool Veterinary Research Ethics Committee (Ref: VREC662).

Samples were collected on three commercial farms during foot-trimming of 133 BDD-affected feet from lactating dairy cattle. Swabs were taken from each hoof knife before trimming (as a negative control) and after trimming. Knives were briefly rinsed in water to remove gross contamination, then the blade was submerged for 20 seconds in one of three disinfectants (1:100 FAM30®, 2% Virkon® and 2% sodium hypochlorite) or water only for comparison. A third swab was taken to provide post-disinfection samples. There was no hoof knife contact with the BDD lesion during trimming in 22 cases, whilst contact was made with the lesion in 111 cases for treatment purposes.

Detection of bacteria from the *Treponema* genus, and from three known BDD-associated pathogenic phylogroups, was undertaken via two methods. Nested PCR of sample swabs taken directly from the hoof knife blades, and nested PCR of samples extracted from cultures given six weeks to grow was carried out according to previously established protocols (2). The former method is more sensitive for detecting *Treponema* DNA and the latter provides a measure of bacterial viability demonstrated by an ability to grow in liquid medium.

Results: Where direct contact was not made with BDD lesions during trimming, 12/22 (54.5%) of swabs taken from hoof knives were positive for at least one of the three pathogenic phylogroups after trimming using nested PCR. After disinfection 10/22 (45.4%) remained DNA positive. In terms of microorganism viability, pathogenic treponemes were detected by nested PCR of cultures after six weeks in 1/22 (4.5%) of these cases after trimming, and disinfection using 2% Virkon® removed culturable organisms.

Where contact *was* made with BDD lesions (n= 111), 100% of swabs taken from hoof knives after trimming and tested directly were DNA positive for at least one of the three pathogenic phylogroups. After disinfection 38/111 (34.3%) of knives remained DNA positive. Pathogenic treponemes were detected by nested PCR of cultures after six weeks in 47/111 (42.3%) of cases after trimming. All three disinfectants (and water alone) were effective at removing culturable organisms (Table 1).

Conclusions: BDD-associated treponemes can be cultivated from hoof knives in 4.5% of cases after foot-trimming of BDD affected feet where no contact is made with the lesion. Where contact has been made with BDD lesions, BDD treponemes are culturable in 42.3% of cases. Rinsing hoof knives briefly in water then disinfecting the blades for 20 seconds in 1:100 FAM30®, 2% Virkon® or 2% sodium hypochlorite was shown to be effective for removing culturable BDD-associated treponemes from hoof knives.

Table 1. The effect of disinfectants on viable treponemes on hoof trimming knives (determined by PCR of cultures), before use, post-trimming and post-disinfection. In all samples knife-BDD lesion contact occurred during trimming.

Disinfectant	PRE-TRIMMING		POST-TRIMMING		POST-DISINFECTION	
	Treponema genus	BDD phylogroups	Treponema genus	BDD phylogroups	Treponema genus	BDD phylogroups
2% Virkon® (n=32)	0/32	0/32	18/32	13/32	0/32	0/32
2% sodium hypochlorite (n=21)	0/21	0/21	10/21	10/21	0/21	0/21
1:100 FAM30® (n=20)	0/20	0/20	12/20	10/20	0/20	0/20
Water (n=38)	0/38	0/38	24/38	14/38	0/38	1038
Total (n=111)	0/111	0/111	64/111 (57.7%)	47/111 (42.3%)	0/111	0/111

References:

1. Sullivan LE, Blowey RW, Carter SD, Duncan JS et al. Presence of digital dermatitis treponemes on cattle and sheep hoof trimming equipment. Vet Rec. 2014;201.
2. Gillespie A, Carter SD, Blowey RW, Evans N. Survival of bovine digital dermatitis treponemes on hoof knife blades and the effects of various disinfectants. Vet Rec. 2019;67.

Keywords: Digital dermatitis, Treponeme, Lameness, Foot-trimming, Disinfection.

LA-08

Comparing the effect of timing of routine early lactation foot inspection and trimming (if required) on fertility performance in high yielding dairy cows

Jonathan Duncan[1], Helen Higgins[2], George Oikonomou[2].

[1]Willows Vet Group, Northwich, United Kingdom; [2]University of Liverpool, Liverpool, United Kingdom.

A randomised, positive-controlled trial to compare the effect of timing of routine early lactation foot inspection and trimming (if required) on fertility performance in high yielding dairy cows.

Objectives: Routine, early lactation foot inspection and trimming (if necessary) is a common preventative strategy used to reduce lameness and improve productivity. The perceived benefits are well accepted across the industry and some evidence does exist to support the use of this intervention (Oultram and others 2017, Griffiths and others 2018). However, evidence regarding the optimal timing of this intervention is scarce. Anecdotally, it is suggested that handling animals for routine foot inspections and treatment (if required) very early in lactation (and therefore around the end of the voluntary waiting period), could provide enough stress to have a detrimental effect on fertility. This randomised controlled trial was designed to assess the subsequent fertility of dairy cows that were randomly allocated at calving to one of two different treatments, receiving early lactation foot inspections and trims at either 26-64 days in milk (early routine inspection +/- trim, EIT), or 76-114 days in milk (late routine inspection +/- trim, LIT).

Materials and Methods: The study was conducted on a single large dairy unit in the UK and all cows were inspected/trimmed by the same qualified foot trimmer who was blinded to the treatment groups. 1,885 were initially randomly allocated (at calving) to one of the two different treatments. Data related to animals' reproductive performance were available from the farm's management software. Multivariable logistic regression and Kaplan-Meyer time to event analyses were used for data analysis.

Results: 1,885 animals were initially randomly allocated to one of the two treatment groups but only 985 of them did actually receive a trim. From these animals, 543 did receive their trim within the correct, randomly allocated, time window. This was due to unforeseen circumstances associated with farm staffing. Of the 543 cows eventually used for data analysis, 304 were in the EIT treatment group and 239 were in the LIT treatment group. Lactation group was a significant predictor of pregnancies per artificial insemination (P/AI) to first service. An effect of treatment group could not be supported for P/AI to first service, time to conception or time to first service analyses. Median time to conception (95% lower and upper confidence limits) was 106 (94 – 112) days for EIT and 112 (97 – 129) for LIT (P = 0.99).

Conclusion: Industry standard timing for early lactation routine inspection +/- trimming is usually at 80-100 days in milk. Our data provide a degree of confidence that subsequent reproductive performance will not be affected, if animals are inspected earlier. An earlier foot inspection and trim if required, may help remodelling of the foot prior to sole ulcer formation, but further work is required in order to test this hypothesis.

Keywords: Dairy Cattle, Lameness, Fertility, Routine Trimming, Conception.

LA-09

Evaluation of the use of ketoprofen for the treatment of digital dermatitis in dairy cattle: a randomised, positive controlled, clinical trial

Konstantina Kasiora[1], Alkiviadis Anagnostopoulos[1], Cherril Bedford[1], Theologia Menka[1], Matthew Barden[1], Bethany Eloise Griffiths[1], Damien Achard[2], Katherine Timms[3], Vinicius Silva Machado[4], Amy Coates[1], George Oikonomou[1].

[1]University of Liverpool, Liverpool, United Kingdom; [2]CEVA Animal Health LTD, Libourne, France; [3]CEVA Animal Health, Amersham, United Kingdom; [4]Texas Tech University, Lubbock, Texas, United States.

Objectives: Digital dermatitis (DD) is one of the main diseases causing lameness in dairy cattle and it is described as an infectious dermatitis of the digital skin that can be painful to touch (Krull et al. 2016). Given that DD is a painful condition, the use of NSAIDs alongside antibiotic treatment may be justified on welfare and possibly on economic grounds; however, this is yet to be proven. The objective of this randomized, positively controlled study was to explore the potential benefits of a single administration of ketoprofen when treating active digital dermatitis lesions.

Materials and Methods: 158 cows that presented signs of active DD (M1, M2 or M4.1 stage) were randomly allocated to either the control or the treatment group. All cows were treated with topical application of oxytetracycline spray. The treatment group additionally received an intramuscular injection of ketoprofen (3 mg/kg, Ketofen 10%, Ceva Animal Health). Cows were mobility scored just before they were treated and then again one week later. Information regarding their daily milk production was also collected. Treatments for each animal from both the control and the treatment group were administered by one person, who also performed the lesion examina-

tion. The mobility score assessor was not involved in treating the cows. Multivariable regression models were used for data analyses.

Results: Animals in the control group were at 2.57 (95% C.I.: 0.82-8.01, *P* = 0.10) times higher odds to be lame at the second evaluation compared to those that received ketoprofen as well. This was a numeric but not statistically significant difference. When only cows that were lame prior to treatment were considered, cows that did not receive ketoprofen were at 20.20 (95% C.I.: 1.40 – 291.29, *P* = 0.03) higher odds of remaining lame a week post treatment comparing to cows that did receive ketoprofen. Cows that received ketoprofen did produce more milk per day the week after treatment than the cows in the control group with adjusted means (± standard error) being 45.35 ± 0.71 and 42.37 ± 0.87 kg per day respectively (*P* < 0.01). The effect of ketoprofen administration was more prominent on cows that were freshly calved and lame at enrolment; these cows produced 58.38 ± 1.85 kg per day the week after treatment if they were in the ketoprofen treatment group comparing to the control ones that produced 47.89 ± 1.81 kg per day (*P* <0.05) .

Conclusions

The addition of ketoprofen in the treatment of active DD lesions appears to have potential benefits for animal welfare as it was associated with an improvement in lameness status and for animal productivity as it was associated with an increased daily milk production. Further research would prove useful to solidify the value of NSAIDs when treating active DD lesions.

References:

Krull AC, Shearer JK, Gorden PJ, Scott HM, Plummer PJ. Digital dermatitis: natural lesion progression and regression in Holstein dairy cattle over 3 years. J Dairy Sci. 2016.

Keywords: Digital dermatitis, Lameness, NSAIDs, Ketoprofen.

LA-10

Investigating the use of thermal imaging as a diagnostic tool for the detection of different stages of Digital Dermatitis in dairy cattle

Alkiviadis Anagnostopoulos[1], Katherine Williams[1], Matthew Barden[1], Bethany Griffiths[1], Cherrill Bedford[1], Mollie Rudd[1], Androniki Psifidi[2], Georgios Banos[3], Georgios Oikonomou[1].

[1]University of Liverpool, Leahurst Campus, Neston, United Kingdom; [2]Royal Veterinary College, North Mymms, Hertfordshire, United Kingdom; [3]Scotland's Rural University College, Roslin Institute Building, Easter Bush, Midlothian, United Kingdom.

Objectives: Bovine Digital Dermatitis (BDD) is one of the major causes of lameness in dairy cattle with increasing economic and welfare importance[1]. The use of Infrared Thermography (IRT) imaging could prove a non-invasive and reliable diagnostic tool for the early detection of BDD lesions. Our aims were to (1) determine how interdigital skin temperature (IST), measured using infrared thermography, was associated with different stages of digital dermatitis (DD) lesions and (2) develop and validate models that can use IST measurements to identify cows with an active DD lesion.

Materials and Methods: This study was conducted on four dairy farms in the UK. Between March 2019 and March 2020, infrared thermographic images of hind feet were taken from 2,334 Holstein cows. We recorded the maximum temperature reading from infrared thermographic images of the interdigital skin between the heel bulbs on the hind feet. A Flir E8 Wi-Fi Thermal Camera was used. Pregnant animals were enrolled approximately 1 to 2 mo precalving, reassessed 1 wk after calving, and again at approximately 50 to 100 d postpartum. At these time points, IST and the clinical stage of DD (M-stage scoring system[2]: M1–M4.1) were recorded in addition to other data such as the ambient environmental temperature, height, body condition score, parity, and the presence of other foot lesions. A mixed effect linear regression model with IST as the dependent variable was fitted. Subsequently, the capacity of IST measurements to detect the presence or absence of an active DD lesion (M1, M2, or M4.1) was explored by fitting logistic regression models, which were tested using 10-fold validation. A mixed effect logistic regression model with the presence of active DD as the dependent variable was fitted first. This model was then restricted so that only explanatory variables that could be practically recorded in a nonresearch, external setting were included.

Results: A total of 2,334 cows were included in this project, providing a total of 12,221 hind feet with lesion records and thermal images for analysis. The mixed effect linear regression model showed that Interdigital skin temperature was associated with DD lesions; compared to healthy feet, IST was highest in feet with M2 lesions, followed by M1 and M4.1 lesions. The average area under the curve for mixed effect logistic regression model was 0.80 when its ability to detect presence of active DD was tested on 10% of the data that were not used for the model's training; an average sensitivity of 0.77 and an average specificity of 0.67 was achieved. Validation of the simplified logistic regression model demonstrated an average area under the curve of 0.78, a sensitivity of 0.88, and a specificity of 0.66 for 1 of the time points (precalving). Lower sensitivity and specificity were achieved for the other 2 time points.

Conclusion: The study showed that IRT imaging can be effective in detecting active BDD lesions. M2 stage lesions resulted in the highest IST which was significantly higher than that of any other stage. Additionally, we highlight the potential for infrared thermography to be used for routine on-farm diagnosis of active DD lesions.

References:

1. Evans NJ, Murray RD, Carter SD. Bovine digital dermatitis: Current concepts from laboratory to farm. Vet J. 2016;211:3-13. doi:10.1016/j.tvjl.2015.10.028.
2. Solano L, Barkema HW, Jacobs C, Orsel K. Validation of the M-stage scoring system for digital dermatitis on dairy cows in the milking parlor. J Dairy Sci. 2017;100(2):1592-1603. doi:10.3168/jds.2016-11365.

Keywords: Digital dermatitis, thermography, dairy cattle.

LA-11

Time-series observations of cattle mobility: accurate label assignment from multiple assessors, and association with lesions detected in the feet.

Konstantina Linardopoulou[1], Lorenzo Viora[1], Francesco Fioranelli[2], Julien Le Kernec[1], Qammer Abbasi[1], George King[1], Elena Borelli[1], Nicholas Jonsson[1].

[1]University of Glasgow, Glasgow, United Kingdom; [2]TU Delft, Delft, Netherlands.

Objectives: To provide accurate diagnostic labels to machine learning algorithms for automated mobility analysis in cattle, we compared repeated subjective lameness scores from multiple assessors with the presence of lesions at repeated hoof examinations. We aimed to determine the extent to which mobility classification by multiple assessors and its variations using video capture was associated with identifying lesions during sequential hoof-trimmer examinations.

Materials and Methods: We recorded videos of 40+ cows fortnightly on 9 visits (405 recordings), and the 4 hoof examinations (HE) performed monthly (176 records). The recorded videos were assessed individually by expert assessors - 3 on visits 1-5 and 4 on visits 6-9. The "AHDB-Dairy" system (scores 0,1,2,3; 0 no abnormality - 3 severe lameness) was used throughout. Evaluators were shown calibration videos (AHDB 0-3) before scoring each visit. Assessors could record a second score if they were not confident in their assessment. A convergent score (ConvS) was constructed for each cow and visit where the maximum agreement was sought from second scores where the first scores differed among assessors. After completing individual scoring for each visit, the assessors convened to discuss each video and agreed to reach a consensus score (CS) for each cow. Each score ConvS and CS were converted to a binary score in which 0/1 became 0 (not lame), and 2/3 became 1 (lame), giving binary convergent scores (ConvSB), and binary consensus scores (CSB). The probability of lameness (P_L) of any animal was estimated with the Bayesian loss function using the 1st AHDB scores. A binary score for the hoof lesions (HLS) was derived; 1= any of white line disease, sole ulcer, digital dermatitis, stones, cracks, underrun; 0=none of these. The scores from all scoring methods were used as the dependent variables in generalized linear models (GLM) with the scores from HLS and lactation number as explanatory variables. The performance (coefficients and *p*-values) of the GLMs were compared among the scoring methods for the visits immediately preceding and following a foot examination. The Bonferroni correction was applied to give alpha = 0.002 as 24 association tests were conducted. We calculated the Cohen's and Fleiss' Kappa *K* inter-rater reliability (IIR) statistic for the individual ratings for each visit.

Results: In total 393 videos were retained for scoring after eliminating unsuitable videos. The IRR between assessors ranged from poor (K = 0.0038) to moderate (K = 0.565) agreement. *K* values and percentage agreement differed significantly between scoring methods (ConvSB> ConvS > CSB > CS) and visits (no consistent pattern observed). The GLM showed a weak effect on lactation number (*p* ranged 0.01-0.64), and only weak associations were seen between lameness and the presence of lesions: 2 out of 24 GLMs showed statistically significant relationships between mobility score and the presence of lesions (p<0.002), both of them from the P_L for the 4th HE (Visit-8~Exam-4 (p=0.0011, coeff=0.31), Visit-9~Exam-4 (p=0.0016, coeff=0.32). The choice of the outcome variable (scoring methods or loss function) did not significantly affect the *p*-values or the coefficients in the GLMs (p>0.05). However, there was a significant negative effect of visits on the *p*-values of the GLMs with HLS (average for all visits, p = 0.030) and a significant positive effect on the coefficients (average for all visits = 0.22).

Conclusions: The agreement among raters when using the AHDB scoring method was poor but was improved by binary transformation and convergent scoring. However, the system used for aggregating scores and assigning labels did not significantly affect the strength of the GLM models. Throughout the study, agreement among raters improved, and the relationship between hoof lesions and all mobility score methods became significantly stronger, as indicated by diminishing *p*-values and increasing coefficient estimates. The addition of an extra scorer after visit5 might also have been a factor. We consider that the loss-function approach to aggregating multiple assessments performed slightly better than the other approaches and is worth further investigation. Our findings highlight the challenges of making reliable mobility assessments in cows to be used as labels for automated lameness detection.

Keywords: Lameness, mobility classification, time-series, labels, automated detection.

LA-12

Can a three-point locomotion scoring system reflect the actual claw health?

Sarah Hertle[1], Isabella Lorenzini[1], Katharina Grimm[2], Bernhard Haidn[1].

[1]Bavarian State Research Centre for Agriculture, Poing, Germany; [2]Hoof Health Associates, Dres. med. vet. Fiedler, Grimm und Kröger, Munich, Germany.

Objectives: The detection of lame cows is the first task when it comes to dealing with lameness on dairy farms. Early identification of lameness and thus of claw problems enables immediate treatment and therefore prevents further disease progression. This not only leads to reduced treatment costs, but also minimises production losses, reduces early cow replacements and increases cows' individual wellbeing. The aim of this study was to validate a three-point locomotion scoring system to evaluate its suitability for future lameness scorings.

Material & Methods: The investigated locomotion score was established as an in practice applicable reference system in 2017 during a project about indirect automatic lameness detection. With this scoring system, cows with an irregular, asymmetric and uneven gait are scored as "lame" (score=3), cows without gait alterations, but showing signs like head bobbing, an arched back or a compensatory posture are considered "unsound" (score=2), and animals who present without any of these features are categorised as "sound" (score=1).

In a first examination, this locomotion score was tested on two different Bavarian dairy farms in a new project on indirect automatic lameness detection. Locomotion scoring was performed one day prior to farm claw trimming by examining video recordings of the cows leaving the milking robot. The results of locomotion scoring were compared to the findings of the farm claw trimming together with a pain test carried out with claw pliers on each claw. A three-level lesion score was assigned to the observations recorded during claw trimming. Score 1 was given to animals with a negative pain test without any visible findings or with minor disorders like sole bleedings, stage 1 digital dermatitis or chronic defects like stage 4 digital dermatitis. Cows with either a positive pain test without findings or the combination of a negative pain test and clinical findings like white line disease, ulcers, tylomas, laminitis or acute digital dermatitis (stages 2 and 4.1) were rated as score 2. Score 3 was given to animals experiencing pain and concurrently suffering from noticeable claw diseases.

The analysed data was comprised of two different datasets, each including two different claw trimming dates. The first dataset included data from 110 cows from November 2020 and May 2021 and included findings documented by claw trimmers as a reference. The second one consisted of data from 115 cows from July and November 2021 and had the results recorded by a veterinarian as a reference.

In a subsequent investigation, the intra- and inter-rater agreement were calculated for the locomotion scoring system using observations from 355 cows on one project farm.

Quadratic weighted Cohen's kappa (K), the 95% confidence interval (CI) as well as the percentage of agreement (PA) were calculated for all datasets of both examinations.

Results: Regarding the comparison of lesion and locomotion score, K was 0.51 (CI=0.34-0.68) for the first dataset and the PA was 66.4%. The results of the second dataset on the other hand showed better results (K=0.72, CI=0.58-0.86, PA=80%), which imply that the actual value might be moderate to almost perfect.

For the intra-rater agreement, the computed PA yielded 93% and the K 0.89 (CI=0.84-0.94), indicating an almost perfect agreement. The inter-rater reliability resulted in a PA=82 % and K=0.72 (CI=0.64-0.81), meaning the inter-rater reliability was substantial to almost perfect.

The examination of the first dataset showed deficits which might have been caused by missing claw health information, as some findings may have not been documented by claw trimmers. More promising results were achieved with the validation of the second dataset and the inter-/ intra-rater agreement.

Conclusion: In conclusion, the validation based on the second dataset and the examination of the inter-/ intra-rater agreement show that in this study the three-point locomotion scoring system was a reliable reference system for claw health. In order to generalise and confirm the presented results additional comparable data sets from other farms and observers should be examined.

The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany. The Federal Office for Agriculture and Food (BLE) provides coordinating support for digitalisation in agriculture as funding organisation.

Keywords: Lameness, Claw Health, Locomotion Scoring.

LA-13

Effectiveness of tildipirosin injectable solution for the treatment of naturally-occurring acute, bovine interdigital necrobacillosis (foot rot) caused by *Fusobacterium necrophorum*

Andre Pacheco De Carvalho[1], Carlos Roberto Da Silva[2], Francisco Barufi[1], Heitor Amaral[1], Luis Vettorato[1], Mauricio Morais[1], Rafael Chiummo[3], Siddartha Torres[4], Rodolfo Mingoti[1].

[1]MSD Animal Health, São Paulo, Brazil; [2]GAIA- Saúde Animal Ltda, Uberlândia, Brazil; [3]MSD Animal Health Innovation, Schwabenheim, Germany; [4]Merck Animal Health, Madison, NJ, United States.

Objectives: Foot rot disorders in cattle are necrotic diseases that affect the connective tissue of the interdigital region and are caused by bacteria, mainly *Fusobacterium necrophorum.* The aim of the study was to evaluate the efficacy of a treatment with a single subcutaneous dose of tildipirosin injectable solution (Zuprevo®) against naturally occurring foot rot.

Materials and methods: Fifty-nine Crossbred bulls (*Bos taurus* × *Bos indicus*), aged between 18 and 24 months and 300 to 500 kg BW, from a commercial feedlot farm, located in Buritizal, São Paulo, Brazil, were included. Animals were randomized into two groups: *Tild-Group* (n=29) were treated with a single subcutaneous administration of tildipirosin at a dose of 4 mg / kg body weight (0.022 mL Zuprevo®/ kg BW); the *Control-Group* (n=30) was treated subcutaneously with 0.9% saline at the same volume (0.022 mL / kg BW). Before and after treatment, both groups were housed in the same paddocks during rainy season. At inclusion, experimental cattle presented lesion scores ≥ 2 (Lesion Score Guide) and lameness scores ≥ 3 (Lameness Scoring Guide). Before treatment, bacteriological samples were collected from the infected hooves of the enrolled cattle. Lesions and lameness were also assessed on Days 2, 4 and 7. On Day 7 another sample for a bacteriological examination was collected from all animals and all samples were evaluated by PCR. Efficacy was determined by a reduction of the lesion and lameness scores and the results of the bacteriological examination of the animals infected by *Fusobacterium necrophorum* 7 days after treatment compared to day 0. Statistical analysis was performed by *Fisher's Exact Test* of *FREQ* procedure of *SAS* (*v. 9.2;* SAS Institute Inc., Cary, NC).

Results: Comparison of the claudication scores between days 0 and 7 revealed that 100% (29/29) of the *Tild-Group* under evaluation presented a reduction of at least one point in the score, whereas the *Control-Group* presented 56.7% (17/30). *Tild-Group* group had 20.69% animals that showed a reduction in lameness and lesion status and other 20.69% that had improvement on their foot rot lesion status, and lesions became milder (*P=0.05*). On the other hand, only one

animal of the *Control-Group* (3.33%) reduced lameness and lesion scores post treatment, and 10% improved their lesion status. Prior to treatment, 68.96% (20/29) and 60% (18/30) of animals were positive for *F. necrophorum* in the *Tild-Group* and *Control-Group*, respectively. After treatment, 37.93% (11/29) and 73.33% (22/30) animals were positive for *F. necrophorum* in *Tild-Group* and *Control-Group*, respectively. *Tild-Group* showed a statistically and clinically significant decrease (*P<0.05*) in the occurrence of *F. necrophorum* 7 days after treatment (P<0.05) compared to day 0.

Conclusion: This study demonstrates the efficacy of tildipirosin in the treatment of naturally occurring *F. necrophorum* related foot rot in cattle under typical feedlot conditions in Brazil.

Keywords: Foot rot, tildipirosin, cross-breed bulls, Brazil.

LA-14

Associations of host genotype with foot skin microbiota profiles and digital dermatitis related bacteria

Veysel Bay[1], Enrique Sánchez-Molano[2], Amy Gillespie[3], Georgios Banos[2], Georgios Oikonomou[4].

[1]Ege University, Faculty of Agriculture, Department of Animal Science, Turkey; [2]The Roslin Institute and R(D)SVS, University of Edinburgh, United Kingdom; [3]Institute of Infection and Global Health, University of Liverpool, United Kingdom; [4]Institute of Veterinary Science, University of Liverpool, United Kingdom.

Objectives: Associations between host genetics and skin microbiota profiles have been shown in humans[1] and mice[2]. We have recently described the bovine foot skin microbiota using 16S rRNA amplicon and shotgun metagenomic sequencing and showed associations with the development of digital dermatitis (DD) in dairy cattle. Here we used genome wide association and regional heritability mapping approaches to provide first evidence for significant interactions between the host's genotype and the bovine foot skin microbiota profiles.

Materials and Methods: Swabs were taken from the heel bulb region of the back-left foot of 259 cows from 3 UK farms 3-4 weeks before calving. 16SrRNA amplicon sequencing was carried out for all samples using the Illumina® HiSeq 2500 platform, and taxonomic assignment of OTUs carried out using QIIME and the RDP classifier. Chao 1, Shannon and Simpson indices were calculated to describe alpha-diversity, and weighted UniFrac distances and non-metric multidimensional scaling values were used to describe beta-diversity.

Cattle genomic DNA samples were genotyped using a 50K SNP chip. Genome wide association analyses (GWA) and regional heritability mapping (RHM) of consecutive 20 SNPs were performed to identify genomic regions associated with various diversity indices and with the relative abundance of genera found to be associated with the development of DD lesions.

Results: Host genotype was not found to be associated with the overall richness and diversity of the foot skin microbiome. However, significant associations were found between the host's genotype and two bacterial genera associated with DD. The heritabilities for relative abundances of *Peptoclostridium* spp. and Treponema spp. were 0.59 ± 0.18 and 0.52 ± 0.00, respectively. One suggestive SNP on BTA6 and one significant SNP on BTA 19 were associated with relative abundance of *Peptoclostridium* spp. Four significant and two suggestive SNPs on BTA1, one suggestive and one significant SNP on both BTA9 and BTA17, one significant SNP on BTA16, and one suggestive SNP on BTA2, BTA6, BTA8, BTA19, BTA21 and BTA29 were associated with relative abundance of *Treponema* spp.

On BTA1, the region associated with relative abundance of *Treponema* spp. explained 9.88% of the total genomic variance and included the genes *GMPS* and *PLCH1*. *GMPS* encodes guanine monophosphate synthetase which plays a role in de novo synthesis of guanine nucleotides; the cyclic GMP was shown to be associated with immune signaling pathways[3]. *PLCH1* is a member of the phospholipase enzyme family that generates the secondary messengers inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) by cleaving phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2). Phospholipases were shown to be involved in inflammation mechanisms[4], especially the expression of *PLCH1* were shown to be downregulated by lipopolysaccharides (LPS)[5] which is found in the outer membrane of Gram-negative bacteria[6]. These associations may explain the role of the genomic region on BTA1 in immune and inflammatory response against the Gram-negative Treponema[7] infections. The region associated with relative abundance of *Treponema* spp. on BTA16 explains 34.78% of the total genomic variance and includes the gene *PTPRC* encoding a transmembrane tyrosine phosphatase which was shown to be upregulated after administration of external bacteria to the intestine of mice[8].

Conclusion: In the present study, DD-linked foot skin microbiome traits were investigated using GWA and RHM approaches, leading to a first understanding of the genomic architecture of these traits.

References:

1. Si J et al. *BMC Genomics*. 2015.
2. Belheouane M et al. *Microbiome*. 2017;5(1):59.
3. Wu J et al. *Science (80-)*. 2013;339(6121):826-830.
4. Lemos MVA et al. *BMC Genomics*. 2016;17(1):213.
5. Lo Vasco VR et al. *Inflammation*. 2013;36(4):862-868.
6. Heumann D & Roger T. *Clin Chim Acta*. 2002;323(1-2):59-72.
7. Holt SC. *Microbiol Rev*. 1978;42(1):114-160.
8. Nerstedt A et al. *Br J Nutr*. 2007;97(6):1117-1127.

Keywords: Digital dermatitis, Microbiome, Genetics, Genomics, Lameness.

LA-15

Association of subclinical ketosis with lameness, locomotion score and hoof lesions in post-partum dairy cows

Alexandros Kougioumtzis[1], Vangelis Tsiamadis[1], Aikaterini Soufleri[1], Nektarios Siachos[1], Georgios Arsenos[1], Georgios Banos[2], Georgios E. Valergakis[1].

[1]Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, Greece, Thessaloniki, Greece; [2]Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, Greece, Scotland's Rural College/Roslin Institute, Edinburgh, Scotland, UK, Thessaloniki, Greece.

Objective: Lameness is a debilitating condition that negatively affects animal welfare and productivity. Subclinical ketosis (SCK) is associated with most metabolic diseases in post-partum dairy cows but its link with lameness is not adequately researched. The aim of this study was to investigate the association of SCK with lameness severity and hoof lesion type in Holstein dairy cows during the post-partum period.

Materials and methods: The study was conducted at a large commercial farm located in Northern Greece and included 288 first parity Holstein cows. All cows were blood sampled and locomotion scored on a five-point scale weekly, during the first 13 weeks of lactation, by an experienced veterinarian. Cows with a locomotion score (LS) ≥2 were considered lame; cows were hoof trimmed by the same veterinarian, who identified, recorded and scored all lesions on a 1 to 3 severity scale (mild, moderate and severe, respectively); appropriate treatment was subsequently provided. Concentration of β-hydroxybutyric acid (BHBA) was measured by a spectrophotometer and SCK was defined as BHBA≥1,100 μmol/L. Lesions were categorized as either claw horn lesions (e.g. sole ulcer, white line disease etc) or infectious (e.g. digital dermatitis, pododermatitis etc). During the study period, cows would be hoof-trimmed and lesions identified and recorded multiple times if needed (a new case was defined as LS≥2, 4 weeks after the previous diagnosis). Cases of lameness were classified in one of the following groups, based on lesion and severity score: a) CHL (n=196): cases with only or predominately claw horn lesions, b) INL (n=153): cases with only or predominately infectious lesions and c) CHL_INL (n=48): cases with both type of lesions, of equal severity. The data set included 3,676 observations (BHBA measurements and locomotion scores) and 397 cases of lameness. For each observation, cows were classified as SCK or non-SCK. Herd level association of SCK with cow lameness, LS and case classification (CHL, INL and CHL_INL) was estimated with Cox regression survival analysis. Kaplan-Meier survival analysis was used to estimate cow median survival time (MST) of repeated observations within each hoof lesion group. All analyses were performed with SPSS ver. 25. Statistical significance threshold was set at P≤0.05.

Results: Incidence of SCK and lameness was 23.96% and 82.29%, respectively. Cows with SCK were 2.7 (1.7-4.7) times more likely to be diagnosed as lame compared to non-SCK ones. Cows with SCK were 4.1 (2.1-8.3) times more likely to be scored with a LS of 3 compared to non-SCK ones; SCK was not significantly associated with LS=-2 and LS=4+5. Cows with SCK were 3.2 (1.7-6.1) times more likely to be diagnosed as lame due to CHL and showed a tendency to be diagnosed as lame due to CHL_INL (P=0.06), compared to non-SCK ones. Impaired locomotion due to INL type was not significantly associated with SCK. Cases classified as SCK+CHL were diagnosed earlier, compared to non-SCK+CHL: (MST median±se) 5.00±1.65 vs. 9.00±0.43 weeks. Cases classified as SCK+INL had a significantly shorter MST (median±se), compared to non-SCK+INL ones: 2.00±1.96 weeks vs. 7.00±0.61. Cases classified as SCK+CHL_INL had a significantly shorter MST (median±se), compared to non-SCK+CHL_INL ones: 2.00±0.82 weeks vs. 7.00±1.01.

Conclusion: Subclinical ketosis significantly increased the odds and severity of lameness; in addition, SCK was associated with claw horn lesions but not with infectious ones. Occurrence earlier in the lactation period in subclinically ketotic cows is expected to aggravate the harmful effects of lameness. Management efforts to reduce incidence of SCK would favor hoof health and cow welfare.

Keywords: Lameness, subclinical ketosis, dairy cows.

LA-16

Genetic selection can reduce the incidence of claw horn lesions in dairy cattle

Matthew Barden[1], Alkiviadis Anangnostopoulos[1], Bethany E. Griffiths[1], Cherry Bedford[1], Marco Winters[2], Bingjie Li[3], Mike Coffey[3], Androniki Psifidi[4], Georgios Banos[3], Georgios Oikonomou[1].

[1]University of Liverpool, Neston, United Kingdom; [2]AHDB, Stoneleigh, United Kingdom; [3]SRUC, Edinburgh, United Kingdom; [4]RVC, London, United Kingdom.

Objectives: Sole haemorrhage (SH), sole ulcers (SU), and white line lesions (WL) are often grouped under the collective term "claw horn lesions" (CHL) (Offer et al. 2000). Claw horn lesions have a high prevalence in dairy cattle (Offer et al. 2000) and, relative to other foot lesions, CHL have been associated with the most severe pain responses (Pastell et al. 2010), economic impacts (Bruijnis et al 2010), and environmental consequences. In 2018, a genetic selection index for lameness, termed "Lameness Advantage", was published by the UK Agricultural and Horticultural Development Board (AHDB). The Lameness Advantage index is calculated using lameness events from farm records (collected via milk recording organisations) in combination with traits from type classification. The objective of this study was to determine the association between the Lameness Advantage genetic index and the frequency of four outcomes: sole haemorrhage (SH), sole ulcers (SU), white line lesions (WL), and lameness during mobility scoring.

Materials and Methods: We enrolled 2,352 Holstein dairy cows from four farms in the UK. Cows were mobility scored and foot lesions recorded at four time points from before calving to late lactation. Over 90% of foot lesion identification and recording was performed by a single researcher. Cows were genotyped and genetic indexes were assigned to each cow following national genetic evaluations. Lameness records and

genetic indexes were successfully matched for 2,107 cows. Four separate multivariable logistic regression models, which included farm and parity as covariables, were used to quantify the association between the Lameness Advantage index and SH, SU, WL, and lameness.

Results: The odds ratios (95% confidence intervals) for one point increase in the Lameness Advantage index were 0.79 (0.72 - 0.86), 0.68 (0.59 – 0.78), 0.94 (0.84 – 1.04), and 0.82 (0.74 – 91) for SH, SU, WL, and lameness, respectively. The same trends were present when the sire's Lameness Advantage index was evaluated in place of the animal's own, although the strength of this association was generally weaker.

Conclusion: The Lameness Advantage index is associated with SH, SU, and lameness, therefore selection on the Lameness Advantage index could be considered in herds aiming to reduce lameness. Where genomic testing of heifers is not conducted, sire Lameness Advantage index may still be effective to reduce SH and SU incidence.

References:

Bruijnis MRN, Hogeveen H, Stassen EN. Assessing economic consequences of foot disorders in dairy cattle using a dynamic stochastic simulation model. J Dairy Sci. 2010 Jun;93(6):2419–32.

Offer JE, Logue DN, McNulty D. Observations of lameness, hoof conformation and development of lesions in dairy cattle over four lactations. Vet Rec. 2000;147(4):105–9.

Pastell M, Hänninen L, de Passillé AM, Rushen J. Measures of weight distribution of dairy cows to detect lameness and the presence of hoof lesions. J Dairy Sci. 2010 Mar 1;93(3):954–60.

Keywords: Genetics, lameness, lameness advantage, claw horn lesions.

LA-17

Hormonal profiles and serum biomarkers for fat mobilisation and their association with sole ulcers in dairy cows

Bethany Griffiths[1], Alkiviadis Anagnostopoulos[1], Matthew Barden[1], Cherrill Bedford[1], Mollie Rudd[1], John Graham- Brown[1], Stuart Carter[1], Androniki Psifidi[2], Georgios Banos[3], Georgios Oikonomou[1].

[1]University of Liverpool, Liverpool, United Kingdom; [2]Royal Veterinary College, London, United Kingdom; [3]Scotland's Rural College, Midlothian, United Kingdom.

Objective: Sole ulcers (SU) are painful, non-infectious lameness causing lesions. Relaxin and insulin have been speculated as playing an important role in their development, through the relaxation of the suspensory apparatus at calving or insulin dysregulation induced damage to the corium. Periparturient fat mobilization has also been implicated in their development. The objective of this study was to evaluate whether serum biomarkers for fat mobilisation and specific hormonal profiles are associated with the odds of a cow displaying a SU in the early lactation period.

Materials and Methods: As part of a larger study, 371 Holstein cows from two commercial dairy farms that were due to calve between April and September 2019 were prospectively enrolled prior to calving. Animals were assessed at four time points relative to calving date; prior to calving (mean: -59 days, SD: 30) immediately after calving (mean: +5 days, SD: 4, fresh), approximately two weeks after calving (mean: +12 days, SD: 4, fresh 2) and in early lactation (mean: +77 days, SD: 10).

At each visit, body condition (BCS) was assessed. The animal was restrained in a foot trimming crush, and all feet assessed for the presence of infectious and non-infectious disease, with lesions graded for severity. Serum samples were collected from each animal at fresh, fresh 2 and the early lactation time points. Animals were excluded from the study if a serum sample was missing. All eligible primiparous animals (60) were enrolled and a further 140 multiparous animals were randomly selected from the rest of the enrolled population. Relaxin, insulin, β-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) were measured using commercially available kits.

Univariable analysis was undertaken and a multivariable logistic regression model was then fitted with presence of a SU at the early lactation check as the outcome. BHB and NEFA concentrations were categorised into normal or elevated based on reference ranges (1.2mmol/l, and 0.7mmol/l respectively). Relaxin and insulin concentrations were analysed as quartiles.

Results: In total, 600 serum samples from 200 animals were analysed for relaxin, insulin, BHB and NEFA at three time points (fresh, fresh 2 and early lactation). Eighteen animals (9%) exhibited a sole ulcer at the early lactation check.

At univariable analyses, parity (P= 0.014), serum NEFA concentrations at fresh 2 (P= 0.014), and serum relaxin concentration at fresh (P= 0.004) were statistically significantly associated with the presence of a SU. The latter highlighting that animals in the first quartile (lowest relaxin concentration) were more likely to display a SU. Trends were also noted; animals with greater serum BHB's at fresh 2 (P= 0.068), and NEFA at fresh (P= 0.078) were more likely to display a SU, as were animals which displayed a SU in the fresh time point (P= 0.067).

Odds ratios (95% confidence intervals) for variables retained within the final multivariable logistic regression model were calculated for an animal exhibiting a SU in early lactation. Animals with elevated BHB concentrations at fresh 2 and NEFA concentrations in early lactation were at 11.12 times (2.07-59.69, P= 0.005) and 3.76 times (0.92-15.35, P= 0.065) higher odds of being affected with a SU (comparing to animals below the elevated levels threshold) respectively. Animals with an insulin concentration in the fourth quartile (highest concentration) in early lactation were 11.57 times (1.19-112.53, P= 0.035) more likely to display a SU than those in the first quartile. Those in the second and third quartile were 5.49 (0.56-53.55, P= 0.14) and 5.86 times (0.57-60.54, P= 0.14) more likely to display a SU than those in the first quartile.

Conclusions: This preliminary analysis has confirmed the importance of previously described risk factors (excessive periparturient fat mobilization) and identified novel associations between serum biomarkers and the development of sole ulcers. Further analysis is required to fully characterise this

association between high serum insulin concentrations in early lactation and the development of SU's that could further our understanding of the disease's aetiopathogenesis.

Keywords: Sole ulcers, dairy, relaxin, insulin.

LA-18

Complexity of the relationship between behaviour, performance, and claw health in dairy cows

Isabella Lorenzini[1], Katharina Grimm[2], Bernhard Haidn[1].

[1]Bavarian State Research Center for Agriculture, Grub, Germany; [2]Hoof Health Practice Drs. Fiedler, Grimm & Krögerr, Munich, Germany.

Objectives: Lameness in dairy cows is a sign of pain and an important welfare issue. Cows tend to hide signs of pain, making visual lameness detection difficult for farmers and vets. Furthermore, research shows that farmers only recognize about a quarter of lame animals on their own farms.

Digitization in dairy farming has the potential to improve health monitoring of individual animals. Previous studies have attempted to combine automatically collected behaviour and performance data to indirectly detect lameness in dairy cows, but have only achieved moderate levels of prediction accuracy, possibly due to the differences between individual farms and animals and also due to the correlation between parameters as well as the interactions thereof.

The aim of this study was to gain a deeper insight into the relationship between claw health, behaviour, and performance in dairy cows to improve indirect automatic lameness detection methods.

Materials and methods: Data was collected between April 2017 and June 2018 on four commercial dairy farms and one research farm in Bavaria. Claw health reference data was collected manually by scoring the locomotion of all animals every fortnight through videos using a three-point score. All cows in the study were fitted with pedometers (ENGS Dairy Solutions, Israel). The pedometers measure activity, lying and feeding behaviour.

The data from the pedometers as well as data from two milking robots were combined into an SQL-database calculating daily values for each parameter and each cow. The reference claw health data and data from monthly milk-yield tests for farms with milking parlours were added to the database.

Data analysis was carried out using RStudio (R version 3.6.2). The data were cleaned, centred, scaled, and balanced using the SMOTE algorithm. A generalised linear model with all variables from the dataset and combinations thereof was run using 10-fold cross validation to find relevant interaction parameters. A random intercept was added for the individual cows and the full multilevel model was reduced by non-significant predictors using backward step regression.

Results: The final dataset contained 71.497 data points from 381 different animals. The final model had 25 predictors, of which 15 were interaction effects. The findings in the study support those found in literature, such as a higher feeding duration (FD, odds ratio OR = 0.51, confidence interval CI 0.49-0.53) and a later stage of lactation (OR = 0.61, CI 0.58-0.64) being a protective factor for lameness and that the ratio between lying duration during daytime and night-time (OR = 1.17, CI 1.13-1.22) and an increased lying duration per day (LD) (OR = 1.42, CI 1.36-1.48) increased the probability of an animal being lame.

The interaction effects were particularly interesting as they offered insight into the complexity of the relationship between the single predictors. The interaction effect between the average daily milk yield (MMY) and FD for example, shows that with mean and above average FD, MMY hardly influences the probability of a cow being lame. With a low FD however, an increased MMY has a strong positive effect on the probability of being lame. Similarly, a higher parity number in combination with a low number of lying bouts (LBN) are associated with a higher risk of lameness, possibly indicating less willingness to stand up often. Cows in their first lactation, however, have a higher probability of being lame if they have a high LBN. This could be an indication of enhanced social stress in heifers after first being integrated in the herd after calving, which affects cows' behaviour and their time budget. If this effect, which leads to spending less time feeding and lying, is more prominent in heifers, it could in turn affect their hoof health more than in older cows.

Conclusions: Using automatically collected behaviour and performance data to detect lame animals could be a promising tool for early lameness recognition. However, using single behaviour and performance parameters to try and predict lameness doesn't reflect the complexity of the relationship between claw health, behaviour, and lactation data. Further research is needed to understand this type of interaction both on a herd and an individual-animal level.

Keywords: Claw health, precision dairy farming, lameness, behaviour, performance.

LA-21

Evaluating the Effectiveness of Organic Therapies for the Treatment of Bovine Digital Dermatitis

Cassie Krebill[1], Jan Shearer[1], H. Morgan Scott[2], Sebastian Umase[3], Ivan Sanabria[3], Jonatan Baron[3], Romina Hernandez[3], Romeo Hernandez[3], Paul Plummer[1].

[1]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, United States; [2]Department of Veterinary Pathobiology, Texas AM &University, College Station, United States; [3]Aurora Organic Dairy, Platteville, United States.

Objectives: Bovine digital dermatitis (DD) is an infectious bacterial disease that causes acute and chronic lameness in dairy cattle. DD has created welfare concerns within the dairy industry since its discovery in the United States during the 1980s. Common effective topical therapies for DD often include antibiotics. This makes it difficult to manage DD in organic production systems while abiding by the United States National Organic Standards Board. The primary objective of

this prospective randomized clinical trial was to identify one or more effective and organic-approved products to provide improved treatment outcomes for DD.

Materials and Methods: Dairy cattle (n=385) with DD lesions were enrolled at D0 on two, similarly managed, commercial organic dairy farms. Cows were blocked by days in milk and lactation number for randomization and assigned to one of four topical treatments: a.) 15 grams of copper sulfate, b.) 20 grams of iodine, c.) 20 grams of honey, and d.) 20 grams of hydrogen peroxide. All treatments were lightly wrapped with a bandage and removed after 3 to 5 days. The assigned treatment was applied on D0, D7, and D14. The cows were evaluated in a hoof-trimming chute on D0, D7, D14, D28, D56, and D112. All data were collected by trained veterinarians and professional hoof trimmers. Metrics of locomotion score (LOCOSCORE: 1 = best, 2, 3, 4, 5 = worst), an adapted digital dermatitis scoring system (DDSCORE: 1 = best, 2 3, 4, 5 = worst), and an algometer pressure reading (ALGOSCORE:1 = worst through, 10 = best) were collected at each evaluation to determine the effect of the assigned treatments on changes in metrics since enrollment. Body condition scores and digital photographs of the lesion were collected and recorded at each evaluation. Treatment failures were switched to a 50:50 copper sulfate and iodine topical solution. Statistical analyses were performed using ordinal (proportional odds) regression for DDSCORE and LOCOSCORE versus linear regression for ALGOSCORE. Farm was treated as a fixed effect while treatment by day effects were modeled in full factorial designs. Margin means were estimated for each treatment at each day. For ordinal outcomes the probability a cow exhibited either score 1, 2, 3, or 4 was the outcome of interest, with increasing probability of lower scores the desirable outcome. For ALGOSCORE, a more rapid rise towards higher scores was desirable.

Results: The results of this study show copper sulfate was clearly the most effective treatment to rapidly and significantly improve all outcome measurements over the 112-day trial. Copper sulfate treatment showed a significant ($P<0.01$) increase in relative odds of becoming a lower category DDSCORE in each post treatment evaluation. With lower scores, the physical appearance of the lesion is drier and more regressed. Cows treated with copper sulfate were estimated to exhibit an ALGOSCORE of 7.1 (95% CI 6.41, 7.69) at D28 compared to 3.4 (95% CI 2.47, 4.26), 4.0 (95% CI 3.16, 4.75), and 2.7 (95% CI 1.91, 3.49) for honey, hydrogen peroxide, and iodine respectively. The ALGOSCORE, or pain-free pressure applied to the lesion, increases as the lesion regresses. The analysis for LOCOSCORE resulted in copper sulfate producing the most rapid recovery and highest probability of an ideal LOCOSCORE of a 1, no visible lameness, throughout each follow-up evaluation. All three statistical analyses showed global significance of treatment (3 degrees of freedom), period (5 degrees of freedom) main effects and their interactions ($p<0.05$).

Conclusion: Of the four organic-approved treatments tested in this trial, copper sulfate was by-far the most effective treatment in decreasing DDSCORE and LOCOSCORE most rapidly as well as increasing ALGOSCORE indicating lesion regression and healing. While the other treatments improved over time, none showed significantly improved changes throughout the four interim evaluation points and there were no significant differences between the non-copper sulfate groups. All cows did show improvement over time, with cows remaining in the study at D112 having improved outcome measures compared to D0; in addition, differences were non-significant ($P>0.05$) among all treatments at D112). However, the effects of culling and switching to the alternative treatment at D28 may have confounded those findings. Copper sulfate is the superior topical treatment to manage bovine DD.

Keywords: Digital dermatitis, locomotion, organic.

LA-22

The Effects of Lameness on Weight Gain and Carcase Classification of UK Finishing Cattle

Jay Tunstall, Helen Higgins, Dai Grove-White, Jo Oultram, Karin Mueller.

University of Liverpool, Liverpool, United Kingdom.

Objectives: Lameness in UK dairy cattle is widely reported to have significant effects on yield, fertility and welfare. However, the effects of lameness of UK beef cattle is unknown. Furthermore, there is minimal information regarding the effects of lameness on beef cattle internationally. There is however evidence suggesting a lower price can be expected at slaughter for cattle having experienced lameness.

The absence of appropriate information regarding the impact of lameness makes treatment and prevention planning more complicated, in particular making it more difficult to determine intervention points. This lack of evidence may potentially lead to lameness being undervalued as an issue, and therefore not treated or prevented adequately.

Knowledge regarding the impact of lameness on production measures would support veterinarian and farmer decision making, highlighting the importance, and justifying treatment and prevention measures. With beef finishing units, Average Daily Live Weight Gain (ADLWG) is useful measure of performance as faster growth will lead to shorter time on farm and generally lower total input costs. Carcase classification is another measure of performance, which represents the conformation and fat coverage at slaughter. Carcass classification will affect the price per Kilogram paid to the farmer.

This study identified the effects of lameness on growth rates and carcass classification of finishing cattle on three UK farms.

Materials and Methods: A longitudinal observation study was carried out on three farms located in England between July 2018 and August 2019. Animals of interest were housed cattle, in the finishing period and being reared for beef production. Three farms were recruited, where eligible animals were weighed and locomotion scored approximately monthly from the start of the study for the duration of their time in the finishing group. Slaughter dates and carcass grading information was obtained.

Results: Farm 3 results have been presented here. On this farm, 275 animals received at least two locomotion scoring

visits before being finished. Of these animals, 265 were Aberdeen Angus (AA) or AA crossbred. The mean age at slaughter was 20.4 months (standard deviation (SD) 1.7 months). 82 of these animals were lame at least one locomotion scoring visit, with 34 being lame for multiple visits. Animals being scored as lame during one or more locomotion scoring visit were more likely to have a lower ADLWG (p<0.001) than animals never having been scored as lame. The ADLWG from first scoring to last scoring was 1.37Kg (SD 0.43) for animals not experiencing lameness, compared to 1.1Kg (SD 0.48Kg) for animals that experienced one or more lameness events. The ADLWG for cattle that were lame at all scoring visits was 0.66Kg (SD 0.59Kg). Details of other farms are discussed, along with carcass grading information and the association with lameness.

Conclusion: The ADLWG was lower for animals experiencing at least one lameness event compared to animals not experiencing a lameness event, which would correlate with production loss data available for the dairy industry. In the dairy industry, some evidence suggests that higher yielding cattle are more likely to become lame than lower yielding cattle. This study doesn't identify whether faster growing beef cattle are more likely to become lame, but if true, this may be masking some of the effects of lameness on ADLWG.

This research suggests that prevention of lame animals may be important to increase growth rates and achieve better carcass classification, increasing productivity and farm profitability.

The authors would like to thank The Animal Welfare Foundation, who supported this research through the Norman Hayward Research Fund.

Keywords: Lameness, Beef, Finishers, Mobility, Locomotion.

LA-23

Comparison of thiamphenicol and oxytetracycline based topical treatments for the control of digital dermatitis lesions in dairy cows: a multicentric randomized clinical trial

Fabien Corbiere[1], Charly De Campos[1], Marc Delacroix[2], François Schelcher[1].

[1]National Veterinary School of Toulouse, Toulouse, France; [2]Boen Veterinary Practice, Boen sur Lignon, France.

Objectives: This trial compared the effect of the topical administration of thiamphenicol (Taf Spray® and oxytetracycline (Oxytétrin®) on the curation of Digital Dermatitis (DD) lesions of various evolution stage.

Materials and methods: A multicentric randomized clinical trial was set up in four flocks with a history of high DD prevalence. The study involved 116 Prim-Holstein and 8 Montbéliardes cows ranging in age from first to fifth lactation and diagnosed with DD lesion on the first examination. On the first examination (D0), hindlimb and forelimb hoofs of included cows were cleaned, washed with clear water, dried and trimmed by a professional hoof-trimmer, and the number, stage (M1-M4) and localization of DD lesions were recorded by a single operator. Stainless steel forceps that allow holding the interdigital cleft open were used for a precise assessment of DD lesions. A multicriteria lameness score was also attributed to each cow on standing position. Photographs of each cow in standing position and of each hoof were submitted to another expert for an independent evaluation. Cows were randomly assigned into either the oxytetracycline group (OTC) or the thiamphenicol (TH) group. The topical treatments were administrated for 3 seconds on each DD lesion by the same operator in a trimming cage, during 3 consecutive days, after the hoofs have been cleaned and dried. For animal welfare and ethical reasons, the study did not include a non-treated control group. DD lesions and lameness were further evaluated at D0+7, D0+14 and D0 +28 days using the same protocol.

All initial M1, M2 or M4 DD lesions that were subsequently scored M0 on D7, D14 or D28 were regarded as cured, and uncured otherwise. The probability of cure was modeled using mixed multivariate logistic models, with the DD lesion as the epidemiological unit. The estimated effect of the treatment group was adjusted on herd, DD lesion stage at D0, hoof level factors (number of DD lesions at D0 observed on the hoof) and cow level factors (number of affected hoofs, lactation number and days in milk at D0). A treatment group x DD lesion stage interaction was tested into the models, and a cow random effect was included. Separate models were fitted for D7, D14 and D28.

Results: In total, 225 DD lesions, including 77 M1-stage (34.2%), 113 M2-stage (50.2) and 34 M4-stage (15.6%) were diagnosed at D0 on 156 foots from 116 cows. A majority of cows (77, 66.3%) had only one affected foot, 37 (31.9%) had two affected feet and only 2 cows had three affected feet. Almost all DD lesions were observed on the plantar surface of the hoof (205, 91.0%) with 49.3% of them located in the interdigital cleft. At D28, the overall cure rates were similar for both treatment groups (OTC: 76.6%; TH: 79.9%, p=0.63) with M1-stage lesions being almost all healed (OTC: 97.1, TH: 92.7%, p=0.62). Adjusting for potential confounders, the cure odd was marginally statistically higher in the OTC group than in the TS group (p=0.04) at D7, but not at D14 (p=0.70) and neither at D28 (p=0.52). No significant treatment group x lesion stage interaction was found. Compared to M1-stage lesions, M2-stage lesions were at a lower odd to be cured at D7 (OR= 0.17; 95%CI: 0.04-0.65, p=0.009), D14 (OR=0.10, 95%CI: 0.06-0.61, p=0.0125) and D28 (OR=0.23, 95%CI: 0.06-0.89, p=0.029). Whatever the date and the treatment group, M4-stage lesions had the lowest cure rate (OTC:35.2%, TH: 27.8% at D28, p=0.72).

Conclusion: In this study the use of thiamphenicol or oxytetracyclin based topical treatments yielded similar cure rates. The high cure rates found for M1-stage lesions was probably partially due to spontaneous healing, that might have been favored the foot washing and drying before all examination and treatment administration. This observation reinforces the pivotal role of foot hygiene in the control of DD. Finally, the fact that only a low proportion of M4-stage lesions was cured after a 28 days' follow-up emphasizes the need to avoid chronicity for a higher probability of positive outcome.

Keywords: Digital dermatitis, topical treatment, randomized clinical trial, thiamphenicol, oxytetracyclin.

LA-24

A study on the association between occurrence of Digital Dermatitis and reproductive performance in a cohort of maiden heifers

Lara Robinson[1], Alkiviadis Anagnostopoulos[2], Lauren Thomas[2], Lucy Gleave[2], Rob Smith[2], Georgios Oikonomou[2].

[1]Daleside Veterinary Group, Wrexham, United Kingdom; [2]Department of Livestock Heath and Welfare, Institute of Veterinary Science, University of Liverpool, Neston, United Kingdom.

Objectives: Digital dermatitis (DD) is an infectious bovine foot lesion first described in the early 1970's[1] as a painful, erosive, wart like lesion. It is a common condition in dairy cattle globally and has been shown to affect heifers too[2]. Given it is a painful condition which has high herd level prevalence, DD is an important disease for the modern dairy herd. Heifers experiencing the disease pre-calving were more likely to experience it again in 1st lactation[2]. They also had significantly lower conception rate and longer days open in the 1st lactation[2]. To our knowledge, there has been no work looking at the association of DD with maiden heifer fertility. The aim of this study was to investigate whether one or more DD events in the 3-month period before and after first service was associated with reproductive performance in maiden heifers.

Materials and Methods: Data were collected from 530 Holstein heifers from one commercial dairy unit in the UK between April 2021 and December 2021. Heifers were enrolled at an average age of 388 days and observed at monthly intervals for a total of 3 observations. At each observation body weight and body condition score were recorded. The hind feet of the heifers were washed with water before being inspected for DD using a mirror with LED lights. DD lesions were scored based on physical appearance using the M-stage classification system. Active lesions (M-stages 1, 2 and 4.1) were treated with topical oxytetracycline spray. Fertility data were collected after the end of the study period. Any animals that became pregnant during the study were not inspected after confirmation of pregnancy. Univariable and multivariable analyses were conducted on the data.

Results: 530 heifers were enrolled into this study of which 502 heifers had 3 inspections for digital dermatitis during the study period. Pregnancy diagnosis data was available for 406 heifers at the time the data was initially analysed. DD period prevalence was 45%.

Univariable regression with conception rate at 1st service as the outcome showed that the variable season during which 1st insemination took place was statistically significantly associated with conception at 1st service (p-value <0.001) and there was a tendency for inseminator to be associated with it too (p-value = 0.08). Two multivariable logistic regression models were run using conception at 1st rate as an outcome variable and, accounting for these confounding variables. Neither having DD at the 1st inspection nor having DD ever during the study was statistically significantly associated with conception rate at 1st service.

Cox proportional hazard analysis with time to conception as the outcome variable showed that season at 1st insemination and season of birth were statistically significantly associated with conception at 1st insemination (p-values < 0.00 and 0.005 respectively). Prevalence of DD at first inspection was not statistically significantly associated with age at conception (p-value 0.30). However, this was a preliminary analysis and a total of 116 heifers were right censored due to no conception data at the time.

Cox proportional hazard analysis showed that DD at 1st inspection was not statistically significantly associated with time to first insemination (p-value = 0.39). Season of birth and daily liveweight gain between birth and 1st observation were statistically significantly associated with this outcome (p-values = 0.004 and <0.001 respectively). As expected, growth rate was strongly correlated to age at 1st insemination (for every 1 kg increase in daily liveweight gain the risk ratio for reaching the first insemination was 9.1 with a 95% confidence interval between 3.66 and 22.88).

Conclusion: This study has shown that DD period prevalence in maiden heifers can be high. A previous study showed that DD events in pregnant heifers increased the risk of future DD events and affected fertility in 1st lactation[2]. Preliminary analysis of our data (data collection is still ongoing) showed that DD was not associated with growth rate or fertility in the studied cohort of maiden heifers.

References:

1. Cheli, R., And C. Mortellaro. La Dermatite Digitale Del Bovino. 8th International Conference On Diseases Of Cattle, 1974. 208-213.
2. Gomez, A., Cook, N. B., Socha, M. T. & Döpfer, D. 2015b. First-Lactation Performance In Cows Affected By Digital Dermatitis During The Rearing Period. Journal Of Dairy Science, 98, 4487-4498.

Keywords: Digital dermatitis, Heifers, Fertility.

LA-25

Modified Thomas splint-cast use in cattle limb fractures: characteristics and predictors of long-term survival of 185 cases (2013-2021)

Linde Gille, Bayrou Calixte, Casalta Hélène, Djebala Salem, Eppe Justine, Sartelet Arnaud.

University of Liège, Liège, Belgium.

Objectives: Traumatic limb fractures are an important cause of premature animal loss. Generally, the most cost-efficient and humane way to handle injured cattle is emergency slaughter on site. However, in the EU, this is only an option if the animal has an acute injury making transport impossible for welfare reasons, is in a further healthy condition, and has not received any medication with a withdrawal period (Eurlex 2004). Furthermore, given the cost involved, it is generally only carried out on animals with a certain weight to avoid incurring more costs than can be gained by slaughtering.

For calves and animals under withdrawal period, salvage options are limited and often expensive. Walking-casts are limited to lower limb fractures and have a weight limit in gen-



eral. Repair trough surgical reduction with internal or external fixation is costly and specialized, also due to the need for general anesthesia. External coaptation methods like the Thomas splint, initially developed for humans, stay relevant to date in a modified form for cattle due to their low cost and relative ease of use on-farm. Studies differ in opinion on their success rate, and relatively few data are available on long-term success to slaughter which makes treatment decisions hard. As such, in order to evaluate survival rate and treatment success, the aim of this study was to describe the long-term outcome and related characteristics of cattle with limb fractures treated with a modified Thomas splint-cast (MTSC), by use of their medical records and national cattle database (SANITEL) data.

Materials and methods: To perform this study, the hospital file of 211 cases of cattle having received a MTSC between September 2013 and December 2021 were retrieved from the clinic database system (SAP). After removal of duplicates (9), MTSC use for other reasons then fracture (5) and animals not retrievable in the national database (12), 185 cases were withheld. Data retrieved included gender, breed, birth date, site of fracture, time since fracture and cause of fracture. From the national herd database (SANITEL), date and type of death was retrieved to perform follow-up until 10 weeks after placement of the MTSC for all animals and a follow-up until death or slaughter for animals deceased.

Results: In 80 % of cases, cattle that received a MTSC were presented for a tibial fracture. Of all animals treated with a MTSC, 42.16 % survived to be slaughtered, 41.08 % died naturally or were euthanized, and 12.43 % was still alive at the time of data retrieval (4.32% was lost to follow-up). Of the animals that did not survive until slaughter, 84.21 % died within 10 weeks after placement of the MTSC, with significantly higher odds of non-survival >10 weeks if their weight was above 275 kg (OR 2.59 $p<0.05$). There was no gender difference on survival. Cause of fracture was recorded in 49 % of cases: the main risks were manipulation (15.7%) and the placement on pasture (13%). Twenty-five percent of fractures where a time since fracture was known were presented two or more days after fracture, with outliers to 15 days after initial fracture.

Conclusions: MTSC have their use in practice as a salvage procedure to raise young animals that suffered from a limb fracture until an acceptable slaughter weight can be reached. The prognosis drops with increasing weight, and placement of MTSC should as such be reserved for young animals below 275 kgs. Animals were presented for cast placement only after two or more days in 25 % of all cases. Even though prognosis was not different in this study, this could be a severe welfare issue for the affected animals.

Keywords: Cattle, Limb fractures, Trauma, Modified Thomas Splint-Cast.

NU-01

Influence of ketosis prevalence during first lactation and subsequently on the cow's performance through its following lactations

Juan Cainzos[1], Cristina Andreu-Vázquez[2], Ramiro Fouz[3], Maria Luisa Barreal[4].

[1]Elanco Animal Health, Greenfield, United States; [2]Universidad Europea de Madrid. Faculty of Biomedial and Health Science. Department of Medicine., Madrid, Spain; [3]Africor Lugo, Lugo, Spain; [4]Laboratorio interprofesional galego de análise do leite (LIGAL), A Coruña, Spain.

Objectives: Ketosis, defined as hyperketonemia (high levels of beta-hydroxybutyrate (BHB) in blood, milk or urine) during first weeks postpartum, is a common and costly disease. The aim of the present study was to evaluate the impact of first milk test ketosis prevalence on first lactation performance as well as on the subsequent lactations' ketosis prevalence and lactation performance.

Materials and methods: Cow information, test-day production data, and milk BHB concentrations were obtained from the Africor Lugo database (Lugo –Spain), including 22018 animals with first calving occurring during 2015. Information from these animals was obtained until May 2019, including farm name and cow ID (coded), date of birth, date of culling, and for each lactation: calving data, lactation number, total milk, fat and protein yield (kg), 305d corrected milk (305d-milk), fat and protein yield (kg), lactation length (days) and days in milk (DIM) and BHB (µmol/L) content at first test. Energy-corrected milk (ECM, kg/d, Santichi et al., 2016 (JDairySci. 99:9263–9270)), for both total ECM (T-ECM) and 305 days ECM (305d-ECM). Milk samples were analyzed by Fourier-transform infrared (MilkoScan FT6000, Foss Electric, Hillerød, Denmark) and ketosis was determined when milk BHB values were ≥ 0.10 mmol/mL (Viña et al., 2017, JAnimPhysiol An N, 101:835–845).Only cows with DIM at first test <26 days were considered, excluding 9445 animals (Santichi et al., 2016, JDairySci. 99:9263–9270). All statistical analysis were performed using JMP 13.1.0 (SAS).

Results: Retrospective analysis of data from 12573 heifers from 1326 farms and first time calving during 2015 indicated that 80.53% (n=9.220), 56.88% (n= 5.244), 10.51% (n=551) and 0.73% (n=4) of animals reached second (2ndL), third (3rdL), fourth (4thL) and fifth (%thL) lactation respectively during the observational period. Ketosis prevalence was 22.99% (n=2632), 19.91% (n=1836), 23.55% (n=1235), 22.87% (n=126) and 0% (n=0) for animals reaching first, second, third, fourth and fifth lactation respectively. Animals developing ketosis had longer lactation period (353.1 vs 346.9 days, p=0.013), yielded less total milk (9836 vs 10114 kg, p<0.001), total protein (318 vs 333 kg, p<0.001), 305d-milk (7909 vs 8286 kg, p<0.001), T-ECM (10481 vs 10759 kg, p=0.003) and 305d-ECM (8315 vs 8703 kg, p<0.001) and tended to lower total fat yield (384 vs 390 kg, p=0.080) during 1stL.

Compared to non-ketotic cows, positive cows on 1stL had higher odds ratio of developing ketosis during 2nL, 3rdL and 4thL (OR= 1.94, 95%CI: 1.73-2.17; 2.19, 95%CI:1.90-2.; and 2.39, 95%CI:1.54-3.71, respectively). During 2ndL, positive cows at 1stL yielded less milk (11138 vs 11506 kg, p<0.001), protein (363 vs 379 kg, p<0.001), fat (354 vs 372 kg, p<0.001), 305-d milk (9195 vs 9579 kg, p<0.001), T-ECM (11891 vs 12374 kg, p<0.001) and 305d-ECM (9712 vs 10193 kg, p<0.001) regardless those cows developed ketosis during 2nL or not. Ketosis prevalence during 2ndL was higher for those cows developing ketosis during 1stL (28.79 vs 17.26%, for ketotic and healthy cows during 1stL respectively, p<0.001). Multinomial regression model, including 1stL 305d-ECM and ketosis prevalence at 1stL and 2ndL (r^2=0.17, p<0.001), indicated that 2ndL 305d-ECM was impaired in a bigger extent when ketosis occurred in 1stL than in 2ndL (9776^{b}; 10121^{a,b}; 10254^{a}; 10122^{a} kg for ketotic animals in 1stL, 1stL and 2ndL, 2ndL and healthy animals, respectively, p<0.001). Contingency analysis indicated that ketosis prevalence during 3rdL was higher for those animals developing ketosis during 1st and 2nd calving compared with animals developing ketosis during first or second and healthy animals in previous lactations (47.4, 31.6, 31.3 and 17.9% respectively, p<0.001). In the same way, 1stL ketotic cows yielded less total milk (10639.6 vs 11178.7 kg; p<0.001), protein (344.3 vs 366.5 kg; p<0.001), fat (412.8 vs 440.6 kg; p<0.001), 305-d total milk yield (9436.5 vs 10076.7 kg; p<0.001), T-ECM (11303.2 vs 12000.4 kg; p<0.001) and 305d-ECM (9962.6 vs 10759.6 kg; p<0.001) during 3rdL than non-ketotic 1stL cows.

Conclusions: Many DHI organizations offer clients an infrared test to detect milk BHB as a herd surveillance test and it has been recommended to monitor ketosis on a herd level when evaluating nutritional management or preventative medicine strategies in herds (Renauld et al., 2018. JDairySci. 102:1–5). Current results indicate a strong impact of a positive result in the first test of the 1stL on cow's performance, not only on the current but also future lactations.

Keywords: Ketosis, Transition, Management.

NU-02

Evaluating plasma methionine in response to feeding three rumen-protected methionine products

Merrick Smith[1], Shane Cronin[1], Javier Mateos[2], Diego Martinez Del Olmo[2], Fernando Valdez[3], Tanya Gressley[1].

[1]University of Delaware, Newark, Delaware, United States; [2]Kemin Animal Nutrition and Health, Herentals, Belgium; [3]Kemin Industries, Inc., Des Moines, Iowa, United States.

Objectives: Rumen protected (RP) amino acid products offer the opportunity for precision feeding of limiting amino acids to ruminant animals. The efficacy of such products is dependent on survival in the rumen and availability in the intestines, necessitating estimates of bioavailability. Plasma methionine (Met) is linearly related to intestinal Met absorption, and, thus, can be used as a tool to assess RP-Met bioavailability. However, rates of ruminal passage and absorption may differ among products, so time of blood sampling relative to feeding may be an important consideration.

The primary objective of this work was to compare the plasma methionine levels of cows fed with three different

RP-Met supplements under similar feeding and experimental conditions. This study examined the comparability of a new RP-Met product to two existing and well-studied products. The comparison was not made against a control diet but again each other as internal controls. The second objective was to determine whether time of blood sampling relative to feeding impacted relative differences among products.

Materials and methods: Ten multiparous Holstein cows, 280 ± 73 days in milk, were used in a replicated 3x3 Latin square design (three complete squares, one incomplete square), with 7-day experimental periods. Treatments consisted of a control diet plus 12 g/d of either RP-Met K (KESSENT® M, Kemin Animal Nutrition and Health, Herentals, Belgium), RP-Met S (Smartamine® M, Adisseo Inc., Antony, France), or RP-Met M (Mepron®, Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany). Amount of Met contained in each of the three products was similar. Cows were fed ad-libitum with 33% of their daily feed allotment and RP-Met treatment provided every 8 h. Milking occurred at twice daily with milk samples collected on days 5-7 of each period. During d 5-7 of each experimental period, blood samples were collected from jugular catheters at 2, 4, 6, and 8 h after the morning feeding. At the end of the experiment, samples were sent to Missouri Agriculture Experiment Station Chemical Laboratories, USA, for amino acid analysis by cation-exchange chromatography.

Prior to statistical analyses, plasma methionine was converted to percentage of total amino acids minus methionine as calculated below:

methionine, % of total amino acids minus methionine = methionine (µg/mL) / (sum of all amino acids [µg/mL] – methionine [µg/mL]) * 100%

Treatment effects on plasma free methionine were evaluated using the GLIMMIX procedure of SAS (SAS Institute Inc., 2011). The model included fixed effects of treatment, period, square, hour of sampling (2, 4, 6, or 8), day of sampling (5, 6, or 7), and all interactions among treatment, hour of sampling, and day of sampling. Plasma free methionine prior to the start of the experiment was included as a covariate, and cow was included as a random effect. Significance was declared at $P < 0.05$.

Results: There was no significant effect of treatment on dry matter intake or production parameters. Plasma Met as a % of total amino acids minus Met was 1.5085, 1.5267, and 1.3622% for RP-Met K, RP-Met S, and RP-Met M, respectively. RP-Met K and RP-Met S were not found to be different (P=0.3420), however RP-Met K and RP-Met M were different (P<0.0001), with RP-Met K yielding greater plasma Met Levels. There was a significant effect of time of sampling on plasma Met as a percentage of amino acids minus Met (P=0.002), due to higher Met at 2 h (1.508%) than 4, 6, and 8 h (1.439, 1.447, and 1.469% respectively). However, the relative differences among treatments remained consistent at all time points.

Conclusions: The relative bioavailability of a new RP-Met product was assessed by comparing plasma Met response to that of existing products. Similarities in plasma Met levels between RP-Met K and RP-Met S treatments would suggest comparative bioavailabilities and bioavailability greater than that of RP-Met M. Differences among products were consistent at all blood sampling times, suggesting that time of sampling relative to feeding is relatively unimportant.

Keywords: Rumen protected methionine, plasma methionine response.

NU-03

Bioavailability of rumen-protected choline chloride sources based on in situ rumen degradability and in vitro intestinal digestibility criteria

Cristian Faturi[1], Maria Ercilda Rodriguez-Prado[2], Fatima Francia[2], Deepak Dubey[3], Sanjeewa Ranathunga[3], Sergio Calsamiglia[2].

[1]Universidad Federal Rural de la Amazonia, Brazil, Brazil; [2]Animal Nutrition and Welfare Service, Universitat Autonoma de Barcelona, Bellaterra, Spain; [3]Kemin Animal Nutrition and Health, Herentals, Belgium.

Introduction: Choline is a vitamin-like substance that interacts very closely with methionine and vitamin B12 metabolism as a methyl donor. It plays an important role in animal production, reproduction, and health (Jayaprakash et al., 2016, Morrison et al., 2018). Choline is rapidly degraded in the rumen, so effective supplementation requires the use of rumen-protected forms that are digestible in the small intestine (Grummer, 2008). There are several commercial protected products in the market, but the amount of choline delivered to the small intestine for absorption (bioavailability) may vary largely between products (Sales et al., 2010, Humer et al., 2019). Therefore, it is important to determine their rumen solubility, potentially degradable fraction, rate of degradation, effective degradation in the rumen and intestinal digestibility. However, this information is often not available. The objective of this project was to evaluate rumen degradability and intestinal digestibility of three different rumen protected choline products.

Mateiral and methods: The study was conducted at the Universitat Autonoma de Barcelona, following approved standard protocols. Rumen-protected choline products were CholiGEM (Kemin Animal Nutrition and Health), product R and product N containing 5.75, 3.23 and 2.71% N (% DM), respectively. Rumen microbial degradation was determined using the in situ nylon bag technique. Samples (2.020.018 g) of each rumen-protected choline product were weighed into nylon bags and incubated in the rumen of a Holstein dry-cow for 0, 2, 4, 8, 16, 24 and 48 h, in duplicate bags and in two consecutive periods. Ruminal degradability was calculated with the exponential function: Y=a+b*(1-exp^(-ct)); where a was the amount of N disappearing from the bag at 0 h; b was the potentially degradable fraction; and c was the degradation rate. The effective degradability of N (EDN) was calculated as: EDN,%= a+[(b*c)/(c+k)]; where k (estimated at 10%/h; Dufreneix et al., 2019) was the rate of outflow from the rumen, and a, b, and c were the same parameters described earlier. For the determination of the intestinal digestion, the in vitro three-step procedure (Gargallo et al., 2006) was used. The undegraded residue after 12 h incubation in the rumen was incubated in vitro in a HCl-pepsin buffer at pH 2.0, 38oC and 1h, followed by a phosphate-pancreatin buffer incubation at pH 7.0, 38oC and 24h.

Results: Fraction a was 27.6, 0.4 and 24.7%; fraction b was 57.1, 5.8 and 73.6%, and fraction c was 0.032, 0.002 and 0.081 /h for CholiGEM, product R and product N, respectively. Effective ruminal microbial N degradation was very low in product R (0.5%), moderate in CholiGEM (41.4%) and relatively high in product N (57.6%). However, intestinal digestion was low in product R (12.2%) compared with CholiGEM (98.4%) and product N (80.9%) resulting in the highest bioavailability for CholiGEM (57.6%), intermediate for product N (34.3%) and lowest for product R (12.1%).

Considering rumen degradation kinetics is not sufficient to define the bioavailability of protected choline chloride sources. Results indicate that intestinal digestibility is a critical step in the evaluation of bioavailability of rumen protected choline sources. There are considerable differences in rumen degradability and intestinal digestibility among rumen protected choline products in the market.

Conclusions: Evaluation of the quality of products requires determination of rumen degradability and intestinal digestibility. Results indicate that bioavailability was very different among commercial sources tested.

Keywords: Rumen protected choline, ruminal degradability, intestinal digestibility.

NU-04

Correlation between feed efficiency and mobilization of fat in dairy Holstein cows during the fresh cow period and early lactation

György Szura[1], Stephanie Schäfers[1], Dirk Von Soosten[2], Ulrich Meyer[2], Jeannette Klüß[2], Gerhard Breves[1], Lena Ruda[1], Sven Dänicke[2], Jürgen Rehage[1].

[1]University of Veterinary Medicine Hannover, Foundation, Hannover, Germany; [2]Friedrich-Loeffler-Institute, Braunschweig, Germany.

Objectives: Feed efficiency (FE), a parameter to estimate the effectiveness of dairy production, gained importance for economic and ecological reasons in recent years. However, after parturition lipomobilization, a consequence of negative energy balance, may compromise estimates of FE. Thus, we aimed to examine the connection between FE and fat mobilization during the fresh cow (FCP) and early lactation period (ELP) in Holstein dairy cows with initially lower and higher condition.

Materials and methods: The study was performed at the Friedrich-Loeffler-Institute (FLI), Braunschweig, Germany. Thirty-one pluriparous German Holstein cows were used to determine the estimated depot mass (eDM) of subcutaneous (SCAT) and total abdominal (AAT) adipose depots by ultrasonography on day (d)-42, 7, 28 and 70 relative to parturition. Cows were allocated into two experimental groups according to the eDM of SCAT on d-42 relative to parturition (low body condition (LBC) group: n=16, mean eDM 8.61 kg; high BC (HBC) group: n=15, mean eDM 15.6 kg). Average daily change (aDC) of adipose mass was calculated for the FCP (d7 to d28) and ELP (d28 to d70). Additionally, dry matter intake (DMI) and lactation performance were recorded, and FE was calculated for FCP and ELP by dividing the energy-corrected milk yield (ECM) with DMI.

Results: The AAT depot had about 2 to 3 times higher mass than SCAT. The HBC cows had greater fat depot masses and mobilized more from both depots during FCP and ELP. The two groups did not differ in DMI, but the HBC group had greater ECM than LBC cows. As consequence, the HBC cows showed better FE compared to LBC cows. Correlation analysis revealed that during FCP, the more SCAT was mobilized the better the feed efficiency was (r^2: 0.18). However, in case of AAT no correlation was found (r^2: 0.01). On the other hand, mobilization of fat from both depots correlated positively with FE (r^2: 0.35 and 0.33, resp.) during the ELP.

Conclusions: During ELP and partly FCP, excessive lipomobilization increased estimated feed efficiency. Results indicate that the parameter feed efficiency may not be suitable for evaluation of performance efficiency in dairy cows during early lactation because it may lead to selection of cows for lipomobilization and possibly subclinical ketosis.

Keywords: Dairy cattle, feed efficiency, fat mobilization, fresh cow period, early lactation.

NU-05

Rumen degradability and intestinal digestibility of different rumen protected lysine products determined in situ and in vitro

Fatima Francia[1], Maria Ercilda Rodriguez-Prado[1], Cristian Faturi[2], Diego Martínez[3], Javier Mateos[3], Sergio Calsamiglia[4].

[1]Animal Nutrition and Welfare Service, Universitat Autonoma de Barcelona, Bellaterra, Spain; [2]Universidad Federal Rural de la Amazonia, Brasil, Brazil; [3]Kemin Animal Nutrition and Health, Madrid, Spain; [4]Universidad Federal Rural de la Amazonia, Bellaterra, Spain.

Introduction: The supply of metabolizable protein to dairy cows is important for optimizing milk and milk protein yields. However, excess MP in dairy cow diet results in a reduced efficiency of N utilization and an increase in the emission of N to the environment (St-Pierre and Thraen, 1999). This problem may be minimized by supplying dairy cows with the correct amount and proportions of essential amino acids. There is consensus that most current diets are deficient in Lysine and Methionine (Schwab and Broderick, 2017). There are several rumen-protected Lysine and Methionine products in the market, but their rumen degradability and intestinal digestibility may vary considerably (Whitehouse et al., 2017). The objective of the current study was to determine the bioavailability of the rumen-protected Lys products in dairy cows using the *in situ* ruminal degradation and the *in vitro* intestinal digestion methods.

Material and methods: The thee rumen-protected Lys products were LysiGEM and LysiPEARL (Kemin Animal Nutrition and Health), and AjiPro L3G (Ajinomoto). Rumen microbial degradation was determined using the *in situ* nylon bag tech-

nique. Samples (0.38±0.01 g) of each rumen-protected Lysine products were weighed into nylon bags. Duplicated bags were incubated in the rumen of a Holstein dry-cow for 0, 2, 4, 8, 16, 24 and 48 h. The process was repeated in two consecutive periods. Degradability of Lysine in the rumen was calculated as N disappearance from the rumen using an exponential function: Y=a+b*(1-⟦exp⟧^(-ct); where a was the amount of N disappearing from the bag at 0 h; b was the potentially degradable fraction; and c was the degradation rate (Mathers and Miller, 1981). The effective degradability of N (EDN) was calculated as: EDN (%)= a+[(b*c)/(c+k)]; where k (estimated at 10%/h; Dufreneix et al., 2019) was the rumen passage rate, and a, b, and c were the same as described earlier. The undegradaded sample residue after 12 h ruminal incubation was used to determine the intestinal digestibility using the *in vitro* three-step procedure (Gargallo et al., 2006): samples were incubated in a pepsin-HCL solution (Sigma 77160) at 39ºC and pH 1.9 for 1 h, followed by a second incubation in a buffer-pancreatin (Sigma P1750) solution at pH 7.75 at 39ºC for 24 h. The in vitro intestinal digestion was calculated as the amount of the sample N (rumen-exposed residue) minus the N remaining after pepsin-pancreatin incubation divided by the amount of sample N.

Results: The soluble fraction (%) was lower in AjiPro L3G (2.6) compared with LysiGEM (20.4) and LysiPEARL (24.6). The potentially degradable fraction (%) was larger in LysiPEARL (70.7) compared with LysiGEM (48.6) and AjiPro L3G (49.1). Rate of ruminal degradation (/h) was highest in LysiPEARL (0.05), intermediate in AjiPro L3G (0.013) and lowest in LysiGEM (0.006). The resulting effective degradation in the rumen (%) was highest in LysiPEARL (48.2), intermediate in LysiGEM (23.2) and lowest in AjiPro L3G (8.2). In contrast, intestinal digestibility was lowest in AjiPro L3G (49.6) compared with LysiGEM (87.3) and LysiPEARL, suggesting that AjiPro L3G was overprotected. Overall bioavailability (%) was highest for LysiGEM (67.1), intermediate for LysiPEARL (50.3) and lowest for AjiPro L3G (45.5). There were differences in ruminal degradation and intestinal digestion among different commercial products. Bioavailability is the result of the combination of the degree of rumen resistance to degradation and the intestinal digestibility of the undegraded fraction. The quality of rumen protected lysine products requires the determination not only of the degree of protection from ruminal degradation, but also the intestinal digestibility of the undegradable proportion of Lysine.

Conclusion: Results indicate that differences in bioavailability from the different Lysine sources may be important and needs to be considered in diet formulation.

References:

Gargallo S., et al., 2006. J. Anim. Sci. 84:2163-2167.

Dufreneix F., et al., 2019. J. Dairy Sci. 102 :3010–3022.

Mathers J.C., Miller E.L. 1981. Br. J. Nutr. 45 ;587-604

Schwab C.G., Broderick G.A., 2017. J. Dairy Sci.100:10094-10112.

St-Pierre N.R., Thraen S.T. 1999. J Anim Sci. 77(suppl. 2):72-83.

Whitehouse et al., 2017. J. Dairy Sci. 100:9585-9601.

Keywords: Rumen protected lysine, rumen degradability, intestinal digestibility, bioavailability.

NU-06

Production performance of lactating dairy cows fed two rumen protected methionine supplements

Fatima Francia[1], Maria Ercilda Rodriguez-Prado[1], Sergio Calsamiglia[1], Marc Piera[2].

[1]Animal Nutrition and Welfare Service, Universitat Autonoma de Barcelona, Bellaterra, Spain; [2]Centre Veterinari Tona, Tona, Spain.

Intorduction: The new formulation systems have refined requirements and supplies of digestible protein and amino acids through the description of different feed protein fractions and digestive processes, and the incorporation of dynamic adaptation (NRC, 2001; INRA, 2018, CNCPS, Van Ambourgh et al., 2015). Despite all these improvements, the efficiency reported at 23.7% 50 years ago (Stone et al., 1960) remains virtually unchanged at 24.0% (Hristov and Huhtanen, 2008). This low efficiency represents a production cost and the emission of excessive N to the environment. Recent evidence indicates that at least part of this inefficiency is associated with the unbalanced supply of essential amino acids (Doepel and Lapierre, 2010). The supply of intestinally available essential amino acids is currently achieved through the use of rumen-protected forms of these amino acids. However, there are large differences in bioavailability of different protected methionine sources (Whitehouse et al., 2017) which may affect performance. The objective of this research is to determine the effect of feeding two different sources of Met on milk production and composition of lactating Holstein dairy cows.

Material and Methods: Ninety-four multiparous lactating Holstein dairy cows from 50 to 110 days in milk were divided in 3 groups to determine the effect of feeding different rumen protected Methionine sources on milk production and composition. Cows were fed a 46:54 forage to concentrate based on corn silage once daily formulated to meet current NRC (2001) recommendations (17.5% CP, 28.4% NDF, 33.2% starch and 4.6% fat and balanced for Lysine). Treatments were the control diet (CTR), and the same diet supplemented with 11.4 g of metabolizable methionine from either KES (KESSENT® M, Kemin Animal Nutrition and Health) or SMT (Smartamine® M, Adisseo Inc.). Experimental animals were blocked by previous milk production, assigned to three different lots and supplied with one of the three treatments. Cows were milked three times daily. After 30 days on treatment, milk production and composition were determined in weeks 6 and 10 postpartum (milk samples taken 3 consecutive days). Data were analyzed using the PROC GLM procedure of SAS as a completely randomized model.

Results: Milk yield (kg/d) was higher (P<0.002) in KES (46.7) than CTR or SMT (43.9 and 44.5, respectively). The 3.5% fat corrected milk (kg/d) was numerically higher in KES and SMT (51.3 and 50.6, respectively) compared with CTR (48.8), but did not reach significance (P<0.11). Milk fat content (%) tended to be higher (P<0.06) in SMT (4.38) than in CTR or KES (4.16 and 4.14, respectively). Milk protein content (%) was higher (P<0.04) in KES and SMT (3.09 and 3.11, respectively) compared with CTR (3.04). Similar effects (P<0.02) were observed for casein (%) (2.40, 2.43 and 2.45 for CTR, KES and SMT, respectively). Milk fat yield (kg/d) was similar among treatments (1.90), but protein yield (kg/d) was higher

(P<0.01) in KES (1.43) compared with CTR and SMT (1.33 and 1.38, respectively). Casein yield (kg/d) was also higher (P<0.01) in KES (1.13) compared with CTR and SMT (1.05 and 1.09, respectively).

Conclusions: Both rumen protected Methionine supplements improved dairy cow performance compared with control, but also significant differences between commercial supplements were observed.

Keywords: Rumen protected methionine, dairy cow performance.

NU-07

Ketosis monitoring in Spain using testday samples

Marcello Guadagnini[1], Gregorio Alcantara[2], Rita Casals[3], Fernando Ruiz[4], Ramiro Fouz[5], Juan Cainzos[6].

[1]Elanco Animal Health, Sesto Fiorentino (FI), Italy; [2]Federación Frisona Castilla y León (FEFRICALE), Leon, Spain; [3]Federación Frisona de Cataluña (FEFRIC), Vic, Spain; [4]Asociación Frisona Cantabria (AFCA), Torrelavega, Spain; [5]Asociación de Control lechero de Lugo, Lugo, Spain; [6]Elanco Animal Health, Alcobendas, Spain.

Objectives: Ketosis (KET) is one of the most impactful diseases in dairy farming and it represents a good indicator of a poor adaptation to high energy demand during the transition period (Duffield T., 2009). β-hydroxybutyrate (BHB) and KET can be monitored in milk (Carrier J., et al., 2004) using testday milk samples (de Roos A. et al., 2007). The main goal of this project was to map KET prevalence across Spain and identify risk factors and consequences.

Materials and Methods: Data from 4 DHI associations across Spain have been joined into one dataset: Cantabria (CANT), Castilla y Leon (CYL), Cataluña (CAT) and Galicia (GAL). The dataset includes 337,759 first tests occurring during 2018 and 2019. Since BHB monitoring best performs in cows ≤25 days in milk (DIM) (Renaud D. L. et al., 2019), samples outside the interval of 5-25 DIM were excluded, so that the final dataset contains 200,593 first tests belonging to 2242 farms. A BHB 0.1 mmol/liter cut-point has been choosen for KET (Viña C. et al., 2017). Variables used to investigate KET risk factors were: fresh season, parity, milk production and somatic cell count (SCC) at first test. First calving age in categories (<24mo; 24-27mo;>27mo), calf outcome (Male, Female, Twins) were also available except for GAL. Mean number of cows controlled per farm and test was divided into quantiles as a proxy of farm size. Calving season effects were evaluated via 2 classes: Atlantic climate regions (GAL and CANT) and the rest of Spain (CYL and CAT). Descriptive statistics were used to describe current KET prevalence across Spain. Multivariate models were built to evaluate specific risk factors for KET and to assess its relationships with milk production and udder health. Statistically significant results were identified using a p<0.05.

Results: 32% of cows were lact=1, 27% lact=2 and 40% lact≥3. 31% of lact=1 calved with <24mo, 41% between 24-27mo and 27% >27mo. Twinning rate was 3%. Overall KET prevalence was 21%: 15% for lact=1, 19% for lact=2 and 27% for lact≥3. Possibly, due to on demand KET monitoring, prevalence for CANT was significantly lower than other regions with 15.25% vs. 21.23% for GAL, 21.58% for CAT and 22.09% in CYL. Running the analysis by farm, 37.1% (n=763) of all farms with at least 20 tests each (n=2055) had a Ketosis prevalence >25%; this percentage varied by region with CANT 17.1%, CYL 40.3%, CAT 38.0% and GAL 39.7%. Mean number of cows controlled per farm and test had a significant impact on KET prevalence: in the Atlantic area the 1st quartile (<5 cows) had a KET prevalence of 27.09%, 2nd quartile (5-7 cows) 20.47%, 3rd quartile (7-11 cows) 17.88% and 4th quartile (>11 cows) 16.62%. In the rest of Spain KET prevalence was: for 1st quartile (<12 cows) 25.7%, 2nd quartile (12-24 cows) 22.13%, 3rd quartile (24-51 cows) 19.66% and 4th quartile (>51 cows) 19.96%. In the Atlantic area, Winter and Spring had the highest KET prevalence, conversely in the rest of Spain Summer and Fall had the highest KET prevalence. Statistically significant differences for KET risk were found across parities (3+ vs. 2, Relative Risk (RR)=1.54 95%CI=1.50-1.58; 3+ vs. 1, RR=2.03 95%CI=1.97-2.08; 2 vs 1, RR=1.31 95%CI=1.27-1.35) and across age at first calving for lact=1 (>27mo vs. <24mo, RR=2.15 95%CI=1.98-2.33; >27mo vs. 24-27mo, RR=1.48 95%CI=1.28-1.59; 24-27mo vs. <24mo, RR=1.44 95%CI=1.34-1.56). Cows with a singleton male had slightly more risk of having KET than cows calving singleton female (M vs F RR=1.05 95%CI=1.01-1.10). Ketotic cows were more prone to have, at the same time, subclinical mastitis (>200 000 cell/ml, Ruegg P. 2017) (KET Yes vs No RR=1.69 95%CI=1.63-1.75). Finally, accounting for parity, farmcode, DIM 1st test, month and year fresh, Ketotic cows yielded 1.29 kg less milk at first test compared to cows without KET (p<0.0001). As it was not possible to account for previous lactation milk production, this milk loss might represents an underestimate of the true milk loss attributable to KET.

Conclusions: KET prevalence and proportion of high risk herds is higher than perceived by much of the dairy industry. Elevated BHB was associated with lower milk production at first test and higher prevalence of subclinical mastitis. The idenfied risk factors can help design better prevention strategies.

Keywords: Ketosis, testday, Spain, dairy.

NU-08

Associations of serum calcium and subclinical hypocalcemia at calving with productive, reproductive and health outcomes in multiparous Jersey cows

Ainhoa Valldecabres Inchaustegui, Noelia Silva-Del-Río.

University of California, Davis, Davis, United States.

The most appropriate blood calcium (Ca) threshold to define subclinical hypocalcemia is still under study. Currently, there is a wide range of suggested definitions, and variability on its reported associations with productive, reproductive and health outcomes, which have not been described in Jersey

cows. Our aim was to evaluate the associations of serum Ca concentration and subclinical hypocalcemia at calving with: subsequent lactation milk and energy-corrected milk yield, fat%, protein%, somatic cells count linear score, mastitis, herd removal, and pregnancy at 1st service and by 150 days in milk (DIM), in 609 multiparous Jersey cows from 2 commercial herds fed acidifying prepartum diet.

Blood samples for total serum Ca concentration determination were collected from the coccygeal vessels at 3 h 10 min (±2 h 17 min) after calving. Monthly test milk yield, fat%, protein% and somatic cells count information up to the 10th test was obtained from the Dairy Herd Improvement Association. Additional information was obtained from herd records. Statistical analyses were conducted with multiple linear, Poisson, log-binomial, and Cox's proportional hazards regression using SAS (version 9.4). Considered explanatory variables for all outcomes were: parity, herd, previous lactation length and 305-days mature equivalent milk yield, dry period length, calving body condition and locomotion scores, calving easiness, and oral Ca supplementation. Additional variables considered were: somatic cells count linear score at test for milk yield, fat%, and protein%; milk yield at test for fat%, protein% and somatic cells count linear score; and DIM at 1st service and breeding code (timed artificial insemination and heat breeding) for pregnancy at 1st service. Serum Ca thresholds among 1.80 and 2.20 mmol/L (7.2 and 8.8 mg/dL) at 0.02 mmol/L (0.08 mg/dL) intervals were used to define subclinical hypocalcemia when serum Ca concentration (in a continuous scale) was associated with the outcome. Serum Ca thresholds that better predicted the outcome (continuous outcomes: smallest *P*-value and most extreme estimate; categorical outcomes: maximized sensitivity and specificity on receiver operating characteristic curve analyses) were chosen to define subclinical hypocalcemia.

Subclinical hypocalcemia (Ca ≤2.18 mmol/L; 8.7 mg/dL) was associated with 1.52 and 1.88 kg/d more of milk and energy-corrected milk yield, respectively ($P < 0.001$). Milk fat% was 0.12 units of percentage higher and milk protein% was 0.06 units of percentage lower per day for cows with subclinical hypocalcemia (Ca ≤1.96 and ≤1.80 mmol/L, respectively; 7.9 and 7.2 mg/dL, respectively), compared to normocalcemic cows during the subsequent lactation ($P = 0.01$ and $P = 0.03$, respectively). Subclinical hypocalcemia was associated with lower 1st service pregnancy risk [Ca ≤2.08 mmol/L (8.3 mg/dL); risk ratio = 0.70; $P = 0.03$] and hazard of pregnancy by 150 DIM [Ca ≤1.90 mmol/L (7.6 mg/dL); hazard ratio = 0.50; $P < 0.001$]. No association was observed among serum Ca concentration, mastitis and herd removal. Similar effects were observed for additional thresholds evaluated.

Establishing a single serum Ca threshold for subclinical hypocalcemia definition based on productive, reproductive and health outcomes doesn't seem feasible. Further studies are needed to elucidate the applicability of a subclinical hypocalcemia definition.

Keywords: Dairy cow, hypocalcemia, transition cow.

NU-09

A prospective cohort study on periparturient muscle tissue mobilisation in high producing dairy cows

Cara Hatfield[1], Robert Smith[1], Jean Routley[1], Rachel Hall[1], William Tulley[2], Bethany Griffiths[1], Andreas Foskolos[3], Georgios Oikonomou[1].

[1]Livestock Health and Welfare, Institute of Veterinary Science, UoL, Leahurst Campus, Neston, Wirral, CH64 7TE, United Kingdom; [2]Epidemiology and Population Health, Institute of Infection and Global Health, UoL, University of Liverpool, United Kingdom; [3]Deparment of Animal Science, University of Thessaly, Karditsa, Thessaly, Greece.

Objectives: Dairy cattle at the onset of lactation experience a period of Negative Energy Balance (NEB). Adipose tissue is mobilised to meet energy demands for milk production. There is evidence that some cows will also begin to mobilise muscle during the dry period prior to fat mobilisation[1]. 3-methylhistidine (3-MH) is a very specific indicator of muscle metabolism in cattle which is not further metabolised nor produced through any other metabolic pathways[2]. The main objective of this study was to investigate the changes in the thickness of the longissimus dorsi muscle in high producing dairy cows in the periparturient period and the possibility to use a commercial ELISA kit for 3-MH measurements as a correlate of changes in muscle thickness.

Materials and Methods: We enrolled 455 cows from three farms with data collected for 500 lactations (312 from Farm 1, 75 from Farm 2, and 113 from Farm 3). Data were collected from each animal on three occasions per lactation: 3-4 weeks before the expected date of parturition (Pre-calving (PC)), 0-10 day's post-partum (Fresh (FR)) and approximately 60-80 days post-partum (Early Lactation (EL)). At each time point blood samples were collected from the coccygeal vein, Body Condition Score (BCS), Muscle Thickness (MT), and Back Fat Thickness (BFT) were also recorded. Muscle and fat thickness were measured using an Easi-Scan ultrasound machine. Longissimus dorsi depth was measured perpendicular to the skin at the fourth lumbar process. Genomically estimated breeding values (GEBVs) were also available for these animals. A commercially available ELISA kit (Abbexa Ltd, 96 test kit) was also evaluated for measurement of 3-MH concentration in bovine serum. Descriptive and univariable analyses were undertaken between variables, before multivariable regression models were constructed. Cox Proportional Hazards Analysis was used for analysis of time to first service and time to conception.

Results: Explanatory variables using MT as an outcome found assessor had a significant effect on MT ($P < 0.0001$). Muscle thickness decreased in the period between the PC and FR measurement. Cows in the lowest Milk PTA tercile had higher MT measurements comparing to the other two terciles but there was no significant Milk PTA tercile by time interaction. Interesting Farm*Time-point interactions were also observed. Higher MT PC was associated with increased MT loss (Estimate -0.39 ± 0.04, $P < 0.0001$). Higher BFT PC was associated with decreased MT loss (Estimate 0.23 ± 0.06, $P < 0.0001$). A longer period between the PC and FR measurement was also associated with increased MT loss (Estimate -0.1 ± 0.03, $P = 0.0006$). Cows in the MT Pre-EL 3rd tercile (cows that had minimal loss of MT or gained MT during the studied period) were served earlier than cows in the 1st tercile

(cows with the greater loss of MT) (Hazard ratio: 1.39, CI = 1.004-1.94, P = 0.046). Additionally, cows in the top fertility index tercile (better genetics for fertility) were served earlier than cows in the lowest fertility index tercile (Hazard ratio: 1.58, CI = 1.17-2.13, P = 0.003).

The 3-MH ELISA produced a good standard curve using assay buffer but the co-efficient of variation was large in duplicate serum samples potentially suggesting an interfering substance within the serum. The polyclonal antibody in the kit was found to be raised against 3-MH conjugated to bovine-serum-albumin (BSA) and the BSA in the serum may have been competing with the 3-MH. Despite trying two different methods of extraction (acetone and 5% sulfosalicyclic acid) previously published for separation of 3-MH from plasma or serum proteins, none gave reliable results, therefore the kits were deemed unsuitable for use.

Conclusions: Although US measurements are an accessible way to measure MT, a more specific method which would avoid assessor effect, such a looking at 3-MH, would have provided further insight into the amount of MT loss occurring. Until a more cost-effective way can be found to monitor muscle catabolism it will be hard to reliably use the current methods of monitoring it on a commercial setting. This is an area of study that requires further investigation.

References:

1. Van der Drift, S. et al. (2012). *Journal of Dairy Science*, 95(9), pp.4911-4920.
2. Houweling, M. et al. (2012). *Journal of Dairy Science*, 95(6), pp.3125-3130.

Keywords: Dairy Cow, Muscle Mobilsation, Periparturien.

NU-10

Magnesium butyrate is a readily available magnesium source in dairy cow nutrition

Bastiaan De Groot[1], Joan Edwards[2], Thomas Schonewille[1].

[1]Utrecht University, Utrecht, Netherlands; [2]Palital Feed Additives, Velddriel, Netherlands.

Objectives: Magnesium (Mg) is an essential nutrient for cows. This means that dairy rations need to supply a sufficient amount of absorbable Mg in order to safeguard the cow's health, for example decreasing the risk of milk fever and grass tetany. Currently, Mg-oxide is widely used to supplement dairy cows with Mg. In dry cow nutrition, however, Mg-butyrate can be considered of interest as an alternative source of supplemental Mg. This is because the use of Mg-butyrate, instead of Mg-oxide, is potentially advantageous. Butyrate, but not oxide, may stimulate the growth of rumen papillae which is instrumental to prevent rumen acidosis. However, the bioavailability of Mg from Mg-butyrate is not known. Therefore, an experiment was conducted with dairy cows to measure the apparent absorption of Mg from Mg-butyrate.

Materials & Methods: Six mid-lactation Holstein Friesian dairy cows were used in an experiment which had two dietary treatments arranged in a cross-over design. The experiment consisted of 2 experimental periods of 14 days each, preceded by a 14-day pre-experimental period to allow the cows to become adapted to the experimental rations. Two different diets were fed during the experiment, with the basal rations being identical and consisting of a low Mg diet without Mg-butyrate (L-Mg, 3.1g Mg/kg dry matter) and a high Mg diet with Mg-butyrate (H-Mg, 3.9 g Mg/kg dry matter). The magnesium content of the diets was modified via a pelleted experimental beet pulp. For the H-Mg group, the experimental beet pulp contained Mg-butyrate (Rumen-Ready®, Palital Feed Additives, Velddriel, The Netherlands) whereas the experimental beet pulp for the L-Mg diet did not. The Mg-butyrate from Rumen-Ready® is completely released and solubilized within the rumen, i.e., it is 100% available for absorption. Both types of experimental beet pulp contained TiO_2, which was used as an inert marker for determination of fecal output. Feed refusals and milk yield were recorded daily during each experimental period, and any feed refusals stored at -18 °C. During the last 4 days of each experimental period, all spontaneously voided feces and urine were collected between 9:00 and 17:00. At the end of each collection day, the individual feces collections were stored at -18 °C whereas the individual urine collections were stored at 5 °C. All individual feces, urine and feed refusal collections were pooled per cow and mixed thoroughly prior to chemical analysis. The Mg content of pooled samples of the feces, urine, feed and feed refusals was measured by means of inductively coupled plasma mass spectrometry, and the titanium concentration of the experimental beet pulp and feces was determined using a spectrophotometer.

Results: Cows offered the L-Mg diet ingested 54.7 g Mg/day while the cows fed the H-Mg diets ingested 66.3 g Mg/day ($P < 0.001$). The fecal excretion of Mg, however, was similar between the two experimental diets ($P = 0.174$). Consequently, apparent Mg absorption was found to be 7.9 percentage units greater ($P = 0.038$) when the cows were fed the diet supplemented with Mg-butyrate. The greater Mg absorption after feeding the H-Mg diet was, however, not reflected by a greater urinary Mg concentration ($P = 0.228$). These results indicate that the availability of Mg from the Mg-butyrate supplemented diet was high (34.1% of intake). The absolute fractional Mg absorption from Mg butyrate (i.e. 8.3 g/d) was at least 1.5 times greater than that of similarly derived values for the fractional Mg absorption from a highly soluble MgO (using previously published data). Thus, it appears that Mg butyrate, relative to MgO, is superior in supplying Mg available for absorption. The fractional Mg absorption from Mg-butyrate was calculated to be 71.6%.

Conclusion: Mg-butyrate is an attractive alternative to supplement dairy rations with Mg.

Keywords: Rumen, ruminant, apparent absorption.

NU-11

Utilising infra-red thermography to evaluate rumen development in dairy youngstock

Nick Britten, Rebecca Hopper, Gareth Pearce.

University of Cambridge, Cambridge, United Kingdom.

Objectives: Timing of weaning is a critical issue for the efficient management of dairy youngstock. Successful transition to a solid diet as soon as possible provides economic advantages due to the high cost of milk or milk replacer products when compared with solid foods. However, successful transition to a solid diet requires sufficient ruminal function to be present before weaning off milk. Accurate determination of ruminal development in calves is therefore an important measure in calf management. Calves with increased solid feed consumption, and therefore more rumen development before weaning show greater weight gain postweaning (1). Currently available rumen function evaluation methods involve direct sampling of rumen fluid or tissue which is both costly and invasive. Infra-red thermography of the left flank has been used to quantify rumen activity in adult cattle with flank temperatures rising 21% after feeding due to increased heat production through fermentation (2). This study evaluated the use of flank temperature to measure rumen function over time in calves between 6 and 105 days of age.

Materials and Methods: The study was conducted in 30 Holstein heifer calves born over a 3-month period at Cambridge University Farm, UK. Calves were housed in individual pens immediately after birth and bottle fed 3l of milk replacer twice daily for one week. Calves were then moved to group housing at an average age of 10±4 days and fed 8l milk replacer per day by free access to a machine feeder (Vario Smart Feeder, Volac) for 40 days before beginning a reduction of 0.5l/day until complete weaning at 56 days of age. Hay and concentrate pellets (18% crude protein) were provided *ad libitum*. After weaning calves were housed in groups of 8 and fed 2.5 kg concentrate pellets per head per day and *ad libitum* hay.

Calves were weighed every 7 days from entry to group housing (T0) for 42 days until weaning (T6) and 42 days post weaning until average age 101±4 days (T12). During weighing the mean maximum surface temperature of the left flank was measured in each calf using an infra-red camera (T335, FLIR Systems, UK).

All data were analysed using RStudio 2022.02.0. Univariate regression was used to quantify the relationship between flank temperature and bodyweight at each time point pre-weaning (T0-T6) and growth rate post-weaning (T6-T12). Variables with a p-value ≤0.1 in the univariate analysis were included in a multivariate model of post-weaning growth rate simplified by stepwise selection based on Akaike Information Criterion (AIC). Recorded cases of pneumonia and diarrhoea were included in the multivariate model to examine the influence of these diseases on growth rate.

Results: Univariate regression demonstrated significant positive correlations between growth rate post-weaning and flank temperature at T0 (r^2=0.146, p<0.05), T1 (r^2=0.336, p<0.01) T2 (r^2=0.208, p<0.05) and a tendency for a positive correlation at T3 (r^2=0.08, p<0.1) and these variables were therefore included in the multivariate model. Stepwise selection based on AIC retained flank temperature at T0, T2 and T3 and occurrence of pneumonia in the final model which accounted for 47.6% of the variance in post-weaning growth rate (adjusted r^2= 0.476, p<0.01).

Conclusions: Measurement of flank surface temperature in calves shortly after introduction of solid feed was a significant predictor of growth rate after weaning - with measurement at one week after the introduction of solid feed (T1) being the largest contributor variable and therefore the best single time for this prediction.

It is suggested therefore that infra-red thermography of the left flank in dairy calves represents a viable method of identifying differences in rumen function between calves of similar age. As the differences in rumen function soon after introduction of solid food identified using infra-red thermography had significant impacts on growth rate post-weaning, this method offers considerable promise as a non-invasive method to identify individual differences in young calves that will have significant commercial implications.

References:

1. Sweeney et al. Journal of Dairy Science. 2010 Jan 1;93(1):148–52.
2. Salles et al. Animal Production Science. 2017 Jun 15;58(11):2032–41.

Keywords: Dairy, Calves, Weaning, Rumen, Thermography.

NU-13

Iron deficiency anemia in whole milk fed calves

Jennifer Allan, Peter Plate, Steven Van Winden.

Royal Veterinary College, London, United Kingdom.

Objectives: This is one of the first studies to assess the prevalence of iron deficiency anemia in whole milk fed dairy calves on farms in the United Kingdom and the effect of a single parenteral iron supplementation on haemoglobin (Hb) levels and daily liveweight gain (DLWG).

Materials and methods: 268 whole milk fed dairy calves from seven farms in the south of England were recruited in the study. Six of the farms were organic, one was non-organic. All calves were blood sampled for total protein and Hb levels and weighed from one to ten days after birth. They were then randomly assigned to either receive or not receive 1 gram of iron as iron dextrane (Uniferon™, Virbac Animal Health) by intramuscular injection immediately after thist first blood sampling and weighing. Calves were again weighed and blood sampled for Hb at around six weeks of age and weighed only at around 12 weeks of age. Hb levels and DLWG were compared between treated and untreated animals.

Results: There were no reported adverse effects to the iron injection in any of the treated calves.

Parenteral iron dextran had a significant effect on DLWG from one to six weeks with an average 52g per day increase

in the treated calves compared to the control group. Iron had a significant effect on Hb concentrations at six weeks and the difference between Hb at week one and week six, with calves in the treated group having a higher average Hb concentration than calves in the control group (112.2 vs 97.0g/l). Over that period Hb levels in the treated group increased by 4.5 g/l while levels in the control group decreased by 10.1 g/l. Calves with higher DLWG and calves in the control group were significantly more likely to have Hb levels below 90 g/l at six weeks: 35 % of calves in the control group (42 out of 120) and 3.4 % of the treated group (4 out of 118) showed Hb values below this level.

There was farm variation in both Hb levels and DLWG differences between groups. The size of the effect on DLWG could not be predicted for any particular farm from either Hb measurement at week one or six.

However, there was a consistent effect of iron across all farms, suggesting a generalized finding.

Conclusion: The current industry recommendations are to feed higher volumes of milk for longer to achieve higher growth rates. On a significant proportion of organic and non-organic farms in the United Kingdom whole milk is fed to to dairy replacement calves, and iron supplementation should be considered in these calves.

Keywords: Calves, anemia, iron, whole milk.

NU-14

Epidemiology of hyperketonemia in first parity Holstein cows. Is subclinical ketosis a problem of fresh cows, only?

Georgios E. Valergakis[1], Alexandros Kougioumtzis[1], Aikaterini Soufleri[1], Nektarios Siachos[1], Georgios Banos[2], Georgios Arsenos[1], Georgios Oikonomou[3].

[1]Laboratory of Animal Husbandry, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece, Thessaloniki, Greece; [2]Laboratory of Animal Husbandry, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece/ Scotland's Rural College/Roslin Institute, Edinburgh, Scotland, UK, Thessaloniki, Greece; [3]Department of Livestock Health and Welfare, Institute of Veterinary Science, University of Liverpool, UK, Liverpool, United Kingdom.

Objectives: Subclinical ketosis (SCK) is a common disorder of dairy cows, associated with several clinical diseases during the post-partum period. Although negative energy balance lasts on average up to 8-10 weeks, commonly recommended herd monitoring programs are mostly limited to the first 3 weeks of lactation. The objective of this study was to explore stage-of-lactation related epidemiologic parameters of SCK.

Materials and Methods: This retrospective observational study was based on two data sets established for two published genetic studies. Data were collected on a large commercial free-stall dairy farm located in northern Greece. The first data-set (A) included β-hydroxybutyrate (BHBA) measurements from 362 primiparous Holstein cows that calved between January 2005 and July 2006 and the second (B) BHBA measurements from 287 primiparous Holstein cows that calved between January 2008 and April 2010. Management conditions were greatly improved during the second period (mainly regarding feeding and housing conditions during the transition period). On both time periods, all cows were blood-sampled weekly, during the first 13 weeks of their lactation. Blood was drawn from the coccygeal vein and samples were left to clot at room temperature for approximately 30 min and then centrifuged at 2,000 ×g. The serum concentration of BHBA was assayed with the use of an enzymatic kinetic method based on the oxidation of BHBA to acetoacetate by β-hydroxybutyrate dehydrogenase; a total of 8,437 samples were analyzed. Subclinical ketosis was defined as a BHBA concentration ≥ 1.2 mmol/L. Prevalence of SCK was calculated for each of the 13 weeks by dividing the weekly number of cows with SCK by the total number of cows tested. Incidence of SCK was calculated as the number of cows with at least one positive BHBA test result, either for the first 3 weeks of lactation (3W) or over the entire 13-week sampling period (OV). Median first positive BHBA test and number of repeated positive test results per cow were also calculated for 3W and OV.

Results: Prevalence of SCK in data-set (A) was 12.2%, 9.7%, 8.0%, 8.6%, 6.9%, 4.8%, 5.7%, 3.3%, 3.0%, 5.2%, 5.0%, 2.5% and 3.0% for the 13 weeks, respectively. Prevalence of SCK in data-set (B) was lower, at 2.1%, 3.5%, 2.8%, 4.9%, 3.5%, 2.1%, 1.4%, 3.1%, 1.7%, 0.3%, 0.3%, 0.3% and 0.0% for the 13 weeks, respectively. Incidence of SCK in data-set (A) was 23.2% (84/362 cows) for 3W, while it was 46.7% (170/362 cows) for OV. Incidence of SCK in data-set (B) was 9.1% (26/287 cows) for 3W and 19.2% (55/287 cows) for OV. Median first positive BHBA test was at week1 and week2 for 3W and data-sets A and B, respectively; it was at week4 for OV for both data-sets. When overall incidence of SCK was high (data-base A) % of cows with ≥2 positive tests were also high, both in 3W and OV (42.9% and 39.4%, respectively). When overall incidence of SCK was lower (data-base B), cows with ≥2 positive tests was only 3.8% in 3W but a considerable 27.3% in OV.

Conclusion: Subclinical ketosis is present well beyond the first 21 days of lactation; actually, overall incidence doubles when the monitoring period extents to 13 weeks post-partum. Therefore, more than half of positive cows would be misclassified as non-ketotic if monitoring was limited to the first 3 weeks of lactation. Testing cows for SCK during the post-fresh transition period may be useful in finding associations with clinical diseases prominent during the first month of lactation, like metritis and displaced abomasum. However, the effects of SCK on reproduction and milk yield would be better evaluated if monitoring continues beyond the post-partum transition period, especially when considering the remarkable presence of repeated positive tests.

Keywords: Dairy cow, subclinical ketosis.

NU-15

Risk factors associated with excessive negative energy balance in commercial United Kingdom dairy herds

Alastair Macrae, Elizabeth Burrough, Julie Forrest, Alexander Corbishley, Geraldine Russell, Darren Shaw.

University of Edinburgh, Roslin, Midlothian, United Kingdom.

Objectives: Numerous studies have shown that excessive negative energy balance (eNEB) in dairy cows, characterized by elevations in blood biochemical parameters such as β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA) and reductions in glucose concentrations in either late pregnancy and/or early lactation, is associated with an increased risk of transition cow diseases (such as ketosis, metritis and displaced abomasum), an increased risk of culling in early lactation, decreased milk production and poorer herd fertility. The objectives of this study were to use the results from a commercial laboratory service specializing in dairy cow biochemical analyses to 1) assess the individual cow risk factors for eNEB in UK dairy herds 2) assess the dietary risk factors for eNEB and 3) assess the relative importance of these different risk factors.

Materials and methods: Between April 2006 and March 2015, blood samples were analysed for BHB, NEFA and glucose from a commercial nutritional monitoring service provided by the Dairy Herd Health and Productivity Service (DHHPS) at the Royal (Dick) School of Veterinary Studies at The University of Edinburgh, UK. For each cow that was blood sampled, additional details were collected of the calving date (milking cows), predicted calving date (dry cows), lactation number, body condition score (BCS) and daily milk yield. Information was standardized for each herd using identical data collection forms. In addition, data was collected of the ration being fed to the cows (in kg fresh weight fed each day).

Results: Following removal of all potential duplicate cows, a final dataset of 69,161 unique individual cows was obtained including biochemical results, individual cow information and feed data. Use of generalized linear mixed-effect models and multivariable classification tree-based models showed that individual cow risk factors for eNEB included: days relative to predicted calving date (dry cows); Days In Milk (lactating cows); Body Condition Score (BCS: lactating cows ≥ BCS 4; OR 2.1); milk yield (around 40 litres per day); parity (first lactation heifers OR 0.46 compared to older cows during lactation); chronic inflammatory conditions as assessed by globulin levels ≥ 50 g/l (OR 0.79 for elevated NEFA values). There was a higher prevalence of eNEB during April to October (OR 1.19), with the lowest prevalence in November. Feeding grass silage and wholecrop (silage made from cereal crops) to dry cows was associated with a reduced prevalence of eNEB, whereas access to grazed grass was associated with a higher eNEB prevalence in both the dry period (OR 1.32) and lactation (OR 1.33).

Conclusions: This study has shown that prevalence of eNEB (as indicated by three separate biochemical measures of energy balance: elevated BHB and/or NEFA values as well as low plasma glucose) in dairy cows is associated with cow level risk factors such as BCS, parity and milk yield. This study has also highlighted a number of dietary risk factors associated with the occurrence of eNEB in dairy cows including feeding of conserved forages such as maize silage and wholecrop to dry cows, and access to grazed grass for both milking and dry cows. Understanding the risk factors associated with eNEB in commercial dairy herds assists in both the implementation of herd monitoring programs and reduction of eNEB in dairy herds, with consequential benefits that could potentially include reductions in transition cow diseases, increased performance and improvements in herd fertility.

Keywords: Dairy cow, BHB, NEFA, glucose, ketosis.

NU-16

Effect of postpartum milking strategy on plasma calcium concentration and risk of subclinical hypocalcemia in multiparous dairy cows

Ainhoa Valldecabres[1], Rubia Lopes[1], Alfonso Lago[2], Cedric Blanc[3], Noelia Silva-Del-Río[4].

[1]University of California - Davis, Davis, United States; [2]DairyExperts Inc., Tulare, United States; [3]Lely International, Nevada, United States; [4]University of California - Davis, Tulare, United States.

The aim of the present study was to evaluate the effect of different postpartum milking strategies on plasma calcium (Ca) concentration and risk of subclinical hypocalcemia in multiparous dairy cows. Additionally, effects of the evaluated milking strategies on colostrum quality and yield, and milk yield and somatic cell count (SCC) at 1st monthly test were assessed. A total of 83 Jersey and Jersey × Holstein crossbreed cows of 2nd to 8th parity, were enrolled in the study before 1st postpartum milking. The study was conducted in a commercial dairy where cows were fed a negative dietary cation-anion difference ration prepartum. Milking strategies implemented during the first 2 days postpartum were: once-a-day milking (M1; cows were milked every 24 h; n = 24), twice-a-day milking (M2; cows were milked every 12 h; n = 21), delayed milking (MD; cows were not milked for the first 24 h, and were milked every 12 h afterwards; n = 19), and restricted milking (MR; cows were milked 3 L every 12 h; n = 19). Blood samples for total plasma Ca analysis were collected from the coccygeal vessels into heparinized vacuum tubes starting before 1st postpartum milking, every 4 h up to 48 h and at 72 h postpartum. Colostrum and transition milk yield was recorded using clear buckets prior to manual homogenization and sample collection for IgG determination at each study milking. Plasma Ca concentration was determined by Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES). Colostrum and transition milk IgG concentration was determined by radio immunodiffusion. First monthly test milk yield and SCC information was obtained from the Dairy Herd Improvement Association. Multiple linear regression with Dunnett adjustment was used to evaluate plasma Ca concentration changes, 1st monthly test milk yield and SCC. Poisson regression was used to evaluate the risk of subclinical hypocalcemia (Ca ≤2.12 mmol/L; Ca ≤8.50 mg/dL).

Prevalence of subclinical hypocalcemia at enrollment was

48%. Overall, plasma Ca concentration was lower and tended to be lower for M2 cows (2.04 mmol/L; 8.18 mg/dL) compared to MD (2.16 mmol/L; 8.66 mg/dL) and MR cows (2.13 mmol/L; 8.54 mg/dL), respectively. Plasma Ca concentration was not statistically lower for M1 cows (2.11 mmol/L; 8.46 mg/dL). Risk of subclinical hypocalcemia during the study period was lower for MD compared to M1 [Risk ratio (95% confidence interval) = 0.63 (0.46 to 0.86)] and M2 cows [Risk ratio (95% confidence interval) = 0.54 (0.39 to 0.76)]. Good quality colostrum (>50 g of IgG/L) was harvested at 1st postpartum milking from ≥80% of the M1, M2 and MR cows, and from 17% of the MD cows. At 2nd postpartum milking, good quality colostrum was harvested form 68% of MR cows. Milk yield and SCC at 1st monthly test were not affected by the milking strategy.

Our results suggest that postpartum plasma Ca concentration and risk of subclinical hypocalcemia may be influenced by the postpartum milking strategy, without negatively affecting subsequent milk yield and SCC. Additionally, the implementation of once-a-day (M1) or restricted (MR) postpartum milking strategies allows to harvest enough good quality colostrum to feed the calves. Postpartum milking warrants further study as a prophylactic strategy for hypocalcemia. Project funded by USDA-NIFA (1013457 CFAH).

Keywords: Dairy cow, hypocalcemia, transition cow.

NU-17

The effect of monensin controlled release boluses on subclinical ketosis, consequent health and production in dairy cows

Rok Marzel[1], Rok Klančnik[2], Klemen Jug[3], Tadej Virk[4], Primož Klinc[5], Jože Starič[5].

[1]VOA Ihan, Kočevje, Slovenia; [2]Veterinary faculty student, Ljubjana, Slovenia; [3]Veterinary faculty student, Ljubljana, Slovenia; [4]Agriculture and Forestry Institut, Celje, Slovenia; [5]Veterinary faculty, Ljubljana, Slovenia.

Objectives: Subclinical ketosis is one of the most important hidden conditions in high yielding dairy cows. It is directly corelated with different management approaches for cows in dry period, transition period and also in later lactation. Subclinical ketosis is usually associated with negative energy balance that occurs in transition period and in first trimester of the lactation. It affects the outcome of transition together with occurrence of diseases typical for this period and is known to have an impact on the most productive stage of lactation. The aim of the study was to determine prevalence of subclinical ketosis (SCK) on a dairy farm and success of preventive intervention using ruminal monensin controlled release boluses. We monitored health status, production, reproduction and culling rate of 110 dairy cows included in this study, to determine the effect of monensin controlled release boluses.

Materials and methods: The study was conducted between years 2017 and 2019 on a dairy farm with about 300 Holstein cows. Total mix ration (TMR) was fed to all the cows according to NRC 2001 recommendations for dry and lactating cows. Average milk yield on the farm was 9926 ± 227 kg/standard lactation with 3,75 ± 0,03 % fat and 3,27 ± 0,03 % protein according to national milk recording control. Only healthy multiparous cows, with the prediction of at least one more lactation, were included in this study. Eighty-five cows were randomly assigned in the control group and 25 cows in test group. Cows in the test group received monensin controlled release boluses (*Kexxtone*®) three weeks prior to calving, because prevalence of SCK in the heard at the time of the study was unknown. Non-esterified fatty acids (NEFA) were measured in all cows three weeks prior to calving. First postpartum check-up was carried out 7-14 days and the second one 28-35 days after calving. Blood samples were taken from all the cows to measure β-hydroxybutyrate (BHB). Reproduction parameters, milk yield and number of culled animals were taken out of the national milk recording database.

Results: SCK threshold for BHB was set at 1,2 mmol/L and for NEFA at 400 µmol/L. NEFA was in normal ranges for all cows 3 weeks before parturition. The first check-up postpartum revealed that 6 % of cows in control group had SCK and none in the test group. At the second check-up 20 % of cows in control group and 8 % in test group had SCK. Two cows in the control group had SCK at both check-ups for BHB. During this study 29 cows were culled, 31 % of those were from control group and 12 % from the test group. Major reason for culled cows was failure to conceive (reproduction problems). There were 14 % of cows from control group and 4 % of cows from test group culled due to reproduction problems. From control group 3,5 % of cows were culled because of metabolic disease and none from the test group. Average days open in test group was 139 ± 72 and 156 ± 72 in control group. Average lactation energy corrected milk (ECM) was 32,0 ± 3,8 kg/day/cow in test group and 31,9 ± 3,9 kg/day/cow in control group.

Conclusions: SCK is present in the herd in ranges established also by other authors. The use of ruminal monensin controlled release boluses can decrease the number of cows with SCK and therefor attribute to better reproductive and metabolic health, which was reflected in the number of cows culled for reproductive failure and metabolic disease in the control group compared to test group. In cows that finished the lactation milk yields were not different between the groups and the average days open ware slightly lower in the test group. Even though ruminal monensin controlled release boluses can be successful in prevention of SCK, they cannot fully prevent it and should be used as an addition to good management and feeding practice.

Keywords: subclinical ketosis, monensin controlled release capsule, Kexxtone®.

NU-18

Expression of ADAMTS-7 in myocardial dystrophy associated with white muscle disease in lambs

Nihat Yumusak[1], Akin Yiğin[2], Pelin Fatos Polat[3], Mustafa Hitit[4], Rahsan Yilmaz[1].

[1]Dept. of Pathology, Fac. Vet. Med., University of Harran, Sanliurfa, Turkey; [2]Dept. of Genetics, Fac. Vet. Med., University of Harran,

Sanliurfa, Turkey; [3]Dept. of Internal Med. Fac. Vet. Med., University of Harran, Sanliurfa, Turkey; [4]Dept. of Genetics, Fac. Vet. Med., University of Kastamonu, Kastamonu, Turkey.

Objectives: White muscle disease (WMD), or nutritional muscular dystrophy, is an acute fatal disease in young farm animals. It generally leads to death associated with heart failure. A-Disintegrin and Metalloproteinase with Thrombospondin Motifs (ADAMTS) genes play an important role in the pathophysiology of chronic inflammations. And also, recent studies have specified the role of ADAMTS-7 gene in cardiovascular system disorders in humans. The aim of the present study was to investigate the role of ADAMTS-7 gene in the pathogenesis of myocardial dystrophy associated with WMD in lambs.

Materials and methods: A total of 341 cardiac tissue samples from lambs with WMD were used in the study. Western-blot, real-time PCR (rt-PCR) and immunohistochemistry were performed for ADAMTS-7 gene expression. Histopathological sections of the samples were stained with hematoxylin-eosin.

Results: RT-PCR revealed that the expression level of ADAMTS-7 was statistically significantly higher in cardiac tissue with WMD compared to the control group ($p<0.05$). Western blot analysis confirmed significantly increased ADAMTS-7 protein level in the hearts of WMD ($p<0.05$). The immunohistochemically a statistically significant in high density of immunopositive cells was found from the in myocytes in which degeneration and necrosis were detected by labeling with ADAMTS-7 ($p<0.05$). Histopathologically revealed myofibrils with irregular borders and significant swelling of cells. Diffuse hyaline degeneration was noticeable in myocytes.

Conclusions: The expression of ADAMTS-7 was significantly upregulated in myocardial dystrophy associated with WMD. Despite to this limited study, if the potential biological mechanisms of ADAMTS-7 in WMD is understood more clearly.

Keywords: ADAMTS-7, myocardial dystrophy, lamb, white muscle disease.

NU-19

On field evaluation of preweaning period growth impact on age at first insemination and survival risk at 500 and 1000 days in dairy heifers

Guillermo Lorenzo[1], David Otero[2], Elisa Cáceres[3], Antonio Jiménez[3], Juan Cainzos[4].

[1]Gando nuevas tecnologías SL., Ferrol, Spain; [2]Nanta, Madrid, Spain; [3]Ceva Salud Animal, S.A., Barcelona, Spain; [4]ABS Progenex, Madrid, Spain.

Previous studies indicated that nutrient intake and growth during the preweaning period can influence a cow's performance later in life[1]. Thus, Average daily gain during the preweaning period (ADGprew) has been stablished as a key parameter to measure the overall health of calves and a threshold of 0.820 Kg/day has been set up as "excellent"[2]. The aims of this study were: a) to evaluate if birth weight and preweaning length have an impact on ADGprew and b) to evaluate AGDprew impact on age at first insemination and survival.

A retrospective study was conducted on a commercial dairy farm in Carral (A Coruña, Spain). All rearing heifers born between March 2017 and November 2021 were included in the study and the following parameters were collected for each animal from the farm's software Gando (Gando Nuevas Tecnologías S.L.): calving date (CALVDATE), weaning date (WEANDATE), first artificial insemination date (1ªIADATE), first calving date (FSTCALDATE) and cull date (CULLDATE), as well as birth weight (BIRTHWGHT, Kg) and weaning weight (WEANINGWGHT, kg). ADGprew (kg/day) and preweaning length (PREWlenght, days) were calculated using Gando. Correlation between BIRTHWGHT and PREWlenght with ADGprew was calculated using a linear regression. Three groups were made based on ADGprew distribution: Bottom (GMD<0.83Kg/d, n=124, 25.75%), Medium (0.83≤GMD<1.02 kg/d, n=245, 50.83%) and Top (GMD≥1.02 kg/d, n=113, 23.44%). Cox Proportional-Hazards Model were performed to evaluate ADGprew group impact on survival at 500 and 1000 days, as well as risk of insemination during first 395 days of life.

BIRTHWGHT, WEANINGWGHT and ADGprew averages were 36.94 ± 4.32 Kg (range: 23 – 46), 95.80 ± 13.86 Kg (range: 56 – 126.5) and 0.912 ± 0.177 kg/d (range: -0.070 – 1.500) respectively. Animals in the Bottom group tended to have an 80% higher risk of elimination at 500 days, with no differences between Medium and Top groups (HR=1.81, 95%CI = 1.08-3.02). In same way, culling risk at 1000 days was higher for animals in the Bottom group compared with Medium group (HR=1.74, 95%CI=1.11-2.74) with no differences between Medium and Top groups. Animals in the Bottom group had a 38% lower risk of being inseminated in the first 395 days (HR=0.62, 95%CI=0.44-0.89) compared to those in the Medium group. Median time to first insemination was 379, 368 and 369 days for Bottom, Medium and Top groups respectively.

Even in a herd with birth-to-weaning growth rates better than those usually described in the literature, differences in survival and age at first insemination were observed as a function of growth during preweaning period.

References:

1. Soberon F, Van Amburgh ME. 2013. Lactation Biology Symposium: The effect of nutrient intake from milk or milk replacer of preweaned dairy calves on lactation milk yield as adults: a meta-analysis of current data. Journal of Animal Science 91 (2), 706–712.
2. Average Daily Gain in Preweaned Holstein Heifer Calves NAHMS Dairy 2014 Study Calf Component. At: https://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy17/adg-preweaned-holstein-heifer.pdf. Visited on March, 10th 2022.

Keywords: preweaning, heifer, survival.

NU-20

Differences in the serum metabolome profile of dairy cows according to the BHB concentration revealed by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR)

Anastasia Lisuzzo[1], Luca Laghi[2], Vanessa Faillace[1], Chenglin Zhu[3], Barbara Contiero[1], Massimo Morgante[1], Elisa Mazzotta[4], Matteo Gianesella[1], Enrico Fiore[1].

[1]University of Padua, Legnaro, Italy; [2]University of Bologna, Cesena, Italy; [3]Southwest Minzu University, Chengdu, China; [4]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy.

Abstract

Objectives: During the transition period, a higher nutrient demand and a reduction in dry matter intake induce to a negative energy balance (NEB). The nonesterified fatty acids (NEFA) are then mobilized by adipose tissue and they may be oxidized into ketone bodies (β-hydroxybutyrate (BHB), acetoacetate and acetone). This condition makes dairy cows more susceptible to metabolic diseases such as ketosis. The BHB concentration is commonly used for the diagnosis in high-yielding dairy cows, with a cutoff of blood BHB value above 1.0-1.4 mmol/L for subclinical ketosis or hyperketonemia without clinical signs. These conditions are not easily identifiable and are frequently related to other diseases that cause economic loss. Metabolomics is a new analytical approach that aims to measure simultaneously the entire metabolite profile of a biologic sample. The aim of this study was to analyze the serum metabolome using ^{1}H-NMR in dairy cows with different levels of BHB.

Materials and methods: Animal care and procedures were in accordance with the European Directive 2010/63/EU and the national law D.L. 2014/26. Furthermore, The Ethics Statement was approved by the Animal Care and Use Committee of the University of Padua (ID number 91/2019 - "BovineOmics" Projects). Forty-nine Holstein Friesian dairy cows between 15 and 30 days in milk were enrolled from a single high-yielding dairy farm. The same total mixed ratio (TMR) was used for all enrolled animals. A cross-sectional experimental design was used. Each animal received a clinical examination by veterinarians at the University of Padua and animals with clinical signs of disease were excluded from the study. The blood samples were collected into tubes containing clot activator to obtain serum for biochemical and metabolomic analysis. According to the serum BHB concentration, the animals were divided into three groups: Group 0 (G0; 12 healthy animals; BHB≤0.50 mmol/L); Group 1 (G1; 19 healthy animals; 0.51≤BHB<1.0 mmol/L); and Group 2 (G2; 18 hyperketonemic animals; BHB≥1.0 mmol/L). The statistical differences for biochemical parameters were performed by one-way ANOVA, whereas a t-test was used to evaluate differences in metabolite concentration. A post hoc pairwise comparison among metabolite concentrations was performed using Bonferroni correction. A robust principal component analysis (rPCA), and the metabolic pathways overrepresentation analysis (ORA) were generated to summarize the structure of the data and to highlight the metabolic pathways influenced by BHB concentration. A p-value<0.05 was accepted, whereas a 0.05≤p-value≤0.10 was considered as trend to significance.

Results: Among biochemical parameters, only NEFA showed a significant difference between groups, with a progressive increment according to BHB concentration. A total of fifty-seven metabolites were identified in serum samples: 27 amino acids and derivates, 10 organic acids, 5 alcohols, 4 carbohydrates, 2 amine and derivates, 2 fatty acids, 2 ketone bodies, 1 sulfone, 1 vitamin, 1 imidazole, 1 nucleoside, and 1 guanidine. The extreme groups (G0-G2) showed a statistical difference for thirteen metabolites, specifically: glutamate, proline, serine, aspartate, isovalerate, and choline showed a significant reduction in G2, whereas 3-hydroxybutyrate, 3-hydroxyisobutyrate, acetate, succinate, 2,3-butanediol, methanol, and methylsuccinate showed a significant increase. In addition, 11 metabolites showed a trend toward significance: lysine, alanine, arginine, formate, pyruvate, and dimethylsulfone were reduced in G2, whereas isoleucine, valine, ethanol, trimethylamine-N-oxide (TMAO), and acetone were increased. The rPCA analysis revealed three different structure of metabolome, with G1 values located between G2 and G0. The ORA analysis identified three metabolic pathways possibly responsible for changes in metabolome profile: lipid metabolism, synthesis of phosphatidylserine, and glycosaminoglycan metabolism.

Conclusions: Metabolomic analysis through ^{1}H-NMR is a useful tool to achieve knowledge about metabolic profiling related to serum β-hydroxybutyrate modifications during the transition period in dairy cows. The metabolic state of our hyperketonemic cows suggests the mobilization of body resources, increased anaerobic fermentation, alteration of lipid metabolism, a potential oxidative stress state, and a possible alteration of inflammatory and healing processes. This study demonstrates that the metabolomic approach can be considered a significant means to achieve knowledge about dairy cow diseases and their pathogenesis.

Keywords: Metabolomics; H-NMR; Ketosis; Dairy cows.

NU-21

The behaviour of dairy cattle in the transition period: Effects of blood calcium status

Rosie Barraclough[1], Darren Shaw[1], Vivi Thorup[2], Marie Haskell[3], Wilson Lee[1], Alastair Macrae[1].

[1]Royal (Dick) School of Veterinary Studies and the Roslin Institute, Edinburgh, United Kingdom; [2]IceRobotics Ltd, Edinburgh, United Kingdom; [3]Scotland's Rural College (SRUC), Edinburgh, United Kingdom.

Objectives: Low blood calcium concentrations at calving can have a detrimental effect on cow health, welfare, and productivity. Currently, there is no reliable method to predict if a cow will develop clinical hypocalcaemia at calving, nor if cows have subclinical hypocalcaemia without analysing blood samples.

This study aimed to use automated behavioural monitoring under commercial farm conditions to investigate the behaviour of both primiparous and multiparous cows 1) pre-partum, with

the aim of predicting calcium status at calving and 2) post-partum, to quantify the subsequent effects of hypocalcaemia on cow behaviour.

Materials and methods: Behavioural data and blood serum samples were collected from 51 multiparous and 21 primiparous Holstein dairy cattle. Blood samples from the coccygeal vein were taken within 24h of calving, and serum was analysed to measure total calcium concentration. Cows were classified into one of three categories: normocalcaemia (serum calcium concentration ≥ mmol/L), subclinical hypocalcaemia (serum calcium concentration below 2.0 mmol/L, absence of clinical signs), and clinical hypocalcaemia (clinical signs and treatment).

An activity sensor (IceQube, IceRobotics Ltd., United Kingdom) was fitted to the right hind leg of cows 3 wk prior to expected calving date. Data for lying time, standing time, number of steps, motion index (total motion), and the total number of standing and lying bouts (postural transitions) were automatically collected and summarised from the time of calving into two datasets: behaviour in 2h periods and 24h periods (behaviour per day). The bihourly dataset was used for the analyses of cow behaviour on the day of calving, and data was analysed in 2h periods from -24 h to 0 h (the time of calving). The dataset containing cow behaviour per day was used to create 2 experimental periods based on the time relative to calving: pre-calving (d −14 to −1), and post-calving (d 1 to d 21). Mixed-effect models were used to analyse cow behaviour in the 14 d before calving (d -14 to d -1), on the day of calving, and the 21 d post-calving (d 1 to d 21).

Results: Between d -14 to d -1, the step count (no./d) of primiparous cows with normocalcaemia decreased by 10.3% and their motion index (unit /d) decreased by 6.1% over the period. The step count and motion index of primiparous cows with subclinical hypocalcaemia remained constant. There were no behavioural differences between primiparous cows with normocalcaemia or subclinical hypocalcaemia on the day of calving. In the post-calving period, step count of cows with normocalcaemia decreased by 6.7% from d 1 to d 21, whilst the step count of cows with subclinical hypocalcaemia decreased by 16.4% ($P < 0.001$). Similarly, the motion index of cows with normocalcaemia decreased by 7.5% from d 1 to d 21, whilst the step count of cows with subclinical hypocalcaemia decreased by 15.5% ($P < 0.001$).

For multiparous cows, there were no behavioural differences between blood calcium status (normocalcaemia, subclinical hypocalcaemia, clinical hypocalcaemia) in the pre-calving period and on the day of calving (d 0). In the post-calving period, cows with clinical hypocalcaemia spent 88 min/d (1.5 h) and 125 min/d (2.1 h) more time lying down compared to multiparous cows with subclinical hypocalcaemia and normocalcaemia ($P < 0.001$). Cows with normocalcaemia had fewer daily postural transitions (18.4 ± 0.5 no. /d; $P = 0.01$) compared to cows with subclinical hypocalcaemia (21.1 ± 0.3 no. /d) and clinical hypocalcaemia (22.0 ± 0.5 no. /d). Cows with clinical hypocalcaemia had 10% fewer steps and 6% lower motion index compared to cows with normocalcaemia, and 6.7% fewer steps and 4% lower motion index compared to cows with subclinical hypocalcaemia ($P < 0.001$).

Conclusions: There was a decrease in activity across the pre-calving period for primiparous cows, and activity could potentially be used to predict subclinical hypercalcaemia in primiparous cows. No behavioural differences between blood calcium status categories were found within the pre-calving period for multiparous cows, suggesting that it would be difficult to predict blood calcium status at calving using lying and activity behaviours. Blood calcium status at calving affected lying time duration, the number of postural transitions, step count, and motion index in the post-calving period for multiparous cows illustrating the profound and long-lasting effects of clinical hypocalcaemia.

Keywords: Hypocalcaemia, transition period, dairy cow, calving.

NU-22

Preliminary results of a metabolic survey for plasma ionized Calcium and Magnesium in dairy herds from the south of Chile

Pedro Melendez[1], Francisca Lopez[2], Jorge Lama[3], Bernardita Leon[3], Pablo Pinedo[4].

[1]University of Georgia, Tifton, United States; [2]Austral University of Chile, Valdivia, Chile; [3]Cooprinsem, Osorno, Chile; [4]Colorado State University, Fort Collins, United States.

Ionized calcium is the biologically active form of calcium; therefore, total calcium might not be the best indicator for hypocalcemia in dairy cows because of the changes in blood pH and protein concentrations. Consequently, the objective of this study was to determine the concentration of plasma ionized calcium and magnesium in cows at parturition from representative dairy herds from the south of Chile. The study was conducted in the southern region of Chile covering an area of 20,544 km2 (214 km length, 96 km wide; -39.82 S, -73.23 W; -40.12 S, - 72.38 W; -41.39 S, -73.46 W; -41.43 S, -72.94 W). Mean temperature during autumn is 7°C while during spring is 10°C, while mean rainfall during autumn is 850 mm, but in spring is only150 mm. The study consisted of selecting at random 11 dairy herds from a pool of 100 herds and sampling 8 cows per herd during the first 12 hours after parturition. All herds were handled under grazing conditions and had a calving distribution of 30% in autumn/winter, and 70% in spring/summer. Herds consisted of Holstein cows (Australian, New Zealand, Europe, and US genetics), artificial insemination breeding, and milked twice a day. Predominant pasture was based on perennial ryegrass (Lolium perenne). Spring calvings received 70% of dry matter from pasture and the rest from a partial mixed ration previous to each milking and concentrate in the milking parlor. Between August and December 2019, a blood sample for plasma collection was taken from a total of 88 cows from the 11 herds. Samples were stored in a cooler and shipped during the same day to a certified veterinary clinical pathology lab (Cooprinsem, Osorno, Chile). Samples were centrifuged and plasma was stored in plastic vials and frozen at -20 °C until analysis. Samples were assessed for ionized Ca and Mg using the Stat Profile® PRIME Plus VET (Nova Biomedical Corporation, Waltham, MA 02454-9141 USA). From the total samples,

26.7% were from parity 1, 19.6% parity 2 and 53.6% parity 3 or more. Total mean ionized Ca concentration was 1.00 mmol/L (range: 0.54-1.24 mmol/L) and ionized Mg was 0.615 mmol/L (range=0.32 – 0.89 mmol/L). Within parity number, the concentration of ionized Ca was 1.064, 1.024, and 0.894 mmol/L for parity 1, 2, and ≥3, respectively. For ionized Mg was 0.63, 0.60, and 0.613 mmol/L, for parity 1, 2, and ≥ 3, respectively. Defining a cut-off value of ionized Ca for subclinical hypocalcemia of 1.10 mmol/L, the total prevalence of subclinical hypocalcemia at day 1 postpartum was 65% with a range of an intra-herd prevalence between 0% and 100%. From the total number of cows (n=88), the prevalence of subclinical hypocalcemia within parity number was 40%, 54.5%, and 86.7% for parity 1, 2, and ≥ 3, respectively. It is concluded that subclinical hypocalcemia based on ionized Ca determination, is a metabolic disorder more common than expected in Chilean southern dairy cattle handled under grazing conditions.

Keywords: Ionized calcium, ionized magnesium, dairy herds, Chile.

NU-23

Very low negative DCAD diet promotes severe metabolic acidosis and alters plasma and urine metabolomics in prepartum Holstein cows

Pedro Melendez[1], Prasanth Chelikani[1], Dhavalkumar Patel[2], Eduardo Garbarino[3].

[1]School of Veterinary Medicine, Texas Tech University, Amarillo, TX, United States; [2]Health Science Center, Texas Tech University, Amarillo, TX, United States; [3]Progressive Dairy Health Service, Clovis, NM, United States.

Several nutritional strategies to prevent clinical hypocalcemia in the US dairy industry and other countries have been focused on feeding anionic diets. These diets reduced urinary pH from 8.5 (normal) to as low as 5.5, which imposes a tremendous acid load on the kidneys. However, the impact of anionic diets on the metabolic status of both the cows and their calves is not well understood, and such levels of over acidification may have detrimental effects on both the dam and her offspring. Consequently, the objectives of this cross-sectional non-intervention observational study were to compare urine and blood parameters between cows consuming a positive DCAD diet (early dry cows, DCAD + 250 Meq/kg DM, n=15) with the same cows consuming a negative DCAD diet (-220 mEq/kg DM) 10 days after moving them from the early dry to the prepartum group. Urine pH and blood metabolites were analyzed by a one-way repeated measures ANOVA for paired samples using the PROC GLM of SAS (2017). Differences in metabolic classes between groups were compared using BH correction (FDR= 0.05; Graph Pad 7.0), and metabolomic analyses was conducted using Metaboanalyst 5.0. Urine pH and blood analytes for the early-dry and prepartum periods, respectively, were as follows: urine pH: 8.18 and 5.33 ($P \leq 0.0001$); blood pH : 7.50 and 7.36 ($P \leq 0.0001$); base excess (mmol/L): 2.46 and -7.79 ($P \leq 0.0001$); lactate (mmol/L): 0.99 and 1.49 ($P \leq 0.05$); HCO3 (mmol/L): 25.65 and 17.45 ($P \leq 0.0001$); sO2 (%): 68.73 and 52.0 ($P \leq 0.01$); TCO2 (mmol/L): 26.59 and 18.39 ($P \leq 0.0001$); pCO2 (mm Hg): 32.62 and 30.68 ($P > 0.05$); pO2 (mm Hg): 37.51 and 29.85 ($P > 0.05$). Parity, BCS and days to parturition effect for all outcome variables were not significant ($P > 0.05$). Importantly, the metabolomics data revealed that only urine concentrations of essential and aromatic amino acids were decreased, and that concentrations of total non-essential amino acids and glucogenic amino acids were increased in plasma and reciprocally decreased in urine, suggesting that the cows fed anionic salts are attempting to meet a high glucose demand by mobilizing gluconeogenic amino acid reserves. The dietary anionic salts exerted marked effects on glycerophospholipids with a reduction in a majority of phosphatidylcholine containing diacyl and acyl-alkyl moieties in plasma and urine. Further characterization of these metabolomic profiles may lead to the development of novel biomarkers for identifying cows susceptible to metabolic alterations.

Keywords: Negative DCAD, metabolic acidosis, urine pH, metabolomics, Holstein.

NU-24

Effect of oral or parenteral iron supplementation in early life on iron concentration and hematological parameters of dairy calves fed commercial milk replacer

Lennart Golbeck[1], Imke Cohrs[2], Esther Humann-Ziehank[3], Walter Grünberg[4].

[1]Clinic for cattle, University of veterinary medicine hannover, Foundation, Hannover, Germany; [2]Educational and Research Centre for Animal Husbandry, Hofgut Neumuehle, Münchweiler an der Alsenz, Germany; [3]LABVETCON-Laboratory Veterinary Consulting, Burgdorf, Germany; [4]Clinic for Ruminants, Justus-Liebig University Gießen, Gießen, Germany.

Objectives: Iron supplementation is common in newborn calves. The objective of this study was to compare the effect of oral and parenteral iron supplemenation to a negative control treatment on iron concentration and hematological parameters.

Material and Methods: Thirty healthy newborn dairy calves were randomly assigned a subcutaneous injecton of Fe^{3+}(1000mg,**INJ**), oral administration of Fe^{3+}(1050mg,**ORAL**) or a sham-treatment without Fe^{3+}(**CON**) within 1h of birth. Calves were then fed whole milk over 5 days(d) and afterwards switched to milk replacer (MR,1500g/d,65mgFe/kg). Blood was collected before treatment, on d1,3,5,7 and once weekly for 7 weeks. Samples were analyzed for hematology and iron concentration.

Results: Red blood cell count declined by ~17% in all groups, reaching a nadir within the first week. Values returned to d0-values by d14 in INJ and ORAL and d28 in CON, and remained constant thereafter. Hemoglobin(Hb) reached a more pronounced nadir at d7(CON) compared to d5 (INJ and ORAL). Hb rose and remained >100g/L from d9 on (INJ and ORAL), CON calves remaind in the range of 80g/L.

Serum-iron peaked at d1 in ORAL (48.5 [46.9-51.7]µmol/L) and INJ (31.0[27.6-37.8]µmol/L) and declined thereafter. A second peak occurred at d7 (ORAL 23.9 [21.2-38.0]µmol/L, INJ 32.2 [20.9-39.1]µmol/L, CON 19.2 [12.6-20.9]µmol/L). Thereafter serum-iron in ORAL and INJ remained above CON.

Conclusions: Oral and parenteral iron supplementation had similar effects on hematological parameters and iron concentrations. Although Hb-values were higher in treated than control calves, hematological parameters remained within reference limits throughout the study in all groups.

Keywords: Iron treatment, hematology, neonatal calf.

NU-25

Variation in Longissimus Dorsi thickness in dry and lactating Holstein dairy cows, and association with early lactation mastitis and sole ulceration

William Tulley, Matthew Barden, Bethany Griffiths, Alkiviadis Anagnostopoulos, Cherry Bedford, Georgios Oikonomou.

University of Liverpool, Liverpool, United Kingdom.

Objectives: Dairy cattle face a drastic increase in nutrient demand at the onset of lactation leading to a period of negative nutrient balance which cattle respond to by catabolism of body tissues. Adipose tissue mobilisation to meet energy demands for maintenance, and milk production and its association with health and fertility have been extensively studied. Studies on the role of muscle mobilization have been limited to small numbers of cows in single herds or experimental settings (Megahed et al., 2019, Van der Drift et al., 2012). While a consistent pattern of muscle mobilisation has been identified, data is lacking from large scale studies involving large numbers of cows across multiple herds. The objectives of this study were to (i) describe changes in longissimus dorsi thickness before and after calving (ii) to identify variables associated with difference in longissimus dorsi thickness and (iii) to describe associations between muscle thickness and early lactation health disorders.

Materials and Methods: A cohort of 2,352 Holstein cattle were prospectively enrolled on four farms and assessed at four different stage pre-calving (PC), immediately after calving (fresh, F), in early lactation (EL), and in late lactation (LL). Cows were grouped by parity as either primiparous or multiparous. At each time point, ultrasonographic images of the longissimus dorsi muscle at the level of the 5th lumbar vertebrae were stored and retrospectively measured according to the method described by Megahed et al (2019). Feet were lifted, and presence and severity of sole lesions was assessed by veterinary surgeons and lesion severity recorded. Mastitis episodes in the first 30 days in milk (CM30) were recorded by trained farm staff. A mixed effects multivariable logistic regression model was fitted to the data, with muscle thickness as the outcome; cow was fitted in this model as a random effect to account for within animal clustering of measurements.

Results: In total, 6,849 observations were available from 2,186 cattle. Significant variables that remained in the final model were parity, farm, CM30, stage, calving season and incidence of sole ulcer in EL, with significant interactions between stage x farm, parity x stage x farm, sole ulcer in EL x stage and parity x stage. Variance components analysis showed that 58% of variation in muscle thickness was due to animal effects.

In agreement with previous studies a greatest decrease in muscle thickness was between PC and F (with model adjusted means and standard error being 36.99 ± 0.90 mm and 32.59 ± 0.91 mm, respectively). Pattern of mobilisation varied by parity with primiparous animals having a greater decrease in muscle thickness between PC and F, and multiparous animals between F and EL. Differences occurred in pattern of change in muscle thickness between farms at different stages. Farms with greater loss in muscle thickness had higher formulated levels of crude and metabolisable protein in early lactation diets, but no consistent difference in pre-calving diets.

Cows with a sole ulcer at EL had significantly lower muscle thickness at EL compared to cows without sole ulcer. A CM30 by stage interaction was observed (p=0.06) with cows with CM30 having a greater decrease in muscle thickness from PC to F.

Conclusion: Decrease in thickness of longissimus dorsi was greatest prior to calving and is not synchronous with mobilisation of body fat (initial analysis; data not shown). Pattern of muscle loss differs between different parities at different lactation stages, and different farms at different lactation stages, suggestive of a management or nutritional influence, although cow factors are highly significant. Pattern of decrease in muscle thickness is also associated with incidence of early lactation mastitis and sole ulceration.

References:

Megahed, A. A., Hiew M.W.H., Ragland D., Constable P.D. (2019) 'Changes in skeletal muscle thickness and echogenicity and plasma creatinine concentration as indicators of protein and intramuscular fat mobilization in periparturient dairy cows', Journal of Dairy Science. American Dairy Science Association, 102(6), pp. 5550–5565. doi: 10.3168/jds.2018-15063.

Van der Drift, S.G.A., Houweling M., Schonewille J.T., Tielens A.G.M., Jorritsma R. (2012) Protein and fat mobilization and associations with serum B-hydroxybutyrate concentrations in dairy cows, Journal of Dairy Science. American Dairy Science Association, 95 pp. 4911-4920. Doi:10.3168/jds.2011-4771.

Keywords: Longissimus dorsi, mastitis, sole ulcer.

NU-26

Factors affecting milk fatty acid composition on Galician Holsteins cows: a field studyFactors affecting milk fatty acid composition on Galician Holsteins cows: a field study

Ruth Rodríguez-Bermúez[1], Ramiro Fouz[1], Margarita Rico[1], Juan Cainzos[2], Francisco Javier Diéguez[1].

[1]Departamento de Anatomía, Produción Animal e Ciencias Clínicas Veterinarias, Universidade de Santiago de Compostela (USC), Lugo, Spain; [2]ABS Progenex, Madrid, Spain.

During the last decades, milk fatty acid composition (mFAc) has gained the interest of manufacturers and consumers as it influences nutritional, physical and flavor properties of dairy products (Bobe et al. 2007). Previous studies reported several factors, including individual variability, genetic parameters, and breed, influencing mFAc (Samková et al. 2012). The aim of the present study was to evaluate type of ration, calving number, lactation phase and season impact on fatty acid composition, including myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1), saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and short chain fatty acid (SCFA) concentration in cow's milk in 25 Galician (NW Spain) commercial dairy farms. 10,098 test-days samples from the 1,557 cows were collected from July 2018 to June 2019 and bronopol-preserved samples from Laboratorio Interprofesional Galego de Análise do Leite (LIGAL, Spain) were received. Quantity of C14:0, C16:0, C18:0, C18:1, SFA, MUFA, PUFA and SCFA, MCFA, LCFA and total FA (in g/100 g of milk), as well as, fat and protein % and milk β-hydroxybutyrate (BHB) concentration (in all the animals from 1st post-partum (PP) test day) were determined individually by infrared-FTIR (Fourier transformed infrared-FTIR, MilkoScan FT6000, Foss Electric, Hillerød, Denmark). On top of this analysis, data from each test-day, including cow ID, date, parity (1st, 2nd and ≥3rd), type of ration (pasture based, grass silage-based total mixed ration (TMR), corn silage-based TMR or grass/corn silage-based TMR), days in milk (DIM) and daily milk yield were recorded. For analysis, animals were classified into five categories: fresh cows without ketosis (DIM ≤ 35 and BHB concentration in the first PP test-day < 0.10 mM/L), fresh cows with ketosis (DIM ≤ 35 and BHB ≥ 0.10 mM/L), peak lactation (DIM > 35 to 90), mid lactation (DIM 91-210) and late lactation (DIM >210).

Data were analyzed descriptively and by means of mixed-effect ordinal regression models. Animals feeding corn silage-based total mixed rations (TMR) and grass/corn silage based-TMR had higher C14:0, C16:0 and SFA concentrations than those feeding pasture-based rations, but lower concentrations of C18:0 and PUFA. Comparing to 1st parity cows, 2nd parity animals had higher C16:0, SFA and SCFA concentrations and 3rd parity cows had higher C18:0, SFA and SCFA. With respect to spring, C14:0, C16:0 and SFA concentrations increased in summer, autumn and winter while MUFA, PUFA and SCFA concentrations decreased. In the case of C18:0 and C18:1, concentrations decreased in autumn and winter. Considering the lactation phase, C14:0, C16:0 and SFA concentrations decreased in fresh cows with ketosis comparing to healthy fresh cows and increased in peak, mid, and late lactation (using again healthy fresh cows as reference). C18:0, C18:1 and MUFA follow the opposite trend. Milk fatty acid profile varies significantly with type of ration, calving number, lactation phase and season. The fact that the fatty acid profile has been associated with animal health, organoleptic properties of milk or even methane production highlights the importance of studying factors that affect its variation.

Keywords: Milk, Fatty acid, holstein.

NU-27

Economic opportunities on prevention of subclinical and clinical hypocalcemia by use of synthetic zeolite

Per Theilgaard[1], Jehan Ettema[2].

[1]ViloFoss, Fredericia, Denmark; [2]SimHerd, Agro Business Park, Tjele, Denmark.

Objective: The objective of this study was to develop a sophisticated and robust tool for dairy farmers, to quantify the impact of using synthetic zeolite (X-Zelit) for prevention of subclinical and clinical hypocalcemia on farm economics.

Material and Methods: Dairy farmers could implement several solutions to decrease subclinical hypocalcemia. The solutions have different costs and efficiency in terms of reducing hypocalcemia. The existing simulation model SimHerd can quantify the economic consequences of changes in management for a dairy herd including youngstock. The expert-version of the model that vets use requires intensive training, and each simulation is time consuming. Therefore, in order for SimHerd to be operational for farmers, a Response Surface Model (RSM) was developed. An RSM is a meta-model that approximately describes the behavior of a certain area of the complex model (SimHerd). The current RSM predicts the change in profit (output) as a function of implementing of synthetic zeolite (X-Zelit) for prevention of hypocalcemia given different levels of disease risk and milk yield, amongst other input parameters. The assumed reduction in hypocalcemia as a result of the implementation was based on scientific literature.

In this project, an RSM was created using four steps.

- First, a simulation experiment with SimHerd, the expert version, was designed, covering the relevant ranges of input parameters like milk yield level, the risk of mastitis, calving interval and calf mortality among others.
- Secondly, simulation of all 160 scenarios from this experiment were done by SimHerd, using 200 replications and a burn-in period of 5 years.
- In the third step, the simulated results (output) were described as a function of the model's input with a linear prediction model. The best model was chosen by the Akaike Information Criterion (AIC) using the stepAIC() procedure in the MASS package of R.
- Finally, the response of the resulting model was explored, when using combinations of input parameters, that were not included in the design (interpolation and extrapolation).

Result: Input parameters of the RSM are herd size, milk yield per cow-year, the herd-level incidence of milk fever, subclinical hypocalcemia, other metabolic diseases, reproductive diseases, culling rate. The output parameters are changes in milk yield per cow-year, replacement rate, life-time yield, number of surplus heifers and economic net return per year and per cow-year. The RSM describes the changes in the output as a function of the input. The equations that make up the RSM contain two-way interactions and quadratic terms. The RSM is used as a web-based calculator (html) embedded on the Vilofoss website, where advisors and dairy farmers can use it.

Table 1. Examples of the RSM for 4 different herds, that are characterized by different levels of yield, hypocalcemia and replacement rate (input). The OUTPUT shows changes in production parameters relative to current production (OUTPUT) when using calcium binder for prevention of subclinical hypocalcemia.

		Herd 1	Herd 2	Herd 3	Herd 4
INPUT	Yield, kg EKM	9.000	9.000	11.000	11.000
	Subclinical hypocalcemia, %	50	70	70	70
	Replacement rate	0.3	0.3	0.3	0.2
OUTPUT, change	Kg ECM	+77	+87	+ 114	+119
	Replacement rate, %	-1.5	-1.8	-1,8	-2.7
	Benefit pr cow, €	54 €	59 €	67 €	71 €

The table shows a larger benefit in case of calcium binding in case the incidence of hypocalcemia is high; the benefit increases with €59 in herd 2 and with only €54 in herd 1. Furthermore, in case the herd has a high milk yield level, the increase in milk yield, and therefore the benefit of the intervention, is also higher (herd 3 versus herd 2). Finally, in case the herd has a lower culling rate and therefore a higher proportion of older parity cows, the intervention shows a larger benefit (herd 4 versus herd 3). Many more factors can be adjusted to explore the benefit in the individual farm.

Conclusion: The developed RSM tool makes it easier for the farmers to see the effects of changes in herd management. This has a potential to improve their decision making, as farmers can improve their economic performance, using the farmers' own key figures and prices.

Keywords: Hypocalcemia, synthetic zeolite, economics.

NU-28

Metabolic profiling of dry cows in practice: three-year results from a routine laboratory

Saskia Van Der Drift, Sietske Haarman - Zantinge, Deon Van Der Merwe.

Royal GD, Deventer, Netherlands.

Objectives: Metabolic parameters can be assessed in close-up dry cows (last 3 weeks before calving) to indicate their risk for postpartum diseases and evaluate transition cow management. In the Netherlands, dairy farmers can submit serum samples from a set of close-up dry cows to the laboratory of Royal GD for analysis of a specific set of metabolic parameters, including non-esterified fatty acids (NEFA), β-hydroxybutyric acid (BHBA), urea, magnesium (Mg), and haptoglobin. Results are presented in a herd report that can be used to improve transition cow management. The objective of this research is to present results from three years of testing close-up dry cows based on samples submitted to the laboratory of Royal GD from 2019 to 2021.

Materials and Methods: Results for metabolic parameters in serum samples of close-up dry cow submitted to the veterinary laboratory of Royal GD (Deventer, the Netherlands) between January 2019 and December 2021 were evaluated. Each submission consisted of samples from a set of 4 to 10 cows (between 21 and 2 days before calving) from one herd. Serum samples were analyzed for concentrations of NEFA, BHBA, urea, Mg, and haptoglobin on a clinical-chemistry analyzer using enzymatic or colorimetric methods. The final dataset contained records of 6798 cows from a total of 1371 submissions (with an average of 5 samples per farm). Data could occasionally originate from cows that were sampled outside the targeted interval of 21 to 2 days before calving, but at a low enough incidence to be insignificant. Cut-off values, based on earlier studies, were: cows with NEFA concentrations ≥0.40 mmol/L, BHBA concentrations ≥0.80 mmol/L, and/or haptoglobin concentrations ≥0.30 g/L were indicated to be at an increased risk for postpartum disease. Cows with urea concentrations ≤3.3 mmol/L were indicated to have a insufficient protein supply. For magnesium concentrations, a cut-off value of ≤0.78 mmol/L was used to indicate increased risk for postpartum hypocalcaemia. The proportion of cows below or above the cut-off value were calculated for each metabolic parameter.

Results: Descriptive statistics are shown in Table 1. Concentrations of one or more metabolic indicators were outside the cut-off values in 50.5% of cows, with urea being the most frequent, at 22.9%, and Mg being the least frequent, at 5.8%.

Table 1. Descriptive statistics for concentrations of NEFA, BHBA, urea, magnesium (Mg), and haptoglobin in serum of 6798 cows (1371 sets of 4 to 10 samples) sent to the laboratory of Royal GD from January 2019 to December 2021, including cut-off values and proportion of results outside the cut-offs.

Parameter	Mean (± SD)	Median	P25 – P75	Min - Max	Cut-off value	% of cows
NEFA (mmol/L)	0.24 (± 0.19)	0.18	0.14 – 0.27	0.10 – 2.07	≥0.40	12.8%
BHBA (mmol/L)	0.6 (± 0.2)	0.6	0.5 – 0.7	0.1 – 5.6	≥0.8	18.4%
Urea (mmol/L)	4.3 (± 1.3)	4.2	3.4 – 5.1	2.0 – 22.3	≤3.3	22.9%
Mg (mmol/L)	0.96 (± 0.12)	0.97	0.90 – 1.03	0.34 – 4.10	≤0.78	5.8%
Haptoglobin (g/L)	0.18 (± 0.36)	0.09	0.07 – 0.14	0.03 – 8.05	≥0.30	9.6%

Conclusions: A substantial proportion of close-up cows were outside the cut-off values for one or more metabolic parameters. Based on the high proportion of cows with low urea results, a suboptimal protein supply in the dry period could be an important risk factor for transition cows in the Netherlands. Testing for metabolic parameters in close-up dry cows can be helpful to evaluate dry cow management and indicate cows at risk for postpartum diseases.

Keywords: Close-up, dry cows, metabolic parameters.

PA-01

Assessment of body weight and weight gain of Eprinomectin or Doramectin injectable strategic control against gastrointestinal helminths on grazing Holstein heifers in Brazil

Luciana Maffini Heller[1], Luiz Fellipe Monteiro Couto[1], Dina Maria Beltran Zapa[1], Gustave Decuadro Hansen[2], Bruno Sivieri De Lima[3], Welber Daniel Zanetti Lopes[1].

[1]Centro de Parasitologia Veterinária, Universidade Federal de Goiás, Goiás, Brazil; [2]Virbac Latam, Santiago de Chile, Chile; [3]Virbac do Brasil, Sao Paulo, Brazil.

Objective: The experiment was conducted to assess the expected body weight and weight gain of distinct strategic deworming control program with injectable eprinomectin or doramectin against gastrointestinal helminths on grazing Holstein heifers in central-west of Brazil from November 2018 until June 2019.

Material & Methods: On November 2018, 150 Holstein heifers with an average age of 5.8 months belonging to the commercial dairy farm located in Jatai (Southwest of Goiania state, Brazil) were enrolled and weighed in order to estimate the weight gain in the last 30 days prior the trial. Dewormer drenches or any other antiparasitic medicine had not been used on the heifers within the previous 120 days. Faecal samples were taken from all heifers before turnout and parasite egg counts ranged from 0 to 300 eggs per gram (EPG). The study population was then stratified with regard to the average daily gain and age, and animals were randomly allocated in 3 homogenous groups of 50 heads as follow, 1) Group 1 (G01) were injected with eprinomectin 2 % at a dose of 1 ml/100kg BW (NEOPRINIL® injectable - Virbac do Brasil), 2) Group 2 (G02) received doramectin 1 % at the dose of 1 ml/50kg BW (DECTOMAX® - Zoetis Brazil), and 3) Group 3 (G03) was designated as negative control group and was not treated with macrocyclic lactones, however, was injected with saline solution as placebo. Treatments were performed for all groups in November 2018 (age ± 5.8 months), February 2019 (age ± 8.8 months) and May 2019 (age ± 11.8 months), completing a study period of 210 days. All enlisted heifers were individually weighed on an electronic scale on day –30 (October 2018), d0 (day of the beginning of treatment), d30, 60 and every 30 days until d210. The weight gain of each animal was calculated considering the difference in bodies weights achieved over the study period.

Results: The average weight of monitored animals from G01 G02 and G03 on day 0 was 147.49 kg, 147.49 kg and 147.53 kg (n.s.), respectively. By d210, same groups showed an average weight of 268.9 kg, 264.6 kg and 258.5 kg, respectively. There was no statistical difference in the average weight obtained between the groups, over the study period. The average weight gain demonstrated by the heifers enlisted in G01, was statistically higher (P≤0.05) to the average weight gain showed by the control animals (G03) on d210. There was no statistically significant difference in average weight gain between the treated groups (G01 and G02) at the end of the observation period.

Conclusion: Dairy replacement heifers represents a drain of cash flow in any dairy farm. Support weight gain with an overall program including strategic deworming can increase the net value of heifers entering the herd. Results of this study confirm that a strategic deworming of grass-fed dairy heifers with injectable macrocyclic lactones improves live weight and weight gain.

Keywords: Eprinomectin, doramectin, helminths, Brazil.

PA-02

On the current resistance status of stable flies from dairy farms in Brandenburg (Germany)

Sophia Reissert-Oppermann[1], Ricarda Daher[1], Jürgen Krücken[1], Burkhard Bauer[1], Wiebke Weiher[2], Peter-Henning Clausen[1], Stephan Steuber[2].

[1]Institute for Parasitology and Tropical Veterinary Medicine, Freie Universität Berlin, Robert-von-Ostertag-Str. 7-13, D-14163 Berlin, Germany; [2]Federal Office of Consumer Protection and Food Safety (BVL), Mauerstr. 39-42, D-10117 Berlin, Germany.

Objectives: Flies play an important role as pests and vectors of pathogens in ruminant production systems. During an earlier study, varying degrees of resistance against insecticides were demonstrated in 58 out of 60 *Musca domestica* field populations collected on dairy farms (Jandowsky et. al., 2010). A further pilot study was carried out to investigate the potential occurrence of resistance in stable flies (*Stomoxys* (*S.*) *calcitrans*) against commonly used adulticides and larvicides on dairy farms in the federal state of Brandenburg.

Material & Methods: Based on a questionnaire survey, 40 dairy farms were selected for an on-farm cross-sectional survey. An bioassay (FlyBox®) was concomitantly used to evaluate the field susceptibility of stable flies against the synthetic pyrethroid deltamethrin. To confirm the preliminary results observed in the on-farm bioassay, stable flies with strong evidence of deltamethrin resistance were collected from 10 selected dairy cattle farms for further investigations.

Using a susceptible reference strain of *S. calcitrans*, the discriminating dose (DD) of deltamethrin (2.3 ng/fly) and the organophosphate azamethiphos (4.9 ng/fly) were calculated. Subsequently, the progeny of the 10 stable fly populations was each tested with 1x, 4x, and 16x of the DD following recommendations of WHO (2016). Furthermore, two insect growth regulators (IGR), the larvicides cyromazine and pyriproxyfen, were evaluated at different concentrations based on the manufacturers' recommendations.

PASA-PCR (amplification of specific multiple alleles) were established with a forward primer matching all known alleles and reverse primers perfectly matching to the wild-type (Leu) or resistance-associated alleles currently known in pyrethroid resistant house and stable flies (e.g. kdr, kdr-his, super-kdr).

Results: The questionnaire survey revealed that pyrethroids are the most frequently used insecticides (78.9 %). The on-farm survey using the cardboard box bioassay (FlyBox®) indicated deltamethrin resistance in all of 40 strains tested (100 %). The topical application of deltamethrin at 1x,

4x, and 16x of the DD provided mean paralysis rates of 22.7 %, 55.3 %, and 87.9 % in the 10 suspect strains at 24 hrs. p. appl. At the highest dose applied (16x) 70 % of the stable fly populations showed paralysis rates < 98% indicating high resistance intensity according to the WHO concept (2016). The topical application of azamethiphos at 1x, 4x, and 16x of the DD revealed mean paralysis rates of 22.7 %, 55.3 %, and 87.9 % at 24 hrs. p. appl. Six out of ten populations (60 %) showed mortality rates below 98 % at the highest dose tested (16x) indicating high resistance intensity according to the WHO concept (2016). The use of the IGRs cyromazine and pyriproxyfen at the recommended concentrations resulted in an inhibition rate of 100 % in all 10 evaluated populations indicating full susceptibility against the two larvicides.

First results of the PASA revealed kdr and kdr-his mutations in *Stomoxys calcitrans* field populations being the second molecular confirmation of kdr resistant stable flies worldwide.

Conclusions: There is an urgent need for re-designing strategies to control fly populations, particularly with regard to any non-strategic use of adulticides. Before applying insecticides (biocides or veterinary medicinal products) it is highly recommended to assess the resistance status of flies in animal husbandries. Insecticides should only be used as the last resort of choice in a cascade of possible control measures such as improved manure management, insect traps and the use of biological control agents. Unfortunately, none of the aforementioned methods alone is sufficient to effectively control flies in dairy farms. Therefore, there is a considerable need for research.

References:

Jandowsky, A. et al. (2010): Occurrence and distribution of insecticide resistance in house flies (*Musca domestica*) on dairy farms in Brandenburg, Germany, Der praktische Tierarzt, 91, 590-598.

WHO (2016): Test procedures for insecticide resistance monitoring in malaria vector mosquitoes, 2nd edt., ISBN 978 92 4 151157 5. www.who.int/malaria/publications/atoz/9789241511575/en/

Keywords: Stomoxys calcitrans, insecticide resistance, kdr, cattle, Germany.

PA-03

Anti-O.ostertagi antibody ODR in bulk tank milk assesses exposure not parasitism

Tom Loughnan[1], David Beggs[2], Peter Mansell[2].

[1]Colac Vet Clinic, Colac, Australia; [2]The University of Melbourne, Melbourne, Australia.

Objective: International research has suggested that anti *O. ostertagi* antibody optical density ratio (ODR) of vat milk can indicate the level of milk production benefit producers will get from applying effective anthelmintic treatments to lactating dairy cows. ODR is an assessment of the magnitude of antibody production specific to *O. ostertagi*. In Australian pastoral dairy systems, lactating cows and young stock graze on pasture for 100% of their life and there will often be a prolonged worm season in dairy regions due to the high rainfall climate. The objective of this study was to look for a correlation between ODR and faecal egg count (FEC) or the presence of parasite eggs in faeces in corresponding cohorts.

Materials and Methods: Fourteen herds in south-west Victoria (Australia) were included in the study. All were commercial dairy enterprises. Milk vat samples were collected during the calving period for ODR. On the same day, faecal samples were collected from recently calved cows (less than 30 days in milk) for individual FEC (sensitive to 2.5 eggs per gram of faeces). Fifteen primiparous and 15 multiparous animals of representative ages were sampled. Farm practices including recent and routine anthelmintic use was recorded for each property and each cohort of animals. Data analysis was then undertaken using the Jamovi statistical package.

Results: Bulk milk ODR was not related to measurable FEC in corresponding herds. Bulk milk ODR was also not related to the proportion of animals in each corresponding cohort with detected parasite eggs in faeces. Bulk milk ODR was strongly correlated to the number of days since most recent anthelmintic application (r^2=0.73).

Conclusion: Anti-*O. ostertagi* ODR is a good measure of likely antibody presence against *O. ostertagi*. However, in pastoral systems in Australia where worms are present on pasture for sustained periods, possibly year-round, it may not be a good measure of current parasite load. ODR should not be used as the sole determinant of anthelmintic application in Victorian pastoral dairy herds.

Keywords: Australia, pasture-based, dairy, ODR, FEC.

PA-04

Prevalence and risk factors for hair loss in outdoor-wintered beef cattle during cold weather conditions

Ylva Persson[1], Katinca Fungbrant[2], Giulio Grandi[1], Wonhee Cha[1].

[1]National Veterinary Institute, Uppsala, Sweden; [2]Farm & Animal Health, Stockholm, Sweden.

Keeping cattle outdoor year-around is an attractive alternative for farmers due to low investment costs. Furthermore, keeping the animals outside can promote animal health and welfare compared to confined settings. In the Nordic countries, the climate can be a challenge though, as extreme cold and harsh wind can negatively affect an animal's thermal balance. The hair is an important parameter in the thermal retention capacity of an animal. Thus, if the hair thins out and bald spots appear, the welfare of the animal can get negatively affected along with the production value of the animal. Therefore, we conducted a repeated cross-sectional study to examine the epidemiology of hair loss in outdoor cattle in Sweden during the winters of 2019-2021. The three main objectives were (1) to describe the prevalence of hair loss in outdoor cattle, (2) to examine the development of hair loss in animals when there was no prophylactic ectoparasite treatment (delousing) ap-

plied, and (3) to investigate the associated factors for the hair loss outcome.

During January-February of 2019-2021, each group of outdoor cattle had an annual visit where they were inspected for hair loss, and group-level data, such as size, breed, and information on delousing treatment (date, drug), was collected along with the inspection parameters in the Swedish control program for outdoor cattle. A subset of the study population, among groups with no prophylactic delousing treatment, was followed up for investigating development of hair loss in animal level. Logistic regression was used to examine the association between collected animal- and group-level factors and status of hair loss with group and farm added as random effects when relevant. All the analyses were done using R.

A total of 463 groups from 75 farms were included in the study. The median size of the groups was 30 animals (range 2-698). 25.7% (n=119) of all the groups had at least one animal with hair loss. Prevalence of hair loss within group varied from 0.6% to 47.9% (mean 8.2%). Thirty-one farms had no hair loss observed. In the final model, group size ($p<0.01$) remained to be significantly associated with the hair loss outcome with the small groups (<14 animals) having lower odds. Also, having at least one dirty cow in the group significantly increased the odds for hair loss (OR=4.34, $p<0.01$), while delousing treatment significantly lowered the likelihood for hair loss in a group (OR=0.43, $p<0.05$). Among the groups with delousing treatment (n= 336), having at least one dirty animal in the group significantly increased the odds to have hair loss outcome (OR=12.64, $p<0.00001$), while delousing the animals before November significantly decreased the odds (OR=0.26, $p>0.05$). For the groups that did not have any prophylactic delousing treatment (n=127), only the big group size (>68 animals) was associated with the hair loss outcome (OR=3.64, $p<0.05$) in the final model.

A total of 3673 animals were included in the groups in which no prophylactic delousing was performed for the study. At the first visit, 249 animals showed hair loss (6.78%) while the proportion increased to 12.02% at the second visit and 18.22% on the third visit, suggesting spread of hair loss within the group over time. In the final model, being >2 years old (OR=11.89, $p<0.001$) and having bedding (OR=4.13, $p<0.001$) were significantly associated with higher likelihood of hair loss in an animal. Also in the final model, compared to Hereford, Angus had significantly higher odds to develop hair loss (OR=8.59, $p<0.05$).

In conclusion, the study showed a wide range of hair loss prevalence between farms and groups. Applying prophylactic delousing treatment significantly decreased the likelihood to develop hair loss in a group while increase in the proportion of animals with hair loss was observed among animals without delousing treatment in the follow-up visits. Along with the statements of veterinarians in the field, these findings suggest ectoparasites, i.e., lice, as a significant cause for the hair loss in these outdoor cattle. Yet, there were farms which had no cows with hair loss during all three years without any delousing treatment, indicating good farm management, like having no dirty cows, and keeping the group size more manageable can also be effective measures against hair loss.

Keywords: Range cattle, lice, ectoparasites.

PA-05

Effect of injectable eprinomectin on milk production and quality of dairy ewes reared semi-intensively and naturally infected with gastrointestinal nematodes

Sofia Afroditi Termatzidou[1], Nektarios Siachos[1], Panagiota Kazana[1], Smaragda Sotiraki[2], Katerina Saratsi[2], Damien Achard[3], Hamadi Karembe[3], Georgios Bramis[4], Georgios Arsenos[1].

[1]Laboratory of Animal Husbandry, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University, Thessaloniki, Greece; [2]Laboratory of Parasitology, Veterinary Research Institute, Hellenic Agricultural Organization Demeter, Thermi, Thessaloniki, Greece; [3]Ceva Santé Animale, Libourne, France; [4]Ceva Hellas Ltd, Alimos, Greece.

Objective: The objective of this study was to investigate the effect of treatment with injectable eprinomectin on milk production and quality of dairy ewes grazing in communal pastures and naturally infected with gastrointestinal nematodes.

Material and methods: One-hundred and fifty (150) clinically healthy adult lactating ewes (2^{nd}-4^{th} month of lactation) from 3 flocks (50 animals per flock) were included. They were raised semi-intensively and had good body condition (BCS ≥2.5), good udder health and negative California Mastitis Test. On day -7, ewes of each flock were randomly allocated in 2 groups of 25 ewes: Control (group C) and Treated (group T). Groups were balanced for faecal egg count (FEC), milk yield record and Maedi-Visna seropositivity. On day 0, ewes in group T received a single subcutaneous injection of eprinomectin at a dose rate of 0.2 mg/kg BW (Eprecis® 20 mg/mL, CEVA santé animale). Ewes in group C were left untreated during the whole experiment. Ewes in group T with a FEC >300 eggs per g on day +60 were given a second injection with eprinomectin at the same dose rate. Faecal samples were individually collected on days -7, 0, +30, +60, +90, +120 for FEC estimations and coprocultures. On days -7, 0, +30, +60 and +90, individual milk yield (MY) was recorded using ICAR approved volumetric milk meters. Moreover, at the same test-days, individual milk samples were collected for chemical composition and somatic cells counts (SCC). Energy corrected milk yield (ECMY) for 6% fat was also calculated. Milk composition [fat (F%), protein (P%) and lactose (L%) content] was determined by infrared analysis (FTIR interferometer) using Milkoscan FT6000 (Foss Electric, Denmark) and SCC were evaluated by flow cytometry using Fossomatic FS (Foss Electric, Denmark). On each test-day, individual fat and protein yield (FY and PY, respectively) were calculated. Total lactation MY (TMY), total ECMY (TECMY), total FY (TFY) and total PY (TPY) were computed according to ICAR recommendations (ICAR, 2016). The effect of treatment on FEC (ln-transformed numeric FEC +1) and milk parameters (MY, ECMY, F%, P%, L%, FY, PY, and Log_SCC) were assessed with mixed models for repeated measures accounting for the random effect of each ewe and each farm. Last, the effect of treatment on TMY, TECMY, TFY and TPY was assessed with general linear models accounting for the random variation within each farm. All analyses were performed with IBM SPSS v.25 (Armonk, NY: IBM Corp.).

Results: The most prevalent parasite at pre-treatment and post-treatment days was *Haemonchus spp*. Treatment had a significant effect ($P<0.001$) on FEC reduction throughout the

trial; the overall efficacy on days +30 and +90 was 97.27% and 98.80%, respectively. In two out of the three farms, 80% and 91.3% of T ewes received a second treatment on day +60, due to high parasitic challenge and burden (FEC >300 eggs per g). Treatment had a significant effect (P=0.033) on MY. Estimated marginal means showed an average benefit of *ca.* 105 mL more milk per test-day for treated ewes compared to untreated ones. No significant effects of treatment were observed on the other parameters, although values were constantly numerically higher for treated ewes compared to control ones. T ewes produced 5.7% more milk (308.8 L vs. 292.1 L, P=0.158), 4.6% more fat-corrected (6%) milk (311.7 L vs. 298.1 L, P=0.236), 2.4% more fat (15.2 kg vs. 14.8 kg), P=0.557) and 6.4% more protein (11.8 kg vs. 11.1 kg, P=0.115) across the whole lactation compared to untreated ones.

Conclusion: In this field trial, injectable eprinomectin had a high overall efficacy against gastrointestinal nematodes, with *Haemonchus* spp. being the most prevalent species identified. Treatment had a beneficial effect on daily milk yield and potentially could lead to higher milk, fat and protein yield during a whole lactation.

Keywords: Eprinomectin, dairy sheep, milk yield, milk quality.

PA-06

Severe eosinophilic dermatitis in Jersey cows infested with *Leptotrombidium* spp (Acari: Trombiculidae)

Marie-Christine Cadiergues[1], Jean-Paul Moulin[2], Marie-Noëlle Lucas[3], Philippe Dorchies[4], François Schelcher[5].

[1]Small Animal Clinic, Université de Toulouse, ENVT - UDEAR, Université de Toulouse, INSERM, ENVT, Toulouse, France; [2]Clinique vétérinaire, Saint-Céré, France; [3]Large Animal Clinic, Université de Toulouse, ENVT, Toulouse, France; [4]Université de Toulouse, ENVT, Toulouse, France; [5]Large Animal Clinic, Université de Toulouse, ENVT - IHAP, Université de Toulouse, INRA, ENVT, Toulouse, France.

Objectives: In cattle, the lesions of eosinophilic dermatitis are attributed to different causes, but mainly to scabies and insect bites (stable flies, horn flies, tabanids, …). Harvest mites, also known as chigger mites, are rarely implicated and this work reports cases observed in Jersey cows. This study describes the clinical and histopathological lesions of several Jersey cows in an organic farming dairy herd in south-west France, that developed a severe outbreak of pruritic dermatitis mid-October suspected to be induced by Trombiculidae.

Material and methods: The herd consisted of 28 cows, including Jersey (21), Bretonne pie noir (3) and crossed Abondance-Montbéliarde (4) breeds. Lactating cows (17) had permanent grazing access except for indoor milking times since June. In addition, they received hay, concentrate and a vitamin/trace mineral supplement. Five 2-year-old and six 1-year-old heifers had been permanently kept in two different meadows, with no contact between them or with adults, since the beginning of the summer. Licking buckets of vitamin/trace mineral supplement were available. No antiparasitic treatment had been administered during the last five months.

Impression smears, multiple skin scrapes, swabs for bacterial culture and skin samples for histopathology were collected from the most affected individuals. Blood samples were taken and haematological parameters, activity of erythrocyte glutathione peroxidase (GSH-Px) and plasma concentrations of inorganic iodine, zinc, copper, vitamins A and E were determined. Jersey heifers were serologically examined for antibodies against *Besnoitia besnoiti*.

Results: Physical examination revealed no abnormalities other than dermatological. Pruritus was moderate to severe. Severe and extensive lesions of alopecia, lichenification and crusting were observed on the jaws, dewlap, face and distal limbs. Crusts overlying yellowish oozing material were present on the perineum and above the udder. Only Jersey cows were affected (10/13 lactating cows and 3/3 2-years-old heifers) with varying degrees of lesions severity. Lesions were more intense in 2-years-old Jersey heifers compared to the adults. The other group of heifers remained unaffected.

Differential diagnoses were parasitic skin disease (psoroptic or chorioptic mange, besnoitiosis, trombidiosis), zinc deficiency, superficial bacterial infection (staphylococcal pyoderma, dermatophilosis).

Cytologic examination of direct skin smears revealed very high numbers of eosinophils but no micro-organisms. Microscopic examination of skin scrapes showed numerous specimens of small-sized Trombiculidae larvae (150 µm x 250 µm) identified as *Leptotrombidium* spp and no other ectoparasite [1, 2]. Histopathological examination of skin biopsy specimens revealed severe hyperplastic, superficial and deep perivascular, and oedematous eosinophilic dermatitis. Bacterial culture yielded *Acinetobacter calcoaceticus* and *Pantoea agglomerans,* gram negative facultative anaerobic strains, considered as contaminants from the soil. Jersey heifers were seronegative for besnoitiosis. Complete blood counts revealed mild to moderate eosinophilia (0.6-1.3Λ10^9/L, reference interval 0.1-1.2Λ10^9/L). GSH-Px activity and plasma concentration of inorganic iodine, zinc, copper, vitamins A and E were in normal range. Final clinicopathological diagnosis was eosinophilic dermatitis likely due to Trombiculidae bites. No treatment was administered. Lesions had completely self-resolved by the end of December in the absence of any specific or concurrent treatment or major modification of the feeding or husbandry conditions. Further complete blood counts and skin scrapes revealed a 20 % reduction of eosinophils and no presence of parasite.

Conclusions: This case report emphasizes the need to include harvest mites in the differential diagnosis of bovine pruritic dermatoses. Around 3000 nominal species of Trombiculidae are known worldwide. Only a few have been identified on cattle [3]. To the best of the authors' knowledge, this is the first report of *Leptotrombidium* sp. in cattle in Europe. A concurrent contributing role of biting flies cannot be excluded. Finding lesions only in Jersey cows remained unexplained, but could be hypothetically consistent with individual genetic susceptibility to develop hypersensitivity reactions [4], or with numerous predisposing breed factors (feeding habits, skin characteristics…).

Selected references:

1. Brennan, J.M. and M.L. Goff, *Keys to the genera of*

chiggers of the western hemisphere (acarina: trombiculidae). J Parasitol, 1977. 63(3): p. 554-66.
2. Shatrov, A.B., et al., *Mouthparts in Leptotrombidium larvae (Acariformes: Trombiculidae).* J Morphol, 2016. 277(4): p. 424-44.
3. Gamboa, R., J. Velazquez, and M. Podesta, *Trombidiosis en bovinos de Costa Rica.* Ciencias Veterinarias, 1983. 2-3: p. 95-98.
4. AHVLA disease surveillance report, *Rhinitis associated with allergic nasal granuloma in Jersey cattle.* Vet Rec, 2012. 171(19): p. 468-71.

Keywords: Jersey cows, eosinophilic, skin, Leptotrombidium, Trombiculidae.

PA-07

Efficacy of a targeted selective treatment in dairy herds affected by clinical dictyocaulosis

Thibaut Lurier[1], Thomas Hilaire[2], Claire Henon[2], Philippe Camuset[3], Gilles Bourgoin[4], Marie-Anne Arcangioli[2].

[1]Université de Lyon, INRAE, VetAgro Sup, UMR EPIA, Marcy l'étoile, France; [2]Université de Lyon, VetAgro Sup, Marcy l'étoile, France; [3]SNGTV, Yvetot, France; [4]Université de Lyon, Université Lyon 1, CNRS, UMR 5558, Laboratoire de Biométrie et Biologie Evolutive, Marcy l'étoile, France.

Bovine dictyocaulosis is a pulmonary disease caused by the presence and development of the strongyle *Dictyocaulus viviparus* in the trachea and bronchi of cattle. This parasite is mostly observed in temperate countries, with potential important clinical and economical impacts. Individuals that already had contact with the parasite normally develop a strong acquired immunity. However, when the first contact with the parasite was poor and the contamination of the environment is high, clinical signs can occur. In such a context of clinical dictyocaulosis outbreak in dairy herds, current recommendations are to treat the whole herd with anthelmintic because of the presence of subclinical infestations. However, current guidelines against anthelminthic resistance recommend the use of selective treatment on adult cattle in order to maintain a refuge helminth population unexposed to anthelmintic. According to the heterogeneity of acquired immunity in a herd, and hence, of sensitivity of animals to parasitic infestation by *D. viviparus*, an early targeted selective treatment of clinically affected and main shedders animals may be effective to control the disease in the herd. Such a strategy was never tested for dictyocaulosis and a field validation is thus necessary to ensure a proper control of ditycocaulosis at the herd level.

Objectives: This study is a pilot study designed to assess the clinical relevance of a targeted selected treatment against *D.viviparus* at the beginning of a dictyocaulosis outbreak in dairy herds.

Materials and methods: In each herd with clinical signs of dictyocaulosis, we performed broncho-alveolar lavage on 6 cows and a pooled McKenna sedimentation on 10 cows of the herd to test for the presence of *D. viviparus*. We implemented a targeted selective treatment in three positive dairy herds by treating with injectable eprinomectine approximately 50% of the dairy cows including clinically affected, primiparous and postpartum cows.The follow-up was implemented one and three months after the targeted selected treatment by performing broncho-alveolar lavage and a pooled McKenna sedimentation. The farmers recorded clinical signs of dictyocaulosis at the herd level during the whole study period.

Results: In the three followed dairy herds, symptoms of dictyocaulosis vanished after the targeted selective treatment. After the treatment, we assessed a dictyocaulosis incidence rate of 2% by month during the whole follow-up, which indicates a low but persistent circulation of the parasite in the herd. Clinical signs of dictyocaulosis remained very low to inexistent in the three herds during the whole study period.

Conclusion: The targeted selective treatment implemented in the three herds of this study was efficient to control dictyocaulosis at the herd levels. To our knowledge, this study is the first to prove the potential interest of a targeted selective treatment to control dictyocaulosis at the herd level. These results should be confirmed in a larger study with more herds.

Keywords: Dictyocaulus viviparus - target selected treatment - dairy cattle - bronchoalveolar lavage - baermann sedimentation.

PA-08

Ctenocephalides felis (cat flea) infestation in neonatal dairy calves managed with deltamethrin pour-on in Australia.

Fei Man Bernice Tsoi, Jan Šlapeta, Michael Reynolds.

Sydney School of Veterinary Science, Sydney, Australia.

Objectives: Fleas are worldwide distributed hematophagous ectoparasites (Lawrence et al., 2019). The cat flea (*ctenocephalides felis*) is considered the most pervasive flea with veterinary significance principally centred around owned dogs and cats (Rust, 2017; Šlapeta et al., 2011). From the veterinary perspective, livestock are rarely recognised as cat flea hosts, or suffer from flea infestations (Araujo et al., 1998; Dryden et al., 1993; Kaal et al., 2006). Here we report on an outbreak of a flea infestation in neonatal dairy calves in eastern Australia.

Materials and Methods: The described events took place in April to June 2018 and included a group of calves (0–12 weeks) on a privately owned dairy farm in eastern New South Wales, Australia, under the care of the University Veterinary Teaching Hospital, Camden, Livestock Unit. The farm operates an all year round calving herd of 600 Holstein-Friesian cows and 400 youngstock.

All calves (n = 25) were individually restrained and their ear tag recorded for numerical identification. The body condition score (BCS) was assessed using the Penn State University method on a scale of 2–5, with 0.25 increments. A capillary refill time (CRT) was evaluated for each calf. Fleas were collected from the sternum and ventral inguinal area of the calves

using a metal flea comb (JW Gripsoft Flea Comb, Australia). Each calf was combed for 90 seconds in each area, 3 minutes per calf. The total flea count for both sites was reported. A commercially available deltamethrin pour-on formula (Arrest Easy-Dose, Virbac Animal Health, Australia) was used according to the registered label (1.9mg/kg). A Flea count was performed immediately before each treatment, and two weeks after the last treatment and the geometric mean calculated for the BCS and flea burden present. The flea reduction post treatment was calculated as a percentage difference between the pre-treatment and post-treatment geometric mean count. Statitical analysis was undertaken using the Mann-Whitney test and exact P values calculated using GraphPad Prism 7.02 (GraphPad Software, CA) with significance level considered at P-value<0.05.

Results: In early April 2018 examination of 15 affected calves (age 0–4 weeks) revealed a geometric mean burden of 41.51 (15–75) fleas (treatment group TA at timepoint 1 {TA1}). The affected calves showed dull and quiet demeanour, with the geometric mean BCS of 2.67 (2–4). At eight weeks (TA4), all calves showed bright, alert and responsive demeanour. The affected treated calves mean BCS was 4.04 (3.75–4.5) and mean count at TA4 was 2.29 (0–2) fleas on affected treated calves (n = 10), representing a 94.8 % reduction from TA1 count. In mid-April 2018, an examination of 10 calves (age<4 weeks) revealed a geometric mean burden of 9.32 (2–33) fleas (treatment group TB at timepoint 1 [TB1]). Demeanour of all calves was bright, alert and responsive. The geometric mean BCS was 3.79 (3–4.25). At four weeks (TB3), all 10 calves showed bright, alert and responsive demeanour. The calves mean BCS was 3.67 (3.25–4). The mean count at TB3 on the treated calves (n = 10) was 0.68 (0–4) fleas representing a 92.7 % reduction compared to TB1 counts.The initial flea burden between TA1 and TB1 (geometric mean) was reduced by 77.5 % in the youngest calf category with no clinical disease apparent and improved BCS from 2.67 to 3.79 in calves at TB1, 4 weeks post first application of pour-on deltamethrin. Both the flea count and BCS scores between TA1 and TB1 were significant (Mann-Whitney test, P-value<0.05).

Conclusion: Deltamethrin is a synthetic insecticide structurally based on natural pyrethrins (Mestres and Mestres, 1992) and causes rapid knockdown effect through paralysis of the insect nervous system. In cattle in Australia, deltamethrin is used for the treatment of lice and flies (Mestres and Mestres, 1992). We took advantage of the existing registered pour-on formulation for cattle, therefore assuring safety, and the knowledge that that the cat flea (*Ctenocephalides felis*), is susceptible to its active ingredient based on previous studies in dogs. The application of deltamethrin pour-on achieved the objective of improving the health and welfare of the calves, through a reduction in the number of fleas.

Keywords: Fleas, Neonatal, Calves, Deltamethrin.

PA-09

Gastrointestinal Parasites in Australian pastoral lactating dairy cows

Tom Loughnan[1], David Beggs[2], Peter Mansell[2], Matthew Playford[3].

[1]Colac Vet Clinic, Colac, Australia; [2]The University of Melbourne, Melbourne, Australia; [3]Dawbuts, Camden, Australia.

Objectives: In Australian pastoral dairy systems, a variety of 'routine' anthelmintic programs are used in lactating cows. These programs vary from no use of anthelmintics to administration of two doses to cattle annually. This suggests that there may be either some overuse potentiating the development of anthelmintic resistance, or underuse with consequential production inefficiencies. We aimed to identify the predominant gastrointestinal parasites in recently calved dairy cows in south-west Victoria, Australia. We also assessed the different anthelmintic management practices used on commercial, pasture-based dairy farms and their effect on measurable worm burdens.

Materials and Methods: The study included 18 commercial, pasture-based dairy farms in south-west Victoria, Australia. Faecal egg counts (FEC) were measured in recently calved cows (<30 days in milk). Fifteen primiparous and 15 multiparous animals were selected based on age (multiparous; 3-4 animals from each age up to 6 years of age) and calving date. Parasitism of individual animals was assessed through ultra-sensitive FEC, sensitive to 2.5 eggs per gram of faeces, in recently calved cows. These animals were sampled for individual FEC and pooled larval cultures (per group of 15 animals). Farm data such as recent anthelmintic use, management and cow body condition score (BCS) at sampling was recorded. Data analysis was undertaken using the Jamovi statistical package.

Results: Thirty-six per cent of all animals and 46% of primiparous cows had FEC of greater than 2.5 eggs per gram (epg). *Ostertagi ostertagi* was the most commonly identified gastrointestinal nematode in all age groups and on all farms. *Cooperia oncophora* were found to be the most frequent *Cooperia spp,* however *C. pectinate* or C. *punctata* were also present on some farms.

Multiparous cows in low BCS post calving are more likely to have a FEC of greater than 5 epg. Herds utilising anthelmintic routinely during lactation or at drying off did not have significantly different mean FEC or proportion of cows with individual FEC > 0 compared to those which did not utilise anthelmintics in lactating cows. Primiparous animals were more likely to have a reduced FEC post calving if the most recent anthelmintic was administered less than 50 days prior to calving.

Conclusion: Measuring the FEC at a sensitivity of 2.5 epg is an effective indicator that parasites are present in primiparous dairy cattle and may still be useful in assessing the presence of infection in multiparous animals if BCS is low. Whilst *O. ostertagi* is the most pathogenic and important gastrointestinal nematode in south-west Victoria, *Cooperia punctata* or C. *pectinata* are more prevalent than previously reported. In some herds these parasite species are still abundant in multiparous animals in moderate levels.

Anthelmintic use in Australian pasture-based systems during lactation or dry off is not likely to be effective at reducing the worm burden of cows in early lactation. Further investigation of timing and value of anthelmintic use in multiparous cattle is warranted.

Keywords: Dairy, roundworms, FEC, Australia, gastrointestinal parasites.

PA-10

Evaluation of the plasma profile and efficacy of eprinomectin 50 mg/mL prolonged-release injection administered at the base of the ear of cattle in Europe

Dietmar Hamel[1], Pascal Dumont[2], Michael Kellermann[1], Valerie Kvaternick[3], Becky Fankhauser[2], Steffen Rehbein[1].

[1]Boehringer Ingelheim Vetmedica GmbH, Rohrdorf, Germany; [2]Boehringer Ingelheim Animal Health USA Inc., Athens, Georgia, United States; [3]Boehringer Ingelheim Animal Health USA Inc.,, North Brunswick, New Jersey, United States.

Objectives: To characterize the plasma profile and the therapeutic and preventive anthelmintic efficacy of eprinomectin 50 mg/mL prolonged-release injection (Eprinomectin-PRI) when administered at 1 mL/50 kg bodyweight (equivalent to 1 mg eprinomectin/kg) to cattle at the base of the ear in Europe.

Materials and methods: Data from one GLP study were used to characterize the plasma profile of Eprinomectin-PRI. In this study, blood was drawn from male and female Simmental cattle in intervals up to 136 days after Eprinomectin-PRI administration and the plasma was analyzed for eprinomectin levels using a HPLC method.

For the characterization of the efficacy, a total of 268 young cattle were included from four experimentally induced (96 animals) and one naturally acquired (16 animals) nematode infection studies (three controlled studies to confirm the therapeutic efficacy against developing and inhibited fourth-stage larval [L4] and adult nematodes; two controlled persistent efficacy studies) and one multicenter field efficacy study (four sites, 156 animals) which were compliant with VICH GCP and WAAVP and VICH anthelmintic evaluation guidelines. The studies included cattle of both sexes and several breeds (Brown Swiss, Hereford, Holstein, Montbéliarde, Pinzgauer, Simmental and various beef crosses) and were conducted in France, Germany and the UK.

In each therapeutic efficacy study, cattle were randomly assigned to groups and received either saline (controls) or Eprinomectin-PRI (treated) when the parasites were developing L4, inhibited L4 and/or or adult nematodes. In the two persistent efficacy studies, cattle were randomly assigned to controls (saline-treated) or two Eprinomectin-PRI-treated groups and were challenged daily with a combination of infective larvae of parasitic nematodes from 80 to 100 or 100 to 120 days after treatment. Percent efficacy was calculated based on the comparison of geometric mean nematode counts of the Eprinomectin-PRI-treated vs. control animals established following necropsy.

In the field study, animals were randomized in a 1:3 ratio to serve as saline-treated control or to be administered Eprinomectin-PRI. Animals were weighed and fecal samples collected at intervals starting pre-treatment through 120 days after treatment for fecal egg and lungworm larval counts. At each site, six sentinel animals grazing in intervals with the study animals were necropsied to characterize the nematode challenge of the study animals.

Results: As expected based on the release characteristics from the Eprinomectin-PRI polymer-based formulation, the eprinomectin plasma profile is characterized by an initial release peak (observed one to five days post administration) declining into a 'trough' region which is followed by a second peak around 80 to 100 days post administration and a subsequent rapid decline. Mean eprinomectin plasma concentrations of >1 ng/mL were observed up to 126 days after Eprinomectin-PRI administration. The basic pharmacokinetic parameters (C_{max}, T_{max}, AUC) were comparable for male and female cattle (P>0.05).

The therapeutic efficacy studies demonstrated Eprinomectin-PRI is efficacious (>90%, P<0.05) against developing L4 *Bunostomum phlebotomum*, *Cooperia oncophora/surnabada*, *C. punctata*, *Dictyocaulus viviparus*, *Haemonchus contortus*, *Nematodirus helvetianus*, *Oesophagostomum radiatum*, *Ostertagia ostertagi/lyrata*, *Trichostrongylus axei* and *T. colubriformis*; against inhibited L4 *Ostertagia* spp., *Cooperia* spp. and *Nematodirus* spp.; and against adult *B. phlebotomum*, *Chabertia ovina*, *Cooperia curticei*, *C. oncophora/surnabada*, *C. punctata*, *D. viviparus*, *H. contortus*, *Nematodirus battus*, *N. helvetianus*, *O. radiatum*, *Oesophagostomum venulosum*, *O. ostertagi/lyrata*, *O. leptospicularis*, *T. axei*, *T. colubriformis* and *Trichuris discolor*.

The persistent efficacy studies demonstrated Eprinomectin-PRI prevents the establishment (>90%, P<0.05) of *B. phlebotomum*, *D. viviparus*, *H. contortus*, *O. ostertagi/lyrata* and *T. colubriformis* for minimum 120 days, and of *C. oncophora/surnabada*, *C. punctata* and *O. radiatum* for minimum 100 days.

Examination of the sentinel animals for characterization of the parasite challenge in the field study identified 18 species of gastrointestinal nematodes, *D. viviparus* lungworms, and *Moniezia* spp. cestodes. At all post-treatment sampling intervals, Eprinomectin-PRI-treated cattle had significantly (P<0.0001) lower strongylid egg and *Dictyocaulus* larval counts than the controls with 98.8% and 100% reduction, respectively, after 120 days of grazing and Eprinomectin-PRI-treated cattle gained significantly (P<0.05) more weight than the controls.

Conclusions: Results of the studies consistently demonstrated that Eprinomectin-PRI can provide high levels of control of all important nematode parasites of cattle including prevention of infection for up to four months following a single administration. The unique biphasic plasma profile supports the efficacy demonstrated in the controlled and field efficacy studies.

Keywords: Eprinomectin prolonged-release injection, cattle, efficacy, parasitic nematodes, plasma profile.

PA-11

Productivity impacts of low to moderate faecal egg counts in growing cattle: Implications from meta-regression of faecal egg count and average daily weight gain

Andrew Hancock[1], Richard Shephard[2], Matthew Playford[3], Sally Oswin[4].

[1]Zoetis, Dublin, Republic of Ireland; [2]Herd Health, Maffra, Australia; [3]Dawbuts, Camden, Australia; [4]Zoetis, Sydney, Australia.

Objectives: Quantify the productivity impacts of low to moderate faecal egg counts (FEC) in growing cattle using the results of a meta-analysis and meta-regression of the association between FEC and average daily weight gain (ADG).

Material and Methods: To quantify the association between strongyle parasite burden and productivity, results were used from a meta-analysis and meta-regression of the relationship between differences in FEC and ADG (Shephard *et al*., under review). In this process, papers from all continents were included, and 27 publications containing 86 groups and 59 comparison ratios were analysed. This analysis identified a 0.127 linear reduction in average daily weight gain ratio for every $\log_{10}$ increase in the difference between comparison and reference group faecal egg count. For example, compared with an animal with FEC of 1 egg/g, an animal with FEC of 10 eggs/g would have 12.7% lower ADG.

This association was then incorporated into a deterministic model, with several input scenarios of starting FEC over two specified growing period durations (Table 1). Average daily weight gains were determined based on the starting FEC, compared with a referent FEC of 1 egg/g, and then extrapolated out for the specified growing period. The starting bodyweight for cattle in all scenarios was set at 100kg.

Results:

Table 1. Two scenarios of defined growing period, and defined target weight, at various starting FEC.

Growing Period 120 days			
Starting FEC (eggs/g)	ADG (kg/day)	Final Weight (kg)	Difference in Final Weight (kg) Compared with FEC of 1 eggs/g
1	1.25	250	-
10	1.09	228.5	-21.5
40	0.99	216.9	-33.1
80	0.94	211.4	-38.6
Target Weight 250kg			
Starting FEC (eggs/g)	ADG (kg/day)	Days to Reach Target Weight (d)	Difference in Days to Reach Target Weight Compared with FEC of 1 egg/g
1	1.25	120	-
10	1.09	139	+19
40	0.99	152	+32
80	0.94	160	+40

Conclusion: These results demonstrate the impact of low to moderate FEC in growing cattle, thereby highlighting the impact of the emerging problem of anthelmintic resistance in cattle and demonstrating the importance of integrated parasite management.

Shephard, RW, Hancock, AS, Playford, M, and Oswin S. A systematic review and meta-analysis of impact of strongyle parasitism on growth rates in young cattle. Under review.

Keywords: faecal egg count, gastrointestinal parasitism, productivity, average daily gain, anthelmintic resistance.

PA-13

Attempts to achieve apparent (in the field) nematocidal efficacy while using popular anthelmintics with varying degrees of anthelmintic effectiveness

Tom Yazwinski, Chris Tucker, Eva Wray.

Animal Science Department, University of Arkansas, Fayetteville Arkansas, United States.

Objectives: The studies reported herein were conducted to further assess the effectiveness of routinely-applied anthelmintic treatment and then attempt to achieve efficacious treatment via means not normally used but which should be put in place if the debilitating effects of nematode parasitism are to be addressed. For more than a decade, it has been well recognized that there has been the lack of a commercially-available, single active, broad spectrum, efficacious anthelmintic for use in ruminants. Producers and veterinarians who have recognized this shortfall, have attempted to achieve effective nematode control in a variety of husbandry (pasture management, dietary inclusions, grazing management, etc.) and treatment (frequency, dose level, targeting, combinations, etc.) schemes. Anthelmintic combinations, formulated as such, represent a logical means of biding our time until a new, efficacious compound becomes available. Said combinations have not become available in the USA, where it is now common that combinations be actuated “animal side”; with the effectiveness of some of these treatment practices evaluated in the studies reported here.

Materials and Methods: In three recent field studies, we looked at apparent anthelmintic efficacies via the fecal egg count reduction test (FECRT) in naturally-infected replacement and stocker cattle; assessing various single and combined anthelmintics. In all instances, at least ten animals were placed in each treatment group, with fecal egg counts recorded for all animals on the days of treatment and again 14 days later. Coprocultures were conducted on all individual fecal samples with egg counts > 20 eggs per gram (EPG).

Results: In the first field study (utilizing beef steers), injectable ivermectin and oral fenbendazole were evaluated at 1X and 2X prescribed dose rates, and as a combination of both at 1X. Neither treatment with ivermectin as the sole anthelmintic resulted in a FECR rate of > 47%. All treatment regimens wherein fenbendazole was used resulted in FECR rates > 99%. In the second field study (utilizing replacement heifers),

1X dose rates of moxidectin injectable, moxidectin topical, ivermectin injectable, doramectin injectable and eprinomectin long-range were evaluated. During the 14-day post-treatment period, fecal egg counts actually rose for ivermectin treated cattle (42%) and for doramectin treated cattle (9%). Treatment with moxidectin topical, moxidectin injectable and eprinomectin long range decreased egg counts by 6, 26 and 56%, respectively. As a follow-up to the second field study and utilizing cattle from the same herd, oxfendazole and levamisole drenches, given separately at labeled dose rate, were evaluated. Both treatments resulted in FECR rates > 98%. For the cattle used in the 3 field studies, *Cooperia punctata* and *C. oncophora* accounted for > 90% of the coproculture larvae harvested on a treatment group basis at both pre- and post-treatment.

Conclusions: It is generally accepted that FECR rates > 90% are necessary in order for an anthelmintic to be assumed to be effective. Given the results obtained herein, it is apparent that the avermectins (ivermectin, doramectin, eprinomectin) are not effective at normal or double dose rates, and that moxidectin is not efficacious at 1X. Fenbendazole, oxfendazole (benzimidazoles) and levamisole (an imidazothiazole) administrations are still proving to be efficacious (> 90% FECR); a guarded assessment of true nematocidal effectiveness given the potential disparity between egg count and worm count reductions. Combinations of a macrocyclic lactone (ML) with a benzimidazole or imidazothiazole also result in acceptable FECR percentages, most likely due to the non-ML in the combination. The implications of these findings, coupled with the findings of many others, are that macrocyclic lactone use in cattle should be augmented with combination. Clearly, gastrointestinal nematodiasis is not being effectively abated with ML use; use that results in: (1), prolonging the deleterious effects of subclinical infections, (2) perpetuation of the selection and propagation of ML-resistant parasites, and (3), continued economic losses (wasted monies spent on non-efficacious products and continued poor animal health and productivity). At the present time, we are clearly past the time of routine "ML remedy" for nematodiasis in herbivores. The looming query at hand is what we do presently to preserve animal health.

Keywords: Cattle Nematodes, Effective Anthelmintics.

PA-14

Evaluation of four protocols for *Haematobia irritans* control and their impact on the productivity of grazing calves on tropical farms in Veracruz

Fernando Livas Calderón[1], Eduardo Ramírez España[1], Miguel Angel Alonso Díaz[2], Chandra Bhushan[1].

[1] Facultad de Medicina Veterinaria y Zootecnia. Universidad Nacional Autonoma de Mexico, Tlapacoyan, Veracruz, Mexico; [2] Facultad de Medicina Veterinaria y Zootecnia. Universidad Nacional Autonoma de Mexico, Tlapacoyan, VER, Mexico.

Objectives: The objective of the study was to compare different chemical control protocols on horn fly infestation (*Haematobia irritans*) and their impact on grazing calves weight gain (*Bos taurus x Bos indicus)* in humid tropical farms of Veracruz, México.

Materials and methods: The study was conducted for 90 days and was carried out 4 farms located in the north-central region of Veracruz, México with a humid warm tropical climate. A total 100 calves (*Bos taurus x Bos indicus* cross) on four farms were included in the study. Farm 1 (T1): 40 calves (mean weight ± SD 174 kg ± 12.0 kg) were poured- on with Flumethrin (Bayer) 0.5% plus Ciflutrina 0.5% (1.0 ml/10 kg of weight) on the back when the average fly infestation exceded 200 flies/animal; Farm 2: 32 calves (mean weight ± SD 109 kg ± 20 kg) were sprayed with 22.47% Cypermethrin (Bayer) spray (1.0 ml of 22.4 % formulation diluted with 1 litre of water) when the average fly numbers infestation exceded 200 flies per animal. Farm 3: 14 calves (mean weight ± SD 126 kg ± 35 kg were tagged with Diazinon (40%) impregnated eartags and were given mineral salt mixed with oral Tetrachlorvinfos larvicide (Bayer) at doses of 780.0 mg/animal/day from the start of trial and pour on application of Flumethrin 0.5% plus Cyflutrin 0.5% (1.0 ml/10 kg weight) on the back of the animal when average fly infestation was greater than 200 flies/animal; Farm 4: 14 calves (mean weight ± SD 134 kg ± 16 kg were given 25.0 g of mineral salt (Bayer) mixed with an oral Tetrachlorvinfos larvicide (97.3%) at a dose of 780.0 mg/animal/day from the start of trial. The animals were weighed and fly counting were done at the beginning and every 15 days until the end of the study. The initial weights (IW), final (FW) and daily weight gains (DWG) of calves and fly infestation (FI) were analyzed using an ANOVA for completely random designs and the means were compared with the Tukey test.

Results: The IW of T1 T2, T3 and T4 were 174.0±12.0, 109.0±20.0, 126.0±35.0 and 134 kg ±16.0 respectively, with no statistical differences between them (P> 0.05). The FW in T1 was 159.7±18 kg with a weight loss each animal in 90 days of 14.3±8.0 kg (P<0.05), coinciding with the high level of *Haematobia irritans* infestation, while the FW in T2, T3 and T4 the calves had a weight 140.0±40 kg, 160.0±25 kg, 165.0±30 kg respectively (P> 0.05). The infestation of flies in each calf for T1, T2, T3 and T4 was 211, 135, 57, 113 respectively, T3 was statistically different from the other treatments (P<0.05). Daily weight gains (DWG) were affected by the level of fly infestation being the highest in T3 with 446.0 g/day/animal (P<0.05) while in T2 and T4 it was 368.0 and 346.0 g/day. In T1 the average weight loss/animal during (90 days) was 14.3 kg ± 9.0 % equivalent to $ 32.5 dollars/calf.

Conclusion: The most effective protocol for the control of *Haematobia irritans* was T3, where a combination of eartags, application of adulticide solution and oral use of a larvicide resulted in higer weight. From return on investment point (ROI) of view protocol (T3) offers a higher economic returns for farmers in the tropics.

Keywords: Horn fly, Haematobia irritans, calves, grazing, weight gains.

PA-15

Seroprevalence and risk factors associated with *Toxoplasma gondii* in breeding ewes from western Mexico

Jaime Alcalá Gómez[1], Leticia Medina Esparza[1], Teódulo Quezada Tristán[2], Carlos Cruz Vázquez[1], Irene Vitela Mendoza[1].

[1]Instituto Tecnológico el Llano Aguascalientes, El Llano, Aguascalientes, Mexico; [2]Universidad Autónoma de Aguascalientes, Aguascalientes, Aguascalientes, Mexico.

The objective of the study was to determine the seroprevalence and risk factors associated with *Toxoplasma gondii* in breeding sheep from western Mexico. 184 blood samples were collected in six municipalities of the state of Jalisco, Mexico. Sampling was performed by venipuncture of the jugular vein using tubes without anticoagulant. To obtain serum, blood samples were centrifuged at 3,000 revolutions per minute for five minutes. Serum was placed in 1.5 mL polystyrene tubes and stored at -20°C until use. Diagnosis was made using the commercial ELISA kit [IDEXX Toxotest Ab Test, IDEXX Laboratories, Inc., Westbrook, Maine], with 100% specificity and 98.9% sensitivity. The manufacturer's recommendations were followed, diluting the sera 1:400; the plates were read at an optical density of 450 nanometers and a cut-off point of > 0.30. Determination of positive and negative cases was performed using xChekPlus software [IDEXX Laboratories, Inc., Westbrook, Maine]. During the visit to the farms, a survey was applied with the objective of identifying possible risk factors, through a logistic regression analysis, calculation of Odds Ratio (OR) and Confidence Intervals (CI). The OR values were estimated using the independent variables that showed statistical significance in the multivariate analysis ($p < 0.05$). The analysis was carried out using the Statistics and Data Science (STATA) v. 10.1 software. An overall seroprevalence of 61.96% (114/184; 95% CI 62.09 – 61.81) was identified. The identified risk factors were: Poor hygiene conditions (OR 12.5; 95% CI 3.4 - 45.1 P<0.05), Presence of cats (OR 9.5; 95% CI 1.9 - 45.7 P<0.05), Presence of others domestic animals (OR 5.7; 95% CI 1.5 – 21.3 P<0.05), Urban context of the farm (OR 9.5; 95% CI 2.5 – 35.4 P<0.05) Public water supply (OR 5.3; 95% CI 1.0 – 26.1 P<0.05). In conclusion, *T. gondii* is an important parasite in sheep farms in western Mexico, identifying a high seroprevalence that may be associated with reproductive losses. The risk factors identified in this study may be useful to veterinarians in the region, farmers, and health personnel to reduce exposure to this parasite and the damage it causes to public and animal health.

Keywords: Parasite, Protozoan, Serology, Sheep Farm, Mexico.

PA-16

Eimeriosis age dynamics in Italian dairy cattle farms

Marcello Guadagnini[1], Alessandra Bazzucchi[2], Marco Gobbi[2], Nicoletta D'Avino[2].

[1]Elanco Animal Health, Sesto Fiorentino (FI), Italy; [2]Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati"(IZSUM), Perugia, Italy.

Objectives: Coccidiosis, or Eimeriosis, is a common clinical or subclinical cause of poor performance, illness, and economic loss in dairy calves (Drackley J., 2008). The prevalence of *Eimeria* infection in cattle is generally high (Daugschies A. et al., 2005). As the epidemiology of *Eimeria* is different from farm to farm due to specific calves raising conditions, proper coccidiosis' control requires an understanding of the life cycle and transmission dynamics (Keeton S. et al., 2018). The aim of this analysis is to describe Eimeriosis' age dynamics in Italian dairy cattle farms by summarizing the results of a coprological diagnostic service (Elancox) developed by Elanco Animal Health together with IZSUM laboratories.

Material and Methods: In this data analysis only dairy cattle farms with *Eimeria* confirmed presence have been included. Individual fecal samples were collected directly from the animal.

Samples were grouped into pools and then processed using FLOTAC® double technique (Cringoli G. et al. 2010) with sucrose and potassium iodomercurate (Rinaldi) solution. For *Eimeria* identification, feces were mixed with 2.5% potassium bichromate solution and placed in a Roux bottle for 2 weeks, at 25 °C, away from light. After incubation, samples with more than 250 *Eimeria* oocyst per gram of feces (OPG) were examined at light microscope 400X and identified using morphological criteria described in literature.

Additional epidemiological data have been collected: number of animals on farm, mean age at weaning, mean calves' age for every pool.

Results: From January to December 2021, 110 farm samples (395 pools), belonging to 105 farms mainly located in Northern Italy, have been analyzed. Herd size averaged 586±558 animals; this variable was further divided in classes for the data analysis: <400 animals (46%; n=50), between 400 and 800 animals (31%; n=34) and >800 animals (23%; n=25).

Each farm sample was composed by 1 to 6 pools. Species identification was possible in 68% (n=75) of the farm samples. According to a previous study by these authors (Guadagnini M. et al., 2021), *Eimeria* oocysts counts above 400 OPG were considered as high: 77% (n=85) of the samples had at least one pool with high oocyst count and 45% had 2 or more high count pools. Overall, 10 species were identified with an *E. zuerni* prevalence of 88% (n=66), *E. bovis* 67%(n=50), *E. alabamensis* 15% (n=11), *E. cylindrica* 44% (n=33), *E. ellypsoidalis* 69% (n=52), *E. auburnensis* 39% (n=29), *E. subspherica* 16% (n=12), *E. wyomingensis* 9%(n=7), *E. pellita* 3%(n=2), *E. brasilensis* 3%(n=2).

In 96% (n=72) of the samples at least one pathogenic species was found (*E. zuerni*, *E. bovis*, *E. alabamensis*). Age at sampling varied between 20 and 350 days, with an average of 107±54 days.

Mean age at first detection of a high OPG count was 85±43 days, with 16% of the farm samples having a high OPG only pre-weaning, 34% only post-weaning and 22% both pre- and post-weaning. Herds with more than 800 animals had higher proportion of samples with at least 1 pool with high OPG (<400 animals= 68%; 400-800 animals= 79%; >800 animals=92%;

p=0.04) and a different distribution of high counts over time (p=0.009) with more pre and post-weaning high counts compared to smaller herds (<400 animals=14%; 400-800 animals=24%; >800 animals=36%) and less pre-weaning only high counts (<400 animals=12%; 400-800 animals=27%; >800 animals=8%). Mean age at weaning was not correlated with the risk of detecting high OPG, and no age pattern was detected for any single Eimeria species. A positive correlation was detected between age at weaning and the risk of detecting high OPG pre-weaning(p=0.02).

Conclusion: Very little knowledge is described in literature regarding Eimeriosis age dynamics, as this aspect seem to be farm specific and highly correlated with housing and management practices. Data presented in this work show that there is wide age range for Eimeriosis, and pathogenic species detection is frequent. No *Eimeria* species pattern by age has been recognized, but rather relevant differences have been described among farm size, with bigger farms having high chance to show high OPG counts and for a longer period. In summary, data confirm the key role of an accurate sampling and diagnostics protocol, to determine farm specific *Eimeria* dynamics and build a control strategy.

Keywords: Eimeriosis, Age, Italy, Cattle.

PA-17

Effect of *Haemonchus contortus* on feed conversion rate, average daily gain and carcass characteristics of Limousine-cross calves

Konstantinos Arsenopoulos[1], George Fthenakis[2], Elias Papadopoulos[1].

[1]Laboratory of Parasitology and Parasitic Diseases, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece; [2]Veterinary Faculty, University of Thessaly, Karditsa, Greece.

Objectives: The objective of the study was to evaluate the potential effects of *Haemonchus contortus* infection on: (a) average daily gain and feed conversion ratio of Limousin-cross male calves and (b) carcass yield and carcass quality at slaughter.

Materials and Methods: The study was conducted for 200 days (April to October 2020) in an intensive-management fattening farm with Limousin-cross male calves in Central Greece. The animals (average age: 8.13 months), which had been naturally infected, were allocated, based on age matching, in two equal (n=12) groups, with animals that underwent anthelmintic treatment (T group) and remained as untreated controls (C group). Standard parasitological examinations were performed throughout the study to monitor the gastrointestinal parasitic burden of all animals at weekly intervals. Five days after group allocation, animals in group T were administered ivermectin (dose rate: 200 μg per kg bodyweight) subcutaneously. Bodyweight of animals was measured at the start and the end of the study. Consumption of feed (concentrate and roughage) by animals of the two groups was calculated daily. At slaughter, carcasses were weighed and assessed by means of the standard EU scale for the classification of carcasses of bovines for conformation class and fat cover class. Statistical analysis was performed to compare results of parasitological examinations, of bodyweight gain and feed conversion ratio and of carcass characteristics.

Results: There were no differences in mean epg counts between the two groups at the start of the study: 545 for T and 550 for C group (p=0.87), with 98% and 97% of larvae in coprocultures identified as *Haemonchus contortus*. After treatment, mean epg counts from T group were 0 until the end of the study, whilst those from C group were >540 epg with ≥97% of larvae in coprocultures identified as *Haemonchus* spp. throughout the study. Animals in T group had a higher bodyweight gain and a better feed conversion ratio than animals in C group: 355.5 kg and 3.85 versus 254.5 and 6.07, respectively (p<0.0001 for all comparisons). Moreover, animals in T group yielded on average heavier carcasses and of better quality that carcasses yielded by animals in C group: 350.5 kg of median U class (very good) with slight fat cover (2) versus 254.5 kg of median R class (good) with average fat cover (3), respectively (p<0.01 for all comparisons).

Conclusions: The study evaluated the effects of *Haemonchus* infection on the production and carcass characteristics of fattening calves in a long-term assessment. The results indicated that *Haemonchus*-infected animals had adverse lower production characteristics (bodyweight gain and feed conversion ratio) of calves and yield lighter carcasses of inferior quality than parasite-free animals.

Keywords: Haemonchus contortus, feed conversion rate, average daily gain, carcass, Limousine breed.

PH-01

Antibiotic resistance and genomic characterization of *Enterobacteriaceae* involved in bovine in mastitis using Nanopore technology

Maria Eugenia Revilla Ruiz[1], Carlo De La Serna[1], Irene Sanchez[1], R. Matamoros Bosco[1], Jose Delgado[1], Emilia Wedel[1], Manuel Ares[1], Natalia Montero,[1], Carlos Martin[2], Víctor García[2], Bruno González Zorn[1].

[1]Antimicrobial Resistance Unit, Department of Animal Health and VISAVET, Veterinary Faculty, Complutense University of Madrid, Madrid, Spain; [2]Calidad Pascual, Madrid, Spain.

Objectives: Bovine mastitis is the most common and costly disease in dairy cattle. Among the causative pathogens, *Escherichia coli* has recently emerged as one of the most prevalent causative agents. Mastitis treatment is mainly based on the use of antibiotics, being one of the main causes for antibiotic use in farms. The use of antibiotics is considered a risk factor in the emergence of antibiotic resistant bacteria. Therefore, the objective of this study was to evaluate the presence of resistance determinants in mastitis pathogens from Spanish dairy farms.

Materials & Methods: More than 10.000 bacterial isolates were recovered over the past 10 years from clinical and subclinical mastitis. The causative agents and their antimicrobial resistance pattern was recorded. Further, mastitis30 isolates from both clinical and subclinical mastitis from different dairy farms in Spain collected during 2019. Bacterial identification was performed using MALDI-TOF (Matrix Assisted Laser Desorption/Ionization- Time of flight). Agar disk diffusion method was used to determine the susceptibility of all isolates. 15 *E. coli* isolates were tested for their antimicrobial susceptibility against fourteen antibiotics via Minimal Inhibitory Concentration (MIC), based on the criteria of the European Comittee on Antimicrobial Susceptibility Testing (EUCAST). All *E. coli* isolates were sequenced using Nanopore technology in order to determine its resistance genes.

Results: Analyzing the trends in antimicrobial resistance over the last 10 years, we can observe the resistance to ampicillin has been maintained in these 10 years. Ampicillin, amoxicillin clavulanic and trimethoprim/sulfamide are the antibiotics with the highest levels of resistance. We observed that the bacteria that is causing the highest number of clinical mastitis is *E.coli*, followed by *Streprococcus uberis*; on the other hand *Streptococcus uberis* and coagulase negative *S. aureus* are the ones that cause subclinical mastitits.

E. coli was detected in 15 samples, *Klebsiella oxytoca* was detected in 2 samples, *Klebsiella pneumonie* was detected in 2 samples. The highest antimicrobial resistance was observed for sulfonamides and tetracyclines due to the presence of the genes *sul1*, *sul2*, *sul3*, *tet*(A), *tet*(B), *tet*(M), $bla_{\text{TEM-1B}}$, $bla_{\text{TEM-1A}}$, $bla_{\text{CARB-2}}$. These genes are related with mobile elements that can favour the dissemination of resistance genes to other bacteria.

Conclusions: The main pathogens that cause mastitis are *Streptococcus uberis*, coagulase negative *Staphylococcus*, *Staphylococcus coagulase positive*, *E. coli* and *Klebsiella spp*. *Staphylococcus coagulase negative*, the bacteria with a higher incidence. The tendency of antimicrobial resistance, shown in the different *E. coli* isolates that cause clinical mastitis, have slightly grown towards penicilin, cephalosporins and fluoroquinolones resistance. This is worrying, as these antibiotics are of great importance for human health, according to WHO and the One Health approach. In the collected isolates during 2019 we can observe an extensive resistance to ampicillin with resistance to to 3rd generation cehalosporins. The genes encoding these resistances are also found in human isolates. The levels of resistance found underline the imortance of reducing the use of antibiotics to preserve their action as long as possible

Keywords: Mastitis, antibiotic resistance, Escherichia coli.

PH-02

Molecular diagnosis of *Coxiella burnetii* in bovine milk tank from small rural properties in Brazil

Simone Baldini Lucheis[1], Thainá Valente Bertozzo[2], Amanda Bezerra Bertolini[3], Suzane Manzini[2], Marcela Alexandrino[2], Maria Eduarda Cavalheiro[2], Jackieline Sampaio Steinle[3], Andresa Xavier Frade Gomes[3], Isabella Neves Aires[4], Nassarah Jabur Lot Rodrigues[2], Maria Izabel Merino De Medeiros[1], Virgínia Bodelão Richini-Pereira[5].

[1]Paulista Agency of Agribusiness Technology - APTA, Bauru, Brazil, Bauru, Brazil; [2]Department of Tropical Diseases and Image Diagnosis, São Paulo State University, Botucatu, Brazil; [3]Department of Animal Production and Preventive Veterinary Medicine, São Paulo State University, Botucatu, Brazil; [4]Department of Biology, São Paulo State University, Bauru, Brazil; [5]Adolfo Lutz Institute, Regional Laboratory of Bauru, Brazil, Bauru, Brazil.

Objectives: Therefore, the aim of this study was to investigate *Coxiella burnetii* in milk from cattle from small rural properties in the state of São Paulo, Brazil.

Material & Methods: Milk samples from bulk tank were collected from 102 family farms and sent to the laboratory for molecular testing. DNA extraction from milk samples was performed and subsequently the polymerase chain reaction (PCR) was performed using specific primers targeting the *C. burnetii* IS1111 transposase region. For the conventional PCR the set of primers Trans1/Trans2 were used and for the nested PCR, the primers N3+ and N4- were used. Then, electrophoresis was performed to estimate possible positive bands, and negative and positive controls were used.

Results: Of the one hundred and two samples collected, 16 (15.6%) were positive for the bacteria by molecular tests. Subsequently, these positive samples were sequenced and compared with sequences in Blastn. The samples demonstrated a similarity level of 98.4 to 100% with the samples in the database.

Conclusion: The presence of the bacterium in milk demonstrates the need for its active research into properties, as it can cause economic losses and infect humans. In addition, it demonstrates the circulation of *C. burnetii* in different municipalities, showing that the bacteria have the potential to

cause outbreaks and cases of Q fever. Therefore, it is necessary that surveillance be carried out continuously so rural producers can be made aware and to draw the attention of public health agencies to the adverse effects of infection by the agent and contamination of food, such as milk and its by-products, in addition to contamination environmental, since *Coxiella burnetii* is reported worldwide.

Keywords: Coxiellosis, Q Fever, Bulk tank, Bovine milk, Molecular diagnosis.

PH-03

Molecular investigation of *Toxoplasma gondii* in raw bovine milk from small rural properties in Brazil

Simone Baldini Lucheis[1], Suzane Manzini[2], Amanda Bezerra Bertolini[3], Thainá Valente Bertozzo[2], Marcela Alexandrino[2], Nassarah Jabur Lot Rodrigues[2], Maria Eduarda Cavalheiro[2], Jackieline Sampaio Steinle[3], Andresa Xavier Frade Gomes[3], Isabella Neves Aires[4], Maria Izabel Merino De Medeiros[1], Virgínia Bodelão Richini-Pereira[5].

[1]Paulista Agency of Agribusiness Technology - APTA, Bauru, Brazil; [2]Department of Tropical Diseases and Image Diagnosis, São Paulo State University, Botucatu, Brazil; [3]Department of Animal Production and Preventive Veterinary Medicine, São Paulo State University, Botucatu, Brazil; [4]Department of Biology, São Paulo State University, Bauru, Brazil; [5]Adolfo Lutz Institute, Regional Laboratory of Bauru, Brazil., Bauru, Brazil.

Objectives: This study aimed to investigate *Toxoplasma gondii* in 102 samples of bovine milk from expansion tanks, in small properties located in different cities of the Midwest region of São Paulo, Brazil.

Material & Methods: Samples from bulk tank milk were collected from 102 family farms. The collection of milk samples was approved by the Ethics Committee on the Use of Animals – CEUA – protocol 1367/2020, of the Botucatu Medical School - UNESP. The samples were submitted to DNA extraction using the commercial GFX Genomic Blood Kit (GE Healthcare) with modifications and subsequently the polymerase chain reaction (PCR) was performed using specific primers targeting the *Toxoplasma gondii* ITS1 region. For the PCR the set of primers NN1 and NN2 were used and for the nested PCR, the primers TgNP1 and TgNP2 were used. The amplicons were subjected to 2% agarose gel electrophoresis, with fragments amplified at 227 bp.

Results: Thirteen samples (12,74%) were positive for *T. gondii.*

Conclusion: The presence of *Toxoplasma gondii* DNA in milk demonstrates the need for investigated into properties, due it can cause economic losses and infect humans. In addition, it demonstrates that circulation of *T. gondii* in different municipalities, showing the potential to cause outbreaks and cases of Toxoplasmosis. Therefore, it is necessary that surveillance be carried out continuously and to draw the attention of public health agencies to the adverse effects of infection by the agent and contamination of food, such as milk and derivatives, as well as contamination environmental, since *Toxoplasma gondii* is reported worldwide. Other diagnostic tests, like bioassay in experimental animals, would be opportune to validate the infection capacity of these samples.

Keywords: Toxoplasmosis, Bulk tank, Bovine milk, Molecular diagnosis, Zoonosis.

PH-04

Antimicrobial resistance profiles of *Escherichia coli* from dairy farms participating on an antimicrobial stewardship educational training program for farm employees

Adriana Garzon[1], Rafael Portillo[2], Gregory Habing[2], Noelia Silva-Del-Rio[3], Betsy Karle[4], Richard Pereira[3].

[1]Department of Population Health and Reproduction, School of Veterinary Medicine,University of California, Davis, Davis, United States; [2]Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, United States; [3]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, United States; [4]Cooperative Extension, Division of Agriculture and Natural Resources, University of California, Davis, Orland, United States.

Objective: The increase in antimicrobial resistant (AMR) bacteria is one of the biggest public health threats of our time. Although current AMR is monitored through reports such as the U.S. National Antimicrobial Resistance Monitoring System integrated report, there is a knowledge gap for on-farm AMR monitoring data. Our goal was to evaluate the antimicrobial resistance of *Escherichia coli* (EC) from pooled fecal samples before and after implementation of an on-farm animal health and diagnostic training program for farm workers in antimicrobial stewardship in adult dairy cattle.

Methods: Pooled fecal pat samples were collected from the hospital pen (cows treated with antimicrobials with a milk withhold period), the fresh pen (1 to 3 DIM) and the mid-lactation pens (90 to 150 DIM) in conventional dairies in CA (n=9) and OH (n=9). Fecal samples were collected as part of a larger study with a quasi-experimental design that assigned farms to training intervention group (TG; 9 per state) or control group (CG; 3 per state). For the TG, farm worker(s) identified as having the task of diagnosis and treatment of adult cows on the farm participated in training program on antimicrobial stewardship practices. Samples were collected at enrollment and three months after completing the intervention. For each pooled sample, EC was isolated. Standard culture, antimicrobial sensitivity testing using the broth microdilution approach, and categorization of isolates as susceptible, intermediate, or resistant was used. Logistic regression models were used to evaluate the association between EC antimicrobial resistance profiles and farm-level factors.

Results: A total of 504 EC isolates were tested for antimicrobial susceptibility. All isolates were susceptible to azithromycin and sulfisoxazole. The antimicrobial resistance more commonly found was against tetracycline (TET, 18.3%), streptomycin (STR, 16.3%), ceftriaxone (AXO, 10.7%) and ampicil-

lin (AMP, 11.1%). Resistance to ceftiofur (XNL) was only found in 1.98% of the isolates, one of the most commonly used drug on the study farms to treat sick cows, while 15.3% isolates were classified as multidrug resistant. Among the most commonly found MDR patterns were streptomycin-ceftriaxone-tetracycline (n=5), streptomycin-chloramphenicol-tetracycline (n=5), and streptomycin-ampicillin-chloramphenicol-tetracycline (n=4).

No significant effect on the proportion of AMR isolates was found associated to the intervention compared to the control farms. However, the univariate analysis showed that samples from OH had a higher proportion of AMR isolates to nalidixic acid (NAL) and TET, while samples from CA had a higher proportion of isolates resistant to XNL. There was also a higher proportion of EC isolates resistant to STR, XNL and TET from the enrollment visit compared to the visit after intervention was completed. Finally, samples from the fresh cow pen had significantly higher proportion of EC isolates resistant to cefoxitin (FOX), STR, AMP and NAL, while samples from the hospital and mid-lactation pens had higher proportion of isolates resistant to XNL and amoxicillin/clavulanic acid (AUG), respectively.

Conclusion: Differences on the antimicrobial resistance profiles of *Escherichia coli* from dairy farms included in this project were detected, and although, there was not a significant difference in the AMR profiles after the educational intervention**,** this supports the need for more research to get a better understanding of the resistome and its changes over time on dairy farms.

Keywords: Antimicrobial resistance, education, cattle.

PH-05

ESBL resistance genes in fecal *E. coli* of calves fed waste milk with antimicrobial residues

Manuel Cardoso[1], Inês Prata[1], Inês Rebelo[2], Telmo Nunes[1], Carla Carneiro[1], Ricardo Bexiga[1].

[1]*Faculdade de Medicina Veterinária, ULisboa, CIISA, Lisboa, Portugal;* [2]*Proleite, Oliveira de Azeméis, Portugal.*

Objectives: Beta-lactamases are enzymes capable of hydrolysing β-lactam antimicrobials, conferring resistance to Gram-negative bacteria. Among β–lactamase enzymes, the extended-spectrum β–lactamase enzymes (ESBL) provide resistance to a wide variety of β-lactam antimicrobials including penicillin and 2nd, 3rd and 4th generation cephalosporins. Infections caused by Gram-negative bacteria harbouring these enzymes are challenging to treat and have increased in incidence in the human as well as in the dairy cattle population. Feeding waste milk to calves, a mixture of excess colostrum, transition milk and non-saleable milk from cows that are being treated with antimicrobials, has been observed to lead to increased antimicrobial resistance in faecal isolates of calves. This study aimed to evaluate the association between feeding waste milk to calves and the occurrence of antimicrobial multi-resistance by extended spectrum β-lactamase enzymes through determining ESBL production by E. coli isolates from 32 dairy farms.

Material & Methods: In each farm, fecal samples were collected from the rectum of five healthy calves in the first month of life and pooled into a single container. Five isolates from each sample were selected and confirmed to be *E. coli* by amplification of the 16S gene. ESBL production was determined phenotypically on 148 isolates from 31 farms by use of antimicrobial susceptibility testing to cefotaxime, ceftazidine both alone and after impregnation with clavulanate. Genotypic confirmation of ESBL production was performed by PCR for the genes blaCTX-M-1, -2, -8, -9 and blaCMY-2. A questionnaire was performed regarding potential risk factors for the emergence of antimicrobial resistance. A univariate analysis was performed to evaluate which risk factors should be included in a multivariable logistic regression. Statistical analysis was performed with the software R®, version 3.5.1, with $p<0.05$ considered statistically significant.

Results: Phenotypically 40 *E. coli* isolates from 15 farms (48.4%) were found, whereas genotypically 55 isolates from 20 farms (64.5%) were found. The questionnaire revealed that 28 out of 31 farms (90.3%) fed waste milk to calves. On all of the farms that had this practice, waste milk comprised milk from animals being treated with antimicrobials and milk originating from animals during the withdrawal period for antimicrobial treatment. For 17 of the farms, waste milk also included milk from animals with high somatic cell counts. On 7 of the 31 farms an antimicrobial was added to milk, either waste milk or milk replacer, in a preventative way. The questionnaire also revealed that the number of different intramammary tubes used as treatment options for mastitis in the past year varied from 1 to 7. The multivariate analysis identified feeding milk with preventative antimicrobial to calves as the main risk factor with this routine leading to a 2.5 times higher risk of calves in their first month of life shedding ESBL-producing *E. coli*. Another risk factor identified by our study was the use of a higher number of antimicrobial treatment options for the treatment of mastitis, which led to a 1.3 times higher risk of calves shedding ESBL-producing *E. coli* on those farms. In our study, feeding waste milk to calves was not significantly associated with an increased risk for the presence of *E. coli* producing ESBL in the faeces of calves in the first month of life, although farms that had this routine, had a 5.2 higher risk of ESBL-producing E. coli.

Conclusion: Feeding waste milk to calves was a very common practice on the participating farms, with it including milk from animals under antibiotic treatment, during the withdrawal period for antibiotic treatments and milk from animals with subclinical mastitis. Some farmers also added antibiotic to waste milk to prevent neonatal calf diarrhea, a procedure that increased significantly the likelihood of calves in their first month of life shedding ESBL-producing *E. coli* in their feces. The other risk factor that significantly contributed to this outcome was the use of a higher number of intramammary antibiotic options to treat mastitis.

Keywords: ESBL, waste milk, calves, antimicrobial resistance.

PH-06

An observational cohort study on antimicrobial usage in dairy farms from Québec, Canada

Hélène Lardé, David Francoz, Marie Archambault, Jonathan Massé, Jean-Philippe Roy, Simon Dufour.

Université de Montréal, Saint-Hyacinthe, Canada.

Quantification of antimicrobial use (AMU) is crucial to measure the impact of intervention programs targeting a more judicious use, to determine associations between usage and resistance, to compare populations and to promote benchmarking.

Objectives: The objective of the study was to describe quantitatively AMU in Québec dairy herds over one year as a whole, then by administration route (intramammary, injectable, oral, intrauterine), and by category based on importance in human medicine according to Health Canada (4 categories: Category I "Very high importance", II "High importance", III "Medium importance", and IV "Low importance"). A secondary objective was to evaluate the effect of herd size, production level, and disease level on AMU.

Material & Methods: Data were obtained from 101 dairy farms randomly selected in 3 regions of the Québec province of Canada (Montérégie, Estrie, Centre-du-Québec) by collecting and recording all empty drug packaging between spring 2017 and spring 2018 (garbage can audit method). The quantity of medicated feed sold during the same period was obtained directly from the feed mills. Antimicrobial usage was reported in number of defined course doses (DCDbovCA) per 100 cows-year (calculated as a whole, by administration route, and by category). The intramammary route was separated between antimicrobial agents (AMs) administered during lactation for treatment of mastitis and AMs administrated at the time of drying-off. The oral route was separated between AMs administered as individual treatments (boluses, oral solutions, etc.) and AMs administered in the feed. The participating dairy producers completed an in-person questionnaire between January and March 2018. The number of dairy cattle in the farm was collected by age group, as well as the amount of milk produced. Questions were asked regarding number of animals experiencing some common diseases during the last 12 months by age group. Effect of herd size, production level, and diseases level on AMU was evaluated by using negative binomial regression models. A predictive model was also built to predict how the level of infectious diseases on a farm could explain its AMU.

Results: The average size of the population was 66.5 cows per farm (range 20-150); 2/101 farms were organic. Overall, a median of 429 DCDbovCA /100 cows-year was used. The most frequent administration was through the intramammary route: median of 161 and 67 DCDbovCA /100 cows-year for lactating and dry cow formulations respectively. A median of 47 and 12 DCDbovCA /100 cows-year was observed for the injectable route and the feed route respectively. The intrauterine route and the oral individual route (other than in the feed) were infrequently used (median of 0 DCDbovCA /100 cows-year). Category II antimicrobials were the most frequently used (median 181 DCDbovCA /100 cows-year), followed by categories III, I, and IV antimicrobials (median of 79, 62, and 23 DCDbovCA /100 cows-year respectively). Category I AMs were more frequently used as intramammary than injectable formulations. A positive and linear association was identified between herd size and global AMU. A positive and non-linear association was identified between diseases level and global AMU. Incidence of diseases only explained 31% of the global AMU, and 2.2% of Category I AMU.

Conclusion: This study provides a detailed description of the AMU over one year on Québec dairy farms. Oral and intrauterine formulations were infrequently collected and other methods of data collection should be considered to obtain a more complete picture of AMU for these formulations. The technique based on garbage can audit could be imperfect and incomplete for reporting on AMU in dairy farms; other methods such as using veterinary prescriptions (or invoices) or treatment records could be used in Québec to estimate the AMU. This study highlights that AMU is positively associated with herd size (mainly for Category IV AMs) and with level of diseases.

Keywords: Antimicrobial usage, dairy cattle, defined course dose, garbage can audit, quantification.

PH-07

Salmonella eradication on 57 dairy cattle farms in Finland

Olli Ruoho[1], Leena Seppä-Lassila[2], Miia Kontturi[2].

[1]Animal Health ETT, Seinäjoki, Finland; [2]Finnish Food Authority, Helsinki, Finland.

Objectives: Finland has a salmonella control program that covers cattle, pigs, and most of the poultry species. The aim of the control program is to maintain *Salmonella* prevalence of all serotypes below 1% in the food chain and thus reduce the risk of human salmonellosis. If *Salmonella* is detected on a farm or at the slaughterhouse sampling, it must be eradicated from the farm. On a dairy farm eradication includes e.g., sampling of animals and the environment, eliminating the possible infection source, and thorough cleaning and disinfection. For the present group insurances through dairies have been available for farms to partly cover the costs of eradication.

Our aim was to investigate *Salmonella* eradications on dairy farms, describe the principles of the eradication process and to clear out the most likely cause of the infection. Based on these findings, it would be easier to advice the prevention and guide the eradication of *Salmonella* on dairy herds.

Materials and methods: Our data included 57 dairy herds with *Salmonella* infection and an eradication that an experienced veterinary advisor (Dipl. ECBHM Olli Ruoho) had planned and guided during 2009–2019. These dairy herds had an average 76 milking cows (range 17-260). The most common serotypes detected were *S.* Typhimurium (n=30), *S.* Enteritidis (n=11) and *S.* Altona (n=4). We calculated the length of the eradication from the start and end date of the official restrictions of the farm prescribed by legislation. Based on environmental and animal sampling at the beginning of the eradication process, we defined the *Salmonella* infection of

the farm as non-existent, restricted, average, or vast.

Results: The eradication lasted 21-533 days (mean 114, median 90). It lasted longer (52-533 days, mean 156, median 120) on 31 farms (54%) where the infection was considered as average or vast at the beginning. Other typical reasons for a prolonged eradication were: if the source of the infection was undetected at the beginning, if salmonella positive animals were not culled in time, if cleaning and disinfection procedures were inadequate or lack of labour leading to exhaustion. On 46 farms (81%) the source of the infection was birds or rodents, and in most of these cases birds. Occasionally, the possible source was *Salmonella* positive animals, humans, or contaminated feed. However, the management of salmonella risk in commercial feeds is at a high level in Finland.

Conclusions: Eradicating *Salmonella* from a dairy farm is possible. However, proper planning is essential as well as working systematically throughout the eradication process. Furthermore, a competent supervision and guidance during the process are important as well as having enough skilled labour on the farm. Nevertheless, the focus should be on *Salmonella* prevention by improving the on-farm biosecurity like protecting the barn and especially feed storage from birds in a way that there will be no easy access to feed. This includes furnishing the barns with anti-bird nets, covering all the feed storage, maintaining the barn and farm area tidy that uneaten feed is taken away regularly, and enhancing the overall pest control. Moreover, the whole sector should discuss the means how to reduce the risk of spreading *Salmonella* infections from dairy farms to calf rearing units and thereby to beef production chain since all calves can not be tested against *Salmonella*.

Keywords: Salmonella eradication, dairy cattle, biosecurity.

PH-08

Fractionation of milk for trace analysis of contaminants and residues

Jan Steils[1], Christian Baumgartner[1], Klaus Schöne[2].

[1]PureMilk analytical GmbH, Pörnbach, Germany; [2]Sartorius Lab Instruments GmbH & Co. KG, Göttingen, Germany.

Objectives: Contamination of dairy products with detergents and disinfectants and their degradation products such as chlorate and perchlorate raise challenges for stakeholders in the dairy food chain, as these substances are a potential threat for food safety. The aim of this work was to develop a passive sample preparation for raw milk, apart from the well-known Quechers or Quppe methods, which does not require any additional extraction agents and enables a cost-effective just-in-time analysis of milk raw materials for chlorate and perchlorate.

Materials and Methods: Unprocessed raw milk was separated into the phases “water”, “fat” and “protein”, using a fractionation unit (FraMiTrACR C/PC®). The separation by FraMiTrACR C/PC® requires the use of a standard table top centrifuge. Different sample volumes were processed, both 10 mL raw milk using a fixed angle rotor at 30 minutes and 2,000 x g and 5 mL raw milk using a "swing out" rotor at 30 minutes and 4,000 x g. For both settings, half of the volume of feed added was recovered as the water phase. For the analysis of the water phase, an ion chromatography system was used, the 930 Compact IC Flex System with Dosin gradient technology for the determination of anions after sequential suppression and conductivity detection (IC-CD). For each determination, 0.25 mL of water phase was injected into the analyser. At the beginning of the development, the anion matrix in the water phase was collected from milk samples that were free of chlorate and perchlorate. To improve the detection limit, the characteristic anion matrix of the water phase has been subtracted when evaluating the results. The chlorate and perchlorate content was determined using spiked samples in standard series and also in native samples. In order to obtain comparability with previously used methods, the investigated raw milk samples were simultaneously analysed in a contract laboratory using the modified Quppe method and liquid chromatography with tandem mass spectrometry coupling (LC-MS/MS).

Results: In direct comparison to well-established sample preparation methods, such as Quechers and Quppe, there is a significant saving in working time and thus personell costs due to the passive sample preparation using a table top centrifuge. In addition, the complete elimination of extraction solvents or other additives leads to further cost savings in the laboratory process chain, simultaneously excluding the risk of unwanted contamination. In the water phase, detection limit of 0.003 mg/kg chlorate and perchlorate could be achieved by using IC-CD. In the analysis of the water phase by means of LC-MS/MS detection limit of down to 0.001 mg/kg chlorate and perchlorate could be achieved.

Conclusions: The application of the fractionation unit (FraMiTrACR C/PC) shows that it is possible to determine defined analytes directly from the water phase of the milk without further preparation. The advantage of this method is that milk samples are prepared in one step, passively and without further additives. This leads not only to a reduction in personnel costs by reducing the active working time of the laboratory staff, but also to a reduction in the costs of operating resources, stock-keeping and stock management of operating resources. Sample contamination is prevented as well, as the sample only needs to be filled into the fractionation unit (FraMiTrACR C/PC) and then closed. The risk of carry-over or contamination within the laboratory can thus be reduced to a minimum. With the method described, it will also be possible, depending on the analytical equipment available, to analyse raw milk just-in-time before processing. It should be emphasised that sample preparation and subsequent analysis using a comparatively low-cost method, IC-CD, led to comparable results to sample preparation using the modified Quppe method and analysis by LC-MS/MS. In the methods compared, the limit of quantification was 0.01 mg/kg chlorate and perchlorate and the detection limit was below 0.01 mg/kg chlorate and perchlorate. This also shows that the sample preparation method can be used universally for many other analytical methods. Basically, the fractionation unit (FraMiTrACR C/PC) opens up new possibilities for residue and contaminant determination in dairy products. More analytes will be investigated with this method in further studies.

Keywords: Fractionation, milk, residues, chlorate, perchlorate.

PH-09

Knowledge, behaviours and attitudes of Scottish dairy farmers towards antimicrobial use

Elena Borelli, Kathryn Ellis, Martin Tomlinson, Emily Hotchkiss.

University of Glasgow - Veterinary School, Glasgow, United Kingdom.

Objectives: Scientific evidence demonstrates that antimicrobial usage (AMU) in animals may contribute to the emergence of antimicrobial resistance (AMR). In the UK, dairy farmers can administer antibiotics without the veterinarian being present and thus, they play a crucial role in the decision making around AMU. Further understanding of how antimicrobials are used in dairy production as well as stakeholder beliefs relating to their use is essential to help ensure responsible AMU. To the authors' knowledge, no work to date has been carried out exploring these aspects in the Scottish dairy sector. Therefore, the aims of this study were: to identify the factors influencing knowledge, AMU and attitudes towards AMR and to determine the barriers and motivators to uptake of best practice recommendations.

Materials and Methods: To address this study, a questionnaire was designed for administration via an online survey; the target population was Scottish dairy farmers (n=840). To inform the design of the questionnaire, a focus group and a workshop were held with representatives of the industry with the aim of eliciting free discussion around the topics of AMU and AMR. As a result of the COVID-19 pandemic, the focus group was held via Zoom, and participants (n=5) were a convenience sample of farmers. The workshop was part of an online agricultural event (Agriscot).

The questionnaire was structured in four main sections. Section One explored farmers' awareness and understanding of antibiotics and AMR. Section Two contained some questions to identify factors that may influence farmers' AMU, as well as the main barriers and drivers for responsible usage. In addition, some specific scenarios of dairy cows' diseases were posed to assess farmers' behaviour. Section Three investigated farmers' attitudes towards AMR and AMU best practices. Section Four covered demographic information of the participants and the farm.

A pilot study was performed using a preselected group of farmers (n=5) to test survey duration and suitability of the questions to the target population. The questionnaire was launched online and was open from May to September 2021. The survey URL was promoted via multiple ways (farming press, social media, veterinary practices, and milk buyers).

Data analysis was performed using R Core Team (2020). Responses were analysed using descriptive statistics. Two outcomes were explored in initial analyses – participant knowledge and AMU. These were derived scales, determined by answers provided in sections One and Two. Univariable logistic regression models were established to explore the association of knowledge and AMU with demographic characteristics, attitudes and other various factors such as frequency of contact with the veterinarian and implementation of AMU best practices. Significant explanatory variables from univariable analysis (P<0.2) were included in multivariable logistic regression models for the two outcomes.

Results: The survey was completed by 61 respondents (7.3 % of the total target population). Overall, farmers expressed good understanding of the phrase antimicrobial resistance (AMR) however, 31 % thought antibiotics were effective against viruses and 25 % against parasites. The vast majority (90%) had implemented practices to reduce AMU, such as written treatment protocols and selective dry cow therapy. Additionally, 70 % expected a further reduction in AMU in the future. Limited knowledge and poor facilities were reported as the main barriers. It was found that veterinarians are usually not consulted before treating animals with antibiotics, even if they are regarded as the most influencing and reliable source of information. The majority of farmers (89 %) thought it was important to reduce AMU on farm; however, only half of them were concerned about AMR in the dairy sector. Future work will look at associations between outcomes, such as farmer knowledge and AMU, and selected explanatory variables.

Conclusions: For dairy practitioners, understanding farmers behaviour and attitudes is essential to engage with them towards antimicrobial usage reduction. These preliminary results suggest farmers' need for more training and support for responsible AMU and veterinary practitioners have a key role on this. Farmers generally agreed on the importance to reduce AMU; however, they often do not perceive AMR as a current threat to their own farm. It is important for veterinarians to consider the different levels of attitude and awareness of farmers in order to implement tailored interventions.

Keywords: Antimicrobial resistance, dairy, farmer behaviour.

PH-10

Antimicrobials resistance in *Escherichia coli* and *Enterococcus fecalis* commensal bacteria in north-eastern Italian dairy farms

Matteo Cornaggia[1], Elena Mazzolini[2], Luca Palazzolo[3], Benedetta Cordioli[3], Alessia Rizzardi[3], Serena Bottin[3], Giulia Zarpellon[3], Angela Guolo[3], Tiziana Ferro[3], Cosetta Bacchin[3], Luca Bano[3].

[1]Istituto Zooprofilattico Sperimentale delle Venezie, Padova - Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna, Brescia, Italy; [2]Istituto zooprofilattico sperimentale delle Venezie, Padova, Italy; [3]Istituto zooprofilattico sperimentale delle Venezie, Treviso, Italy.

Objectives: Antimicrobial resistance (AMR) is of public and animal health concern worldwide.

Aim of our work was to identify an AMR indicator to classify healthy dairy herd according to antimicrobial susceptibility (AST) in indicator bacteria *Escherichia coli* (EC) and *Enterococcus fecalis* (ENT) towards an harmonized list of antimicro-

bials as suggested (European Food Safety Agency (EFSA), 2019). The overall farm health condition or prevalence of animal diseases were not considered yet the animal category (whether milking cows (VL), dry cows (VA), heifers (M) and calves (V)) of the isolate origin was analyzed to identify within heard different source of AMR.

Materials and methods: Thirty-three dairy farms were enrolled in this study, caring that both small and larger farms located in foothills or ground level of the Veneto and Friuli-Venezia Giulia regions of Italy were selected. According to preliminary analysis (data not shown in this paper) in each herd the following sampling protocol was applied: individual fecal samples were collected VL, VA, M and V to isolate 20 *Escherichia coli* (EC) and 10 *Enterococcus fecalis* (ENT). Isolates were AST with broth microdilution (CLSI VET01, 2020) using a commercial kit EUVSEC3 and EUVENC (ThermoFisher, US) to determine the minimum inhibitory concentration (MIC). MICs were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) epidemiological cut-off value (ECOFFs;www.eucast.org/mic_distributions_and_ecoffs/). Isolates displaying MICs above the ECOFF were classified as non-wild type. Antimicrobial resistance index (ARI) was calculated for each bacterial isolate by dividing the number of resistances by the number of antimicrobials tested to provide descriptive statistics of the AMR herd indicator as reported (Agnoletti, 2018).

Results: 620 EC isolates and 229 ENT were AST assessed. The percentage of non-wild strains for each animal categories for each active ingredient are summarized in table 1 and table 2 for EC and ENT, respectively. EC isolates showed high proportions of non-wild phenotype towards sulfamethoxazole and tetracycline. In calves EC non-wild phenotypes are largely diffuses when compared to the other animal categories. Among ENT, non-wild phenotypes were mainly observed for gentamicin (dry cows), erythromycin (calves) and tetracycline (calves).

The herd level indicator ARI ranged from 0 to 0.14 (mean 0.08) for *E. coli* towards 13 antimicrobials tested (presumptive

Tables.

Antimicrobial	A	L	M	V
Sulfamethoxazole	35/68(51.5)	131/254(51.6)	69/160(43.1)	103/138(74.6)
Trimethoprim	2/68(2.9)	5/254(2)	6/160(3.8)	29/138(21)
Ciprofloxacin	1/68(1.5)	2/254(0.8)	3/160(1.9)	28/138(20.3)
Tetracycline	3/68(4.4)	14/254(5.5)	15/160(9.4)	71/138(51.4)
Meropenem	0/68(0)	0/254(0)	0/160(0)	0/138(0)
Nalidixic Acid	1/68(1.5)	0/254(0)	3/160(1.9)	24/138(17.4)
Azithromycin	0/68(0)	0/254(0)	0/160(0)	0/138(0)
Cefotaxime	1/68(1.5)	4/254(1.6)	0/160(0)	8/138(5.8)
Chloramphenicol	2/68(2.9)	3/254(1.2)	1/160(0.6)	19/138(13.8)
Tigecycline	0/68(0)	0/254(0)	0/160(0)	0/138(0)
Ceftazidime	0/68(0)	0/254(0)	2/160(1.2)	2/138(1.4)
Colistin	0/68(0)	1/254(0.4)	0/160(0)	0/138(0)
Ampicillin	4/68(5.9)	8/254(3.1)	8/160(5)	53/138(38.4)
Gentamicin	0/68(0)	0/254(0)	1/160(0.6)	6/138(4.3)
Presumptive_ESBL	1/68(1)	4/254(2)	2/160(1)	8/138(6)

Table 1. Number of non-wild strains of E. coli divided for animal category.

Antimicrobial	A	L	M	V
Gentamicin	26/33(79)	40/73(55	8/16(50	60/107(56)
Chloramphenicol	0/33(0)	5/73(7)	0/16(0)	21/107(20)
Ampicillin	0/33(0)	1/73(1)	0/16(0)	1/107(1)
Vancomycin	0/33(0)	0/73(0)	0/16(0)	0/107(0)
Teicoplanin	0/33(0)	0/73(0)	0/16(0)	0/107(0)
Erythromycin	10/33(30)	15/73(21)	3/16(19)	38/107(36)
Quinuopristin_Dalfopristin	4/33(12)	9/73(12)	2/16(13)	9/107(8)
Tetracycline	14/33(42)	19/73(26)	2/16(13)	73/107(68)
Tigecycline	0/33(0)	0/73(0)	0/16(0)	0/107(0)
Linezolid	0/33(0)	2/73(3)	0/16(0)	0/107(0)
Daptomycin	3/33(9)	4/73(5)	0/16(0)	3/107(3)
Ciprofloxacin	0/33(0)	0/73(0)	0/16(0)	2/107(2)

Table 2. Number of non-wild strains of *E. fecalis* divided by animal category.

ESBL were defined as being not wild for at least one among cefotaxime and ceftazidime) and 0.04 to 0.29 (mean 0.13) for *E. faecalis* towards (12 antimicrobials tested).

Conclusions: Microbiologic resistance against molecules widely employed in Veterinary Medicine (sulphonamides, tetracyclines, aminoglycosides, macrolides, and phenicols) was detected in the sampled dairy herds. Yet with the exception of fluorchinolones, EC isolated showed low levels of microbiologic resistance against antimicrobials classified of highest critical importance for human therapy (hstCIA; WHO, 2020). However, enterococci displayed high levels of microbiologic resistance towards erythromycin and gentamycin. Compared to adult bovines, including heifers 6 months aged, isolates from calves were keener to microbiologic resistance, which may be attributable to inappropriate farmer behavior of feeding calves with waste milk from cows treated with antimicrobials. Calves must be considered when monitoring AMR to avoid underestimate data.

References:

Agnoletti F, et al. Longitudinal study on antimicrobial consumption and resistance in rabbit farming. International Journal of Antimicrobial Agents 2018.

Keywords: Antimicrobial resistance, dairy cows, calves.

PH-11

Critically important antimicrobials are not needed for treating clinical mastitis in lactating dairy cows

Diego Nobrega, Syed Naqvi, Herman Barkema.

University of Calgary, Calgary, Canada.

Objectives: There is ongoing debate regarding whether critically important antimicrobials (CIAs) should be used to treat infections in food-producing animals. Mastitis accounts for majority of worldwide antimicrobial usage (including CIAs) in dairy cattle. Objectives of this network meta-analysis (NMAs) were to evaluate whether critically important antimicrobials (CIAs) were necessary to treat non-severe clinical mastitis (CM) caused by the most commonly isolated bacterial pathogens worldwide. The literature reporting on bacteriological cure (BC) rates of antimicrobials used to treat lactating dairy cows with non-severe CM was analyzed using a set of networks.

Material and Methods: On March 30, 2019, the databases CAB Abstracts, Web of Science (all databases), MEDLINE, Scopus (Elsevier) and PubMed were screened for potentially relevant articles reporting on pathogen-specific BC rates of antimicrobials for treating non-severe CM in lactating dairy cows. Two reviewers reviewed all titles and abstracts independently. Prior to analyses, categories of antimicrobial treatment protocols, supportive therapy and pathogens were generated. Antimicrobial treatment protocols were grouped using categories defined a priori based on the WHO 5th revision of Critically Important Antimicrobials for Human Medicine and route of administration (systemic and intramammary). A frequentist approach using generalized linear models applied using graph theory was used to develop networks. Five NMAs were carried out according to most commonly reported bacteria causing CM worldwide (*Staphylococcus aureus*, *Escherichia coli*, non-*aureus* staphylococci, environmental streptococci and *Klebsiella* spp.). Potential sources of heterogeneity and inconsistency, including use of supportive therapy, were assessed using sensitivity analysis.

Results: Our search strategy yielded 9,173 records, from which 30 studies were included. Out of 30 studies, 25 were randomized controlled trials. *Staphylococcus aureus* was the most frequently studied pathogen (22 studies), whereas relatively few studies (n = 8) reported on BC of *Klebsiella* spp. There was no evidence supporting the need of CIAs for treating non-severe CM caused by most commonly reported bacteria worldwide; no protocol including the use of CIAs had superior BC rates of non-severe CM than protocols relying on non-CIAs. Additionally, there was no evidence to support use of antimicrobials for treating non-severe CM caused by *Klebsiella* spp. or *E. coli*, as the probability of BC was similar for treated versus untreated cows.

Conclusions: To our knowledge, this is the first systematic review comparing efficacy of CIAs and non-CIAs for treating non-severe CM caused by the 5 most commonly isolated bovine mastitis pathogens worldwide. Antimicrobials other than CIAs were equally effective to treat non-severe CM in dairy cows. In addition, antimicrobial treatments did not alter BC rates of non-severe CM caused by the Gram-negative bacteria *E. coli* and *Klebsiella* spp., although the relatively low number of studies reporting on BC rates of CM caused by *Klebsiella* spp. warrants further investigation. Due to the comparable efficacy of CIAs and non-CIAs and assuming all other variables impacting safety, choice and use of antimicrobials are equal, no adverse effects in terms of animal health and welfare should be expected by ceasing use of CIAs for treating non-severe CM in dairy cattle. Findings from this study will be important to inform public strategies aimed to promote antimicrobial stewardship in veterinary medicine.

Keywords: Antimicrobial resistance, antimicrobial use, critically important antimicrobials, dairy cows, mastitis.

PH-12

Longitudinal study of fecal commensal Gram-negative bacteria resistant to critically important antimicrobials found in healthy lactating dairy cattle on three farms from Île-de-France

Vincent Plassard[1], Sophie Granier[2], Philippe Gisbert[3], Yves Millemann[1].

[1]Alfort National Veterinary School and AnTiBioresistance Mission, Maisons-Alfort, France; [2]Anses Fougères and AnTiBioResistance Mission, Fougères, France; [3]CEVA, Libourne, France.

Objectives: The specific objectives of the project were to evaluate on a convenient sample of three dairy cattle farms the baseline intestinal colonization with commensal gram-negative bacteria resistant to carbapenems and 3rd generation

cephalosporins (3GC) using specific selective media and normalized methods for evaluation of antibiotic resistance.

Materials and methods: Three peri-urban dairy farms were selected because they are included in a regular reproductive follow-up by Alfort Bovine Theriogenology Unit. The study lasted 1 complete year. Basically, 10 healthy producing cows were sampled in each herd on a monthly basis. The samples consisted in rectal swabs, easy to make and to proceed rapidly afterwards in the lab. In addition, the bulk tank filter was sampled monthly in 2 out of 3 herds. The selective used media aimed at selecting for Gram-negative bacteria resistant to 3rd generation cephalosporins and carbapenems. For that purpose, we used 2 commercial media (Carba and Oxa48) (ChromID®, Biomérieux) and one especially prepared media on the basis of scientific literature (MacConkey with 1 mg cefotaxime /L). Isolates were identified through a Maldi-Tof device, and resistance to critically important antibiotics was confirmed via disk diffusion antimicrobial susceptibility testing implemented on Mueller Hinton agar according to EUCAST recommendations.

Results: In farm A, the average monthly percentage of sampled cows harboring bacteria resistant to 3rd generation cephalosporins was over 15% (table 1). This percentage was highest in June at 70%. Among the 16 resistant isolates collected during the year, antimicrobial susceptibility revealed that none was resistant to carbapenem, but all were displaying an AmpC or an ESBL phenotype. AmpC producers all belonged to *Enterobacter* genus; one ESBL producer belonged to the *Pseudomonas* genus, and all others belonged to the *Escherichia* genus. All these ESBL producers were multi-drug resistant (MDR) bacteria according to the classification of Magiorakos *et al.*

In farm B, only one resistant bacterium was isolated on one cow in June; this was a *Pseudomonas aeruginosa* exhibiting an AmpC-producing phenotype. By contrast, the monthly sampling of bulk tank filters revealed the presence and persistence almost entirely throughout the year of AmpC-producing *Pseudomonas aeruginosa* in the milking machine (table 2). All of these isolates were resistant to imipenem and MDR.

In farm C, no bacterium resistant to 3rd generation cephalosporins or carbapenems was isolated in cattle during the year. As in farm B, the monthly analysis of bulk tank filters also revealed the presence and persistence throughout the year of AmpC-producing *Pseudomonas aeruginosa*. Only 20% of these isolates were resistant to imipenem, yet all of them were still considered MDR.

	Farm A	Farm B	Farm C
Total number of phenotypically resistant bacteria	75	21	36
Number of presumptive ESBL and/or AmpC producers (%)	16 (21.3%)	1 (4.8%)	0 (0%)
Number of presumptive ESBL producers (%)	14 (18.7%)	0 (0%)	0 (0%)
Number of presumptive AmpC producers (%)	2 (2.7%)	1 (4.8%)	0 (0%)
Number of MDR bacteria (%)	14 (18.7%)	1 (4.8%)	0 (0%)

Table 1: number of resistant bacteria harbored by sampled healthy dairy cattle in each farm during the year 2018.

	Farm B	Farm C
Total number of phenotypically resistant bacteria	83	117
Number of presumptive ESBL producers (%)	0 (0%)	0 (0%)
Number of presumptive AmpC producers (%)	12 (14.5%)	10 (8.5%)
Number of MDR bacteria (%)	12 (14.5%)	10 (8.5%)

Table 2: number of resistant bacteria isolated in bulk tank filters for farms B and C during the year 2018.

Conclusions: The intestinal colonization baseline of healthy dairy cattle by Gram-negative bacteria resistant to 3GC or carbapenems on a convenient sample seems to vary greatly between farms. By monitoring this resistance for a whole year on a monthly basis, we observe an increase of the proportion of resistant bacteria during summer. These data will be confronted to the antimicrobial consumption data as well as pasture and fertilization conditions in these farms.

The presence in the milking machine of AmpC-producing phenotype *Pseudomonas aeruginosa* in Farms B and C needs to be confirmed and investigated further by determining the support of this resistance. The milking industry would be greatly impacted in terms of public health image should this reservoir be confirmed.

Acknowledgements: Christelle Gandoin and Corinne Bouillin are gratefully acknowledged for expert technical assistance.

Keywords: Antimicrobial, critical, cattle, baseline, France.

PH-13

Monitoring antimicrobial consumption in the Dairy Sector in Portugal - system structure and results

João Niza-Ribeiro.

ICBAS-UP, Porto, Portugal.

Objectives: Monitoring antimicrobial (AM) consumption in the dairy sector is relevant to assess the quantity, quality and reasons for antimicrobials usage.

The presentation describes the Portuguese system for antimicrobial consumption (PSAC) of the dairy sector and presents first results on antimicrobial consumption patterns from three years of farm activity.

The PSAC provides data for the ESVAC system, records and analyse the data to provide benchmark information to farmers and veterinarians, help in establishing the baseline for future target setting at farm level and serves also scientific purposes.

Material & Methods: The PSAC is a conjoint initiative from the Portuguese Veterinary Authority (DGAV), the Portuguese Dairy Farmers Association (ANABLE) and Faculty of Biomedical Sciences Abel Salazar- Porto University (ICBAS-UP).

Antimicrobial consumption (AMC) data used by PSAC is supplied by the BOVNIFOR ® system. This is a WEB base information system that harbours more than 70% of the dairy farms in Portugal: The Continent and Azores Islands. It is operated at national level by the dairy farmers' association ANABLE. Farms in PSAC adhere on a voluntary basis and are selected from the database of BOVINFOR ® in order to represent, stratified by region and by size, the Portuguese dairy sector. The objective is to reach 250 dairy farms.

BOVINFOR ® MEDICAMENTOS records all batches of AM purchased by the farms using the system; the registry at the farm, of AM and other medicines prescribed by the veterinarian is compulsory in Portugal. Beside AM consumption, PSAC also collects from BOVINFOR ® data from every animal present at the farm the last day every month, categorized as milking cow, dry cow or by age group otherwise – from calf to heifer; milk production for every milking cow and somatic cell count are also collected.

Data treatment involves the calculation of Defined Daily Doses for animals (DDDvet) and Defined Course Doses (DCDvet) after the ESVAC recommendation, 2016. The consumption of each AM substance per farm and the average weight of animals' is calculated for the entire year after ESVAC, 2016. In the analysis DDDvet or DCDvet are expressed by 100 cow/day or cows/year. Other metrics are also calculated, like DDDvet or DCDvet per ton of milk produced yearly. Analysis is performed for injectable, oral, intramammary and intrauterine formulations. The use of Critically Important (CIA) AM is analysed separately.

Standardized costing of AMC within the herds is performed attributing an average cost per product after yearlly consultation of the market. Total quantity of products acquired per year provides basis for calculation.

Descriptive statiscical analysis of the data is performed as well as Principal Component Analysis to look for dominant consumption patterns.

Reporting is available on line, to users, using an interactive web-based dashboard. The dashboard allows for the farms have a benchmark with farms from the same region and size category. Standardized costing of AMC per farm, per cow in milk/year and per ton of milk is calculated and presented in dashboard.

Results: The structure and operation of the PSAC is presented and discussed. Advantages and limitations of treating data from AM purchasing rather than from consumption, as well as the methodology for cost calculation are discussed.

Data from more than 100 farms of AMC and heterogeneity among farms is analysed. The variation in DDDvet or DCDvet reaches fivefold. The profile of AM substances used is wide even when the same prescriber is involved: the farmer seems to play an important role deciding the AM substances used. The use of CIA is analysed and doesn't seem to play important role in higher production of better SCC.

The dashboard will be explored in the presentation and the way forward is discussed.

Conclusion: The PSAC approach involves the relevant stakeholders: veterinary authorities, farmers, farm veterinarians and academia which has so far proven to be a very positive solution.

Data analysis suggests high heterogeneity of AMC among herds offers ample opportunities for reduction and improvement . Analysis of the use CIA raise interesting hypothesis about the relevance of systematic use and deserves investigation in the future.

The use of interactive web-based dashboard is a popular solution for the farnmers and veterinarians involved.

Keywords: ESVAC, Dairy, Antimicrobial Consumption.

PH-14

Evaluation of the longitudinal effect of metaphylaxis treatment of preweaned dairy calves with enrofloxacin or tulathromycin on the susceptibility of antimicrobial resistant fecal *E. coli*

Richard Pereira[1], Craig Altier[2], Julie Siler[2], Sabine Mann[3], David Jordan[4], Warnick Lorin[5].

[1]Department of Population Health and Reproduction, College of Veterinary Medicine, University of California Davis,, Davis,CA, United States; [2]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell, University, Ithaca, NY, United States; [3]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell, University, Ithaca,NY, United States; [4]Division of Primary Industries, Industry & Investment NSW, Wollongbar, NSW, Australia; [5]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell, Ithaca,NY, United States.

Objective: The objective of this study was to longitudinally quantify *E. coli* resistant to ciprofloxacin and ceftriaxone in calves treated with enrofloxacin or tulathromycin for the control of bovine respiratory disease. Dairy.

Material and Methods: Calves 2 to 3 weeks old were randomly selected and enrolled in each study group: (1) receiving single label dose of enrofloxacin (ENR)(n=22) ; (2) receiving single label dose of tulathromycin (TUL)(n=23); or (3) serving as a control and not receiving an antimicrobial treatment (CTL)(n=20). Calves were housed in individual hutches and at approximately 60 days of age weaned and housed in group pens. Fecal samples were collected immediately before the administration of the antimicrobial treatment and at days 2, 4, 7, 14, 21, 28, 56, and 112 days after beginning treatment. Samples were used for qualification of *E. coli* using a selective hydrophobic grid membrane filter (HGMF) master grid. Kruskal-Wallis test was used to compare proportion of *E. coli* resistant to ciprofloxacin and proportion of *E. coli* resistant to ceftriaxone by treatment group over time.

Results: ENR had a significantly higher proportion of *E. coli* resistant to ciprofloxacin when compared to the CTL and TUL at time points 2, 4 and 7. At time point 28, a significantly higher proportion of *E. coli* resistant to ciprofloxacin was observed only when compared to CTL. TUL had a significantly higher proportion of *E. coli* resistant to ciprofloxacin when compared to the CTL at time points 2, 4 and 7. Lack of significant difference in shedding of ciprofloxacin resistant *E.coli* in ENR and TUL compared to CTL was because of lower total

CFU/g of feces shedding of ciprofloxacin resistant *E.coli* in these treatment group. None of the treatment groups resulted in a significantly higher proportion of *E. coli* isolates resistant to ceftriaxone.

Conclusion: Our study identified that treatment of calves at high risk of developing BDR with either enrofloxacin or tulathromycin resulted in a consistently higher proportion of ciprofloxacin resistant *E. coli* in fecal samples.

Keywords: ENrofloxacin; prohylaxis; dairy calves; BRD.

PH-15

Effect of heat and pH treatments on degradation of ceftiofur in whole milk

Richard Pereira, Adriana Garzon, Pramod Pandey, Lisa Tell, Sharif Aly, Robert Poppenga.

University of California Davis, Davis,CA, United States.

Objectives: Waste milk (milk that contains drug residues and high somatic cell counts) feeding practices of preweaned dairy calves have been implicated as a potential source for disseminating antimicrobial resistant bacteria among animals and the environment. Two interventions that have shown potential for degrading antimicrobial drugs in milk are heat and pH treatment. The aim of this study was to evaluate the effect of heat and pH treatments on the degradation of ceftiofur and ceftiofur free acid equivalents in milk at concentrations previously found in waste milk on dairy farms by spiking saleable pasteurized whole milk with ceftiofur sodium.

Material and Methods: Three heat treatments of ceftiofur sodium spiked milk were evaluated for their ability to degrade ceftiofur: 63°C for 30 minutes (LTLT), 72°C for 15 seconds (HTST) and 92°C for 20 minutes (HTLT). Two pH treatments of ceftiofur sodium spiked milk were evaluated: pH 4.0 (LpH) and pH 10 (HpH). Control samples spiked with ceftiofur sodium were kept at room temperature and samples collected at corresponding times for heat and pH treatments. Four treatment replicates were performed for each treatment group. Ceftiofur was quantified in milk samples using liquid chromatography mass spectrometry (LC-MS/MS) and ceftiofur free acid equivalents (CFAE) were measured using high-performance liquid chromatography (HPLC).

Results: HTLT resulted in a degradation of 35.24% of the initial concentration of ceftiofur. Ceftiofur degradation did not differ between control and the remaining two heat treatment groups (LTLT and HTST). HpH resulted in degradation of the 95.72 and 96.28% of the initial concentration of ceftiofur and CFAE, respectively.No significant changes in the degradation of ceftiofur or CFAE were observed for control or LpH treatments.

Conclusion: In conclusion, our study results were that alkalinizing milk to pH 10 and heating milk to 92°C for 20 minutes degraded ceftiofur and CFAE in spiked simulated waste milk demonstrated promising potential as treatment options for degrading ceftiofur and CFAE in waste milk, and further research is needed to evaluate the viability for implementation of these treatments in dairy farms.

Keywords: waste milk; drug residues; antimicrobial resistance.

PH-18

Antimicrobial sensitivities of mastitis pathogens amongst herds with different dry cow therapy usage history

Scott McDougall[1], David Dymock[2], Amanda Kilby[2], Joanne Holter[3].

[1]Cognosco, Morrinsville, New Zealand; [2]SP Animal Health Ltd., Upper Hutt, New Zealand; [3]SP Animal Health Ltd, Upper Hutt, New Zealand.

Objectives: Antimicrobial use in animal production faces increasing scrutiny due to concerns about potential selection for antimicrobial resistance. Intra-mammary dry cow therapy (DCT) is the largest single indication for antimicrobial use on dairy farms in New Zealand (Compton and McDougall 2014). DCT containing the beta lactams cephalonium, cloxacillin or cloxacillin/ampicillin in combination, are the most commonly-used in New Zealand. However, the relationship between the use of cephalonium or ampicillin/cloxacillin-based DCT and the development of antimicrobial resistance has not been examined in New Zealand. The objective of this study was to compare the distribution of Minimum Inhibitory Concentrations (MICs) for common mastitis pathogens from cows in herds exposed to cephalonium or cloxacillin-based DCT, relative to isolates from cows on organic farms where there has been no recent antimicrobial exposure.

Material and Methods: Herds were selected on the basis of not having used any antimicrobial for at least three years (organic; n=7 herds) or having used either cloxacillin/ampicillin dry cow therapy (cloxacillin/ampicillin; n=11 herds) or cephalonium DCT (cephalonium; n=8 herds) as the predominant DCT in the preceding three years.

In each of these herds, quarter-level milk samples (n=793) were collected from all cows (n=984) with an SCC >200,000 cells /mL at the most recent herd test. Cows that had been treated with antibiotics for mastitis in the 30 days prior to sampling were excluded. The MIC of coagulase-negative Staphylococci (CNS), *Staphylococcus aureus*, *Streptococcus uberis* and *Streptococcus dysgalactiae* isolates were determined using a broth microdilution plate which included 10 antimicrobials (Mastitis plate, Trek Diagnostics, CMV1AMAF).

The MIC values were categorised as sensitive or resistant using Clinical and Laboratory Standards Institute (CLSI) categories (where available) and the effect of herd DCT exposure on the MICs within each pathogen/antimicrobial combination was analysed using the nonparametric Kruskal Wallis rank test and by ordinal logistic regression.

Results: For CNS isolates (n=240), 13% and 32% were resistant to ampicillin and to penicillin, respectively. Less than 3% of the CNS isolates were resistant to the other 7 antimicrobials tested (including 0.8% resistant to cephalothin). Isolates from organic herds had a lower risk of being in a higher MIC category for ampicillin (OR=0.14) and penicillin (OR=0.18) than isolates from cephalonium herds. For 29% and 35% of

isolates were resistant to ampicillin or penicillin, respectively. Resistance was detected in 0.3% of *S. aureus* isolates to erythromycin and 1.2% of isolates were resistant to cloxacillin. Isolates from organic herds had lower MICs for tetracycline than those from cephalonium (OR<0.01) herds and had lower MICs for ampicillin and penicillin than for cloxacillin/ampicillin exposed herds (both P<0.05). The MICs for ampicillin and penicillin were lower (P<0.05) in cloxacillin exposed herds than cephalonium exposed herds but were not different between organic and cephalonium herds. For *S. dysgalactiae* (n=50), 2% of isolates were resistant to ceftiofur, 4% were resistant to erythromycin, 2% were resistant penicillin/novobiocin, 2% were resistant to pirlimycin, and 8% were resistant to tetracyclines. There were insufficient isolates of *S. dysgalactiae* to analyse the effect of DCT-exposure on MIC. Amongst *S. uberis* isolates, 1.1% were resistant to erythromycin, 1.6% were resistant to pirlimycin, and 0.5% were resistant to tetracycline. Organic herds had lower MIC's for ampicillin, ceftiofur, cephalothin, penicillin, pirlimycin, sulphadimethoxine, and tetracycline than isolates from cephalonium herds, and similarly organic herd isolates had lower MICs for ampicillin, ceftiofur, cephalothin, penicillin, pirlimycin, and tetracycline than those from cloxacillin/ampicillin herds.

Conclusions: Antimicrobial resistance was detected amongst some bovine mastitis pathogen/antimicrobial combinations. Higher MICs were observed for some pathogen/antimicrobial combinations amongst isolates drawn from herds with a history of exposure to either cloxacillin or cephalonium DCT, compared with isolates from organic herds. It should be noted that these differences in MIC distribution generally occurred at MIC values below clinical cutpoints as defined by CLSI, such that the antimicrobials are likely to remain clinically effective. Bimodal MIC distributions for some antimicrobials within some bacterial species were observed in organic herds suggesting that recent DCT usage is not the only factor affecting MICs. Given the observed findings, further work is required to determine if indeed exposure to DCT is in fact causal of elevated MICs, and whether reduction or removal of DCT from herds alters the MIC of mastitis pathogens from herds.

Keywords: Antimicrobial resistance, dry cow treatment, dairy cow.

PH-19

Antimicrobial resistance of *E. coli* in bulk tank milk from dairy farms in Germany – A 10 year perspective

Bernd-Alois Tenhagen, Katja Alt, Annemarie Käsbohrer, Jens-André Hammerl, Mirjam Grobbel.

German Federal Institute for Risk Assessment, Berlin, Germany.

Objectives: The objective of this study was to compare current antimicrobial resistance (AMR) in *E. coli* from bulk tank milk in German dairy herds with AMR of *E. coli* from previous years collected in the same sampling framework. While the risk of consumers due to AMR in *E. coli* from bulk tank milk is typically low as most of the milk is heat treated before marketing and consumption, it gives a good indication of the overall resistance situation in the dairy herds and may mirror antimicrobial use.

Material and Methods: The study was carried out in the framework of a national monitoring program on zoonotic bacteria and antimicrobial resistance in the food chain in Germany. Randomly chosen dairy herds (n=471) were included in this study in 2009, 2010, 2014 and 2019. A new random selection was carried out in every year, i.e. different herds were tested over the years. In 2014 an additional sampling frame included organic dairy herds. No. of conventional herds was distributed across the country proportionate to the number of dairy cows in the respective federal state. Data for 2019 are still subject to further validation. Final data will be presented at the conference.

Escherichia coli were isolated from bulk tank milk by regional state laboratories using routine methods. Isolates were submitted to the National Reference Laboratory for Antimicrobial Resistance (NRL-AR) for antimicrobial susceptibility testing (AST) using the broth microdilution method according to CLSI guidelines. For the comparison, minimum inhibitory concentrations were evaluated based on epidemiological cut off values (ECOFF) provided by EUCAST and Commission Implementing Decision 2013/652/EU. Fourteen antimicrobials were included in the testing annually. However, due to a change in the panel of antimicrobials only ten antimicrobials (gentamicin, chloramphenicol, cefotaxime, ceftazidime, ciprofloxacin, nalidixic acid, ampicillin, sulfamethoxazole, trimethoprim and tetracycline) were available for comparison across all years, while some antimicrobials were only included in the first two years (kanamycin, streptomycin and florfenicol) and replaced by others (azithromycin, tigecycline and meropenem) in 2014. Colistin was tested in all years. However, concentrations tested in 2009 and 2010 did not cover the current ECOFF. Therefore results from those years could not be included in the analysis.

Results: Overall, antimicrobial resistance in the tested *E. coli* was low in all years, with more than 80 % of the isolates susceptible to all antimicrobials. The highest proportion (95.9 %) of fully susceptible isolates was observed in isolates from organic farms (n=74) that were tested in 2014. The lowest rate was seen in 2010 with 75.8 % fully susceptible isolates. Overall, highest resistance rates were observed to sulfamethoxazole (10.0 %), tetracycline (6.2 %) and ampicillin (5.9 %), while resistance to azithromycin, tigecycline and meropenem (tested only in 2014 and 2019) was absent. Resistance to third generation cephalosporins (cefotaxime and ceftazidime) was absent in 2009 and in isolates from organic farms that were additionally tested in 2014. Highest resistance rates to these substances were observed in 2019 (6.9 % and 5.7 %) while overall resistance rate was low (1.9 %). Resistance to fluoroquinolones (2.3 %) was highest in 2010 (5.3 %) and was only absent in isolates from organic farms in 2014. Resistance to colistin was observed in one isolate from a conventional farm in 2014, but absent in isolates from organic farms in 2014 and dairy farms in 2019.

Conclusions: Overall results indicate a constantly low rate of AMR in *E. coli* from bulk tank milk in Germany over the years. On the other hand, observed resistance patterns have been including antimicrobials of highest priority to human

medicine indicating room for further improvement.

Keywords: Antimicrobial Resistance, Bulk Tank Milk, Dairy, E. coli.

PH-20

Quantifying, benchmarking and rationalising medicines use in the UK beef industry

David C. Barrett[1], Kristen K. Reyher[1], Jon G. Massey[1], Rachel Adams[1], Judith L. Capper[2].

[1]Bristol Veterinary School, University of Bristol, Bristol, United Kingdom; [2]Livestock Sustainability Consultancy, Harwell, United Kingdom.

Objectives: There is growing awareness of the threat of antimicrobial resistance (AMR) to animal, human and ecosystem health, therefore livestock industries must demonstrate a clear intent to reduce, replace and refine antimicrobial use (AMU). Individual farmers and veterinarians must also demonstrate responsible, evidence-based medicine use to maintain productivity, animal health and public health, and to ensure consumer confidence in animal welfare and food safety. A whole-system approach to livestock health is required, incorporating measuring and improving on-farm medicines use, which is facilitated by benchmarking between time periods, farms and industry sectors. Various medicines benchmarking metrics are already in use within livestock production (e.g. mg AMs/kg liveweight, defined daily dose (DDD), etc.), with the relevance, applicability and adoptability of each metric depending on the quality and availability of input data. However, within the UK livestock industry, beef producers are uniquely heterogenous, with farms spanning a wide range of cattle breeds, herd sizes, production systems and marketing strategies, which poses significant challenges for medicines benchmarking.

This study analysed on-farm medicine use on beef operations within and across UK beef sectors in order to develop medicines metrics and methodologies for on-farm data collection, recording and benchmarking; and to inform the development of a national electronic medicine data recording hub (eMH) for UK cattle.

Materials and methods: A multimodal approach with industry input at every stage was employed, using farmer questionnaires in conjunction with on-farm medicine and veterinary prescribing records to assess the quality and quantity of medicines data available on UK beef farms and to develop adoptable, appropriate and effective data collection methodologies. Focus groups involving farmers, veterinarians, suitably-qualified persons (SQPs) and beef industry stakeholders were convened, with participatory methodology used to gain insight into opportunities for and barriers to data collection.

Results: Although keeping detailed on-farm medicine records is a legal requirement, no standard approach exists for UK beef operations, with considerable variation in the quality, accessibility and extent of data recorded on farms. Medicines records obtained in this study were shown to be consistent with existing databases. However, they were often confounded by multiple beef operations on a single holding or the co-presence of sheep, pigs or dairy operations. Furthermore, many beef operators did not weigh their cattle and growing cattle moved between operations without medicine records being simultaneously transferred. It was therefore difficult to calculate accurate livestock weight-based metrics (e.g. mg AMs/kg cattle liveweight), dose-related metrics (e.g. DDD) or individual animal metrics (total AM use over the animal's lifetime/kg beef produced). Nevertheless, novel metrics that allowed cattle producers to benchmark and compare medicines use between cattle groups or timepoints (e.g. percent of cattle treated with a specific medicine) and allowed AMU to be quantified and compared were both achievable by, and applicable to, the UK beef industry, providing that the denominator could be accurately determined. Consequently, a standard beef cattle unit (SBCU) was developed, based on characteristic and representative cattle liveweights according to cattle breed (beef or dairy), age, sex and system (intensive or extensive). The most appropriate metrics for AMU benchmarking were determined to be:

- Total AMU (mg/SBCU)
- Total Highest Priority Critically Important Antimicrobial (HPCIA) (mg/SBCU)
- Total non-HPCIA (mg/SBCU)

The most-appropriate metric for benchmarking other medicines use (e.g. vaccines, parasiticides, steroids, etc.) was the percent of the at-risk population treated with the medicine per annum. These novel metrics have since informed national AMU standards for the beef sector and the cattle eMH.

Conclusion: Establishing and adopting on-farm medicines use metrics will encourage farmers and veterinarians to improve animal health and responsible medicines use. However, it is also recognised that there is value in augmenting the core benchmarking metrics recommended in this study with more accurate farm-specific information as well as in moving in the future to recording lifetime health and medicine administration information. In future, electronic cattle identification, data collection apps and the development of an integrated data hub that can include birth dates, movements, live/slaughter weights, laboratory tests, herd health status, medicine administration and treatment outcome would add value for the industry.

Acknowledgements: This work was partially funded by the Agriculture and Horticulture Development Board (AHDB). Discussion group participation and expert input from the project's industry stakeholder group plus numerous veterinarians, SQPs and farmers is gratefully acknowledged.

Keywords: AMR, Medicine, Beef, Cattle, Metric.

PH-21

An outbreak of Salmonella Typhimurium RDNC in a dairy herd

Alan Murphy.

Animal and Plant Health Agency, Bury St Edmunds, United Kingdom.

Objectives: An advisory visit and a subsequent zoonosis sampling exercise were conducted on a dairy herd following the isolation of Salmonella Typhimurium RDNC in the adult herd. The aim of the advisory visit was to establish a cause for acute milk drop in 80% of the adult herd. When confirmed as being due to Salmonellosis a second visit was carried out to epidemiologically assess the site and establish the likely route of entry for the bacteria into the herd. This visit was also an opportunity to reinforce the zoonotic risk posed by the organism.

Materials and Methods: An acute episode of milk drop was reported over one weekend in a 120 cow dairy herd with 80% of the herd affected. The bulk tank volume dropped by over 50% and the private veterinary surgeon involved requested an advisory vist by an Animal and Plant Health Agency Veterinary Investigation Officer. At the time of this initial visit the cows were observed to be dull, with vague signs of abdominal discomfort and inappetance. Pyrexia had been reported in a small proportion and supportive treatment had been administered. Walking the grazing area of the cows, casts of intesinal mucosa were observed with frank blood staining also.

Blood and faecal samples were submitted for assessemt with the primary differential being alimentary tract disease.

Following isolation of Salmonella Typhimurium RDNC from all faecal samples, an advisory zoonosis visit was conducted. This resulted in a detailed overview of the entire farm, its management and a substantial amount of environmental sampling.

Results: The blood results were unremarkable.

The faecal samples collected all yielded Salmonella Typhimurium RDNC.

The environmental samples collected demonstrated widespread contamination of the adult cow and feed areas, with Salmonella Typhimurium RDNC

Whole genome sequencing confirmed the isolate to be one of concern in an ongoing human health issue under investigation by Public Health England.

Conclusion: This was an unusual presentation of Salmonellosis in a group of adult cattle, given the morbidity and acute onset. At no point was disease observed or suspected in other age groups in the herd and it was considered that this was a measure of the robust biosecurity already in place. Though additional means to enhance this were suggested. The cows recovered within a few weeks with moderate losses. This comprised of one abortion, one cow death and three cases of tail tip necrosis. No further losses were reported some three motnhs later and a full return to expected yields was reported. The strain invoved in this case had been the subject of an information leaflet published by APHA in Novemner 2018 following a significant issue of mortlality in sheep flocks and associated human health incidents. The latter had been linked to the consumption of lamb and mutton. One of the flocks involved in this case was near to this dairy farm. At the advisory visit it was established that the outbreak coincided with the opening up of a maize silage clamp, which had been heavily contaminated by wild birds. Sampling of the feed area recovered the Salmonella of interest and it was proosed that the most likely route of entry onto the farm had been wild bird movements from another infected premisies. The provison of the maize silage via a TMR had then enabled large scale innoculation of the adult herd in one event, leading to the uniformity of the clinical signs.

Salmonella Dublin is routinely reported as the predomnant serotype of concern in the bovine currently. As veterinary surgeons we must always be aware that other strains can be just as significant or even more so on an individual farm basis. New and emerging threats such as this with, established human health significance; are timely reminders that we should never become complacent in the control of this bacteria.

Keywords: Zoonosis, Salmonella Typhimurium RDNC, Dairy.

PH-22

There's more work to do: New Zealand Dairy Farmers' understanding of One Health, Antimicrobial Resistance, and the Restricted Veterinary Medicines process

Kurt Arden[1], Richard A Laven[2], Kristina R Mueller[2].

[1]Royal Veterinary College, London, United Kingdom; [2]Massey University, School of Veterinary Sciences, Palmerston North, New Zealand.

Objectives: Veterinarians play a key role in managing the threat of antimicrobial resistance in animal and human populations, not only by adopting antimicrobial stewardship in their treatment and prescribing decisions, but also through education of stakeholders. The Restricted Veterinary Medicines (RVM) procedure under the Agricultural Compounds and Veterinary Medicines (ACVM) Act 1997 is one method that veterinarians in New Zealand use to monitor antimicrobial use on dairy farms and provides an opportunity for veterinarians to introduce farmers to the One Health model.

The aim of this study was to generate a pilot set of data on the opinions and understanding of New Zealand Dairy Farmers with regards to 'One Health', 'Antimicrobial Resistance (AMR)' and the 'Restricted Veterinary Medicines' operating plan. Furthermore, it aimed to allow a greater understanding of how these topics impact dairy farmers' lives and to lay the groundwork for further research in this globally important area.

Materials and methods: A questionnaire including 55 questions (40 open questions and 15 closed questions) was distributed to a convenience sample of dairy farm clients of the Massey Farm Services Clinic. The questionnaire was then completed via in-person interviews with the principal researcher. The study period was from September 2021 to November 2021. Results were entered into a database and processed with Microsoft Excel.

Results: 15 out of 16 farms participated in the survey (response rate of 93.75%). The average herd size of this study was 181 cows. Only one farm reported being aware of the term 'One Health' and none of the interviewees could define the discipline. In comparison, 73% of interviewed farms were aware of antimicrobial resistance and could attempt to define it. 53% of clients interviewed were unaware of the existence of the New Zealand Veterinary Association's (NZVA) 'Antimicrobial Traffic Light system' and only 33% considered following the traffic light system when choosing antibiotic treatment. 53% of respondents did not use antibiotics as first line treatment

without seeking veterinary advice, 80% of which said they had decreased their antibiotic use over the past 5 years.

Conclusions: The World Health organization has categorized AMR as one of the top threats facing public health and created the global action plan on AMR in 2015. The NZVA antimicrobial traffic light system dividing antimicrobials into three groups (green, orange, and red) was launched in 2016, encouraging preferential use of green light antibiotics which are less important to human health compared to orange or red rated antibiotics. New Zealand Veterinarians have a key role to play within this plan and by following the NZVA traffic light system and using the RVM process as tools to combat AMR, the New Zealand veterinary industry is in a strong position to keep AMR at the low levels we currently see in large animal veterinary practice. However, ensuring all relevant parties understand the terms that are used to achieve One Health goals is crucial for success and for ensuring buy-in from farmers. This study identified a lack of understanding of the term 'One Health' in farmers which is concerning. Furthermore, in 2021, a 'traffic light system' for the COVID-19 response was launched in New Zealand which could cause confusion of terms, especially when using search engines for example.

Overall, farmers interviewed in this study found the RVM of use to their farm; providing opportunity for clinical veterinarians to communicate the key themes of One Health and Antimicrobial Resistance to receptive clientele. This study has laid the groundwork for future research into farmer opinions of AMR and One Health and allow all those who work in this field to collaborate on this important global health threat.

Keywords: Antimicrobial resistance, One Health, farmer survey, antimicrobial traffic light system.

PT-01

Temperatures of pharmaceutical storage areas in large animal veterinary practice vehicles in the winter

Elizabeth R. Homerosky[1], Jeff D. Ondrak[2], Meredyth L. Jones[3], Virginia R. Fajt[4].

[1]Veterinary Agri-Health Services, Ltd., Airdrie, Canada; [2]Great Plains Veterinary Educational Center, University of Nebraska, Clay Center, United States; [3]Oklahoma State University, Stillwater, United States; [4]Texas A & M University, College Station, United States.

Objective: Many manufacturers recommend that non-refrigerated products be stored at room temperature (20–25 °C). Large animal veterinary practice vehicle storage units are commonly outfitted with an adjustable heater to prevent freezing as ambient temperatures in many locations in North America drop below these temperatures in winter. The authors have previously evaluated storage units in vehicles in the summer, so the objective of this study was to measure storage area temperatures in the winter to evaluate the effectiveness of heaters and the extent to which temperatures fall outside the manufacturers' recommended range.

Materials and Methods: A convenience sample of six vehicles from an ambulatory beef cattle practice in Southern Alberta was used. Ambient temperature and temperatures in two storage areas (close to the heater and far from the heater) in each vehicle were recorded from November 1, 2018 – February 28, 2019, at 15-minute intervals using self-contained, battery operated temperature recording devices.

Results: The lowest and highest overall temperatures recorded in a storage unit were -29.7 °C and 62.6 °C, respectively. The mean temperature recorded across all six storage units was 16.8 °C near the heater and 11.6 °C far from the heater, while ambient temperature averaged 2 °C. During the 120-day data collection period, temperatures below freezing were recorded between 3-48 days for each of the six vehicles. Readings far from the heater fell within the recommended range for room temperature 11.3% of the time, whereas only 5.6% of readings near the heater fell within the recommended range.

Conclusion: Temperatures in practice vehicle storage units were outside recommended pharmaceutical storage temperatures a significant portion of the winter. Furthermore, there is a discernable variation between storage areas near and far from the heater. Research is needed to determine the extent to which these fluctuations outside the manufacturers' recommended storage temperatures impact efficacy of stored pharmaceuticals.

Keywords: Drugs, vet box, heater, frozen.

PT-02

Efficacy of an oral solution of paromomycin for the treatment of newborn dairy calves with cryptosporidiosis. Results from a comparative European multicentric field study

Damien Achard[1], Béatrice Besche-Barbazanges[1], Gaëlle Pagny[1], Jean-François Collin[1], Dejan Cvejić[2].

[1]Ceva Santé Animale, Libourne, France; [2]Klifovet AG, Munich, Germany.

Objective: Cryptosporidiosis is a frequent parasitological infection of mammals including but not limiting to humans and neonatal ruminants. In newborn calves, cryptosporidiosis has been repeatedly identified as a major contributor of neonatal diarrhoea, a dominant calfhood disease with detrimental health and economic consequences. Current treatment options for clinically affected newborn calves are limited. A recent meta-analysis work reported halofuginone and paromomycin as valuable oral treatment options while recognizing that halofuginone can present important safety issues and that data is insufficient to fully support the use of paromomycin (*Brainard et al.,* 2020, 2021). To address this lack of evidence, a study under field conditions was performed in dairy newborn calves clinically affected by cryptosporidiosis comparing a new dose regimen of an oral paromomycin solution (Gabbrovet Multi®, Ceva Santé Animale) to a reference product based on halofuginone (Halocur®, MSD).

Material and methods: A GCP compliant study was performed according to a blinded, randomized, positive controlled, multicentric design in 4 commercial farms located in Germany, Hungary, and Portugal. The farms enrolled had a recent history of cryptosporidiosis and complied with several conditions such as vaccination for BVDV and neonatal diarrhea. Each newborn calves in this study were required to meet the following criteria for enrollment: age between 3-14 days, faecal score ≥ 2, negative rapid test for *E. coli*, coronavirus, rotavirus, and positive rapid test for *C. parvum*. Any calf that presented with diarrhoea for > 24 hours or that has been previously exposed to antibiotics, parasiticides or probiotics was excluded. Animals were randomly allocated to two treatment groups. Calves in group A received 150 mg paromomycin sulfate/ kg b.w., once daily, for 5 days by oral route while calves in group B were orally administered 100 µg halofuginone/ kg b.w., once daily, for 7 days. The following clinical and parasitological parameters were monitored at fixed times during the 21 days of the study: fecal score (0-3), general health observation (0-3), hydration score (0-3), oocyst counts (number of oocysts per gram of dry faeces), bodyweight and mean daily body weight gain (MDBWG). Percentages of calves cured at day 8 and MDBWG between day 0 and day 21 were the main criteria to evaluate the efficacy in both treatment groups. Other criteria such as parasitological cure at day 3, 5, 7, 14 and 21 and time to clinical cure from day 2 to day 8 were also assessed. The statistical unit was the calf and the 5% level of significance ($p < 0.05$ for two-sided tests) was used to assess statistical differences.

Results: Three hundred thirty-four dairy calves, mostly Holstein-Friesians, with a mean age of 9.1 days and a mean body weight of 41.8 kg were enrolled in this study. 165 calves

were allocated to treatment group A, 169 in the treatment group B. At inclusion, the treatment groups were found comparable. 94.6% (156/165) of newborn calves in group A and 87.6% (148/169) in group B were considered clinically cured by day 8. Clinical cure rate in group A was found superior to those observed in group B (lower bound of the 95% CI >0). In addition, MDBWG was higher for calves in group A in comparison to calves in group B, with a difference between groups of 0.0405 kg/day (p = 0.0086, best model); that is a bodyweight advantage of 850 g at the end of the 3 weeks follow-up period. Calves in group A were found to be cured faster in comparison to calves in group B. More precisely, the probability of healing first was 56.75% in favor of group A. The qualitative assessment of oocyst counts (positive / negative) assessed at each time point showed no significant difference, except on Day 7 (76.4% and 87.0% for negative calves in group A and B respectively). Overall, seven adverse events were documented from seven animals in group B but were not found to be related to the treatment.

Conclusion: In this European field study, daily oral treatment with 150 mg/kg of paromomycin (Gabbrovet Multi®) for 5 days was found safe and highly effective to cure sick dairy calves with cryptosporidiosis and to control their oocyst burden. In addition, this new treatment regimen was found superior to the current reference treatment based on halofuginone.

Keywords: Dairy calves, cryptosporidiosis, treatment, paromomycin, field study.

PT-03

Effects of early treatment with non-steriod anti-inflammatory drugs (NSAIDs) against bovine respiratory syncytial virus (BRSV)

Sara Hägglund[1], Eve Laloy[2], Ignacio Alvarez[3], Katarina Näslund[4], Yongzhi Guo[3], Sara Rosenberg[3], Mansour Naghibi[3], Gabriella Hallbrink Ågren[3], Haleh Yazdan Panah[3], Ganna Shevchenko[5], Jonas Bergquist[5], Anna Hillström[6], Edouard Timsit[7], Jean Francois Valarcher[3].

[1]Swedish University of Agricultural Sciences, Uppsala, Sweden; [2]Laboratoire VETODIAG, Saint-Pierre-en-Auge, France; [3]Swedish University of Agricultural Sciences, Uppsala, Sweden; [4]National Veterinary Institute, Uppsala, Sweden; [5]Uppsala University, Uppsala, Sweden; [6]University Animal Hospital, Uppsala, Sweden; [7]Ceva Santé Animale, Libourne, France.

Objectives: Non-steroid anti-inflammatory drugs (NSAIDs) are increasingly used as a single and early treatment against respiratory disease caused by bovine respiratory syncytial virus (BRSV). These drugs reduce fever, but consistent reduction of pulmonary inflammation has not been demonstrated [1]. While some of the induced mechanisms are anti-inflammatory, others might augment inflammation in the lung and delay the healing process. The drawbacks include increased levels of leuokotriene B4, a chemoattractant for neutrophils, and decreased levels of prostacyclin and prostaglandin E2, which mediate a switch from leukotriene to lipoxin production. On the other hand, the production of lipoxin and resolvins, which are important for lung epithelial repair, can also be triggered by aspirin, by another pathway [2][3]. The effect of aspirin is higher on COX-1 *versus* on COX-2, whereas the opposite is valid for meloxicam. The objective of this study was to map and compare the inflammatory events following early treatment with these different drugs against BRSV in calves.

Material and methods: Fifteen 3-8-week-old dairy calves were allocated to three groups, according to age, bodyweight and BRSV-specific maternal antibodies, and were infected with BRSV by aerosol. The calves received either 0,5 mg/kg meloxicam (MET) subcutaneously on D4 post infection (PI, n=5), 20 mg/kg acetylsalicylat-DL-lysin (ASP) intravenously on D4 and D5 (n=5), or no treatment (n=5, CRT).

Clinical signs of disease were scored and peripheral capillary oxygen saturation was measured on a daily basis. Bronchoalveolar lavages (BAL), plasma and nasal swabs were repeatedly collected until post mortem examination of lungs on D7 PI. The extent of macroscopic lung lesions were quantified and histological analyses were carried out. BRSV was detected in swabs and BAL by RT-qPCR. Inflammatory cells were counted in BAL and BAL proteins were semi-quantified by liquid chromatography and tandem mass spectrometry. Eicosanoids were analysed in plasma and BAL by liquid chromatography triple quadrupole tandem mass spectrometry.

Results: The calves developed mild to moderate clinical signs of respiratory disease. All calves shed virus from D2 or D3 throughout D7. No significant difference was observed with regard to clinical signs, virus shedding or extent of lung lesions. The two calves with the most severe clinical signs and most extensive pathological lesions, had been treated with ASP and MET, respectively. Meloxicam significantly increased the proportion of neutrophils in BAL compared to aspirin (24h after treatment) and compared to no treatment (72h after treatment). Histopathological, proteomic and metabolomic data will additionally be presented at the meeting.

Conclusions: In contrast to aspirin, treatment with meloxicam increased the influx of neutrophils in the lungs of calves infected with BRSV, compared to controls 7 days after infection and 3 days after treatment. Neither aspirin, nor meloxicam had a curative effect on clinical signs or lung lesions. The effect of these drugs will be analysed by taking into consideration systemic and local eicosanoids, as well as proteins in BAL fluids and histology. The results might contribute to evidence-based practical handling of acute outbreaks of BRSV in the field, as well as to the general understanding of pulmonary inflammation, needed for development of efficient drugs.

References:

1. Francoz, D. et al. Evidence related to the use of ancillary drugs in bovine respiratory disease (anti-inflammatory and others): are they justified or not? *Vet Clin North Am Food Anim Pract* 2012, *28*, 23-38, vii-viii, doi:10.1016/j.cvfa.2011.12.003.
2. Zheng, S., et al. Lipoxin A4 promotes lung epithelial repair whilst inhibiting fibroblast proliferation. *ERJ Open Res* 2016, *2*, doi:10.1183/23120541.00079-2015.
3. Gilligan, M.M., et al. Aspirin-triggered proresolving mediators stimulate resolution in cancer. *Proc Natl*

Acad Sci U S A 2019, *116*, 6292-6297, doi:10.1073/pnas.1804000116.

Keywords: BRSV, inflammation, NSAID, Eicosanoids, BAL proteome.

PT-04

Evaluation of the clinical effects of epidural butorphanol in cattle

Tomas Trucco[1], Mazzini Ruben[2], Cristian Leiva[2], Oscar Garnero[2], Reza Seddighi[3].

[1]Veterinaria Trucco, Private Practice, Argentina; [2]Universidad Nacional del Litoral, Facultad de Ciencias Veterinarias, Argentina; [3]University of Tennessee, College of Veterinary Medicine, United States.

Introduction and Objectives: Epidural administration is one of the commonly used regional blocks in bovine anesthesia for pain control. Caudal epidural anesthesia is commonly utilized in veterinary medicine to allow diagnostic, obstetrical, and surgical intervention in the perineal region of large animals, while allowing animals to remain in the standing position. Local anesthetics are the most frequently used drugs for producing analgesia by epidural injection, but other classes such as α-$_2$ adrenoceptor agonists, ketamine, and opioids have also been widely used. It has been shown in several species that opioids and alpha-adrenergic agents produce selective caudal epidural analgesia via binding to the spinal receptors. Stimulation of these spinal receptors results in the inhibition of rostral transmission of nociceptive (pain) impulses. Thus, a potential advantage of these agents is the selective sensory blockade, without the unfavorable depression of motor or autonomic neurons. Although many opioids have been evaluated and clinically used for epidural analgesia in cattle, there are no clinical trials on the use of butorphanol (BTL) epidurally in cows. Therefore, the objectives of this study were to determine the sedative and analgesic effects of butorphanol administered epidurally (C1-C2 intercoccygeal space) in standing cattle.

Methods: Five 2 - 3 year-old Holstein heifers (mean 14.6 months ± 0.7); weighing 423 ± 41 kg were used in this study. During the experiment, cows were restrained in a chute. The skin area over the first intercoccygeal (Co1-Co2) space was identified and aseptically prepared. Epidural puncture was performed with an 18-gauge, 38 mm needle, that was directed at the right angle to the general contour of the croup. The correct needle placement in the epidural space was confirmed by hanging-drop technique and the lack of resistance during administration of the injectate. Each animal received epidural anesthesia with butorphanol at a dose of 0.02 mg/kg BW. For each animal, butorphanol was diluted in saline (0.9%) to a final volume of 5 ml. Sedation, ataxia, and analgesia were assessed before butorphanol administration and at 5 min intervals after epidural administration for 60 min, and every 15 min thereafter for 120 min. Analgesia was tested by applying a standard noxious stimulus (skin pinching using a kocher hemostat) and subjectively scored based on a 3-point scale: *1)* no response *2)* depressed response; and *3)* normal response. The onset, magnitude, and duration of caudal epidural analgesia were also determined. Sedation was subjectively evaluated by the attitude of the cow, including the response to noise, carriage of the head, and the presence of excessive salivation. Ataxia was evaluated by observing the position of the pelvic limbs, swaying and leaning against the chute, or any knuckling of the hindlimbs. Physiological variables including heart rate (HR), respiratory rate (RR), rumen contraction (RM), and rectal temperature (RT) were assessed before epidural administration (baseline) and at 15 min intervals thereafter for 120 min. Data were analyzed using descriptive statistics and presented as mean ± SEM.

Results: Caudal epidural anesthesia was produced in all heifers following the administration of BTL. The epidural injection was easy to perform and well tolerated by all animals. Caudal epidural analgesia ranged from the tail, vulva, perineum, paralumbar fossa, flank fold and last rib in all heifers. Time to the onset of analgesia was 15 ± 2 minutes and duration of epidural analgesia was 69 ± 7.2 minutes. In all cattle, mild to moderate sedation (slight lowering of the head carriage and lower lip and ptyalism) was noted for 68 ± 6 minutes. No signs of ataxia were detected. No significant differences in heart rate, respiratory rate, rumen motility and rectal temperature were observed between measurements before and after epidural administration.

Conclusion: Butorphanol (0.02 mg/kg) administered epidurally to adult cattle produced adequate cutaneous analgesia and mild sedation without affecting the cardiopulmonary and rumen motility at the doses used in this study.

Keywords: Bovine, opioids, epidural, pain, butorphanol.

RB-01

Recombinant glycoprotein with eCG-like activity did not increase the incidence of multiple ovulation in beef cattle in a 5-day CoSynch protocol

Alejandro Rodriguez[1], Sebastian Maresca[1], Sebastian López Valiente[1], Lucas Gelid[2], Maria Guillermina Bilbao[3], Karen Moran[3], Gabriel Franco[4], Pedro Ezcurdia[1], Santiago Pérez Wallace[5], Ana Meikle[6], Julian Bartolome[4].

[1]Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Cuenca del Salado, Argentina; [2]Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Anguil, Argentina; [3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Facultad de Ciencias Veterinarias Universidad Nacional de La Pampa, Argentina; [4]Facultad de Ciencias Veterinarias Universidad Nacional de La Pampa, General Pico, La Pampa, Argentina; [5]Syntex S.A., Buenos Aires, Argentina; [6]Laboratorio de Endocrinología y Metabolismo Animal, Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay.

Objectives: The objective was to compare the effect of a glycoprotein with eCG-like activity on the incidence of multiple ovulation in *Bos taurus* beef cattle synchronized with a 5-d CoSynch plus progesterone protocol.

Material & Methods: Angus heifers of 22-month-old and suckled cows were submitted at random to either a 5-d progesterone CoSynch with reCG (reCG; n=140) or a 5-d progesterone CoSynch without reCG (Control; n=141). The body weight (BW; mean±SD) and body condition score (BCS, scale 1-9) of 22-month-old heifers of Herd 1 (n=95) and Herd 2 (n=97) were 377±21 Kg and 382±30 Kg, and 5.2±0.4 and 6.3±0.5 respectively, while suckled cows in Herd 3 (n=89) had 460±46 Kg and 5.2±0.5 units of BCS. The day of TAI was considered as Day 0 of the experiment. On Day -8, BCS was recorded and presence, number and location of CL (cyclicity) was determined based on ultrasonography. On the same day, all animals received 100 µg of GnRH analog (Gonadoreline acetate, 2 mL, im, Prolusyn®, Syntex) and a 1.0 g of progesterone intravaginal device (DIB®, Syntex). Ovulation in response to initial injection of GnRH was evaluated by the number and location of CL on Day -3 in relation to Day -8 based on ultrasonography. Also, on Day -3, all animals received 0.500 mg of sodium closprostenol (2 mL, im, Ciclase®, Zoetis) at device removal and were assigned randomly to receive 300 IU (1.5 mL, im, reCG, Syntex) in heifers and 400 IU (2 mL, im, reCG, PCT/EP2019/073277, Syntex) in cows of reCG or remain as untreated controls. On Day -2 animals received a second dose of closprostenol and finally on Day 0 (72 h after DIB removal), received 100 µg of gonadoreline acetate concurrent with TAI. On Day 7, the number of ovulations (single = 1; multiple ≥ 2) in response to synchronization of ovulation protocol was determined by ultrasonography. Data was analyzed using SAS (Statistical Analysis System). Optimal BCS was considered in heifers ≥6 and in cows ≥5. Baseline comparisons were established evaluating the distribution of cows in both treatment groups using a Chi-square test (Proc Freq, SAS system®). The effect of treatment group (reCG *vs.* control), herd (1, 2 and 3), BCS (<optimum *vs.* ≥optimum), cyclicity (anestrus *vs.* cyclic) and ovulation in response to initial injection of GnRH (yes *vs.* no) on incidence of multiple ovulation was evaluated by univariate analysis with Chi-square test (Proc Freq, SAS system®). In addition, the effect of treatment adjusted by the rest of the variables and interactions on the incidence of multiple ovulation was evaluated by multiple logistic regression models (Proc Logistic, SAS system®) using the backward elimination procedure.

Results: There was no difference in the distribution of cows by herd (P=0.58) and cyclicity (P=0.96) between treatment groups. At the beginning of the experiment (Day -8), 85, 84 and 89 % of heifers and suckled cows from herd 1, 2 and 3, respectively, were cyclic. The percentage of heifers and suckled cows that ovulated in response to initial injection of GnRH was 38 and 55%, respectively. Two suckled cows did not ovulate in response to second injection of GnRH. A 7.2% (20/279) of animals had multiple ovulation in response to synchronization of ovulation protocol. In the univariate analysis, the incidence of multiple ovulation was not affected by treatment group [6.5% (9/139) and 7.9% (11/140) in reCG and control group, respectively; (P=0.65)]. There was also not affected by herd (P=0.90), BCS (P=0.33), cyclicity (P=0.59) and ovulation in response to initial injection of GnRH (P=0.76). In the multivariable analysis, the incidence of multiple ovulation was also not affected by treatment group (P=0.81).

Conclusions: In conclusion, the addition of reCG in a 5-d CoSynch plus progesterone protocol did not affect the incidence of multiple ovulation in *Bos taurus* beef cattle.

Keywords: reCG, 5-day CoSynch, Multiple ovulation.

RB-02

Prevalence and risk factors of purulent vaginal discharge and cytological endometritis in French beef cows

Vincent Herry[1], Magadalena Claes[2], Lamia Briant-Amirat[3], Clara Bourel-Conroy[4], Nicolas Masset[5].

[1]SELAS EVA veterinary clinic, Reseau Cristal, Argentonnay, France; [2]SELAS EVA, veterinary clinic, Reseau Cristal, Argentonnay, France; [3]Laboratory of Biotechnology and Reproductive Pathology, ONIRIS: The National Veterinary, Food agriculture, and food hygiene school of Loire Atlantique, Nantes, France; [4]MSD Santé Animale, Beaucouzé, France; [5]Clinic for Ruminants, CHUV, Oniris, Nantes, France.

Objectives: The primary objective of the study was to determinate the prevalence of purulent vaginal discharge (PVD) and cytological endometritis (CE) in French beef cows and to find out cow-related risk factors associated with PVD and CE. A secondary objective was to evaluate the performance of leukocyte esterase test strips for the diagnosis of CE.

Materials and methods: This prospective cohort study was conducted in 6 herds of Charolais and Blonde d'Aquitaine breeds from December 2020 to April 2021 in the region of New Aquitaine in France. The cows included underwent a thorough examination of the reproductive tract at 30 ± 15 days post-partum that consisted in chronological order of i) a visual observation of vaginal mucous secretion using the Metricheck device (Simcro Technology, Hamilton, New Zealand) ii) endometrial sample using a double-protected cytobrush, iii)

ultrasonographic examination in order to determine the diameter of the cervix, the presence of the uterine contents and to describe the ovarian structures.

Immediately after the endometrial sampling, the cytobrush was gently rolled onto a microscopic glass slide which was subsequently colored (May-Grünwald-Giemsa) in order to determine of the percentage of polymorphonuclear (PMN) leukocytes. The cytobrush was cut and placed in a tube containing 1 mL isotonic sodium chloride (NaCl 0,9%) to perform a leukocyte esterase test (Multistix 10 SG; Siemens, Saint-Denis, France).

The cows were also blood sampled between 30 to 15 days before the expected date of calving and between 7 and 24 days after calving to determine the blood concentration of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate acid (BHBA) respectively. The body condition score (BCS) was determined before calving and at the time of reproductive examination. Some additional data regarding the calving (degree of assistance or caesarian section), post-calving diseases (uterine prolapse, retained fetal membranes, puerperal metritis) or the calves (twinning, birth weight, vitality after birth) were gathered. Risk factors associated with PVD or CE were identified using multivariable logisitic regression.

Results: A total of 182 beef cows of Charolais (n=52) and Blonde d'Aquitaine (n=130) breed were enrolled in the study among which 66 were primiparous (36.2%) and 116 were multiparous (63.8%). The prevalence of PVD and CE defined as a percentage of PMNs superior to 6% were 7.1% and 45.0%, respectively. Herd prevalence of PVD and CE was highly variable. Risk factors positively associated with PVD and CE were the presence of a post-partum disease (*i.e.* retained fetal membrane or vaginal prolapse; OR = 5.93 (1.01-30.77) and OR = 7.0 (1.16-134.26) for PVD and CE respectively) and the birth weight of the calves (*i.e.* ≥ 53 kg, OR= 3.94 (1.09-15.99) or ≥ 49 kg, OR= 2.28 (1.19-4.43) for PVD and CE respectively). No association were found between occurrence of PVD or CE and the breed, the herd, cervix diameter, pre-partum blood NEFA concentration, post-partum BHBA, BCS at calving or BCS variation around calving or the presence of a corpus luteum at the time reproductive tract examination. The percentage of PMNs varied positively with the PVD score, but the correlation between PVD and CE was not significant. The leukocyte esterase test was significantly correlated with the % PMNs, and using a threshold of 6% PMNs for the definition CE, the sensibility, specificity, positive predictive value, negative predictive value of the leukocyte esterase test was 0.64, 0.66, 0.60 and 0.69 if the result is "≥1+" and 0.31, 0.94, 0.81 and 0.63 if the result is "≥2+". Besides PVD or CE was not correlated with noticeable ultrasonographic changes of the uterine contents.

Conclusion: Prevalence of PVD was not previously reported in beef cows and appeared lower than observed in dairy cows. Cytological endometritis, which is not strongly correlated with PVD is much more prevalent in this study than previously reported. Beef cows that gave birth to heavy calves or suffered post-partum disease (uterine prolapse or retained fetal membrane in this study) might benefit a thorough evaluation of "uterine health" before the breeding period because they have higher risks to be affected by PVD or CE. In comparison with conventional cytology, the performance of reagent leukocyte esterase strip as an alternative test was relatively moderate to good.

Keywords: Beef Reproduction Endometritis.

RB-03

The influence of a natural Neospora caninum infection substantiated by serum antibody levels on the semen quality of Belgian Blue bulls

Celien Kemel[1], Matthieu Salamone[1], Hans Van Loo[1], Catherine Latour[2], Sébastien Vandeputte[2], Jozefien Callens[3], Miel Hostens[4], Geert Opsomer[1].

[1]University Ghent, Merelbeke, Belgium; [2]AWE Group, Ciney, Belgium; [3]DGZ Vlaanderen, Torhout, Belgium; [4]University Utrecht, Utrecht, Netherlands.

Objectives: Worldwide, *Neospora caninum* (*N. caninum*), a cyst-forming protozoan parasite, is considered as one of the most important causes of bovine abortion/stillbirth. Even though studies have shown an association of *N. caninum* and the failure of reproductive performances in female animals, the importance of *N. caninum* on bull fertility has been neglected. The presence of *N. caninum* DNA in semen, however, indicates that *N. caninum* can as well infect the male reproductive system. Recent literature showed that sperm concentration, motility, and viability were significantly lower in testes tissue of slaughter bulls with natural neosporosis. To the best of our knowledge, no longitudinal studies are available in which the association between sperm quality parameters and peripheral *Neospora* antibody titers has been assessed. Our study aimed to assess the potential impact of a natural infection with *N. caninum* on the sperm quality of fresh and frozen/thawed semen of Belgian Blue Bulls located in Belgium, taking sperm samples harvested over a 1-year period into consideration.

Materials and methods: The present study took place from January 3rd till December 31st 2020. In total 92 Belgian Blue bulls kept for breeding purposes, spread over three locations of the Artificial Insemination Centre "Association Wallonne De L'Elevage" (AWE, Ciney, Belgium) were used in this study. Every two months, each bull was serologically screened for *N. caninum* antibodies. Only semen data of bulls that were six times negative or positive for *N. caninum* antibodies, were included in the study.

Semen ejaculates were collected with an artificial vagina twice weekly. In total 10.478 ejaculates of the 92 bulls were included, with 9.537 fresh samples originating from *Neospora* negative (n=84) and 941 from positive (n=8) bulls. Outcome variables assessed for the fresh samples included: concentration, volume, and subjective motility. Only semen samples with more than 60% of total sperm motility and concentrations higher than 0.3x 10^9 sperm cells/ml (n= 7.685 being 7.018 originating from *Neospora* negative bulls and 667 from positive bulls) were further extended and frozen. Immediately after thawing, total motility, progressive motility, and morphology were assessed.

Sperm parameters were used as the dependent variable of

interest. Generalized linear and binomial mixed models were used for statistical analysis of each outcome variable. Initially, individual explanatory variables age, herd, Temperature Humidity Index (THI) at the moment of sperm production as well as collection, and season of sperm production and collection, as well as the *Neospora caninum* antibody test results were tested in univariable models for each outcome variable. Akaike information criterion (AIC) values were used to select explanatory variables to build the multivariable model, where the *Neospora caninum* test result was forced in all models.

Results: Results revealed an overall apparent seroprevalence of *Neospora caninum* of 9,2% in our tested bull group.

For fresh semen: volume (negative *N. caninum* samples: 6,60 ml ± 2,55 ml; positive *N. caninum* samples: 6,28 ml ± 3,28 ml) and concentration (negative: 1.395,23x10^6 sperm cells/ml ± 493,77 x10^6 sperm cells/ml; positive: 1.471,53 x10^6 sperm cells/ml ± 532,41x10^6 sperm cells/ml) were normally distributed, where the subjective motility (negative: 64,29% ± 14,71%; positive: 61,21%± 12,94%) was right skewed. After freezing: total motility (negative: 45,11% ±11,60%; positive: 44,47% ± 12,49%), progressive motility (negative: 26,28% ± 8,17%; positive: 27,11% ± 8,81%) and normal morphology (negative: 96,03%± 2,53%; positive: 95,90%± 2,38%) were normally distributed.

Although there were numerical differences in the descriptive results for some outcome variables, no significant associations were detected between natural neosporosis, substantiated by ELISA-antibody levels, and any of our tested outcome variables on fresh and frozen/thawed semen samples.

Conclusions: Results suggest no significantly negative association between *N. caninum* and any of the tested outcome variables in the fresh nor in the frozen-thawed semen samples. Current evidence that *N. caninum* seropositive bulls have lower semen quality or semen production is therefore unlikely. Based on these results, there appears to be no reason to advise against the rearing and use for mating of bulls that are seropositive for *N. caninum*, but extensive field fertility studies should further confirm this.

Keywords: Belgian Blue Bulls; Neospora caninum; semen quality parameters.

RB-04

Effects of single treatment with low doses of hCG in timed AI protocols on: follicle growth dynamics, ovulatory dispersion and ovulation rate on Nellore cows

Henderson Ayres[1], Joao Paulo Barbuio[1], Carlos Antônio De Carvalho Fernandes[2], Francisco Barufi[1], Heitor Amaral[1], Rodolfo Mingoti[1].

[1]MSD Saude Animal, São Paulo, Brazil; [2]Biotran Biotecnologia Animal LTDA, Alfenas, Brazil.

Objectives: Postpartum anoestrus is very prominent in Nellore cows and has a negative effect on reproductive results in the breeding session. The objective of this study was to evaluate follicle growth dynamics, ovulatory dispersion and ovulation rate in postpartum Nelore (*Bos taurus indicus*) cows receiving low doses of hCG (Chorulon™ MSD- Brazil) vs eCG (Folligon™ MSD- Brazil) after progesterone device withdrawal on a timed AI protocol.

Materials and methods: A total of 42 anestrous (absence of CL at the first day of the FTAI protocol) Nelore cows, with average BCS of 2.73±0.05 (scale 0-5) and at 42 to 83 days postpartum were treated on day 0 with 2 mg of estradiol benzoate (Fertilcare Sincronização™, MSD-Brazil) and received an intravaginal progesterone (P4) device (Fertilcare 600®, MSD). On day 8, 12h before device withdrawal, an ultrasound evaluation was performed. Cows were homogeneously randomized to four treatment groups according to the highest follicle diameter. At the moment of the intravaginal P4-device removal all cows were treated with 0.5 mg Cloprostenol (Ciosin®, MSD), 1 mg of estradiol benzoate, and then randomized to receive either saline (Control Group, n = 8), 100 IU of hCG (Chorulon®, MSD, hCG-100 Group, n = 14), 150 IU of hCG (Chorulon®, MSD, hCG-150 Group, n = 11) or 300 IU of eCG (Folligon®, MSD, eCG Group, n = 9). Subsequently, ultrasound examinations were performed at 12h intervals until 96h post P4-device removal, in order to evaluate follicular growth and time of ovulation. Data for ovulatory dispersion and ovulation rate were analyzed by least-squares *ANOVA* with the *PROC GLIMMIX* procedure of SAS (*v. 9.4; SAS Institute Inc., Cary, NC*). Orthogonal contrasts were used to compare follicle growth means (C1=Control vs Gonadotropins; C2 = eCG vs hCG; C3=100 IU of hCG vs 150 IU of hCG).

Results: There were no differences in time to ovulation and distribution of ovulations across time among groups (51.6±0.82h; *P>0.95*). There were no differences in ovulation rate [83,3% (35/42); *P=0.81*]. Dominant follicle growth within 24h periods was greater on cows treated with gonadotrophic hormones than the Control Group [gonadotropins = 2.28 mm d^{-1}; Saline = 0.18 mm d^{-1}; *P=0.001* (C1)], but hCG and eCG treatments had similar results [hCG = 0.96 mm d^{-1}; eCG = 0.75 mm d^{-1}; *P=0.81* (C2)] and different doses of hCG (100 IU vs 150 IU) had similar growth [150 IU of hCG = 1.05 mm d^{-1}; 100 IU of hCG = 0.75 mm d^{-1}; *P=0.26* (C3)].

Conclusion: In conclusion, different doses of hCG and eCG treatments resulted in similar dispersions between the first and last ovulations, and similar ovulation rates and follicular growth development rates after P4-device removal. Both gonadotrophic treatments (hCG and eCG) resulted in higher follicular growth rates than follicles in cows that did not receive a gonadotrophic stimulus.

Keywords: Anestrous, Bos indicus, Gonadotrophic, Postpartum, Ovarian Activity.

RB-05

Reproductive program in Avileña-Negra Iberica breed heifers (AECRANI) for an age programmed to the first birth of 2.5 years

Pablo Iglesias Santiago[1], Mar González Muñoz[2], Javier López Cordero[2], P. Herraiz Espinosa[2], Almuneda Cabezas Albéniz[3], Vicente Jimeno Vinatea[4].

[1]INSAVET - Investigación y sanidad veterinaria, Salamanca, Spain; [2]Asociación Española de Criadores de Ganado Vacuno Selecto de Raza Avileña Negra Ibérica AECRANI, Ávila, Spain; [3]Facultad de Veterinaria, Universidad Complutense, Madrid, Spain; [4]E.T.S.I.A.A.B., Universidad Politécnica, Madrid, Spain.

Objectives: To evaluate the reproductive efficiency of an assisted reproduction program based on estrus synchronization and fixed time artificial insemination (FTAI) in heifers of the Avileña-Negra Iberica breed (ANI) for an average age programmed at the first birth of 30 months. The reproductive model consisted of an estrus synchronization and through a Presynch + CIDR Cosynch and FTAI protocol with natural mating (NM) after 15 post-insemination.

Materials and methods: The program has been conducted with a sample of 94 ANI heifers from five livestock, located in the provinces of Salamanca, Ávila and Segovia (Castilla y León).

At the moment of insemination, all the heifers from the sample complied with the maturity and development index proposed by AECRANI (table 1).

Table 1. Recommendations AECRANI of the maturity and development index in heifers of ANI breed, for PPE of 30 months of age (PV_A = 650 kg, A_A = 1.40 m).

Age (months)	Maturity index (%)	Development index (%)	Phase
0	6,5	57	Birth
3	18,2	65	
7	33,6	74	Weaning
9	39,2	80	Recribed
12	47,6	85	Recribed
14	52,0	85	Recribed
16	56,4	88	Puberty
19	63,0	89	Coverage
21	67,4	90	Gestation
27	78,8	100	
30	85,0	100	1 postpartum week
LW_A = Adult reproductive live weight; WH_A = Adult reproductive withers height			

The average age of the heifers at insemination was 18.4 ± 2.9 months, with an average live weight of 395.7 ± 56.5 kg, withers height of 127.4 ± 4.1 cm and an average corporal condition of 3.0 ± 0.4 points. The average age, average live weight, withers height and corporal condition of the heifers of each livestock at the moment of artificial insemination (AI) are shown in table 2.

Table 3 describes the synchronization protocol (Presynch + CIDR Cosynch) applied to the heifers of the different livestock in the study.

After 15 days of the AI, the stallions were introduced in the different livestock to review by means of natural mating (NM) the heifers that were not pregnant in the AI. The diagnosis of pregnancy was made by ultrasonography 70 days post-AI, thus confirming the pregnant heifers.

Results: Through the reproduction program used in this study, the average gestation rate in all livestock was 72.9%.

Table 2. Age, live weight, withers height and corporal condition of the ANI heifers of the different livestock studied at the time of AI.

	Livestock I	Livestock II	Livestock III	Livestock IV	Livestock V
Number of heifers	34	11	12	20	17
Age, months	18,2±1,1	18,0±1,5	19,4±4,2	15,62±0,3	21,6±3,4
Live weight, kg	395,5±43,6	420,1±26,5	346,8±41,8	354,6±29,6	463,2±52,7
Withers Height, cm	125,5±3,7	126,6±3,4	126,8±3,4	127,6±3,9	131,8±2,9
Corporal condition	2,8±0,4	3,1±0,2	3,1±0,3	2,9±0,2	3,3±0,3

Table 3. Estrous synchronization and protocol in ANI heifers.

Presynch + CIDR Cosynch		14 d	Day 0	5d	Day 5	1d	Day 6	2d	Day 8
			Aplication CIDR				Retired CIDR		FTAI
	$PGF_2\alpha$		GnRH		$PGF_2\alpha$		$PGF_2\alpha$ + eCG		GnRH

Table 4. Percentage of pregnant heifers after the application of the reproduction program with IATF.

	Livestock I	Livestock II	Livestock III	Livestock IV	Livestock V
Number of heifers	34	11	12	20	17
Age AI, months	18,2±1,1	18,0±1,5	19,4±4,2	15,62±0,3	21,6±3,4
Pregnant rate, %	91,2	63,6	91,7	65,0	52,9

Table 4 shows the gestation rate in the different livestock that have taken part in the study.

Conclusions: The use of a reproductive program based on the estrus synchronization and "Presynch + CIDR Cosynch" and IATF, in combination with the introduction of stallions for natural mating 15 days after insemination, is a recommended method in ANI heifers at the first birth of 30 months of age, given the excellent rate of pregnant heifers obtained in this study.

Keywords: Avileña-Negra, Synchronization, Insemination, Heifers, Birth.

RB-06

An outbreak of congenital goiter and chondrodystrophy among calves born to spring-calving beef cows

Elizabeth R. Homerosky[1], Megan Johnsen[1], Mei Steinmann[2], Carling Matejka[2], Michael J. Jelinski[1].

[1]Veterinary Agri-Health Services, Ltd., Airdrie, Canada; [2]University of Calgary, Calgary, Canada.

Objectives: An outbreak of concurrent congenital diffuse hyperplastic goiter and chondrodystrophy occurred at a commercial cow-calf operation in southern Alberta during spring 2018 (Homerosky et al., 2019). All affected calves were born to mature Angus cows and sired by one of two half-sibling mature Red Angus bulls. Matings during the previous four breeding seasons resulted in apparently normal calves. The objective was to investigate non-genetic etiologies of congenital goiter and chondrodystrophy.

Materials and methods: The herd was on pasture until November 2017 when they were transitioned to a ration comprised of wrapped high-moisture barley greenfeed bales and mixed grass hay bales. No supplemental trace minerals or vitamins were provided during the winter feeding period. Twenty of 35 calves born between April 5th and June 5th, 2018 were affected with goiter and generalized weakness in the presence or absence of varying degrees of chondrodystrophy. Incidence of stillbirths and neonatal deaths (N=7) and severity of clinical signs decreased as calving season progressed. All dams were in good body condition and appeared clinically healthy.

Results: Three deceased affected calves submitted for additional diagnostics tested negative for bovine herpesvirus, Leptospira spp., Neospora caninum, and bovine viral diarrhea. Liver concentration for iron was high, manganese was low, and cobalt was deficient in multiple calves. Cobalt may serve as a proxy for iodine as these two minerals must be supplemented continuously to cattle in Alberta. As such, iodine deficiency was determined to be the cause of goiter in these calves. The herd was treated by providing trace minerals with added ethylenediamine dihydroiodide.

Conclusions: Although congenital goiter and chondrodystrophy are observed globally in several species, there is a paucity of reports describing concurrent manifestations, especially in calves. In the current outbreak, it is unclear if hypothyroidism impaired normal fetal growth and development or if multiple concurrent mineral deficiencies predisposed these calves to goiter and chondrodystrophy independently.

Keywords: Iodine, manganese, mineral, deficiency, hypothyroidism.

RD-01

Effect of treatment of phantom cows with a progesterone-based synchrony programme

Greg Chambers[1], Mat O'Sullivan[2], Chris Compton[3].

[1]Zoetis New Zealand, Auckland, New Zealand; [2]Veterinary Centre, Oamaru, New Zealand; [3]Massey University, Palmerston North, New Zealand.

Objectives: To determine the effect of a progesterone-based synchrony programme on the daily hazard of conception and the probability of being pregnant at the end of the seasonal mating period in cows not observed in oestrus within 35–49 days of insemination and that were diagnosed non-pregnant (phantom cows) on seasonally-calving New Zealand dairy farms. Secondary aims were to determine the phantom cow prevalence and estimate the proportion of phantom cows with a functional corpus luteum (CL) at enrolment.

Material & Methods: Phantom cows from 14 New Zealand commercial dairy farms were enrolled in a randomised controlled trial. Cows that were artificially inseminated ≤14 days after mating start date and were not subsequently detected in oestrus were presented for pregnancy diagnosis approximately 49 days after mating start date. Non-pregnant cows were diagnosed as phantom cows and randomly allocated to treatment and control groups. A milk sample was collected for progesterone assay to determine the presence of a functional CL. Treatment consisted of an injection of buserelin and insertion of an intravaginal device containing progesterone on day 0, injections of dinoprost and equine chorionic gonadotrophin and removal of the intravaginal device on day 7, injection of buserelin on day 9, and fixed time artificial insemination on day 10. Treatment group cows were then mixed with bulls for the remainder of the seasonal mating period. Cows allocated to the control group were mated naturally by bulls. Statistical models were constructed to determine the effect of treatment on the daily hazard of conception and the probability of being pregnant at the end of the seasonal mating period.

Results: A total of 378/4,214 (9.0%) cows presented for pregnancy diagnosis were diagnosed as phantom cows. A functional CL was diagnosed in 257/362 (71.0%) phantom cows. Median predicted enrolment to conception intervals were 33 (95% CI= 30-45) and 30 (95% CI= 28-33) days respectively for cows in the control and treatment groups respectively. The odds of being pregnant at the end of mating were 1.70 (95% CI= 1.34-2.17) times greater for treated phantom cows than untreated phantom cows. Estimated marginal mean proportion pregnant at mating end date were 59.5 (95% CI= 47.9-70.1) % and 71.5 (95% CI= 62.6-79.0) % for control and treatment group cows respectively.

Conclusion: Treatment with a progesterone-based synchrony programme significantly increased the probability of phantom cows being pregnant at the end of the seasonal mating period.

Keywords: Phantom cow, dairy cattle, reproduction.

RD-02

Evaluating the cost-effectiveness of diagnosing and treating phantom cows in seasonal-calving dairy herds

Greg Chambers[1], Mat O'Sullivan[2], Carolyn Gates[3].

[1]Zoetis New Zealand, Auckland, New Zealand; [2]Veterinary Centre, Oamaru, New Zealand; [3]Massey University, Palmerston North, New Zealand.

Objectives: The objective of this study was to explore the cost-effectiveness of using a progesterone-based synchrony program to manage phantom cows on seasonal-calving dairy farms. Phantom cows were defined as cows that had been artificially inseminated ≤14 days after mating start date (MSD), were not subsequently detected in estrus, and were diagnosed non-pregnant at a pregnancy diagnosis conducted approximately 49 days after MSD.

Material & Methods: Decision-tree analysis was applied to data from a previous randomized controlled trial in which phantom cows (n=378) from spring-calving dairy farms were randomly allocated to an untreated control group or were immediately treated with a 10-day progesterone-based synchrony program with fixed-time artificial insemination. The net economic return of treating all cows presented by the farmer for pregnancy diagnosis that were diagnosed non-pregnant was compared to no intervention. The net return was therefore calculated per cow present at MSD because the decision trees followed all cows present at MSD through to mating end date to account for farmers inadvertently presenting ineligible cows for pregnancy diagnosis and possible treatment. Probabilities, costs and benefits of reproductive outcomes were based on published data and expert opinion. The effects of key parameters on the economic return were tested by sensitivity analysis.

Results: Phantom cow intervention delivered a net return of NZ $4.51 per cow present at MSD. The sensitivity of pregnancy diagnosis, proportion of ineligible cows presented by the farmer for pregnancy diagnosis and the prevalence of phantom cows were highly influential on the net economic return from phantom cow intervention.

Conclusion: These findings suggest that treatment of phantom cows in seasonal-calving dairy farms using a progesterone-based synchrony program is economically viable based on the current model assumptions. Accurate cow selection and pregnancy diagnosis are essential to success. Veterinarians can improve the net economic return by selecting farms likely to have a higher prevalence of phantom cows based on the presence of observable risk factors.

Keywords: Phantom cows, Reproduction, Estrus Synchronization, Economics, Decision Tree Analysis.

RD-03

Embryo production in dairy cattle after superovulation with gonadotrophin preparation- effect of variability in FSH/LH ratio-

Santiago Fuentes Ibañez[1], Ana Sierra Toral[2], Eugenio Liébana[3], Juan Carlos Boixo Pérez-Holanda[4], Fernando De La Fuente Crespo[5].

[1]Aberekin, S,A., Derio, Vizcaya, Spain., Derio, Vizcaya, Spain; [2]Selection and Animal Reproduction Center (CENSYRA), Villaquilambre, León, Spain., Villaquilambre (LEÓN), Spain; [3]Embryo Transfer Unit of Cantabria, Torrelavega, Cantabria. Spain, Torrelavega, Cantabria, Spain; [4]Faculty of Veterinary Medicine, University of León, León, Spain, LEÓN, Spain; [5]Department of Animal Production. Faculty of Veterinary Medicine, University of León, León, Spain, LEÓN, Spain.

Introduction and objectives: Ovarian superstimulation of dairy heifers and cows is a practical tool to increase offspring from females of high genetic merit. The success of superovulation and embryo recovery in cattle depends on numerous factors but one of the most significant limiting factor has been the high between-individual variability, in the ovarian response to gonadotropin stimulation. (Mikkola & Taponen, 2017).

Currently the gonadotrophins preparations approved for veterinary use derive from porcine and ovine pituitary tissue. One of the disadvantage resulting from the nature of the origin of the products is inconsistency among batches. This variation in bioactivity affects embryo yield (Kanitz et al. 2002).

The more important question is the possible biological effect of the so called FSH/LH ratio. Variability in FSH:LH ratios among different batches of one product of commercially available gonadotrophins is a very important challange that deserves to be considered. Because of this, the objective of this study was to compare the outcome of embryo recovery after superovulation with purified porcine pituitary extracts with different FSH:LH ratios, using a standard protocol of eight injections in a decreasing dose in cows and heifers.

Materials and methods: Data of 465 superovulations and subsequent embryo collections from Spanish dairy farms were analyzed. Donor animals were cows (n= 222) (47.74%), and heifers (n= 243) (52.26%) of Holstein breed.

Superovulation was induced by eight intramuscular injections of gonadotrophin preparation, at 12-hour intervals over 4 days. Declining doses of 650 to 1000 IU in total was administered for cows, whereas heifers received 500 to 750 IU. Embryos were collected 7 days after inseminations by transcervical uterine flushing according to the IETS guidelines.

Donors were randomly divided into three groups (I, II and III), according to treatment they had received because of different FSH:LH ratio in follicle-stimulating hormone preparation:

Group I (T1): (n= 362) Donors stimulated with a batch in which FSH:LH ratio was ≥ 1

Group II (T2): (n= 56) Donors stimulated with a batch in which FSH:LH ratio was <1 to ≥ 0.9

Group III (T3): (n= 47) Donors stimulated with a batch in which FSH:LH ratio is < 0.9

Statistical analyses: With the aim of studying factors that influence on number total recovered embryos variation; number viable embryos and number of quality 1 embryos variation, statistical analyses were carried out using lineal models (GLM procedure in SAS version 9.1.3, SAS Institute Inc., Cary, NC). The variance factors analyzed were: type of donor (heifer or cow) and treatment (T1, T2, T3).

Results: The average values for the analyzed variables were: number of total embryos recovered (10.28±0.32), number of viable embryos (5.66±0.23) and number of quality 1 embryos (2.83±0.12).

The donor type factor (heifer of cow), was not significant for the three variables analyzed, however the treatment effect was significant for the number of viable embryos ($p < 0.05$) and for the number of quality 1 embryos.

There was no effect (P=0.08) of different FSH:LH ratios of gonadotrophins on mean of **total embryos recovered** between Groups I, II, III (10.53 ± 0.37 vs. 9.73 ± 0.94 vs. 9.25 ± 1.03 respectively).

There was a main effect of different FSH:LH ratios used for superovulation in present trial (P = 0.0004), on **viable embryos** obtained between Group I (FSH:LH ratio ≥1), with highest value (6.06 ± 0.26) and group III (FSH:LH ratio < 0.90) with lowest (3.26 ± 0.73). The mean showed of Group II (FSH:LH ratio: <1 to ≥0.90) (5.07±0.67) tended (P=0.07), respect to Group III value.

There was also a significant effect of the treatment on the number of **quality 1 embryos:** donors of Group III had lesser number (1.81 ± 0.38) than Group II (2.68 ± 0.35) (P=0.09) and Group I (2.98 ± 0.13) (P=0.0043).

Conclusions: The number of viable and quality 1 embryos recovered resulted in significantly increased in donors stimulated with a batch of gonadotrophin preparation in which FSH:LH ratio was ≥ 1 respect to those obtained from females superovulated with a batch which ratio of FSH:LH was < 0.9.

These results suggested that relative high LH contamination in gonadotrophin preparations impairs the ovarian response to superstimulation, resulting in decreased yield of viable and quality 1 embryos.

Keywords: FSH/LH ratio, SOV, embryo, cattle.

RD-04

Quantification of reproductive hormone use in Dutch dairy herds: "From no treatment to excessive use"

Savannah Van Der Laan[1], Bart Van Den Borne[2], Peter Vos[1], Hilde Aardema[1], Tine Van Werven[1].

[1]Utrecht University, Utrecht, Netherlands; [2]WUR, Wageningen, Netherlands.

Objectives: Globally, reproductive hormones are used in the dairy industry to increase reproductive efficiency and, hence, to increase the longevity of dairy cows. The number of treatments seems to vary significantly between herds, but exact numbers on reproductive hormone use are lacking for most countries. Variation between countries appears to be highly dependent on a farm's management system, including the type of calving pattern used. In the Netherlands, the

calving pattern is year-round and, consequently, hormonal treatments tend to be prescribed at the cow level based on diagnostics rather than by using timed artificial insemination protocols at herd level.

Consumers' growing interest in food safety and cases of pharmaceutical product misuse have resulted in scepticism among the public regarding the use of antimicrobials and hormones in modern farming, although direct negative effects of reproductive hormones are not described. Transparency in the process of milk production, including insight into key performance indicators and medicinal treatments, is a strong and effective tool in influencing social opinions and acceptance of modern dairy farming.

The aim of this study was to estimate reproductive hormone use on year-round calving dairy farms under Dutch circumstances and to obtain insight into a number of herd-level factors that are associated with this reproductive hormone use.

Materials and methods: The use of three commonly used reproductive hormones, namely prostaglandins, GnRH and progesterone, and associated herd-level factors were studied on 760 Dutch dairy farms selected from 5 veterinary clinics. From 2017 to 2019, data on the registered sales of reproductive hormones were collected and converted into the number of reproductive hormone doses based on the prescribed amount of product per treatment on the package leaflet. The total number of doses was expressed as the annual number of reproductive hormone doses per 100 adult dairy cows. Additional herd-level information, including milking system and participation in veterinary herd health management (VHHM) programs, was available for 2019. Due to the excess of zeros in the dataset (i.e., a substantial number of nonusers), zero-inflated negative binomial models were used to associate herd-level factors with the use of reproductive hormones.

Results: In the entire study period from 2017 to 2019, 5.8% of the dairy farms did not use any reproductive hormones, varying between 0.0 and 10.3% per veterinary clinic. This proportion of nonusers was around 13.5% on an annual basis. Prostaglandins were the most frequently used reproductive hormone in Dutch dairy cows (62.9%), followed by GnRH (33.1%) and progesterone (4.0%). In 2019, the total number of reproductive hormone doses per 100 adult dairy cows ranged from 0 to 248. The mean number of doses per 100 adult dairy cows was 40.6, and its median was 32.8. Similar observations were made regarding the distribution of reproductive hormone use in 2017 and 2018. Furthermore, farms participating in a VHHM program used more reproductive hormones than farms that did not participate in such a VHHM program and these farms were also a minority in the nonuser group. Technologies, such as pedometers and automatic milking systems, also had an effect on reproductive hormone use. The presence of pedometers or activity monitors on farms was more common in the group of farmers with reproductive hormone use. Farms with an automatic milking system used more reproductive hormones than farms equipped with a conventional milking system, while organic herds used less reproduction hormones. Lastly, hormone use was more common in larger herds compared to smaller herds.

Conclusions: The veterinary clinics and farms varied regarding their use of reproductive hormones, indicating room for optimization regarding the use of hormones on a number of farms. Farm characteristics were associated with greater use of reproductive hormones. This study is a first step to achieve transparency in the Dutch dairy industry by providing an objective overview of reproductive hormone use on dairy farms and its association with a number of herd-level factors. Further research will be needed to estimate the effect of reproductive hormone interventions on cow level and, hence, the impact on reproductive performance of the herd.

Keywords: Hormone treatment, reproduction, dairy.

RD-05

Progesterone based heifer re-synchrony in an extensive pasture grazing system: A case study

Kristen Baxter[1], Luke Smyth[2], Gregory Chambers[3].

[1]Zoetis New Zealand, Christchurch, New Zealand; [2]Oamaru Veterinary Services, Oamaru, New Zealand; [3]Epivet Ltd., Te Awamutu, New Zealand.

Objective: The objective of this study was to determine if a synchrony/re-synchrony programme would prove effective in achieving a pregnancy rate of >85% in 3 cycles.

New Zealand dairy heifers are typically raised in extensive pasture based grazing systems. These systems often preclude artificial insemination (AI) from being used throughout the mating period due to the inability to carry out extended periods of daily heat detection. However, with the eradication of Mycoplasma bovis currently underway in New Zealand, some farmers are reluctant to use bulls of often unknown provenance for their heifers.

Materials and Methods: 140 nulliparous approximately 15-month old dairy heifers from a single farm were used for the study. All heifers received gonadotrophin-releasing hormone (GnRH) intramuscularly (I/M) on Day -9, a progesterone (P4)-releasing intravaginal device [1.38g CIDR®] from Days -9 to -2, prostaglandin F2α (PGF) I/M on Day -2 and a second dose of GnRH I/M as well as fixed time artificial insemination (FTAI) on Day 0 (Co-Synch). On Day 14, all heifers were re-synchronized with insertion of a new progesterone device, which was removed at Day 21. From Day 22-26 heifers were mated to detected heat. On Day 35, all heifers were pregnancy tested using rectal ultrasonography, and divided into three groups. All heifers diagnosed as pregnant were removed from the programme (Group 1). All heifers not diagnosed pregnant, but which had been mated at Days 22-25 (Group 2: possibly pregnant) were treated with a new progesterone device from Day 35 to Day 42. These devices were removed at day 42, and heifers were mated to detected heat for Days 43-46. Heifers which were not visibly pregnant at Day 35 and which had also not been mated on day 22-26 (Group 3) received another Co-Synch programme followed by FTAI on day 44. The final pregnancy test was carried out on Day 74.

Results: 123/135 (91%) of heifers which completed the trial conceived over the 46-day mating period. The submission rate (heifers mated/heifers not pregnant and available for mating) was >90% in both re-synchrony cycles. The conception

rate (heifers pregnant/heifers mated) in cycle 1 (Day 0) was 64%, in cycle 2 (Days 22-26) was 52% and in cycle 3 (Days 42-46) was 67%.

Conclusions: The heifer re-synchrony programme used was successful in achieving a >85% pregnancy rate in three cycles of mating in an extensive pasture based grazing system.

This study was approved by the Kaiawhina Animal Ethics Committee, Palmerston North, New Zealand. CIDR is a registered trade mark of InterAg. ACVM No. A4559

Keywords: Dairy cattle, synchrony, re-synchrony, heifer, progesterone device.

RD-06

Association between infectious diseases with late embryonic losses in supplemented grazing dairy cow

Luis Ernesto Quintero Rodriguez[1], Germán Domínguez[2], María Fiorella Alvarado[3], Gabriel Traverría[3], Rodolfo Luzbel De La Sota[1], Laura Vanina Madoz[1], Mauricio Javier Giuliodori[1].

[1]Instituto de Investigaciones en Reproducción Animal (INIRA), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata (FCV-UNLP), 30, Argentina; [2]Actividad Privada, 4, Argentina; [3]Centro de Diagnóstico e Investigaciones Veterinarias, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, 15, Argentina.

Objectives: To evaluate the association between Bovine Viral Diarrhea (BVD) and Infectious Bovine Rhinotracheitis (IBR) with the odds for late embryonic losses (LEL) in supplemented grazing dairy cows in Argentina.

Materials and Methods: A prospective cohort study was carried out in a commercial dairy herd, located in Carlos Casares, Buenos Aires Province, Argentina (35°37′ S, 61°22′ W) to assess the associations between seroprevalence and seroconversion to BVD and IBR with the odds for LEL. Cows having embryo with no heartbeat, or with detached membranes or floating structures including embryo remnants detected by ultrasonography (US) at 28-42 days post-AI were defined as LEL, whereas cows diagnosed with positive pregnancy by US 28-42 d post-AI were considered as non-LEL. A total of 92 cows were selected for the study (46 LEL and 46 Non-LEL). All the cows were bled twice 28 days apart (0 and 28 d). The day 0 corresponded to the day when every cow with LEL or non-LEL was diagnosed. Serological titers were determined by Virus Neutralization Test (titers were from 1:2 up to 1:2048, and from 1:2 up to 1:512 for BVDV and IBR, respectively). Seroconversion was considered when titers at d 28 were 3X higher than at d 0. The conceptus from LEL cows was sampled (day 0) with an insemination pistol attached to a 10 mL syringe, stored in a vial with RNAlater, and transported to the laboratory. Subsequently, the aspirated conceptuses from LEL cows that seroconverted were processed for virus identification by PCR. Logistic regression models were used to assess the association between serological titers for BVD and IBR (as continuous predictors) with the odds for LEL, and the association between seroconversion to BVD and IBR (yes vs. no) with the odds for LEL. Logistic models were run with Proc Glimmix of SAS with binomial distribution and logit link function.

Results: At d 0, the seroprevalences for BVD and IBR were 100% (46/46) for LEL and non-LEL cows. At d 28, the seroprevalence for BVDV was also 100% (46/46) for LEL and non-LEL cows, whereas the seroprevalence for IBR was 93.4% (43/46) and 82.6% (38/46) for LEL and non-LEL cows, respectively. Seroconversion to BVD was 20.0% (9/46) and 6.6% (3/46) for LEL and non-LEL cows, whereas seroconversion to IBR was 4.4% (2/46) and 2.2% (1/46) for LEL and non-LEL cows. The odds for LEL was associated with BVD at d 28 (P = 0.03) given that the risk increased 3.44 times per 1 SD of increment in titer over the mean. The odds for LEL was also associated with seroconversion to BVD (P = 0.09) given that the risk was 3.27 times higher in cows that seroconverted than in herd-mates that did not. Conversely, neither IBR at d 28 nor seroconversion to IBR were associated with the odds for LEL. Finally, the BVD virus was identified in all the conceptuses from LEL cows that seroconverted (9/46).

Conclusion: We concluded that seroconversion to BVD is associated with increased risk for LEL in grazing dairy cows. In addition, the BVD virus can be detected in conceptuses from cows diagnosed with LEL that seroconverted to BVD 28 d later.

Keywords: Bovine Viral Diarrhea, Infectious Bovine Rhinotracheitis, Late embryonic loss, Dairy cows.

RD-07

Risk factors for anovulation and its association with reproductive performance in a herd of grazing dairy cows

German Ariel Dominguez[1], Santiago Gerardo Corva[2], Eduardo Ravera[1], Santiago Martin Perez Wallace[3], Mauricio Javier Giuliodori[2], Rodolfo Luzbel De La Sota[4].

[1]Private Practice, Venado Tuerto, Santa Fe, Argentina; [2]Facultad de Ciencias Veterinarias-UNLP, La Plata, Argentina; [3]Zoetis Argentina SA, Villa Adelina, Argentina; [4]Facultad de Ciencias Veterinarias-UNLP; CONICET, La Plata, Argentina.

Objective: The objectives of this study were to assess the risk factors for anovulation and its association with reproductive performance in a herd of grazing dairy cows.

Material and Methods: A retrospective cohort study, including a total of 15,846 records of cows calving from January 1st, 2010 to December 31st, 2016, from a dairy farm in Argentina (~2800 milking cows) was run. Included records were those having an episode of anovulation diagnosed by ultrasonography at 50-64 day postpartum (dpp, n=3,975) and those not having an episode of anovulation that were considered as cyclic (n=11,871). The odds for anovulation at 50-64 dpp was tested with a Logistic Regression Model that included the fixed effects of calving season (spring [SP], summer [SU], autumn [AU], winter [WI]), parity (PAR 1, 2, 3+), and disease status during the first 50-64 dpp (healthy [HEALTHY, no health events], reproductive [REPRO, retention of fetal membranes, puerperal metritis, clinical endometritis, and pyometra],

non-reproductive [NOREPRO, clinical mastitis, clinical lameness], both [BOTH]). Additionally, the association between anovulation (yes vs. no) with reproductive performance was tested with a Kaplan-Mayer survival analysis through calving to conception interval (DOPEN).

Results: The occurrence of anovulation at 50-64 dpp was 25.1%. The risk for anovulation (odds ratio [OR], 95 percent confidence interval [95%CI]) was higher in cows that calved in autumn (1.388 [1.235-1.558]), winter (1.717 [1.525-1.929]) and spring (2.339 [2.080-2.631])than in cows that calved in summer (P<0.001). The risk was lower in cows of parity 2 (0.845 [0.765-0.935]) and 3+ (0.988 [0.898-1.087])than in those of parity 1 (P <0.001); and lastly, the risk for anovulation tended to increase with REP events (1.088 [0.977-1.211]), and increased with NREP events (1.609 [1.454-1.780]) and with both events (1.715 [1.501-1.960]; P <0.001). Regarding reproductive performance, anovulatory cows had a more DOPEN than cyclic cows (164.56±2.32 vs. 123.20±1.11; P<0.001). The REPRO events (126.08±1.84), NOREPRO events (144.23±1.84), and BOTH events (149.51±3.03) had more DOPEN than HEALTHY cows (123.20±1.11, P<0.001).

Conclusions: In conclusion, the risk for anovulation is affected by season, given that it is higher in cows calved in autumn, winter, and spring than in those calved in summer; and by parity, due to the risk is higher in primiparous cows multiparous ones. In addition, anovulatory cows have a longer calving to conception interval than cyclic herd mates.

Keywords: Anovulation, risk factors, reproductive efficiency, days open, grazing dairy cows.

RD-08

Relationship between reproductive performance and Q-fever in dairy herds in Brittany (France)

Vincent Jegou[1], Grégoire Kuntz[2], Bertrand Maynard[1], Jérôme Caudrillier[3], Philippe Gisbert[1].

[1]Ceva Santé Animale, Libourne, France; [2]GDS Bretagne / Innoval, Poufragan, France; [3]Evolution / Innoval, Noyal sur Vilaine, France.

Objectives: *Coxiella burnetii* is a small intracellular bacterium responsible for Q Fever in animals and humans. In cattle, Q fever is a major cause of abortion: 2nd cause according to the French observatory of the causes of abortion in ruminants (OSCAR). But this disease is also responsible for low fertility.

GDS Bretagne is a farmer association helping farmers to manage sanitary issues, including abortions and fertility disorders. Evolution is an Artificial Insemination Company operating in the same area. These two companies together with Ceva Santé Animale, carried out a study to compare reproduction performances in dairy herds depending on the reason for abortion.

Material and methods: 831 herds with abortions were included between November 2018 and May 2020. Causes of abortions were determined using a combination of PCR on vaginal discharge and serology on blood of animals from the same cohort. In the case of two or more aborted animals had positive PCR results for *Coxiella burnetii*, Q fever was considered as a highly probable cause of abortion. If only one PCR result was positive, the blood serology of other animals was taken into account. If at least 50% of serology tests were positive, Q fever was considered the cause of abortion with medium probability.

An annual summary of reproductive performances was made for each herd and crossed with the reason for abortion. Fertility of cows and heifers, stillbirth, culling rate, calving interval, age at first calving were studied.

ANOVA was applied to explain reproductive criteria.

Results: Finally, data were available for 642 herds.

12 % of the abortion events were due to Q fever with medium or high probability.

In herds where *Coxiella burnetii* was detected as a highly probable cause of abortion, the fertility rate at first insemination was 6.99 points lower than the average for herds in the same area and during the same period (40.16% and 47.16% respectively, p=0.023). If herds with a medium probability of Q fever abortion are also included, the fertility rate remains lower than that of herds without Q fever abortion (41.42% and 47.16% respectively, p=0.013). In herds with low probability of Q fever, the first insemination was almost twice as likely to be successful as in herds with a medium or high probability of Q fever (OR=1.92 CI95% [1.07 - 3.45]).

The study also highlighted that the calving interval was extended by 4.7 days in Q fever infected herds (420 d vs 415 d).

No difference was highlighted for the other criteria (fertility of heifers, culling rate, stillbirth, and age at first calving).

From an economic point of view, the cost simulator developed by the Vets school of Nantes (Bilan de santé du troupeau laitier - bioepar.org) showed that this drop in fertility rate was 23 euros per cow per year, excluding the cost of abortions.

Conclusion: Dairy herds affected by Q fever abortions have lower cow fertility than others. We showed that the disease has a global impact on reproduction and not only on abortions. This finding is in accordance with some data already published on the involvement of *Coxiella burnetii* in fertility, metritis/endometritis and retained placenta.

It appears that a control of the disease including both biosecurity measures and vaccination could be of interest in such herds to improve fertility.

We focused herds with abortions due to Q fever. This study could be complemented by another one which would consist in the detection of *Coxiella burnetii* in herds with degraded fertility, even in the absence of abortions.

Keywords: Q fever, fertility, cattle, reproductive performance.

RD-09

Evaluation of the impact of multifactorial parameters to improve activity-based heat detection systems

Hila Kroll[1], Doron Bar[1], Juan Pedro Campillo[1], Monika Ptaszynska[2].

[1]Allflex Livestock Intelligence, Netanya, Israel; [2]MSD Animal Health, Milton Keynes, United Kingdom.

Objectives: Heat detection devices have traditionally been using changes in the activity pattern of female cows. This is either done via recording of simple parameters such as the number of steps, detection of specific movements (mounting behavior), or by detailed analysis of intensity and direction of the animal's movements. Inclusion of other parameters could lead to an improvement in heat detection algorithm accuracy. The restless behavior of cows in estrus is associated with a decline in their rumination time. This was demonstrated after the introduction of rumination monitoring collars (Bar and Solomon, 2010) and confirmed in later studies (Reith and Hoy, 2012). Inclusion of the normal estrous cycle pattern and duration of behavioral heat should further increase the accuracy of heat detection and monitoring. In order to increase heat detection accuracy, Heat Index algorithm, based on the above mentioned parameters, was developed for an automated monitoring system. The objective of this study was to compare the accuracy of estrus detection in dairy cows using Heat Index versus activity data only algorithm.

Materials & Methods: A total of 15,000 activity increase alerts with a very low threshold (about 25% activity index increase) were collected from about 3,000 cows in 7 commercial farms, with all cows on farm wearing monitoring collars (Allflex® Livestock Intelligence™) throughout the lactation.

A novel notation method was developed defining a "gold standard" to evaluate heat detection efficiency under field conditions. All heat alerts resulting in inseminations leading to a confirmed pregnancy were classified as True Positives. All episodes of increased activity recorded in pregnant cows were classified as True Negatives. It is important to note that the system user does not receive the false positive alerts because the system does not present pregnant cows in the "Cows ready for AI" report. From these two populations, a Receiver Operating Characteristic (ROC) curves were drawn for an algorithm based on activity change alone, or Heat Index, combining several parameters.

Results: The analysis of True Positives (TP) against False Positives (FP) revealed a significant added benefit of using the Heat Index algorithm compared to the one based on the activity increase only. At the relevant interval (95-98% sensitivity), the use of Heat Index algorithm led to a reduction of false heats by 28% and 36%, respectively. At 95% specificity, activity only algorithm discarded 51% (1839/3617) FP heats while Heat index algorithm discarded 79% (2857/3617) TP heats. At 98% specificity, activity only algorithm discarded 26% (940/3617) FP heats while Heat index algorithm discarded 62% (2242/3617) TP heats; Heat Index ROC curves also demonstrated an improvement of 1% in sensitivity of heat detection, at the default detection level threshold.

Conclusions: The Heat Index algorithm (Allflex® Livestock Intelligence™) shows a better performance in heat detection in dairy cows compared to algorithms based on activity patterns only, with significantly superior specificity while preserving, and even slightly improving the detection sensitivity.

This brings significant benefits to the dairy farmers using monitoring system with heat detection ability. The use of Heat Index algorithm decreases the risk that animals that are not in heat are selected for insemination, which reduces wastage of semen and unnecessary expenses on service. Although more limited, improved sensitivity of heat detection with Heat Index algorithm means fewer cows in heat and eligible for insemination are missed. Consequently, improved heat detection accuracy would contribute to improved farm reproduction performance, reduction of days open and intervals between calvings.

Keywords: Heat detection, monitoring, heat index, estrus.

RD-10

Pastoral dairy bull procurements and management effects on fertility

Kate Mitchell[1], David Beggs[2], Peter Mansell[2].

[1]Scottsdale Veterinary Services/University of Melbourne, Scottsdale, Tasmania, Australia; [2]University of Melbourne, Melbourne, Australia.

Objective: Dairy herd bulls are utilised on most farms as a backup to AI in the southern Australian dairy system. The current recommendations for their management include the purchasing and movement of bulls onto farm 2-3 months prior to their use. This recommendation is from the Bull Power study completed in Queensland & New South Wales, Australia which assessed beef bull reproductive performance following transportation to new properties and changes in management conditions.

We aimed to assess dairy bulls' reproductive performance; both prior to sale and following movement onto their destination property under new management conditions. Links between stress associated with transportation & management changes, semen motility, morphology and scrotal circumference will be investigated.

Methods: Holstein-Friesian & Jersey bulls (n=34) were examined using a veterinary bull breeding soundness evaluation (VBBSE) on their farms. Bulls were then transported to new properties and a VBBSE was repeated at 20 days, 50-70 days and 120-150 days following arrival. At each VBBSE semen samples were collected via electroejaculation and submitted for morphological examination by a registered morphology laboratory.

Management practices on the property of origin and new property were noted for each of the bulls. Recorded management practices included mixing with existing bull teams, workload, and nutrition.

Results: Preliminary results show that following movement onto a new property and changes to management (BBSE 20d) sperm morphology & scrotal circumference are temporarily negatively affected, these deficits are resolved in the follow-

ing BBSEs (50-70d, 120-150d). Some correlation between distance travelled and the effect on semen morphology and scrotal circumference is evident in preliminary assessment of the data set.

Conclusion: The current recommendations for dairy herd bulls throughout Australia are based on data obtained from a study of beef bulls completed in 2005. This work aims to give appropriate guidelines for minimum timing of procurement prior to use as well as an indication of the likely stress associated with mixing of bull teams.

Keywords: Bull, fertility, scrotal circumference, semen morphology, dairy herd bull.

RD-11

The effect of integrin binding domain peptide and C-terminal fragment of osteopontin on restoring the endometrial EGF abnormality in repeat breeder dairy cows

Takashi Tanida[1], Takayoshi Tagami[2], Yojiro Yanagawa[3], Seiji Katagiri[3].

[1]Laboratory of Theriogenology, Department of Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan; [2]Laboratory of Molecular Enzymology, Division of Fundamental AgriScience Research, Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan; [3]Laboratory of Theriogenology, Department of Clinical Sciences, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan.

Objectives: In fertile cows, epidermal growth factor (EGF) concentrations in the endometrium show two peaks on days 2-4 and 13-14 of the estrous cycle. The absence of EGF peaks in the endometrium has been linked related to reduced fertility and around 70% of repeat breeder (RB) dairy cows lost endometrial EGF peaks. Intravaginal infusion of seminal plasma osteopontin (OPN) restored the endometrial EGF profile and fertility in RB cows. However, the mechanism by which OPN exerts this effect is unknown. OPN can be cleaved by thrombin, dividing into N-terminal and C-terminal fragments. The N-terminal fragment has integrin binding domain (RGD sequence and SVAYGLK sequence adjacent to RGD). While the C-terminal fragment interacts with CD44. The objective of this study was to evaluate whether the integrin binding domain peptide and the C-terminal fragment of OPN restore the endometrial EGF abnormality in RB cows.

Material & Methods: 271 RB dairy cows were used in this study. They were examined for endometrial EGF concentrations on day 3 (day 0 = estrus) and cows showing low concentration (< 4.70 ng/g tissue weight) were used. Then, all cows were synchronized for estrus. Each cow was infused with either 10 ml phosphate-buffered saline (PBS) alone (n = 85) or one of the following samples prepared in 10 ml PBS into the vagina within 4 to 12 hours after detection of estrus; recombinant full length OPN (rOPN, 32 nmol, n = 121), recombinant C-terminal fragment (C-rOPN S162-N278, 46 or 53 nmol, n=25) and synthetic peptide (peptide G151-K161, 32 nmol or 320 nmol, n = 20). Then, endometrial EGF concentrations on day 3 of the estrous cycle were examined for the second times.

Results: The normalization rates of the endometrium EGF profile (≥ 4.70 ng/g tissue weight) were higher in the cows infused rOPN (59.5%, n = 72) and 320 nmol peptide G151-K161 (55.0% n = 11) than PBS (27.1%, n = 23) (P<0.05). The normalization rates were similar between rOPN and 320 nmol peptide G151-K161. While the normalization rates were similar in the cows infused C-rOPN S162-N278 (12.0%, n = 3) and 32 nmol peptide G151-K161 (30.0%, n = 6) to PBS. Endometrial EGF concentrations after treatment were higher in the cows infused with rOPN [5.91 (3.15-7.05), median (interquartile range)] than PBS [2.71 (1.91-5.34)] (P<0.05), but were similar in the cows infused with C-rOPN S162-N278 [1.91 (1.71-2.62)], 32 nmol [3.15 (1.81-5.20)] and 320 nmol [5.30 (1.94-6.35)] peptide G151-K161 to PBS. In the cows infused with peptide G151-K161, endometrial EGF concentrations after treatment tended to be higher in the cows infused 320 nmol peptide than 32 nmol peptide (P<0.1).

Conclusion: Integrin binding domain peptide of OPN has function to restore the endometrial EGF abnormality in RB dairy cows. However, the peptide needed a larger amount in mole than rOPN to restore the endometrial EGF profile. Cells having integrin might have a role to normalize the endometrial EGF profile by OPN. On the other hands, the C-terminal OPN fragment may have less or no function to normalize the endometrial EGF profile in RB dairy cows. This may indicate that CD44 might not be a main targeting receptor for normalization of the endometrial EGF profile by OPN.

Keywords: Osteopontin, Epidermal growth factor (EGF), Endometrial biopsy, Repeat breeder dairy cows.

RD-13

Reproductive Prolapse in Cows in Western Thailand: Grades, Severity and Treatment

Smit Srisomrun, Pipat Arunvipas, Niorn Ratanapob.

Kasetsart University, Kamphaengsaen, Nakhon Pathom, Thailand.

Objectives: This study determined grades, severity and treatment of reproductive prolapse in cows in western Thailand.

Materials and methods: This study investigated the reproductive prolapse cases submitted to Kasetsart University Veterinary Teaching Hospital Kamphaengsaen, from January 2016 to November 2019. The data were collected from out-patient department records and a questionnaire and analyzed by STATA.

Results: There were 90 cases submitted during the study period, 72 cases (80%) were beef and 18 cases (20%) were dairy cattle. Forty-nine cows (54%) were raised in the free stall system and 32 cows (36%) were raised in the grazing system. Only 11 of affected cows (13%) were heifers. One-fourth of the reproductive prolapse cases found before calving occurred during the last trimester of pregnancy.

Grade of prolapse was divided in 5 grades. There were 20

vaginal prolapse (grade 1 and 2) cases (22.22%), 52 cervical prolapse (grade 3) cases (57.78%) and 18 uterine prolapse (grade 4 and 5) cases (20%). A few factors were associated with the severity of reproductive prolapse. More severe prolapse cases were found in beef cattle less than in dairy cattle ($P < 0.001$). Most of the severe cases were found after calving ($P < 0.001$). All uterine prolapse (grade 4 and 5) cases were found after calving and almost all of these cases (17/18) occurred within the first day after calving.

The treatment protocols were cleaning the reproductive tract to decrease risk of infection, performing epidural anesthesia, replacing the reproductive organs and suturing the vulva lips with Bühner technique, applying tincture-iodine at the suture site and injecting an anti-inflammatory drug. Calcium solution was administered before replacement in cows suspected hypocalcemia. Every case was rechecked 1-7 days after treatment depended on severity of the reproductive prolapse, and there was no recurrent prolapse.

Conclusions: The reproductive prolapse cases submitted in this study were beef cattle more than dairy cattle. However, most reproductive prolapse cases in dairy cattle were uterine prolapse which is related to post-partum hypocalcemia.

Keywords: Reproductive prolapse, cows, grade of prolapse.

RD-14

Comparison of three different reproductive management strategies for lactating dairy cows emphasizing detection of oestrus or synchronization of ovulation and Timed Artificial Insemination (TAI)

Richard Vazquez[1], Katie Denholm[1], Pedro Pinho[2], Federico Randi[2], Lorenzo Viora[1].

[1]University of Glasgow, Glasgow, United Kingdom; [2]CEVA Sante Animale, Loudeac, France.

Objectives: The aim of this study was to compare three reproductive management strategies for first insemination of lactating dairy cows: Ovsynch; PRID-synch, both using oestrus detection and subsequent timed artificial insemination (TAI); and Double-Ovsynch at TAI.

Materials and methods: A total of 1681 Holstein cows from two commercial Scottish dairy farms were randomised by parity to three different reproductive strategies for first AI. For the first two protocols, cows were eligible to be inseminated by detected oestrus between 50 and 70 days in milk (DIM) and were subsequently enrolled in either one of the following two protocols:

1. Ovsynch (n=541), GnRH (G1) at 70±3 DIM, PGF2α (PG1) 7 d later, PGF2α (PG2) 24h later and GnRH (G2) 32h later, followed by FTAI 16 h after;
2. PRID-synch (n=562), GnRH (G1) + PRID at 70±3 DIM, PGF2α (PG1) 7 d later, PGF2α (PG2) + PRID removal 24h later and GnRH (G2) 32h later, followed by FTAI 16 h after.

For the third protocol, Double-Ovsynch (n=578), 53±3 days after parturition, cows received an injection of GnRH, PGF2α 7 d later and GnRH 3 d after, as presynchronization. Then at 70±3DIM, the breeding Ovsynch including an injection of GnRH (G1), PGF2α (PG1) 7 d later, PGF2α (PG2) 24h later and GnRH (G2) 32h later, followed by FTAI 16 hours later.

Ovarian monitoring was performed by transrectal ultrasonography at 43±3 and 50±3 DIM in all animals, and 70±3 (G1) and 77±3 (PG1) DIM only for the cows that were synchronized. Pregnancy was diagnosed by transrectal ultrasonography between 28 and 35 days after AI.

Results: Twenty-eight-day pregnancy rates for first AI was similar among treatment groups: Ovsynch (44%; 162/365), PRID-synch (41%; 158/383) and Double-Ovsynch (45%; 160/353). Cows with a corpus luteum (CL) at 77±3 DIM (PGF1) were 3.23 times more likely to be pregnant regardless of the treatment group ($p<0.001$). Primiparous cows were more likely to be pregnant at 28 days than multiparous cows in all treatment groups (Ovsynch 59% vs 35%; PRID-synch 47% vs 38%; and Double-Ovsynch 55% vs 39%) ($p<0.001$).

Conclusions: There was no significant difference in pregnancy rate at 28 days among treatment groups, which is consistent with previous comparable studies (Fricke et al., 2014, Robichaud et al., 2016). This may have been due to cows in the first two protocols being mated to observed oestrus on commercial farms where heat detection efficiency and P/AI on standing oestrus were good (57% and 46% respectively); which may have over-inflated the 28 days pregnancy rate in these two groups; however, these results are reflective of the expected performance of the protocols in a commercial setting. Primiparous cows submitted to Double-Ovsynch did not have higher pregnancy rate compared with the other two strategies in contrast to other published reports (Souza et al., 2008; Borchardt et al., 2016).

Keywords: Dairy, reproduction, oestrus, synchronization, TAI.

RD-15

Effect of puerperal metritis occurrence on reproductive efficiency and milk production in grazing dairy cow

Santiago Corva[1], German Dominguez[2], Eduardo Ravera[2], Mauricio Giuliodori[1], R. Luzbel De La Sota[1].

[1]Facultad de Ciencias Veterinarias-UNLP, La Plata, Argentina; [2]Private Practice, Venado Tuerto, Santa Fe, Argentina.

Objective: The objective of this study was to estimate the effect of puerperal metritis (PM) occurrence on reproductive efficiency and milk production in a dairy farm in Argentina (~2800 milking cows).

Material and Methods: An observational retrospective study, including a total of 16856 records of cows calving from January 1st, 2010 to December 31st, 2016, was used. At parturition, calving assistance was recorded (ACAL; normal, slight or severe assistance, c-section, abortion), and retention of fetal membranes (RFM) was recorded. All cows were exam-

ined for the diagnosis of PM (0-3) during the first two weeks after calving. Only cows diagnosed with PM3 (enlarged uterus and a fetid watery red-brown uterine discharge and signs of systemic illness [decreased milk yield, dullness, or other signs of toxemia]) were treated (TRT) with ceftiofur (6.6 mg/kg BW, SC; Excede, Zoetis Argentina). Cows were rechecked a week later, and cows with PM0 were diagnosed as clinically cured (CCURE), and cows with PM1-2 were diagnosed as partially cured (PCURE). Only cows with PM3 were treated again with the same protocol until clinically cured or culled from the herd. After three weeks, post-partum cows were examined for diagnosis of clinical endometritis (CE, 0-3). After having a 50 days voluntary waiting period (VWP), cows were AI at detected heat. Reproductive efficiency was measured by percent of IA cows by 80 (AI80) days postpartum (dpp), percent of pregnant cows by 100 (PRE100) and percent of open cows by 200 (OPE200) dpp, percent of pregnancy losses to first pregnant AI (LOSS1P), and interval from calving to first IA (C1AI) and to conception (ICC). Also, milk production (kg) at 150 (MP150) and 305 (MP305) days of lactation were measured.

Results: During the first week of calving, the occurrence of PM0-PM3 was 47.72%, 14.04%, 22.99%, and 15.25% (8043, 2366, 3874, and 2571/16854, respectively). First lactation cows had higher occurrence of PM3 compared to 2+ lactation cows (22.96% [1510/6578] vs 10.32% [1061/10276]; P<0.01), and cows calving during the spring had higher occurrence of PM3 compared to cows calving the rest of the year (17.45% [634/3634] vs 11.94% [1937/16218]; P<0.01). Of a total 2571 cases of PM3 diagnosed, 2439 had registered TRT information and were used for further analysis. The CCURE rate 24.89% (607/2439) and the PCURE rate was 53.59% (1307/2439). About 18.49% (451/2439) of the cases did not respond to TRT and remained PM3; and 3.03% (74/2439) of the cases were TRT but were not rechecked by the veterinarian. Furthermore, 39.66% of PM3 persisted as CE after 21 dpp. The interval calving to PM3 TRT was 8.81±0.77 d. Retrospectively, cows with PM3 had more ACAL and RFM compared to cows without PM0 (52.98% [1362/2571] vs 72.63% [5842/8043], P<0.01; 14.16% [364/2571] vs 2.00% [161/8043], P<0.01). Also, more cows with PM3 failed to persist in the herd for a subsequent lactation compared to PM0 cows (27.15% [698/2571] vs 26.00% [2091/8043], P<0.01). Cows with PM3 had a lower IA80 and PRE100, and higher OPE200 compared to PM0 cows (58.37 [1465/2510] vs 64.55 [5036/7802], 30.89 [767/2483] vs 37.59 [2897/7706], 30.74% [710/2310] vs 25.64 [1843/7188]; P<0.01, respectively). Furthermore, cows with PM3 had 4% more pregnancy losses compared to PM0 cows (30.12% [635/2108] vs 25.97% [1742/6709], P<0.01). Pregnancy rate to first IA (IC1AI) and day open (ICC) were 3 and 10 days longer for PM3 compared to PM0 cows (76.43±0.41 [n=2260] vs 73.67±0.22 [n=7108]; 134.42±1.43 [n=1982] vs 124.34±0.77 [n=6379]; P<0.01, respectively). Cows with PM3 produced 460 kg and 675 kg less of milk compared to PM0 cows (5055.91±11.82 [n=6961] vs 4595.62±19.55 [n=2335]; 9016.34±37.35 [n=1750] vs 9691.85±22.58 [n=4834]; P<0.01, respectively).

Conclusions: Cows with PM3 have more pregnancy losses, more days open, and produced less milk at 150 and 305 days of lactation compared to PM0 cows. The clinical cure rate and partial cure rate for PM3 cows were low, and many PM3 cows developed CE after 21 dpp.

Keywords: Puerperal metritis, treatment, reproductive efficiency, milk production, dairy cows.

RD-16

Purulent vaginal discharge score and its association with subsequent reproductive performance in seasonal-calving, pasture-based dairy cows

Eber Rojas Canadas[1], Mary M Herlihy[2], Jonathon Kenneally[2], Francis Kearney[3], Jim Grant[4], Pat Lonergan[5], Stephen T Butler[2].

[1]The Ohio Sate University, columbus, United States; [2]Teagasc, fermoy, Republic of Ireland; [3]Irish Cattle Breeding Federation, Bandon, Republic of Ireland; [4]Teagasc, Ashtown, Republic of Ireland; [5]University College Dublin, Belfield, Republic of Ireland.

Objectives: Uterine infection diminishes reproductive performance in cattle. The use of Metricheck® to assess purulent vaginal discharge (PVD) may help to identify uterine pathological condition. The objective of this study was to evaluate PVD as a predictor of failure of reproductive performance in pasture based dairy cows to simplify diagnostic tools for uterine abnormalities and to reduce costs. Secondary objective was to determine the agreement between Metricheck® with ultrasound examination of the uterus as a tool to identify post-partum uterine abnormalities in dairy cattle. Materials&Methods: First and second lactation dairy cows (n=2,600) located in the province of Munster in Ireland were enrolled in the study. Postpartum examinations were performed at week 3 (wk3,14 to 27 days in milk) and week 7 (wk7, 42 to 55 days in milk) after calving. Purulent vaginal discharge was established as follows: PV1= clear mucus; PV2= flecks of pus within clear mucus; PV3= 50% purulent material. Ultrasound uterine score examination (8.5 MHz transrectal transducer Ibex Pro, Ibex®, Colorado, USA) was assessed as follows: G1: spoke wheel-shaped lumen; G2: small volume (>2 mm, ≤5 mm) of fluid of mixed echogenicity; G3: Moderate volume (>5 mm, ≤10 mm) of fluid of mixed echogenicity; G4: Large volume (>10 mm) of mixed echogenicity fluid. During the breeding season all animals were served after estrous detection. A post-breeding visit was carried out once between 34-50 days after farm mating end date to determine pregnancy status and foetal age. The reproductive parameters included in the study were: Submission rate (SR21), pregnant to first service (P/AI1), pregnant within 21, 42 and 84 days of onset of breeding (P21, P42, P84, respectively) and the Mating Start day Conception Interval (MSD-CI). Fisher's Exact Test was used and was supplemented by multiple logistic regression using the LOGISTIC procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC) to calculate odds ratios and predicted probabilities. The concordance between PVS and USE was assessed using Cohen's Kappa Coefficient with the FREQ procedure of SAS. Results: At wk 3, 18.7% (486/2600), 30.4% (790/2600), 26.9% (699/2600) and 24.0% (625/2600) of animals were diagnosed as having PV1, PV2, PV3 and PV4; and 2.2% (59/2600), 25.1% (653/2600), 60.5% (1574/2600) and 12.0% (314/2600) of animals were diagnosed as having G1, G2, G3 and G4 ultrasound uterine score, respectively. At wk7, 40.4% (1051/2600),

39.6% (1030/2600), 16.6% (434/2600) and 3.2% (85/2600) of animals were diagnosed as having PV1, PV2, PV3 and PV4; and 27.3% (669/2445), 48.3% (1182/2445), 23.3% (572/2445) and 0.8% (22/2445) of cows were diagnosed by ultrasonography as having G1, G2, G3 and G4, respectively. There was no association between PVD at wk3 (P=0.54) and wk7 (P=0.84) and SR21. Conversely, animals classified as having a PV4 at wk 3 and wk7 had reduced P/AI1 (both *P*<0.05; - 8 and -11 percentage points) compared with animals classified as having PV1. On the same way, cows classified as having PV3 and PV4 were less likely to achieve P21(*P*=0.01; -9.2 and -10.6 percentage points), P42 (*P*=0.008; 4.8 and 7.9 percentage points) P84 (*P*=0.003; only PV4, -5.6 percentage points) and the mean MSD-CI was 4.19±0.24 and 5.03±0.19 d longer (*P*<0.0001) compared with animals diagnosed as having PV1, respectively. Similarly, reproductive performance and MSD-CI were associated with PVS at wk7. Animals classified as having PV4 had less likelihood of P42 (*P*=0.005; -15.35, -12.25 and -14.11 percentage points) and P84 (*P*=0.01; -8.61, -6.67 and -7.11 percentage points) compared with animals having PV1, PV2 and PV3, respectively. MSD-CI was longer (*P*=0.04) in animals classified as having G4 uterine score compared with cows classified as having PV1, PV2 and PV3 (7.4 ± 0.2, 6.1 ± 6.18 and 6.4 ± 0.15 d, respectively).

The Kappa coefficients at week 3 and week 7 postpartum were 0.17 and 0.13, respectively, indicating slight to fair agreement between PVD and ultrasound uterine score examination as diagnostic techniques for uterine abnormalities.

Conclusion: The observations arising from this study provide evidence that PVD was strongly associated at both wk3 and wk7 postpartum in seasonal-calving, pasture-based lactating dairy cows. Purulent vaginal score evaluation at wk3 and wk7 post-partum identified an unfavorable association between PV3 and PV4 and reproductive performance. On average, the PVD scores were lower than ultrasound examination scores in the early postpartum period.

Keywords: Uterine-infection, dairy-cattle, fertility, ultrasonography.

RD-17

Effects of thermal stress on pregnancy characteristics in dairy cows

Ioannis Nanas[1], Katerina Dadouli[2], Thomas - Markos Chouzouris[1], Konstantina Stamperna[1], Eleni Dovolou[1], Georgios S. Amiridis[1].

[1]Univ. of Thessaly, Vet Faculty, Dept of Obstetrics & Reproduction, Karditsa, Greece; [2]Univ. of Thessaly, Faculty of Medicine, Larissa, Greece.

Objectives: Due to global warming, the adverse effects of heat stress (HS) on dairy cattle welfare and production are gradually extending in northern latitudes. The combination of high ambient temperatures and relative humidity (THI) in dairy cows decreases appetite, milk yield, estrous expression, estrus length and pregnancy rates of inseminated animals. The extensive use of cooling systems for the abatement of the negative effects of HS, proved to be successful in preserving milk production, but their impact on fertility records are rather negligible. This is related to the high sensitivity of the maturing oocyte and the early embryo to the elevated temperatures as well as to the modifications that increased temperatures bring about at follicular, oviductal and uterine environment.

The trophoblastic cells of the bovine placenta express a series of glycoproteins named Pregnancy Associated Glycoproteins (PAGs) whose concentration is steadily increased with pregnancy progression, culminating at calving. PAG concentration of inseminated cattle is used as a biochemical marker of pregnancy and a reliable indicator for the wellbeing and functionality of the placenta. Progesterone (P4) is of paramount importance for pregnancy establishment and preservation, however, the limits between optimal and suboptimal concentrations is difficult to be accurately defined, as P4 concentrations are related to various environmental and metabolic factors.

Here, we focused to estimate the association between circulating concentrations of PAG, P4 and the early embryo mortality rates during summer and winter.

Materials and Methods: Two trials were conducted in two Holstein dairy farms of central Greece. Both farms are equipped with effective cooling systems comprising electronic assessment of THI and automatic activation of fans and sprinklers. In addition, according to thermal load all lactating cows had intensive cooling (soaking–drying) two to four times daily.

The purpose of the first trial was the quantification of the early embryonic mortality rates under HS or thermoneutral conditions. In 2018, a total of 279 cows were used (144 during the winter, group W, and 135 during the summer, group S). On days 24-25 post AI, all cows were diagnosed as pregnant by transrectal ultrasonography; on days 34-36 they were re-examined, and a blood sample was withdrawn for PAG determination.

The second trial lasted from 2014 to 2019 and aimed at PAG levels evaluation in 6109 blood samples submitted for routine pregnancy diagnosis. All samples were collected 29 to 36 days after breeding and assayed by a commercial ELISA kit (DG29, Conception Animal, Canada). According to THI, the samples were allotted into group S THI≥76 n=2388, or into group W THI≤68, n=3721. A sample was characterized as positive, ambiguous, or negative (PAG ≥1000pg/ml, 500 to 999pg/ml or <500, respectively).

Progesterone concentration was also determined in 50 pregnant animals from each group in serum samples collected on days 31 to 33 post AI by a commercial ELISA kit (DRG, Germany).

Results: In trial 1, the pregnancy maintenance rate differed significantly (p=0.03) among groups (136/144 - 94.4% and 81.5% - 110/135, for groups W and S, respectively). According to the PAG concentrations all animals were characterized as pregnant.

In trial 2, PAG levels on days 33 to 36 after breading were significantly higher (p=0.043) in group S (2546.8±63.1 ng/ml) compared to those of group W (2385.51±42.4 ng/ml).

Progesterone was lower (p<0.05) in group S (6.76±0.96ng/ml) compare to W (8.66±1.27).

The proportions of ambiguous pregnancies were lower

(p<0.001) in group S (7.88%) compared to W (11.10%).

Conclusions: From the data presented here we infer that during summer months the embryos that can survive HS develop a well-functioning placenta. This is in concert with our in vitro studies where we provided evidence that blastocysts produced from oocytes exposed to HS have high expression of genes related to placentation. On the other hand, the combined results of embryo mortality and progesterone might be indicative that during the winter, slow developing or weak embryos have more chances to survive.

Acknowledgements: Work co-financed by the EU and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code:T-1EDK-01078, the Summer Cow).

I.N is financially supported by IKY (project MIS-5000432, co-financed by Greece and E.U.-ESF).

Keywords: Heat stress, progesterone, PAGs, early embryonic death, dairy cattle.

RD-18

The effect of *coxiella burnetii* inactived vaccine on reproductive parameters and milk yield in Holstein cows

Gulnaz Yilmazbas-Mecitoglu[1], Guleycan Egesu Yildiz[1], Kerem Kucuk[1], Baris Guner[2], Abdulkadir Keskin[1], Ahmet Gumen[1].

[1]Bursa Uludag University, Bursa, Turkey; [2]Balikesir University, Balikesir, Turkey.

Objectives: The aim of this study was to determine the effect of *Coxiella burnetii* inactivated vaccine on reproductive parameters and milk yield in Holstein cows.

Material & Methods: A total of 575 pregnant cows (165-170 days of pregnancy) were examined serologically and distributed to the research groups. While the cows were serologically positive formed the Positive Control (n=174, PosC) group, the cows that were serologically negative for *Coxiella brunetii* were randomly assigned into two groups: The cows were vaccinated formed the Negative Vaccine (n=175, NegV) and were not vaccinated served as Negative Control (n=226, NegC) The cows in the NegV were vaccinated 3 weeks apart with *Coxiella burnetii* inactivated vaccine (4 ml, s.c. Coxevac®, Ceva, Istanbul, Turkey) and serological examinations were repeated 3-4 weeks later to determine the efficacy of the vaccine. During the study, pregnancy, birth, postpartum, and lactation processes of the cows were monitored. At the end of voluntary waiting period, the cows were inseminated with modified G6G protocol for the first service and the cows whose unable to get pregnant were received resynchronisation protocol. Pregnancy examinations performed by ultrasonography.

Results: The abortion rate was 2.7% (6/226) in NegC, 1.1% (2/175) in NegV, 0.6% (1/174) in PosC group. Due to the different reasons 21 cows were culled thus 554 cows were monitored for postpartum period; The incidence of stillbirth was 1.9% (4/210) in NegC and 3.5% (6/170) in PosC groups, no stillbirth cases were observed in the NegV (0/169) group. Fetal membrane retention was 14.5% (31/214), 10.0% (17/170) and 9.4% (16/170) in NegC, NegV and PosC groups, respectively. The incidence of metritis was 8.4% (18/214) in NegC, 10.6% (18/170) in NegV and 7.1% (12/170) in PosC groups. The total of 477 cows were able to be evaluated for reproductive parameters since there were cows (n=98) that culled for different reasons. First service conception rate was 29.8% (54/181) in NegC, 30.1% (43/143) in NegV, 34.4% 52/151 in PosC groups. Number of inseminations per pregnancy were 1.56 (128/82) in NegC, 1.39 (96/69) in NegV and 1.44 (89/88) in PosC groups. Embryonic loss rates were determined as 6.0% (5/83) in NegC, 9.2% (7/76) in NegV and 11.1% (11/99) in PosC groups. The rate of repeat breeder cows was determined as 4.9% (9/181) in NegC, 2.8% in NegV (4/144) and 4.6% (7/152) in PosC groups. The data belongs to the cows were completed for the first 100 days; In the NegC (n=184), NegV (n=147) and PosC (n=154), the total milk yield in the first 100 days was recorded as 4739.65 ± 46.56 kg, 4772.81 ± 55.27 and 4752.82 ± 47.92 kg; mean peak yield was recorded as 56.21 ± 0.50 kg/day, 56.39 ± 0.53, and 55.91 ± 0.56 kg/day. While the mean peak day was 64.03 ± 2.13 DIM in NegV, it was 67.07 ± 2.43 DIM in PosC, which was higher (P<0.05) than in Neg C (61.28 ± 1.74 DIM) groups.

Conclusion: In conclusion, it was found that the cows have no *Coxiella burnetii* antibody at the beginning of the study (NegC) had numerically higher abortion and retention of fetal membrane rates compare to other cows in NegV and PosC groups which included the cows whether had natural or acquired immunity. Stillbirth and embryonic loss rates were numerically higher in PosC group compare to the other groups. Also it was found that the milk peak day of the cows in PosC group was seen later than of the cows in other groups.

Acknowledgement: This research is supported by The Scientific and Technological Research Council of Turkey (TUBITAK, Project No.119O952).

Keywords: Cow, Coxiella brunetii, inactivated vaccine, reproductive parameters, milk yield.

RD-19

HH1 double-carrier embryos develop normally to elongated conceptuses

Alejandra C Quiroga, Priscila Ramos-Ibeas, Ismael Lamas-Toranzo, Óscar González-Recio, Pablo Bermejo-Álvarez.

INIA-CSIC, Madrid, Spain.

Objectives: Intensive genotyping of Holstein population has identified deleterious haplotypes impacting fertility. These haplotypes are never found in homozygosity indicating that double-carrier (i.e., homozygous) embryos die before birth. However, the developmental stage when embryonic or fetal loss occurs remains unknown. Timing of developmental arrest is relevant for reproductive management, as embryonic losses occurring before maternal recognition of pregnancy lead to luteolysis and recovery of estrus cycle, whereas later losses ex-

ert a much greater impact on conception intervals. The objective of this study was to determine if homozygous embryos for Holstein Haplotype 1 (HH1) are able to develop to elongated conceptuses, which trigger maternal recognition of pregnancy.

Material & Methods: *In vivo* produced embryos were recovered at Days 9 (D9, expanded blastocysts), 11 (D11, ovoid conceptuses) or 14 (D14, elongated conceptuses) post-fertilization from superovulated HH1-carrier cows (2 cows/stage) inseminated with semen from a HH1-carrier bull. Embryos were fixed in 4 % paraformaldehyde for 10 min and kept at 4 ºC until analysis. D9 embryos were subjected to immunostaining with anti-CDX2 to determine trophectoderm (CDX2+) and inner cell mass (CDX2-) cell number. Total and epiblast cell numbers were determined on D11 conceptuses by immunostaining for SOX2. Conceptus and embryonic disc length were measured on D11 and D14 conceptuses. Finally, Sanger sequencing was performed to determine the genotype of each embryo: non-carrier (NC), carrier (C) or double-carrier (DC).

Results: Day 9 embryos showed Mendelian distribution of alleles (5:8:4 for NC:C:DC). At that stage, genotype did not determine blastocyst cell counts (TE: 98±7 vs. 106±4 vs. 105±5; ICM: 20±1 vs. 21±1 vs. 22±2; for NC, C and SC, respectively, ANOVA p>0.05). Day 11 conceptuses also showed Mendelian distribution of alleles (4:12:5 for NC:C:DC). Conceptus or embryonic disc size was also similar across genotypes (conceptus length 535±84 vs. 546±89 vs. 446±68 µm, disc length 106±13 vs. 107±12 vs. 105±7 µm, for NC, C and DC, respectively, ANOVA p>0.05) and no differences were noted on total or SOX2+ (epiblast) cells (total cells 1262±209 vs. 1291±352 vs. 951±282; SOX2+ cells 50±4 vs. 54±10 vs. 50±7; for WT, Hz and KO, respectively, ANOVA p>0.05). Finally, Mendelian distribution was also unaltered by D14 (2:3:3 for NC:C:DC) indicating that DC embryos are able to develop to elongated conceptuses. A significant cow effect was noted on conceptus and embryonic disc length at D14, but genotype did not influence any of these parameters (conceptus length 7.9±6.1 vs. 5.6±5 vs. 5.7±2.7 cm; disc length 0.52±0.11 vs. 0.44±0.16 vs. 0.57±0.13 cm, for NC, C and DC, respectively, two-way ANOVA p<0.05).

Conclusion: HH1 double-carrier embryos develop normally to elongated conceptuses, suggesting that the developmental arrest induced by the causative mutation occur after maternal recognition of pregnancy, leading to a significant increase in days open.

Keywords: Embryo; haplotype; fertility; elongation; HH1.

RD-20

Effect of clinical and subclinical mastitis treatment with meloxicam on clinical cure and fertility of dairy cows in Ecuador

Christian Albuja Arroba[1], Joel Hernádez Cerón[2], Ramiro Rearte[3], Rodolfo Luzbel De La Sota[3].

[1]Facultad de Medicina Veterinaria y Zootecnia, Universidad Central de Ecuador, Quito, Ecuador; [2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad de México, Mexico; [3]Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata & CONICET, La Plata, Argentina.

Objective: To study the effect of clinical and subclinical mastitis treatment with meloxicam on clinical cure rate and fertility of dairy cows in Ecuador.

Material and Methods: Holstein cows from two dairy herds from Pichincha, Ecuador, were used in a study that began in March 2020 and ended in October 2021. Cows with 35-150 days in milk (DIM) had a California Mastitis Test (CMT) done every two weeks and cows with a CMT score 2 (CMT2), score 3 (CMT3), or with clinical mastitis (CM) were enrolled. Cows with odd ear tags were assigned to the control group (CON, n=204), and cows with even tag numbers were assigned to the treatment group (TRT, n=189). Cows with CMT2 in the TRT group were administered meloxicam (0.5 mg/kg BW, Metacam® Boehringer Ingelheim, Ecuador), whereas cows in the CON group remained untreated. Cows with CMT3 and CM in the TRT group were administered an intramammary antibiotic treatment (tetracycline 200mg, neomycin 250 mg, bacitracin 2000 UI, prednisolone 10 mg, Mastijet Fort®, MSD Salud Animal, Mexico) and meloxicam (0.5 mg/kg BW); whereas cows in the CON group were administered the intramammary antibiotic but not the meloxicam treatment. After treatment, cows had a CMT done every two weeks to determine the clinical cure rate (complete, CMT0; partial, CMT reduced; worst, CMT increased or maintained). After a voluntary waiting period of 55 days (d), cows were synchronized with cloprostenol (Ciclase DL®, Zoetis, Ecuador) and AI at detected heat. Cows not detected in heat by 80 DIM were synchronized and timed AI. In addition, for a cow to enroll in the study, the CMT2-3 and CM event had to take place in a window of time of 30 d before 45 d post-AI. Transrectal ultrasonography was used to diagnose pregnancy status at 30±3d post-AI, and those cows diagnosed pregnant were reconfirmed pregnant at 60±3d post-AI. The clinical cure rate (CCR) to CMT2, CMT3+CM at 30 d post-treatment were examined. Pregnancy rate at 30d (PREGR), pregnancy losses at 60 d (PLOSS), calving to conception interval-days open (CCI), number of services per conception (SPC), and culling rate (CULLR) were also analyzed with logistic regression (PROC GLIMMIX, SAS 9.04).

Results: The CCR for CMT3+CM in the TRT group was higher compared to the CON group (72.5% [58/60] vs. 57.3% [43/75], P=0.036), but was similar between CMT2 TRT and CON groups (62.5% [152/243], P=0.73). The PREGR and PLOSS were similar in TRT and CON groups for CMT3+CM (41.9%, P=0.44; 36.76%, P=0.53), and CMT2 (47.22%, P=0.56; 32.35%, P=0.35). The CCI for CMT3+CM in the TRT group was shorter than the CON group (100.66±11.08 vs. 133.23±11.79 d, P=0.04). Also, the SPC for CMT3+CM in the TRT group was lower than the CON group (1.69±0.32 vs. 2.62±0.34, P=0.05). Conversely, the CCI and the SPC were similar between CMT2 TRT and CON groups (118.50±10.95 d, P=0.46; 2.19±0.24, P=0.21). The CULLR was reduced in CMT2 TRT cows compared to CON cows (5.50% [6/109] vs. 15.5% [20/129], P=0.01), but was similar in CMT3+CM TRT and CON cows (14.19% [22/155], P=0.52).

Conclusion: Cows with CMT3+CM that received antibiotic treatment plus meloxicam had a higher clinical cure rate than cows that received antibiotic therapy alone. Furthermore,

these cows had fewer days open and lower AI per conception than the untreated cows. In addition, cows with CMT2 had no benefit of meloxicam treatment on clinical cure rate or reproductive performance, but meloxicam treated cows had a lower culling rate during lactation.

Keywords: Clinical mastitis, subclinical mastitis, meloxicam, fertility, clinical cure.

RD-21

Injuries of the bovine birth canal after eutocia

Theresa Maria Scheu[1], Michael Berger[2], Imke Cohrs[1], Christian Koch[1], Axel Wehrend[2].

[1]Educational and Research Centre for Animal Husbandry, Hofgut Neumuehle, Münchweiler an der Alsenz, Germany; [2]Clinic for Obstetrics, Gynecology and Andrology with Veterinary Ambulance, JLU Giessen, Giessen, Germany.

Objectives: While there is numerous of literature describing the involution of the uterus after unassisted birth, there is little information available about possible alterations of the soft birth canal in cows and heifers. So the aim of this study was to describe potential injuries of the soft birth canal post partum (p.p.).

Material and Methods: For this purpose, 50 Holstein-Friesian cows (25 heifers und 25 cows) were assessed vaginally using a speculum within eight hours after birth and on day 1, 5, 10, 15 and 21 p.p.. Special attention was given to lacerations, hematomas, hyperemia and suffusions in the area of the cervix, vagina, hymenal region, vestibulum vaginae and vulva.

Results: Immediately after birth all animals presented lacerations in the dorsal commissure of the vulva, which had healed off completely until day 15 p.p. in approximately 50% of the heifers, and on day 10 p.p. in approximately 70% of the cows. Additionally, all heifers and 41% of the cows presented with lacerations of the hymenal region, which had healed almost completely by day 15 in 75% and 99%, respectively. Hyperemia and suffusions were present in all heifers in this region. While the hyperemia had disappeared by day 10 p.p, suffusions were still present in approximately 45% of the animals on day 21 p.p..

Conclusions: In summary, there are injuries in the soft birth canal even after unassisted birth and it is possible to describe a unique injury pattern. Particularly heifers have significant injuries in the area between the vulva and the hymenal region; however, these heal entirely within a short period of time.

Keywords: Laceration, hematoma, hymenal region, vulva.

RD-22

Influence of different factors on fertility of dairy cows submitted to fertility protocols for first insemination postpartum and for resynchronization

A. Francisco Álvarez Baldor.

AFRIVEPA, SOC COOP, Santa María del Páramo, Spain.

Objectives: The use of "fertility protocols" for artificial insemination (AI) at fixed time adding improvements to Ovsynch®, as presynchronization, use of a progesterone device and two doses of prostaglandin F2α (PG), has showed increases of conception rate in comparison to the normal Ovsynch protocol or natural estrus. At commercial farms level, the length of the protocol is something important so it can be long for first insemination postpartum but should be as short as possible in the case of resynchronizing open cows in order to reduce the number of days open.

In this study we tried two protocols for first AI: the G6G protocol, modified to end as Cosynch (with the last GnRH dose at the same time of AI), with 6 handlings, that lasts for 18 days, and a 7 d Cosynch plus progesterone device, with 4 handlings, that lasts for 10 d. For resynchronizing open cows we used a Resynch protocol (modified for administering the last GnRH at the same time of AI) starting with a GnRH 7 d before pregnancy diagnosis, with 4 handlings, that lasts for 3 d from the diagnosis of open cow. We analyzed some factors that could affect these protocols to understand how they can be used to improve reproduction efficiency in comercial dairy farms

Materials and methods: The study was conducted during 2019 in 12 dairy farms located in León, Spain. A total of 994 synchronization protocols were performed and the cows were explored by a veterinarian at the moment of insemination to evaluate the size of the follicle and the signs of estrus at uterine and ovarian level to inseminate only cows with estrus signs.

The cows enrolled for first AI were treated as "Group G6G" (n=421): day 0 PG, day 2 GnRH, day 8 GnRH, day 15 PG, day 16 PG, and day 18 AI plus GnRH; "Group progesterone device" (n= 227): day 0 GnRH + insertion of progesterone device, day 7 PG and progesterone device removal, day 8 PG, day 10 AI plus GnRH. In a subgroup of cows in groups "Progesterone device" and "G6G", blood samples were collected at the beginning of the Cosynch, at the moment of the first prostaglandin of the Cosynch and at the moment of AI. The cows that didn't show return in estrus, were enrolled as "Group Resynch" (n=346) receiving GnRH 7 d before the examination and if diagnosed "open" the day of the pregnancy diagnosis, they were treated: day 0 PG, day 1 PG, day 3 AI plus GnRH.

Data were analyzed with Medicalc® and the different chi-square test were performed for every variable.

Results: The synchronization rate was evaluated as percentage of cows accepted for AI and was high for the three groups: "G6G" 84.03%, "Progesterone device" 88.33% and "Resynch" 94.02% (p=0,057) with progesterone concentration profiles corresponding with that findings: beginning of Cosynch 2,16 ng/ml, first PG 2,90 ng/ml and moment of AI 0,32 ng/ml. The conception rate was high in the three groups: "G6G" 44.89%, "Progesterone device" 40.53% and "Resynch"

49,42%, (p=0,11). The median days to AI were significantly different in inseminations with diagnosis "pregnant" or "open": open 121.47 and pregnant 134.08. (p=0.007). The conception rate of different follicle sizes were 10 to 15: 40.43%, 16 to 20: 40.00% and > 20: 30.77 (p=0.45%). The conception rate of different parities were 1: 49.86%, 2: 44.73%, 3 : 39,13% and cows with 4 or more calvings 49.26%. (p=0.08).

Conclusions: The numerical differences in conception rate between protocols could have been influenced by the days in milk at AI so, taking into account the high synchronization rate and fertility of these fertility protocols, they can be implemented at farm level, the longer ones for first post-partum AI considering their easiness of compliance, and the shorter one for a fast resynchronization of open cows.

The decreasing conception rate by parity have been previously reported, but the high conception rate in cows with 4 or more calvings could be influenced by the retention of the most fertile cows, showing that the retention of some old fertile cows could extend the general longevity of farms.

Keywords: Fertility, protocol, post-partum, resynchronization, insemination.

RD-23

Adiponectin and Chemerin in the endometrium of postpartum dairy cows with cytological endometritis

Gonçalo Pereira[1], Ricardo Bexiga[1], João Chagas E Silva[1], Elisabete Silva[1], Christelle Ramé[2], Joelle Dupont[2], Patrice Humblot[3], Luis Lopes-Da-Costa[1].

[1]CIISA - Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal; [2]INRAE, UMR85 Physiologie de la Reproduction et des Comportements, 37380, Nouzilly, France; [3]Division of Reproduction, Department of Clinical Sciences, SLU, Uppsala, Sweden.

Objective: To evaluate the relationship between the gene transcription and expression of Adiponectin (ADIPOQ, ADIPOR1 and ADIPOR2) and Chemerin (RARRES2 and CMKLR1), and the inflammatory status of the uterus of postpartum dairy cows.

Materials and methods: High-yielding postpartum dairy cows (n=36) without puerperal disease were retrospectively allocated to groups: i) Healthy (H; n=6), without cytological endometritis (CE) at 25 and 45 days postpartum (dpp) and pregnant at first AI; ii) CE-H (n=19), with CE at 25 dpp, but that recovered by 45 dpp; iii) CE-CE (n=11), with persistent CE until 45 dpp. Endometrial cytology was assessed from uterine swabs taken with a cytobrush device at 25 and 45 dpp (cut-off values: 18 % and 5 %, respectively). At 45 dpp, a low volume lavage followed by centrifugation, allowed to obtain cellular pellet and supernatant samples of each uterine horn. An endometrial biopsy was also taken from each uterine horn. Gene transcription of *ADIPOQ* and its receptors (*ADIPOR1* and *ADIPOR2*), and of chemerin (*RARRES2*) and its receptor (*CMKLR1*) was analysed in the cellular pellet by quantitative real-time PCR. Protein expression was analysed by immunohistochemistry (IHC) in endometrial biopsy samples and protein production (ADIPOQ and RARRES2) by ELISA in the supernatants.

Results: Transcription levels of *ADIPOQ* and *ADIPOR2* were higher ($p < 0.001$) in CE-CE than in H and CE-H cows, whereas *ADIPOR1* mRNA levels were lower ($p < 0.05$) in CE-CE cows than in H cows. Transcription levels of *RARRES2* and *CMKLR1* were higher in CE-CE than in H and CE-H cows ($p < 0.01$). Positive immunostaining for ADIPOQ was observed in the luminal and glandular epithelium, endothelial cells, stroma and inflammatory cells of all cows. However, CE-CE cows exhibited a stronger staining than H and CE-H cows. Staining for ADIPOR1 was only observed in the luminal and glandular epithelium, whereas ADIPOR2 also stained in stroma and inflammatory cells. Staining for RARRES2 and CMKLR1 was observed in all endometrial compartments of all cows. However, RARRES2 staining showed a stronger signal in CE-CE than in H and CE-H cows. Uterine fluid ADIPOQ and RARRES2 concentrations were higher ($p < 0.001$) in CE-CE than in H and CE-H cows.

Conclusions: At 45 dpp, cows with persistent CE show up-regulated gene transcription and protein expression of Adiponectin and Chemerin in the endometrium, compared with healthy cows and cows that recovered from CE. Results support a local production of these mediators, and a relationship with the inflammatory status of the postpartum uterus of dairy cows. The local production and signalling of these adipokines prompt for an autocrine and/or paracrine role in the inflammatory response exhibited by cows with subclinical endometritis. This turns these adipokines into potential biomarkers of endometrial inflammation, namely to identify cows at risk of persistent subclinical endometritis. However, further studies are warranted to clarify their role in the establishment of subclinical endometritis.

Funding: FORMAS (Grant No 2015-00888) and CIISA UIDP/CVT/00276/2020. Gonçalo Pereira has a PhD grant from FCT (SFRH/BD/130923/2017).

Keywords: Adiponectin, Chemerin, Endometritis, Cow.

RD-24

The Effect of Postpartum Metabolic Diseases on Fertility in Lactating Dairy Cows

Gulsum Selcuk Kaya[1], Serdar Dursun Ak[1], İsmailİlik Kocaer[1], Ahmet Gumen[2], Gulnaz Mecitoglu[2], Abdulkadir Orman[2].

[1]Atasancak Dairy Farm, Denizli, Turkey; [2]Bursa Uludag University, Bursa, Turkey.

Postpartum (PP) metabolic disorders may become more important for the modern high producing lactating dairy cows. The aim of this study was to evaluate the effect of PP metabolic disorders on fertility in dairy cows. The study was conducted in a dairy farm with 5000 lactating dairy cows and milk yield was 11.148 kg for 305 d in 2019. Primiparous and multiparous cows were evaluated for metabolic disorders such as Retained Placenta (RP), Septic metritis (SM, Fever >39.4˚C

and reddish vaginal discharge with fetid odor after DIM 5), Milk fever (MF), Ketosis (BHBA higher than 1.2 ng/ml, DIM 3-7) and Displaced abomasum (DA). In multiparous cows, BCS (at the close up period) and its relation to PP metabolic disorders were also evaluated. All cows were timed inseminated (TAI at 75-81 DIM) with Double Ovsynch protocol for first service and second service were done by Resynch protocol (GnRH-7d-GnRH-7d-PGF-1d-PGF-56h-GnRH-16-20h TAI). Pregnancy diagnosis were done at the time of the first PGF (30-36 d after TAI) by ultrasonography. In multiparous cows mean BCS was higher (P<0.01) in the cows with PP metabolic disorders compared to the cows without metabolic disorders (BCS 3.42, BCS 3.37, respectively). Pregnancy rate after first (33.0%), and second (29.5%) service were higher (P<0.002) in multiparous cows without PP metabolic disorders compared to the multiparous cows with at least one metabolic disorders (21.7%, 19.0% respectively). Pregnancy rate of multiparous cows after first service was negatively effected (P<0.001) in the cows with RP, SM, and ketosis, tended to be effected (P<0.09) with MF, but not effected with DA. In addition, cows with ketosis had also lower (P< 0.03) pregnancy rates after second services. However in primiparous cows pregnancy rate was lower (P<0.03) only in cows with Ketosis. Thus, cows with higher BCS at close up period at higher risk of having PP metabolic disorders and cows with some of the metabolic disorders such as RP, SM and ketosis in early PP period having more effect on fertility at first service. The negative effect of ketosis on fertility may be more prominent compared to other PP metabolic disorders, because it's long lasting affect and in both primiparous and multiparous cows. Interestingly, primiparous cows less effected from metabolic disorders than multiparous cows except ketosis.

Keywords: Metabolic disorders, Fertility, lactating dairy cows.

RD-25

Key performance indicators used by dairy consultants during the evaluation of reproductive performance in a first visit

Ramon Armengol[1], Lorenzo Fraile[1], Àlex Bach[2].

[1]Universitat de Lleida, Lleida, Spain; [2]Marlex, Barcelona, Spain.

Objectives: The purpose of this study was to (1) describe the KPI that consultants specialized in dairy reproduction use to assess the reproductive status of a conventional dairy farm in a first visit, (2) categorize the different KPI according to their importance to the consultants, and (3) identify primary KPI that could be universally used in first visit to a conventional dairy farm.

Materials and methods: An online survey was sent worldwide and answered by consultants specialized in dairy reproduction (n=49) to find out the most suitable parameters in a first visit approach. The survey was comprised of 190 questions, 178 of them rated from 0 (irrelevant) to 10 (maximum importance) points. The questions were divided into 5 sections: 1) consultant and farm model, 2) general data of the farm, 3) cow reproduction, 4) postpartum and metabolic disease, and 5) heifer reproduction. The median, range, and 95% confidence interval were determined for each question. Afterwards, a multivariate analysis, using between-group linkage via Ward´s hierarchical clustering was conducted to generate clusters of consultants according to their response pattern. Lastly, a Chi-square test was conducted to assess the association between years of experience of the consultant and farm size within the clusters generated in each section of the questionnaire.

Results: The majority of the consultants considered 27 parameters to be highly important to analyze in a first visit.

The parameters that consultants specialized in dairy reproduction mostly consider as primary KPI are:

General data of the farm: Culling rate (%), pregnant cows (%), average DIM (d), cows culled for reproductive reason (%), 305-d yield (Kg), herd status for BVDV (present/absent) and % of 1st lactation cows in the herd.

Cows' reproduction: First service CR (%), overall pregnancy rate (%), voluntary waiting period (d), CR (%), heat detection rate (%), days open (d), cows not pregnant >200DIM (%), 21d. pregnancy rate (%), CR of the first service in 1st lactation cows, CR of the first service in multiparous cows (%), CR of the 1st lactation cows (%), CR of the multiparous cows (%), calving to first service interval (d), CR synchronized cows (%) and percent conceiving of served (%).

Heifers' reproduction: Heat detection rate (%), CR (%), CR of the first service (%), age at first calving (d) and heifers culled for reproductive reason (%).

Conclusion: Consultants never use only one single primary KPI to evaluate any of the presented sections, but they use several of them (in variable quantitative range). Despite the presence of a great number of parameters likely to be KPI to assess the reproductive performance of a farm, consultants clearly prefer parameters that provide information about heat detection, fertility and when the pregnancy is achieved regarding to the production cycle of a dairy cow.

Consultants also show high interest in getting a general overview of milk production, the farming efficiency and the heifer rearing process but the farm size and the years of experience of the consultant is other factor influencing in the type and numbers of parameters chosen to be KPI.

The parameters rated with the highest importance (rate 10) that could be considered for an easy, fast and universal use in a first visit to assess the reproductive status were: First service CR (%), overall pregnancy rate (%) and 21d pregnancy rate (%) for cows and age at first calving (d) for heifers.

Parameters that monitor postpartum and metabolic diseases are not considered necessary to evaluate the reproduction performance of a farm in a first visit.

Keywords: survey analysis, dairy consultant, reproductive performance, first visit, KPI.

RD-26

Elevated tail: clinical sign for the early diagnosis of vaginal pathologies

Angel Revilla Ruiz[1], Susana Astiz[2], Raquel Patrón Collantes[1], Juan Vicente González-Martín[1].

[1]Universidad Complutense de Madrid, Madrid, Spain; [2]Animal Reproduction Department, National Institute of Agronomic Research (INIA), Madrid, Spain.

Elevated tail is a feature seen around defecation and urination. It is a clinical sign present in pathologies related to reproduction, urinary and digestive systems. In reproduction, this sign has been observed with calving contusions and hematomas of the vagina; with minor vaginal traumas after mating or vaginal explorations; and in cases of necrotic vaginitis, vestibulitis, and vulvitis postpartum. The sign occurs within 1–4 days post calving and it may persist for 2–4 weeks, until vaginitis resolves, except in cases of vaginal lacerations, pneumo, and urovagina in which the raised tail may stay in time. Vaginal disorders such as pneumovagina and urovagina reduce the fertility of the cow and they are conditions frequently underdiagnosed. In large farms with intensive reproduction management systems, it is uncommon to perform complete reproductive exploration of cows, including the vagina, of all cows, before first service. However, some cows can keep vaginal problems since calving and are diagnosed at the earliest as repeat breeder with >200 days in milk, as non-pregnant after≥3 services, when the correcting surgery may be not any more profitable.

Objectives: The present study assesses the usefulness of the sign of "elevated tail" as an indicator to detect in an easy and fast way, cows with vaginal pathologies. Our hypothesis was that selecting those post calving cows raising their tail, for a complete vaginal exploration during routine farm visits, will provide an early diagnosis of vaginal pathologies, allowing an efficient treatment of them, without notable extra work, reducing the culling rate due to reproduction.

Materials and methods: We worked in three Spanish dairy farms of 73, 263 and 250 cows in milk/farm, respectively, during one routine reproductive control. The cows that showed elevated tail at the visit (excluding those which were defecating, urinating, less than a day calvingor had recently been inseminated), were completely explored. This exam included rectal palpation, ultrasound exam and vaginal exploration, trying to identify the type of problem and vaginal content. The presence of air, urine, other vaginal content, and tears was recorded.

Results: A total of 586 cows in milk were present during these visits; 28 raised their tail (4.78%). In 7 cases out of 28 (25%) no pathologies were diagnosed, which would make a sensibility for the sign used as diagnoses method of 75% with 25% of false negatives. The remaining 21 animals (75%) showed the following reproductive problems: postpartum vaginitis in 6 cases (21.42%); pneumovagina 6 (21.42%); urovagina 4 (14.29%), pneumo and urovagina 3 (10.71%) and vaginal laceration 2 (7.14%).

Conclusions: Our preliminary results seem to indicate that the clinical sign of an elevated tail may allow to efficiently select, with a high probability (≈75%), those cows with vaginal pathologies for a further and complete examination. This method will allow their prompt treatment, reducing the culling rate due to reproduction. Further studies will be implemented to describe the validity of this clinical sign as a diagnosis method of vaginal disorders in dairy cows.

Keywords: Tail, elevated, urovagina, pneumovagina, diagnosis.

RD-27

Uterine contractility patterns of early puerperal hypocalcaemic dairy cows without and with fetal membrane retention

Arpad Csaba Bajcsy[1], Gergő Kürtös[2], Laura Zámbó[3], János Tibold[4], Imre Élö[4], István Mádl[4], Ottó Szenci[5].

[1]University of Veterinary Medicine Hannover, Foundation, Hannover, Clinic for Cattle, Germany; [2]Turul Veterinary Clinic and Pharmacy, Gödöllö, Hungary; [3]Animal Hospital, Veszprém, Hungary; [4]Bonafarm Group, Agroprodukt, Pápa, Zsigaháza, Hungary; [5]MTA-SzIE Large Animal Clinical Research Group, Üllő, Hungary.

Objectives: Characteristics of the early postpartum uterine contractility based on the measurement of intrauterine pressure (IUP) changes in hypocalcaemic cows without and with retained placenta during the first two days after calving has been compared in a field study.

Materials and methods: Myometrial contractions of altogether 31 early puerperal Holstein-Friesian cows at a large-scale Hungarian dairy farm were repeatedly measured using an open tip catheter system, which was suitable for digital intrauterine pressure (IUP) recording. Cows were divided into four groups according to their initial coccygeal venous blood Ca^{2+}-concentrations, which was measured on site between 14 and 17 hours after calving (ABL™ 77 portable blood gas and electrolyte analyser, Radiometer, Copenhagen, Denmark) and upon their status regarding placental expulsion. Cows that had lower than 1.06 mmol/l blood Ca^{2+} concentrations at this first blood withdrawal either belonged to Group HC-NRFM (hypocalcaemic, no retained fetal membranes, n=12) or to Group HC-RFM (hypocalcaemic, retained fetal membranes, n=6), while others that had at least 1.06 mmol/l blood Ca^{2+}, belonged either to Group NC-NRFM (normocalcaemic, no retained fetal membranes, n=8) or to Group NC-RFM (normocalcaemic, retained fetal membranes, n=5). Initially, a 4-hour IUP recording was performed, starting between 14 and 17 hours after calving, from which only the first hour was used for the recent analysis (labelled as pp12). In 12-hour intervals, three further recordings took place with a duration of 1 hour for each (labelled as pp24, pp36 and pp48, respectively). IUP data were collected from the previously pregnant uterine horn and were analysed using a semiautomatic, operator-made software (LabVIEW® 5.0, National Instruments, Austin, TX). Mean contraction frequency (FREQ), amplitude (AMP), duration (DUR), mean and total area under the pressure curves (AUC and TAUC) of the pressure cycles were calculated. At the end of each recording

session a further blood withdrawal has been made to determine Ca^{2+} concentrations.

Beside discovering possible associations among values of various IUP parameters and blood Ca^{2+} concentrations by the use of a regression analysis, repeated measures analysis of variance was used to calculate group- and time-related differences. In case of significance at P<0.05, Tukey post-hoc tests were performed to explore where significance between groups, recording times or their interactions appeared (STATISTICA v. 6.1., Statsoft, Inc., Tulsa, OK).

Results: All hypocalcaemic cows had a mild to moderate level of hypocalcaemia with the lowest initial Ca^{2+} concentration of 0.74 mmol/l in two cows. In all IUP parameters time dependent significant declines were found (P<0.001) when neither RFM nor Ca^{2+} status were distinguished. When both the effect of RFM and Ca^{2+} status were considered, also none of the parameters showed in their values an overall significant difference at P<0.05, which would have been attributed to either of these group differences. However, at pp12, hypocalcaemic cows with RFM had higher TAUC values, representing a more active uterine contractility than their NRFM alternatives (P=0.0064). At pp12 TAUC was significantly higher in cows with RFM (P=0.0014) and at pp24 AUC also in such cows (P=0.0325) without considering the initial levels of blood Ca^{2+}. However, without considering the time effect, despite consequently higher mean values in all IUP parameters in RFM cows, the differences were statistically not significant at P<0.05. When the grouping effect according to initial Ca^{2+} levels has not been taken into account, except DUR, all IUP parameters showed significantly higher overall means in cows with RFM than in cows without (FREQ: P=0.0017, AMP: P=0.0079, AUC: P=0.0020 and TAUC: P=0.0001), while the level of such hypocalcaemia did not influence uterine contractility. Blood Ca^{2+} has significantly changed by declining from pp12 to pp24 and gradually increasing thereafter (P=0.012), but among cows with and without RFM, no difference was found regarding this parameter.

Conclusions: Dairy cows with mild or moderate hypocalcaemia during the first two days postpartum showed rather similar uterine contractility patterns with a significant time related decline, however, cows with RFM had higher uterine mechanical activity, irrespective of their calcium status within the mentioned range.

Keywords: Cattle, Uterine contractility, Hypocalcaemia, Placenta retention.

RD-28

Novel methods to analyse mucus. Use of FTIR-spectroscopy to examine bovine cervical vaginal mucus

Ida Beate Løken[1], Volha Akulava[1], Achim Kohler[1], Volha Shapaval[1], Michael Morris[2], Adam Martin[1].

[1]Norwegian University of Life Scienses, Ås, Norway; [2]Univerity of the West Indies, Port of Spain, Trinidad and Tobago.

Objective: Cervical vaginal mucus (CVM) has important functions related to reproduction and reproductive disease in cattle. To facilitate further study of CVM, an appropriate method of storing and processing CVM samples needs to be developed. The aim of this study was to compare the effects of different methods of storage and processing of CVM on the spectra obtained by Fourier Transform Infrared (FTIR) spectroscopy.

Material and Method: 16 CVM samples were taken from 15 Norwegian Red dairy cows housed at Livestock Production Research Centre at the Norwegian University of Life Sciences. The cows were selected based on convenience of the likelihood that they would be able to provide 2ml of CVM when sampled.

The cows were secured, and their vulvas cleaned with a chlorhexidine solution, before being dried with paper towel. A Metricheck™ device was used to sample CVM. The samples were transferred to Eppendorf® tubes. All samples underwent light microscopy to characterise ferning patterns before freezing and after thawing.

The samples were separated into two equal groups (1 and 2) and processed as follows: 1) Samples were further split into two groups and frozen (FRO) or frozen and freeze-dried (FD). 2) Samples were split into two groups and frozen (FRO) or kept fresh (F).

FRO: Samples were frozen in a freezer at -20°C.

FD: samples were frozen in a freezer at -20°C before being freeze-dried in a Labconco freeze-drier using high vacuum at -52°C. The samples were mixed with approximately 100µl water/g dried sample and 70g of acid washed glass beads (710-1, 180µm) /g sample before disrupting.

F: Fresh samples were treated directly after sampling.

Once processing was complete, the FRO and FD were thawed at room temperature. The F and FRO groups underwent one of five treatments. These were: no treatment, three durations of ultrasound treatment (5s, 10, s and 15s), and disruptor treatment. The FRO and F disruptor-treatment involved 0.5ml of mucus applied to 250mg of acid washed glass beads. All disruptor samples were run on a work program (5500 rpm, 6 cycles à 20s, 20s pause) in a Precellys Evolution disrupter-machine. For the ultrasound treatment, 0.5ml of mucus was placed on ice and homogenized with an ultrasonic homogenizer a 2mm tip was inserted 1cm into the mucus with amplitude of 40%. Five second exposures were applied once, twice or three times, giving samples exposed 5, 10 and 15 seconds.

After treatment, all samples were transferred to IR-light-transparent silicon 384-well microplates (Bruker Optik, Germany), approximately 10µl/ well, with 3 replications, and dried at room temperature. FTIR spectroscopy was performed using a high-throughput screening eXTension (HTS-XT) unit coupled to a Vertex 70 FTIR spectrometer (both Bruker Optik, Germany) allowing performance of high-throughput screening (HTS) transmission mode measurements. The spectra were recorded in the region between 4000 and 500 cm−1 with a spectral resolution of 6 cm−1 and an aperture of 5.0 mm.

A general analysis of the whole spectra was performed before the different informative regions were analysed. Spectra in the 1654 cm−1-region <0.2 or >2.5, were removed from the analysis. The data were pre-processed using a second de-

rivative calculated with the Savitzky-Golay algorithm, window size 11, second polynomial order. Three informative regions were split as follows: lipids (3050–2800 cm−1 and 1800–1700 cm−1), proteins (1700–1500 cm−1) and polysaccharides (1200–700 cm−1). The whole spectra and the informative regions were all pre-processed using Extended Multiplicative Signal Correction (EMSC) and analysed with principal component analysis.

Results*:* Light-microscopy showed little, or no, change in fern ing patterns before and after freezing. All the preparation methods improved handling and homogenisation compared to the F group. Scatterplots show that the variable individual cow had a greater effect on the spectra (general and informative groups) than the preparation method. However, variation exists between preparation methods within cow. The F samples show most inter-cow variation, followed by the ultrasound treated samples. Whilst the disruptor samples show least variation.

Conclusions: Provisional results reveal that a homogenising technique may be useful in measuring changes in CVM. Further work needs to be performed to identify the optimal dilution for sample analysis. Treatment with disruptors appears to provide a better result than ultrasonographic treatments, although this needs further study. Freezing does not seem to alter the results.

Keywords: Cervical vaginal mucus, bovine, Fourier Transform Infrared spectroscopy.

SP-01

Health and production benefits in veal calves born from NCD and BRD vaccinated cows

Magdéléna Chanteperdrix[1], Béatrice Mounaix[1], Marlène Guiadeur[1], Christophe Martineau[1], Sébastien Assié[2], Sylvie Masselin[1], Christophe Le Nouvel[3], Frédéric Leboeuf[3], Geert Vertenten[4], Thibault Jozan[3].

[1]Institut de l'Elevage, Paris, France; [2]Oniris, site de la Chantrerie, Nantes, France; [3]MSD Santé Animale, Beaucouzé, France; [4]MSD Animal Health, Boxmeer, Netherlands.

Objectives: Neonatal calf diarrhea (NCD) and bovine respiratory disease (BRD) are the most important health problems in veal calves. Given the increased pressure to reduce intensive antimicrobial use, vaccination may be an efficient measure to reduce the use of antibiotics in the treatment and prevention of BRD and NCD in young animals. Passive immunity transfer from vaccinated dams may be applied for the prevention of NCD whereas BRD protection can be provided by vaccinating the calves but also by passive immunity transfer.

The objective of this study was to measure the benefit of vaccinating pregnant cows against NCD and BRD (Rotavec® Corona and Bovilis® Bovipast RSP) on the health and production in veal calves.

Materials and methods: 211 calves that received adequate colostrum from cows vaccinated with inactivated multivalent vaccines against NCD (Rotavec Corona) and BRD (Bovilis Bovipast RSP) (V2+) and 220 calves without known history (V-) were equally divided over 10 veal farms at an age of at least 14 days and compared for clinical NCD, BRD, weight and general condition at 3 (d3) and 45 (d45) days post arrival, antibiotic treatments, mortality and carcass weight.

Results: At d3, V2+ (11.4%) had less clinical BRD compared to V- (19.1%) (P=0.028). The weight at d3 was higher (P=0.026) in V2+ compared to V- and the calves in V2+ were in better condition. The mortality in V2+ (2.8%) was significantly (P=0.01) lower than V- (11.8%). The odds of mortality in V2+ was 4.57 times lower than in V- (P=0.01). V2+ received about one antibiotic treatment less compared to V- (P=0.02) (3.5 versus 2.9). All other measured parameters were not statistically significant different, which is not surprising for NCD at d3 and d45 post arrival as NCD occurs mainly before arrival at the veal unit.

Conclusions: This study clearly describes several health and production benefits in veal calves receiving adequate colostrum from mother cows that were vaccinated against NCD and BRD (respectively with Rotavec Corona and Bovilis Bovipast RSP). It reinforces the interest to strengthen the relationship between the dairy and veal sector, in order for health investments in dairy calves (e.g. vaccination) to be appreciated by the benefiting veal producers.

Keywords: Veal, mother vaccination, BRD, scours, benefits.

SP-02

Age at First Calving in UK Jerseys; Impact on First Lactation 305-day Milk Yield, Lifetime Daily Milk Yield, Calving Interval and Survival to Second Lactation

Fiona Boothby[1], David Grove-White[2], Georgios Oikonomou[2], Jo Oultram[2].

[1]Vale Veterinary Group, Cullompton, Devon, UK, Cullompton, United Kingdom; [2]University of Liverpool, Leahurst campus, United Kingdom.

Objectives: To determine the association between Age at Fist Calving (AFC) and production, fertility and survivability in Jersey heifers, and to compare this with published literature on Holstein heifers

Material and methods: Lactation records from 7255 pedigree Jersey heifers from 471 UK milk recorded herds, calving for the first time from 1st January 2009 to 31st December 2010, were examined. Heifers were grouped by AFC into 4 groups comprised of AFC 18-24 months, 25-26 months, 27-29 months, and 30-36 months. Mixed effects multivariable regression modelling and binary logistic regression modelling was used for data analysis.

Results: Increased Lifetime daily yield (LDY) was significantly associated with a reduced AFC; heifers calving at 18-24-month-old produce 9.87 kg milk/day (mean) (CI: 9.64-10.11) (P=<0.000001), nearly 1 litre more than those calving at 30-36 months (mean 8.94kg; CI: 8.72-9.16). Calving UK Jersey heifers at 24 months or below is optimal. There was some evidence that lower AFC may be associated with enhanced fertility; heifers calving at 25-26 months had a significantly lower mean calving interval (391 days; CI 386-397) than those calving over 30 months (399; CI 394-405) (P=0.03). Heifers with an AFC over 30 months were less likely to survive to second lactation than those with an AFC 18-24 months; odds ratio 1.75 (CI 1.38-2.23) (P=<0.0001) more likely to fail to reach second lactation than heifers calving at younger ages.

Season of first calving had a significant impact. Spring calving heifers produced significantly less milk per day (9.87kg; CI: 9.64-10.1) than Autumn (10.18; CI: 9.94-10.41) or Winter calvers (10.07; CI: 9.84-10.3) (P=<0.01). Heifers that calved in the winter had a significantly shorter calving interval (389 days; CI: 394-383) (P=<0.01) than heifers calved in any other season.

Conclusion: The results of this study show the importance of achieving a low AFC which was associated with increased lifetime daily milk yield, improved reproductive performance and increased odds of calving for the second time. Calving heifers over 30 months is associated with significantly poorer survivability, fertility and lifetime production parameters. The impact of AFC on Jersey production, fertility and longevity traits shows a similar to trend to that seen in the Holstein, though potentially on a lesser scale.

Keywords: AFC, Jersey, heifers, UK, dairy.

SP-03

A randomised control trial to explore the effect of overall space allowance in adult dairy cow housing on reproductive performance, behaviour and milk yield

Jake Thompson[1], Christopher Hudson[1], Jonathan Huxley[2], Robert Robinson[1], Kathryn Woad[1], Nicola Bollard[1], Jasmeet Kaler[1], Martin Green[1].

[1]University of Nottingham, Nottingham, United Kingdom; [2]Massey University, Palmerston North, New Zealand.

Objectives:

- To support the evidence base on optimal methods to house dairy cows.
- To evaluate the impact of living space on dairy cow reproductive performance, behaviour and production.
- To evaluate the extent to which the impact depends on parity and days in milk.

Materials and Methods: A randomised [1:1], controlled, long term (364 day), parallel-group, cross-over study designed to evaluate the superiority/ inferiority of a spatial intervention was undertaken. This research was conducted in accordance with government regulations under a Home Office License, in a unique, purpose-built facility, which allowed precise measurement and configuration of the housed area. The study had a cross over design to account for differences between pen location.

Cows were matched based on parity and days in milk (±3days for cows ≤ 2nd parity, ±5days for cows ≥ 3rd parity) then randomly allocated into a control group (total: 9m^2 per cow, living space: 3m^2 per cow; based on GB median) and high space group (total: 14m^2 per cow, living space: 6.5m^2 per cow; based on GB upper 95%ile). The environment was controlled and matched for each group, excluding floor space allowance. A mirror image set-up was used to place the water trough area, electric brush and mineral access. Feed-face length, cubicle stocking density and design were identical. Both groups experienced the same management routines.

Data collection included insemination data and key reproductive events (e.g. conception), ultrasound scanning in early lactation, anti-Mullerian hormone (AMH) blood sample at week 5 for ELISA and milk sampling (3x per week at 14-84DIM) for progesterone ELISA. Production data was recorded via Lely Astronaut A4's, to provide daily yield per cow, alongside rumination time and weight. Cows were fitted with location sensors providing position and acceleration data for each cow using a novel wireless sensor system (Omnisense Series 500 Cluster Geolocation System) every 7s. Direct comparisons were made between time budgets for both groups. Key behavioural activities analysed were time spent in designated areas such as loafing areas, freestall and environmental enrichment use.

Data analyses were undertaken in R statistical software, including survival analysis, mixed-effects-modelling, non-linear regression and bootsampling methods.

Results: Compared to cows in the commercial average space (control group), cows in the high-space group produced more milk per 305-day lactation (first parity cows; 12,235L vs 11,592L, P<0.01, parity >1 cows 14,746L vs 14,644L, P<0.01).

There was a difference in median time to conception between groups; the control group median was 101 days (95%CI: 82-143 days) and high-space group 134 days (95%CI: 105-202 days), with a P-value of 0.02. A Cox-proportional hazards model revealed a reduced time to conception in the control compared to high-space group (hazard ratio 0.6, 95%CI: 0.35-0.94, p=0.03). Pregnancy rates (number of conceptions/inseminations) in the control vs high-space group were 40% and 25% respectively (p=0.049). Commencement of luteal activity (based on milk progesterone concentrations) showed no significant difference between groups; medians were 39 days for control and 38 days for high-space groups. No differences in time to first service, week 5 AMH concentrations, endometritis scores or antral follicle counts between groups were detected.

Cows with greater living space availability spent more time in lying (64 minutes/d) and feeding (10 minutes/d) areas, and less time in passageways (67 mins/d).

Conclusions: This is the first long term study in dairy cows to demonstrate that increased living space results in meaningful benefits in terms of productivity and behaviour. However, the cows with greater living space took significantly longer to conceive than cows in the control group, despite no detectable changes to underlying reproductive physiological parameters. Living space is a basic requirement for all housed dairy cows yet its fundamental impact on physiology, health, reproduction and welfare has been scarcely researched. It is likely that additional living space will be of benefit to adult dairy cows but further research is required into generalisability and cost-effectiveness of providing increased space to ensure it's sustainable.

Keywords: Dairy cow, housing, production, behaviour, reproduction.

SP-04

Motivation of dairy farmers to engage in primary prevention: current situation, drivers, and perceived constraints

Marijn Stok[1], Jantijn Swinkels[2], Nadia Lahaye[1], Merel Martena[1], Geert Vertenten[2], Tine Van Werven[1], Ruurd Jorritsma[1].

[1]Utrecht University, Utrecht, Netherlands; [2]MSD Animal Health, Boxmeer, Netherlands.

Objective: While bovine veterinary medicine was traditionally focused on treatment of diseased animals, disease prevention is currently generally accepted as the preferred strategy, as this approach is favorable for animal welfare, highly appreciated by the general public, and also economically attractive for farmers. Instead of allowing diseases to occur and prevent it from getting worse (tertiary prevention), or to prevent it from re-occurring in the future (secondary prevention), primary prevention, is implemented before the occurrence of any clinical or subclinical disease. Despite the benefits of primary disease prevention, it remains challenging for many veterinarians to convince farmers to focus on it. This study was designed to explore underlying psychological constructs to be

able to understand why farmers do or do not (yet) engage in primary preventative practices. Such underlying factors can both facilitate and hinder the performance of primary prevention practices.

Materials & Methods: We conducted an online survey in 15 Dutch veterinary practices. We used three established frameworks in our study: the Transtheoretical model, the Theory of Planned Behavior, and the Capabilities-Opportunities-Motivation-Behavior model.

The survey included close-ended questions probing the relevant psychological constructs from the three guiding frameworks, open-ended questions to allow respondents to provide additional in-depth information about either claw or calf health, and questions about specific preventative behaviors. Responding farmers were randomly assigned to questions on the prevention of claw or calf diseases. For claw health, the explored specific behaviors were keeping floors dry and clean; preventive claw trimming; and preventing overcrowding – for calf health, these specific behaviors were feeding colostrum within 2 hours after birth; using a separate maternity unit; and cleaning the calf hutches after every use.

Results: Respondents were 226 dairy farmers: 111 were assigned to questions on the primary prevention of claw disease, while the remaining 115 completed questions on the prevention of calf disease.

Most dairy farmers appeared to be in the action and maintenance stages of change, with about 70% already engaging in primary preventative behavior regarding claw or calf health. In addition, dairy farmers viewed preventative behaviors in general highly favorably and were highly motivated to perform primary preventative behaviors. Together, this shows a strong willingness to engage in primary prevention to promote claw and calf health among the target group. Results also indicated that there are factors which hamper farmers' ability to consistently engage in all targeted behaviors. These were, primarily, the limited importance of unsupported social norms (i.e. seeing other farmers engage in preventative behaviors and feeling like performing preventative behaviors is supported by others), confined available resources (i.e. time, equipment, facilities), and low habitualness of the behavior (the extent to which a behavior is engrained and performed almost automatically). These are thus important factors to target with tailored interventions, since improving upon them is likely to lead to increased preventative behavior.

Conclusion: We found that participating farmers had a strong willingness to engage in primary prevention, which could be supported by increased the importance of social norms, available resources, and engraining of preventive behavior.

Keywords: Drivers, constraints, primary prevention, dairy farmers.

SP-05

BRD vaccination strongly reduces the use of antibiotics in Dutch dairy calves

G. Bart Wille[1], Henk Kuijk[2], Geert Vertenten[2].

[1]Slingeland Dierenartsen, Varsseveld, Netherlands; [2]MSD Animal Health, Boxmeer, Netherlands.

Objectives: A substantial portion of antibiotics applied in the dairy sector are used for controlling respiratory diseases (BRD) in young stock. Vaccination against BRD pathogens may be a strategy to reduce antibiotics, and therefore an optimization for long-term performance of dairy cattle. In this field observational study, the antibiotic use in young stock on several Dutch dairy farms was measured depending on the used BRD vaccination program.

Materials and Methods: 250 Dutch dairy farms were involved. In 159 farms calves were treated against BRD and/or vaccinated against BRD. These 159 'BRD' farms were divided in 3 groups depending on the BRD vaccination protocol. Group A did not vaccinate, group B vaccinated only in autumn with an inactivated multivalent BRD vaccine (Bovilis® Bovipast RSP) and group C vaccinated all year round with the same vaccine. The antibiotic treatment percentage (TP) was calculated as the number of calves of 60kg bodyweight that could theoretically be treated with the amount of antibiotics actually used in 2017 to treat BRD in young animals divided by the number of calves born in 2017. Groups were compared for the number of farms that had an antibiotic treatment percentage higher than 20 (TP>20).

Results: Only 63 (40%) of the 159 'BRD' farms had a BRD vaccination program. Group A, B, and C had respectively 96, 36 and 27 farms. The TP>20 was respectively 84%, 47% and 26% for group A, B and C ($p<0.001$). The TP>20 was significantly different between group A and B ($p<0.001$) and between group A and C ($p<0.001$), but not between group B and C ($p=0.14$). The odds to have TP>20 is 83% lower in group B compared to group A, and 94% lower in group C compared to group A ($p<0.001$).

Conclusion: This study clearly demonstrates the potential of vaccination with a multivalent inactivated BRD vaccine (Bovilis Bovipast RSP) to reduce the antibiotic use in dairy young stock. In this study, a strong reduction was seen in the antibiotic treatment percentage of farms using this vaccine, with the strongest reduction on farms vaccinating all year round.

Keywords: BRD vaccination, reduction antibiotics, dairy calves.

SP-06

Color values as a potential tool for the assessment of raw milk quality and its technological aptitude in cheesemaking

Javier Caballero-Villalobos[1], Eoin G. Ryan[2], José M. Perea[3], Elena Angón[3], Orla Keane[1], Ana Isabel Garzón Sígler[3].

[1]Teagasc - Animal & Bioscience Research Department, Dunsany (Co. Meath), Republic of Ireland; [2]University College Dublin - School of Veterinary Medicine, Dublin, Republic of Ireland; [3]Universidad de Córdoba - Departamento de Producción Animal, Córdoba, Spain.

Objective: This study aims to determine milk hygienic status, composition and coagulation parameters by measuring its color values in order to assess if colorimetry could be used as an effective, quick and economical tool to predict *in situ* quality of raw bovine milk.

Material & methods: This study included the analysis of 1045 individual milk samples from 20 dairy farms located in the Republic of Ireland. Milk samples were collected at similar moments of lactation from heifers and cows from a of wide range of parity groups and with different health statuses. Milk was analyzed using the CIELAB space, which is considered the traditional method for an objective quantification of food color and has been previously used to evaluate bulk tank milk (Jiménez Sobrino et al., 2018). This system expresses color as 3 values: L* (lightness), a* (red/green value) and b* (blue/yellow value) (ISO/CIE 11664-4, 2019). In addition, CIELCh (the cylindrical representation of the CIELAB system) combines lightness with 2 other additional values to represent color: C* (chroma or saturation) and h* (hue). The color of the milk can vary greatly and depends to a large extent on its physical structure, the fat content and the pigmentation due to the presence of carotenoids and riboflavins. Color values of milk were measured with a PCE-CSM2 Color Meter (PCE Instruments Ltd., Southampton, UK) by placing the lens directly over a capsule containing the milk sample, following the methodology used by Figueroa et al. (2019).

Results: After statistical analysis, variables that showed a higher discriminant ability were L*, a* and b*. Preliminary results reveal that color values of milk reflect to a large extent variation in milk composition and the efficiency of the coagulation process. Color values seem to show a predictive ability similar to traditional technologies for quantification of milk quality, such as infrared spectroscopy technologies (for determination of milk composition), or the *Formagraph* lactodinamograph (for monitoring milk coagulation). However, colorimetry appears to provide lesser information on raw milk hygienic quality.

Conclusions: Colorimetry of milk has proved to be a useful tool to reflect variations in milk quality. This method can therefore be considered as an effective test to supplement conventional analysis. In addition, further development and implementation of this test as a low-cost portable technology in farms or dairies would allow better control of raw milk and contribute to the development of precise product characterization (at hygienic, nutritional and technological level).

Acknowledgements: This study has received funding from the Irish Research Council and the European Union's Horizon 2020 Research & Innovation programme through the CAROLINE Cofund scheme, under the Marie Skłodowska-Curie grant agreement No. 713279.

References:

Figueroa et al. (2019). J. Dairy Sci. (Accepted for publication)

ISO/CIE 11664-4:2019. Colorimetry - Part 4: CIE 1976 L*a*b* colour space.

Jiménez Sobrino et al. (2018). Ital. J. Anim. Sci. 17:477–488.

Keywords: Dairy cattle, chromaticity, milk quality, coagulation.

SP-07

Post-partum cow milk microbiota: effect of dry cow antimicrobial treatment

Richard Pereira[1], Carl Basbas[2], Sharif Aly[3], Emmanuel Okello[3], Betsy Karle[1], Erika Ganda[4], Terry Lehenbauer[3], Noelia Silva-Del-Rio[5], Deniece Williams[3], Martin Wiedmann[6].

[1]University of California Davis, Davis, United States; [2]University of California Davis, Davis CA, United States; [3]University of California Davis, Davis,CA, United States; [4]PennState, University Park, PA, United States; [5]University of California Davis, Davis, CA, United States; [6]Cornell University, Ithaca,NY, United States.

Objective: Given the increased scrutiny of antimicrobial use in livestock due to potential selection for antimicrobial resistance in bacteria, research on the effects of antimicrobial usage on the mammary gland and milk microbiota is vital. The goal of this study was to evaluate the effect of dry cow antimicrobial therapy on the udder milk microbiota by comparing the microbial populations in milk at dry-off (~60 days before calving) and post-partum from cows receiving intra-mammary antibiotic infusion and cows that did not receive therapy.

Methods: Aseptic composite milk samples were collected as part of a selective dry cow therapy trial from 3 commercial dairy farms in California's Central Valley for the purpose of the current study. Samples were stored at -20ºC. Cows with clinical signs of mastitis at enrollment (dry-off) were excluded. Milk samples from 23 cows from the intra-mammary therapy (IMT) group receiving either cephapirin benzatine or sodium cloxacillin and 27 cows from the control (CTL) group that did not receive any IMM therapy were utilized for our study. All cows were sampled at dry-off (DRY) and 4-11 days post-partum (FRESH). Whey and fat was separated from milk, and DNA was extracted using DNeasy PowerSoil Kit (QIAGEN). Library preparation and 16S rRNA gene sequencing of the V4 hypervariable region was conducted using the Illumina Miseq platform.

Results: Initial stepwise discriminant analysis between IMT and CTL group at DRY and FRESH time points did not show significant difference in the abundance of the microbial populations at the phyla level. The 4 most common phyla for any sample point or therapy group were Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria. Further analysis

will evaluate differences in microbiota abundance at different time points and therapy groups for lower taxonomic ranks. Richness and Shannon diversity will also be calculated.

Conclusions: Our initial analysis indicated that IMM dry cow therapy may have minimal impacts on the microbiota at the phylum level. Further analysis of the data will determine if this trend continues at lower taxonomic ranks.

Keywords: Dry cow treatment; milk microbiota; antibiotic resistance.

SP-08

Male Lidia cattle morphometric study using photogrammetry technique

Juan Manuel Lomillos Pérez[1], Marta Alonso[2].

[1]CEU Cardenal Herrera, Valencia, Spain; [2]Universidad de León, León, Spain.

Objectives: Lidia cattle constitute an autochthonous breed whose production has great economic and social importance in Spain, Portugal, southern France and some Latin American countries. In Spain, it is the most numerous autochthonous bovine breed and the second, in censuses, after the Friesian. Although in recent years the knowledge about the different genetic variety or *encastes* of Lidia breed has increased substantially, still there are only a few morphometric and zoometrical studies because the difficulty of applying the techniques commonly used in other cattle breeds. The particular patterns of behavior called *bravura* and the extensive rearing conditions made impossible the handling and restraining necessary for the realization of traditional manual measures using standard zoometric tools such as sticks, non-elastic measuring tape, compass, goniometers and calipers.

Considering all of the previously mentioned difficulties the aim of this study was to carry out a first approach to the morphological characterization of the males of Lidia breed using a novel technique for zoometric measurements, called photogrammetry.

Materials and Methods: A total of 184 bulls, 4 to 6 years old, from 21 herds representatives of the 15 genetic lines Miura, Pablo Romero, Veragua, Murube, Santa Coloma-Buendía, Santa Coloma-Graciliano, Gamero Cívico, Conde de la Corte, Atanario-Lisardo, Domecq, Torrestrella, Núñez, Albaserrada, Vega-Villar and Navarra, were used in this study. Farms were chosen for their genetic purity and their clearly belonging to an *encaste* reflected in the racial prototype.

The animals were photographed from a distance between 10 to 15 meters, to minimize the risk for operator and the disturbance of the animals, with a three cameras photogrammetric equipment, patented by our research group, adapted for both arena and farm conditions. The photographs were further processed and transformed into three dimension files with software PhotoModeler Scanner 2010© for Windows©. For each bull 20 standardized morphological measurements were obtained following the reviewed literature standars. A descriptive statistical analysis, ANOVA and determination of Pearson correlation coefficients of the measurements were performed using SPSS® 19.0 package for Windows©.

Results: Fighting bulls can be described as mid-sized compared with bovine species with a mean height at withers of 128 ± 7.7 cm with significant differences between the smallest *encastes* Vega-Villar (111 ± 3.5) and Navarra (113 ± 6.9) and the biggest Miura (136 ± 6.3) and Pablo Romero (135 ± 2.8). There are significant correlations between the heights at withers, loins, rump and tail, with the highest correlation value between height at rump and height at tail (0.96). The mean value of body length of Lidia bull (150 ± 10.8) is shorter than other autochthonous Spanish breeds, with significant differences between Miura (162 ± 4.4) and Vega-Villar (127 ± 7.7). Males of Lidia breed presented a harmonic morphostructural model with 59% of their parameters positively correlated. The *encastes* Núñez, Domecq, Gamero Cívico, Albaserrada and Santa Coloma (Gracialiano and Buendia lines) presented similar morphological characteristics with mean values of heights and greater exterior length of the horns with a maximum mean value of 72 ± 2.7 cm in the Gamero Cívico animals. These *encastes* shared a common phylogenetic provenance (Vistahermosa).

cm Farms were chosen for their genetic purity and their clearly belonging to an encaste reflected in the racial prototype, in which animals were analyzed 17 morphological measurements standardized.

Conclusions: The results showed that Lidia males present smaller dimension than other autochthonous Spanish breeds with a considerable internal variability of the parameters evaluated and a high relation between these morphometric measures and the characteristic morphology for each *encaste* reported in the descriptions of the breed made visually.

Keywords: Lidia cattle, bull, fighting bull.

SP-09

Using cattle tracing system databases to monitor cattle production efficiency, unnecessary carbon emissions and financial losses at herd, sector and national level

Timothy Geraghty[1], Andrew Barnes[2], Ian Archibald[2], Lesley Wylie[2], John Berezowski[3], Mary Vickers[4], Steven Thomson[2].

[1]SRUC, Aberdeen, United Kingdom; [2]SRUC, Edinburgh, United Kingdom; [3]SRUC, Inverness, United Kingdom; [4]AHDB, Kenilworth, United Kingdom.

Objectives: All sectors of the economy are required to reduce carbon emissions. Methane emissions, primarily from ruminant enteric fermentation, account for over 50% of agricultural emissions in the UK. Improving livestock production efficiency reduces carbon output and achieves financial gain and is the most cost-effective carbon mitigation strategy available to producers. Cattle Tracing Systems (CTS) databases are a legal requirement in the EU and UK and contain the dates of birth, death and movements of all cattle in the state. CTS databases have been underutilised for monitoring production efficiency. We present a novel method of analysing CTS da-



tabases to measure production efficiency of the entire cattle population and present results from CTS data from England and Scotland over 6 years.

Materials and methods: CTS datasets for Scotland and England for the years 2015-2020 were included. Animal-day of life (*d*) was considered an economic input relating directly to carbon emissions (enteric methane) and cost of production (variable on-farm costs). Slaughter (*S*) and new calf registration (*B* for the calf born, *C* for the dam calving) were considered productive output, and death on farm (*D*) as loss. A comprehensive model of optimal efficiency accounting for growth, fertility, slaughter of breeding stock and death on farm was derived from accepted Key Performance Indicators in terms of d required prior to or between productive events: birth to slaughter (*B*-*S*=600d, growth); birth to first calf registration (*B*-*C1*=730d, growth); inter-calving interval (*Cn*-*Cn*+*1*=365d, fertility); final calf registration to slaughter (*Cf* – *S*=365d, slaughter of breeding stock) and prior to death on farm (*B*-*D*=all life up to 600d, death). Actual data was compared to the optimal model with days meeting threshold considered productive (required to achieve an economic output) and those above thresholds as non-productive (leading to no additional output). Non-productive days were considered as resource waste and were sub-classified as being due to growth, fertility, slaughter of breeding stock or death according to the threshold broken. Crude conversion factors of 5.5KgCO2e and £1.50 cost per animal day were used to quantify the associated resource waste. A basic hierarchical logical process was used to categorise herd types within the CTS database into one of six primary systems: seasonal dairy, non-seasonal dairy, beef breeder, beef breeder-finisher, beef finisher and grower. Total productive efficiency was calculated at the herd, sector and national level as:

Production Day Efficiency PDE%= (∑⟦productive animal days⟧)/(∑⟦total animal days⟧)

Results: The dataset contained 14.9 billion *d* in total, of which 11.15 billion (74.9%) were classified as productive. Total *d* declined from 2.49 billion in 2015 to 2.42 billion in 2020 and PDE% increased from 74.3% in 2015 to 76.4% in 2020 (only 2018 had lower PDE% than the preceding year). Total non-productive days declined from 642 million in 2015 to 575 million in 2020 (only 2018 had more non-productive days than the preceding year) equating to reduced waste emissions of 369,000t CO2e and a saving of over £100million.

54.8% of non-productive days were due to slow growth, 21.0% due to death, 20.2% due to sub-fertility and 3.8% due to late slaughter of breeding cows. Total non-productive days reduced for all sub-categories over the period, most notably in growth (12.8% reduction). Non-productive days due to death increased in 2018 (5.2% higher than in any other year). There were marked differences in PDE% between sectors and herds within sectors. Dairy herds showed the greatest improved PDE% (3.6%) while beef breeding herds had no change in PDE%.

Discussion: This novel method can derive a comprehensive measure of cattle production efficiency from CTS datasets, track efficiency over time, quantify improvements in terms of carbon emissions and financial gain, and offer valuable insight into causes of sub-optimal productivity. Technical efficiency of the cattle herds in England and Scotland improved between 2015 and 2020, leading to reductions in waste CO2e emissions and financial savings. Efficiency improvements were not evenly distributed between sectors with the beef breeding sector showing no improvement. Production efficiency reduced in 2018 most likely due to an extreme weather event demonstrating the further potential of this model as a syndromic surveillance tool. The method needs to be further improved by measuring performance that is better than the optimal model and improving the conversion method to better quantify resource waste.

Keywords: Efficiency, carbon, data, production, statutory.

SR-01

Pseudotuberculosis, how to find the enemy hidden inside doors

Lina Costa[1], Belén Huerta[2], Ángela Galán-Relaño[3], Lídia Gómez-Gascón[3], Anabela Almeida[4], Inês Viegas[5], Alfonso Maldonado[3].

[1]Polytechnique Institute of Portalegre, Portalegre, Portugal; [2](2) Department of Animal Health - Faculty of Veterinary Medicine - University of Córdoba, Córdoba, Spain; [3]Department of Animal Health - Faculty of Veterinary Medicine - University of Córdoba, Córdoba, Spain; [4]Vetdiagnos - Veterinary diagnostic laboratory, Cantanhede, Portugal; [5](4) ICAAM - Institute of Mediterranean Agricultural and Environmental Sciences, Institute of Advanced Research and Training, University of Évora, Évora, Portugal.

Objectives: The actual prevalence of CLA (caseous lymphadenitis) in small ruminant flocks is underestimated in many countries and continues to spread without data and information about its real economic impact. The difficulty in the accurate identification of the causative agent in internal subclinical cases allows the disease to spread within and between flocks. This research intends to assess the utility of an ELISA (enzyme-linked immunosorbent assay) test in the detection of internal subclinical cases of CLA in farms and to simultaneously add data on the seroprevalence of the disease in Portugal.

Materials & Methods: In this research, we selected a field population of 82 flocks of small ruminants (55 sheep and 27 goat flocks).The procedure was carried out on the farm and in a regional slaughterhouse in the province of Alentejo in southern Portugal. Sera randomly collected from 756 small ruminants (approximately 10 samples per holding), 70% sheep (528/756) and 30% goats (228/756) were screened for antibodies against *Corynebacterium pseudotuberculosis* using the ELISA technique based on a recombinant phospholipase D (ELITEST CLA # CK105A®). The animals showing internal lesions (n = 58) were sampled and a conventional PCR assay was made for the identification of *C. pseudotuberculosis* in pyogranulomatous lesions and for the cross-sectional study to assess the performance of the ELISA test. The ELISA assay results were subjected to frequency analysis (prevalence of the disease). The same method was applied to the data regarding the species, productive aptitude, production regime, and dimension (size) of the herd. The performance of the ELISA test was evaluated by association tests, namely, $\chi 2$ (Chi-square – Fischer's Exact Test) with a 95% confidence interval. The statistical analysis was performed with SPSS (Statistical Package for Social Sciences) 22.

Results: In this investigation, we found a prevalence of CLA of 34% (258/756), with the ELISA test showing a low specificity, 78% (35/45) and high sensitivity, 100% (13/13). The descriptive analysis of the results suggests that the proportion of disease higher in goats ($\chi 2$ (1, N = 756) = 30.773, $p < 0.01$). Regarding productive aptitude, dairy farms appear to be more susceptible to CLA ($\chi 2$ (1, N = 756) = 17.458, $p < 0.01$); the same propensity appears to exist regarding the production regime, with the farms in the non-extensive regime (intensive and semi-extensive, approximately 6% of the total sample) showing an increased propensity for the disease ($\chi 2$ (2, N = 756) = 22.561, $p < 0.01$). When the results of the ELISA are analyzed regarding the size of the farm, the data are also statistically significant. This data suggests that farms without the reported disease have an average of 85 animals, whereas farms with confirmed disease have an average of 171 animals ($p < 0.001$). This observation appears to mean that CLA is more prevalent in medium to large farms (50 - 200 animals and > 200 animals). By performing a herd-level analysis (considering an infected farm with at least one animal positive for the ELISA), of the 82 farms in the study, 66 had at least one positive animal, leading to a value of 80% (66/82) of affected holdings. The performance of the ELISA test technique (ELITEST CLA # CK105A®) was evaluated by determining the positive predictive value (PPV) and negative predictive value (NPV). In this analysis, the ELISA test allowed the detection of 57% of subclinical animals, which was confirmed by the *post-mortem* examination and conventional PCR assay performed in pyogranulomatous lesions. In the group of animals in which both tests were performed (PCR and ELISA) (n = 58), 57% of the animals positive for the ELISA test were positive for the PCR assay (13/23), and all the negatives, 100%, in the ELISA test were negative for the PCR assay (35/35).

Conclusion: This is the first prevalence study of CL in small ruminants carried out in Portugal. This research clarifies an actual problem and pointed out the importance of CLA in small ruminant herds in Portugal showing that CLA is a prevalent disease in small ruminants, with 34% seroprevalence. Finally seems to demonstrate that the ELISA test is a good diagnostic tool for use in CLA eradication programmes, as can detect a valid proportion of subclinically infected animals.

Keywords: Caseous lymphadenitis, ELISA, Portugal, seroprevalence, small ruminants.

SR-02

Prevalence of visceral Caseous Lymphadenitis (CLA) in sheep herds of Aragón, Spain

Héctor Ruiz[1], Asier Albite[1], Cristina Baselga[2], Luis Figueras[3], Amaia Torre[1], Delia Lacasta[1].

[1]Facultad Veterinaria de la Universidad de Zaragoza, c/ Miguel Servet 177, 50013, Zaragoza, Spain; [2]Exopol S.L., Pol. Río Gállego D8, San Mateo de Gállego, Spain; [3]Gabinete Técnico Veterinario S.L., Zaragoza, Spain.

Objectives: Caseous lymphadenitis (CLA) is an infectious-contagious pathology caused by *Corynebacterium pseudotuberculosis* biovar ovis which appears frequently in herds. When this agent affects small ruminants, it can develop a visceral clinical form that mostly affects adult animals, or a superficial clinical form, which appears more frequently in young animals. However, its subclinical appearance, especially of the visceral form, makes it difficult to diagnose it in clinical practice.

Thus, although this pathology is widespread, many times reminds underdiagnosed and is not easily identified as a cause of loss of productivity in the sheep flocks.

The main objective of the present survey was to study the relevance of Caseous Lymphadenitis as a cause of early cull-

ingin sheep herds of Aragón region, Spain, and to analyze the main clinical presentations of the disease.

Material and methods: The Ruminant Clinical Service (SCRUM) of the Veterinary Faculty of Zaragoza, Spain, receives animals from the Faculty´s area of influence which can be culling animals belonging to collaborating farms or clinical cases referred by veterinarians. In the present survey, 483 culling animals received during the years 2017, 2018 and 2019 were analyzed.

All the received animals were subjected to the same protocol. After arrival, a rigorous clinical examination was performed and blood samples were taken to carry out a blood count test. Subsequently, depending on the preliminary diagnoses, necessary ancillary tests, such as ultrasonography, thermography or computed tomography, were carried out. Finally, all the animals were humanely sacrificed to conclude with the pathological examination. Then samples of lesions and affected organs were taken for microbiological, biomolecular and histopathological analysis. The isolation of the causative agent was carried out in the laboratories: EXOPOL diagnóstico y autovacunas S.L. and in the Agroalimentary Laboratory of the Government of Aragón and histopathology was performed at the Pathological Clinical Service of the Veterinary Faculty of Zaragoza.

The animals included in the present study as positives to CLA were those diagnosed observing compatible lesions at necropsy and subsequently with etiological confirmation.

Finally, all the data were recorded in computer programs, such as MIcrosoft Office Excel 2010 and IBM Statistics Base 22.0, which allowed the statistical study of that data.

Results: The results show that 31.06% of the animals analysed showed CLA lesions (150/483) that were subsequently confirmed by *C. pseudotuberculosis* isolation.

Attending to the clinical form of presentation, 74.67% of the animals suffered the visceral clinical form, while only 21.33% were affected by the superficial form of the disease. The remaining 4.00% of the animals showed both clinical presentations simultaneously. That result was expected due to the average age of the studied animals that was 5.90 ±1.73 years.

However, the most relevant data of this study was that obtained from the analysis of CLA as cause of early culling in sheep. Eighty-two animals out of the 150 animals diagnosed with CLA (54.67%) presented this pathology as the sole cause of culling, which could justify the loss of productivity. Moreover, when these 82 animals were analyzed regarding the 483 culling studied sheep, it was showed that 16.98% of the culling animals were discarded by this pathology as the sole cause of culling, demonstrating the importance of this disease as a cause of culling in sheep herds. The visceral clinical form was the main presentation of the disease, accounting 14.70% of the total, while the superficial clinical form was only de 2.18%.

Regarding the visceral clinical form, the primary location was that related to the respiratory system, with affection of mediastinic lymphnode, lungs or both simultaneously. Likewise, the concurrent affection of several organs, such as liver-lung liver-kidney, or other variants appeared in a significant number of animals.

Conclusions: It can be concluded that caseous lymphadenitis is a relevant diseaseproducing loss of productivity and early culling in sheep in Aragón region. The visceral form was the most frequently found, this being of great importance due to the difficulty of the clinical diagnosis of this presentation of the disease, which leads to the disease being frequently underdiagnosed.

Keywords: Caseous Lymphadenitis, sheep, visceral, prevalence.

SR-03

Evaluation of the effect of a topical wound anaesthesia formulation on concentration of serum amyloid A in lambs where the tail was excised surgically

Marta Borobia[1], Aurora Ortín[2], Maria Teresa Tejedor[1], Marta Ruiz De Arcaute[2], Luis Miguel Ferrer[2], Delia Lacasta[2], Héctor Ruíz[1], Peter Andrew Windsor[3].

[1]Facultad de Veterinaria. Universidad de Zaragoza, Zaragoza, Spain; [2]Facultad de Veterinaria. Instituto IA2 (Universidad de Zaragoza-CITA), Zaragoza, Spain; [3]Faculty of Veterinary Science. University of Sydney, Sidney, Australia.

Objectives: Tail-docking is a routine global husbandry procedure conducted in lambs to reduce myiasis risk and improve fertility. It is often performed without pain relief, although use of general anaesthesia has been advocated, at an exorbitant cost to farmers. There is an urgent need to find a practical and affordable approach acceptable to farmers that avoids or markedly reduces animal suffering. This study evaluated the effect of a topical anaesthetic 'spray-on' wound management formulation containing the local anaesthetics lignocaine and bupivacaine, plus cetramide and adrenalin, in a gel matrix (Tri-Solfen®, Bayer Animal Health, Gordon, NSW, Australia). Impacts of treatments on concentration of the major acute phase protein serum amyloid A (SAA) in lambs, where the tail was excised surgically, with and without use of general anaesthesia, are reported.

Material & Methods: This study was approved by the Ethical Commission of the University of Zaragoza. Forty-four 45-day-old Rasa Aragonesa female lambs with similar weights in a farm in Zaragoza (Spain) were recruited for this study and divided into 4 equal cohorts (n=11): in Group A the tail was excised using a scalpel without using anaesthesia or any additional treatment; in Group B the tail was surgically excised under general anaesthesia; in Group C the tail was removed with a scalpel without prior anaesthesia and treated immediately with Tri-Solfen®; in Group D the tail was surgically excised under general anaesthesia and treatment with Tri-Solfen® applied before suturing of peri-wound skin to close the wound. Blood samples were collected (and sera were obtained and stored at -20 ºC until analysed) prior to tail docking (T1), and at 5hr (T2) and 48hr (T3) intervals, plus 7 days later (T4). The concentration of SAA was assessed using a solid phase sandwich ELISA kit (PHASE TM Serum Amyloid A Assay, Tridelta Development Ltd., Maynooth, Ireland). Before statistical analysis, a logarithmic transformation (10log) was used to normalize SAA concentration. Statistical analysis were performed using IBM

SPSS statistics version 26 (2019) software (IBM, Armonk, NY, USA). For each procedure (with or without anaesthesia), a two-way mixed ANOVA was run to understand the effects of treatment (with or without Tri-Solfen®) and time (T1 to T4) on SAA concentration.

Results: In cohorts tail-docked under general anaesthesia (B & D), SAA concentrations increased from T1 to T2, achieving maximum values at T3 and decreasing by T4, with T3 significantly higher ($p<0.01$) than at T1, T2 and T4. These findings are consistent with previously reported significant elevations of SAA 48 hours after a noxious stimulus. The cohort tail-docked with Tri-Solfen® (D) had consistently lower SAA levels than the cohort without this wound treatment (B), although statistically significant differences were not determined between these cohorts ($p=0.604$). In cohorts tail-docked without general anaesthesia (A & C), SAA concentrations of the untreated group (A) displayed similar trends to Groups B and D. However, the SAA concentrations in the Tri-Solfen® treated group (C) failed to achieve the maximum concentrations at T3 and were similar to T1 and T2, decreasing by T4. These results appear to indicate that treatment with Tri-Solfen® avoided the elevation of SAA concentrations that is expected 48 hours after the surgery. Although statistically significant differences were not detected between lambs treated and non-treated with Tri-Solfen® ($p=0.913$), this is considered most likely due to insufficient sample size of each cohort.

Conclusions: These results and clinical evidence of pain relief presented elsewhere, are encouraging, suggesting that surgical tail-docking without general anaesthesia but wounds are immediately sprayed with Tri-Solfen®, is an affordable and efficacious method of conducting the procedure, although use of larger sample sizes for SAA estimates is recommended to confirm these findings.

Keywords: Lambs, tail-docking, welfare, topical anaesthesia, serum amyloid A.

SR-04

Effect of a topical wound anaesthesia formulation on the cortisol responses of lambs undergoing tail docking

Aurora Ortín[1], Juan José Ramos[1], Marta Borobia[2], María Teresa Tejedor[2], Enrique Castells[3], María Pérez[2], Hector Ruiz[2], Peter Andrew Windsor[4].

[1]Facultad de Veterinaria. Instituto IA2 (Universidad de Zaragoza-CITA), Zaragoza, Spain; [2]Facultad de Veterinaria. Universidad de Zaragoza, Zaragoza, Spain; [3]Centro Clínico Veterinario, Zaragoza, Spain; [4]Faculty of Veterinary Sciences. University of Sidney, Sidney, Australia.

Objectives: Tail-docking is a routine global husbandry procedure conducted in lambs to reduce myiasis risk and improve fertility. It is often performed without pain relief, although use of general anaesthesia has been advocated, at an exorbitant cost to farmers. There is an urgent need to find a practical and affordable approach acceptable to farmers that avoids or markedly reduces animal suffering. This study evaluated the effect of a topical anaesthetic 'spray-on' wound management formulation containing the local anaesthetics lignocaine and bupivacaine, plus cetramide and adrenalin, in a gel matrix (Tri-Solfen®, Bayer Animal Health, Gordon, NSW, Australia). Impacts of treatments on serum cortisol concentrations in lambs, where the tail was excised surgically, with and without use of general anaesthesia, are reported.

Material & Methods: This study was approved by the Ethical Commission of the University of Zaragoza. Forty-four 45-day-old Rasa Aragonesa female lambs with similar weights in a farm in Zaragoza (Spain) were recruited for this study and divided into 4 equal cohorts (n=11): in Group A the tail was excised using a scalpel without using anaesthesia or any additional treatment; in Group B the tail was surgically excised under general anaesthesia; in Group C the tail was removed with a scalpel without prior anaesthesia and treated immediately with Tri-Solfen®; in Group D the tail was surgically excised under general anaesthesia and treatment with Tri-Solfen® applied before suturing of peri-wound skin to close the wound. Blood samples were collected prior to tail docking (T0), and at 30 min (T1), 5hr (T2) and 48hr (T3) intervals post-tail excision, and sera were obtained and stored at -20 ºC until analysed. Serum cortisol concentrations were determined using a competitive ELISA assay (Salivary Cortisol ELISA SLV-2930, DRG Diagnostics, Marburg, Germany). A logarithmic transformation (10log) was used to normalize serum cortisol concentration before statistical analysis, which were performed using IBM SPSS statistics version 26 (2019) software (IBM, Armonk, NY, USA). For each procedure (with or without use of general anaesthesia), a two-way mixed ANOVA was run to understand the effects of treatment (with or without Tri-Solfen®) and time (T0 to T3) on serum cortisol concentration.

Results: In cohorts tail-docked under general anaesthesia (B & D), serum cortisol concentration did not change significantly over time ($p=0.250$). Moreover, serum cortisol concentration did not differ significantly between the cohort treated (D) or not treated with Tri-Solfen® (B) ($p=0.919$). In cohorts tail-docked without general anaesthesia (A & C), serum cortisol concentrations peaked at 30 min post tail removing and decreased thereafter, being T1 value significantly higher than T0, T2 and T3 values ($p<0.001$). However, in the Tri-Solfen® treated group (C) serum cortisol concentrations at T1 and T2 were lower than in the untreated group (A), although statistically significant differences were no detected between the groups ($p=0.162$).These results seem to indicate that treatment with Tri-Solfen® reduced the cortisol response elicited after the surgery without general anaesthesia. Low statistical power due to small sample size could have prevented from finding significant differences between A and C cohorts. The elevated cortisol response in lambs tail-docked without general anaesthesia likely reflects a combination of factors, including the effect of handling, restrain and potentially haemorrhage, in addition to pain.

Conclusions: These results, and clinical evidence of pain relief presented elsewhere, are encouraging, suggesting that surgical tail-docking without general anaesthesia but where wounds are immediately sprayed with Tri-Solfen®, is an affordable and efficacious method of conducting the procedure, although use of larger sample sizes for serum cortisol estimates is recommended to confirm these findings.

Keywords: Lambs, tail-docking, welfare, topical anaesthesia, cortisol.

SR-05

Predicting energy balance in pre-partum dairy ewes by ultrasound measurements of backfat and *longissimus dorsi* thickness

Sofia-Afroditi Termatzidou[1], Nektarios Siachos[1], Georgios Valergakis[1], Zoe Polizopoulou[2], Danai Sklaveniti[1], Georgios Bramis[1], Georgios Arsenos[1].

[1]Laboratory of Animal Husbandry, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University, Thessaloniki, Greece; [2]Companion Animal Clinic, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University, Thessaloniki, Greece.

Objective: The objective of this study was to investigate the association of ultrasound measurements of backfat thickness (BFT) and *longissimus dorsi* muscle thickness (LDT) with energy balance in pre-partum dairy ewes. The aim is to identify practical, on-field applicable and non-invasive methods to assess energy status.

Material and methods: The study was conducted in four commercial dairy sheep flocks, each keeping one of the four main breeds reared in Greece. Fifty purebred ewes were pre-selected from each flock, based on ultrasound pregnancy diagnosis at day 60 after ram introduction; a total of 177 ewes (Chios, n=46; Frizarta, n=40; Lacaune, n=42 and Assaf, n=49), 3 to 5 years old, that remained pregnant and clinically healthy until lambing, were enrolled in the analysis. Body condition score (BCS) was assessed by palpation in the lumbar region and ultrasound measurements of BFT and LDT thickness were performed using a 5-MHz linear transducer, both at -30 days (-30d) and -15 days (-15d) prepartum. The probe was placed perpendicular to the vertebral column between the transverse processes of the 3rd and 4th lumbar vertebrae. Each time, three measurements were performed and the mean value (in mm) was calculated. The sum of BFT and LDT (TOTAL_T), the LDT: BFT ratio (RATIO_T) on each timepoint and the difference in BFT (Δ_BFT) and LDT (Δ_LDT) between the two measurements (-30d minus -15d) were also calculated. A blood sample was collected at -15d from each ewe and serum was separated within 2h of collection by centrifugation (15 min × 2500*g*) and stored at -20°C until analysis. Beta- Hydroxybutyrate (BHB) and non esterified fatty acids (NEFA) were measured with an automated chemistry analyzer (Vitalab flexor E., Vital Scientific N.V., Netherlands), using commercially available kits (BHB: Ben biochem. enterprise, Milano, Italy; NEFA: Randox Laboratories Limited, UK). Negative energy balance status was defined as serum BHB >0.8 mmol/L or NEFA >0.3 mmol/L and treated as a binary variable. The receiver operating characteristic (ROC) analysis was used to define thresholds for fat and muscle reserves and mobilization parameters to predict elevated BHB or NEFA status. All variables with a significant area under the ROC curve were then entered in a binary logistic regression as categorical variables (using the thresholds obtained from the ROC curves) to assess the association of fat and muscle reserves and mobilization parameters with BHB and NEFA status.

Results: Δ_BFT and TOTAL_T(-15d) were significant predictors of BHB status (R^2=0.272). Ewes with a BFT loss had 6.7 times (P<0.001) increased probability to have serum BHB >0.8 mmol/L; probability increased quadratically as Δ_BFT increased (R^2=0.486; P<0.001). Moreover, ewes with TOTAL_T(-15d) ≤25.6 mm, which corresponds to an average BCS of 2.25-2.50, had 5.3 times (P<0.001) increased probability to have serum BHB >0.8 mmol/L; probability decreased quadratically as TOTAL_T(-15d) increased (R^2=0.425; P<0.001). Δ_BFT and BFT(-30d) were significant predictors of NEFA status (R^2=0.316). Ewes with Δ_BFT >0.65 mm had 3.2 times (P=0.002) increased probability to have serum NEFA >0.3 mmol/L; probability increased linearly as Δ_BFT increased (R^2=0.441; P<0.001). Moreover, ewes with BFT(-30d) >6.86 mm, which corresponds to an average BCS of 2.75-3.00, had 6.7 times (P<0.001) increased probability to have serum NEFA >0.3 mmol/L; probability increased quadratically as BFT(-30d) increased (R^2=0.915; P<0.001).

Conclusion: Ultrasound measurements of BFT and LDT during the last month of gestation can predict quite accurately the energy status of dairy ewes. As expected, ewes that start losing fat a month before lambing and with a poor BCS at -15d are at higher risk to enter in negative energy balance. Moreover, ewes with a high BCS during the last month of gestation are at greater risk of mobilizing fat reserves pre-partum. Ultrasonography, a non-invasive technique, appears useful in monitoring energy balance during the critical pre-partum period.

Keywords: Energy balance, dairy sheep, ultrasound.

SR-07

Q fever in sheep: Long-term control of infection by vaccination of gimmers

Benjamin Bauer[1], Britta Janowetz[2], Vanessa Turowski[2], Christina Ambros[2], Michaela Alex[2], Sabine Roth[3], Ursula Domes[2], Jens Böttcher[2].

[1]now University of Veterinary Medicine Hanover, Foundation, Clinic for Swine and Small Ruminants, Hanover, Germany; [2]Bavarian Animal Health Service, Poing, Germany; [3]State Veterinary Office, Karlstadt, Germany.

Introduction: Small ruminants are regarded as a major source of human infection with *Coxiella burnetii* (Cb). In summer 2012 a Q fever outbreak was observed in a sheep flock with 800 adult ewes. End of 2012 we got involved in this case in order to implement a control program. At that time, we assumed that most of the sheep were already infected or immune. Yearly primary vaccination of gimmers (replacement ewes) with Coxevac™ (Ceva Santé Animale) has been introduced as a long-term measure. The vaccine is not licensed for sheep; therefore, a monitoring of vaccination was implemented. This case-report describes the long-term effect of a primary vaccination of gimmers until 2020.

Methods: Gimmers were vaccinated twice three weeks

apart (primary vaccination), no further revaccination was performed.

The following groups were ear-tagged for control purposes, animals were randomly selected:

1. 20-30 vaccinated gimmers each year (VG13, 14, 15 etc.),
2. As a positive control 30 ewes were vaccinated in 2013 (VE13).
3. Each year 30 unvaccinated gimmers were selected into a group of sentinels (S). Additionally, gimmers before first vaccination were included. After seroconversion animals were removed from the group.

Direct pathogen monitoring was performed by PCR-testing of vaginal swabs collected hours after parturition and nasal swabs. Indirect monitoring was based on blood samples from sentinels which were tested for seroconversion.

The immune response (PhI/PhII-antibodies, IFN-γ-Recall Assay (RA)) was assessed before and after primary vaccination. The ratio of titers (PhI, PhII) or IFN-γ-reactivity was assessed as n-fold (nx) increase. Quantitative PCR, PhI- and PhII-antibody tests and IFN-γ-RA were performed as previously described (Böttcher et al., 2013; Boettcher et al., 2017).

Animals which had been primary vaccinated in 2013 (n=6 i.e. VG13 and VE13), 2014 (n=3), 2015 (n=10), 2016 (n=11) and 2017 (n=10) were once revaccinated in 2018 and the immune response was assessed before and 3 weeks after revaccination.

Data were analysed by MedCalc Statistical Software version 19.1.3 (MedCalc Software bv, Ostend, Belgium; https://www.medcalc.org; 2019). Groups were compared by Kruskal-Wallis-test.

Results: From November 2012 until February 2014 the rate of positive vaginal and nasal swabs was 78/268 and 67/263, respectively. The mean pathogen load in positive samples was $10^{2,6}$ and $10^{1,6}$ Cb per vaginal and nasal swab, respectively. Thereafter swabs were tested negative, the numbers of analysed vaginal/nasal swabs per year were: 2014: 69/62, 2015: 68/39, 2016: 105/42, 2017: 158/40, 2018: 86/40, 2019: 49/- and 2020(Jan): 97/-.

Percentages of annual seroconversion (PhII-titer >100) in sentinels were: 2013:16.8% (n=95); 2014: 25% (n=52); 2015: 5,6% (n=53); 2016: 1,3% (n=76); 2017: 2,6% (n=77); 2018: 0% (n=105) and 2019: 0% (n=95).

Groups VE13 and VG13 responded well after vaccination. E.g., VG13 showed an n-fold increase of PhI-, PhII-titers and IFN-γ-reactivity of 116x, 168x and 6x, respectively. In contrast, gimmers born after shedding had ceased (VG15-19) showed only weak immune responses after vaccination. The n-fold increase of PhI-, PhII-titers and IFN-γ-reactivity e.g. in VG16 was only 1x, 2,5x, 2,7x, respectively.

Available animals in groups VE13, VG13-17 were revaccinated once in 2018. In all groups a similar strong n-fold increase of PhI-, PhII-titers and IFN-γ-reactivity was observed after revaccination (e.g., VG16: PhI-titer (11x), PhII-titer (38x) IFN-γ (33x - in this case IFN-γ was determined as pg/ml).

Conclusions: Two years after an outbreak *C. burnetii* was still detected in vaginal and nasal swabs, however, pathogen load was very low. Although seroconversion in sentinels was even detected until 2017, it did not result in shedding at parturition. Vaccination preferentially boostered an existing immunity, because gimmers born since 2015 showed only a weak immune response after vaccination. However, despite this weak immune response these animals showed a strong immune response after a single revaccination even three years after primary vaccination. Consequently, primary vaccination of gimmers is a cost-efficient long-term measure to control *C. burnetii* in sheep. We do not know if it protects from infection, however, at least in case of urgency revaccination of such a sheep flock would rapidly increase the herd-level immunity.

Acknowledgements: This study was financially supported by the Free State of Bavaria and the Bavarian Joint Founding Scheme for the Control and Eradication of contagious Livestock Diseases.

References:

Böttcher, J. et al. (2013) Berl Münch Tierärztl Wochenschr 126, 427-435.

Böttcher, J. et al. (2017) J Vet Med Res 4(9):1106.

Keywords: Coxiella, vaccination, sheep, immunity.

SR-08

Influence of housing and management on claw health of Swiss dairy goats

Sailer Lisa-Marie[1], Joan-Bryce Burla[1], Patrik Zanolari[2], Katharina Friedli[1].

[1]Federal Food Safety and Veterinary Office (FSVO), Centre for Proper Housing of Ruminants and Pigs, Tänikon, Switzerland; [2]Clinic for Ruminants, Vetsuisse Faculty University of Bern, Bern, Switzerland.

Objectives: Dairy goats are commonly housed on deep bedding without continuous access to a hard ground, at least during winter periods. Due to the lack of claw horn wear on abrasive surfaces, goats have a high prevalence of overgrown wall horn, even if trimmed several times a year (Hill et al.,1997; Kofler, 2016). Overgrown wall horn can result in claw lesions and lameness, impaired locomotion behavior and welfare, as well as reduced milk yield (Christodoulopoulos, 2009). However, only scarce literature is available on the characteristics and health of goat claws. Moreover, in contrast to dairy cows, claw trimming in goats is less organized and little is known about the quality of claw trimming on Swiss farms. The aim of this study was to obtain greater knowledge on the characteristics and health of goat claws, and on the influence of the over grown wall horn on the goats locomotion behavior.

Material & Methods: Data was collected on 28 dairy goat farms all over Switzerland during two identical farm visits in autumn 2018 and spring 2019. During each visit, the same veterinarian recorded claw condition (claw length, claw width, overgrown claw horn, abnormal claw forms) and claw lesions (e.g. toe ulcer, foreign body, white line lesion) of 12 randomly selected goats during routine claw trimming. All findings have been documented for each animal on a “claw card”. In case of

a suspicion for an infectious claw disease (particularly *Dichelobacter nodosus* or *Treponema spp.*), swab samples were taken for further examination. Locomotion activity of the 12 goats was recorded over a period of three days, once just before and once two weeks after the claw trimming. Additionally, the farmers completed a questionnaire on housing conditions and management practices on their farms in order to identify potential risk factors that may contribute to claw problems.

Results: Preliminary, descriptive results (final results available by summer): In total 336 goats were included in the study. In spring and autumn all goats showed at least two feet with overgrown claw horn. 0,72% of the examined claws showed no overgrown claw horn, 58.31% showed moderate overgrown claw horn and 40.97% showed sever overgrown claw horn. The severity of overgrowth was greater in spring than in autumn. Animals that had been grazing in alpine regions during the summer time, showed less overgrown claw horn. Claws with sever overgrown claw horn showed more hematomas than the ones with moderate and no overgrown claw horn. Suspicious infected claws were checked for Dichelobacter nodosus and Treponema sp. with interdigital swabs, all were tested negative.

Conclusions: Claw health, different trimming regimes, the influence of overgrown wall horn on claw lesions, the goats locomotion activity, and potential risk factors for overgrown wall horn were evaluated and compared between autumn and spring. Final results from this study will provide important information for dairy goat farmers, contribute to prevent claw problems, and thus promote goat welfare.

References:

1. G. Christodoulopoulos. Foot lameness in dairy goats. Research in Veterinary Science (2009) 86, 281-284.
2. N. P. Hill, P. E. Murphy, A. J. Nelson, N. Mouttotou, L. E. Green, K. L. Morgan. Lameness and foot lesions in adult British dairy goats. Veterinary Record (1997) 141, 412-416.
3. J. Kofler. Klauenpflege bei Schaf und Ziege. Klauentierpraxis (2016) 24, 61-68.

Keywords: Goats, claw health, housing, locomotion activity, animal welfare.

SR-09

Vaccinating pregnant ewes with an iron regulated protein (IRP) vaccine could be a suitable strategy to control ovine respiratory complex

J Gutiérrez[1], F Hernández[2], A Tirado[2], Jl Blasco[1], G Vertenten[3], L Fraile[4], L Elvira[1].

[1]MSD Animal Health, Salamanca, Spain; [2]Cerromonte Farm SL, Ávila, Spain; [3]MSD Animal Health, Boxmeer, Netherlands; [4]Department of Animal Science, University of Lleida, Lleida, Spain.

Objectives: Ovine Respiratory Complex (ORC) is the leading cause of death in all types of sheep farms, times of the year, regions, and breeds. It is one of the main causes of morbidity and mortality in lambs with high prevalence and economic consequences (González, 2018). Pneumonia in sheep is a disease complex involving interactions between host factors (immunological and physiological conditions) and different infectious agents (viruses, bacteria and mycoplasmas). *Mannheimia haemolytica* is a key bacterium that causes lung damage whilst viruses and mycoplasmas are considered predisposing agents for the disease. Indeed, *M. haemolytica* causes serious outbreaks of acute pneumonia in neonatal, weaned and growing lambs, as well as calves and goat kids (Ackermann and Borgden, 2000). Very young lambs are often affected, with very short time to protect them through active immunization (Lacasta et al, 2008).

Inactivated vaccines based on the iron regulated proteins (IRP) of *M. haemolytica* have been shown to induce a protective immune response (over 80%) from 6 weeks after the first dose of primary vaccination (Lacasta et al., 2008), and offer cross-protection to serotypes not included in the vaccine (Gilmour et al, 1991). The concept of prenatal vaccination to increase maternal immunity in newborn lambs during the first weeks of life is currently accepted for the prevention of neonatal diarrhea; however, for ORC, very limited information is available.

Therefore, the purpose of this study was to evaluate the titers of specific antibodies against *M. haemolytica* in the colostrum of vaccinated dams and their passive transfer in lambs.

Materials & Methods: A blinded and randomized study was performed in a Spanish dairy sheep farm with 4,300 Lacaune sheep. Two hundred pregnant ewes were randomly allocated to one of two experimental groups: Vaccinated (Ovilis® Ovipast, MSD Animal Health) and Negative Control (PBS administration). The first 59 lambing ewes were followed (31 control and 28 vaccinated) with a collection of serum and colostrum samples within 6 hours after lambing. At least 200ml of colostrum was collected to feed the lambs with a nasopharyngeal tube within 6h after birth, followed by a second administration of the same amount 6–12h after birth. Subsequently, a sample of lamb`s serum was collected at 24-48 hours of life and at the age of 4-5 weeks (just before weaning).

To monitor the immune response, the titer of specific antibodies against *M. haemolytica* was quantified in the serum and colostrum samples at the Centre for Diagnostic Services in Boxmeer (MSD Animal Health, NL) using an in-house ELISA test for *M.haemolytica* IgG. Additionally, colostrum quality and passive transfer in lambs were monitored with a Brix refractometer.

A non-parametric means comparison test was used (Wilcoxon and Kruskal-Wallis test) to compare the antibody titers between experimental groups (vaccinated versus control) in both colostrum and serum with p=0.05.

Results: At lambing, the *M. haemolytica* antibody levels in vaccinated ewe's serum were significantly increased versus the control group ($p<0.0001$). Although no significant difference in brix-measured colostrum quality was found among groups ($p>0.05$), the specific level of *M. haemolytica* antibodies in the colostrum significantly increased in the vaccinated group versus the control one ($p<0.0001$). Moreover, the level of antibodies present in the colostrum was corelated with the titer in the ewe's serum suggesting a transfer of serum antibodies to colostrum.

Finally, no significant differences were observed in degree of passive transfer between the lambs of the two groups (born to vaccinated versus control ewes) as measured with Brix refractometer in lamb serum at 24-48 hours of life ($p>0.05$). This result suggests that the passive antibody transfer was similar between experimental groups. The serum of lambs from vaccinated dams had significantly higher level of antibodies against *M. haemolytica* than control ones ($p<0.0001$). Moreover, at weaning this difference was still significant ($p<0.001$) and showing a correlation with the initial level of antibodies in the lambs ($p<0.0001$). An interesting point to emphasize is the correlation between the *Mannheimia* antibody titers at dam level (serum at lambing and colostrum) and lamb level (serum after colostrum intake and at weaning).

Conclusion: Vaccination of pregnant ewes with an iron regulated protein (IRP) vaccine (Ovilis® Ovipast) is a suitable strategy to provide maternally derived immunity as an aid to control ovine respiratory complex in young lambs.

Keywords: Ewes, ovine respiratory complex, pneumonia, vaccination, lambs.

SR-10

Microbial agents associated with infectious keratoconjunctivitis in Swedish reindeer

Karin Wallin Philippot[1], Lena Ström[2], Julia Bonevik[2], Jenny Frössling[3], Charlotte Berg[4], Jonas Johansson Wensman[3], Anna Omazic[5], Ylva Persson[3].

[1]National Veterinary Institute and SLU, Department of Clinical Sciences, Uppsala, Sweden; [2]Swedish University of Agricultural Sciences, Department of Clinical Sciences, Uppsala, Sweden; [3]National Veterinary Institute and SLU, Uppsala, Sweden; [4]Swedish University of Agricultural Sciences, Skara, Sweden; [5]National Veterinary Institute, Uppsala, Sweden.

Background: Infectious keratoconjunctivitis (IKC) is a multi-factorial, transmissible and severe ocular disease that affects ruminants worldwide. The disease in semi-domesticated Eurasian reindeer (*Rangifer tarandus tarandus*) was first reported for more than 100 years ago. Both isolated cases and outbreaks occur, mainly during the colder period of the year, and primarily affecting calves and yearlings. Intensified herding interventions (increased handling, transport, and supplementary feeding of reindeer), raising the stress level of the animals, may contribute to the present increase in appearance of IKC in Sápmi. Previous studies indicate that cervid herpesvirus 2 (CvHV2) works as a primary causative agent. In addition, the bacterium *Chlamydia pecorum* was isolated for the first time during an outbreak of IKC in 2016 in a Swedish herd of reindeer, but its role in developing of IKC remains unclear. Many different bacteria have been isolated from reindeer with IKC, such as *Moraxella bovoculi*, *Escherichia coli* and *Staphylococcus* spp. which all may play a role in the progression of disease. Further investigations, including prevalence, pathogenesis, and risk factors for IKC in semidomesticated reindeer is therefore needed.

Objectives: The objective was to study the presence of potential pathogens in samples from reindeer in Sweden with and without clinical signs of IKC and examine which possible secondary bacterial ocular infections followed the suspected primary pathogens CvHV2 and *Chlamydiaceae*.

Materials and Methods: Herds (n=37) with ongoing outbreaks of IKC in Swedish reindeer 2019-2021 were selected for participation in the study. A referral form was developed and implemented to gather information on clinical signs from affected reindeer. Conjunctival eye swabs (eSwab, Italy) were collected from reindeer with (n=170) and without (n=30) clinical signs of IKC, sent to the National Veterinary Institute in Sweden (SVA), and analyzed for the presence of CvHV2 and *Chlamydiaceae* with real-time PCR. In addition, routine general aerobic bacteriological culturing was performed. No specific ethical permission was needed since the animals were sampled as part of routine veterinary investigations for medical reasons prior to treatment and for clinical evaluation and with the approval of the owners.

Results: The preliminary results revealed a proportion of 24.3% for CvHV2 (45/185) and 33.5% for *Chlamydiaceae* (59/176). Only 4.6% (8/174) of the samples tested positive for both CvHV2 and *Chlamydiaceae* simultaneously, whereas 50.0% (87/174) of the samples tested negative for CvHV2 and *Chlamydiaceae*.

Bacterial cultivation from 186 samples revealed bacterial growth in 91.9% (171/186) with no specific infection in 43.0% (80/186) of the samples. The most frequent isolated bacteria were *Pseudomonas aeruginosa* and *Staphylococcus aureus* cultivated in 11.8% (22/186) and 11.3% (21/186) of the samples, respectively. *Moraxella* spp. and *Klebsiella pneumonia* were revealed in 8.6% (16/186) and 8.1% (15/186) of the samples. Mixed flora was revealed in 81.7% of the samples (152/186). Data are to be investigated with regards to bacterial growth in reindeer with and without clinical signs.

In reindeer with clinical signs of IKC (n=170), there was an association between the presence of *Pseudomonas aeruginosa* and CvHV2, compared to *Chlamydiaceae*. On the contrary, *Klebsiella pneumoniae* and *Moraxella bovoculi* were associated with the presence of *Chlamydiaceae* and not CvHV2. Finally, *Staphylococcus aureus* was more common in samples that tested positive for *Chlamydiaceae* than for CvHV2 and among samples tested negative for both CvHV2 and *Chlamydiaceae*. The most common signs among infected reindeer, reported in 31% of the herds, were epiphora (clear eye flow), leading to a wet cheek, followed by shedding of pus and red mucosa/conjunctivitis, reported in 21% and 15% of the herds, respectively. Data will be complemented prior to the conference and only preliminary results are presented here.

Conclusions: In this study we hypothesized that CvHV2 and *Chlamydiaceae* act as primary pathogens of IKC in reindeer. Preliminary results support CvHV2 to be the primary cause since it was only isolated in reindeer with clinical signs, which was not the case for *Chlamydiaceae*. In addition, secondary bacterial infections seem to differ depending on previous findings of CvHV2 or *Chlamydiaceae*, which could be of use when deciding on treatment strategy and to improve our understanding of the pathogenesis and risk factors. However, to conclude whether detected *Chlamydiaceae* in general was preceded by CvHV2 requires further investigation.

Keywords: Cervid herpes virus 2, Chlamydiaceae, bacteria, eye disease, reindeer.

SR-11

Flock sensitivity and specificity of pooled fecal qPCR and pooled serum ELISA for screening ovine paratuberculosis

Corbiere Fabien, Mathevon Yoann, Foucras Gilles.

UMR INRAe-ENVT IHAP, Toulouse, France.

Objectives: The aim of our study was to evaluate the flock sensitivity and specificity of fecal qPCR and serum ELISA using pooled samples for screening paratuberculosis in French sheep.

Materials and methods: Using individual feces with low or high qPCR Ct values from ewes sampled in 14 infected flocks, a total of 555 pools of size 5, 10 and 20 were created by diluting individual materials in negative feces and analysed using a commercial IS900 qPCR kit. The relative performances of pooled serum ELISA analysis were evaluated based on the analysis of 181 different pools of size 5 and 10, composed of individual serum samples of various individual S/P values. Finally, a simulation study was carried out to evaluate the performances of 16 screening strategies at flock level, with varying pool size (5 to 20) and number (5 to 60).

Results: For pools of size 5, 10 or 20, individual fecal samples with low Ct values were invariably detected. Conversely fecal samples with high Ct values were associated with a lower detection rate in both pools of size 5 (87.0% to 90.0%), 10 (63.0% to 70.7%) and 20 (46.7% to 60.0%). After lowering the decision threshold to 25% and 15% for serum pools of size 5 and 10 respectively, the pooled serum ELISA relative sensitivity ranged between 62.2% and 100.0% depending on the composition of the pools.

At the flock level the use of pooled serum ELISA led to false positive detection rates ranging between 37.6% and 91.8% in paratuberculosis free flocks and prevents its further use in that context. For infection prevalence ≤ 5%, the flock sensitivity based on pooled fecal qPCR ranged between 39.0% (5 pools of size 10) and 99.9% (300 sampled individuals, with pools of size 5,10 or20), and was always above 93% when the infection prevalence was greater or equal to 15%.

Conclusion: We conclude that pooled-fecal qPCR but not pooled-serum ELISA could be a useful tool to detect sheep flocks infected with paratuberculosis.

Keywords: Paratucerculosis, pooled samples, faecal qPCR, Serum ELISA, flock sensitivy and specificity.

SR-12

Diagnostic accuracy of a digital Brix refractometer for assessing colostrum quality and failure of passive immune transfer in neonatal lambs

Corbiere Fabien[1], Rousseau Frederic[2], Gadenne Sophie[1], Jacquet Amélie[1], Rousset Anne-Lise[1], Gautier Jean-Marc[3].

[1]National Veterinary School of Toulouse, Toulouse, France; [2]Clinique Vétérinaire d'Amikuze, Béhasque-Lapiste, France; [3]IDELE, Toulouse, France.

Objectives: This study aims ta assessing the diagnostic performances of a digital Brix refractometer (Hanna HI 96801) for the evaluation of colostrum quality and failure of transfer of passive immunity (FTPI) in sheep.

Materials and methods: Colostrum samples from 330 meat ewes were collected within 6 hours after lambing and frozen immediately until analysis. The immunoglobulins G1 (IgG1) concentration was assessed by radial immunodiffusion (RID). The colostrum total fat content was assessed by the Gerber method. In addition, 230 plasmas were collected in 2- to 4-day old lambs and their IgG1 concentration measured by RID. All colostrum and plasma samples were assessed by the digital Brix refractometers. Test sensitivity (Se) and specificity (Sp) were calculated using RID as the reference standard with failure of passive immune transfer (FTPI) defined as IgG < 10.0 g/L. The IgG<50 g/L threshold was used to mark poor quality colostrum. Receiver operating characteristics curves were created and optimal cut-offs values for each refractometer were selected based on the maximization of the Youden's J statistic.

Results: The Pearson correlation coefficient between RID and %Brix results on lamb plasma was high (r=0.91). The RID and %Brix results on colostrum samples were also highly correlated (r=0.79). A significant influence of the colostrum fat concentration on the %Brix value was evidenced, with an increase of 10 g/L of fat leading to an average 0.5% increase on the Brix scale. For the detection of colostrum with IgG1 concentration lower than 50g/L, the optimal decision threshold value was 24.0 % Brix (Se=87%; Sp=91%) for the Hanna Brix refractometer. For the detection of FTPI in lambs (plasma IgG1<10 g/L) the optimal decision threshold was 8.3% Brix (Se=97% and Sp=87%).

Conclusion: Brix refractometry appeared to provide reasonably accurate results for the assessment of colostrum quality and FTPI in sheep. The optimal decision cut-offs were similar to those established in cattle. For on-farm use, a digital Brix refractometer can be a valuable and inexpensive tool to help producers and veterinary practitioners in their lamb health management programs.

Keywords: Colostrum, transfer of passive immunity, digital refractometer, sensitivity, specificity.

SR-13

Detection of Corynebacterium pseudotuberculosis as an etiologic agent causing polyarthritis in lambs

Héctor Ruiz[1], Mar Juní[1], Marcos Pons[2], Oihane Alzuguren[3], Paula Cano[1], Juan José Ramos[1].

[1]Facultad Veterinaria de la Universidad de Zaragoza, c/ Miguel Server 177, 50013., Zaragoza, Spain; [2]Centro de servicios clínicos veterinarios Osca, Avda. del Cinca 55, 22300, Barbastro, Spain; [3]EXOPOL diagnósticos y autovacuna S.L., Pol. Río Gállego D/8 50840, San Mateo de Gállego, Spain.

Objectives: Polyarthritis is one of the most important pathologies in lambs, both in lactation and during fattening period, due to the implications that this pathology has in the economy of the farm, as it produces growth losses and an increase in mortality. Polyarthritis can be caused by some different microorganisms such as *Streptococcus dysgalactiae*, *Erysipelothrix rhusiopathiae, Trueperella pyogenes,* etc.

Corynebacterium pseudotuberculosis is the etiologic agent of the caseous lymphadenitis (CLA) which usually affects small ruminants. The most frequent locations of the pyogranulomas caused by *C. pseudotuberculosis* are in superficial lymph nodes, in the superficial clinical form of the disease, or in deep lymph nodes such as mediastinic and internal organs, in visceral clinical form.

In this paper is described the first presentation of *C. pseudotuberculosis* as microorganism responsible for polyarthritis in feedlot lambs.

Materials and methods: The Ruminant Clinical Service (SCRUM) of the Veterinary Faculty of Zaragoza, Spain, receives animals from the Faculty´s area of influence which can be culling animal belonging to collaborating farms or clinical cases referred by veterinarians. Two fattening lambs coming from two different clinical cases of polyarthritis were referred to the SCRUM during 2019 in order to reach a final diagnosis of the cause of the outbreak.

Both animals were subjected to the same protocol of all the animals received at the service. They were submitted to a rigorous clinical examination and blood samples were taken to carry out a blood count test. Subsequently, thermography and arthrocentesis of the affected joints were made in both animals. In addition, a computed tomography (CT) was also performed in one of the animals. Finally, the lambs were humanely sacrificed to conclude with a complete pathological examination with sampling of affected joints. Samples obtained from arthrocentesis and *post-mortem* study were quickly submitted to the laboratory in order to perform a microbiological and biomolecular analysis. The isolation of the causative agent was carried out in the laboratory EXOPOL diagnóstico y autovacunas S.L.

Results: The clinical examination of the lambs showed that one of the lambswas affected in the tarsal joint of the right hindlimb and the other in the tarsal joint of the left hindlimb. The animals were lame and reluctant to move and they also showed a clear stunted growth. The affected joints were hot, swollen and painful.

Hematology revealed a clear leukocytosis with neutrophilia in both animals. These parameters can be indicatives of an acute bacterial infection. Likewise, thermography showed an increase in the temperature of the joint of the affected limbs in comparison with the unaffected joints. Finally, computed tomography (CT) scan performed in one of the lambs revealed an important enlargement of the popliteal lymph node of the affected limb that partially collapsed the adjacent vein, which hinders the venous return.

Pathological findings confirmed an important purulent arthritis in the affected joints in both lambs.

In Both analyzed animals a massive and pure isolation of *Corynebacterium pseudotuberculosis* was obtained in the affected joints, in both samples, those of arthrocentesis and those taken after necropsy.

Conclusions: It can be concluded that *Corynebacterium pseudotuberculosis* can cause polyarthritis in lambs, therefore, this microorganism should be included in the list of microorganisms associated with this disorder in lambs. However, the reason why *C. pseudotuberculosis* is able to migrate to the joint and produce this pathology is still unknown.

Keywords: Corynebacterium pseudotuberculosis, polyarthritis, lambs, arthrocentesis.

SR-14

Major concerns of the small ruminant farmer on St. Kitts

Jerry Roberson[1], Ninian Cameron-Blake[2], Hilari French[2], Souvik Ghosh[2].

[1]Long Island University College of Veterinary Medicine, Brookville, United States; [2]Ross School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis.

Introduction: Small ruminants make up a large percentage of livestock on St. Kitts but their numbers are declining, estimated to be less than 10,000 head. Because many of the small ruminant farmers of St. Kitts do not own pasture, their herds and or flocks will typically be allowed free range. This complicates production and disease management practices that could help provide improved sustainability. Small ruminant productivity can be increased but only after understanding, addressing and correcting the major issues and concerns of the small ruminant producer on St. Kitts.

Objectives: 1) Investigate the concerns and management practices of the St. Kitts small ruminant producer and 2) Prioritize and provide possible solutions.

Materials and Methods: Fifty small ruminant producers were solicited via telephone or in person to participate in this study. A questionnaire was administered on site to the producers to better comprehend herd and flock management practices. Information was gathered regarding general farm statistics, nutrition, marketing, reproduction, disease occurrence, disease treatment and or prevention methods and facilities. Producers were asked to list their top three major concerns. Producers were also asked to list the top 3 major disease concerns.

Results: The results indicate that predation by dogs af-

fected 50% of small ruminant producers. The second most common issues (36%) were genetic improvement and obtaining medications for both treatment and prevention. The fourth most common issue was theft, 30% of farms. Other major issues were nutrition (26%), general disease issues (24%), fencing/pens (24%), internal parasites (22%), external parasites, predominatley ticks (22%) and availability of land/pasture (22%). Other issues that were listed < 10% of the time were, dermatophilus, livestock transport, herd health, housing, wild fires, trauma (hit by bus) and marketing. Specific diseases were not among the top four concerns but the primary specific disease concerns were internal parasites (76%), ticks (72%) and dermatophilus (36%). Other diseases or clinical conditions mentioned as concerns (< 20% of the time) were upper respiratory diseases (nasal bots), abortion, contagious ecthyma, mange, spiders, foot issues, tetanus, sudden death and frothy mouth.

Conclusion: The major issues affecting the small ruminant farmers were unexpected as disease issues were not in the top four (e. g. internal parasites). Efforts directed toward decreasing dog predation and theft would benefit the small ruminant farmer. Genetic improvement may be increased by importation of select sheep and goats to the island. Making medications easily, economically and readily available would benefit the St. Kitts small ruminant producer.

Keywords: Sheep, goat, problems, survey.

SR-15

Assessment of the impact of a biofilm-embedded bacteria-based vaccine against Staphylococcus in suckler flock ewes on lambs' development and mammary gland score

Anaïs Dubray[1], Benoit Bolkaerts[1], Christine Baricalla[1], Leonard Théron[2], Nathalie Kirschvink[1].

[1]University of Namur, Veterinary department, Narilis, Namur, Belgium; [2]Hipra Benelux, Gent, Belgium.

Objectives: To evaluate the effect of a vaccine against staphylococcal mastitis in suckler flock ewes by assessing lambs' growth and mammary gland score in vaccinated and non-vaccinated ewes. The used vaccine (VIMCO©, Hipra) included an antigen based on a bacterin of Staphylococcus aureus strain, expressing the exopolysaccharide poly-N-acetylglucosamine (PNAG), which is involved in biofilm formation by these bacteria. It was hypothesized that the vaccine would reduce incidence of subclinical mastitis and thereby improve suckler lambs growth.

Material and methods: 200 pregnant Ile-de-France ewes were divided in two similar groups based on their parity and age. One group underwent vaccination against mastitis by 2 intramuscular vaccine (VIMCO©) injections at 5 and 2 weeks before lambing (Vacc), the second group served as non-vaccinated control (non Vacc). Their suckling lambs were weighed at birth, at 48 hours of age and at weaning, allowing to calculate daily weight gain (DWG) and age at weaning. Cases of clinically detectable mastitis were recorded. At weaning, each ewe underwent mammary gland inspection. A mammary gland score taking into account retromammary lymph nodes (normally sized versus hypertrophy), left-right gland asymmetry and presence or absence of nodules was established for each ewe. Data were analyzed by ANOVA in order to assess the effect of vaccination on lambs' weight at 48 hours of age, weight at weaning, DWG at weaning, clinical mastitis incidence and mammary gland score. A p-value below 0.05 was considered as significant. Data from ewes whose lambs died or needed continuous bottle feeding were withdrawn from analysis, leading to 96 non-vaccinated ewes aged of 3.77±1.78 years with 158 lambs and to 93 vaccinated ewes aged of 3.76±1.66 years with 146 lambs.

Results: While weight at birth did not differ between groups (Non Vacc: 4.72±0.95 kg *versus* Vacc: 4.81±0.77 kg, p = 0.95), weight at 48 hours after birth was significantly higher in lambs raised by vaccinated ewes (Non Vacc: 5.03±0.95 kg *versus* Vacc: 5.25±0.89 kg, p < 0.05). At weaning around 74 days of life, weight and DWG were significantly higher in lambs suckling vaccinated ewes (weight : Non Vacc: 29.39±4.78 kg *versus* Vacc: 30.63±4.39 kg, p <0.05; DWG : Non Vacc: 0.34±0.06 kg/day *versus* Vacc: 0.35±0.05 kg/day, p <0.05). Mortality of newborn lambs equaled 4.5 % and did not differ between groups. Regarding udder evaluation of vaccinated and non-vaccinated ewes, the mammary gland score did not differ between groups (Non Vacc: 1.19±0.73 *versus* Vacc: 1.18±0.81, p = 0.89). Intramammary small-sized nodules were detected in 14% of non-vaccinated ewes and in 15% of vaccinated ewes whereas minor udder asymmetry occurred in 40% of non-vaccinated and in 38% of vaccinated ewes. Clinical mastitis occurred in 1 non-vaccinated and 2 vaccinated ewes.

Conclusions: The low prevalence of clinical mastitis in the flock made not possible to asses the impact on detectable signs of mastitis. However, suckler lambs' growth increased by 4% as demonstrated by increased DWG and weight at weaning in lambs raised by vaccinated ewes.

Keywords: Mastitis, vaccine, suckler lambs, daily weight gain.

SR-16

Chronic Coenurosis in sheep: Spontaneous remission of clinical signs and role of CT and MRI in the diagnosis and follow-up

Jorge Gimeno, Maria Pérez Barcia, María Climent, Ricardo De Miguel, María Teresa Ortiz, Alba Magdalena, Enrique Castells, Amaia Escudero, Luis Miguel Ferrer.

Facultad de Veterinaria Zaragoza, Zaragoza, Spain.

Coenurosis is a condition caused by Coenurus cerebralis, the larval stage of Taenia multiceps, and causes enormous economic losses in husbandry production. C. cerebralis mainly develops in the central nervous system of sheep and other ungulates whereas T. multiceps inhabits the small intestine of wild and domestic canids, being the dog the main definitive host. Proper diagnosis of Coenurus cerebralis infection is

challenging and although epidemiological and clinical studies are useful to guide the diagnosis, a post-mortem examination is decisive to confirm the suspicion. In alive animals, a presumptive diagnosis is usually made in the light of the neurological examination, but clinical signs observed are usually non-specific. In the present study, a case of spontaneous clinical remission of chronic cerebral coenurosis is presented for the first time and the role of computed tomography (CT) and magnetic resonance imaging (MRI) in the diagnosis and follow up is studied.

Five animals presenting neurological clinical signs were referred to the Clinical Ruminant Service (SCRUM) of the University of Zaragoza. All animals were adult young sheep (1-3 years old) and they belong to a meat sheep flock reared in a semiintensive production system. No treatments were applied to the animals prior to its reference to the SCRUM. Exhaustive clinical examination of the five animals was performed, making special emphasis on neurological examination. Although a huge variety of neurological clinical signs were observed, the most common symptoms were circling, frequent bleating, separation from the flock and visual impairment. A CT scan of the five affected animals was performed with a Bivro CT scan of two slides (General Electric Healthcare). CT imaging revealed the presence of single fluidfilled cystic structures that occupied 40-55% of the cranial cavity, comprising and atrophying the adjacent encephalic structures. In the light of clinical signs and CT images, diagnosis of chronic coenurosis was stablished. Four of the five animals were humanely sacrificed and the diagnosis was confirmed at the postmortem evaluation, where big intracranial parasitic cyst with multiple protoscolices were found associated with severe focally extensive pressure atrophy of the brain.

As one of the animals was pregnant, it was hospitalized until it raised the lamb. During this time, that lasted 7 months, clinical signs remitted spontaneously in the absence of any treatment application. To clarify this outstanding clinical finding both, CT and MRI (Hitachi AIRIS Mate 0.2T, Blue Star E&E) exams were performed. This second CT revealed a marked retraction of the parasitic cyst. In addition, a severe increase in tisular density was found in contrast with the fluid-filled Coenurus cyst showed in the first CT. MRI confirmed the location of the Coenurus and helped to further characterize the morphological abnormalities in the cranial cavity. Interestingly, MRI revealed that the area previously occupied by the parasitic cyst had been replaced by encephalic tissue and a mass of undefined consistence. After weaning, the ewe was humanely sacrificed. Post-mortem examination of the brain showed focally extensive polioencephalomalacia localized in the right occipital lobe. At cut section, shrunken remnants of the parasitic cyst were found as a nodular greyish mass, which distorted the white matter, expanding and compressing adjacent structures. Tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin wax for histopathological evaluation. Microscopically, degenerate coenurus were characterized by a disrupted 300µm thick hyaline tegument, partially mineralized and filled by granular acellular eosinophilic refringent material and multiple round basophilic structures. The parasite cyst was surrounded by moderate edema and a rim of abundant foamy macrophages, eosinophils, lymphocytes and plasma cells. The final diagnosis of chronic coenurosis with cyst collapse and larval death was assessed.

In this report, CT and MRI revealed outstanding results in the detection of the parasitic cysts. Moreover, valuable information of the surrounding nervous structures was obtained. In regards of these results, both techniques can be proposed as the gold standard for the diagnosis of the chronic phase of this condition. Furthermore, CT and MRI can become key tools for the diagnosis of ovine encephalic affections and the development of new therapeutical approaches in sheep. To date, this is the first report of the imaging follow-up of spontaneous clinical remission, suggesting the role of optimal management in the outcome of the chronically affected animals.

Keywords: Ovine, coenurosis, computed tomography, magnetic resonance imaging.

SR-17

A topical anaesthestic wound formulation diminishes pain responses of lambs at tail-docking

Aurora Ortin[1], Juan José Ramos[1], Delia Lacasta[1], María Teresa Tejedor[1], Luis Miguel Ferrer[1], Enrique Castells[2], Marta Ruiz De Arcaute[1], Inés Rubira[3], Peter Windsor[4].

[1]Facultad de Veterinaria. Instituto IA2 (Universidad de Zaragoza-CITA), Zaragoza, Zaragoza, Spain; [2]Centro Clínico Veterinario S.L., Zaragoza, Spain; [3]Gabinete Técnico Veterinario S.L., Zaragoza, Spain; [4]Veterinary Faculty. University of Sydney., Sydney, Australia.

Objectives: Tail-docking is a routine global husbandry procedure conducted in lambs to reduce myiasis risk and improve fertility. It is often performed without pain relief, although use of general anaesthesia has been advocated, at an exorbitant and prohibitive cost to farmers. There is an urgent need for a practical and affordable approach acceptable to farmers that markedly reduces animal suffering. We evaluated use of a topical anaesthetic 'spray-on' wound management formulation containing the local anaesthetics lignocaine and bupivacaine, plus cetramide and adrenalin, in a gel matrix (Tri-Solfen®, Bayer Animal Health, Gordon, NSW, Australia). Impacts of treatments on pain response in lambs, where the tail was excised surgically, with and without use of general anaesthesia, are reported.

Materials and methods: This study was approved by the Ethical Commission of the University of Zaragoza. Female Rasa Aragonesa 45 day-old lambs (n = 44) with similar weights in a farm in Zaragoza (Spain) were recruited for the study. The 4 equal cohorts (n=11) of lambs with: the tail excised with a scalpel without local or general anaesthesia and without subsequent stitching (Group A); the tail excised under general and local anaesthesia with stitching to close the wound (Group B); the tail excised with a scalpel without prior anaesthesia and without stitching but treated immediately with Tri-Solfen® (Group C); the tail excised under general and local anaesthesia with stitching to close the wound following immediate treatment of the wound with Tri-Solfen® (Group D).

Pain-related behaviour was assessed using a numerical rating scale (NRS) developed previously. A trained scientist blinded to treatment observed the lambs immediately (B0),

2.5h (B1) and 5h (B2) after tail docking. Individual lambs were ascribed an NRS score between 0 and 3, where: 0 = no pain-related behaviour; 1 = mildly abnormal posture, gait or behaviour, including mild kyphosis without hyperextension of hindlegs, ventral recumbency with hindlegs partially extended or mild stiffening of gait without overt limping or leg dragging; 2 = moderately abnormal posture, gait or behaviour, including 'statue standing' with head down and prominent kyphosis, moderate stiffening or slowing of gait or hyperextension and/or abduction of hindlegs, ventral recumbency with hindlegs fully extended; 3 = displaying severely abnormal posture, gait or behaviour, including marked agitation with twisting or writhing, high frequency of postural change from lying to kneeling or standing, distressed vocalisation, lateral or prostrate lying, kneeling, dog sitting or tremors, shaking or lip curling. Data were analyzed using the Mann –Whitney U test.

Results: In cohorts tail-docked under general anaesthesia (B & D) the mean of the NRS pain score at B0 was 2.364 for untreated animals and 2.045 for animals treated with Tri-Solfen® ($p>0.05$). However, in cohorts tail-docked without general anaesthesia (A & C), the mean of the NRS pain score at B0 was lower, at 1.273 for untreated animals and 0.682 for treated lambs ($p = 0.013$). The differences between the two methods of tail docking at B0, regardless of whether the lambs were treated or not with Tri-solfen®, also showed highly significant differences ($p = 0.004$). The group not undergoing surgical stitching suffered less pain immediately after the procedure, especially if treated with Tri-solfen®. These results accord with those previously published but where tails were removed by a hot-iron (Lomax et al. 2010).

By 2.5hrs after tail docking (B1), the NRS scores had begun to equalize, although differences were still observed between treatments. In cohorts without Tri-solfen® (A & B) the mean of the pain response was 1.136 and 1.091, whereas in cohorts treated with Tri-solfen® (C & D) the mean was 0.864 and 0.818, respectively ($p > 0.05$). Finally, at 5 hours (B2) most of the animals, independent of the group they belonged to, no longer displaying pain behaviours.

Conclusions: These findings and additional clinical evidence of pain relief presented elsewhere are encouraging, suggesting that surgical tail-docking without general anaesthesia but where wounds are immediately sprayed with Tri-Solfen® is an affordable method of alleviating pain in lambs undergoing tail docking.

Keywords: Sheep, topical anaesthesia, pain, tail-docking.

SR-18

Effect of a topical anaesthetic formulation on wound healing after tail-docking in lambs

Luis Miguel Ferrer[1], Delia Lacasta[1], Aurora Ortín[1], María Pérez[1], María Teresa Tejedor[1], Juan José Ramos[1], Calasanz Jiménez[2], Peter Windsor[3].

[1]Facultad de Veterinaria. Instituto IA2 (Universidad de Zaragoza-CITA), Zaragoza, Zaragoza, Spain; [2]Gabinete Técnico Veterinario S.L., Zaragoza, Spain; [3]Veterinary Faculty. University of Sydney., Sydney, Australia.

Objectives: Tail-docking is a routine global husbandry procedure conducted in lambs to reduce myiasis risk and improve fertility. It is often performed without pain relief, although use of general anaesthesia has been advocated, at an exorbitant cost to farmers. There is an urgent need to find a practical and affordable approach acceptable to farmers that avoids or markedly reduces animal suffering. This study evaluated the effect of a topical anaesthetic 'spray-on' wound management formulation containing the local anaesthetics lignocaine and bupivacaine, plus cetramide and adrenalin, in a gel matrix (Tri-Solfen®, Bayer Animal Health, Gordon, NSW, Australia). Impacts of treatments on wound healing in lambs, where the tail was excised surgically, with and without use of general anaesthesia, are reported.

Materials and methods: This study was approved by the Ethical Commission of the University of Zaragoza. Female Rasa Aragonesa 45 day-old lambs (n = 44) with similar weights in an intensively-housed farm in Zaragoza (Spain) were recruited for the study. The 4 equal cohorts (n=11) of lambs with: the tail excised with a scalpel without local or general anaesthesia and without subsequent stitching (Group A); the tail excised under general and local anaesthesia with stitching to close the wound (Group B); the tail excised with a scalpel without prior anaesthesia and without stitching but treated immediately with Tri-Solfen® (Group C); the tail excised under general and local anaesthesia with stitching to close the wound following immediate treatment of the wound with Tri-Solfen® (Group D). Following the procedure, the animals were examined daily for 15 days, with lesions photographed. This enabled analysis of wound healing after tail-docking and the detection of secondary infections. Data were analyzed using survival analysis (Kaplan- Meyer method and Breslow test), evaluating the days until the wound is found infected.

Results: In the period analyzed, 73% (16 of 22) of lambs in the two cohorts not undergoing surgical stitching (A & C) and 91% (20 of 22) of lambs in the two cohort undergoing surgical stitching (B & D), developed signs of wound infection. Further, the mean of days on which the animals showed no signs of wound infection was significantly later in the two groups not undergoing surgical stitching (A:10.2 & C:11.5) versus those where stitching occurred (B:5.2 & D:4.6). The median, being the day when half of the lambs displayed infection and half did not, was: A:2 days; B: 3 days; C: 4 days; and D: 1 day. Although only significant differences were found when comparing the two methods, with and without stitching, among those treated with Tri-Solfen®, it was observed that the best results was in cohort group C, being lambs whose tail was excised without stitching and the wounds were treated with Tri-Solfen®.

Conclusions: These results and those presented elsewhere are encouraging, suggesting that surgical tail-docking without general anaesthesia but where wounds are immediately sprayed with Tri-Solfen® is an affordable and efficacious method of conducting the procedure, since this topical anaesthesia wound treatment formulation reduces infections to a certain extent, hastening wound healing.

Keywords: Sheep, topical anaesthesia, wound healing, tail-docking.

SR-19

Study of the use of bronchoalveolar lavage as a live diagnostic method to detect clinical pulmonary Maedi Visna

Alba Magdalena[1], Andrea Menjón[1], Marcelo De Las Heras[1], Marta Borobia[1], J.l Arnal[2], Julen Elorza[1], Delia Lacasta[3].

[1]Facultad de Veterinaria de Zaragoza, Zaragoza, Spain; [2]Laboratorio Agroambiental de Aragón, Zaragoza, Spain; [3]Instituto IA2 (Universidad de Zaragoza-CITA), Zaragoza, Spain.

Objectives: Maedi-Visna virus (MVV) is a lentivirus that infects, mainly, sheep older than two years, causing a multi-systemic and slow progressive syndrome, inducing a chronic inflammation of the lung, mammary gland, central nervous system and joints. It causes relevant economic losses worldwide and due to the lack of vaccines and treatments, a suitable diagnosis is decisive to develop control programs.

The main objective of this survey was to evaluate the usefulness of the molecular study of bronchoalveolar lavages using PCR techniques to improve the live diagnosis of the pulmonary form of Maedi Visna disease (MV). In order to investigate the efficacy of this diagnostic method, the MV compatible lung lesions found after the necropsy of the studied animals were analyzed and lung tissue samples were also collected to perform a molecular diagnosis of the MVV.

Material and methods: One hundred and fifty-five culling sheep coming from four collaborating farms were analyzed at the Ruminant Clinic Service of the University of Zaragoza. All the animals were subjected to the same protocol: complete clinical examination with special emphasis on the respiratory system was performed and a blood sample was collected to carry out a MVV serological analysis. Subsequently, prior to the animals being euthanized, a bronchoalveolar lavage was accomplished to obtain a sample of the lower respiratory tract in order to be analyzed by PCR. Finally, post-mortem pathological study was carried out at the Pathological Service of the Veterinary Faculty of Zaragoza and lung tissue samples were taken for molecular studies. Samples obtained in bronchoalveolar lavages and post-mortem collections (portion of lung parenchyma) were analyzed in EXOPOL diagnóstico y autovacunas S.L. by quantitative PCR (qPCR). The specific EXOone Maedi Visna - CAEV oneMIX kit developed by the EXOPOL laboratory was used. The samples were considered positive when the Cq value (quantification cycles) was ≤38.

The data were recorded in SPSS STATISTICS 22.0. computer program and the WinEpi web platform was used to estimate sensitivity and specificity values and predictive values.

Results: Lungs with compatible MV lesions (12.25%) were those that presented these four lesions: lung enlargement, general grayish discoloration, grey subpleural dots and a significant increase in mediastinal lymph nodes size. Lungs suspicious of having MV (22.60%) were considered to be those with 2 or 3 of these lesions and lungs not compatible (65.15%) those with 1 or none compatible lesions. The main lesion found was lung enlargement (43.90%), followed by the discoloration (32.90%), increased size of mediastinal lymph nodes (23.90%) and grey subpleural dots (12.25%).

Regarding bronchoalveolar lavages, 16.10% of the analyzed animals were MVV-positive by PCR. Therefore, 77.80% of animals with MV-compatible lesions and 20.60% of the animals classified as suspected gave a positive PCR result for MVV in bronchoalveolar fluid. Only 3.10% of the lungs classified as non-compatible were MVV-positive in bronchoalveolar lavage. The results of positive predictive value (PPV) and negative predictive value (NPV) related to the presence or absence of macroscopic lesion have a value of 82.40% and 96.00%, respectively.

On the other hand, 29.73% of the lung parenchyma analyzed tested positive by PCR. In this case, 89.50% of the lungs with lesions compatible with MV, 37.10% of the suspicious and 14.90% of the non-compatible ones showed positive results in parenchyma. Thus, PPV was 54.80% and NPV was 97.60%. These results could suggest that the PCR test of lung parenchyma also detects infected animals that do not show obvious MV macroscopic lung lesions.

Conclusions: Bronchoalveolar lavage is revealed as a suitable technique, little invasive and innovative, for the *in vivo* diagnosis of the pulmonary form of Maedi Visna disease, with adequate predictive values both positive and negative.

Keywords: Bronchoalveolar, lavage, diagnose, pulmonary, Maedi.

SR-20

Evaluation of different reproductive management protocols to advance pregnancy onset in sarda ewe-lambs at their first reproductive season

Antonio Spezzigu[1], Francesca Daniela Sotgiu[2], Cristian Porcu[2], Giovanni Molle[3], Fiammetta Berlinguer[2].

[1]Embryo Sardegna Reproduction and Fertility, Perfugas, Italy; [2]Department of Veterinary Medicine, University of Sassari, Sassari, Italy; [3]AGRIS Sardegna, Sassari, Italy.

Objectives: For a successful dairy production Mediterranean dairy sheep breeding aims to initiate the mating season in late spring for adult ewes with lambing in late autumn. The ewe-lambs born in autumn are usually mated during the reproductive season of the following year (late August – December). This scenario imposes a different management strategy for the ewe-lambs which respond poorly to the male effect and tend to have poorer response to synchronization protocols when compared to adult ewes. Thus, the reproductive management of ewe-lambs relies mostly on natural mating, where young ewes are kept with the males in a single flock. This practice leads to a long lambing season with the bulk of the ewe-lambs lambing in March-April (pregnancy onset October-November). In addition, ewe-lamb's reproductive and productive performance are lower compared to adult ewes.

The present study aimed to assess if the ram effect alone or associated with a GnRH administration, are able to advance pregnancy onset in dairy ewe-lambs of different weight, when entering the first reproductive season. Treatments were tested in different groups having low, medium and high live weight. The different outcome between protocols was evaluated by comparing the number of days from treatment to pregnancy onset.

Materials & Methods: On mid-July, 131 prepubertal ewe-lambs were selected from a single flock. Ovarian status was evaluated by transrectal ultrasonography to ensure the absence of cyclicity. The animals were divided in three groups according to live weight (live weight ± SE; high weight: HW, 39,1 ± 0,3, n=35; medium weight: MW, 33,8 ± 0,2, n=64; low weight: LW, 28,2 ± 0,3, n=32). Within each weight group, animals were randomly allocated into two subgroups: GnRH, treated with a GnRH analogue (gonadorelin, 40 µg/head; 0.8 ml of Cystoreline®, Ceva Salute Animale, Italy); CTR, which was the untreated control. Both subgroups were exposed to the ram effect. GnRH groups following 1 week from the treatment administration, were evaluated again by ovarian ultrasound scanning. Ewe-lambs showing corpora lutea were injected with a PGF2α analogue (cloprostenol, 100 µg/head, PGFVeyx, Vexy-Pharma GmbH, Germany) and then placed with crayon marked rams. Ewe-lambs not showing signs of ovulations were injected with a second dose of GnRH. After 7 days, the ewe-lambs were checked again and the ones showing corpora lutea were injected with a PGF2α analogue, while the remaining received a third injection of GnRH. After all ewe-lambs were exposed to fertile rams. Ewe-lambs in the CTR, after the initial ultrasound scanning, were managed as single flock with fertile rams fitted with crayon markers.

Ewes mating behaviour was checked daily for 120 days starting from rams introduction. The mated ewe-lambs, once identified, were removed from the flock. Pregnancy was diagnosed within 30 days after mating using transrectal ultrasonography. During the trial, data collection was supported by a farm management software developed by Sementusatech®. The efficacy of the protocols in anticipating pregnancy onset was determined by assessing differences between groups in the number of days (from Day 1 - treatment start to pregnancy onset) needed to reach the 25%, 50%, 75% of flock pregnancy rate.

Results: There was a difference in the timing of pregnancy establishment between groups ($p<0.005$). The GnRH-MW group showed the best performances in reaching the threshold pregnancy rates of 25, 50 and 75% ($P<0.05$) when compared to the other groups. Both LW groups had poorer performances ($P<0.05$). The administration of GnRH had no effect in advancing the onset of pregnancy in the HW group (GnRH-HW) when compared to (CTR-HW). Both groups showed a delay in reaching the 25% pregnancy rates compared to the GnRH-MW group, but thereafter HW groups were able to catch up.

Conclusions: The current study showed that the hormonal protocol can improve reproductive performances in ewe-lambs at their first reproductive season by advancing pregnancy onset and by increasing fertility rates in medium weight ewe-lambs. The ram effect alone could be used with ewe-lambs that have already reached the optimal weight at the onset of the breeding season.

Keywords: Puberty, male effect, GnRH, ultrasound, dairy sheep.

SR-21

Effect of a topical anaesthetic formulation on viral load in lambs naturally infected with orf virus

Delia Lacasta[1], Ramsés Reina[2], Juan José Ramos[1], Luis Miguel Ferrer[1], Silvia Martínez[1], Alfredo A. Benito[3], María Teresa Tejedor[1], Irache Echevarria[2], Peter Windsor[4].

[1]Veterinary Faculty of Zaragoza and IA2. University of Zaragoza, Zaragoza, Spain; [2]Institute of Agrobiotechnology (CSIC-Gobierno de Navarra), Mutilva, Spain; [3]Laboratorios EXOPOL, diagnóstico y autovacunas, San Mateo de Gállego, Spain; [4]Faculty of Veterinary Science. University of Sydney., Sydney, Australia.

Objectives: Orf is a highly contagious eruptive skin condition of sheep and goats, caused by a Parapoxvirus with a worldwide distribution. It affects mainly lambs and kids, with more serious outbreaks often associated with intensive husbandry, causing significant financial losses to livestock production. It is also a zoonotic disease, affecting mainly people via direct or indirect contact with infected animals. Vaccination remains the preferred option to control the disease. However, currently in Spain and many other countries, no orf vaccine is available. The treatment of this disorder referred to as Contagious Ecthyma and Scabby Mouth, involves standard hygiene practices and management of presumptive secondary infections.

The wound dressing formulation, Tri-Solfen® (Animal Ethics Pty Ltd, Australia) is registered for use in sheep in Australia, cattle in Australia, New Zealand and Laos, and is in the process of being registered to be used in cattle and pigs in Europe and other countries. This formulation offers numerous advantages over current therapies, particularly as it has been confirmed as providing pain relief and more rapid healing of lesions (Windsor et al, 2016). The formulation contains two local anaesthetics (lignocaine and bupivacaine), adrenalin and an antiseptic (cetramide) in a gel formulation that creates a barrier effect with a pH of ~2.7. This numbs the pain of lesions, reduces their infectivity, hastens healing, potentially reduces weight loss, and the antisepsis properties avoids the need for other treatments, including antibiotics.

This study explores potential antiviral roles and healing properties of Tri-Solfen® in orf naturally infected lambs through viral genome real time PCR quantification and tissue culture in ovine primary cells.

Materials and methods: This study was approved by the Ethical Commission of the University of Zaragoza. Fourteen one-month-old Rasa Aragonesa lambs, naturally infected with orf, were recruited from a farm where an outbreak of orf disease was occurring. The animals were selected at the early stages of the infection when lesions were initially advised and divided into two cohorts: Group A (n=11) consisting of animals with orf lesions treated with Tri-Solfen® and Group B (n=3), a control group without treatment.

Cotton swabs were obtained before treatment (T0) and days 1 (T1), 3 (T2) and 5 (T3) post-treatment, then submitted to direct DNA extraction and real-time PCR quantification (Exopol) or to incubation with primary tissue cultures from ovine skin fibroblasts (OSF) and T-immortalized goat embryonic fibroblasts (TIGEF). Orf virus quantification was performed

by real time PCR on DNA from cultured cells at day 0 and 5 post-treatment. Data were analyzed using the non-parametric Wilcoxon test for paired samples and by T-Student's test for unrelated samples.

Results: In the study carried out using quantitative PCR, no significant differences were found between day 0 pre-treatment (T0) and day 5 post-treatment (T3) (p=0.722). However, when the viral load was assessed in primary tissue cultures of ovine skin fibroblasts (OSF) and T-immortalized goat embryonic fibroblasts (TIGEF), there was a reduction in both groups between T0 and T3 that was significant in the OSF cell cultures (p<0.05).

Conclusions: These results suggest that despite the presence of the viral DNA in the orf lesions at 5 days post-treatment, this may belong to inactivated virus as the viral load obtained after cell culture of the samples of the treated animals was significantly less than that obtained from controls. These findings suggest that as treatment of orf lesions with Tri-Solfen® reduces the viral load present in lesions, such therapy may also alter the clinical progression and transmission in outbreaks of Contagious Ecthyma.

Keywords: Sheep, orf virus, topical anaesthetic formulation, viral load.

SR-22

Clinico-serological and molecular study of *peste des petits ruminants* in sheep and goats in Al Muthanna province/ Iraq

Karima Al Salihi.

Al Muthanna university, Samawah, Iraq.

Objectives: This study intends to investigate the *peste des petits ruminants* virus (PPRV) infection in sheep and goat using Clinico-serological and molecular tools in Al Muthanna province/ Iraq.

Materials and Methods: In October 2017 outbreak was occurred in sheep and goats in Al Muthanna province/ Iraq. There were obvious deaths both in young and adult sheep and goats during the period extended from October/ 2017 to March 2018. According to the case history, clinical symptoms, and post-mortem investigations, the PPR virus was accused. Blood samples were collected from the jugular vein of totally 75 animals of the infected flocks (50 sick and 25 healthy animals) using sterile vacutainer tubes (without anticoagulant), and the serum was extracted after centrifugation and kept at -20 ^{O}C deep freeze in the laboratory until the test is conducted. Competitive Enzyme-Linked Immunosorbent Assay (C-ELISA) was down to detect the presence of antibodies against PPRV. Mouth swabs (epithelial tissue from the lesions) from sick animals were collected, kept in a frozen box, and sent to the laboratory. RNA was extracted from all samples according to the instructions using the RNeasy_ Mini Kit (Qiagen, Germany). The extracted RNA was kept at (- 120 ^{O} C) until amplification procedures. Later on, the RNA samples were used for cDNA synthesis, and specific two sets of primers were used for the detection of PPRV fusion (F) gene and nucleocapsid (N) gene.

Results: Typical clinical signs of PPRV have obliviously appeared on the sick animals including fever, loss of appetite, marked depression, erosive stomatitis, ocular catarrhal inflammation, profuse diarrhea. Some cases revealed fetid& blood-stained and respiratory signs that included nasal mucous discharge, different stages of bronchopneumonia and mouth breathing and erosions of the nasal cavity. The post-mortem examination of few numbers of dead animals showed congested trachea, red lung hepatization with area firm to touch, particularly in the cardiac and anterior lobes and enlargement of pulmonary and mesenteric lymph nodes accompanied with congestion of small intestinal mucosa. The serological test shows positive results in 90 % (45 out 50) and 48 % (12 out of 25) of sick and healthy animals respectively. The results of molecular analysis revealed 10 positive samples for PPRV, and 7 F genes (PPRV/ Al Muthanna / 2017) were genetically close to the (PPR \ Kurdistan 2012), (KF478924) and (JF 274480) strain previously determined in Kurdistan/ Iraq, Turkey and Egypt respectively with homogeneity reached to 95% nucleotide sequence. Moreover, three samples were genetically identical for the N gene of %100 (PPR \ Kurdistan 2012), %99 (DQ840197), %99 (FJ 795511), and %97 (DQ 840190) strain from Kurdistan/ Iraq, Saudi Arabia, Emirate and Israel respectively.

Conclusions: In conclusion, PPRV was diagnosed in small ruminants in Al Muthanna province, and the diagnosis was done according to clinical sings & post-mortem examination and confirmed by serological C-ELISA test and RT-PCR molecular technique. Moreover, the results of the molecular technique revealed the close genetic relationship between local PPRV strain and strains isolated previously from neighboring countries. Another molecular epidemiological study is recommended by the author to determine the origin of PPRV in Iraq.

Keywords: Peste des petits, Sheep, Molecular , Iraq.

SR-23

Use of thermography for the diagnosis of chronic proliferative rhinitis in sheep and for the differential diagnosis of the first case affecting the dorsal tubinate

Aude Levacher, Carmen Ceresuela, Silvia López-Tamayo, Juan Pedro Bueso, Oiane Alzulguren, Ana Rodríguez, Luis Figueras, Luis Miguel Ferrer.

Facultad de Veterinaria de Zaragoza, Zaragoza, Spain.

Introduction: Chronic proliferative rhinitis (CPR) is an upper respiratory tract disease that affects sheep and is associated with Salmonella enterica subsp. diarizonae serotype 61:k:1:5:(7). It may be unilateral or bilateral, and, although minor changes have been seen in dorsal turbinate, the ventral nasal turbinate is always affected, which makes it possible to observe the proliferative tissue emerging from the nostril.

In the following case it is described the use of a thermal

imaging camera in the diagnosis of an atypical case of chronic proliferative rhinitis in sheep. Contrary to what is normally observed, this time the dorsal turbinate and ethmoidal area where mainly affected.

Materials and methods: The Small Ruminant Clinical Service of the Veterinary Faculty of Zaragoza, Spain, (SCRUM) received, in 2018, a Lacaune ewe with severe upper respiratory tract clinical signs. The 3-year-old female presented a very low body condition, despite no loss of appetite, and extreme weakness. The ewe also showed bilateral seromucous nasal discharge, an upper airway snoring and inspiratory dyspnea. Moreover, a nasal deformation in the middle area of the nose was clearly present.

In order to obtain more information, further complementary imaging techniques such as thermography, x rays and computed tomography scanner (CT) were carried out.

Results: Thermography revealed an increase in the temperature of both nasal cavities, corresponding to the difficulty of the cold air passing through, and a hottest area in the middle part of the nose, which corresponded with the area of the swollen dorsal turbinate. Thereafter, CT of the cranial region enabled us to obtain a clear image of the damaged tissue, showing the increase in size of swollen dorsal turbinate and the partially affected ethmoidal area. Along with these, two lateral radiographic projections were made, one on each side of the affected area, and an increased opacity was observed inside the dorsal nose chamber. All this images were compatible with chronic proliferative rhinitis although affecting, for the first time in such a strong extent, the dorsal nasal turbinate and the ethmoidal area.

After the death of the animal, a post-mortem examination was performed. When sectioning the head sagittally, an inflammation of the ventral and dorsal nasal turbinates of both nasal cavities were observed. When the dorsal turbinate from the right side was removed, different polypoid formations could be observed.

Histopathological study of the swollen turbinates revealed a thickened nasal mucosa and an epithelium composed of several layers of disorganized epithelial cells. All the tissue presented a chronic inflammation. The lamina propia was expanded and densely infiltrated by plasma cells and a few macrophages. The intracellular presence of the bacteria in the epithelium and the lamina propia was also confirmed by immunohistochemistry of the affected tissue.

Both the microbiological (culture) and molecular (qPCR) study of the samples taken from the affected tissue of both turbinates revealed a massive presence of Salmonella enterica subsp. diarizonae, which was typified as serovar 61:k:1,5,(7).

Discussion/conclusions (literature; acknoledgments): Chronic proliferative rhinitis is becoming a more commonly diagnosed disease, not only in Spain, but also in other countries in Europe and America. To date, in all the descriptions made of this disease the ventral turbinate was always affected unilaterally or bilaterally and only minor changes were observed in dorsal turbinate or ethmoidal area. In the case here reported, a detailed description is made of the condition of the dorsal and partially ethmoidal turbinate, showing how this bacterium can deepen the respiratory tract, affecting more seriously internal structures.

The use of thermography, a cheap and handle diagnostic imaging technique, can be very helpful to locate the affected area, thereby facilitating clinical diagnosis of upper respiratory tract diseases of sheep, especially at the beginning of the process.

Keywords: Chronic, proliferative, rhinitis, thermography, dorsal.

SR-24

Vaccination against ovine footrot in lactating ewes has only a transient effect on milk production

Gaëlle Porcheron[1], Frédéric Leboeuf[2], Raphaël Jaquet[3], Guillaume Vautrain[4], Julien Visse[4], Geert Vertenten[5].

[1]CEBIPHAR, Fondettes, France; [2]MSD Santé Animal, Beaucouzé, France; [3]Veterinary Practitioner, Andouillé, France; [4]Clinique Vétérinaire Velvet, Lacaune, France; [5]MSD Animal Health, Boxmeer, Netherlands.

Objectives: A multivalent inactivated vaccine against ovine footrot (Footvax®, MSD Animal Health) is indicated for the active immunization of sheep against footrot caused by *Dichelobacter nodosus*. The need of a non-restrictive claim on lactating ewes becomes obvious to reduce the use of antibiotics, to avoid milk discard in relationship with milk antibiotic residues and to avoid footrot related discomfort.

The aim of the study was to assess the immediate impact on milk production of vaccination against footrot in lactating ewes.

Materials & Methods: Forty-four lactating ewes were included and randomised (milk production) in one farm with no history of footrot. Twenty-two ewes were vaccinated subcutaneously with the inactivated multivalent footrot vaccine (Footvax®, MSD Animal Health) on D0 and D28 and twenty-two received a saline solution (negative control or NaCl group) on the same days. During the period following the vaccine injections, the milk production was individually measured daily for 42 days after the first injection.

Results: First administration of the vaccine (D0):

Cumulative milk yield by ewe (L/Ewe) was 2.9% lower in the vaccinated group (38.6 ± 4.9 L and 39.8 ± 6.1 L respectively) from 0 to 14 days after vaccination. Moreover, both groups showed similar time curve of mean milk yield during the first 2 days after product administration. After that, the average daily milk yield in the vaccinated group was slightly below the average daily milk yield in the NaCl group until 12 days after vaccination when milk production was recovered in the vaccinated group compared to the NaCl group.

Second administration of the vaccine (D28):

Time curve of mean milk yield per ewe in the vaccinated group decreased sharply the day after product administration until D31 (i.e. 3 days after vaccination) compared to the curve in the NaCl group. There was a significant decrease in milk yield of 8.8% in the vaccinated group compared to the NaCl group from D28 till D42. The milk yield in the vaccinated group

increased to the same yield of the NaCl group from D32 to D39, demonstrating once again that vaccination led only to transient milk reduction and was fully recovered 11 days after vaccination.

The second vaccination can be considered as a worst-case safety scenario which will in veterinary field practice not that often occur in lactating ewes, because the basic repeated vaccination schedule is normally given to lambs at a younger age.

Conclusion: Vaccination against ovine footrot (Footvax®, MSD Animal Health) in lactating ewes led to a transient and slight decrease in milk production of 2.9 %, which recovered around 11 days after the first administration. From this, it is clear that the milk loss due to vaccination is very moderate compared to potential footrot related negative consequences such as economic impact, extra antimicrobial use and extra labour.

Keywords: Ovine footrot, vaccination, lactation, transient effect, anti microbial reduction.

SR-25

The impact of veterinarian-farmer engagement on sheep lameness: a clinical impressions study

Alexandra Ashworth, Rebecca Cole, Hannah Mckerrow, Bridget Girvan, Liz Cresswell.

MSD Animal Health, Milton Keynes, United Kingdom.

Objectives: The objective of the study was to encourage vets and farmers to engage with reducing sheep lameness in UK flocks and to increase implementation of the 'Five Point Plan' (developed by Food Animal Initiative farms in 2014 and adopted as the UK national strategy for tackling lameness in sheep).

Materials & Methods: Between January and June 2020 veterinary practices across the UK were approached by convenience sampling to assess interest in participation in a clinical impressions study. Veterinarians were required to carry out a visit to each enrolled farm to work through the Five Point Plan and identify measures which could be taken to reduce lameness on an individual flock basis. A veterinary assessment of lameness prevalence was undertaken and where possible, farm medicine and treatment records consulted to establish antibiotic usage. Individual flock-level data were retained by the veterinarian to maintain confidentiality. The participating farms were provided with a primary course of *Dichelobacter nodosus* vaccine (Footvax®, MSD Animal Health) to be administered to each animal in the flock. A subsequent visit was carried out by the veterinarian 6-9 months later to reassess lameness, and medicine and treatment records. An anonymised questionnaire was distributed to all participants to gain feedback on farmer perceptions of the impact of the study on lameness in their flocks.

Results: Twenty-seven veterinary practices were recruited. Veterinarians from these practices enrolled a total of 70 farms. Average flock size was 630 with a range from 60 to 2300 breeding ewes. In total 44,000 sheep were vaccinated. During the 6-9 months of the study, this targeted approach more than halved average flock lameness from 13% (range 3-40%) to 5% (range 1-15%). This is compared to a national reduction in lameness prevalence over 17 years from 11% (2004) to 3.2% (2021). Among the participating farmers, 33% were previously unaware of the Five Point Plan, while 54% considered there were difficulties in implementing parts of the Five Point Plan on their farm.

The majority of participating farmers (97%) said they found the project and subsequent veterinary engagement useful and 95% said they would continue to implement the Five Point Plan. Moreover, 82% of farmers stated that they would continue to vaccinate their sheep against footrot.

Conclusion: Significant gains can be made in a short period of time with concentrated focus between vet and farmer on lameness in sheep flocks. The Five Point Plan has been demonstrated to be a highly effective way in which to reduce lameness and this study demonstrates that farmers value veterinary engagement in this area. It highlights a simple, significant opportunity for veterinarian-farmer engagement to increase the use of the Five Point Plan and reduce lameness on sheep farms.

Keywords: Sheep, lameness, footrot, vaccination.

SR-29

Cost-benefit analysis of ultrasonographic pregnancy diagnosis in dairy ewes in Greece: a simulation study.

Georgios E. Valergakis, Panagiota Kazana, Sofia-Afroditi Termatzidou, Aikaterini Soufleri, Nektarios Siachos, Georgios Arsenos, Alexandros Kougioumtzis.

Laboratory of Animal Husbandry, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece, Thessaloniki, Greece.

Objectives: Ultrasonography is widely used for pregnancy diagnosis in sheep; however, in order to be cost-effective, pre-lambing feeding strategies must be accordingly adapted. The objective of this study was to perform a cost-benefit analysis of ultrasonographic pregnancy diagnosis (UPD) in dairy ewes in Greece, based on different fertility outcomes and feeding management strategies.

Materials and Methods: This simulation study was based on available local milk recording schemes' data. For 100 ewes entering the milking period and after accounting for barren ones, abortion during the last 2.5 months of gestation, losses at lambing and ewe culling at the end of the lamb-rearing period due to mastitis and low milk production, a flock size of 120 ewes at the beginning of the breeding period was considered. The 2.5-month breeding period was divided in 4 successive conception sub-periods (CSP), each lasting 17 days. Fertility outcomes included 2 levels of barren ewes (5% and 10%) and 3 conception patterns in respective CSPs: a) early (E: 82.5%-17.5%-0.0%-0.0%), medium (M: 51.0%-41.0%-8.0%-0.0%) and delayed (D: 25.0%-35.0%-28.0%-12.0%). For the simula-

tion, UPD was performed twice, 45 days after the start of the breeding season and 30 days after its end; pregnancy status and number of fetuses would therefore be established for all ewes. At the first UPD visit, all ewes would be examined while at the second one, only those previously found empty or dubious (10% of positives). Each UPD visit was charged 20€ plus 0.75€ per ewe examined, resulting in total expenses of 140€, 155€ and 180€, for the 3 conception patterns, respectively. Barren ewes were removed from the simulated flocks immediately after the second UPD visit and no feed was allocated to them thereafter, nor any expense on preventive health management (vaccinations, treatments for parasites etc; 1.5€ per ewe) was considered. Pregnant ewes of all fertility outcomes were virtually allocated in either 2 or 3 feeding groups. When allocated in 2 groups, and for the 3 conception patterns, these consisted of: a) E, ewes conceiving at the first and second CSP, b) M, ewes conceiving at the first and second+third CSP and c) D, ewes conceiving at the fisrt+second and third+fourth CSP. When allocated in 3 groups: a) E, ewes conceiving at the first CSP bearing a single lamb, those conceiving at the first CSP bearing twin lambs and those conceiving at the second CSP, b) M, ewes conceiving at the first, second and third CSP, and c) D, ewes conceiving at the fisrt+second, third and fourth CSP. The feeding plan included a maintenance period (starting from the second UPD until 6 weeks before presumed lambing), and a 3-period step-up pre-lambing program each lasting 2 weeks as proposed by INRA-2019. Rations were formulated according to INRA-2019 recommendations using alfalfa hay, wheat straw, corn grain, wheat bran, soybean meal and a mineral/vitamin supplement. Cost of maintenance, step-1, step-2 and step-3 rations (for ewes carrying twin lambs) were 0.24, 0.29, 0.36 and 0.44€, per ewe per day, respectively. For conception pattern E, cost for single-lamb carrying ewes were 0.29, 0.33 and 0.39€, for step-1, step-2 and step-3 rations, respectively.

When combining CSPs, feeding of step-3 ration started with the earliest lambing ewes and lasted until the end of this sub-group's lambing period. All the above fertility scenarios were compared to identical ones with no UPD and no culling of barren ewes; the start of the 3-period step-up feeding program for all ewes was based on the starting date of the breeding period and the step-3 ration was fed to all ewes until they lambed. Costs were calculated accordingly. Results are reported as differences per 100 ewes between UPD and no pregnancy diagnosis scenarios.

Results: Net benefit of UPD for the E pattern with 2 feeding management groups was 200€ and 480€, for 5% and 10% barren ewes, respectively. Forming 3 groups would increase benefit to 240€ and 530€, respectively. For conception pattern M, net benefit was 300€/580€ and 330€/610€, respectively. For conception pattern D, net benefit was 240€/460€ and 510€/760€, respectively.

Conclusion: Ultrasonographic pregnancy diagnosis is cost-effective when results are used to adapt grouping and feeding management. When conception is delayed, the formation of 3 feeding groups is highly recommended.

Keywords: Ultrasonography, pregnancy diagnosis, sheep.

SR-30

Surgical treatment of abomasal impaction in goat

Ana Paula Abreu Mendonça, Leticia Hirata Mendes, José Ricardo Barboza Silva, Nathalia Cardoso De Sousa, Lucas Vinícius De Oliveira Ferreira, Juliana De Moura Alonso, Carlos Alberto Hussni, Ana Liz Garcia Alves, Marcos Jun Watanabe, Celso Antonio Rodrigues.

School of Veterinary Medicine and Animal Science, São Paulo State University - FMVZ - UNESP, Botucatu, São Paulo, Brazil, Brazil.

Information about disorders of abomasum in goats are scarce and the aim of this report is to describe the success of abomasotomy as a treatment of abomasal impaction in a goat. An 8-month-old goat weighing 33kg was referred to the School of Veterinary Medicine and Animal Science - UNESP, Brazil with an apathy and decreased appetite four days ago. According to clinical history, the diet consisted of crushed hay *ad libitum* and 300g of ration bran. Clinical examination revealed apathy, pale mucous membranes, ruminal hypomotility, reduced faecal output, tense abdomen with distension of the ventral quadrants and loss of ruminal stratification. Increased chloride ion concentration (67 mEq/L) evidenced by rumen fluid analysis was indicative of abomasum-ruminal reflux and as well as compatible with the observed metabolic acidosis. Transabdominal ultrasonography revealed dense hyperechoic content in ventral field of the left abdomen corresponding to the location of all abomasum, thus suggest an abomasal impaction. The animal underwent exploratory laparotomy by the left flank, which revealed an empty rumen, distended and compacted abomasum occupying much of the abdominal cavity. In contrast to the treatments described, by severe distension of abomasum, we opted for an abomasotomy followed by emptying of the viscera, removing about 7 kg of the digest. In addition to constant monitoring, antimicrobial medication with ceftiofur (5mg/kg; IV once daily; six days) associated with gentamicin (6.6mg/kg; IV; once daily; six days) and analgesic with flunixin meglumine (2.2mg/kg; IV; once daily; four days) was performed postoperatively vitamin B1 (2mL; SC; once daily; five days), dexamethasone (0.2mg/kg; IV; once daily; two days), transfaunation and calcium replacement for six days. Furthermore, was performed the prokinetic to stimulate abomasal emptying like bromopride (20mg; IM; three times a day; three days), metoclopramide (0.2mg/kg; SC; three times a day; two days), and promethazine (0.5mg/kg; IM; twice daily; four days). Although the goat defecated normally on the second postoperative day, abomasal emptying remained reduced to auscultation. Thus, with clinical suspicion of posterior functional stenosis, the atropine test and radiographic examination of the thorax and abdomen were performed, and they revealed no changes. After 14 days after surgery, the goat no longer presented abomasal motility changes, being discharged with recommendations to provide good quality food and water. The abomasotomy associated with viscera emptying and supportive therapy resulted in the successful treatment of a goat affected by abomasal impaction in the present study.

Keywords: Abomasotomy, abomasal disorder, small ruminant.

SR-31

Obstructive rhinopathy associated with chronic pithomycotoxicosis in sheep

Marina Pomar[1], Clara Aguado[1], Helena Bertran[1], Laia Del Olmo[1], Pablo Quílez[1], Sixena Villellas[1], Ane Rivas[2], Ane Garziandia[3], Raúl A. Reséndiz[1], Enrique Castells[4], Vicente González[5], Marcelo De Las Heras[1].

[1]Facultad de Veterinaria Universidad de Zaragoza, Zaragoza, Spain; [2]Veterinary Practitioner, Basque Country, Spain; [3]Gomiztegi koop, Basque Country, Spain; [4]Centro Clínico Veterinario, Zaragoza, Spain; [5]Centro de Investigación y Tecnología Agroalimentaria de Aragón, Zaragoza, Spain.

Objective: Two sheep were received at the Clinical Service of Ruminants of the University of Zaragoza (SCRUM) with respiratory and dermatologic clinical signs. A complete clinical examination was performed, emphasising the respiratory tract and skin lesions to arrive at a final diagnosis.

The practitioners reported that most of the flock animals presented upper tract respiratory clinical signs.

The objective of this work is the clinical and aetiological study of a case characterised by an inspiratory dyspnea, which affected a herd kept in an extensive regime, located in the north of Spain.

Materials and methods: The sheep came from a dairy farm located in Anzuela, Basque Country, and were reared in an extensive production system, grazing most of the year, except the winter months.

After their arrival at the SCRUM, the protocol was as follows: clinical examination, highlighting in detail the signs or lesions presented by the animal, haematology, biochemistry, computed tomography and postmortem examination.

Result: The animals showed inspiratory dyspnea with a characteristic respiratory noise, had bilateral serous nasal discharge and an enlarged submandibular lymph nodes. In one of the animals, different skin lesions with alopecias and scabs in the dorsal area of the face, eyelids and ears were noticed. Blood biochemistry showed an elevation in gamma-glutamyl transferase, alanine aminotransferase, and lipase.

The computerised tomography scan revealed an increase of the soft tissue on the rostral part with variable degrees of obstruction of the meatuses of the nasal cavities in both sheep. Both nasal cavities were generally blocked with different degrees of severity. Intravenous injection of contrast did not result in the differentiation enhancement.

Necropsy detected intense liver atrophy and fibrosis associated with chronic pithomycotoxicosis in the studied sheep. It confirmed slight elevations and roughness on the alar folds and close nasal areas in all sheep. Histopathology of the nasal lesions using histochemical and immunohistochemical techniques to study connective tissue, identified moderate to severe arteriosclerosis in the small arteries investigated with fibrosis and oedema. These lesions were similar to the ones described in blood vessels of the liver in chronic pithomycotoxicosis and our cases. The results of this study suggest a direct action of the sporidesmin on this area of the nasal cavity.

Conclusions: The clinical and postmortem findings suggest that both lesions, respiratory and dermatologic, were caused by the sporidesmin produced by the fungus *Pithomyces chartarum*. Pithomycotoxicosis (facial eczema) has been well studied as a seasonal hepatogenous photosensitisation of sheep caused by the ingestion of sporidesmin contained in the spores of the fungus *Pithomyces chartarum*. However, to the knowledge of the authors, respiratory clinical signs have not been reported to date associated with this disease.

Keywords: Sheep, pithomycotoxicosis, facial eczema.

SR-32

The Effects of Oxytocin and PGF2α Injections on Semen Quality and Libido in Buck

Cigdem Cebi Sen[1], Koray Tekin[2], Beste Cil[2], Ergun Akcay[2].

[1]Dep. of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine University of Harran, Şanliurfa, Turkey, Sanliurfa, Turkey; [2]Dep. of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine University of Ankara, Ankara, Turkey.

Objectives: The use of assisted reproductive techniques in goat breeding provides additional advantages for cryopreservation and artificial insemination. In some cases, certain animals constantly have a high libido and good semen quality for evaluation, freezing or insemination, while others are reluctant for collection and have low quality ejaculates with decreased volume and concentration or other inadequate spermatological characteristics. During the last decades, specific hormones (oxytocin, prostaglandins, testosterone and GnRH) were introduced to increase the sperm output, quality of male-related reproductive deficiencies and to regulate the breeding activity. The aim of the present study was to evaluate the effects of exogenous oxytocin and PGF2α on seminal quality and libido sexualis in bucks.

Materials and methods: This study was conducted according to ethical laws and regulation of Ankara university animal experiments local ethics committee. 20 Norduz bucks (3-4 years of age) were barned at Research Farm of Ankara University, Faculty of Veterinary Medicine (40°05'53.5"N 32°37'19.6"E). The bucks were maintained under the constant nutritional regime and with water ad libitum. To investigate the role of these hormones on male fertility, semen samples from 20 Norduz bucks (3-4 years of age) were collected with an artificial vagina twice a week with five replications in breeding season. Bucks were randomly assigned to five groups, control group was administered with 2 mL of sodium chloride, 0.9% (w/v)) i.m., whilst the experimental groups were administered with oxytocin 10 IU, i.v. (Group 1, n = 5), oxytocin 20 IU, i.v. (Group 2, n = 5), PGF2α 5 mg, i.m. (Group 3, n = 5) or PGF2α 10 mg, i.m. (Group 4, n = 5) 20 min before each collection. The behavioural signs of libido (leg kicking, sniffing, vocalization, flehmen reaction, mounting without thrust and mounting with ejaculation) were recorded as the total score for libido. Semen volume, total sperm motility, sperm concentration, sperm morphology, membrane integrity and pH were recorded.

Results: According to obtained results, there was not any

statistical difference between the dosage groups regarding motility. However, in terms of semen volume, concentration, abnormal spermatozoa rate, intact membrane rate, and libido test, results were statistically significant among the different dose groups (P<0.05). Average semen volume of Group two (1.34±0.16 mL) was found significantly higher than other groups, whereas mean values of PGF2α groups (Group-3 and Group-4) were found lower than control value (P<0.05). When the concentration was taken into account, Group two has statistically higher mean value than PGF2α groups. For abnormal spermatozoa rate, a significant increase in Group one was observed regarding other groups. A significant decrease of mean intact membrane percentages was evident at Group four comparing to group one. A significant increase in libido evaluation scores for oxytocin groups (3.04±0.64; 3.2±0.5) was observed The bucks, which were administered PGF2α, were reluctant for mating and showed a decrease in libido. Thus, it might have enhanced hyperthermic and psychological stress in animals. Therefore, the duration of ejaculation was longer in PGF2α groups than the other groups.

Conclusions: In conclusion, administration of 20 IU oxytocin twenty min before the semen collection have increased both the semen volume and the concentration of spermatozoa in bucks. Administration of oxytocin has increased the libido although there were not any improvements in semen quality. In contrast to oxytocin, PGF2α administration has led to a decrease of libido and has detrimental effects on semen quality. We concluded that administration of oxytocin stimulates sexual behaviour and performance in bucks.

Keywords: Buck, Libido, Oxytocin, PGF2α, Semen quality.

SR-33

Effects of hydrogenated fat-embedded calcium gluconate on performance and intestinal parameters in growing lambs

M.V. Sanz-Fernández[1], I.P.C. De Carvalho[1], D.J. Seymour[1], J.M. Bello Dronda[2], J. Doelman[1], J. Martín-Tereso[1].

[1]Trouw Nutrition, Amersfoort, Netherlands; [2]Nanta, Tres Cantos, Spain.

Introduction: Upon fermentation in the hindgut, the prebiotic gluconic acid and its salts have shown benefits on growth in monogastric species, attributed to changes in fermentation patterns and volatile fatty acid (VFA) production.

Objectives: The objective of the current study was to evaluate the effects of rumen-protected hydrogenated fat-embedded calcium gluconate (HFCG) on performance and intestinal parameters in a model of growing ruminants.

Materials and methods: A total of 300 male Rasa Aragonesa feedlot lambs were enrolled in a dose response study divided in 2 identical rounds. Within round, animals were blocked by body weight at arrival (20.1 ± 2.0 Kg) and randomly assigned to pens corresponding to 1 of 5 HFCG supplementation doses (0, 0.4, 0.75, 1.5, or 3 g/lamb/d). After 20 d of supplementation, lambs were slaughtered at a commercial abattoir and cecum digesta was collected from 2 representative lambs per pen. Data was analyzed using the MIXED procedure of SAS Studio (v3.81) with pen as the experimental unit, dose as fixed effect and block as random effect. Orthogonal contrasts were included to test for linear and quadratic trends, and control (dose 0) vs. HFCG (rest of the doses).

Results: There were no differences on final body weight or average daily gain. Cecum digesta pH, dry matter content, and ammonia concentration were not different among treatments. Butyrate and total VFAs molar concentrations increased (22 and 10%, respectively; $P \leq 0.15$) in HFCG-supplemented lambs compared to controls. However, no differences in VFA concentrations were detected when normalized to dry matter content.

Conclusions: Overall, these results show signs of altered hindgut fermentation in response to HFCG supplementation. We hypothesize that the application period was too short to adequately detect differences in response to supplementation, as it has been previously demonstrated that relatively long treatments (˷56d) are necessary to influence hindgut ecology and improve performance in beef and dairy cattle.

Keywords: Gluconate, hindgut, ruminants.

SU-01

Lateral Approach to Subtotal Bulla Osteotomy and Horizontal Ear Canal Ablation to ameliorate head tilt due to Chronic Otitis Media attributed to *Mycoplasma bovis* infection

Mike Kerby.

Delaware Veterinary Group, Castle Cary, Somerset, United Kingdom.

Objective: Otitis media caused by *Mycoplasma bovis* infection is well recognised in young calves in the UK and most of the World (Foster *et al*., 2009) ,usually resulting in head tilt which may not respond to antimicrobial therapy with or without Non-steroidal anti-inflammatory drugs (NSAID's).This paper describes a surgical approach to attempt amelioration of the degree of head tilt in a pedigree Guernsey heifer and is believed to be the first such recorded in the UK.

Materials and Method: A 5 month-old Guernsey heifer with head tilt of 3 months' duration was referred in early February 2015 having been diagnosed with otitis media in November 2014 when seroconversion to *Mycoplasma bovis* was reportedly demonstrated by the referring veterinarian and having undergone various antimicrobial treatments with and without NSAID's.

On initial examination, the degree of head tilt to the horizontal was approximately 75 degrees to the right. Otoscopy revealed a ruptured tympanic membrane.Radiographs showed an increased density within the right tympanic bulla but no abnormalities in the cervical vertebrae.

A lateral approach to subtotal bulla osteotomy (adapted from a technique described in dogs by Smeak and Inpanbutr,2005) along with horizontal ear canal ablation was performed under general anaesthesia.

Induction was with Xylazine (0.2 mg/kg Rompun, Bayer plc) and Ketamine (0 mg/kg. Narketan, Vetoquinol UK Ltd. followed by intubation and maintenance with Oxygen and Isoflurane (Merial Animal Health Ltd.).The right auriculo-palpebral nerve was anaesthetised pre-operatively with Procaine Hydrochloride (Adrenacaine, Norbrook) . Flunixin meglumine was given intravenously (2.2 mg/kg, Cronyxin , Bimeda).

The horizontal ear canal cartilage was identified by palpation and a 15 cm incision made in a dorso-ventral direction caudal to the base of the ear.This cartilaginous ear canal was dissected out - care was taken to avoid the Facial Nerve emerging from the Stylomastoid Foramen located ventral to the mastoid process sited at 11 o'clock relative to the osseous ear canal (the stylomastoid foramen is separated from the External Auditory Meatus (EAM) by a distinct,readily palpable, sharp bony ridge along the caudal edge of the EAM).

Blunt dissection of the soft tissue was performed from the lateral and ventral aspects of the Tympanic Bulla with elevators.The ventro-lateral floor of the osseous EAM was then removed using rongeurs; the soft tissue lining of the EAM was teased from the osseous EAM compartment ; bone removal was continued ventrally into the ventral tympanic cavity.

The area where the Facial Nerve runs was protected and the palpable sharp bony ridge removed at the caudal edge of the osseous ear canal followed by continued bone removal on the caudolateral aspect of the Tympanic Bulla.The interior of the Tympanic Cavity was then visualised with a dark green-brown coloured membranous lining (it is transparent in the normal state).

Curettage of the thickened diseased membranous lining of the tympanic bulla was performed (particularly difficult off the medial wall) and an in-dwelling fenestrated cannula and Penrose drain inserted within the Tympanic Cavity, the former exiting through the skin rostro-dorsal to the incision site at the ear pinna level and the latter exiting ventral to the incision site .

Post-operative treatment comprised twice daily 1% povidone-iodine solution flushing for 4 days, 6 days NSAID's[2.2 mg/kg Flunixin meglumine daily for 3 days(Cronyxin, Bimeda) then 0.5 mg/kg Meloxicam (Metacam, Boehringer Ingelheim Ltd.) on day4 after surgery] and 10 days antimicrobial cover [8.75 mg/kg amoxicillin/clavulanic acid (Synulox RTU, Zoetis UK Ltd.)].

The irrigation cannula was removed on day 5 post-operatively and the Penrose drain on day 7 (when no further fluid was draining).

Results: On the day of discharge (Day 13 post surgery) the degree of head tilt had reduced to approx. 25 degrees and 6 weeks post-operatively was 15 degrees (reported by the referring veterinarian) and 10 degrees by August 2015.

Conclusion: This procedure can be considered as a viable option to correct major head tilt deviation in cases due to chronic otitis media.

References:

Foster,A.P.,Naylor, R.D.,Howie, N.M., Nicholas,R.A.,Ayling,R.D. (2009) *Mycoplasma bovis* and otitis in dairy calves in the UK *Vet.J.* **179** (3):455-457.

Smeak D.D. and Inpanbutr N. (2005) Lateral Approach to Subtotal Bulla Osteotomy in Dogs. *Compendium* pp 377-384.

Keywords: Head tilt correction, Mycoplasma bovis.

SU-02

Association between hyperglycemia and survival in adult cattle affected by acute gastro-intestinal disorders

Isabella Nicola, André Desrochers, Sylvain Nichols, David Francoz, Marie Babkine, Julie Berman, Gilles Fecteau.

Université de Montréal, Saint-Hyacinthe, Canada.

Objective: To evaluate the association between glycemia and short-term outcome in adult cattle with acute gastro-intestinal disorders (AGID).

Material and methods: We reviewed medical record of adult dairy and beef cattle (> 24 months), presented to the Veterinary Teaching Hospital of the Université de Montréal between January 1st, 2015 and December 31st, 2019 and diagnosed with an AGID. The type and diagnosis of the AGID (strangulating, non-strangulating and haemorrhagic bowel syndrome (HBS)) were established based on the clinical examination, the abdominal ultrasonography findings, the sur-

gery and/or the necropsy findings. All cattle with a serum biochemistry profile, including glycemia, performed at admission were included. Exclusion criteria were: cattle treated with dextrose, propylene glycol or dexamethasone prior to admission.

Glycemia, heart rate, packed cell volume, urea, creatinine, total proteins, lactates and category of lesion (strangulating, non-strangulating, HBS) were evaluated as possible predictors for short-term outcome (discharge or not from the hospital) in a logistic regression model. The association between category of lesions and clinical as well as laboratory findings was evaluated with a general linear model followed by Tukey's post-hoc tests. *P* was set at 0.05.

Results: Overall, 197 records were evaluated and 112 were selected. Median (interquartile range (IQR)) values of glycemia for animals with positive (79%) and negative outcome (21%) were 6.7 mmol/L (IQR: 5.3-9.3) and 8.15 mmol/L (IQR: 6.6-10.7), respectively. Median values of glycemia for animal with strangulating (26%), non-strangulating (59%) and HBS lesions (15%) were 7.4 mmol/L (IQR: 5.8-9.3); 6.65 mmol-/l (IQR: 5.3-9.3) and 8.8 mmol/L (IQR: 7.3-10.7), respectively. Glycemia was associated with non-survival in the univariable analysis (OR 1.16; 95% CI: 1.035, 1.30, $p < 0.05$), but not in the multivariable analysis. In the multivariable analysis, only decreased total proteins (OR 0.94; 95% CI: 0.89, 0.99), increased creatinine (OR 1.01; 95%CI: 1.001; 1.017) and category of lesions (HBS OR 1.56 compared to strangulating lesions 95% CI: 1.12, 19.96) were associated with non-survival ($p < 0.05$). Interestingly, urea and glycemia were significantly higher with HBS compared to non-strangulating lesions ($p < 0.05$). Urea was significantly higher with HBS compared with strangulating lesions ($p < 0.05$).

Conclusion: Glycemia is an easily obtainable parameter in field setting. It was not a predictor for short-term survival in our study. However, glycemia was increased in cases with a negative outcome and specifically for HBS. Further prospective studies are needed in order to elucidate the validity of glycemia as a prognostic tool in different type of acute gastro-intestinal disorders.

Keywords: Hyperglycemia, gastro-intestinal disorders, surgery, haemorrhagic bowel syndrome, cattle.

SU-03

Perineoplastic surgery for the treatment of pneumovagina in dairy cows

Jacek Mrowiec[1], Dawid Król[2], Oskar Pietrasina[3].

[1]Hipra Polska Sp. z o. o., Warszawa, Poland; [2]Wroclaw University of Environmental and Life Sciences, Wrocław, Poland; [3]Ovet, Koźmin Wielkopolski, Poland.

Objectives: The infertility associated with anatomical disorders seems to be greatly underestimated in bovine medicine. The genital tracts injuries and scars are the main source of anatomical and physiological dysfunction often caused by peri-calving disturbances. Pneumovagina is a condition associated with air aspiration into vagina, usually caused by the leakiness of vulva. It could lead to excessive starching of vagina, accumulation of urine and mucosal irritation conducted chronical inflammation of genital track causing infertility. Surgery targeted on restoration of labial closure could be an effective method of pneumovagina treatment.

The aim of this study was to evaluate the efficacy of perineoplastic surgery as a method of treatment of pneumovagina in dairy cows.

Materials and methods: The study was performed between November 2018 to February 2020 in two regions of Poland in 36 family farms on 60 dairy cows with diagnosed pneumovagina. Before every surgery, rectal palpation and ultrasound examination of genital track were performed to exclude other pathologies than pneumovagina. In the presence of pathological discharge together with pneumovagina, intrauterine antibiotic infusion (gentamycine, 20 mg/animal) was performed before operation. Premedication before surgery was performed with xylazine (2 mg / 100 kg of body weight (BW)) and epidural anesthesia with procaine (20 mg / 100 kg BW). The incision was carried out at the border between the skin and mucous membrane of the labia from the dorsal symphysis to the bottom of the designated area. Then mucosa and submucosal tissue were prepared to create the vault limiting new, reduced labial closure. In simply case there was no tissue removal. In more complicated cases, scar tissue was resected and labial structure was reconstructed. Then, the dissected tissues were sutured with three layers of sutures with absorbable PGA monofilament USP 2. The first layer was made with Lambert's or Cushing's suture to connect the vulva mucosa. The second one was joined with a straight or interrupted suture. The skin was connected with an intradermal suture. At least a two-week recovery period was recommended after surgery before insemination.

Results: By the end of February 2020, pregnancy was confirmed in 41 out of 60 operated cows (68 %). In 19 patients (46 % of pregnant cows), the first artificial insemination (AI) after the procedure was successful, in some cases two, three or four AIs were necessary. There was no postoperative complication as extended healing, oedema or abscesses. Among all cows already delivered in 4 cases there was a vulvar rupture during parturition following perineoplastic surgery.

Conclusions: Perineoplastic surgery is an effective method of pneumovagina treatment in dairy cows. It allows to restore labial closure without creating large scar limiting vulvar elasticity.

Keywords: Pneumovagina, surgery, bovine.

SU-04

Teat lesions – Retrospective data evaluation of 116 German Holstein Cows – Case collection of the Clinic for Ruminants and Swine Leipzig

Kaiser Matthias, Starke Alexander.

Clinic for Ruminants and Swine, Leipzig, Germany.

Objective: The management and treatment of teat injuries represent major challenges in modern dairy farming. The objective of this study was to create guidelines for practicing veterinarians for the treatment and management based on the evaluation of a large data set.

Materials and Methods: The data from patients from the Clinic for Ruminants and Swine were analysed in a retrospective study. The location and type of injury, initial and postoperative medication and treatment and outcome were analysed.

Results: The teat canal was the most common location of injuries (70.8%), and closed injuries (without an external wound) were the most frequent type (59.8%). Of all teat canal injuries, 54.2% were internal and did not involve the skin. Most of these injuries were treated under theloscopic guidance directly after initial examination irrespective of concomitant disorders, such as thelitis (except severe acute), cysternitis and mastitis. The majority of treated teats (87.4%) had functional healing and could be milked, 7.5% of injured teats were amputated and the remaining had functional healing after a second surgery. After at least 700 days post-treatment, owners were asked about the outcome. Fifty percent of the nonsurgically treated cows and 21.5% of the surgically treated cows had been slaughtered because of complications associated with the treated teat or the corresponding quarter. High-volume caudal epidural anaesthesia using xylazine provided adequate analgesia during surgery. A tilt table was found to be very convenient and safe for both the cow and the surgeon and useful for restraint of standing cows or cows in lateral recumbency. ***Conclusion and Clinical Relevance:*** Teat injuries are commonly seen in dairy practice. A successful outcome can be obtained when surgical or medical treatment is given promptly and appropriate postoperative therapy and monitoring are carried out.

Keywords: Cattle, Surgery, Udder, Pain management.

TE-01

Use of a serious game in teaching bovine veterinarians clinical communication skills: a pilot study

Linda Dorrestein[1], Jolanda Jansen[2], Tessa Plagis[2], Caroline Ritter[3], Herman Barkema[1].

[1]Department of Production Animal Health, Faculty of Veterinary Medical Sciences, University of Calgary, Calgary, Canada; [2]St Anna Advies, Nijmegen, Netherlands; [3]Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Canada.

Objectives: Effective communication can aid bovine veterinarians in improving client relationships in herd health consultancy, improving client satisfaction, adherence to veterinary advice and patient welfare and health. Communication skills are often taught using traditional role-play training, which has limitations, e.g., time constraints, assessments and cost. The Veterinary DialogueTrainer (VDT) is a new tool mitigating these barriers. It is a serious game platform using 'digital role-play' with avatars, developed to enhance and assess learning outcomes, improve use of learned skills, and increase cost-effectiveness of communication training. The objective of this pilot study was to determine suitability and applicability for using the VDT.

Materials and Methods: Twenty-four Finnish bovine veterinarians participated in a communication training using the VDT for training and assessment. After playing the simulation participants received scores and feedback on selected conversation options. VDT scores measure multiple aspects of communication on a 0-100% scale, based on motivational interviewing methodology and Calgary-Cambridge guidelines. Participants completed the provided simulation at least twice.

Results: Mean (±SD) number of attempts participants played the scenario was 4.1 (±2.6), with highest total score reached after a mean of 3.7 (±2.0) attempts. All participants scored <50% at the first attempt of playing the scenario, whereas most participants (n=22) increased their initial score at the second attempt. Mean score increased from 15% (±14%) to 43% (±29%) (paired T-test, $p < 0.001$) and the majority (n=17) of participants was able to reach a score >80% after 4.0 (±1.6) attempts.

Conclusion: Communication skills scores increased when using the VDT. Whether increased communication skills measured through VDT will improve communication skills in practice is subject of further study. However, based on these results there is likely a benefit of using the VDT in teaching and monitoring veterinary communication competencies and preparing for offline role-plays and real-life conversations in veterinary practice.

Keywords: Dairy cattle, veterinary communication, serious games, herd health management.

TE-02

Veterinary student attitudes towards careers in the production animal sector

Emily Payne, Kate Cobb, John Oldham, John Remnant.

University of Nottingham, Sutton Bonington, United Kingdom.

Objectives: Challenges with recruiting and retaining veterinarians in rural and farm animal practice are widely reported. The aim of this study was to identify the factors influencing veterinary undergraduate students' interest in pursuing a career in production animal veterinary practice.

Materials and methods: An electronic questionnaire was distributed to all veterinary students in the United Kingdom and Republic of Ireland. The questionnaire contained questions collecting demographic data, likert style items, and categorical questions on interest in farm animal careers and factors influencing these decisions. There were also free text questions used to gather qualitative data on these topics. The results were analysed using a mixed methods approach, with inductive thematic analysis used to interpret the qualitative data and descriptive statistics performed on the quantitative data.

Results: There were responses from 1146 veterinary students (representing a response rate of approximately 20%), with responses received from all veterinary schools and all years of study. Free text responses were provided by over 90% of respondents.

The factors most identified as influencing the respondents' desire to work in farm practice or not were their personal interests, and their experiences on extra mural studies (work experience carried out as part of the veterinary course). There were differences between factors listed as positive and negative between those keen to pursue a farm animal career and those that that weren't ($p<0.05$), although in both groups working conditions were the most frequent factor that didn't appeal)

Thematic analysis generated seven major themes relating to decision making regarding farm animal veterinary practice: relationships, preferences and interest, fit, direct experience, fear, ethics and values and lifestyle. These themes will be described and illustrated with quotes.

Conclusions: Veterinary students' decisions whether to pursue a career in farm animal practice are heavily influenced by their own personal interest and their experiences seeing practice. There is a perception that working conditions in farm practice are less favourable than other sectors. This provides an opportunity to correct misconceptions as well as identifying aspects of farm and rural veterinary practice that might need addressing to enhance recruitment of new graduate veterinarians.

Keywords: Education, student, mixed methods, questionnaire, motivation.

TE-03

Ensuring the veterinary profession meets the needs of livestock agriculture

John Remnant.

University of Nottingham, Sutton Bonington, United Kingdom.

Objectives: Recruitment and retention of farm animal and rural veterinarians is a challenge globally. This represents a threat to the welfare and productivity of farmed animals, as well as a threat to the viability of existing farm animal and rural veterinary businesses. The objective of this study was to explore approaches used to address these challenges internationally.

Materials and methods: The author received funding from the Nuffield Farming Scholarships Trust to investigate the "farm vet pipeline". Exploring challenges and solutions before veterinary training (outreach and admissions), during training (training and inspiring), after training (recruitment and retention), and in veterinary businesses (services to farms). Interviews with veterinary students, producers, veterinary academics, and private veterinary practitioners were carried out. Interviews were conducted in The Netherlands, Canada, USA, Australia, and New Zealand. Themes were identified that applied at all stages of the pipeline.

Results: Challenges with recruitment and retention of farm veterinarians were present in all areas visited. Several themes and areas that could be improved applicable at all steps of the pipeline were identified. The themes identified were 1. values, 2. inclusivity, 3. flexible and out-of-hours working, 4. collaboration, 5. herd health, and 6. technology.

Conclusions: Letting farm veterinarians conduct work that aligns with their values will improve retention. Highlighting these values to potential and current veterinary students will increase interest in farm animal careers. Throughout the areas visited, the farm animal sector was not always seen as being welcoming to all demographic groups. Increasing inclusivity at all stages of the pipeline is key to a sustainable profession. The lack of flexible working patterns and need for out-of-hours working are seen as negative downsides of farm and rural veterinary practice. Taking steps to facilitate flexible working and manage the burden of out-of-hours work will improve inclusivity and make the sector more desirable to veterinary students as well as those thinking of leaving the sector. Many of these changes will be easier by improving collaboration and delegation with other members of the animal health team. Herd Health, or population medicine, will also enable these changes – this type of work aligns better with veterinarians values and tends to take place over a more traditional working day.

Keywords: International, recruitment, retention, business model, education.

TE-04

Do students feel well prepared for the digital transformation in dairy farming?

Karina Weimar[1], Wolfgang Heuwieser[2], Michael Iwersen[1], Marc Drillich[1].

[1]Clinical Unit for Herd Health Management in Ruminants, University Clinic for Ruminants, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna,, Vienna, Austria; [2]Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany.

Objective: Digital technologies from milking and feeding robots to animal-attached accelerometers and others are increasingly used on dairy farms worldwide. A wide range of data about the health, behaviour and performance of cows is captured and processed by use of algorithms and artificial intelligence with the aim to gather additional information and improve the management of individual cows and herds. In this study, we used an online survey to collect the opinion and acceptance of the use of sensor technologies on dairy farms among students of veterinary and agricultural (livestock) sciences.

The main objective of the study was to evaluate whether the participants felt well prepared for the digital transformation in dairy farming by the universities.

Material & Methods: The online survey was created with SurveyMonkey (American polling company, San Mateo, USA) and included i) demographic data, ii) questions about the knowledge of the participants of today's dairy farming, iii) assessments of sensor technologies, iv) associations based on image impulses, v) visions and expectations of dairy farming in the future. Finally, the participants were asked whether they felt well prepared for the digital transformation in dairy farming by their universities. Students from eight universities of agricultural sciences and six universities of veterinary medicine from Austria, Germany and Switzerland took part in the survey. The administration or the student body of the universities distributed the link to the survey and a covering letter by email to all students. The survey was open for response for six weeks from November to December 2021. Most of the answers were exported directly from SurveyMonkey to Excel (MS Excel 2016, Microsoft Cooperation, Redmond, USA) and analysed. The question whether the participants felt well prepared for the digital transformation in dairy farming by the universities was an open question. Therefore, the answers had to be classified into five categories before the evaluation. The main focus at this point will be on the categories 1 and 2. Category 1 contains the answers with "yes", the participants feel well prepared for the digital change. Category 2 contains the 'no' answers, the participants do not feel well prepared for the digital change.

Results: A total number of 429 students took part in the online survey. Of these, 296 students (69 %) stated that they were studying veterinary medicine and 133 agricultural sciences (31 %). Overall, 25.9 % of the students felt well prepared for the digital transition during their studies, while 42 % said they were not well prepared. In this context, no significant differences are found between the universities in Austria, Germany,

and Switzerland, but between students of veterinary medicine and agricultural sciences. Whereas 46.6 % of veterinary students did not feel well prepared for the digital transformation, this proportion was 32.3 % in agricultural sciences. Only 20.6 % of veterinary students felt well prepared. This proportion is significantly greater (37.6 %) in agricultural students. In the first and second semesters, more students felt well prepared than in higher semesters.

Conclusions: Digitalisation and digital transformation in livestock farming should be given greater consideration in the education of students in order to better prepare them for their future careers. This applies to both the universities of veterinary medicine and agricultural sciences. More detailed results of the survey will be presented at the conference.

Keywords: Survey, students, digital technologies, dairy farming.

TE-05

Improving dairy cattle veterinarians' communication skills - challenges and opportunities for training in Motivational Interviewing

Catarina Svensson[1], Lars Forsberg[2], Ulf Emanuelson[1], Alison M Bard[3], Kristen K Reyher[3], Hans Wickström[4].

[1]Swedish University of Agricultural Sciences, Uppsala, Sweden; [2]MICLab AB, Stockholm, Sweden; [3]Bristol Veterinary School, University of Bristol, Bristol, United Kingdom; [4]MeetMe Psykologkonsult AB, Gothenburg, Sweden.

The importance of communication skills are increasingly being recognized in the veterinary profession. For dairy cattle veterinarians to adopt a preventative role, a better understanding of and skills in how to facilitate client behavior change are needed. Motivational Interviewing (MI) is an evidence-based and client-centered communication methodology that is increasingly being adopted in human medicine and psychology. MI has been shown to be effective in stimulating client behaviour change such as reducing smoking or alcohol abuse and to adopt life style changes. It may therefore be of relevance in veterinary herd health management (VHHM).

Objectives: This presentation aims to describe results from an evaluation of a 6-month MI training program for dairy cattle veterinarians involved in VHHM and discuss challenges and opportunities in the communication training.

Materials and methods: Communication skills of 38 Swedish dairy cattle veterinarians involved in VHHM (who volunteered to participate in the study) were measured before and after MI training using audio recordings of role-play conversations with professional actors. Each veterinarian participated in three role-plays before and three after the MI training. The role-plays covered scenarios typically encountered within VHHM.

Recordings were coded using the MI Treatment Integrity Code 4.2.1 (MITI), a well-recognized instrument to measure communication skills in MI. The MITI identifies frequency counts of ten verbal behaviours and assessments of four global scores. Besides the word content, tone of voice, loudness and tempo are also evaluated. MITI specifies six summary measurement derived from the original 14 variables. The proportion of veterinarians reaching suggested thresholds of moderate MI skills was calculated.

Veterinarians gathered in groups of four to eight persons at six workshops (in total 36 hrs) spread out over a 6-month period. Between workshops participants were expected to read 30-70 pages from the main MI handbook, to practice their new skills and record conversations. At the end of each workshop, participants were requested to fill in a brief questionnaire about their experiences and the relevance to their work of their newly gained knowledge and skills. The questionnaires comprised of two parts, one using a 6-point Likert scale and one using open-field questions.

Descriptive statistics were calculated using Microsoft Excel. The effect of training on MI skills was evaluated using hierarchical regression models.

Results: In total 31 (82%) of the veterinarians completed the training program and participated in role-plays both before and after training. All veterinarians improved their communication skills in at least one MITI variable and there were significant improvements in all but three of the 16 statistically evaluated MITI variables. After training, veterinarians reduced the amount of information given and were less persuasive, paying more attention to exploring how clients perceived their advice and being more eager to investigate clients' expectations of the consultancies and to acknowledge clients' knowledge. Taken together, MITI results indicated that veterinarians adopted a more client-orientated approach after training. The proportion of veterinarians reaching suggested thresholds of moderate skills increased from 0 to 29% after training. The program was particularly successful in reducing so called non-MI-adherent behaviours (persuation, confrontation), utterances known to negatively impact the effect on outcome behaviours.

The mean (25-75th percentiles) of the veterinarians' Likert scores (where 1=low and 6=high) for the relevance of MI skills in their work was 5.1 (5.0-6.0). The mean (25-75th percentiles) of the veterinarians' overall satisfaction with the program was also 5.1 (5.0-6.0). Participating veterinarians reported difficulties to find sufficient training opportunities in professional work situations. One challenge identified by the research team was to reach higher levels of skills in a majority of participants.

Conclusions: It was a challenge for this educational concept to provide sufficient training for a majority of participants to reach high skills. MI training was perceived useful and relevant by participating veterinarians and the training program was successful in improving veterinarians' communication skills in VHHM.

Keywords: Motivational Interviewing, education, communication skills, veterinary herd health management, advisory services.

TE-06

Plan to Communicate, Communicate to Plan. How can evidence-based communication training help vets improve farmer engagement in herd-health planning? Tips and first opinion practitioner experience

Andrea Turner.

Langford Vets, Bristol, United Kingdom.

Objectives: To highlight opportunities for better vet-farmer communication and information sharing regarding herd health, as described in the literature.

To summarise evidence-based communication and engagement techniques which can be used by vets to improve herd health planning outcomes.

To describe how evidence-based communication and engagement techniques have been used in a first-opinion farm animal practice (in Somerset, UK) to improve farmer engagement with herd health planning.

To make vets aware of communication and engagement techniques and signpost to further training opportunities in this area.

Materials and methods: There is increasing interest and awareness in the veterinary profession regarding communication techniques and methods of engaging clients to promote positive behaviour change. In the case of farm animal medicine and herd health planning, vet–farmer communication and information sharing are crucial in order to achieve meaningful and sustainable behaviour change in farmers and farm workers which can have maximal impact on improving herd health.

Three PhD theses (Morgans, 2019, Rees, 2019) completed at the University of Bristol Vet School by Dr Gwen Rees, Dr Lisa Morgans and Dr Alison Bard will be summarised. These theses explored farmer decision making, farmer engagement through peer-to-peer learning and vet-farmer communication (Motivational Interviewing; (Bard *et al.*, 2017)), specifically relating to antimicrobial use and herd health planning. The author (a full-time practicing first opinion farm vet) who has had training in the facilitation of farmer peer-to-peer learning and Motivational Interviewing (from Dr Lisa Morgans and Dr Alison Bard, respectively) will describe the ways in which the findings of these theses can, and have been applied and used in first-opinion farm animal practice to engage farmers in herd health planning.

Results: Experiences from, and 'tips' for the practical implementation of the outcomes of each of the theses described will be shared by the author, specifically relating to how these outcomes can help vets engage farmers in herd health planning, including;

- How 'what farmers really want from their vets' (as identified by the work of Dr Gwen Rees) can be achieved practically through farm-specific protocols, laminated posters, farm-specific herd health calendars and practice protocols.
- How facilitated farmer meetings and farmer peer-to-peer learning (as studied by Dr Lisa Morgans) can be used to create co-produced herd health plans and the successes and pitfalls of doing this in practice.
- How Motivational Interviewing techniques (as studied by Dr Alison Bard) can be used in herd health planning meetings, including how the author has used these techniques both successfully and not-so-successfully.

Conclusions: The way in which vets communicate and engage with farmers can have huge implications for the success of herd health planning as sustainable and positive behaviour changes need to be elicited in both farmers and farm workers for herd health plans and related management changes to be implemented successfully.

The practical application of communication and engagement techniques studied in academic settings can vary for different practitioners in different settings. Evidence-based communication and engagement strategies have been implemented by the author in her first-opinion farm animal practice in relation to herd health planning. It is hoped that by sharing practical experience of these techniques, practicing farm vets will be inspired to apply these evidence-based strategies to their own practice. Signposting practicing vets to these communication strategies and helping them to apply these techniques to their own practice is essential if the veterinary profession is to move forwards in engaging farmers in meaningful herd health planning.

References:

Bard, A., Main, D., Haase, A., Whay, B., Roe, E. & Reyher, K. 2017. Partnership or persuasion? A qualitative investigation of veterinary communication in the pursuit of client behaviour change. PLoS ONE, Vol 12.

Morgans, L. 2019. A participatory, farmer-led approach to changing practice around antimicrobial use on UK dairy farms. University of Bristol.

Rees, G. 2019. Understanding Prescription Veterinary Medicine Use on UK Dairy Farms. University of Bristol.

Keywords: Coomunication, Training, Herd health, Motivational Interviewing, Facilitation.

TE-07

The use of the virtual-problem-based learning in veterinary education during SARS-CoV-2 (COVID-19) pandemic emergency: a resource or a disadvantage?

Fabiana Micieli, Giovanni Della Valle, Chiara Del Prete, Paolo Ciaramella, Jacopo Guccione.

University of Napoli Federico II, Department of Veterinary Medicine and Animal Productions, Napoli, Italy.

Objectives: The COVID-19 global pandemic emergency is forcing Veterinary College all over the world to modify deeply the traditional teaching approach employed. Due to the nationwide lockdown, the social distancing and the restrictions adopted in our country to reduce the virus spread, the Department of Veterinary Medicine of Napoli decided to replace the "traditional and in presence" hours dedicated to the clinical, pre-graduating, veterinary medical training with multiple sessions of clinical virtual-problem-based learning (v-PBL). In vet-

erinary education, problem-based learning (PBL) represents one of the most popular and flexible teaching systems able to replicate and simulate real-life experiences. This prospective cross-sectional case-control study aimed to evaluate the students' perception of the v-PBLs compared to the traditional veterinary clinically training (t-VCT).

Materials and methods: All the fifth-year students who completed the t-VCT (76 students) or the clinical v-PBL (46 students) represented the study population. The t-VCT consisted of supervised management of clinical cases admitted at the Veterinary Teaching Hospital or performed in the field under academic-staff supervision. The v-PBL consisted of genuine clinical case shared by tutors throughout an online platform. For each case, in the v-PBL group the time was approximately divided as follow: 1h of case-introduction by the tutor; 8h of self-learning and problem-solving activities (consisting of 15 to 20 progressive questions and activities related) and 4h of virtual group-discussion activity between students and tutor. To evaluate the student perception of the teaching methods, a survey with 18-Likert and two open-end questions was delivered to all the students in the study population. The latter was divided into five sections: "demographic data", "satisfaction", "clinical skills", "supervision" and "student's perceptions of the training".

Results: The survey was completed by 49% of the students (36.8% and 69.6% for t-VCT and v-PBL, respectively). Cronbach's alpha coefficient for internal consistency for the questionnaire was 0.9 (acceptable). Overall, the students' satisfactions degree regarding the training experiences was high in both groups. Nevertheless, the v-PBL group overall felt less satisfying as compared to the t-VCT group. The students of v-PBL group perceived that they could not improve their practical clinical skills through online sessions. All students of the v-PBL group had not suggestions to improve the v-PBL although they emphasized how it could be employed as support of traditional practical activities, in the future. All the students considered the trainings correctly focused on relevant learning objectives and the task clearly explained. Moreover, despite the social distancing imposed, the students in both groups express an overall satisfaction for the supervision activities performed by the academic staff.

Conclusions: Stimulating the integration of knowledge and lifelong learning skills replicating life experiences the v-PBLs represented an attractive curricular alternative for veterinary education in a period in which the conventional didactic systems have been deeply changed by the COVID-19 pandemic emergency. Moreover, it should be considered to integrate the traditional practical teaching approach with PBL to increase motivation and physiological arousal in students.

Keywords: Sars-cov-2, veterinary students, virtual-problem-based learning, veterinary teaching.

TE-08

FarmSkills4Vets - A virtual dairy farm brings veterinary herd health advisory into the home office of students and sparks their interest in cattle practice

Linda Dachrodt[1], Carsten Gazivoda[1], Martina Hoedemaker[1], Heidi Arndt[2], Svenja Woudstra[3].

[1]Clinic for Cattle, University of Veterinary Medicine Hannover, Germany; [2]Institute of Animal Science, University of Hohenheim Stuttgart, Germany; [3]Section for Production Nutrition and Health, University of Copenhagen, Denmark.

Objectives: Veterinary herd health advisory is a central element of modern veterinary medicine. Despite the importance, practical farm visits can be conducted with students only occasionally during their education at universities due to the limited resources. In addition, there is an increasing trend that fewer and fewer graduates want to work in bovine practice after their studies. The aim of the project FarmSkills4Vets was therefore (1) to develop a virtual dairy cow farm where students can learn the basics of veterinary herd health advisory, and (2) to evaluate if such a new learning environment might increase the students interest in bovine practice.

Material and methods: The virtual dairy farm was created on the basis of 360°photo images from real dairy farms. The individual scenes showed typical areas of a dairy farm, e.g., the housing area for calves and youngstock, compartments in the cowshed like feeding bunk, lying cubicles, maternity pen, and area for sick cows as well as silo facilities and milking parlor. The students step into the boots of the advising veterinarian and learn the basics of veterinary herd health advisory by exploring photos, videos and audio files in a self-directed way. Integrated questions and quizzes stimulate them to apply the learned skills directly.

From August to September 2021, students from the 2nd to the 10th semester of the University of Veterinary Medicine Hannover had the opportunity to test the virtual dairy farm. For the evaluation, 2 questionnaires were designed: Before the visit of the virtual dairy farm, the students were asked about their interests, prior knowledge, and self-assessment (pre-evaluation). Following the visit of the virtual farm, the students had to evaluate FS4V, especially the format, content, and realization, and were again asked to self-assess their skills and knowledge (post-evaluation). In the first test phase, 192 students completed the tour through the virtual dairy farm and evaluated FS4V.

Results: Almost all students enjoyed the visit of the virtual dairy farm (94.8 %) and were sure that this format can help to better understand and apply the specialist knowledge (96.3 %). Furthermore, students felt better prepared for instructed or independent conduction of veterinary herd health advisory. A large percentage of students (68.0 %) indicated that the virtual farm visit had increased their interest in cattle medicine. In addition, the visit of FS4V woke the interest in cattle practice in some students who had indicated in the pre-evaluation that they had not yet decided on what to specialize later (12.0%) or were more interested in small animal or equine medicine (4.7 %). Furthermore, students indicated that they would appreciate to have such a format for teaching

other veterinary topics, such as animal welfare and food safety, as well as veterinary herd health advisory of other animal species, such as pigs and poultry.

Conclusion: The authors regard such a format as a good opportunity to transfer the scientific knowledge in a practical and vivid way. In addition, a playful transfer of knowledge can help to increase the interest in cattle medicine and thus, also win cattle veterinarians for the future.

The authors thank the participating farmers and the Ministry of Science and Culture of Lower Saxony for financial support (“Innovation plus” 2020/2021, No.053).

Keywords: Virtual dairy farm, education, knowledge transfer.

UH-01

Selective and deferred treatment of clinical mastitis in seven New Zealand dairy herds

Andrew Bates[1], Richard Laven[2], Olaf Bork[3], Merlyn Hay[4], Jess Mcdowell[5], Bernardita Saldias[6].

[1]Vetlife Scientific Ltd, Temuka, New Zealand; [2]School of Veterinary Sciences, Massey University, New Zealand; [3]Mastaplex Ltd, Dunedin, New Zealand; [4]Vetlife Oamaru, Oamaru, New Zealand; [5]Vetlife Temuka, Temuka, New Zealand; [6]Centre for Dairy Excellence, Geraldine, New Zealand.

Objectives: This study focused on evaluating the ability of a novel on-farm diagnostic system for bovine mastitis (Mastatest©) to control antibiotic usage whilst achieving equivalent bacteriological and clinical cure rates alongside long term individual somatic cell count (**ISCC**) outcomes as conventional treatment choices.

Mastitis is the most frequent reason for antibiotic use in New Zealand dairy cattle and technologies reducing this use contribute to responsible product stewardship. Rapid identification of pathogen and antibiotic susceptibility facilitate targeted treatment but currently involve a minimum 24 hours delay. Studies from confinement systems where Gram-negative organisms are responsible for a significant proportion of mastitis, indicate selective treatment can reduce antibiotic use without reducing clinical or bacteriological cure. However, in New Zealand's seasonal, pastoral dairy system, mastitis is dominated by Gram-positive organisms and if treatment is deferred, it is vital both short- and long-term clinical health outcomes are not compromised.

Materials and methods: Mastatest© is an on-farm or clinic diagnostic system for bovine mastitis indicating the pathogen and its antibiotic sensitivity within 24 hours of sampling.

Mild to moderate mastitis cases in the 100 days after calving in 6,467 cows from 7 farms were milk sampled and randomly allocated to a positive control group (non-selective treatment) or a culture-based treatment using Mastatest©. All milk samples were processed on-farm.

For the positive control, the quarter was treated immediately with 3 treatments of procaine penicillin every 12 hours. For the selective treatment group, treatment was delayed for 24 hours and then informed by pathogen and antibiotic sensitivity from the Mastatest© result. Gram-negative and no-growth quarters were untreated. Gram-positive quarters were treated with the antibiotic for which the lowest in vitro antimicrobial sensitivity was reported.

Re-sampling was carried out from affected quarter(s) approximately 21 days after initial diagnosis and cultured for bacterial identification. Clinical recurrence within 60 days and ISCC data was recorded at herd tests over the duration of the lactation. Antimicrobial usage and days of milk withhold pending clearance of residues were also noted.

Results: There was no difference in bacteriological or clinical cure rate between the two treatment groups. Out of 535 quarter cases, 451 (84%) were bacteriologically cured at re-sampling and 43 (8%) were re-diagnosed with clinical mastitis within 60 days of the original diagnosis. Bayesian models predicted no difference in the cure proportion by treatment group but the probability of a bacteriological cure for cows infected with *Staph.aureus* was significantly less than that for *Strep. uberis* in both groups. There were numerical differences in the median bacteriological cure proportion by farm, but the coefficients spanned zero and overlapped. There was no evidence for a significant interaction between treatment group and farm, nor between treatment group and pathogen.

Final herd test ISCC - 225,000 cells/mL (95% predictive interval (PI) =25,000-4,543,145) - and days of milk withhold from supply - 5.7 days per quarter case (95% PI=1.0-6.5) - did not differ between groups. There were numeric differences in the median predicted ISCC by pathogen and farm, but the coefficients spanned zero and overlapped. There was no evidence for a significant interaction between treatment group and farm, nor between treatment group and pathogen.

Antibiotic usage was 24% less (95% PI = 12-47%) in the selective group with the model predicting that the there was a 98% chance that antibiotic usage in the selective group (1.3 daily doses per case, 95%PI=1.1-1.6) was less than in the non-selective group (1.7, 95%PI=1.4-1.9).

Conclusions: This study suggests that on farm decisions about deferred treatment of mastitis using Mastatest© to identify the intramammary pathogen can reduce the antimicrobial usage with no loss in bacterial or clinical cure and with no effect on ISCC over the lactation.

Keywords: Mastitis, deferred treatment, antibiotic sensitivity, Mastatest.

UH-02

Udder health in German dairy heifers – risk factors during first days of lactation

Martin Tho Seeth[1], Julia Nitz[2], Volker Krömker[3].

[1]Chamber of Agriculture Lower Saxony, Udder Health Service / Hannover University of Applied Sciences and Art, Faculty II, Microbiology, Oldenburg / Hannover, Germany; [2]Hannover University of Applied Sciences and Art, Faculty II, Microbiology, Hannover, Germany; [3]University of Copenhagen, Faculty of Health and Medical Sciences, Department of Veterinary and Animal Sciences, Section for Production, Nutrition and Health, Copenhagen, Denmark.

Objectives: Heifer mastitis is of great importance in many German dairy farms. Intramammary infections (IMI) prior to calving and during the early lactation period may influence the development of the mammary glands, the future milk production, udder health and related culling hazard negatively (Piepers et al., 2009). Up to now, the exact time of infection has not been identified. However, knowing this time point would be of major importance in order to reduce the rate of new IMI by possibly switching off the main risk factors. The aim of this study was to evaluate the exact moment of IMI in dairy heifers during early lactation and to demonstrate the most important risk factors.

Material and methods: In total, 279 Holstein Frisian heifers reared on 3 German dairy farms were included in this study. The average bulk milk SCC during the test period was

150,000, 180,000 and 260,000 cells / ml, respectively. From September 2017 until March 2018 quarter milk samples were collected from all four udder quarters for cyto-microbiological diagnosis at two time points. The samples were obtained 3 and 17± 3 days after calving in order to define the postpartum IMI status. Isolated NAS strains were differentiated using MALDI-TOF-MS (Bruker, Bremen). The farmers observed the heifers during the first 100 days of lactation and documented all signs of clinical mastitis.

Results: No pathogen could be detected in 80.2% (n = 725) of the udder quarters three days after calving and in 85.8% (n = 776) of the udder quarters 17 days after calving. This means that on day 17 after calving an IMI could be detected in 129 udder quarters. Based on the infection status on day 3 after calving, 83.0% (n = 107) of these 129 quarters suffered from a new infection. A latent infection could be detected in 17% (n = 22) of these udder quarters. 80.4% of the udder quarters suffered from an infection caused by NAS at day 3 p.p. showed a somatic cell count of >100.000 cells/mL. The probability of IMI caused by contagious mastitis pathogens increased with elevated SCC. Heifers showed an increasing probability of IMI with NAS and coryneforms on day 17 postpartum when being at a higher age at calving. Heifers with high milk yields showed less IMI caused by environmental pathogens 17 days postpartum and fewer clinical mastitis in the first 100 days of lactation. However, animals with high milk yield showed increased IMI caused by NAS and coryneforms pathogens on day 3 postpartum. Udder edema was associated with an increased risk of IMI caused by NAS and coryneforms on day 3 postpartum. A strong association between udder health of dairy heifers and teat-cup fell-offs with the result of sudden air ingress could also be shown. Animals from farms with increased problems during milking showed an increased probability of IMI on days 3 and 17 postpartum.

Conclusion: This study reveals that an elimination of pathogens is possible during early lactation. However, there is a risk of new infection at the same time. In addition to the period before calving and calving itself, the period between the 3rd and 17th day of lactation plays an extremely important role for the udder health of dairy heifers. For this reason, the risk factors specific to this stage of early lactation must be taken into account in order to apply a modern udder health management to dairy farms.

Keywords: Mastitis, intramammary infections, dairy heifers.

UH-03

In vitro penicillin sensitivity of *Streptococcus ssp.* isolated from quarter milk samples of Bavarian dairy cows (2018 - 2019)

Reglindis Huber-Schlenstedt, Laura Macias-Luaces, Ulrike Sorge.

Bavarian Animal Health Service, Poing, Germany.

Objective: The objective was to evaluate the *in vitro* antimicrobial resistance against penicillin of *Streptococcus (S.) uberis, S. agalactiae, S. dysgalactiae,* and *S. canis* of routine quarter milk samples.

Material and methods: In 2018 and 2019 the Bavarian Animal Health Services received quarter milk samples from whole dairy herd tests as well as individual cows. The laboratory used standard culture methods in accordance with guidelines of the German veterinary association to diagnose infections in quarter milk samples. The identification of *Streptococci* was based on colony morphology, hemolysis, esculin hydrolysis, camp factor as well as gram strain. Further differentiation of esculin-positive *Streptococci* was conducted with an in-house method that utilized *Enterococcus* selective agar plates and a disc test against Penicillin and Rifampicin. Strains of unclear results were identified by MALDI-TOF-MS (microflex™ MALDI Biotyper™, Bruker Daltonik, reference database V.3.3.1.0., Bruker Daltonik GmbH, Bremen Germany). A subset of aforementioned *Streptococci* was tested for sensitivity to common antibiotics using with the method of MIC (minimum inhibitory concentration) (mastitis 3 plat, Merlin Diagnostika GmbH). Breakpoints for sensitivity are based upon CLSI Vet01-A4 (2015) and CLSI Vet01S 5th edition. As a breakpoint for penicillin for the indication “mastitis cow” does not exist, an older breakpoint (CLSI M31-A2; AVID, 1998) was used instead. Furthermore, intermediate results were reported as resistant. Results were summarized by descriptive statistics.

Results: A total of 854,782 quarter milk samples from 218,336 cows of >7,000 individual dairy farms were analyzed in the laboratory in 2018-2019. These included samples from subclinical (based on California Mastitis Test (CMT), n= 213,174) and clinical cases (n= 17,642). Mastitis pathogens were found in 18.7 % (n= 159,900) of all submitted samples.

S. uberis was the most commonly isolated mastitis pathogen of clinical samples (2018: 35.0%, n= 2,566; 2019: 34.4%, n= 2,280). Additionally, *S. uberis* was the second most commonly isolated pathogen in samples of subclinical cases in both years (2018: 23.9%, n= 12,178; 2019: 23.1% n= 9,778) - only surpassed by Coagulase Negative *Staphylococci* (2018: 29.5%; 2019: 30.4%).

Of 7,345 *S. uberis* tested, exceedingly few isolates (n=47) had a breakpoint >0.125 for penicillin (2018: 0.8%, n= 33; 2019: 0.4%, n= 14). The vast majority had a breakpoint <0,125 for penicillin and thus was considered susceptible to penicillin *in vitro*. All tested isolates of *S. agalactiae* (n= 900) and *S. canis* (n= 355) had a breakpoint <0.125 for penicillin - i.e., none were considered resistant to penicillin. Only five (0.2%) of 2,684 tested *S. dysgalactiae* were considered resistant to penicillin as they had a penicillin breakpoint above >0.125 (2018: 0.1%, n= 1,444; 2019: 0.2%, n= 1,240).

Conclusion: Only sporadic *Streptococci* isolates had a breakpoint >0.125 and were resistant to penicillin *in vitro*. The vast majority of tested *Streptococci* had a breakpoint <0.125 for penicillin. Therefore, penicillin should continue to be the first choice for antibiotic therapy of *S. uberis* and esculin-negative Streptococci in Bavaria (Southern Germany).

Funding Source: Bavarian Ministry for Food, Agriculture and Forestry and the Bavarian Joint Founding Scheme for the Control and Eradication of contagious Livestock (Bayerische Tierseuchenkasse).

Keywords: Mastitis, streptococci ssp., penicillin sensitivity.

UH-04

Monitoring Udder Health on Routinely Collected Census Data: Evaluating the Effects of a Changing Antimicrobial Policy in the Netherlands

Christian Scherpenzeel[1], Kristel Van Den Heuvel-Van Den Broek[1], Inge Santman-Berends[1], Gerdien Van Schaik[2].

[1]Royal GD Animal Health, Deventer, Netherlands; [2]Royal GD Animal Health & Utrecht University, Deventer, Netherlands.

Objectives: Antibiotic resistance is becoming increasingly important and given its potential association with antimicrobial use (AMU), continuous actions are taken to reduce AMU in the livestock industry. One of the most important measures to avert preventive antibiotic usage was implementation of selective instead of blanket dry cow therapy in 2013. In the Netherlands, both AMU and udder health parameters are monitored in the Dutch cattle health surveillance system (CHSS) based on routinely collected census data (17,000 dairy herds). The aim of our study was to determine whether we could monitor the effect of changing AMU on udder health in Dutch dairy farms.

Material and methods: In total, six udder health indicators are monitored on a quarterly basis i.e. bulk milk somatic cell count (BMSCC), prevalence and incidence of high SCC cows (HSCC), herds with a >25% incidence of HSCC primi- and multiparous cows during the start of the lactation and herds with a >25% prevalence cows with a consistent HSCC before and after calving. The association between udder health key indicators and AMU was analysed using population average multivariable regression techniques with appropriate distributions and link functions. In addition, other variables that may be related to udder health were included in the models.

Results: With the implementation of general regulations aiming to decrease AMU, the Animal Defined Daily Dosage (DDDA) in dairy herds decreased from 5.78 in 2009 to 4.03 in 2013 (SDA, 2014). After implementation of SDCT, DDDA in dairy herds decreased further to 3.06 in 2017 (SDA, 2018). Our results showed that both implementation of regulations for AMU in general (2009) and implementing selective dry cow therapy rather than blanket treatment (2013), did not result in a deterioration in most udder health indicators (Fig. 1a). The only indicator that was somewhat negatively influenced by the implementation of SDCT was the percentage of herds with >25% new HSCC in multiparous cows during the start of lactation (Fig. 1b).

Even though most udder health parameters were not negatively influenced, the results of the multivariable models showed that not applying antimicrobials at all resulted in a higher BMSCC (+15.6 x10^3 cells/mL (95% CI: 15.1-16.1)), a higher incidence and prevalence of HSCC cows (OR=1.6) and a higher probability to have >25% cows with a new HSCC during the start of lactation.

Conclusion: The results indicate that even though AMU was decreased with 47% (from 5.78 DDDA in 2009 to 3.06 DDDA in 2017), a decrease in udder health indicators was not observed. Thus, application of SDCT did not seem to affect the general udder health in dairy herds. However, in dairy herds that did not use any antimicrobials the SCC of cows was significantly higher compared to the herds with an average AMU. In conclusion, it is advisable to closely monitor the effects on animal health when new policies to reduce AMU are implemented. The results provide confidence that AMU reducing measures lead to more prudent use of antimicrobials without jeopardizing animal health.

Keywords: Udder health, Antimicrobial use, Census Data, Selective Dry Cow Therapy.

UH-05

In vitro antimicrobial resistance of bacteria isolated from clinical mastitis cows in Germany

Luis Leon[1], Christian Fidelak[2], Thomas Breuer[1], Hans-Georg Schoen[3].

[1]Zoetis Germany, Berlin, Germany; [2]Bovicare GmbH, Potsdam, Germany; [3]University of Applied Sciences Osnabrueck, Osnabrueck, Germany.

Objectives: The goal of this study was to analyze the clinical mastitis pathogens resistance to routinely used antibiotics by analyzing samples collected across different regions in Germany.

Material and Methods: Quarter milk samples of cows with clinical mastitis were analyzed at a milk quality laboratory (bovicare GmbH, Germany). The samples were processed for bacteriology, SCC and resistance against the most frequently intramammary used antibiotics / antibiotic combinations: Amoxicillin and Clavulanic acid (AMC), Cefquinome (CEFQ), Cefoperazone (CFP), Cephapirin (CEPA), Cephalexin and Kanamycin (CKN) and Penicillin (P). The samples were submitted by 308 local veterinarians during September 2015 and February 2020. The median of samples per vet was 4. The samples were examined following NMC guidelines. Resistance was determined using agar diffusion method, evaluation of the inhibition-zone-results (S: susceptible, I: intermediate, R: resistant) was done using literature and companies' recommendations. The resistance results were statistically analyzed (IBM-SPSS; version 26.0) using Cochran-Q-test (S+I versus R) followed by Bonferroni-Dunn post hoc test. The Kruskall-Wallis-test with the Bonferroni-Dunn correction was used for analysis of the region´s effects. Differences consider statistically significant when P<0.05.

Results: 3,137 quarter bacteriological positive samples were tested for resistance. From East Germany were 488 (47%) collected, 853 (28%) from South and 796 (25%) from Northwest Germany. The most frequent pathogen was *S. uberis* (36.9%), followed by *S. aureus* (12.8%), *E. coli* (11.9%) and CNS (10.1%). This pathogen distribution is related in the literature. The results showed a significant difference of the in vitro susceptibility between the antibiotics tested (p=0.000). The resistance situation for the *S. uberis*-Isolates was: CKN^{a} (9%), CEFQ^{ac} (7%), P^{bc} (6%), CEPA^{bc} (6%), CFP^{d} (2%), AMC^{d} (1%). No differences across the regions were found. For *S. aureus* the distribution of resistant-isolates was: P^{a} (25%), CKN^{b} (7%), AMC^{b} (6%), CEPA^{b} (5%), CEFQ^{bc} (3%), CFP^{c} (2%). Regional differences were found only for P (less resistance in East

Germany). The frequency of resistant *E. coli*-isolates was: P[a] (100%), CEPA[b] (77%), CKN[c] (30%), CEFQ[d] (4%), CFP[d] (3%), AMC[d] (3%). Regional differences were found only for CNK (less resistance in Southern Germany). Considering all pathogens, the resistance situation was as follows: P[a] (32%), CEPA[b] (22%), CKN[c] (16%), CEFQ[d] (8%), CFP[e] (5%), AMC[e] (4%). There are differences between regions for P (more resistances in Northern) and CEPA (less resistances for the East Germany isolates).

Conclusion: Large number of isolates showed resistance against P, CEPA and CKN confirming the hypothesis that some gram positive and gram-negative mastitis isolates are not susceptible to often used antibiotics. AMC shows an activity against the most important gram-positive and gram-negative pathogens. AMC is an effective alternative to cephalosporines 3rd (CFP) / 4th generation (CEFQ). Similar results were published in Germany and other countries (Zieger et al. 2014, Leon et al. 2015, Leon et al. 2020, Bolte et al. 2020).

Keywords: Mastitis, resistance, pathogens, antibiotics, Germany.

UH-06

Analysis of udder health indexes of dairy operations from Galicia, Spain

Manuel Angel Fernandez Sanchez[1], Ana Benitez Sánchez[1], Carmen Castella[2].

[1]Mafer S.A., Lugo, Spain; [2]VIRBAC Espana, Barcelona, Spain.

Objectives: The objective of this study was to process data from a dairy herd improvement (DHI) organisation of Galicia (Spain) to identify key risk factors of intramammary infection (IMI) and changes in these factors over a seven-year time series.

Material & Methods: Data were retrieved from the DHI organisation in Galicia (Federación Frisona Gallega, FEFRIGA) which monitors 2545 farms (July 2019). Base values of several udder health indexes (UHI) were estimated from this database. Then a sub-set of 254 farms was selected at random to establish individual time series for the UHI (01/2012 to 12/2019); this represents a total of 18563 control-days. Selected farms resorted to a consultancy service (veterinarian) to improve quality control. The Somatic Cell Count (SCC) decision-making threshold was 200 x103cells/ml (>200 x103=infected, ≤200 x103=healty). UHI considered in this study were:

- Prevalence
  - [PL1] PREVALENCE (first lactation cows: L1): L1 > 200 x103cells/mL in current control/total number L1 in current control
  - [PF] PREVALENCE (Flock level): Cows > 200 x103 cells/mL in current control/total number of cows in current control.
- Incidence rates
  - [IF] INCIDENCE (Flock level): cows previous control (C1) ≤ 200 x103 cells/mL and current control (C2)>200 x103 cells/mL /cows C1 ≤ 200 x103cells/mL and with presence in C2.
  - [IL1C1] INCIDENCE (First Lactation: L1) – First Control (C1): L1 C1 > 200 x103cells/mL current control /number of L1 C1 in current control.
  - [IDP] INCIDENCE (dry period): > 200 x103cells/mL first control after parturition with < 200 x103 cells/mL before dry period / < 200 x103cells/mL before dry period
- Cure rates
  - [CL] CURE (Lactation): number of cows with C1>200 x103 cells/mL and C2 (second control) ≤200 x103 cells/mL /number of cows C1>200 x103 cells/mL
  - [CDP] CURE DRY PERIOD (After dry period): number of cows C1 ≤200 x103 cells/mL and >200 x103cells/mL in previous control /number of cows C1, with >200 x103 cells/mL in previous control.
- **C**hronicity [CHR]
  - **C**ows >200 x103 cells/mL. in last two controls/Total infected in the previous month (>200 x103 cells/mL).

A selection of risk factors that can impact UHI were considered. These factors were:

- Average daily milk yield/cow/day (L/cow/day)
- Farm Size (<40, 40-65 or >65 heads)
- Bedding (organic/inorganic)
- Number of daily milkings/cow/day (2 or 3)
- Dry cow therapy (yes or no)
- Grazing (yes or no)
- Pre-dipping (yes or no)

Logistic regression was used as a statistical model for the analysis of factors to model the probability of a certain class. The autoregressive integrated moving average (ARIMA) analysis has been used for the study of the time series and to predict future points in the series (forecasting). ARIMA is actually a class of models that 'explains' a given time series based on its own past values, that is, its own lags and the lagged forecast errors, so that equation can be used to forecast future values. Data were processed with Statgraphics Centurion XVI software (Statgraphics Technologies, Inc., The Plains (VA), USA).

Results: The logistic regression enlightened the influence (either positive or negative) of every UHI (Table 1). Only significant associations (P<0.05) are presented.

The ARIMA analysis led to the conclusions that it does exist an over year tendency and a season effect for every single UHI with the exception of IDP.

Conclusions: Statistical studies on DHI data can verify the seasonability and trends in the evolution of data in time series. Also, this study of major factors that influence the udder health indexes can give guidance on the way to improve the udder health status.

Keywords: Udder health; cure rate; ARIMA.

Table 1. Significant (P<0.05) associations between risk factors and magnitude, and change (increase/decrease) in selected Udder Health Indexes (UHI).

Risk factors		**UHI**	
Item	**Magnitude**	**Decrease**	**Increase**
Milk yield	Higher production	PF, PL1, IF, IL1C1, IDP, CHR	CDP, CL
Farm size	Larger farms	CDP	PF, IL1C1, IDP
Bedding (small farms)	Inorganic	PF, PL1, IF, IDP	
Milking frequency	3x day	PF, PL1, CDP, CHR	IDP, CL
Dry cow therapy	No DCT	CDP, CL	PF, PL1, IF, IL1C1, CHR
Grazing	No grazing	PF, PL1	CDP
Pre-dipping	No teat dipping	CDP, CL, IL1C1, IDP	CHR

UH-07

Spread of livestock associated MRSA CC398 in German dairy herds

Arne Schnitt[1], Tobias Lienen[1], Heidi Wichmann-Schauer[1], Christiane Cuny[2], Bernd-Alois Tenhagen[1].

[1]German Federal Institute for Risk Assessment, Berlin, Germany; [2]Robert Koch Institute, Berlin, Germany.

Objectives: Livestock associated methicillin resistant Staphylococcus aureus (LA-MRSA) often belong to clonal complex (CC) 398 and may colonize and infect different animal species including humans (Cuny et al 2015). In both, animals and humans, LA-MRSA were rarely associated with clinical infections. In dairy cows, MRSA prevalence is low especially in comparison to pigs (Schnitt and Tenhagen 2019). However, LA-MRSA ST398 can cause mastitis in dairy cows (Falk 2018). Mastitis caused by MRSA is a problem in veterinary medicine, since most antibiotics approved for mastitis and dry cow therapy are β-lactams. Therefore, MRSA monitoring and prevention strategies are needed for dairy herds. This study aimed to determine the occurrence and spread of LA-MRSA on preselected German dairy farms.

Materials and methods: Based on previous phenotypic MRSA detection, 20 German dairy herds were selected and visited in 2018-19. Eight farms used automatic milking systems and 12 farms used milking parlours. From dairy cows, quarter milk samples (QMS) and bulk tank milk (BTM) was collected. Additionally, swab samples from calves, heifers, dust, teat liners, pigs, farm personnel and suckers from automatic calf feeders were analysed. In total, 3396 samples were screened for the presence of MRSA using a two-step selective enrichment protocol. All presumptive MRSA isolates were further analysed by MALDI-TOF, *mecA*/*mecC* PCR, *spa*-typing, SCC*mec*-typing and certain isolates were selected for next generation sequencing (NGS). A structured questionnaire and on farm observations were used to collect data on milking hygiene, biosecurity and general herd management.

Results: Prevalence: MRSA prevalence was 23.6% (47/199) in milk-fed calves, 13.6% (26/191) in samples from heifers, 8.6% (16/187) in post-weaning calves and 7.9% (47/595) of cows carried MRSA in QMS. In total, 68/2384 (2.9%) QMS were tested positive, indicating that multiple quarters were affected in some cows. As an indicator for mastitis, the average somatic cell count (geometric mean) was higher in QMS from MRSA affected quarters (357,000 cells/ml) in comparison to all quarters (114,000 cells/ml) (p<0.05). In BTM, MRSA was detected on 12 farms. In the environment, LA-MRSA was detected in dust, teat liners and automatic calf feeders. In samples from farm personnel, MRSA was detected on 4/7 farms.

Characterization of isolates: All MRSA isolates carried the *mecA* gene and belonged to CC398. Moreover, most isolates were identified as SCC*mec*-type V and *spa*-types t011 and t034. Results from NGS revealed additional genes that mediate resistance against aminoglycosides, macrolides, trimethoprim, tetracycline and lincosamides. Determination of virulence-associated genes in LA-MRSA isolates predicts low risk for human infections.

Potential risk factors: Improper milking hygiene procedures were observed on all farms with MRSA detection in milk (n=12). Two farms did not perform any udder cleaning and on one farm, one udder towel was used for all cows. Five MRSA-positive farms had no cluster disinfection and on one farm disinfection was only sporadically performed. Two farms did not do any post dipping and on three more farms post dipping was ineffective. Five MRSA affected farms kept both cattle and pigs at the same facility. However, MRSA was detected in only two pig barns and on one farm MRSA genotypes were different.

Conclusions: On 20 German dairy farms, the highest MRSA prevalence was detected in nasal swabs from young calves. Therefore, future MRSA monitoring programs should include samples from young stock. Improper milking hygiene as well as MRSA carrying humans and replacement heifers may enhance the spread of LA-MRSA CC398 within and between dairy herds. The role of pigs as a potential reservoir for LA-MRSA that may be transmitted to dairy cows needs to be further investigated.

References:

Cuny C., Wieler L.H., Witte W. (2015): Livestock-Associated MRSA: The Impact on Humans; Antibiotics 4(4):521-543.

Falk R. (2018): Unusual outbreak of mastitis caused by methicillin resistant Staphylococcus aureus (MRSA) in a dairy herd in winter 2018. Animal Health Report—International Dairy Federation 9–10. https://www.fil-idf.org/wp-content/uploads/2018/09/IDF-Animal-Health-Report-Web.pdf accessed February 21, 2020.

Schnitt A. & Tenhagen BA. (2019)*: Risk Factors for the Occurrence of Methicillin-Resistant Staphylococcus aureus in Dairy Herds: An Update. Foodborne Pathog Dis. doi:10.1089/fpd.2019.2638.*

Keywords: Mastitis, antimicrobial resistance, MRSA, Staphylococcus aureus.

UH-08

Milk Somatic Cell Count in Buffaloes and its Relation to Intramammary Infections, Parity, and Stage of Lactation

Kanchan Arya, Baljinder Kumar Bansal, Dhiraj Kumar Gupta, Sikh Tejinder Singh.

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India.

Objectives: Milk somatic cell count (SCC) is a key component of regulations for milk quality and is an indicator of udder health in dairy animals. The present study determines the physiological level of milk SCC in buffaloes and explores its relation to quarter infection, parity, and stage of lactation.

Materials and Methods: The study was conducted in two parts. The first part of the study determined the physiological levels and effect of quarter infection and parity on the milk SCC in buffaloes. In this, the quarter foremilk (QFM) and udder composite milk samples from ninety-one buffaloes of different parities were analyzed for bacteriology (Microbial Procedures of the National Mastitis Council) and SCC (using somatic cell counter SomaScope Smart, DELTA Instruments, The Netherland). The second part evaluated the effect of stage of lactation on the milk SCC. Here, 39 freshly calved buffaloes were enrolled and studied for quarter infection and milk SCC at four different stages of lactation viz., fresh calving (3-5 days of calving), early lactation (35-42 days), mid-lactation (120-135 days) and late lactation (210-225 days). The health status of quarters was defined as per International Dairy Federation criteria i.e. simultaneously considering the microbiology and SCC of QFM. The threshold value of QFM SCC was taken as ≤100×10^3 cells/ml for defining the healthy quarters The Data was stored in Microsoft Excel and analyzed by Chi-square test (Fisher's Exact Test), ANOVA with post HOC (Tuckey's method), Spearman's correlation, t-Test and Logistic regression with backward elimination using SAS version 9.3 (SAS Institute, Cary, USA). The level of significance was set at $p < 0.05$.

Results: The evaluation of quarter health in buffaloes under study revealed 7.99% specific subclinical mastitis, 11.57% nonspecific subclinical mastitis, and 7.71% latent infections. 72.73% of the quarters were found healthy. Animal wise, 20.88% of udders were positive for specific subclinical mastitis in at least one quarter. The average milk SCC in buffaloes was found 100×10^3 cells/ml (range 74-126) at the quarter level and 105×10^3 cells/ml (range 58-151) at udder level. The 77% of buffaloes had SCC ≤100×10^3 cells/ ml, with 7% showing >200×10^3 cells/ml. The milk SCC was found significantly ($p<0.05$) higher in the infected quarter (356×10^3 cells/ml) than in healthy quarters (52×10^3 cells/ml). The distribution of intramammary infections (IMI) to milk SCC showed 9.59%, 20.51% and 65.62% infections in quarters with QFM SCC of 0-100, 101-200 and > 200 ×10^3 cells/ml, respectively. The streptococci IMI resulted in highest milk SCC (mean 710×10^3 cells/ml) followed by *Staphylococcus aureus* (464×10^3 cells/ml) and coagulase-negative staphylococci (224×10^3 cells/ml) as compared to 52×10^3 cell/ml of healthy uninfected quarters ($p<0.01$). The *corynebacteria* infections initiated no significant reaction (mean SCC 54×10^3 cells/ml). The milk SCC increased with the advancing parity; average cell counts being 168×10^3 cells/ml for 3-4th parity buffaloes as compared to 84×10^3 cells/ml for 2nd parity and 48×10^3 cells/ml for 1st parity animals ($p<0.01$). The milk SCC showed a significant positive correlation of 0.30 with the 305-day lactation milk yield ($p< 0.01$). The evaluation of milk SCC *viz.-a-viz.* stage of lactation revealed the average milk SCC as 118×10^3 cells/ ml at calving, which decreased to 69×10^3 cells/ml at 35-42 d ($p<0.05$), and then increased to 80×10^3 cells/ml in mid- and 94×10^3 cells/m in late- lactation. The occurrence of quarter infections was minimum (10.25%) in freshly calved animals, which increased to 21.15% in early lactation, 22.91% in mid-lactation and 31.03% in late lactation (Chi-square = 58.00, df = 03, $p < 0.01$).

Conclusions: Assessment of present results in buffaloes in terms of our similar studies in HF×Sahiwal crossbred cows under parallel dairy management exposes that buffaloes relatively harbor fewer quarter infections and possess lower milk SCC. Thus, the physiological threshold of milk SCC in buffaloes probably may be defined at 100×10^3 cells/ml and considered pathological when >200×10^3 cells/ ml.

Keywords: Buffalo, Milk Somatic Cell Count, Intramammary Infection, Parity, Lactation.

UH-09

Selective dry cow therapy: the potential role of a novel biomarker panel

Lorenzo Viora[1], Theo Pepler[2], Emily L. O'reilly[2], Nicola Brady[2], Ruth N. Zadoks[3], P. David Eckersall[2].

[1]University of Glasgow, School of Veterinary Medicine, University of Glasgow, United Kingdom; [2]Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, University of Glasgow, United Kingdom; [3]School of Veterinary Science, Faculty of Science, University of Sydney, University of Sydney, Australia.

Objectives: The aim of the study was to evaluate whether the combination of protein biomarkers will improve the diagnostic accuracy for selective dry cow therapy compared to somatic cell count (SCC) and California mastitis test (CMT).

Material and Methods: Two commercial dairy farms (900 and 600 milking cows respectively) were enrolled in the study. Quarters milk samples were collected aseptically from cows at the time of dry-off based on CMT results to enable a case-control study design. Each sample was tested for SCC, standard bacteriology combined with matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-ToF MS) and protein concentrations of five potential biomarkers*.

Standard bacteriology wwas performed using blood agar incubated at 37°C in aerobic conditions and examined after 24 hours. Plates without growth were considered negative for mastitis-associated pathogens (bacteria absent) and those with three or more morphotypes were considered contaminated and excluded from the analysis. Samples yielding one or two morphotypes were considered indicative of infection and isolates were submitted for species identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS). The results from the MALDI-ToF

MS were considered definitive.

The five biomarkers were combined in a classification tree model to predict bacteria presence. Its performance was compared to the SCC (≥ 200 000 cells/ml) and CMT results (> 0). Sensitivity (Se), specificity (Sp) and accuracy in distinguishing between bacteria presence and bacteria absent samples were calculated.

Results: Of 209 quarter milk samples, 14 (6.8%) were contaminated and excluded from the analysis. Of the remaining 195 samples, 108 (55%) had bacteria present and 87 (45%) did not.

The Sp of the biomarker classification tree model (63%; 95% confidence interval: 53%-73%) was significantly greater than of SCC (39%; 30%-50%) or CMT (25%; 17%-35%). The Se of the biomarker model (73%; 64%-81%) was similar to that of SCC (79%; 70%-85%) and significantly lower than for CMT (90%; 83%-94%). Accuracy of the biomarker model (69%; 62%-75%) was not significantly different compared to SCC (61%; 54%-68%) and CMT (61%; 54%-68%).

Conclusion: Selective dry cow therapy (DCT) is a treatment strategy designed to reduce antimicrobial usage, with selection largely based on SCC or clinical mastitis history, which are used as proxies for current infection status. There have been limited attempts to evaluate whether measuring inflammatory biomarkers in milk results in more accurate selection of infected cows for DCT.

We evaluated whether a combination of protein biomarkers in a classification tree model improved the diagnostic accuracy compared to SCC and CMT. The biomarker model has greater Sp at the cost of reduced Se.

Further investigations will show whether additional inflammatory biomarkers, used independently or in conjunction with other data such as SCC, could refine decision making at dry-off.

Keywords: Selective dry cow therapy, biomarkers, antimicrobial reduction.

UH-10

Diversity of selected staphylococci and streptococci isolated from milk samples in a dairy cow herd with low prevalences

Svenja Woudstra[1], Nicole Wente[2], Yanchao Zhang[2], Stefanie Leimbach[2], Maya Katrin Gussmann[1], Carsten Kirkeby[1], Volker Krömker[1].

[1]University of Copenhagen, Copenhagen, Denmark; [2]University of Applied Sciences and Arts Hannover, Hannover, Germany.

Background and objective: Most mastitis cases worldwide are caused by staphylococci and streptococci. Farm-specific strategies for the prevention and control of intramammary infections with these can be developed based on the results from the regular microbiological analysis of milk samples from clinical and subclinical mastitis cases. The dominant organisms on a farm determine if the focus should be on improving milking practices or the hygiene in the cows´ environment. This approach is based on the traditional categorization into contagious pathogens (e.g. *Staphylococcus (Staph.) aureus*) that are mainly transmitted during milking, and environmental pathogens (e.g. *Streptococcus* (*Strep*.) *uberis*) that have their reservoirs in the environment. The diversity of a bacterial species (i.e. number of different strains) in a herd is indicative of the sources of infections. If the diversity is low, a common source of infections is assumed (contagious transmission or an environmental hotspot). When the diversity is high, independent infection events are most probable. For some pathogens like *Strep*. *dysgalactiae,* it remains unclear if they spread rather contagiously or if cows become infected from environmental sources. Additionally, for some species (e.g. *Strep*. *uberis*) the traditional categorization has been challenged in recent decades. Furthermore, most studies have investigated the diversity of mastitis-causing pathogens in herds that are experiencing mastitis outbreaks or an unsatisfactory udder health situation. Therefore, the objective of the present study was to describe the diversity of selected staphylococci and streptococci isolated from milk samples in a herd with low prevalences.

Material and methods: From June to October 2020, one Swedish dairy cow herd was visited 10 times with 14-day intervals. At each visit, quarter foremilk samples from all lactating cows were collected aseptically according to DVG guidelines and transported immediately to the laboratory [1]. From each sample, 10µl were streaked onto esculin blood agar. Microbial growth was evaluated after 24 and 48 hours incubation at 37°C. Preliminary species identification was based on morphological and biochemical characteristics, and final species identification conducted by MALDI-TOF MS. For all isolates identified as *Staph*. *aureus*, *Staph. epidermidis*, *Strep*. *dysgalactiae* or *Strep. uberis*, strain typing based on RAPD-PCR was conducted. All isolates with the same banding pattern were considered to be the same RAPD-type. A quarter was considered infected if at least 100 colony-forming units per milliliter were detected and infections ended when at least two consecutive samples were negative for the respective strain type.

Results: In total, 8056 milk samples from 263 cows were collected. From 82 (1.0%), 91 (1.1%), 106 (1.3%) and 114 (1.4%) of all samples *Staph. aureus*, *Staph. epidermidis*, *Strep. dysgalactiae* and *Strep. uberis* were isolated, respectively. For *Staph*. *aureus,* five different RAPD-types were found. Of all *Staph. aureus* infections (n=48), 81% (n=39) were due to the same strain. All other strains caused maximum 8% of all *Staph*. *aureus* infections. For *Staph. epidermidis,* 45 different strains were isolated. None of these strains caused more than 5% of all infections (n=55). Seven different strains of *Strep. dysgalactiae* were isolated. One of these caused 50% (n=18) of all (n=36) observed infections. Two other strains were isolated from 19% (n=7) and 11% (n=4) of the *Strep*. *dysgalactiae* infections. All other strains caused maximum 8% of all *Strep*. *dysgalactiae* infections. The *Strep. uberis* isolates belonged to 18 different RAPD-types. 24% (n=10) of all *Strep. uberis* infections were caused by the same RAPD-type and another 15% (n=6) and 12% (n=5) were caused by two other RAPD-types. All other *Strep*. *uberis* strains caused less than 10% of all infections.

Conclusions: For *Staph*. *aureus*, *Strep*. *dysgalactiae* and *Strep*. *uberis*, dominating strains were isolated. This confirms the contagious nature of *Staph. aureus* even in this herd with a low occurrence and indicates that also *Strep*. *uberis* and *Strep*.

dysgalactiae infections can spread mainly contagiously or via environmental hotspots. In contrast, no dominating strain was found for *Staph*. *epidermidis*. Therefore, most infections with *Staph. epidermidis* seemed to have occurred due to independent infection events.

Acknowledgements: This work was funded by FORMAS (Swedish Research Council for Sustainable Development, Stockholm, Sweden; grant numbers: 2019-02276 and 2019-02111).

References:

1. DVG. (2018). Leitlinien zur Labordiagnostik der Mastitis - Probenahme und mikrobiologische Untersuchung [Guidelines for laboratory diagnostics of mastitis - Sampling and mikrobiological investigation]. Deutsche Veterinärmedizinische Gesellschaft e.V, Giessen, Germany. ISBN: 978-3-86345-454-8.

Keywords: bovine mastitis, strain diversity, Staphylococcus spp., Streptococcus spp., mastitis pathogen categorization.

UH-12

Applying internal teat sealants at drying off; does full versus partial insertion of the tube cannula matter?

Cherry Bedford[1], Philippa Mahen[1], Kath Aplin[2], George Oikonomou[1].

[1]Livestock Health and Welfare, Institute of Veterinary Sciences, University of Liverpool, Liverpool, United Kingdom; [2]Boehringer Ingelheim Animal Health UK Ltd, Bracknell, United Kingdom.

Objectives: Internal teat sealant (ITS) and intramammary antibiotic application tubes can often be used with either a short or a long insertion cannula. Leelahapongsathon et al (2016) found that intramammary infection in early postpartum was significantly associated with full cannula insertion for the administration of antibiotic dry cow therapy (ADCT). There is a lack of published work on the effect of using full (FI) or partial (PI) cannula insertion for the administration of ITS. Our randomised control trial aimed to test the hypothesis that FI could increase the risk of introducing new infections into the udder leading to higher somatic cell counts (SCC) post-calving and a greater incidence of mastitis post calving comparing to PI.

Materials and methods: Three pedigree Holstein UK dairy farms were selected to take part in the study over a period of six months. The farmers selected which cows were to be dried off each week and dictated whether each cow would receive internal teat sealant only (ITS, Ubroseal® Boehringer Ingelheim Animal Health UK Ltd) or intramammary antibiotic and internal teat sealant (AB+ITS). Cows were then randomised to receive ITS or AB+ITS via either FI or PI of the cannula/e. The facilitator was blinded to the insertion type until the cow was enrolled. One farm opted to only allow enrolment of cows receiving ITS as the antibiotic tubes used at dry off did not have the option of partial cannula insertion. The facilitator was trained in best practise aseptic technique for drying-off cows by three different experts in the field. All farms milk recorded monthly and the SCC data collected was collated along with incidence of mastitis within 30 days of calving. Cure rates (cows with SCC>200K cells/ml before drying off having a first test of SCC<200k cells/ ml after calving) and new infection rates (cows with SCC<200K cells/ml before drying off having a first test of SCC>200k cells/ ml after calving) were calculated from these data. Univariable and multivariable regression analyses were employed for data analysis.

Results: 287 cows were included in the study, 47% of the cows received full insertion of the cannula/e (n = 135), 30% of the cows received AB+ITS as allocated by the farmers (n = 86). There was no evidence to allow us to reject the null hypothesis; there was no difference in post-calving SCC, new infection rates, cure rates, or mastitis incidence when comparing FI versus PI. With regards to cows with low SCC before drying off, cows receiving PI were 1.01 times as likely to have high SCC post calving as cows receiving FI (95% confidence interval (CI): 0.42 to 2.46, P = 0.98). Cows in their second or greater lactation and cows calving in the Spring or Summer were more likely to acquire a new infection compared to cows in their first lactation and cows calving in the Autumn respectively. Factors associated with a high SCC post calving were: calving season, infection status before drying off, and lactation group; treatment (PI vs FI) was not statistically significantly associated with this outcome either. PI versus FI was also not associated with the cure rate post calving (cows receiving PI were 1.45 times as likely to have low SCC post calving as cows receiving FI; 95% CI: 0.30 to 7.06, P = 0.65). Cows in their first lactation were 9.86 times more likely to cure an infection comparing to older cows (95% CI: 0.83 to 117.62, P = 0.07). Cows in their second or greater lactation were 5.23 times more likely to be diagnosed with clinical mastitis the first month after calving comparing to cows in their first lactation (95% CI: 1.34 to 20.31, P = 0.02). Treatment (PI vs FI) was not associated with mastitis incidence.

Conclusions: In conclusion this study showed that when the correct aseptic technique is used for drying cows off there is no difference in post-calving infection status or mastitis incidence when comparing FI versus PI.

Acknowledgements: This study was funded by Boehringer Ingelheim Animal Health UK Ltd. The funder was involved in the study design but not in data collection and analysis.

References:

Leelahapongsathon K, T. Piroon, W. Chaisri, and W Suriyasathaporn. 2016. Asian Australas. J. Anim. Sci. Vol. 29, No. 4 : 580-585

Keywords: Udder health, drying off, internal teat sealants, mastitis, somatic cell count.

UH-13

Risk of clinical or subclinical mastitis following infusion of an internal teat sealant alone at the end of lactation in low somatic cell count cows

Scott McDougall[1], John Williamson[2], Khaled Gohary[1], Jane Lacy-Hulbert[2].

[1]Cognosco, Morrinsville, New Zealand; [2]DairyNZ, Hamilton, New Zealand.

Objectives: To identify risk factors for subclinical and clinical mastitis in dairy cows in the lactation following treatment with a bismuth subnitrate internal teat sealant (ITS) alone.

Methods: Cows (n=1,614 in total) from 36 herds were selected at random from within each herd from those cows with a maximum herd test somatic cell count (SCC) of <250,000 cells/mL and having had no history of clinical mastitis in the current lactation. In the last week of lactation, cows were assessed for the presence of teat end hyperkeratosis, milk samples were collected from each quarter following aseptic teat end preparation for microbiology, and a tube of ITS was infused into each quarter after the last milking. Herd owners (n=22) monitored for clinical mastitis in the first 60 days of the subsequent lactation. Herd-level risk factors including average bulk milk SCC in the last month of lactation, lactational cow-case clinical mastitis incidence, and cow-level risk factors including age, breed, presence of a major pathogen intramammary infection, milk yield at the last herd test of the lactation, and maximum SCC across lactation were considered as potential risk factors in multilevel binary logistic regression analyses.

Results: A total of 8.9 (95%CI=7.5-10.4) % of cows had subclinical mastitis as defined as a SCC >200,000 cells/mL at the first herd test in the subsequent lactation. Animals with a major pathogen infection at dry-off were more likely to have subclinical mastitis in the next lactation than those without a major pathogen (OR=4.7 (95%CI=2.29-9.65; p<0.001), animals >3-years-old were more like have a subclinical mastitis than 3-years-olds (OR=3.16 (95%CI=1.70-5.88), p<0.001), there was a positive association between cow-level milk yield at the last herd test of lactation and subclinical mastitis (OR=1.07 (95% CI=1.01-1.13), p=0.02), and there was a positive association between the natural log of the maximum SCC in the preceding lactation and subclinical mastitis in the next lactation (OR=1.54 (95%CI=1.13-2.10), p=0.01). The overall clinical mastitis incidence was 3.1 (95%CI=2.1-4.4)% in the first 60 days after calving in the next lactation. Cows from herds with a lactational cow-case incidence of >0.24 were at higher risk of clinical mastitis than cows from herds with a lactational cow-case incidence of <0.158, with cows from herds with cow case-clinical mastitis incidence intermediate not being different from these two categories. Cows yielding >15 L/day at the last herd test were more likely to be diagnosed with clinical mastitis than cows yielding <10 L/day, or 12.1 to 15 L/day, with those cows yielding 10 to 12 L/day intermediate between these categories. There was a positive linear association between the log maximum SCC in the preceding lactation and the risk of clinical mastitis (OR=1.96 (95%CI=1.09-3.54), p=0.03).

Conclusions: Both herd-and cow-level risk factors were identified for subclinical and clinical mastitis in the lactation following the infusion of an ITS. Hence cow selection criteria for infusion of ITS should consider herd level cow-case clinical mastitis incidence, cow-level milk yield at last herd test, as well as individual cow SCC.

Keywords: Internal teat sealant, mastitis, risk factors.

UH-14

Impact of clinical mastitis on the reproductive performance of eight Spanish dairy herds

Demetrio Herrera Mateo[1], Lorenzo Fraile[2], Oriol Franquesa[1], Laura Hurtado[1], Gemma Benaiges[1].

[1]Q-Llet, Seva, Spain; [2]Facultad de Veterinaria-UdL, Lleida, Spain.

Objective: The objective of this study was to determine if cows experiencing clinical mastitis within the period calving-conception, had worse reproductive performance than cows with no clinical mastitis during this period.

Material and Methods: Data from dry off and pregnant cows were collected in eight commercial Spanish dairy herds from September-18 to January-20. Data collected for every animal was lactation number, calving date, conception date, mastitis occurrence and mastitis date, days in milk to first clinical mastitis case and number of inseminations per pregnancy. A total of 4763 animals were analyzed, 443 cows with mastitis before conception and 4320 with no clinical mastitis before conception as a control group.

Calving-conception interval (CCI) and number of inseminations (NI) per pregnancy were used as indicators to compare reproductive performance between the two groups.

The association between the presence of mastitis with continuous non-normally distributed variables (CCI and NI) was analyzed using a non-parametric test (Wilcoxon test) using SAS V.9.1.3 (SAS institute Inc., Cary, NC).

Results: The average calving conception interval was 39.7 days longer for cows suffering mastitis ($\geq$1 cases) before conception than non-affected ones (P<0.001).

Cows suffering at least one clinical mastitis case before conception need on average 1.1 more inseminations to get pregnant than cows with no clinical mastitis before conception (P<0.001).

Conclusions: As shown in previous scientific studies, results are indicating a strong association between the occurrence of clinical mastitis before conception and the overall reproductive performance in Spanish commercial dairy herds.

Keywords: Mastitis, reproduction.

UH-15

Study of the effects of the administration of a phytogenic core on the decrease of milk production at dry-off in dairy cows

Plichart Gaetan[1], Gwydion Pinon[2], Raphaël Guatteo[3].

[1]Biodevas Laboratoires, Savigné l'Evêque, France; [2]Vet&Sphère, Quintin, France; [3]Oniris, Nantes, France.

Objective: Drying off, defined as the cessation of milking at the end of lactation in dairy cows, is an essential step allowing mammary involution, renewal of mammary epithelial cells and even the treatment of persistent intramammary infections.

However, it can lead to complications such as mammary edema, source of pain and discomfort, and milk losses, which can lead to mastitis. One of the main risk factors for such complications is milk yield at drying-off. Reducing milk yield quickly should prevent these complications and improve welfare, especially for cows still producing more than 20 kg of milk at drying off.

Materials and methods: The study, approved by of an ethical committee, consisted of a randomized double-blind, multicenter trial to evaluate the effectiveness of a phytogenic complementary feed on the reduction of milk yield in cows during the two days following drying off, compared to a placebo. Based on the hypothesis of a 50% reduction in milk loss, the most visible factor for a farmer, for a statistical power of 90%, with a precision of 5%, one hundred and six Prim'Holstein cows producing an average of 21.9 kg of milk on the day of drying off, located in 4 farms in Sarthe (France), were randomly allocated into 2 groups. Cows in group A received orally after their last milking 100 mL of a placebo while cows in group B received 100 mL of phytogenic extracts composed of artichoke (Cynara scolimus) and chaste tree (Vitex agnus-castus) (Parlac®, Biodevas Laboratoires).

Milk yield was evaluated indirectly before the last milking (D0), then 24 (D1) and 48h (D2) afterwards by two parameters: the mechanical nociceptive threshold measured with an algometer reflecting the mammary engorgement and the morphology of the udder, evaluated by the distance between the teats. Milk loss and rumination (through rumination collar system) time were also described and compared. The statistical unit was the cow. A Student's t test was performed on nociceptive threshold and rumination time, a mixed model with random effect was performed on teat distance and a Chi² test was performed on the percentage of cows with milk loss.

Results: At D0, no difference was found in the characteristics of the animals of the 2 batches, confirming the randomization as effective. At D2, the nociceptive threshold was not significantly different between the 2 groups. At D1, cows in group B tended to have a lower rear teat distance than those in group A (50.56 mm vs. 60.67, p-value = 0.1). The variation in rear teat distance was +18.34 mm for cows in group A and +12.20 mm for those in group B (p-value = 0.071). The total distance between all teats was significantly lower for group B (403 mm vs. 445 mm for group A, p-value = 0.035) reflecting less mammary engorgement. The percentage of cows with milk loss was significantly lower for group B (23% vs. 47% for group A, p-value = 0.017). In both groups, rumination time decreased, but less for cows in group B compared to group A, with -18 min and -52 min of decrease respectively, p-value = 0.06.

Conclusion: This study showed that PARLAC® reduces milk loss in the 2 days following drying off, reducing then mammary engorgement, and improving the well-being of dairy cows (rumination less affected). It provides a natural solution for farmers who implement a sudden drying off and wish to reduce the milk production of high-yielding cows to limit the risk of complications.

Keywords: Dry-off, phytogenics, dairy, bovine.

UH-16

MALDI-TOF Bacterial Subtyping for the Rapid Detection of Methicillin-resistant *Staphylococcus aureus* in Milk Bacteriology

Sara Fusar Poli[1], Martina Penati[1], Valentina Monistero[1], Clara Locatelli[1], Renata Piccinini[1], Paolo Moroni[2], Maria Filippa Addis[1].

[1]University of Milan, Lodi, Italy; [2]Cornell University, Ithaca, NY, United States.

Objectives: *Staphylococcus aureus* is one of the main pathogens involved in the development of contagious bovine mastitis and its presence is usually more associated with cases of subclinical than clinical mastitis. This pathogen shows low recovery rates, also due to antimicrobial resistance. A high priority has been given to the spread of methicillin-resistant *S. aureus* (MRSA), as it can have important implications for animal but also human health. Most of the MRSA strains present the *mecA* gene, held in a staphylococcal cassette chromosome (SCC*mec*) and acquired by horizontal gene transfer. The *mecA* gene is responsible for the synthesis of an alternative penicillin-binding protein (PBP2A), which is characterised by a low affinity for beta-lactam antibiotics, but also associated with a lower affinity to other antimicrobial classes. MRSA isolates are defined as multidrug-resistant. Recently, matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) has become the reference method for the identification of microbial species and can also be applied for the detection of antibiotic resistance. The aim of this study was to assess the performance of the Bruker MALDI Biotyper (MBT) system's subtyping module for the rapid identification of MRSA isolated from milk. After the species identification of *S. aureus*, the subtyping module identifies MRSA by detecting a peak at 2413 ± 2 m/z, which corresponds to PSM-mec, a small phenol-soluble modulin that is encoded in the SCC*mec* locus. We compared phenotypic and genotypic analyses of *S. aureus* strains with results obtained with the MBT subtyping module to establish the diagnostic accuracy of this method in routine practice.

Materials and methods: A retrospective study was carried out on 63 isolates of *S. aureus* from 27 different farms, collected during the diagnostic activity in the microbiology laboratory of the Department of Veterinary Medicine at the University of Milan. These isolates were previously classified as phenotypically resistant to methicillin based on susceptibility to oxacillin, a specific active substance routinely used for screening beta-lactam antibiotic resistance in *S. aureus* isolates. *S. aureus* strains were genotypically investigated for the presence of *nuc* and *mecA* genes by PCR. Species-level identification of *S. aureus* was confirmed by MALDI-TOF MS with the MBT system using the direct transfer method; a log (score) ≥ 2.0 is the threshold for species-level identification. All identified *S. aureus* isolates with a score ≥ 2.0 were immediately processed by the subtyping module of the MBT Compass software to detect a specific peak at 2413 ± 2 m/z corresponding to the PSM-mec.

Results: Genotypic analysis of 63 *S. aureus* isolates showed that all were positive for *nuc*, which is recognised as highly specific for *S. aureus*. Phenotypic resistance to oxacillin was confirmed by *mecA* gene carriage. All the 63 original

isolates were identified as *S. aureus* with a score ≥ 2.0 and therefore processed with the subtyping module, but only 11 strains were subtyped as 'presumptive PSM-positive MRSA'. The MBT detected only 17.5% of all MRSA identified by standard susceptibility tests and molecular analysis. The sensitivity reported was in agreement with previous results. In particular, the PSM-mec peptide detected by MALDI is only present in the SCC*mec* II, III and VIII cassette types.

Conclusion: MALDI-TOF MS is a rapid and accurate technique for bacterial identification, comparing to laborious and time-consuming biochemical tests and reliable but expensive PCR-based methods. On the other hand, its application to detect methicillin resistance in bovine *S. aureus* isolates is affected by limited sensitivity. However, it can be a valuable warning tool for the presence of MRSA in the herd, as this information is provided by the MBT subtyping module simultaneously with the identification of *S. aureus* without requiring further user intervention, sample processing, or additional materials. Future studies will be required to assess its specificity.

Keywords: Mastitis, Staphylococcus aureus, MRSA, MALDI-TOF.

UH-17

Test characteristics of differential cell counts to identify subclinical intramammary infections estimated by Bayesian latent class analysis

Tine Van Werven[1], Steven Sietsma[2], Mirjam Nielen[1], Gerrit Koop[1].

[1]Utrecht University, Faculty of Veterinary Medicine, Utrecht, Netherlands; [2]University Farm Animal Practice, Harmelen, Netherlands.

Objectives: Prudent use of antibiotics requires that the treatment of subclinical mastitis is based on the treatment of cows with a confirmed intramammary infection (IMI). Because subclinical mastitis is defined as an infection in absence of visible changes in milk or udder appearance, the use of a diagnostic test is needed. The three most commonly used tests are: 1) somatic cell count (SCC), 2) bacteriological culture (BC) and 3) polymerase chain reaction (PCR). All these tests are imperfect, and lack either sensitivity (BC) or specificity (PCR) or both (SCC). In this study, we investigate the performance of VETSCAN DC-Q™, an automated differential leukocyte device, which uses the different leukocyte counts in combination with the total SCC to diagnose IMI. A model that accounts for classification error is the latent class model, which allows estimation of the sensitivity (Se) and specificity (Sp) of different tests assuming no gold standard test.

The objective of our study is to determine the test characteristics of the VETSCAN DC-Q™ to detect quarter level IMI in combination with quarter level SCC (QSCC) at a threshold of 100.000 cells/mL, BC and PCR in cows with a new elevated cow level SCC just after calving (≤ 30 days), early lactation (> 30 days) and in cows with an elevated SCC in the last 4 weeks before drying off.

Material & Methods: The VETSCAN DC-Q™ has three different settings based on DIM of the animals to be tested. To validate the three different settings of the VETSCAN DC-Q™ a total of 449 cows, i.e. 1796 milk samples on quarter level, were collected on 8 Dutch dairy herds. Inclusion criteria were a 4-weekly DHI- participation and high new IMI rate. Cows were selected on first elevated cow level SCC (≥ 200.000 cells/mL) in current lactation or with elevated SCC within 4 weeks before dry off. Quarter samples were taken aseptically before cluster attachment, within one week after DHI sampling. Within 6 hours, these samples were split and QSCC (Delaval DCC) and VETSCAN DC-Q™ test were performed, BC and PCR samples were stored at -18°C and these tests were later performed at two different laboratories. Bayesian latent class models were built to determine Se and Sp of the 4 tests, assuming no gold standard test.

Results: Results of BC and PCR were classified as minor or major pathogens. Of the 1794 samples analysed 23% tested positive by BC, 42% tested positive by QSCC at a threshold of 100.000 cells/mL (QSCC>100), 8% was positive for a major pathogen in PCR, 68% was positive for a major and/or a minor pathogen in PCR, and 41% tested positive by VETSCAN DC-Q™. The most commonly isolated pathogens by BC were *Streptococcus uberis* (8% of all samples) and *Coagulase negative Staphylococcus spp.* (6%).

Results from the latent class analysis model that estimated Se and Sp of identifying IMI caused by major or minor pathogens showed VETSCAN DC-Q™to have a Se varying from 0.64-0.90 and a Sp of 0.63-0.86 for the three different lactation periods. These estimates were almost similar to the test characteristics of standard QSCC>100. Overall test characteristics were substantially better than those from BC, which had very low Se (0.37) and PCR, which lacked Sp (0.51). For diagnosing IMI caused only by major pathogens, the Se of the VETSCAN DC-Q™ varied between 0.90-0.98, and Sp for “Early Lactation” and “30+ DIM” was 0.71 and 0.76, respectively, but Sp dropped to 0.27 in the category “Dry Off”.

Conclusion: Altogether, our results suggest that the VETSCAN DC-Q™ has good test characteristics for diagnosing intramammary infections, but the added value over an undifferentiated QSCC measurement remains to be demonstrated.

Usability of the VETSCAN DC-Q™ will depend on the goal of the test. For screening cows with new IMI in early lactation in order to treat infections before they become clinical or chronic, it is a useful and convenient tool, with fair to near excellent Se and Sp at a quarter-level. For selecting cows at the end of lactation for selective dry cow therapy (SDCT) based on the presence of only major pathogens, the results of the VETSCAN DC-Q™ might lead to a higher use of antibiotics than necessary, because of the low Sp.

Keywords: Differential cell count; dairy; subclinical mastitis; Bayesian latent class analysis.

UH-18

CellCheck Dry Cow Consults-supporting the adoption of selective dry cow therapy in Ireland

Finola Mc Coy[1], Catherine McAloon[2], Michelle McGrath[1], Eoin Ryan[2], Javier Caballero Villalobos[3].

[1]Animal Health Ireland, Carrick-on-Shannon, Republic of Ireland; [2]University College Dublin, Dublin, Republic of Ireland; [3]Universidad de Cordoba, Cordoba, Spain.

Objectives: While blanket dry cow therapy has been commonplace in Ireland for many years, the recent European Veterinary Medicines Regulation (2019/6) means that preventive antibiotic usage in groups of animals, including dairy cows at the end of their lactation, is no longer acceptable. Hence, adopting a selective approach to dry cow treatment will require both a change in mindset and practice, for many Irish farmers and their prescribing veterinary practitioners. To facilitate this, CellCheck, the Irish national mastitis control programme, developed a Dry Cow Consult (DCC). The objective of this DCC was to enable farmers to engage with their nominated vet, to develop farm-specific selective dry cow treatment (SDCT) plans, where appropriate.

Materials and methods: DCCs have been delivered as part of the Targeted Advisory Service on Animal Health (TASAH) funded through the Rural Development Plan 2014-2022. Veterinary practitioners must first complete relevant training, delivered through the CellCheck programme, to be eligible to deliver a consult. To be considered eligible for the free service a dairy farmer must meet the following criteria:

- Average bulk milk tank somatic cell count (SCC) for the previous 12 months <200,000 cells/mL.
- At least 4 whole herd milk recordings in the previous 12 months.
- Members of HerdPlus, which is the Irish Cattle Breeding Federation's (ICBF) information service, providing herd-owners with performance data.

The purpose of the eligibility criteria was to identify farms that have some evidence of good mastitis control, as well as the necessary information and records to support decision-making and planning. During the three-hour consult, milk recording results and farm records are reviewed, as well as current practices when drying off cows and dry cow management, to help identify and resolve any potential risks associated with a selective dry cow strategy. Animals potentially suitable for receiving internal teat sealant only at drying off are identified. Participants of a DCC were invited to register for a Dry Cow Review the following year. Since 2018, 933 Consults and 268 Reviews have been completed.

Whole herd milk recording results, for the last recording before drying off and the first recording of the following lactation, were available for participating herds. Treatment records (both dry cow and in-lactation) were available, although were not complete for all participating herds. Telephone interviews were carried out with the participants of the consult service in 2018 (n=19). Audio recordings were professionally transcribed verbatim and analysed qualitatively using an inductive thematic analysis.

Results: Quantitative analysis showed that, although there was a slight increase in early lactation SCC in the group of cows treated exclusively with teat sealer at drying off, average SCC levels remained low (<200,000 cells/ml) and similar to those of cows treated with intramammary antibiotics at drying off. The CellCheck-recommended selection criteria for administering teat seal only at drying off were not always followed, with some farmers still including a small number of cows with a history of high SCC (>200,000 cells/ml) in this group. Results also showed that, while a considerable number of animals from each herd were eligible for SDCT, farmers were still reluctant to fully engage in this practice and maintained a cautious attitude.

The qualitative analysis identified 6 barriers and 6 facilitators to implementing SDCT. Barriers to SDCT included a significant fear of a resulting rise in mastitis incidence, infrastructural limitations, a perceived lack of availability of preventative advice, as well as peer influence. Facilitators to implementing SDCT included adopting a gradual approach to SDCT, regulatory pressure, high standards of farm hygiene, education and the use of targeted veterinary consults. Despite cited negative influences, peer influence can be utilised to encourage the farming community to change this particular behaviour.

Conclusions: SDCT has proven to be effective when used in cows with an adequate udder health status. While selective dry cow therapy is now a requirement under current Veterinary Medicines Regulations, for most dairy farmers in Ireland it will require a change in mindset and practice. Educating farmers on good drying-off routines is essential in order to build confidence and create awareness about the safety of moving to SDCT. Though there are challenges to face, engagement with professional support will be important and can be successfully facilitated through structures such as the Dry Cow Consult.

Keywords: Drying off, selective dry cow therapy, behaviour change.

UH-20

Gene expression of the teat canal epithelium in dry and lactating cows

Shirli Notcovich[1], Norman Williamson[1], Kristene Gedye[1], David Wheeler[2], Axel Heiser[3], Jimena Yapura[1], Ynte Schukken[4], Cord Heuer[5].

[1]Massey University, School of Veterinary Sciences, New Zealand; [2]Department of Primary Industries, NSW, Australia; [3]AgResearch, Palmerston North, New Zealand; [4]Wageningen University, GD Animal Health, Netherlands; [5]Massey University, Epi Centre, New Zealand.

Objectives: A keratin plug is formed in the teat canal during the dry period and protects the mammary gland against infections. However, mastitis incidence during the early dry period is high. Information about the variation of gene expression in the teat canal epithelium could provide insights into the physiology of the keratin plug formation during the early dry period.

The objective of this study was to identify differences in

gene expression in teat canal biopsy samples obtained at different time points during the early dry period and early lactation.

Materials and methods: In a first study (Experiment 1), the gene expression of teat canal samples from five cows in late lactation to early dry period were analysed. Samples from Day 0 (dry off day) and Day 11 of the dry period were compared using RNA-Seq. Genes differentially expressed between Day 0 and Day 11 samples were selected for further analysis. The RNA-Seq results were used to select 42 relevant genes including nine keratin genes of interest that were analysed for variation of gene expression in Experiment 2 by Nanostring nCounter. Repeated teat canal biopsy samples obtained from eight cows on days 0, 7, and 21 of the dry period, and on approximately days 10 and 25 after calving (Day 70 and 90) were also analysed. Oral mucosa samples were taken at the same time-points to serve as controls.

Results: Experiment 1 identified 36 differentially expressed genes of the teat canal epithelium at dry off (Day 0) and Day 11. Twenty-two of the genes were downregulated. Gene ontology enrichment analyses revealed that these genes were mainly related to binding, adherence, and RNA transcription factors. Analysis of individual gene functions showed that they were genes involved in mitosis and immune response pathways. Results from Experiment 2 showed different patterns of expression of the genes between the teat canal and the oral tissue (control) over time. Genes NR4R, KRT17, CSRP2, KIF23, TRPS1, LOC107131159, and TFPI2 were downregulated 7 days after drying off whereas RBBP8NL, PKIB, MARKS, TLR5, BAZ1A, IRX3, KRT4, PADI1, PENK, RND3, SERPINA1, SYT17 only presented differences after calving ($p<0.05$) in the teat tissue. The expression levels of the genes in a comparison of the results of both methods (Illumina and Nanostring) coincided, suggesting a decrease in gene expression after drying off in the teat canal related to cellular functions such as binding, adhesion, mitosis and immune response.

Conclusion: Modern gene expression analysis of the experiments reported in this study suggests that cell proliferation and immune response of epithelial tissue in the teat canal decrease during the early dry period and there is a major reactivation of gene expression after calving. The pathways affected might contribute to the higher incidence of mastitis during the early dry period. To our knowledge, this is the first study to investigate changes in gene expression of the teat canal epithelium during the early dry and lactating period. Further detailed studies on the physiology of the teat canal and keratin plug formation would be required for developing strategies to improve the immune response of cows against mastitis in the early dry period with the aim of decreasing the incidence of mammary infections.

Keywords: Keratin plug, dry off period, gene expression, teat canal.

UH-21

Early detection of mastitits using ultrasonographic teat and udder tissue changes and measuring echogenicity using an innovative method

Omranjit Singh, Dhiraj Kumar Gupta, Swaran Singh, B.K. Bansal.

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India.

Objective: Traditionally, the echogenicity of a tissue is expressed as anechoic, hypoechoic isoechoic and hyperechoic. However, this method is very subjective. So, a new method was devised to assess the level of echogenicity of a tissue with the help of software ImageJ. As mastitis is of high economic importance early diagnosis is necessary and USG can help in early diagnosis by measuring the changes in teat and udder tissue. So, the experiment was done to measure echogenicity of infected udder tissue and to evaluate the ultrasonographic changes due to intra-mammary infection of dairy cows.

Materials and Methods: Ultrasonography was conducted just before milking and quarter health status was determined based on milk SCC and bacteriological findings to determine the udder and teat tissue changes with respect to quarter health status. The ultrasound scans of teat structure were performed with a portable ultrasound scanning system (Sonosite M-Turbo) using a 10-5 MHz linear transducer by water bath technique or cup method. The teat tissue measurements were later done using the software ImageJ. The USG picture of udder tissue captured in RGB format was converted into greyscale (8-bit) format by using the same software. And in greyscale there are 256 shades ranging from pure black (0) to pure white (255). Division was done into 8 Grades(Grade1-8) , so that grade 1 has pixels ranging from 0 to 31, grade 2 had pixels ranging from shade 32-63 and so on till grade had shade 224 to 255.The software was used for the first time for the measurement of echogenicity.

A total of 32 apparently healthy lactating HF crossbred dairy cows, having 121 functional teats, were ultrasonographically scanned at university dairy farm. Quarter foremilk samples were aseptically collected and immediately transferred to the laboratory. Ultrasonographic readings such as teat canal length (TCL), teat cistern diameter (CD), teat wall thickness (TWT), overall teat diameter (OTD), the teat diameter at the level of the Furstenberg rosette (FTD) and associated changes in udder were recorded. The isolation and identification of microbial organisms from milk samples was done as per standard microbial procedures of National Mastitis Council (Brown *et al* 1969). Bromothymol blue (BTB) paper test, California Mastitis Test (CMT), electrical conductivity and pH of milk were performed. The analysis of milk samples for somatic cell count (SCC) (Delta Instruments, The Netherlands) was also done.

Results: Upon comparison of teat tissue measurements in relation to quarter health status, the TCL and CD were observed to be significantly increased in specific mastitis quarters compared to that of healthy ones. While TWT increased in quarters with latent infection and specific mastitis compared to healthy ones. The BTB score was significantly different in both non-specific mastitis and specific mastitis quarters, when

compared to both healthy and latent infection quarters. Similar observations were noted with CMT score, EC, pH and Log_{10}SCC values. The TWT was found to be significantly more in quarters with SCC 5-10 lac cells/ml of milk and in quarters with SCC > 10 lac, when compared with quarters having <2 lac and SCC 2-5 lac /ml of milk. The TCL, TWT and FTD were significantly more and CD less in infected teats in comparison to non-infected ones. Log_{10}SCC and CMT were significantly positively correlated with TWT and negatively with CD. During the measurement of echogenicity it was noted that pixels of higher grades (more echogenicity) i.e. G7 and G8 were absent from healthy quarters. And mean Log_{10}G1 was significantly lesser and Log_{10}G3 significantly higher for quarters with specific SCM thus suggesting that the amount of normal tissue (less echogenic) reduced due to infection and the echogenicity was also increased. The amount of pixels in G5 was significantly lesser in healthy and specific mastitic quarters as compared to quarters with latent infection.

Conclusion: It can be concluded from the results that **t**eat tissue changes can be detected in sub-clinically affected teats with the help of ultrasonography and Echographic measurements of teat were well correlated with milk inflammatory parameters so, they can be taken into consideration while assessing for infection. Grading, as indicated by our findings, can be used to measure echogenicity. The data is small, however with further valuation this technique can be standardized for better accuracy.

Keywords: Ultrasonography, teat, udder, tissue changes, echogenicity.

UH-22

The potential of intramammary administered cephapirin and cephalonium to select for ESBL-producing *E. coli* in the bovine gut and in dairy manure

David Speksnijder[1], Nina Kusters[1], Arjen Timmerman[1], Jantijn Swinkels[2], Pleun Penterman[2], Volker Krömker[3], Andrew Bradley[4], Nadine Botteldoorn[5], Aldert Zomer[1].

[1]Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands; [2]MSD Animal Health, Boxmeer, Netherlands; [3]Department of Veterinary and Animal Sciences, University of Copenhagen, Copenhagen, Denmark; [4]Quality Milk Management Services Ltd, Easton, United Kingdom; [5]Animal Health Care Flanders, Torhout, Belgium.

Objectives: Selection and spread of Extended Spectrum Beta-lactamase (ESBL)-producing Enterobacteriaceae within animal production systems and potential spill over to humans is a major concern. Orally applied cephalosporins can select for antimicrobial resistant organisms in the gastrointestinal tract. There is however limited research on the effect of non-oral, locally applied, antimicrobials on the selection of resistant organisms in the gastrointestinal tract. First generation cephalosporins are widely used to treat and prevent intramammary infections in dairy cows. We studied the potential effects of low doses of cephapirin (CP) and cephalonium (CL) to select for ESBL producing *E. coli* in fresh faecal fermentations and in the manure of adult dairy cows as a model for intramammary application of cephapirin, because the minimal concentration which still selects for resistance (MSC) is unknown in a complex environment like the gastrointestinal tract.

Materials and Methods: A literature search has been conducted, to determine the test conditions for the MSC testing and to predict the maximum expected concentrations of CP in the intestinal content and in the manure of dairy cows after intramammary treatment.

Three different laboratory experiments have been conducted on samples from The Netherlands, Germany, Belgium and the United Kingdom.

1): Competition assay in rich culture media at 37^{0}C. From every country, 3 ESBL and 3 non-ESBL *E. coli* isolates from dairy farms have been collected. A 1:3 ratio mixture of these ESBL and non-ESBL isolates under nine different conditions have been tested for their potential to select for Cefotaxime (CTX) resistance as an indicator for ESBL selection: no antibiotics baseline; no antibiotics (blank condition); 0.25 µg/ml CTX; 0.04, 0.4 and 4.0 µg/ml of CL; 0.08, 0.8 and 8.0 µg/ml of CP. After 6 h of incubation the percentage of resistant colonies to CTX in each of the different test conditions have been assessed to determine the MSC of CP and CL for ESBL producing *E. coli*.

2): Competition assay in fecal fermentations at 37^{0}C. Freshly derived manure samples from 10 different healthy dairy cows were pooled and were spiked with the 3 ESBL and 3 non-ESBL *E. coli* isolates at 10^7 CFU/ml in 9 different tubes containing anaerobic standard ileal efflux medium. A comparable approach as in experiment 1 has been followed to determine the MSCs for ESBL producing *E. coli* in freshly derived fecal fermentations of dairy cows.

3): Competition assay in dairy manure. Manure pit samples from each country where taken. The same procedures were being followed as in experiment 2, however at a temperature of 17^{0}C.

Results: In experiment 1, a significant (p=0.007) increase in CTX resistant colonies was seen between a 0.8 µg/ml (45% resistant) and a 8.0 µg/ml (96% resistant) concentration of CP. No significant increase from 0.08 (38%) to 0.8 µg/ml was observed. A significant (p=0.023) increase in CTX resistant colonies was seen between a 0.4 µg/ml (41% resistant) and a 4.0 µg/ml (90% resistant) concentration of cephalonium.

In experiment 2, a borderline significant (p=0.079) increase in CTX resistant colonies was seen between a 0.8 µg/ml (44% resistant) and a 8.0 µg/ml (75% resistant) concentration of CP. The results were skewed by the unexpectedly low number of CTX resistant colonies from the Belgian samples. In the experiment with different concentrations of CL (0.04, 0.4 and 4.0 µg/ml), no significant differences in CTX resistant colonies was observed (respectively 78%, 88% and 91%).

In experiment 3, no increases in CTX resistant colonies have been observed in the CP and CL group at each of the tested concentrations.

Based on available literature on pharmacodynamics and pharmacokinetics of CP and CL in dairy cows, the maximum expected concentration of CP and CL in the intestinal content of adult dairy cows after intramammary treatment according to the label will most probably not exceed 0.29 µg/ml. The expected maximum concentration of CP and CL in manure pits of

average dairy farms will not exceed 0.03 µg/ml.

Conclusions: We found that the expected concentrations of cephapirin in the gut and manure pits are at least 10 fold lower than the MSC of cephapirin to select for CTX resistance in the different environments. Thus, the potential of intramammary administered cephapirin to select for ESBL-producing *E. coli* in the bovine gut and in manure pits seems low.

Keywords: Antimicrobial resistance, intramammary, cephapirin, cephalonium, dairy cow.

UH-23

Comparison of endotoxin concentrations among commercially available Gram-negative, lipopolysaccharide core-antigen vaccines used to control bovine mastitis

Norman Stewart[1], Scott Nordstrom[1], John Champaign[1], Alison Hurwitch[1], Barbara Markley[2], Joe Hogan[3].

[1]Merck Animal Health, Madison, NJ, United States; [2]Beacon Diagnostics, East Falmouth, MA, United States; [3]Ohio Agricultural Research and Development Center, the Ohio State University, Wooster, United States.

Objective: Lipopolysaccharide core-antigen vaccines have been developed to provide cross-protection against a variety of Gram-negative bacterial pathogens capable of causing clinical mastitis in dairy cows. Though a variety of products have been shown to reduce the severity and duration of coliform intramammary infections, these products differ in formulation including adjuvants and carrier solutions. A compositional difference of potential concern is a variation among products in endotoxin concentration. Adverse effects attributed to parenteral administration of endotoxin to cows include reproductive failures, anorexia, and decreased milk production. The purpose of this study was to quantify the endotoxin concentrations in Gram-negative, lipopolysaccharide core-antigen vaccines used to control bovine mastitis.

Materials & Methods: Four commercially available bacterins tested for endotoxin were vaccine A (Bovilis®-J5; Intervet, Inc.), vaccine B (Enviracor™ J-5; Zoetis, Inc.) vaccine C (Endovac-Dairy®; Endovac Animal Health) and vaccine D (J-Vac®; Boehringer Ingelheim Vetmedica, Inc.). Nine bottles of each vaccine, each bottle from a unique lot/serial number, were tested for endotoxin concentration by the *Limulus* amebocyte lysate assay using kinetic turbidimetric detection.

Data were analyzed using a linear mixed model for a complete block study design. A single sample from each vaccine lot/serial number was the experimental unit, and a random intercept term was included in the model to account for the lack of independence among samples within replicates. A fixed effect of vaccine, with four categories, was included in the model. The data were not normally distributed so they were transformed (log10) prior to analysis with a model fitted with a normal (Gaussian) and identity link. The model was fitted with restricted maximum likelihood estimation, Kenward-Roger degrees of freedom and Newton-Raphson and Ridging optimization procedures (Proc GLIMMIX SAS 9.4). When the main effect of vaccine product was significant (P <0.05), pair-wise comparisons were made using the Tukey-Kramer method to adjust for multiple comparisons.

Results: The overall effect of vaccine product was significant (P <0.05); rejecting the null hypothesis the endotoxin levels were equal among products. Similarly, pair-wise comparisons of all vaccine products indicated that all means differed significantly (P values <0.01). Model-adjusted mean for vaccine A was 1.43 $\log_{10}$ EU/ml (range 0.53 to 2.09 $\log_{10}$ EU/ml) vaccine B 3.77 $\log_{10}$ EU/ml (range 3.62 to 3.90 $\log_{10}$ EU/ml), vaccine C 4.90 $\log_{10}$ EU/ml (range 4.65 to 5.19 $\log_{10}$ EU/ml) and vaccine D 5.54 $\log_{10}$ EU/ml (range 5.34 to 5.66 $\log_{10}$ EU/ml).

Conclusion: Gram-negative, lipopolysaccharide core-antigen vaccines used to control bovine mastitis differed in endotoxin content with a difference greater than 13,000-fold in endotoxin units per milliliter between the lowest and highest concentrations.

Keywords: Endotoxins, vaccination, bovine mastitis, Gram-negative.

UH-24

Treatment of clinical mastitis: intramammary or in combination with parenteral administration of penicillin?

Line Svennesen[1], Alice Skarbye[1], Michael Farre[2], Carsten Kirkeby[1].

[1]University of Copenhagen, Frederiksberg C, Denmark; [2]SEGES, Skejby, Denmark.

Objectives: Antibiotics prescribed for dairy cows in Denmark are mainly penicillin products for parenteral mastitis treatment (DANMAP, 2020). However, it may be possible to reduce the amount of penicillin used, if intramammary (IMM) treatment is sufficient as an alternative to parenteral or combined IMM and parenteral treatment which is the first choice for Danish veterinarians (Wilm et al., 2021). This study aimed to investigate if IMM administration of penicillin was non-inferior to IMM combined with parenteral administration of penicillin regarding bacteriological cure of non-severe clinical mastitis cases caused by Gram-positive bacteria.

Materials and methods: The study was carried out as a non-inferiority longitudinal randomized clinical trial in 12 Danish dairy herds from May 2020 to June 2021. The treatment protocols evaluated were **A**) IMM treatment for three days, and **B**) both IMM and parenteral treatment for three days. The IMM treatment comprised 600,000 IE benzylpenicillinprocain administered in the affected quarter once per day for three days. The parenteral treatment comprised 15,000 IE penethamate-hydroiodid/kg body weight administered in the muscle (IM) once per day for three days. Treatment A and B were randomly assigned to cows with non-severe clinical mastitis caused by a Gram-positive bacteria. The farm personnel used an on-farm test to get an indication of this classification (Gram-positive/negative) within the first day of symptoms and before the onset of antibiotic treatment. In the meantime, all clinical mastitis cases were treated with ketoprofen in a dose of 3 mg/kg body

weight. Subsequently, the quarter milk samples were cultured and pathogens were identified using MALDI-TOF. A case was included if one or two bacterial species were identified, and at least one of those were Gram-positive. Bacteriological cure was evaluated on two follow-up samples, two and three weeks after ended treatment. A case was deemed to be cured if the pathogens present in the clinical sample were not detected in any of the follow-up samples. A non-inferiority analysis was carried out with a margin of 15%.

Results: Of almost 1,800 clinical mastitis cases registered, 347 were eligible for evaluation of bacteriological cure based on treatment A or B. The results showed that the cure rate depended on the pathogen. The overall cure for treatment A was 76% [95% CI: 69.2-82.5] and the overall cure for treatment B was 83% [95% CI: 76.8-88.3]. Treatment A was not non-inferior to treatment B with a margin of 15% across all bacterial species. However, the majority (183) of included mastitis cases involved *Streptococcus uberis* infection, either alone or in combination with another species. When including only these cases in the analysis, the cure rate for treatment A was 83% [95% CI: 74.1-90.1], and the cure rate for treatment B was 81% [95% CI: 70.9-88.3]. Thus, treatment A was non-inferior to treatment B for cases with *Streptococcus uberis*.

Conclusions: Penicillin administered IMM for three days is no worse than penicillin administered both IMM and parenteral for three days when it comes to bacteriological cure of non-severe clinical mastitis caused by *Streptococcus uberis*. Implementation of this result in Danish dairy herds can reduce antibiotics used for clinical mastitis treatment. However, the reduced treatment was not found non-inferior across all bacteria species. This underlines the importance of accurate mastitis diagnostics and the need to adjust treatment protocols to the herd's specific mastitis pathogens, in order to facilitate prudent use of antibiotics.

The research project was funded by the Danish Milk Levy Foundation.

References: DANMAP. 2020. Danmap 2020 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. ISSN 1600-2032.

Wilm, J., L. Svennesen, E.Ø. Eriksen, T. Halasa, and V. Krömker. 2021. Veterinary Treatment Approach and Antibiotic Usage for Clinical Mastitis in Danish Dairy Herds. Antibiotics 10:189. doi:10.3390/antibiotics10020189.

Keywords: Mastitis, treatment, intramammary, cure.

UH-25

Identification of cows with delayed milk ejection using milk flow recordings

Paola Bacigalupo Sanguesa, Pamela Ruegg, Roger Thomson, Bo Norby, Ronald Erskine.

Michigan State University, East Lansing, United States.

Objectives: The objective of this study was to identify an interval of milk flow, from a parlor recording system, that would best identify individual cows with normal and delayed milk ejection and for that time interval, to determine the milk flow cutoff with the best specificity to identify cows with normal milk ejection.

Materials and methods: We connected digital recorders to measure vacuum (VaDias, BioControl) in the short milk tube and mouthpiece chamber of two liners during individual milking in 4 farms, and we collected milk flow dynamics from milking recording system software (DeLaval DelPro Software and SCR MC200 Series Milking Control Systems), for 529 cows. We obtained milk ejection time (seconds) after unit attachment by analyzing the milking vacuum data, as a proxy for milk flow, for each cow. Cows were categorized as either having delayed (> 30 s) or normal (≤ 30 s) milk ejection. Individual milk ejection time and milk ejection category were matched to milk flow during four time intervals: ≤ 15 s, 16-30 s, 31-60 s, and 61-120 s. We performed univariate and logistic regressions for each milk flow interval using milk flow as the dependent variable and milk ejection time and milk ejection category as independent variables, respectively, on SAS. Finally, we calculated sensitivity and specificity for milk flow cutoffs of 1.8, 2.3, and 2.7 kg/min (4, 5, and 6 lb/min) liters per minute.

Results: Univariate (table 1) and logistic regressions (table 2) of the four-time intervals on ejection time and milk ejection category, respectively, identified milk flow between 31-60 s as the best time interval to predict delayed milk ejection (R-square 0.33, P <.0001; LS means milk flow of 1.7 kg/min and 3.6 kg/min for delayed and normal milk ejection cows, respectively (P <.0001). For the 31-60 interval, a cut-off of 1.8 kg/min had the best specificity of 0.87, and a sensitivity of 0.64.

Conclusions: Delayed milk ejection lowers milking efficiency and can decrease milk production, impair teat health, and can negatively impact animal welfare. Producers and veterinarians can use of on-farm technology that measures individual milk flow dynamics to identify and monitor delayed milk ejection incidence in their herds, allowing them to look for underlying causes and intervene when needed to improve and maintain milking efficiency.

Table 1. Regressions of milk flow time intervals and milk let-down time.

Milk flow time interval (s)	R-Square	P-value
0-15	0.0125	0.01
16-30	0.0735	<.0001
31-60	0.3288	<.0001
61-120	0.0087	0.03

Table 2. ANOVA of milk flow time intervals and milk ejection category.

Milk flow time interval (s)	Delayed milk ejection LS Means	Normal LS Means	P-value
0-15	0.89	1.14	0.002
16-30	2.05	2.96	<.0001
31-60	1.67	3.6	<.0001
61-120	3.93	4.02	0.51

Keywords: Milk flow, delayed milk ejection.

UH-26

Emergence of cloxacillin resistance in *Streptococcus uberis*

Heather King[1], John Williamson[2], Jane Lacy-Hulbert[3], Yvette Macpherson[1], Ali Karkaba[1], Scott McDougall[1].

[1]Cognosco, Morrinsville, New Zealand; [2]DairyNZ, Hamilton, New Calcedonia; [3]DairyNZ, Hamilton, New Zealand.

Objective: To assess whether there has been a change in penicillin and cloxacillin minimum inhibitory concentrations (MIC) across time (2003 to 2018) in bovine *Streptococcus uberis* isolates.

Materials and Methods: *S. uberis* from clinical and subclinical mastitis cases from one farm during 2003/04 and 2018/19, were re-isolated from a preservation media (LSPQ Preservation Medium, Fort Richard Laboratories Ltd, Auckland, New Zealand). Isolates were confirmed as *S. uberis* based on Gram stain, catalase reaction, cleaving of esculin, SF broth, cleavage of inulin, and MALDI-TOF confirmation for non-conclusive reactions. MIC was determined using semi-quantitative penicillin and cloxacillin test strips (Liofilchem, Roseto degli Abruzzi, Italy) placed on a lawn of *S. uberis* grown on Mueller Hinton CLSI agar plates with sheep blood (Fort Richard Laboratories Ltd, Auckland, New Zealand). Differences in distribution of MIC for penicillin and cloxacillin between the 2003 and 2018 sample sets were assessed using frequency distributions and histograms, and visually assessed for a bimodal distribution. The MIC_{50} and MIC_{90} and their 95% confidence intervals were calculated. The key outcome variable was the epidemiological cut-off value (ECOFF), which was determined visually. Explanatory variables included: year 2003 (n=112) vs 2018 (n=44), clinical (n=46) vs subclinical (n=110) mastitis, spring (n=128) vs other (n=28) seasons, and timing of sampling relative to calving (≤4 days vs >4 days). Bivariate (chi-squared and univariate logistic) and then multivariable linear mixed regression models were used for analyses. Manual stepwise model building approach was used and variables with a $p > 0.05$ were removed from the final model, so long as the odds ratio for other variables in the model did not vary by greater than 20%.

Results: The MIC_{50} and MIC_{90} were 0.047 (interquartile range 0.03-0.09) and 0.19, respectively for penicillin, and 0.25 µg/mL (interquartile range 0.19-1), and 2 µg/mL respectively for cloxacillin. There was a bimodal distribution for the MIC values for penicillin and cloxacillin, and the ECOFF were defined as 0.064 and 0.38 µg/mL for penicillin and cloxacillin, respectively. A total of 28% of the isolates were above the ECOFF points for both penicillin and cloxacillin. The proportion of MIC values above the ECOFF increased from 23% to 40% for both penicillin and cloxacillin between 2003 and 2018. In the final model the proportion of isolates above the ECOFF increased over time (OR=1.06, CI 95% 1.08-1.11, p=0.02) and (OR=1.07, CI 95% 1.02-1.13, p=0.01) for penicillin and cloxacillin, respectively; and with a higher proportion above the ECOFF for samples collected outside of spring (OR= 2.95, CI 95% 1.03-10.7, p=0.06) and (OR= 3.02, CI 95% 1.05-11, p=0.059) for penicillin and cloxacillin, respectively.

Conclusion: The proportion of *S. uberis* isolates above the ECOFF for penicillin and cloxacillin increased between 2003 and 2018. While this data is from only one farm, it supports data from MIC estimates for *S. uberis* isolates from bulk tank milk across New Zealand which demonstrate increasing cloxacillin MICs (McDougall *et al.* 2018). In New Zealand, cloxacillin is widely used for therapeutic treatment during lactation, and prophylactically at dry off. It was commonly used in the herd from which these isolates were drawn. Isolates from later in lactation had higher MIC. Whether this is due to survival of these isolates following treatment or is associated with persistence and host adaptation remains to be determined. The clinical significance of the highest MICs observed i.e. 0.38 µg/mL for penicillin, and 4 µg/mL for cloxacillin remains unclear. However, a recent report suggests that MICs for oxacillin >0.5 µg/mL tended to be associated with reduced bacteriological cure following therapy of clinical *S. uberis* cases (McDougall *et al.* 2020).

References:

McDougall S, Castle R, MacPherson Y, Karkaba A, Graham L. The DAIRYANTIBIOGRAM; A novel way to monitor antimicrobial sensitivities of mastitis pathogens in dairy herds. In: *Proc Dairy Cattle Veterinarians of the New Zealand Veterinary Association*. Hamilton, New Zealand Vetlearn, NZVA, 2018.

McDougall S, Clausen L, Ha H-J, Gibson I, Bryan M, Hadjirin N, Lay E, Raisen C, Ba X, Restif O, Parkhill J, Holmes MA. Mechanisms of b-lactam resistance of *Streptococcus uberis* isolated from bovine mastitis cases. *Veterinary Microbiology*, 108592, 2020.

Keywords: Mastitis, S. uberis, MIC, Resistance.

UH-27

An Investigation into Optimising Antibiotic Use through Selective Use of Dry Cow Treatment at the Quarter Level

Andrew Bradley[1], Katharine Leach[2], James Breen[3], Barbara Payne[2], Vikki White[2], Martin Green[3], Jantijn Swinkels[4].

[1]Quality Milk Management Services Ltd / University of Nottingham, Wells, United Kingdom; [2]Quality Milk Management Services Ltd, Wells, United Kingdom; [3]University of Nottingham, Sutton Bonington, United Kingdom; [4]MSD Animal Health, Boxmeer, Netherlands.

Objectives: Blanket use of antibiotic dry cow therapy (DCT) was a cornerstone of the Five Point Plan. More recently, the selective use of antibiotic DCT in combination with blanket use of an internal teat sealant (ITS) has been successfully implemented in the UK. Historically, cow level application of antibiotic DCT has been advocated, primarily because quarters are not independent within cows and therefore an increased risk of infection has been perceived in 'uninfected' quarters in 'infected' cows. However, there is evidence that this lack of independence is less marked with 'environmental' than 'contagious' mastitis pathogens. The aim of this study was to investigate the utility of a quarter level approach to DCT in well managed low SCC herds with a low prevalence of contagious mastitis pathogens.

Materials and Methods: Farms with a history of low bulk

milk SCC were invited to participate in the study. Cows, within herds, were stratified as 'infected' or 'uninfected' (using SCC and clinical mastitis history), before being randomly allocated to one of three treatment groups: CLT, QLT0 and QLT1. The CLT (Cow Level Treatment) group were allocated, using SCC and clinical mastitis history, into animals eligible for the use of ITS alone (Cepralock™) or ITS in combination with antibiotic DCT (CEFA-SAFE™) - importantly this decision was applied at the cow level (all quarters within a cow receiving the same treatment). Within the QLT0 (Quarter Level Treatment - CMT>0) and QLT1 (Quarter Level Treatment - CMT>1) groups, quarters within cows were allocated (based on a California Mastitis Test (CMT) score of >0 or >1 respectively) to receive ITS alone (score below the threshold) or ITS in combination with antibiotic DCT (score above the threshold) depending on the quarter CMT score at drying off.

The bacteriological status and SCC of all quarters were assessed at drying off and within a week post calving and for SCC 7-14 days post calving. All cows were monitored for clinical mastitis from dry off until 100 days post calving.

Univariable and multivariable analysis were undertaken to assess the effectiveness of the three approaches for their impact on dry period outcomes including the likelihood of a quarter being pathogen free at calving. The impact on clinical mastitis was also assessed.

Results: 807 cows from six herds were recruited; 401 were defined as 'infected' and 406 as 'uninfected' by historic SCC and clinical mastitis data. The overall prevalence of infection at drying off was low with only 10.5% of quarters culturing a major pathogen. Minor pathogens were prevalent with 52.8% of quarters culturing positive for these species.

In cows defined as 'infected' at drying off, analyses suggested that there were no significant differences between treatment groups in the likelihood of a quarter being positive for a major pathogen or any pathogen post calving (p>0.05). In cows defined as 'uninfected' at drying off, whilst there was no difference in the likelihood of a quarter being culture positive for a major pathogen, quarters in QLT0 group (antibiotic DCT administered to quarters showing a 'trace' reaction on the CMT) were at significantly lower odds of being culture positive for a minor pathogen post calving than quarters in cows receiving a teat sealant alone (OR 0.658; 95%CI 0.488-0.889).

No significant differences in the proportion of quarters affected by clinical mastitis were observed between any of the treatment groups.

Antibiotic use was assessed with respect to the number of cures effected by treatment. In cows defined as 'infected' at drying off, cow level treatment achieved the highest 'cure' rate of major pathogens (97.7%) but was associated with the highest level of antibiotic tube usage/cure (13.66 tubes/major pathogen cure); the QLT1 group had the lowest tube usage (5.3 tubes/cure) but also the lowest cure rate (89.0%). In cows defined as 'uninfected' at drying off, the 'self-cure' was 100% in the CLT and QLT0 groups. Three quarters failed to 'cure' in the QLT1 group, though these were due to infections with pathogens against which antibiotic DCT would not have been effective.

Conclusions: In low SCC herds, with a low prevalence of contagious pathogens, there appears to be an opportunity to reduce antibiotic DCT use by selecting treatments at the quarter level without adversely affecting udder health.

Keywords: Udder Health, Dry Cow Therapy, Antibiotic, Mastitis.

UH-28

Impact of selective dry cow theraphy on farm udder health

Almudena Tato Mosquera, Noelia Mourazos Garcia, Carlos Noya Couto, Francisco Sesto Perez, José Luis Miguez Vázquez, Margarita Penelas López.

SERAGRO S.C.G, Galicia, Spain.

Objectives: The aim of the present retrospective observational study was to compare how the use of selective dry cow therapy (SDCT) influences udder health instead of blanket dry cow therapy (BDCT) in a set of dairy cattle farms impacts udder health.

Materials and methods: This study was carried out from January 2017 to December 2018, with the implementation of the SDCT in 25 selected dairy farms of Friesian cattle that traditionally applied BDCT. These farms were located in Galicia (North-western Spain) and associated with the Milk Quality Service.

Selection criteria

In order to be selected, farms must have had a sustained constant annual somatic cell counts(SCC) <250,000, percentage of monthly clinical mastitis <3% and no contagious isolates.

In cow selection, for the use of internal sealants only, no intramammary antibiotic therapy, the criteria were that the last three months of SCC <200,000, absence of clinical episode records at least in the last three months and negative result to the California mastitis test at the time of drying.

Furthermore, key factors for the selection of the farm were considered knowing their epidemiology, as well as good management practices around the dry period . At the cow level, it was considered important that the animals reach drying with the anatomical integrity of the sphincter in good condition.

Udder health parameters

After the SDCT implementation during the years 2017 and 2018, for evaluating herd udder health we used: changes in somatic cell count and percentage of monthly clinical mamitis, both parameters related to the subsequent lactation, comparing BDCT (2016) and SDCT (2018).

Also two rates of dry period performance were used: prevalence of infections at calving (percentage of cows with first postpartum SSC> 200.00 with respect to total calves) and rate of new infections at calving (percentage of animals that when drying had a SCC 200,000 and that in the first postpartum control had a SCC >200,000 with respect to the total cows that were dried with a control lower than SCC 200,000).

Data analysis

All data were stored in our own software "Gesgando". To

compare the monthly SCC of the 25 farms in 2016 and 2018, a linear regression model was developed. The Naperian logarithm of monthly SCC was used as a response variable and year, as an explanatory variable, taking into account month and farm as random effects. To compare the monthly percentage of clinical mastitis, a Poisson regression model with farm and month as random effects and year as an explanatory one has been performed.

Results: From January 2017 to December 2018, 2,210 cows were dried applying the selective dry cow therapy (SDCT), only 635 received intramammary antibiotic drying, that is, 71.27% of the cows only carried internal sealant, with the consequent antibiotic reduction.

The results obtained with respect to SCC were 159 cells/mL in tank (2016) to 166 cells/mL (2018). The percentage of average monthly clinical mastitis moved from 1.66% (2016) to 1.48% (2018). The effectiveness rates of drying therapy: prevalence of infection at calving 18.29% (2016) and 21.75% (2018), and rate of new infections at calving 16.4% (2016) and 17.76% (2018).

When analyzing the data, it is observed that the monthly SCC shows significant differences (pvalue <0.001) between years, being higher in 2018 (166.62 ± 42.59) than in 2016 (158.85 ± 38.68), on the contrary, percentage of monthly clinical mastitis shows a reduction (p- value <0.001) from 2016 (2.39 ± 2.93) to 2018 (1.57 ± 2.19).

Conclusion: The transition from BDCT to SDCT resulted in a huge reduction in the number of antimicrobials used on dairy herds without having a clear deleterious effect on udder health regarding general data obtained. When managed appropriately the dry and transition period and selected cows with optimal udder health can be successfully dried off without the use of intramammary antimicrobials.

The increase in the SCC after the new therapy implementation with a no clinical mastitis rate associated, maybe must be addressed with an improvement and even higher requirements in the conditions of dry cow management in a global context of antibiotic reduction.

Keywords: Cow, dry, selective, theraphy, health.

UH-29

Internal teat sealant retention after selective dry cow therapy in dairy cows

J. M. Swinkels[1], A. Deterink[2], M. Holstege[2], A. Schmenger[3], G. D. Kempe[2], T. Bruggink[2], P. Penterman[1], V. Kromker[4].

[1]MSD Animal Health, Boxmeer, Netherlands; [2]Royal GD Animal Health, Deventer, Netherlands; [3]Hannover University of Applied Sciences and Arts, Hannover, Germany; [4]Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

Objectives: Internal teat sealants (ITS) are increasingly used in the EU for prophylaxis of new IMI in the dry period due to their efficacy and the concerns for prophylactic use of antibiotics (AB). ITS require no milk withdrawal time, and residues may enter the bulk tank and end up in cheese, affecting the image of the dairy industry. Current ITS residue data are mainly based on visual presence, and objective data of their weight in time after calving are lacking. The primary objective was to determine the quantity of ITS excreted in milk, when used alone or combined with AB, during the first week after calving.

Materials & Methods: The study was conducted on 3 farms in NL, and 4 in DE, selected on availability of DHI records, a conventional milking system, milking 2x/d and willingness to participate and comply to the protocol. Cows were treated with AB (Cefa-safe®, MSD Animal Health) in all 4 quarters only when the last monthly DHI test before dry-off (DO) showed a cow SCC > 200k cells/ml (high SCC). All cows were dried off with ITS (ShutOut®, MSD Animal Health). At DO, teat end callosity score, body condition score, milk leakage and udder pressure were measured.

Cow eligibility at DO included pregnancy, expected DO period > 32 d, > 3 functional quarters, milk yield > 5 L, good general health, and absence of clinical mastitis and concomitant treatment within 30 d of DO. During the 1st 7 DIM, 50 ml of milk was collected pre-milking at each milking, centrifuged, and the amount of remaining ITS and milk solids weighed. ITS in mg/milking was calculated by deducting the tubes total weight from the final 2 milkings, assuming no TS was left in the tubes (± 2SD), from the weight of the tube including all its content (ITS + milk solids). Quarter milk samples were collected aseptically prior to the final milking and DO treatment, and at 3 DIM, to determine etiology and SCC. Clinical mastitis events and general health were monitored from D0 to 30 DIM. ITS retention and treatment effects will be evaluated using generalized linear mixed models.

Results: A total of 103 cows were included in the preliminary analysis, 76 cows were 'high SCC' at DO, and treated with ITS+AB, and 27 cows were 'low SCC' at DO and treated with ITS alone. At DO, 63.5% of quarters were cultured positive and most identified pathogens were Non-aureus staphylococci (NAS, 20.8%), Staph. aureus (5.9%) and Lactococcus species (2.1%). At 3 DIM, 48% of quarters were cultured positive, and most identified pathogens were NAS (12.3%), Staph. aureus (1.4%), E. coli and yeast (both 1.2%). Bacteriological cure or new infection rate analysis is ongoing. Just prior to the 1st milking, 76.6% of quarters (n=423) contained ITS > minimum detection limit, quickly declining to 26.7% at the 2nd milking, 8.3% at the 3rd milking, and at 1.8% at the 14th milking. The ITS excretion curve analysis in g/milking is ongoing. Differences could be detected between the ITS retention when combined with AB or not.

Preliminary efficacy analysis of ITS was based on 219 quarters, 98 with a qSCC < 200k cells/ml and 121 qSCC > 200k cells/ml at DO. Comparing qSCC data (n=219) at DO and at d3 after calving, using a qSCC of 200k cells/ml as a threshold for imm infection, prevention of new infection rate, cure rate, new infection rate and rate of failure to cure, was 74.5%, 71.9%, 25.5%, and 28.1%, respectively. Generalized linear mixed models to identify risk factors for ITS retention and ITS efficacy are currently developed and the analysis is ongoing.

Conclusion: ITS residues were detected in 76.6% of quarters at the 1st milking after calving, declining to < 10% of quarters at the 3rd milking, and < 2% of quarters at the

14th milking. Differences in ITS excretion were detected when combined with AB or not. This study shows the importance for farmers to comply to EU regulations to not enter colostrum in the bulk milk tank and to install milk filters to prevent ITS residues from entering the bulk tank.

Keywords: Teat sealant, dry cow therapy, dairy cattle.

UH-30

The use of cabergoline at dry-off increased milk production in a commercial dairy herd in Brazil

Philippe Gisbert[1], João Rodrigues[2], Carla Azevedo[1], Fernanda Viscione[2], Juan Muñoz[1], Alexandre Souza[3].

[1]Ceva Sante Animale, Libourne, France; [2]Ceva Saúde Animal, Paulínia, Brazil; [3]Cargill, Campinas, Brazil.

Cabergoline is a dry off facilitator that reduces milk production at dry-off by inhibiting prolactin secretion. It has been recently demonstrated that one single injection of cabergoline at dry-off effectively reduces milk leakage by 82% and new intramammary infections by 21% (Hop et al, 2019), accelerates mammary gland involution and improves its immune response (Boutinaud et al, 2016, 2017). Interestingly, recent literature has reported also an increase in milk production in the following lactation, in those cows where prolactin was inhibited in the first days after dry off (Lacasse, 2015). However, and to the authors knowledge, no studies to date have investigated the impact of the use of cabergoline at dry-off on dairy cow's milk production in the subsequent lactation and under Brazilian field conditions.

Objectives: The objective of this study was to investigate, under Brazilian field conditions, the benefits of reducing prolactin secretion at dry-off on the subsequent lactation performance with one single administration of cabergoline at dry-off (Velactis®, Ceva Santé Animale, Libourne, France).

Materials and methods: The trial was conducted from March 2019 to February 2020 in a commercial Holstein dairy herd in upper-Southwest Brazil in Minas Gerais State. A total of 417 Holstein cows, producing 27,9 ± 3.8 kg at drying off, were dried-off abruptly and treated with intramammary antibiotic therapy (Bovigan-VS®, Bayer Animal Health, Brazil) plus an internal teat seal (Ememast Selante®, Boehringer Ingelheim Animal Health, Brazil) at dry-off. Cows were randomized to two experimental treatments, as follows: 1) Cabergoline 5.6 mg (5mL, Velactis®, Ceva Santé Animale, France) given intramuscularly to cows with even ear tags, or 2) Control (saline, 5 mL) given intramuscularly to cows with odd ear tag numbers. Immediately after treatment, cows were moved to a new dry cow pen and kept in a far-off diet for approximately 4 weeks. Then, 3 to 4 weeks before calving all cows were moved to a close-up prepartum pen and fed an anionic-based diet until calving. Total daily milk production was recorded once within the week prior to drying off, and throughout lactation at 20-day intervals during milk testing performed from calving until 200 DIM, the records on incidence of retain placenta, clinical mastitis, and breeding records were retried from the herd's management software (Ideagri®, Brazil) and the edits in the final database included in the data analysis. Statistical analyses were performed using the SAS 9.4.

Results: There was a significant effect of treatment ($P < 0.05$) on milk production, in which cows treated with Cabergoline produced significantly more milk from calving to 200 DIM (Cabergoline: 7395 Kg vs Control: 7017 Kg; $P = 0.03$). There were no differences between experimental groups in the proportion of cows with clinical mastitis within 30 (Cabergoline: 6.0 ± 0.04 vs Control: 6.0 ± 0.05; $P = 0.95$) or 100 days post calving (Cabergoline: 13.9 ± 0.05 vs Control: 12.0 ± 0.04; $P = 0.71$). Similarly, treating cows with Cabergoline at dry off did not influence proportion of cows having retain placenta (Cabergoline: 11.4 ± 0.4 vs Control: 11.6 ± 0.3; $P = 0.97$) and proportion of cows conceiving by 150 DIM (Cabergoline: 69.0% vs Control: 65.5%; $P = 0.45$).

Conclusions: Treatment with Cabergoline at dry-off effectively improved milk production in Holstein cows. In addition, findings from the current trial indicate that cows treated with Cabergoline at dry-off produce in average 1.89 kg/day more milk than control cows. This represents extra income for the farmers and provide data to help producers evaluate best return on investment of differing technologies available that can be used at the time of dry-off.

Keywords: Dry-off, Mastitis, Dairy Herd, Milk Production, Cabergoline.

UH-31

Productive and reproductive impact of subclinical mastitis by prototheca in a commercial dairy farm in central Spain

Natividad Pérez -Villalobos[1], Marina Cañaveras[2], Cristina Andreu[1], Raquel Patrón[3], Susana Astiz[4], Juan Vicente González[3].

[1]Universidad Europea de Madrid, Villaviciosa de Odón, Spain; [2]Clínica Olfato, Madrid, Spain; [3]TRIALVET SL, UCM, Madrid, Spain; [4]INIA, Madrid, Spain.

The genus *Prototheca* is a microalgae, unicellular, aerobic and achlorophyllic and ubiquitously distributed in commercial dairy farms. Although it is becoming an emerging and important disease in dairy cattle and despite of producing a fatal mastitis, this disease has been scarcely studied in terms of epidemiology, costs and specifically regarding the subclinical infections of this pathogen.

Objectives: Therefore, the aim of this study was to quantify the concrete consequences of the subclinical protothecal mastitis, regarding yield (predicted 305-day milk yield, fat and protein yields) and udder health (mean somatic cell count).

Matherial&methods: The retrospective and observational study was carried out 2016-2018 due to a natural outbreak at a commercial dairy cattle farm in central Spain, with 900 animals in milk and 500 heifers of which approximately 30% of the total herd was infected with the algae. Animals with clinical mastitis, without previous lactation to the outbreak (heifers) or cows that standed in the same lactation (due to a long lactation), or without available data were discarded from the study. A to-

tal of 418 cows were included in the study and based on the analysis of its milk (PCR t first screening and microbiological culture at periodical revisions) were distributed into the group of healthy or infected cows (39.23 and 60.77% of the animals, respectively). Yield, udder health and reproduction data during the previous lactation to the infection were compared within each individual cow to those to the lactation during the infection. The reproductive rates studied through collective farm data were the days in lactation at the first insemination as well as at the first heat, the average number of empty days and the interval between births. Absolute yield values during the lactation of the infection of infected and healthy cows were also compared. Saphiro-Wilk test determined the non-normality of the data and non-parametric tests (Mann Whitney U) were used to analyze the differences.

Results: The protothecal mastitis produced significantly lower projected milk yield to 305d in the infected cows (P<0.001) when compared to non infected cows (median of 8,847.26L vs 11,281.66 L) while non-infected cows showed no significant differences with respect to their previous lactation. The somatic cell count was 485.85 and 1,392.81 for healthy and infected cows (P<0.001). There was no significant difference in fat and protein yields with respect to the previous lactation (P=0.01).

In addition to the consequences due to the productive outcomes, we observed that collective reproductive results showed that the reproductive performance of the farm worsened between January 2016 and January 2017, when comparing the days to the 1st artificial insemination, the days to the first heat, the average number of open days and the interval between births. However, when analyzing the farm data for January 2018, these indicators showed an improvement that indicate that the measures taken were in the right way. Nevertheless, there were an obvious decrease in the number of total inseminations as well as in the intention to inseminate in the cows that were identified as positive.

Conclusions: In conclusion, our data indicate that the productive losses caused by subclinical mastitis produced by *Prototheca* in dairy cattle are very high, with a great impact on the quality of the milk produced and a possible impact on reproduction, which requires individual analysis of the data to be clarified. To all these consequences we must add the impact of this type of infection on the prevention and control measures used to control the outbreak. Finally, we consider of interest to continue with the analysis of the data regarding subsequent lactations in the positive surviving population to study their evolution.

Keywords: Mastitis, Prototheca.

UH-32

Procaine penicillin for mastitis - Have we forgotten how good it is and is it getting even better?

Mick Clews[1], Matt Wells[2].

[1]Herd Health Services, Western Australia, Australia; [2]Virbac NZ, Hamilton, New Zealand.

Objectives: This study compares outcomes of clinical mastitis treated with intramammary procaine penicillin and the market leading cloxacillin and examines if MIC can explain differences in case cure rates.

Material & Methods: 16,500 cows from 35 seasonal calving dairy herds were monitored for clinical mastitis. Pre- and post-treatment samples were cultured, isolates identified, and MIC determined using Liofilchem® test strips. Cows were treated with either 1) cloxacillin (CL) 5 times s.i.d., 2) procaine penicillin (PP) 3 times b.i.d. ,or 3) the PP treatment followed by further treatment with a product containing both PP and CL (PC) 3 times s.i.d. starting 48 hours after initial case diagnosis. Treatment regimens were selected to conform with the New Zealand registered use. Case cure rate (CCR) (both clinical and bacteriological) was measured.

Results: Of the 767cases S. uberis (SU) was most commonly isolated (43.0%), followed by S. aureus (SA) (15.29%). In the case of SU, PP had a higher CCR than CL (76.83% vs 61.25%, p=0.01). In the case of SA, the combined PC treatment had a higher CCR than CL (40.96% vs 24.36%, p=0.02) and PP tended to be better than CL (CCR = 40%, p=0.08). The MIC50 and MIC90 of CL (0.125 and 0.250µg/ml) were higher than those of PP (0.023 and 0.190µg/ml) in SA, and much higher in SU (0.380 vs. 4.0, and 0.047 vs. 0.190µg/ml, for CL and PP, respectively). There was no difference in MIC50 for cases that cured vs. non-cured regarding either treatment. CCR for SU cases treated with CL, was lower though for isolates when MIC was ≥2µg/ml (p=0.04).

Conclusion: The MIC values for PP in this study were lower than previous studies in NZ and fit a trend of significant decrease in regards both SU and SA over the last 30 years. 32% of SU isolates had a MIC of CL ≥2µg/ml which is higher than the minimum inter-treatment concentration (ITCmin) achieved in milk. The MIC for all other isolate/treatment combinations was below the ITCmin. Individual MIC was only predictive of clinical outcome when it was >ITCmin. Better case outcome, supported by lower MIC (particularly in the predominant SU) and a New Zealand Vet Assoc. classification as a more responsible medicine class, support the intramammary use of procaine penicillin over cloxacillin for clinical mastitis.

Keywords: Procaine penicillin, cloxacillin, mastitis, treatment.

UH-33

Farmer and veterinarian perception on udder health management

Michael Farre[1], Carsten Thure Kirkeby[2], Tariq Halasa[3], Erik Rattenborg[4], Henk Hogeveen[5].

[1]KU SUND, Vejen, Denmark; [2]KU SUND, Copenhagen, Denmark; [3]Novo Nordisk, Bagsværd, Denmark; [4]SEGES Innovation, Århus, Denmark; [5]Wageningen University, Wageningen, Netherlands.

Objectives: Mastitis is the most costly disease in dairy herds in developed countries (Hogeveen et al., 2019) and a significant reason for compromised animal welfare (von Key-

serlingk et al., 2009). We have much knowledge on mastitis and udder health management, allowing for evidence-based improvement. In recent years, motivation and perception of the farmer have received increasing attention, with communication research (Jansen et al., 2010b; Jansen et al., 2010a), to successfully implement procedures such as the NMC 10-point plan(NMC, 2016). As a consultant, the veterinarian has a crucial role in udder health management, and their behavior has been described (McDermott et al., 2017). However, the effect of veterinary consultancy depends on the interaction between farmer and veterinarian. Because perceptions of udder health can differ between farmer and herd veterinarians, it is crucial to identify potential gaps in agreement to understand the impact on the outcome BMTSCC.

Material and methods: The participating dairy farmers and veterinarians were enrolled on the Danish National Cattle Data Base data. The criteria for participating were: DHI recording, > 90 % Holstein cows, parlor milking, conventional herd, herd size > 100 cows located in the western part of the country. The dairy farmers were contacted first and encouraged to participate in an anonymous survey conducted in a larger research project on udder health management. Next, the herd vet was contacted and encouraged to participate. The study enrolled a total of 88 herds with their herd veterinarian. The survey included questions regarding cooperation between farmer and herd veterinarian and the consulting methods by the vet. Veterinarians received the questionnaire by email and were reminded if not responding within two weeks. The dairy farmers filled out the questionnaire during the visit from the researcher. The answers were predominantly based on the Likert scale (Likert, 1932) to measure agreement between dairy farmers and herd veterinarians.

Results: The response percentage was 100 % for the dairy farmers and 94 % for the herd veterinarians. Initial analysis was an agreement based on Cohens Kappa and weighted Kappa value. Focusing on some of the results, the farmer regards the vet as most important in handling udder health in the dairy herd, with 91 % answering agree or strongly agreeing to this question. Also, in converting Evidence-Based Scientific Data to operational consulting, 74 % of the farmers agree or strongly agree about the vet`s effort. The data will be further analyzed, focusing on the agreement between the farmer and the vet, linked to the outcome BMTSCC as a proxy of udder health. This will high light where the herd veterinarian needs to focus to maintain the role as resource in the area udder health management in competition with other professionals and equipment supplier.

Conclusions: The initial results highlight differences in perception between farmer and herd veterinarians regarding udder health. Identifying potential obstacles is essential because the farmer sees the herd veterinarian as critical in udder health consulting. The vet translates evidence-based knowledge in udder health management to apply practical solutions for the farmer.

References:

Henk Hogeveen, W.S. and C.A.W. 2019. Production Diseases Reduce the Efficiency of Dairy Production_A Review of the Results, Methods, and Approaches Regarding the Economics of Mastitis.

Jansen, J., R.J. Renes, and T.J.G.M. Lam. 2010a. Evaluation of two communication strategies to improve udder health management. J. Dairy Sci. 93:604–612. doi:10.3168/jds.2009-2531.

Jansen, J., C.D.M. Steuten, R.J. Renes, N. Aarts, and T.J.G.M. Lam. 2010b. Debunking the myth of the hard-to-reach farmer: Effective communication on udder health. J. Dairy Sci. 93:1296–1306. doi:10.3168/jds.2009-2794.

von Keyserlingk, M.A.G., J. Rushen, A.M. de Passillé, and D.M. Weary. 2009. Invited review: The welfare of dairy cattle—Key concepts and the role of science. J. Dairy Sci. 92:4101–4111. doi:10.3168/JDS.2009-2326.

Likert, R. 1932. A technique for the measurement of attitudes.. Arch. Psychol. 22 140:55.

McDermott, M.P., M.A. Cobb, V.A. Tischler, I.J. Robbé, and R.S. Dean. 2017. Evaluating veterinary practitioner perceptions of communication skills and training. Vet. Rec. 180:305. doi:10.1136/vr.103997.

NMC. 2016. Recommended Mastitis Control Program. Natl. Mastit. Counc. www.nmconline.org/docs/NMC/checklist.pdf. 1–2.

Keywords: Management, udder health, social science.

UH-34

Fly repellency using deltamethrin reduces intramammary infections, stress and fatigue indicators of dairy ewes under intensive management

Konstantinos V. Arsenopoulos[1], Georgios Sioutas[1], Eleutherios Triantafillou[2], Athanasios I. Gelasakis[3], Elias Papadopoulos[1].

[1]Aristotle University of Thessaloniki, Thessaloniki, Greece; [2]Vet Analyseis, Veterinary Microbiological Laboratory, Larissa, Greece; [3]Agricultural University of Athens, Athens, Greece.

Objective: The aim of the study was to assess the fly repellency effect of deltamethrin and link it to the occurrence of common bacterial intramammary infections (IMI), milk somatic cell counts (SCC), the serum cortisol (SC) and creatine kinase (CK) levels (stress and fatigue indicators, respectively) during the fly season.

Materials and Methods: The study was conducted in one intensively reared dairy sheep flock of Lacaune breed, located at Thessaloniki (Central Macedonia, Greece), between June and July 2020. Fifty multiparous ewes were randomly assigned in two similar groups (n=25 ewes per group); deltamethrin treated group (D group) and control group (C group). Group D ewes were individually dressed on their back with deltamethrin 10% (Deltanil® 10 mg/mL, Virbac Hellas, Greece) on day 0. The enumeration of the fly burden was carried out by direct observation of the animals on day 0 and two more times with 15-days intervals (i.e., day 15 and 30) to assess the repellency effect of deltamethrin. Moreover, 10 fly traps (5 per group) with sticky surface were set in predefined locations of equal distances within the pens at the level of the ewes. Individual blood samples were collected at the forementioned time points, while an electrochemilu-

minescence immunoassay method and spectrophotometry were used to estimate SC and CK concentrations, respectively. On every sampling occasion, milk samples were collected from each individual ewe and were transported to the laboratory to be microbiologically assayed, emphasizing on the isolation of *Staphylococcus aureus* and Coagulase-negative Staphylococci (CNS). Moreover, SCC were estimated using an automatic high-throughput analyzer. Mixed linear regression models were built to estimate the random effect of the ewe and the fixed effects of deltamethrin treatment and sampling occasion on the logarithmic values of flies, *S. aureus* CFU (Colony-forming units), CNS CFU, SCC, as well as SC and CK levels. All statistical analyses were performed using SPSS (v23).

Results: Deltamethrin treatment was associated with a reduced number of flies (mostly *Musca domestica*) landed on treated ewes, compared to untreated ones ($p<0.05$). Also, the application of deltamethrin was associated with decreased colony-forming units in the case of CNS IMI ($p<0.05$) and SCC in the milk ($p<0.001$). Likewise, SC and CK levels were significantly lower in the deltamethrin-treated ewes ($p<0.001$).

Conclusions: Deltamethrin application was associated with decreased colony forming units of CNS, isolated from IMI cases. Furthermore, the reduction in the logarithm of SCC in the deltamethrin-treated group implies that fly repellency using deltamethrin favours the health status of the mammary gland possibly via the reduction of pathogens transferred by flies and the inflammation caused by them. Finally, reduced SC and CK concentrations in the deltamethrin treated ewes support the assumption that fly-repellency effect of deltamethrin facilitates the control of flies and contributes to a more welfare-friendly environment for intensively reared dairy ewes.

Keywords: Dairy ewes, deltamethrin, fly, mastitis.

UH-35

Bulk-tank somatic-cell count as an indicator for mastitis-infection rates and fertility in the herd

Viktoria Neubauer[1], Franz-Ferdinand Roch[2], Walter Obritzhauser[3], Martin Wagner[2], Beate Conrady[4].

[1]Austrian Competence Centre for Feed and Food Quality, Safety, and Innovation, FFoQSI GmbH, 3430 Tulln, Austria; [2]Unit for Food Microbiology, Institute for Food Safety, Food Technology and Veterinary Public Health, University of Veterinary Medicine, 1210 Vienna, Austria; [3]Unit of Veterinary Public Health and Epidemiology, Institute of Food Safety, Food Technology and Veterinary Public Health, University of Veterinary Medicine, 1210 Vienna, Austria; [4]Department of Veterinary and Animal Sciences, University of Copenhagen, 1870 Frederiksberg C, Denmark.

Objectives: Some dairies in Austria use stricter milk-quality parameters than other EU and non-EU countries. Farmers only receive enhanced payment for the highest milk quality, if the bulk-tank milk somatic-cell count (SCC) is <200,000 cells/ml, and the aerobic mesophilic count (AMC) is <50,000/ml. The background of more strict thresholds is that the regular 400,000 cells for shipping milk are rather high compared to the maximum SCC of 150,000 found in a healthy udder. The objective of this study was to show that a bulk tank SCC of <200,000 is an indicator for better udder health, in terms of mastitis-infection rates, and better herd-management, indicated by fertility and nutritional-related parameters, compared to higher SCC in the bulk tank.

Materials and methods: Data from 24 Austrian dairy farms (on average 36 cows with 8470 kg milk/cow/305 days) were collected for two years. Bulk-tank somatic-cell count was used to assign the farms to low SCC herds (LSCC; >200,000 cells/ml in maximum 10% of the tests), and high SCC herds (HSCC; >200,000 cells/ml in more than 10% of the tests). None of the farms ever exceeded the maximum threshold of 400.000 cells for shipping milk. From the herd data of at least 20 routine-milk tests, individual cow data (i.e., SCC, number of lactations, days in milk, protein-urea class, waiting time, days open, calving interval) were extracted. The individual cow SCC were assigned to SCC-classes (class1 ≤50,000; class2 ≤100,000; class3 ≤200,000; class4 ≤400,000; class5 >400,000 cells). Class 1 and 2 were considered as healthy, class3 as suspect, class4 and 5 as infected. Cows were considered chronically infected with being at least two consecutive tests infected, or three consecutive tests suspect or infected. The new-infection rate was calculated using the first milk-testing SCC of the lactation. SCC classes before and after the dry-off period of the same cow were used to calculate the infection or healing rate during the dry period. Statistical analysis was done using Mann-Whitney-U, Moods-Median, or chi²-Test, including posthoc tests, depending on the variables analyzed and data distribution. HSCC and LSCC were the fixed effects, and the evaluated herd data and mastitis indicators as dependent variables.

Results: LSCC had more cows in SCC-class1 (51%) and fewer cows in class3 (15%), 4 (7%), and 5 (6%) than HSCC (28, 23, 15, 13%, respectively; $P<0.01$). HSCC had more chronically infected cows (44%) than LSCC (20%; $P<0.01$). LSCC had more healthy cows and less infected cows before dry-off than HSCC (55 vs. 31%, 22 vs. 41%, respectively; $P<0.01$). New-infection rate and suspect animals at the beginning of the lactation were higher in HSCC than LSCC (24 vs. 12%, 15 vs. 8%, respectively; $P<0.001$). A higher new-infection rate on HSCC was seen for both primiparous ($P=0.06$) and multiparous ($P<0.01$) cows. The proportion of cows staying healthy during the dry period was twice as high in LSCC than in HSCC (48% vs. 24%; $P<0.01$), and of staying infected was higher in HSCC (15 vs. 5%; $P<0.01$). The waiting days, days open, and calving intervals were shorter in LSCC than HSCC ($P<0.01$). LSCC had more cows in the optimum protein-urea class, reflecting optimal feeding regimen than at HSCC (37 vs. 31%, $P<0.01$).

Conclusion: The study showed that a change to a bulk tank SCC of <200,000 is an indicator for lower mastitis disease rates in the individual cows. The more optimal fertility parameters and feeding regimen prove that LSCC farms also have better general herd management. Better udder health and optimal management are indicators for overall better animal health on these farms. Therefore, quality programs should consider lower thresholds for the bulk tank milk to value the work of LSCC farms and support the improvement of HSCC farms to contribute to better dairy-cow health.

Keywords: New infection rate, Healing rate, cow health, udder health.

UH-36

Dry period management and new high somatic cell count during the dry period in Dutch dairy herds under selective dry cow therapy

Betsie Krattley[1], Lotte Huybens[2], Mirjam Nielen[3], Tine Van Werven[3].

[1]ULP Harmelen, Harmelen, Netherlands; [2]Vet Clinic IJmond Noord, Castricum, Netherlands; [3]Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands.

Objective: The aim of this study was to describe the current management practices that could influence udder health during the dry period and to investigate the associations between management and new high SCC during the dry period. In 2008 the Netherlands started to improve transparency and decrease the use of antimicrobials in animal husbandry. Prevention of new high SCC during the dry period via antimicrobial dry cow treatment (DCT) was no longer allowed in animals with a low SCC before the dry period. An increase of new high SCC during the dry period was expected in dairy cows without antimicrobial protection, but was not observed.

Materials & Methods: In 2018 an online questionnaire was conducted among 1,942 Dutch dairy farms using 12 different veterinary clinics. The questionnaire asked about the management of dry cows at the start of and during the dry period, and around calving, considerations in the use of DCT, and knowledge of the 2012 guidelines for selective DCT. A total of 690 farmers (36%) responded to the questionnaire. Data on new high SCC during the dry period, use of antimicrobials for intramammary DCT and mastitis treatment, herd size, and milking system were available from other sources. Descriptive statistics were used to evaluate the associations between the different variables. For analysis of new high SCC on herd level, explanatory variables were analyzed using a generalized linear mixed model.

Conclusion: Respondents indicated that the most important management factor to reduce the risk of new high SCC was reduction of milk yield before dry-off. The variables associated with a lower proportion of new high SCC on herd level during the dry period were the use of dip or spray after drying off, a higher animal-defined daily dose of intramammary antimicrobials for DCT, the use of DCT in low-SCC cows based on SCC or mastitis history, correct knowledge of the guideline, and awareness of importance of low infection rate and good hygiene during dry-off. The variables associated with a higher proportion of new high SCC on herd level were dry cow housing other than cubicles and a higher animal-defined daily dose for intramammary antimicrobials for mastitis. This research clearly indicates that farmers can balance limited use of antimicrobials at dry-off with management measures to maintain good udder health during the dry period.

Keywords: Dry cow management, selective dry cow therapy, new high SCC.

UH-37

Efficacy of a ready-to-use udder care product based on lactic acid for daily teat disinfection after milking by spraying

Tiago Tavares[1], Elien Claeys[2], Lawrence Grab[2], Korneel Decroos[2], Elisabet Rodriguez[3].

[1]CID LINES, An Ecolab Company, Lisbon, Portugal; [2]CID LINES, An Ecolab Company, Ieper, Belgium; [3]CID LINES, An Ecolab Company, Sant Joan Despí, Spain.

Objectives: The goal of the present trial was to assess the efficacy of a ready-to-use udder care disinfectant based on lactic acid (Kenolac® SD, PT3 biocidal product-type, from CID LINES, An Ecolab Company) for daily teat disinfection after milking by spraying.

Material & Methods: Two studies were designed to assess the efficacy of the product. In the laboratory study, Kenolac® SD disinfectant efficacy was tested according to the European Norms (EN 1040 and EN 1656) against mastitis causing bacteria (test conditions: in presence of organic matter, in 5 minutes contact time).

Test Organism	Concentration	Contact time	Temperature	Interfering substance
Bacteria (EN1656) Gram + *Staphylococcus aureus* (ATCC 6538) *Streptococcus ub*eris (ATCC 19436) Gram - *Escherichia coli* (ATCC 10536) **Yeast (EN1657)** *Candida albicans*	Ready to use	5 mins	30 °C	1% skim milk Dirty conditions

In the field study, the impact of two methods of application of Kenolac® SD on the microbiological contamination of the teats was evaluated: spray and dip. The samples are taken during milking. Kenolac® SD was used after each milking with a sprayer or a dip cup, respectively (recommended dosing regimen: 5 mL per cow per application). After dipping Kenolac® SD on the teats, waiting for 3 minutes contact time was required.

The reduction of bacteria growth was evaluated for the two application methods. The scoring system was based on bacteria count (colony-forming unit-cfu/ml): the bacterial reduction for Staphylococcus spp. and Coliforms count. These bacteria are a good indicator of the mastitis infection pressure, from contagious and from environmental sources.

Results: Laboratory trial showed that Kenolac® SD reduced the number of viable microorganisms tested by more than 5 log (bacteria) and 4 log (yeast) during 5 minutes of contact at 30°C under dirty conditions (1% skim milk).

Statistical analysis of the microbiological results obtained at the field trial showed similar results for the spray application than for the dip application regarding percentage of

bacteria reduction on the teats after milking. A high microbiological reduction was achieved for spray application for total *Staphylococcus spp* (93,83%, n=114), *Staphylococcus aureus* (92,94%, n=114) and Coliforms (99,47%, n=114). For dip application, reduction was 90,76% (n=119), 91,4% (n=119) and 98,22% (n=119), respectively.

Conclusions: The efficacy of Kenolac® SD, ready-to-use udder care biocide based on lactic acid, has been demonstrated to be efficacious in reducing the amount of microorganisms involved in dairy cow mastitis, when applied by spraying the teats after milking.

Keywords: Mastitis, disinfection, teat dip, teat spraying, lactic acid.

UH-38

Bovine Ischaemic teat necrosis: an observational study investigating the potential farm level risk factors and economic impact on GB dairy farms.

Hayley Crosby-Durrani[1], Al Manning[2], Roger Blowey[3], Joao Sucena Afonso[1], Stuart Carter[1], Nicholas J. Evans[1], Joseph W. Angell[4].

[1]University of Liverpool, Neston, United Kingdom; [2]Quality Milk Management Services, Wells, United Kingdom; [3]Appithorne, Minsterworth, United Kingdom; [4]Wern Vets CYF, Ruthin, United Kingdom.

Bovine ischaemic teat necrosis (ITN) is an emerging disease affecting the teats of dairy cattle with many affected animals being culled due to the disease process. Little is known around the epidemiological and economic data that can inform control strategies.

Objectives: The aim of this observational study was to investigate farmer-reported experiences, identify potential farm-level risk factors ad provide an estimate on the economic impact of ITN on GB dairy farms.

Materials and methods: In January 2018, a postal questionnaire with an online and telephone option was sent to a random sample of 1855 Great Britain (GB) dairy farmers. Descriptive statistics were utilised to understand the main at-risk animals and the potential outcomes of the disease. The economic impact was estimated based on the different clinical outcomes. Univariable and multivariable logistic regression models were used to explore associations between the presence of ITN on farm and various risk factors.

Results: A usable response rate of 12.3% was obtained. Fifty-one percent (95% confidence interval (CI): 44.4 - 57.8%) of farmers reported having experienced ITN on their farm between 1985-2018. Rising numbers of farms indicated ITN is an emerging disease with 46.3% of farmers reporting the first case in the three years up to 2018. At the animal level, 47.3% (95% CI: 38.7-55.9%) of cases occurred during the first lactation and 78.9% (95% CI: 75.2-82.6%) within the first 90 days in milk. Only 20.8% (95% CI: 15.9-26.4%) of cases were reported to recover, whereas 22.8% (95% CI: 17.8-28.5%) of cases required culling. The remaining cases experienced complications such as loss of a teat and/or mastitis. From these data, the cost of ITN, through production losses and expenditure, was estimated to be £1121 per farm per year. The costs were estimated at £720, £860 and £2133 for recovered, complicated and culled cases, respectively. The presence of udder cleft dermatitis (UCD) (odds ratio 2.80; 95% CI: 1.54-5.07; p-value <0.01) and chapped teats (odds ratio 6.07; 95% CI: 1.96-18.76; p-value <0.01) in the milking herd were associated with the presence of ITN at farm-level.

Conclusions: This is the first national questionnaire of ITN within GB and highlights the association of UCD and chapped teats with ITN at farm-level. While there are many limitations and potential bias around farmer questionnaires these findings highlight several key areas for further disease investigation and possible intervention.

Keywords: Bovine; Ischaemia; Necrosis; Questionnaire; Risk factors; Dairy.

UH-39

The use of cabergoline at dry-off reduces the probability of milk leakage and dry-off stress-related behaviours in large commercial dairy herds in Torreón, Mexico

Philippe Gisbert[1], Juan Munoz[1], Oclydes Barbarini[1], Rolando Morales[2], Fernanda Quinones[2], Carla Azevedo[1].

[1]Ceva Santé Animale, Libourne, France; [2]Ceva Salud Animal, Mexico City, Mexico.

Objectives: Cabergoline is a dry-off facilitator that reduces milk leakage by 81% and new intramammary infections by 21% (Hop et al, 2019), accelerates mammary gland involution and improves its immune response (Boutinaud et al, 2016, 2017), and improves lying time (Bach et al, 2015). However, no studies to date have investigated the benefits of the use of cabergoline at dry-off on dairy cows under Mexican field conditions. The objective of this study was to investigate, under Mexican field conditions, the effect of one single administration of cabergoline (Velactis®, Ceva Santé Animale, Libourne, France) at dry-off on milk leakage incidence and frequency of dry-off stress-related behaviours.

Materials and methods: The trial was conducted in 2018, and involved 322 Holstein- Frisian dairy cows from 3 modern commercial dairy farm in the area of Torreón. Cows were enrolled in the trial if they were 210 days in gestation and daily milk production was equal or more than 25kg the day before dry-off. All cows were dried off abruptly and randomly assigned to one of two groups: treatment group (n = 161) received a single intramuscular injection of 5.6 mg of cabergoline after last milking at 217 days of gestation (dry period length of 53 days) and the control group (n=161) was dried off at 210 days in gestation (dry period length of 60 days) and left untreated. Cows were observed at 24- and 48-hours following treatment for a period of 2 hours. The incidence of milk leakage and frequency of observation of dry-off stress-related behaviour (agitation, increased vocalization, waiting in front of the gates to the milking parlour, aggressive behaviour) were recorded during both periods. Chi-square test was used to determine if

relation between variables was significant.

Results: The results demonstrated that cows in cabergoline group were less likely to have milk leakage than cows in control group (1.9% vs. 13.4%, P < 0.01). Moreover, cows treated with cabergoline were less likely to express at least one dry-off stress-related behaviour than cows in control group (9.5% vs. 29.8%, P < 0.01).

Conclusions: It is the first time that the impact of cabergoline on milk leakage and dry-off stress-related behaviour is evaluated in Mexican commercial dairy farms. Our data provide evidence that a single injection of cabergoline at dry-off significatively reduces the incidence of milk leakage and stress-related behaviour in dairy cows and therefore improves management, udder health and welfare of the dairy cow.

Keywords: Cabergoline, dry-off, Milk leakage, Mexico, Cow.

UH-40

The impact of herd size and milking technology on milk production in dairy cattle units

Dorottya Ivanyos[1], Attila Monostori[2], Csaba Németh[2], István Fodor[1], László Ózsvári[1].

[1]Department of Veterinary Forensics and Economics, University of Veterinary Medicine Budapest, Budapest, Hungary; [2]Livestock Performance Testing Ltd, Gödöllő, Hungary.

Objectives: The aim of the study was to survey the milking technology, and to assess the relationship between the milking technology, the herd size, and the milk production parameters on commercial dairy farms.

Material and Methods: The milking technology was surveyed by using a questionnaire on 417 Hungarian dairy herds with 177,514 cows in 2017, and it was compared with their official farm milk production data. The surveyed farms were categorized according to their size (Group 1: 1-50, Group 2: 51-300, Group 3: 301-600, and Group 4: >600 cows) and to their milking parlour types (herringbone, parallel, carousel, and others). The relationships were analysed by multivariate linear models, one-way ANOVA, and Fisher's exact test. Pairwise comparisons were performed by Tukey's post hoc tests.

Results: The number of farms was 40 (9.6%) in Group 1, 140 (33.6.0%) in Group 2, 142 (34.0%) in Group 3, and 95 (22.8%) in Group 4. The most commonly used type of milking parlours was the herringbone (296 farms, 71.0%), followed by the parallel (62 farms, 14.9%), the carousel (40 farms, 9.6%), and others (19 farms, 4.6%). Herds with different milking parlour types significantly differed in herd size (p<0.001), except for the parallel vs. other parlour types. The occurrence of parallel and carousel parlour systems decreased with the herd size.

The number of milking stalls per farm increased with the number of cows (p<0.001). Farms with herringbone parlour had significantly smaller number of milking stalls than the farms with parallel (p=0.022) and the carousel (p<0.001) parlours.

Herd size was significantly associated with the number of daily milkings (p<0.001). The number of daily milkings increased with the herd size, 60.0% of the farms with more than 600 cows milked the cows three or four times per day. The type of milking parlour was significantly related to the number of milkings per day (p<0.001). On dairy farms using herringbone parlours the cows were mostly milked two times a day, but where parallel or carousel milking parlour was installed the occurrence of this milking practice diminished.

Herd size was associated with average daily milk production per cow (p<0.001) and the average daily milk yield (p<0.001). As the herd size increased, so did the average daily milk yield (p<0.001) and the average daily milk production per cow (p<0.001). Herd size was significantly related to SCC (p<0.001), with the largest farms having the best quality milk in terms of somatic cell count.

The type of milking parlour was related to the average daily milk yield (p=0.039), and showed a tendency with average daily milk production per cow (p=0.062). Dairy farms using parallel milking parlours had significantly larger average daily milk production per cow (p=0.033) and average daily milk yield (p=0.019), than farms using herringbone parlour. The type of milking parlour tended to be associated with SCC (p=0.061). Herringbone parlours resulted in the highest average SCC, although no significant differences were found between the different parlour types.

Conclusion: The dairy herd size in Hungary has a huge range and large differences can also be seen in the milking technology (e.g. parlour type, number of milking stalls, and daily milkings) and milk production parameters (e.g. daily milk yield, SCC) between farms. However, majority of the surveyed farms still use herringbone parlours, but over 600 cows the parallel and carousel milking systems also play a significant role. Our findings show that the herd size has a greater impact on the milk production parameters than the type of milking parlour. The larger dairy farms may have better and newer housing, feeding conditions, and milking technologies, which could allow for higher milk production, however the average SCC was high in all size groups.

Acknowledgement: The Project was supported by the European Union and co-financed by the European Social Fund: (1) EFOP-3.6.1-16-2016-00024; (2) EFOP-3.6.2-16-2017-00012; and (3) EFOP-3.6.3-VEKOP-16-2017-00005.

Keywords: Dairy, milking technology, milking parlour, udder health, milk production.

UH-41

Determining the Cut-off value of Bovine Leukemia Virus proviral load based on the severity of clinical mastitis

Aiko Watanabe[1], Hironobu Murakami[2], Tomoya Hirose[3], Seiichi Kakinuma[4], Koki Murao[4], Kaori Ohmae[4], Hirohisa Akamatsu[5], Takahiro Seto[6], Yasunori Shinozuka[7], Yoko Aida[8], Kazuhiro Kawai[7].

[1]School of Veterinary Medicine, Azabu University. Kakinuma Veterinary Hospital, Sagamihara, Honjo, Japan; [2]School of Veterinary Medicine, Azabu University, Sagamihara, Japan; [3]Viral Infectious

Disease Unit, RIKEN, Wako, Japan; [4]Kakinuma Veterinary Hospital, Honjo, Japan; [5]Akamatsu Farm Clinic, Fujinomiya, Japan; [6]Shizuoka Prefecture Livestock Research Institute, Fujinomiya, Japan; [7]School of Veterinary Medicine, Azabu University. Azabu University Mastitis Research Center, Sagamihara, Japan; [8]Viral Infectious Disease Unit, RIKEN. Nakamura Laboratory, Baton Zone Program, RIKEN Cluster for Science, Technology and Innovation Hub, Wako, Japan.

Objectives: Severe bovine mastitis causes considerable economic losses in the dairy industry due to significantly lowered milk production and an increase in cow mortality. Enzootic bovine leukosis (EBL) is caused by the bovine leukemia virus (BLV), and its prevalence in Japan has been steadily increasing. It has been reported that the proviral load (PVL), i.e., the proviral copy number of BLV in the blood of BLV-infected cows increases as the disease progresses, and that the prevalence of BLV tends to be higher on farms where the majority of cows have high PVL. In addition, previous studies have shown that the recurrence of bovine mastitis and morbidity rates were significantly higher on farms with a high prevalence of BLV compared to farms with uninfected cows. Further, other studies have demonstrated a strong association between the severity of clinical mastitis and PVL. Taken together, these findings imply that PVL may be associated with other diseases. Although methods for diagnosing and screening for PVL based on DNA concentration and cell number have been developed, the application of PVL as a criterion for evaluating the risk of clinical mastitis severity has not yet been examined. The purpose of this study was therefore to establish whether PVL values could be used to assess clinical mastitis severity using two quantitative polymerase chain reaction (qPCR) methods. The first method is targeting the BLV pol gene among evaluations based on DNA concentration (DNA-based qPCR), and the second method uses the Coordination of Common Motifs (CoCoMo) algorithm targeting the BLV long terminal repeat (BLV-CoCoMo-qPCR) among the evaluations based on cell number (Cell-based qPCR).

Materials and Methods: Milk and blood samples were collected from a total of 63 lactating Holstein-Friesian dairy cows with clinical mastitis from 13 dairy farms. The severity of clinical mastitis among all cases was classified as mild (milk abnormalities; n=32) or severe (milk abnormalities, udder abnormalities and general symptoms; n=31). DNA-based qPCR and cell-based qPCR were used to determine the PVL at the onset of clinical mastitis. Utilizing receiver operator characteristic curve analysis and the Youden index, and the severity of clinical mastitis as the dependent variable (outcome) and PVL as the independent variable, we estimated PVL cut-off values to estimate the onset of severe clinical mastitis for both the DNA-based qPCR and cell-based qPCR methods. Using cut-off values determined by DNA-based qPCR and cell-based qPCR as classification criteria, we classified PVL into the following three groups: High PVL (above cut-off value), Low PVL (below cut-off value), and Negative (PVL below detectable limits), and evaluated short- and long-term prognoses after the onset of clinical mastitis. Specifically, short-term prognosis was assessed by measuring milk somatic cell count in milk (SCC) on day 1, 7, 14 and 21, and long-term prognosis by estimating 6-year survival rates.

Results: The cut-off value was 18.4 (copies/10 ng DNA) (AUC (area under the curve): 0.731, sensitivity: 0.781, specificity: 0.71) using DNA-based qPCR, and 16,777 (copies/10^5 cells) (AUC: 0.731, sensitivity: 0.531, specificity: 0.903) using cell-based qPCR. Nosignificant differences were observed in either SCC or the 6-year survival rate among the three groups classified using the cut-off value; High-PVL group (DNA- based qPCR; n=34, Cell-based qPCR; n= 21), Low-PVL group (DNA-based qPCR; n=20, Cell-based qPCR; n=32) and Negative group (DNA-based qPCR; n=9, Cell-based qPCR; n=10).

Conclusions: Using DNA- and cell-based qPCR assays to determine PVL cut-off values and then estimate the severity of clinical mastitis was moderately accurate; no difference in accuracy was observed between the methods. The results implied that the cut-off value determined by both methods can be used to evaluate the risk of severe mastitis. In evaluating short- and long-term prognoses, no significant differences were observed among the High-PVL, Low-PVL, and Negative groups. The results suggested that the cut-off value may not be effective for predicting short- or long-term prognoses, but that it can be used as a criterion for evaluating the risk of severe clinical mastitis.

Keywords: Bovine leukemia virus, Cut-off value, Proviral load, Severity of mastitis.

UH-42

Comparison of various diagnostic tests for early diagnosis of mastitis in dairy goats

Rakesh Kumar, Dhiraj Kumar Gupta, B.K. Bansal, Shukriti Sharma.

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India.

Objectives: Subclinical mastitis (SCM) is one of the most important infectious diseases in goats that affect 5-30% goats. Early detection of mastitis followed by preventive activities in controlling the infections becomes of utmost importance. Parameters like somatic cell count (SCC), California Mastitis Test (CMT), electrical conductivity (EC), pH, milk composition and N-acetyl-β-D-glucosaminidase (NAGase) have been evaluated for diagnosis of SCM. SCC has been accepted as the most effective index of mammary inflammation in dairy herds to evaluate yield and quality of milk. The CMT roughly estimates the number of cells of the immune system and epithelial cells in a given milk sample. CMT levels correlate well with SCC levels found in caprine milk. During mastitis, NAGase activity has been found to be reliable for the detection of mastitis pathogen induced IMI. Measurement of electrical conductivity (EC) provides another way to detect mastitis. Concentration of lactose in milk decreases slightly in case of inflammation, making it promising to be applied as an indicator of IMI. If sensitivity and accuracy are the most important factors in mastitis diagnosis methods, the milk NAGase activity test and lactose content in milk together have the highest likelihood to be the most reliable. However, no single method is completely reliable in detecting subclinical mastitis.

Materials and methods: A cross sectional study was carried out involving 200 apparently healthy randomly selected lactating beetal crossbred goats from 10 loose dairy goat flocks across the state. The flocks were visited during morning hours and the Quarter foremilk (QFM) milk samples were aseptically collected. The analysis of milk samples for SCC was done using milk somatic cell counter (DELTA Instrument, The Netherland). Results were expressed in × 10^3cells/ ml. CMT was conducted by as per standard method described by Pandit and Mehta (1969). The EC using Electrical Conductivity Meter (Mettler Toledo), pH by digital pH meter and fat, protein and lactose content in milk using Milk analyser were recorded. The NAGase activity was measured using the spectrophotometric method of Kitchen *et al* (1978) with some modifications. The tests applied for diagnosis were correlated with each other. Sensitivity (SE), Specificity (SP), Likelihood Ratio (LR), Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of these tests were evaluated. Discriminating Functional Analysis of the diagnostic tests was done at cut-off points where the tests are having maximum SE and SP to see the efficacy of test to diagnose subclinical mastitis.

Results and Conclusions: The pearson correlation test was applied to find out the correlation among different diagnostic tests. The CMT, lactose content, pH, NAGase and Log_{10}NAGase were significantly ($p < 0.01$) positively correlated with SCC. At cut-off value of 700 and 750 (×10^3 cells/ml), SCC was having comparatively high SE (51.40, 52.00), SP (89.45, 89.19), LR (4.87, 4.81), PPV (0.38 each) and NPV (0.63 each), respectively. The SE, SP, LR, PPV and NPV at 600 (×10^3 cells/ml) were same as those at 700 (×10^3 cells/ml). So, we can consider 650/700 or 750 as most suitable cutoff value to differentiate infected udder halves from non-infected ones. NAGase activity at cut-off value of 25 and 30 (nMoles/ml/min) showed moderately high SE, SP, LR , PPV and NPV. So, we can consider either of these cut-off values as most suitable to diagnose intra-mammary infection. For EC, pH and Lactose the cut-off values 6.4, 6.4 and 4.6, respectively were found most suitable to differentiate infected udder halves from non-infected ones.

At cut-off values of 750 **(**×10^3cells/ml) for SCC, 25nMoles/ml/min for NAGase, 6.4 for EC, 6.5 for pH and 4.4% for lactose, the Discriminating Functional Analysis showed that SCC, NAGase, EC, pH and lactose could be able to discriminate the positive cases with negative ones by 85.16%, 66.36%, 60.82%, 69.23% and 49.94%, respectively.

Keywords: Diagnosis, mastitis, goats, SCC, NAGase.

UH-43

Field trial to compare efficacy of two commercially available *Escherichia coli* J-5 vaccines against clinical coliform mastitis

Norman Stewart[1], Scott Nordstrom[1], John Champaign[1], Alison Hurwitch[1], Joe Hogan[2].

[1]Merck Animal Health, Madison, NJ, United States; [2]Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, United States.

Objective: The first 100 days of lactation is a period of physiological transition for dairy cows, critical to the health and production for animals during the current and future lactations. Clinical coliform mastitis during this period has potentially negative impacts on the performance and longevity of a cow in the herd. The use of *Escherichia coli* J-5 bacterins before parturition and in early lactation has been shown to reduce the severity and duration of clinical coliform mastitis. The purpose of the current trial was to compare the efficacies of two commercially available *Escherichia coli* J-5 vaccines for reducing the incidence of clinical coliform mastitis during the first 100 days of lactation.

Materials & Methods: Cows enrolled in the trial were from a commercial herd of Holsteins. Within each projected treatment allocation week, cows were ranked based on their previous lactation milk production, within parity group, and then randomly assigned to one of the two treatment groups. Heifers were randomly allocated only by their electronic identification numbers. Experimental animals within a treatment group were immunized with one of two different commercially available bacterins: either vaccine 1 (Enviracor™ J-5; Zoetis Inc.) or vaccine 2 (Bovilis® J-5; MSD Animal Health Intervet Inc.). Both bacterins were administered at approximately 60 d prior to anticipated calving (day of drying off for cows), approximately 30 d after the primary immunization and on day 14 of lactation. All immunizations were 5 ml subcutaneous approximately 100 mm anterior of the scapula on the neck.

Clinical mastitis was diagnosed by farm personnel and quarter foremilk samples collected for bacteriological analyses prior to antibiotic therapy. The number of clinical cases was determined retrospectively by an investigator blinded to treatment codes using all reports of clinical signs and culture results of milk samples. Dairy-Comp 305 recording system was used to collect milk production following vaccination during lactation, first service pregnancy rates, percentage of pregnancies lost, and mortality and culling data for comparisons between treatment groups.

Results: Health and production data for 506 animals in the vaccine 1 group and 479 in the vaccine 2 group were analyzed. Rate of clinical mastitis caused by coliform bacteria during the first 100 days in milk for animals immunized with vaccine 1 was .0444 cases/ 100 cow-days compared with 0.0183 cases/ 100 cow-days for cows vaccinated with vaccine 2 ($P < 0.05$). Rate of total clinical mastitis also differed between treatments with 0.1138 cases/100 cow-days in vaccine 1 cows and .0690 cases/100 cow-days in vaccine 2 cows ($P < 0.05$). Rates of clinical cases caused by environmental streptococci, coagulase-negative staphylococci, other pathogens and bacteriologically- negative clinical cases did not differ between vaccine treatment groups. Percentage of animals that died or culled in the first 100 days in milk did not differ ($P > 0.05$) between cows immunized with vaccine 1 (4.4%) and vaccine 2 (3.8%). Percentage of cows pregnant after first artificial insemination service was 33.9% for vaccine 1 cows and 35.8% for vaccine 2 cows ($P > 0.05$). Pregnancy loss was 7.1% for cows in the vaccine 1 groups and 5.8% for those in the vaccine 2 group ($P > 0.05$). Daily milk production did not differ between vaccine groups for the two days prior to the immunization on day 14 of lactation, day 14 of lactation, or the three days after the immunization.

Conclusion: The efficacy of vaccines differed in reducing clinical mastitis caused by coliform bacteria during the first 100 days of lactation. Use of vaccine 2 reduced the rate of clinical coliform mastitis 2.4-fold greater than use of vaccine 1. Incidence of death and early culling, first service conception rate, and pregnancy losses did not differ between animals in the two vaccine groups. Milk production did not differ between groups on the days surrounding immunization on day 14 of lactation.

Keywords: Field efficacy trial, vaccination, coliform mastitis, E. coli, J-5 vaccines.

UH-44

Streptococcus agalactiae: new insights about a long-known mastitis pathogen

Annet Heuvelink[1], Manon Holstege[1], Christian Scherpenzeel[1], Stefan Bollen[2], Michel Swarts[1], Theo Lam[3].

[1]Royal GD, Deventer, Netherlands; [2]Utrecht University, Utrecht, Netherlands; [3]Royal GD / Utrecht University, Deventer, Netherlands.

Introduction and objectives: *Streptococcus agalactiae* is one of the first pathogens that was described as having harmful consequences for udder health in dairy cows. Until some decades ago it was considered a strictly cow-bound pathogen that was highly contagious within a herd, led to serious consequences with respect to subclinical and clinical mastitis, but was relatively easy to manage when adapting the traditional five point mastitis prevention scheme. At some point in time, in some regions *S. agalactiae* was almost considered eradicated. In recent years, however, several scientific publications indicated the pathogen was still present and even increased in prevalence. Voices from practice in the Netherlands endorse this, while indicating that the pathogen is not as harmful as it used to be in previous times. In our laboratory at GD we found that some *S. agalactiae* isolates had a surprising non-hemolytic character. This led to the question whether one of the most long-known pathogens in dairy mastitis, *S. agalactiae*, had changed over time, and whether enough attention is paid to this pathogen.

Material and methods: In the standard operating procedure (SOP) at GD, bulk milk samples from dairy farmers participating in the voluntary udder health surveillance program are examined on modified Edward's medium (EDW) for the presence of *S. agalactiae*. Based on the apparent changed hemolytic character of some *S. agalactiae* strains combined with the experience that EDW is sometimes difficult to read for the presence of *S. agalactiae*, it was decided to also use *Brilliance* GBS agar (SOP + GBS) for a one year period. Herd-level results on the prevalence of *S. agalactiae* based on the SOP and based on the SOP + GBS were compared. Additionally some management characteristics as well as the antimicrobial usage (AMU) in *S. agalactiae* positive herds based on the SOP, based on the SOP + GBS and *S. agalactiae* negative herds were compared.

Results: Bulk tank milk samples were collected during 10 sampling rounds from a one-year period from August 2020 until July 2021 in on average 1,896 herds, varying from 1,443 to 2,180 herds per sampling round. The prevalence of *S. agalactiae* per sampling round based on SOP + GBS varied from 2.4% to 2.8% of samples. By adding GBS to the SOP, 25% up to 72% more *S. agalactiae* positive samples per sampling round were found, indicating GBS has a significant added value in *S. agalactiae* diagnosis of bulk milk samples. Comparing 101 herds that were positive for *S. agalactiae* during a one-year period, the pathogen was found on GBS in 93 herds and on EDW in 84 herds at some point in time. In the 93 herds positive on GBS*, S. agalactiae* was also found on EDW in three or more samples in 28 herds. In the 84 herds positive on EDW*, S. agalactiae* was also found on GBS in three or more samples in 51 herds. This indicates GBS seems to have more added value in finding *S. agalactiae* than EDW, but that combining both media is needed to optimize sensitivity.

Disucsion and conclusion: Comparing udder health between herds positive for *S. agalactiae* in the SOP approach to those in the SOP + GBS approach during the first four rounds of the study did not reveal significant differences. Comparison of AMU revealed that total AMU in adult cows as well as intramammary AMU was higher for *S. agalactiae* positive herds than for negative controls, with a lower total and intramammary AMU for herds that were only positive in the SOP + GBS approach than those found in the SOP approach.

In conclusion it seems that, contrary to reports from the start of this century*, S. agalactiae* is still present in modern dairy farms, but may behave less aggressive. Specific attention should be given to its diagnostics, in which *Brilliance* GBS agar has an added value. It may be so that *S. agalactiae* cultured on GBS only behaves different from those also cultured on EDW. This needs further study, including analysis of culture results and possibly genome sequencing.

Keywords: Mastitis, streptococcus agalactiae, diagnosis.

UH-45

Presence of Antibiotic Resistance Genes in Staphylococci Isolated from Bovine Subclinical Mastitis

Zafer Cantekin[1], Gamze Ozge Ozmen[1], Melek Demir[1], Zeynep Yılmaz Er[1], Kemal Gurturk[2], Hasan Solmaz[3], Ismail Hakki Ekin[2], Dilek Ozturk[4], Ahmet Gozer[1], Yasar Ergun[1].

[1]Hatay Mustafa Kemal University, Hatay, Turkey; [2]Yuzuncu Yil University, Van, Turkey; [3]Karabuk University, Karabuk, Turkey; [4]Burdur Mehmet Akif Ersoy University, Burdur, Turkey.

Resistance to antimicrobial agents is an important problem in the dairy industry and poses a threat to human health. Antibacterial resistance in staphylococci, especially methicillin resistance, hampers the treatment and control of staphylococcal infections, which are prevalent in dairy cows. The aim of this study was to identify antibiotic resistance genes in staphylococci obtained from cases of bovine subclinical mastitis in three provinces (Burdur, Hatay and Van) in Turkey. In total, 283 isolates (Burdur, *n* = 36; Hatay, *n* = 47; Van, *n* = 200 iso-

lates) were studied. The isolates were first identified as *Staphylococcus aureus* and/or non-aureus staphylococci (NAS) by conventional phenotypic methods, and the species was then confirmed by a multiplex polymerase chain reaction (PCR). A simplex PCR assay was performed to detect antibiotic resistance genes (*mecA*, *mecC*, *aacA-aphD*, *ermA*, *ermB*, *ermC*, *tetK*, *tetM* and *blaZ*). Among the isolates from all three provinces, the *blaZ* gene was the most prevalent antibiotic resistance gene, present in 43 out of 156 (28%) NAS isolates, 27 out of 127 (21%) *S. aureus* isolates and 25% of the all isolates, whereas *tetM* was the most prevalent gene in the Hatay isolates, detected in 64% of all *Staphylococcus* isolates. The *mecA*-gene was present in 10% of the NAS, and in 3% of the *S. aureus* isolates. The *mecC* and *ermA* genes were not detected in any of the isolates. This shows that antimicrobial resistance, as determined by PCR, is common in *Staphylococcus* isolates from mastitis in Turkey, and warrants systematic treatment protocols as well as the implementation of preventative strategies to reduce antimicrobial usage.

Keywords: Bovine mastitis, antimicrobial resistance, Staphylococcus.

UH-46

Effects of selective dry cow therapy approach in an Italian commercial Dairy Farm

Laura Filippone Pavesi, Giulia Sala, Valentina Vailati, Clara Locatelli, Claudia Pollera, Valerio Bronzo.

University of Milan, Department of Veterinary Medicine and Animal Sciences, Lodi, Italy.

Objective: The dry period in dairy cows is a crucial period for udder health. It provides an opportunity to treat pre-existing intramammary infections (IMI), using intramammary dry therapy, but leave the udder in a high-risk situation of exposure to new IMI. Blanket dry cow therapy (BDCT) consists in treating all quarters of all cows with antimicrobials at the dry off. This therapy was widely used by dairy farmers. Due to changes in epidemiology and increasing antibiotic resistance, efforts are being made to reduce the use of non-essential antibiotic treatments. The aim of this study was to investigate changing in milk's somatic cells count (SCC) values, IMI trend and milk yield between cows treated with selective dry cow therapy (SDCT) and cow treated with BDCT, in order to assess the usefulness of administration of antimicrobial treatment to seemingly healthy udders.

Materials and methods: The selected herd was monitored from October 2020 to September 2021. The enrolling criteria, as described in literature, were no clinical mastitis during previous lactation, average SCC value lower than 200.000 cell/ml along the lactation and no IMI from major pathogens. These data have been taken from dairy herd improvement (DHI) controls of the previous and actual lactation, while the presence of major pathogens was detected at dry-off milk sample. According to literature, as major pathogens, we considered *Staphylococcus aureus, Streptococcus dysgalactiae, Streptococcus agalactiae, Streptococcus uberis* and *Escherichia coli*. The enrolled cows were randomly assigned to SDCT group or BDCT group. The animals in SDCT group were treated only with internal teat sealant (Easiseal™, Fatro, Italy) while the cows in BDCT group were treated with intramammary antibiotic treatment (Orbenin extra™, Zoetis, Italy) and internal teat sealant. 10 days after calving, samples of milk from individual quarters was collected from all enrolled cows and the animals were monitored up to 100 lactation days for the onset of clinical mastitis. Data for milk yield have been taken from the first three DHI controls post-partum. Data were collected on a spreadsheet (Excel™) and, about bacteriological and SCC analyses, they were analyzed using SPSS 27.0 statistical software (IBM, SPSS, Armonk, USA). The frequencies of mammary infections, before dry off and post-partum, were compared by $\chi 2$ test, while the SCC, after the assessment of normal distribution of data by means of the Shapiro-Wilk test, was compared by means was compared by a non-parametric U-Mann Whitney test because the data were not normally distributed. Statistical significance was considered for $p<0.05$.

Results: Forty-six cows were included in the study due to meeting the inclusion criteria and 24 cows were assigned to SDTC group and 22 cows to the BDTC. Results showed a non-statistically difference of IMIs incidence in post-partum in the SDTC group in comparison with BDCT group. Within the BDCT group, there was a statistically decrease in post-partum IMIs ($p=0.033$) respect pre-partum IMIs, despite the presence of 3 quarters infected with major pathogens at post-partum milk sampling. No statistically difference was recorded for SCC in both treatments' groups before dry-off and post-partum. Using only SCC values and milk yield coming from DHI controls, we observe a non-significant difference in milk yield, both before dry-off and after calving, for two treatments, while SCC was significantly higher in BDCT group vs SDTC group ($p=0.036$) before dry-off, but non-significant differences were observed in SCC after calving in both experimental groups.

Conclusion: This study confirms that it is possible to apply selective dry off therapy without risk of new infections or increase of SCC at calving, considering cows without IMI from major pathogens and SCC values <200,000 cells/ml in the previous lactation period. Therefore, the use of selective dry-off has been confirmed as an effective method for reduction the use of antibiotics in dairy farms in a One-Health perspective. Moreover, milk yield was not affected by the absence of antibiotic treatment at dry-off.

Keywords: Cattle, Mastitis, Selective dry cow.

UH-47

Barrier Characteristics of Three External Teat Sealants to Prevent Bacterial Penetration Under In Vitro Conditions Using Rubber Calf Feeding Nipples

Sofie Piepers[1], Igor Van Den Brulle[2], Geoff Ackaert[3].

[1]Ghent University, FAc. Vet Medicine, Gent, Belgium; [2]Ghent University, Fac. Vet. Medicine, Ghent, Belgium; [3]Inovet, Arendonk, Belgium.

The main objective of this study was to evaluate the barrier characteristics of a new external teat sealant for dry cows (Ubera® Dry, Inovet, Arendonk, Belgium) in preventing teat penetration by 3 common major mastitis pathogens (*Escherichia coli*, *Staphylococcus aureus* and *Streptococcus uberis*) compared to two commercially available external teat sealants via a novel *in vitro* simulation model using rubber calf feeding nipples. All feeding nipples were filled with a sterile cotton plug soaked in sterile Broth Heart Infusion medium and were treated as follows: teat 1 and teat 5 were sealed with Ubera® Dry (Inovet, Arendonk, Belgium), teat 2 and teat 6 were sealed with T-Hexx® Dry (Huvepharma Livestock, Missouri, US), teat 3 and teat 7 were sealed with UDDERgold® Dry (Ecolab Food and Beverage Division, Minnesota, US), and teat 4 and 8 remained unsealed and served as positive and negative controls, respectively. After drying, teats 1 to 4 were immersed in a suspension of *E. coli* (i.e. experiment 1), *Staph. aureus* (i.e. experiment 2) or *Strep. uberis* (i.e. experiment 3) (≥ 1.5 x 10^8 CFU/mL) for 24 hrs whereas teats 5 to 8 were not exposed to one of the bacterial suspensions. All external teat sealants adhered well to the rubber teats. All cotton plugs collected from the teats that were not exposed to either *Escherichia coli, Staph. aureus* or *Strep. uberis* (teats 5 to 8) remained culture-negative except for one (due to contamination). Of the teats that were exposed to the major mastitis pathogens, all cotton plugs collected from the teats dipped with Ubera® Dry and T-Hexx® Dry remained also culture-negative for the mastitis pathogen they were exposed to. The cotton plugs of the teats that were sealed with UDDERgold® Dry while being exposed to *E. coli* and *Strep. uberis*, resulted in positive cultures for the respective bacteria as demonstrated using strain-typing. The cotton plugs collected from the teats that were not sealed with an external teat sealant and that served as positive controls became culture-positive for the mastitis pathogens they were exposed to. In conclusion, Ubera® Dry showed comparable and superior barrier performances against the penetration of *E. coli*, *Staph. aureus* and *Strep. uberis* compared to T-Hexx® Dry and UDDERgold® Dry, respectively, under *in vitro* conditions using a novel simulation model. A large-scale clinical trial is needed to evaluate the adherence, duration of adherence and barrier performances of Ubera® Dry against intramammary infection under field conditions.

Keywords: Transistion management, udder health, external teat sealant.

UH-48

Economic consequences of an evidence-based mastitis therapy concept

Anne Schmenger[1], Stefanie Leimbach[1], Volker Krömker[2].

[1]Hannover University of Applied Sciences and Arts, Hannover, Germany; [2]Kopenhagen University, Kopenhagen, Denmark.

Objectives: The economic significance of targeted evidence-based mastitis treatment protocols is unclear. On the one hand, a greater diagnostic effort is required before therapy, and on the other hand, certain treatments are dispensed with. In a field study in which an evidence-based mastitis therapy system was established on dairy farms, the main economic aspects were recorded to carry out not only a medical evaluation but also an economic assessment.

Material and methods: In a three-year field study, the effects of establishing a targeted mastitis therapy concept based on clinical scoring, the consideration of the animal's individual mastitis history and the application of an on-farm test before therapy selection were examined on 1392 mastitis cases in 5 dairy farms in Northern Germany. All clinical mastitis cases and treatment data were recorded over the entire duration of the study. After analyzing the existing conventional therapy concepts of the farms, the mastitis therapy concept and the on-farm culture mastDecide® were introduced. Three treatment groups were compared: the conventional treatment group – before introducing the new concept (n = 483), the targeted therapy group (n = 506) and the modified targeted therapy group (n = 403), including the cases in which farmers deviated from the therapy concept. The investigated approach did not lead to any change in bacteriological and cytological cure rates, new infection rates or recurrence rates. The two targeted treatment groups hardly differed in terms of cures and treatment effort (P> 0.05).

Results: The application of the test and the implementation of the targeted therapy concept as recommended resulted in a saving of 67.4 % of intramammary antibiotic doses and 63.8 % of systemic antibiotic doses compared to the conventional treatment. Furthermore, this procedure led to a lower application effort (- 56.3 %) and a reduction of milk that could not be delivered (-25.1 %). These savings were offset by increased costs due to the on-farm tests used and the time required for using the on-farm test. Included in the cost calculation are: the amount of milk discarded, the time spent on rapid diagnosis and treatment of the animals, the cost of the rapid test and the cost of pharmaceuticals. For the average case of mastitis treated with a targeted treatment concept, the costs in total were lower by 33.20 €. The reduction of costs depended on the pathogen group and amounted to 25.4 % of the costs of standard therapy for Gram-negative microorganisms, 22.9 % for Gram-positive microorganisms and 29.1 % for samples without bacterial growth, respectively.

Conclusion: With the help of the targeted evidence-based mastitis therapy concept, a reduction of antibiotic doses by more than 60 % and of costs by 25.7 % were achieved with unaffected cure rates.

Keywords: Mastitis, treatment, economics, targeted.

UH-49

Impact of mastitis degree and moment of occurrence in milk production in dairy cattle

Nayara Magalhaes Goncalves[1], Almudena Molinero Arguello[2], Juana C. C. Chagas[3], Phd Marcos Inácio Marcondes[4].

[1]UNIFORM-Agri BV, Belo Horizonte, Brazil; [2]UNIFORM-Agri BV, Pamplona, Spain; [3]Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences (SLU),

Skogsmarksgränd, 90183, Umeå, Sweden; [4]Dairy Cattle Production and Nutrition, Universidade Federal de Viçosa, Brazil.

Objectives: Mastitis is the disease that causes the most loss in the dairy industry, even though there are already many studies regarding its prevention. Due to its gravity, mastitis can promote early culling animals, high costs with medicines, milk withdraw and decreased milk production and quality. Thus, we aimed to describe the impact of mastitis on milk production, based on mastitis degree and moment of occurrence.

Materials and methods: In this retrospective study, we used data from five dairies in the Netherlands and the databank consisted of 26,228 monthly milk test records from 1,230 cows, from September 2014 to January 2020. Lactations with less than five records were removed from the dataset. Mastitis was considered as affecting milk yields if observed at least 30 days before the milk test day, and was scored as 1) mild mastitis, and 2) acute mastitis. Then, we evaluated the effect of mastitis based on the drop in milk yield following three steps. Firstly, we removed all mastitis records from the data and fitted a wood's curve (WC) for each cow and lactation number. Secondly, we returned the mastitis data to the databank and tested the effect of mastitis in each WC parameters following the model: $MY = (1 + 1 \times Mast) \times Wk^{2 + 2 \times Mast} \times e^{-(3 + 3 \times Mast) \times Wk}$, where 1, 2 and 3 were parameters determined for each cow, 1, 2 and 3 were the effect of mastitis on MY, Mast is the binary occurrence of mastitis, and Wk is the week of lactation. The mastitis score (MS) was tested on all parameters. Lastly, a general WC was fit including in the general WC to estimate the MY loss throughout the lactation. Farm was included as random effect in all models. The model was run using PROC NLMIXED (SAS University edition) and parameters were considered different when $P < 0.05$.

Results: A total of 205 cows had, at some point of their life, a mastitis case recorded. There were 137 cows with mild mastitis and 68 with acute mastitis. Number of lactation when mastitis occurred varied from 1st lactation to 11th lactation. Also, there was a big variability of occurrence among week of lactation (2nd to 43th weeks). The mastitis level did not impact 1 ($P = 0.223$) but did affect 2 ($P = 0.026$) and 3 ($P = 0.047$). Additionally, Mast did impact 1 parameter ($P = 0.001$), thus one equation was fit for each mastitis degree as follows: Mast = 1) $MY = (31.860 + 0.966 \times Mast) \times Wk^{0.162 - 0.075 \times Mast} \times e^{-(0.023 - 0.0056 \times Mast) \times Wk}$; and Mast = 2) $MY = (31.860 + 0.966 \times Mast) \times Wk^{0.162 - 0.143 \times Mast} \times e^{-(0.023 - 0.012 \times Mast) \times Wk}$. Cows with mastitis had lower milk yield at lactation peak when compared with healthy cows. Cows with mastitis grade 1 had a peak production of 34.5 kg/d, and a peak of 32.0 kg/d with mastitis grade 2, while healthy cows had, on average, 37.1 kg/d of milk production at peak. Our analysis revealed that 50% of cows with mastitis grade 1 had a milk yield loss from 2.4 to 5.0 kg/day and cows with mastitis grade 2 had a milk yield loss from 4.1 to 8.0 kg/day. Week of lactation where mastitis occurred had a strong impact in milk production. Highest losses were detected between weeks 10th and 14th of lactation, and percentage of total milk loss during this period was up to 8.5% for mastitis grade 1 and 16.5% for mastitis grade 2.

Conclusions: In conclusion, despite a large variance in milk yield responses to mastitis, we demonstrated that mastitis results in a reduction in milk yield regardless of severity, but this drop is greater in acute mastitis cases. Additionally, the reduction in milk yield due to mastitis is related to the time of occurrence and is greater at weeks 10-14th of lactation, with losses up to 16,5% of milk yield.

Keywords: Mastitis, milk losses, dairy cattle.

UH-50

Negatively controlled, randomized clinical trial comparing label intramammary use of amoxicillin to ceftiofur hydrochloride for treatment of bovine clinical mastitis caused by Gram-positive pathogens

Tiago Tomazi[1], Matthew Sumnicht[1], Ana Carolina C.h. Tomazi[1], Josiane C.c. Silva[1], Leonardo Bringhenti[1], Livia M. Duarte[1], Marjory X. Rodrigues[1], Jantijn M. Swinkels[2], Lowell Midla[3], Scott Nordstrom[3], Rodrigo C. Bicalho[1].

[1]Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, United States; [2]Global Ruminants, MSD Animal Health, Boxmeer, Netherlands; [3]Merck Animal Health, De Soto, United States.

Objectives: This negatively controlled field trial aimed to compare clinical, microbiological and performance outcomes of label use of a narrow spectrum antimicrobial (amoxicillin) with a wide spectrum antimicrobial (ceftiofur hydrochloride) for treatment of non-severe clinical mastitis (CM) caused by Gram-positive bacteria.

Material and methods: After microbiological culture results, lactating dairy cows with non-severe CM (without systemic symptoms) caused by Gram-positive bacteria were assigned to two protocols: AMOX - three infusions with 62.5 mg of amoxicillin (Amoxi-Mast®, Merck Animal Health) performed ~12 hours apart; CEFT - five infusions with 125 mg of ceftiofur hydrochloride (Spectramast®, Zoetis) performed ~24 hours apart; NEG-CTR – quarters assigned to this group did not receive any interventions until five days after diagnosis. Before the onset of the study a randomized list was created to pre-assign all pregnant animals to one of treatment groups; approximately 90% of cows were assigned to one of antibiotic treatment groups (~45% in each protocol) and 10% to the negative control.

Duplicate milk samples taken before treatment (day 0) and on days 3, 5, 8 and 14 ± 3 after enrollment for analysis of milk composition, somatic cell count (SCC), total bacterial count (TBC) and next-generation sequencing of 16S rRNA gene and quantitative PCR (qPCR). Multivariate logistic regression models were created to evaluate dichotomized outcomes such as clinical cure (CC) and bacteriological cure (BC) at 14 ± 3 days after enrollment and quarter-level CM recurrence by the same species up to 90 days after treatment. Repeated-measures analysis of ANOVA was conducted to analyze the effect of treatment on milk production, SCC, composition, TBC, bacterial relative abundance (RA; based on microbiota) and 16S rRNA gene copy numbers (based on qPCR).

Results: A total of 477 quarter-cases of CM were evaluated: 198 assigned to AMOX, 223 to CEFT and 56 to NEG-CTR. The most frequent isolated species were *Streptococcus*

uberis (60.6%), *Strep. dysgalactiae* (19.3%), *Streptococcus* spp. (8.4%) and non-*aureus* staphylococci (6.1%). The overall CC (based on least square means) was 84.1% for AMOX and 89.0% for CEFT, and no significant statistical difference (*P*=0.15) was observed between groups. Likewise, no difference (*P*=0.10) between groups was observed on the evaluation of BC (AMOX = 58.2%; CEFT = 66.4%). Moreover, we found no statistical differences (*P*>0.05) between treatments when comparing CC and BC according to the mastitis-causing species (i.e., *Strep. uberis*, *Strep. dysgalactiae* or combination of other pathogens). Compared to antibiotic treated groups, quarters assigned to NEG-CTL had higher CFU, 16S rRNA gene copy numbers, and *Streptococcus* RA until day five after enrollment. Moreover, milk samples from NEG-CTL cows had lower fat and lactose contents, and higher total protein than antibiotic-treated cows up to the third test day after enrollment. A substantial reduction of bacterial load (CFU and qPCR) and *Streptococcus* RA was found for quarters receiving antibiotic therapy. Quarters treated with AMOX had higher CFU on days 5, 8 and 14 after enrollment compared to CEFT. In addition, the relative abundance of *Strep. uberis* was higher on day 14 after enrollment for AMOX-treated quarters than for those assigned to CEFT group, which may be related to the duration of treatment. Linear score of SCC (LSSCC) was higher for AMOX-treated cows than for those treated with CEFT in the first test day after CM, but no differences were observed in the second and third test days following CM. Higher milk production was observed for cows assigned to AMOX group compared with CEFT-treated cows until the third test day after enrollment. No significant differences between groups (*P*=0.92) were found on CM recurrence (9.0% for CEFT; 9.3% for AMOX), and on survival of cows in the herd after treatment (*P*=0.51).

Conclusion: Two-day protocol with three intramammary infusions of amoxicillin (narrow spectrum antimicrobial) had similar CC and BC than five administrations (once a day) with ceftiofur hydrochloride (wide spectrum), and no difference was observed on the risk of CM recurrence and cow survival. However, quarters treated with 5-day ceftiofur protocol had higher reduction of milk CFU than quarters treated with amoxicillin up to 14 days after treatment. Antibiotic use remains an indispensable strategy for treatment of Gram-positive bacteria, since untreated quarters remained with high CFU, gene copy numbers and *Streptococcus* spp. RA than quarters receiving any of the antimicrobial protocols.

Keywords: Treatment of clinical mastitis, dairy cows, microbiome, Gram-positive mastitis pathogens.

UH-51

Antibiogram of pathogens isolated from milk of mastitic dairy buffaloes (*Bubalus Bubalis*) of Punjab and association of California Mastitis Test Scores with intramammmary infection status

Dhiraj Kumar Gupta, C.S. Randhawa.

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India.

Objectives: To determine the antibiogram of pathogens isolated from milk of mastitic dairy buffaloes and to evaluate the California Mastitis Test (CMT) as an indicator of intramammary infection (IMI) in lactating dairy buffaloes.

Materials and methods: A total of 1489 milk samples from 474 mastitic buffaloes were subjected to CMT and microbial culture in Mastitis Laboratory, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. Isolates were confirmed by standard biochemical characterization and further subjected to antimicrobial sensitivity testing (AST). A total of 14 antibiotics belonging to 7 groups were used to study the drug resistance pattern. Further, the CMT was evaluated as an indicator of intramammary infection (IMI) in lactating dairy buffaloes using chi square test.

Results and conclusion: Of these, 643 (43.18%) quarters were culturally positive which comprises Coagulase-negative staphylococci (68.90%), Coagulase-positive staphylococci (27.06%), *Streptococcus spp.* (1.24 %) and Gram Negative organisms (2.80%). A total of 846 quarters revealed no growth. A total of 14 antibiotics belonging to 7 groups were used to study the drug resistance pattern. Overall antibiotic sensitivity test revealed highest susceptibility to Ceftriaxone+tazobactum (86.97%) followed by Ceftriaxone+sulbactum (78.91%) and Cefaperazone (73.13%), while least resistance was observed against penicillin (30.00%) followed by ampicillin (31.14%) and amoxicillin (38.81%). Coagulase-negative staphylococci (CNS) were the most frequently recovered bacterial species accounting for 68.90% of all isolates. Out of 54 CNS isolates antibiotic sensitivity testing was done only in 48 isolates, which revealed highest susceptibility to Ceftriaxone+sulbactu followed by Cefaperazone and gentamicin while resistance was observed against penicillin, amoxicillin and ampicillin. An IMI was identified in 43.18% of quarters. For buffaloes without evidence of clinical mastitis, the sensitivity of a CMT score more than or equal to trace in predicting an IMI on a quarter basis was assessed. CMT score with IMI was 1.21±0.076 and without IMI was 0.89±0.047. For quarters without evidence of clinical mastitis, the sensitivity of a CMT score ³ trace in predicting an IMI on a quarter basis was 0.48.

Keywords: Antibiogram, mastitis, buffaloes, AST, CMT.

UH-52

A Randomized Non-Inferiority Study Evaluating the Efficacy of Two Commercially Available Teat Sealants in Dairy Cows

Patrick J Gorden[1], Jantijn Swinkels[2], Michelle P Buckley[3], Jenna Bayne[4], Gustavo S Silva[1], Tiago Tomazi[5], Brian E Miller[5], Sandra M Godden[6].

[1]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA 50011, Ames, United States; [2]MSD Animal Health Global Ruminants, Boxmeer, Netherlands; [3]Department of Veterinary Microbiology and Preventative Medicine, Iowa State University, Ames, IA 50011, Ames, United States; [4]Auburn University College of Veterinary Medicine, Auburn, AL 36849, Auburn, United States; [5]Merck Animal Health, Kenilworth, United States; [6]Department of Veterinary Population Medicine, University of

Minnesota, St. Paul, MN 55108, St. Paul, United States.

Objectives: The primary objective of this study was to compare the efficacy of a new internal teat sealant (ShutOut™, Merck Animal Health, Madison, NJ, USA) (SO) to the current US industry leader (Orbeseal®, Zoetis, Parsippany, NJ, USA) (ORB). This comparison was based on a non-inferiority evaluation of quarter-level new infection risk during the dry period, with secondary comparisons of cure risk, incidence of clinical mastitis at the cow level during the first 120 days in milk, as well as cow-level performance in early lactation based on milk production, somatic cell count (SCC), and risk for removal from the herd.

Materials & Methods: This study was conducted on six commercial dairy farms (two in Iowa and four in Minnesota) and one university dairy (Iowa State University). On all farms, cows were randomly assigned to treatment groups, blocked by farm on the day of enrollment. Eligibility criteria included an expected dry period of 30-90 days, at least three functional quarters, body condition score >2.0 out of 5, and a lameness score <4 out of 5. Cows designated to be culled early in the subsequent lactation were also ineligible. Cows were excluded from enrollment if they had received any antimicrobial treatment within 14 days of dry off.

Study personnel collected aseptic, quarter-level, duplicate samples for routine aerobic culture immediately prior final milking and treatment at dry-off, and again within 14 days of calving, to evaluate risk of new intramammary infection (NIMI) and cure of existing intramammary infection (CIMI). Following milking machine detachment, all quarters received 500 mg of cloxacillin benzathine (Orbenin DC, Merck Animal Health, Madison, NJ, USA) followed by their assigned sealant. Post-milking procedures and dry period care were executed per individual farm protocols. Animal performance was also monitored for 120 days post-calving using Dairy Herd Improvement Association (DHIA) electronic records and owner captured clinical mastitis events as well as culling and death loss. Effect of treatment on dry period CIMI and NIMI in the first 14 DIM were evaluated using generalized linear mixed models.

Results: At dry off, 65.1% of quarters were not infected with no difference between groups (P = 0.16). During the dry period, the least square means new infection risk was SO=0.266 [CI 0.233, 0.303] vs ORB=0.276 [CI 0.243, 0.313], with no difference identified between treatment groups (P = 0.646). In post-fresh samples, least square means of cured infections were also not different between treatment groups SO=0.966 [CI 0.927, 0.984] vs ORB=0.949 [CI 0.902, 0.974] (P=0.259). When evaluating cow-level events within the first 120 days, there was no difference in clinical mastitis risk with a probability of ORB cows developing an infection of 0.122 and 0.115 for SO cows (P=0.74). Risk of culling was also similar between treatment groups with ORB cows experiencing a probability of being affected of 0.111 and SO cows at 0.073 (P=0.699). Finally, death rate did not differ between treatment groups with a probability of 0.019 for ORB treated cows vs 0.022 SO cows (P=0.956).

Conclusions: No difference was identified in quarter-level new infection and cure risks during the dry period in our comparison of two commercially available internal teat sealants. Cow-level events including culling, clinical mastitis, and death within the first 120 DIM also showed no differences between treatment groups.

Keywords: Teat sealant, dry cow therapy, dairy, mastitis.

UH-53

Cabergoline as a tool to reduce the incidence of milk leakage and facilitate dry-off management in large dairy herds. Results in Saudi Arabia

Ahmed Hamdy[1], Aarafa Mohammed Shamia[2], Mohamed Barghout[2], Imad B. Saleh Alhamid[2], Juan Munoz Bielsa[1], Alaa Fattouh[1], Carla Azevedo[1], Cyrille Chevtzoff[1].

[1]Ceva Santé Animale, Libourne, France; [2]Nadec Dairy Farm, Saudi Arabia, Saudi Arabia.

Objectives: The incidence of milk leakage (ML) has been proven to be a risk factor for acquiring new intramammary infections. Moreover, Zobel *et al*. (2013) have shown a relationship between the milk production at dry-off and the percentage of milk leakage. Usually many farms decide to perform a gradual dry-off procedure in order to decrease the milk production at the time of dry-off. However, this results in losses of milk production, extra time, labour costs and welfare issues due to nutrient restriction and social regrouping. In this study, dry-off procedure was done abruptly to evaluate if cabergoline treatment can facilitate the dry-off procedure and decrease milk leakage. The objective of this study was to investigate the effect of cabergoline (Velactis® Ceva Santé Animale, Libourne, France) on the incidence of milk leakage after dry-off in a large commercial dairy farm in Saudi Arabia.

Materials and methods: 861 dairy cows located in one commercial dairy farm (NADEC dairy farm) in Saudi Arabia were enrolled the day of the dry-off and followed up until 48-52 hours after. All cows were dried abruptly, without changing in feeding or milking frequency. 431 cows were treated with a single intramuscular injection of 5.6 mg cabergoline after last milking and 430 cows didn't receive a treatment and were considered as control group. The animal distribution was done in order to have similar proportion of primiparous and multiparous animals between the two groups (respectively 38.28% and 61.72% for the treated group and 38.37% and 61.67% for the control group). All levels of milk production cows were included if animals met the inclusion criteria. Milk leakage was observed at three different time-points after the dry-off: 20-24, 30-24 and 48-52 hours after. The study was randomized and blinded. The drug administrator was different from the person who did the milk leakage observations. The individual cow was the experimental unit and the quarter was the data collection. Data analysis was performed by using STATA® (version 14.0) software. Number of quarters leaking milk per cow in each group was estimated with corresponding 95% confidence interval.

Results: Overall, the percentage of cows with milk leakage was lower in cabergoline treated (12%) compared with control (49%) cows (P < 0.001). Cows in the control group

were 7.19 times more likely to show milk leakage compared with cabergoline treated cows When comparing the data by parity (adjusted on milk production, visit and teat score), the percentage of cows leaking milk was lower in cabergoline treated compared to control cows, both for primiparous (1% vs. 17%) and multiparous (5% vs. 35%) cows (P < 0.001). Also, the risk of having milk leakage was not statistically different between level of milk_production (adjusted on parity, visit and teat score): percentage of cows leaking milk was lower for cabergoline group compared to control group (P < 0.001) in cows producing <17kg (2% vs. 17%), 17-24.5kg (3% vs. 22%), 24.5-31kg (3% vs. 30%), and more than 31kg (3% vs. 33%).

Conclusions: It is the first time that milk leakage incidence is evaluated in a large and well managed commercial dairy farm in Saudi Arabia. Our data provide evidence that a single injection of cabergoline decreases significantly the percentage of milk leakage after dry-off, reducing risk factors for udder health in both primiparous and multiparous cows and for different levels of milk production. Considering these results, cabergoline can be a useful tool to be used in largest dairy farms to optimize the dry-off management, to decrease the milk production at the time of dry-off and consequently reduce the risk for new intramammary infections after the dry-off.

Keywords: Cabergoline, Dry-off, Cows, Milk leakage.

UH-54

Oral fully oxidized beta-carotene increases spontaneous bacteriological cure and reduces risk of subsequent clinical mastitis in cows with subclinical mastitis

Scott McDougall.

Cognosco, Morrinsville, New Zealand.

Objective: To determine if oral supplementation with fully oxidised beta-carotene (OxBC) would improve spontaneous bacteriological cure rate in lactating dairy cows with subclinical mastitis.

Materials and Methods: Cows (n=4 herds) with an elevated somatic cell count (SCC; >200,000 cells/mL) and which had not been treated with antimicrobials or nonsteroidal anti-inflammatory drugs in the preceding 14 days had milk samples collected following aseptic teat end preparation for culture and quarter-level SCC determination. Cows with one or more quarters that met the enrolment criteria (i.e. had quarter-level SCC >200,000 cells/mL and had one or two distinct bacterial species isolated upon culture) were blocked by lactation number (primiparous vs multiparous), ranked on herd test SCC, and randomly assigned to treatment (i.e. 0.3 g of OxBC, in the form of commercial product OxC-beta Livestock 10%, incorporated into 0.5 kg of a cereal based pelletised feed, and fed once daily for 42 days) or control (0.5 kg of the pelletised feed without the OxBC). Milk samples were collected from enrolled quarters at 21 and 42 days after initiation of treatment for microbiology and SCC determination. Cows were observed daily for evidence of clinical mastitis (i.e. presence of flecks or clots in the milk and/or heat and swelling of the mammary gland). Quarters were defined as having undergone spontaneous bacteriological cure if the bacterial species present prior to an initiation of treatment were not cultured in either of the post enrolment milk samples. A multilevel multivariable binary logistic regression model was used to determine the effect of treatment (i.e. OxBC vs control), and including potential covariates such as age, bacterial species (coded as major pathogens i.e. *Staphylococcus aureus* and Streptococcus spp. vs others), days in milk, quarter position within cow (i.e. fore vs rear glands), and pre-treatment quarter level SCC. Quarter level SCC were natural log (ln) transformed before analysis in a generalised linear mixed model with treatment and Day (i.e. Days 0, 21 and 42) and their interaction as fixed effects.

Results: Data from 77 vs 79 cows and 135 vs 128 quarters were available for analysis from the control vs treatment feeding groups, respectively. Treatment groups did not differ in terms of age, breed, cow-composite SCC prior to treatment, days in milk at enrolment, or frequency of bacteria associated with intramammary infection (all P>0.8). More quarters from cows fed OxBC underwent spontaneous resolution of bacterial infection compared with quarters from cows fed the control feed (13.9% (95% CI 4.1-23.7) vs 6.9% (95% CI 4.8-9.1) for quarters from OxBC fed vs control fed cows, respectively; odds ratio = 2.18 (95% CI 1.14-4.17); P=0.02). There was no effect of treatment (P=0.34) or day (P=0.12), nor was there a treatment by day interaction (P=0.17) for the quarter-level ln SCC. Fewer of the quarters in cows fed OxBC had clinical mastitis in the 42 days post initiation of feeding compared with quarters from control fed cows (1/129 (0.78% (95%CI 0.02-4.24)) vs 6/135 (4.44% (95%CI 1.65-9.42)). The odds ratio of mastitis diagnosis in quarters from cows fed OxBC was 0.17 (95%CI 0.03-0.82) relative to quarters from cows fed the control diet.

Conclusions: This study demonstrated that oral supplementation with fully oxidised beta-carotene (OxBC) of dairy cows with subclinical mastitis resulted in a higher spontaneous cure of existing intramammary infection and a reduced risk of subsequent clinical mastitis. The potential mechanism for this effect may be enhanced innate immune response associated with upregulating the numbers of pattern recognition receptors including toll -like receptors 2 and 4 (Johnston *et al.* 2014). This compound provides a non-antimicrobial approach to reducing the prevalence of intramammary infection in dairy herds.

References:

Johnston JB, Nickerson JG, Daroszewski J, Mogg TJ, Burton GW. Biologically active polymers from spontaneous carotenoid oxidation: a new frontier in carotenoid activity. *PLOS ONE* 9, e111346-e, doi:10.1371/journal.pone.0111346, 2014.

Keywords: Subclinical mastitis, control, beta-carotene.

UH-55

Farmer´s attitude, level of application and challenges to implement selective dry cow therapy on Spanish dairy farms

C. Carbonell[1], N. Charfeddine[2], M. Marcos[1], L. Elvira[1].

[1]MSD Animal Health, Salamanca, Spain; [2]CONAFE Technical department, Madrid, Spain.

Objectives: The dry period is crucial for udder health in the dairy cow. Indeed, this period is at the same time an opportunity to cure intramammary infections (IMI) and a risk for new IMI (Bradley and green 2004). For this reason, blanket dry cow therapy (BDCT), regardless the cow infection status, was included in the 5-point plan for mastitis control (Neave et al., 1969). However, in the last years, there is an increasing public health concern in EU about antimicrobial resistance. Consequently, a new European veterinary medicines regulation (EU 2019/6) has restricted the prophylactic use of antimicrobials. Hence, BDCT is not allowed anymore on European dairy farms since February 2022.

Selective dry cow therapy (SDCT) involves only treating infected cows with antibiotics at dry-off. Numerous studies (Kabera et al., 2021, Rowe et al., 2020, Swinkels et al., 2021) and previous experience (Vanhoudt et al., 2018) have shown that SDCT is an alternative strategy to reduce total antimicrobials usage (AMU) without negative consequences on udder health. Nevertheless, a good dry cow management (DCM) is crucial for a successful implementation of SDCT (Roodenburg et al, 2020).

In Spain, there is a lack of knowledge about DCM and SDCT implementation in the dairy farms.

Then the purpose of this study is to provide insight into (1) farmer´s knowledge, level of implementation and attitude towards SDCT in Spanish dairy farms just before new EU regulation becomes mandatory, (2) DCM and possible challenge to implement SDCT and (3) the relationship between SDCT, BDCT and the main key performance indicators related with dry cow udder health.

Materials and methods: The study was based on a survey performed to farmers belonging to Dairy Cow National Association (CONAFE) between August and December 2021. It consisted of fourteen questions to collect their knowledge about SDCT and the new regulation, their dry off strategy and DCM. To avoid bias, all the interviewers were previously trained and couldn't be involved in the milk quality of the farm.

A random sample of 450 farms representative of the different farm sizes and regions included in the DHI program were selected. At the end of the survey, we asked them for authorization to use their DHI data to calculate the main KPI related with the dry off period: cows infected at dry-off; new infections rate, cure rate and chronic rate at calving; and percentage of healthy cows at calving.

Results: A total of 401 questionnaires were completed, 390 agree to respond and 364 authorized to share their DHI data. Surprisingly, 39.7% of farmers have not previously hear about SDCT, shortly before it becomes mandatory. This highlights the lack of communication between the different stakeholders involved in the dairy segment. Furthermore, a 33.3% of farmers already knew about SDCT but didn't apply it, mainly due to their fear to worsen their udder health status.

On the other hand, 21.8% of farmers were already applying SDCT. More than a half for two or more years (51.8%) and only a very low percentage just starting in the last 12 months (9.4%). The AMU reduction was very high with a 45.9% of farms treating less than a 25% of cows with antibiotics at dry off.

One of the main challenges to implement SDCT, was related with the high level of production of cows at dry off. Farmers estimate around a 39.7% of cows still produce more than 25 litres at dry off. This feeling was confirmed by DHI data, with 40.7% of cows producing more than 25 liters of milk in the milk control previous to the dry off.

As expected, no statistical differences were found in the main KPI indicators of udder health related with the dry off between SDCT and BDCT farms (19.1 and 18.5% new IMI and 67.1 and 69% cure rate for SDCT and BDCT, respectively)

Conclusions: In conclusion, many Spanish dairy farms will have to get out of their comfort zone and move towards SDCT in a short time. It will be a great challenge for both dairy farmers and vets, with 78% of dairy farms still using BDCT in 2021. Hopefully, as those ones starting with SDCT they will also reach it successfully.

Keywords: Selective dry cow therapy, udder health, farmer attitude, dry cow management.

UH-56

Bacterial identification of milk samples from small family farms in the Pontal do Paranapanema region, State of São Paulo, Brazil

Mario Augusto Reyes[1], Jeferson Carvalho[1], Natalia Gaeta[1], Alessandra Nassar[2], Gisela Gregoria Choque[1], Lilian Gregory[1].

[1]Departamento de Clinica Medica, Universidade de São Paulo, Brazil; [2]Laboratorio de Bacteriologia Geral, Instituto Biologico de São Paulo, Brazil.

Objectives: The objective of this study was to identify bacteria from milk samples from cows in rural São Paulo, in the Pontal do Paranapanema region.

Materials and methods: 2,431 samples were obtained from 616 animals from different small family milk productions in the Pontal do Paranapanema region, State of São Paulo, Brazil. The milk samples were cultured on blood agar for 48 hours to observe the growth of bacterial colonies, after which the colonies that grew were stained with the "Gram" staining technique for observation and identification under the microscope.

Results: We observed that from the 2,431 samples, 46.11% (1.121 / 2.431) did not show bacterial growth, 26.28% (639/2431) showed only growth of Staphylococcus sp., 12.58% (306/2431) showed only growth Bacillus sp., 3.82% (93/2431) showed only growth of Corynebacterium sp., 3.16% (77/2431) showed only growth of Enterobacteria, 2.5%

(61/2431) showed growth of Bacillus and Staphylococcus sp., 0.98% (24/2431) showed only E.coli growth, 0.86% (21/2431) showed only Enterobacter gergoviae growth, 0.7% (17/2431) showed growth of Corynebacterium and Staphylococcus sp., 0.53% (13/2431) showed growth of Corynebacterium and Bacillus sp., 0.37% (9/2431) showed growth of Non-fermenting Gram Negative Bacteria (GNNF), 0.12% (3/2431) presented growth of Enterobacteria and Staphylococcus sp., 0.04% (1/2431) presented Yeasts and Bacillus and E.coli and Staphylococcus sp.

Conclusions: It concluded, we can say that Staphylococcus, Bacillus, Corynebacterium sp. and Enterobacteria are bacterial agents that cause mastitis and play an important role in the health of the mammary gland, in addition to the fact that studies of this type should continue to be carried out, in order to better understand what type of bacteria are affecting the production of family milk in Brazil .

Keywords: Production, bovine, cattle, hygiene.

UH-57

Antimicrobial susceptibility of Staphylococcus sp. isolated from bovine milk samples from family dairy farms in the Pontal do Paranapanema region, State of São Paulo, Brazil.

Mario Augusto Reyes[1], Jeferson Carvalho[1], Natalia Gaeta[1], Alessandra Nassar[2], Lilian Gregory[1].

[1]Departamento de Clinica Medica, Universidade de São Paulo, Brazil; [2]Laboratorio de Bacteriologia Geral, Instituto Biologico de São Paulo, Brazil.

Objectives: The objective of the study was to determine the antimicrobial susceptibility of Staphylococcus sp. isolated from samples of bovine milk production of small family dairy, located in the Pontal do Paranapanema region in São Paulo, Brazil.

Material and methods: 648 colonies of Staphylococcus sp. obtained from bovine milk samples from small family dairy farms were tested for antimicrobial sensitivity using the disk diffusion test, approved by the Clinical and Laboratory Standard Institute (CLSI). The following antibiotics were used: amoxicillin (25 µg), ampicillin (10 µg), cephalothin (30 µg), chloramphenicol (30 µg), enrofloxacin (10 µg), streptomycin (10 µg), gentamicin (10 µg), neomycin (30 µg), penicillin (10 IU), sulfazotrim (25 µg) and tetracycline (30 µg).

Results: We observed that 15.43% (100/648) of the colonies found were resistant to eight antibiotics simultaneously: amoxicillin, cephalothin, chloramphenicol, enrofloxacin, streptomycin, penicillin, sulfazotrim and tetracycline. In addition, 29.06% (186/648) of colonies were resistant to five antibiotics simultaneously: amoxicillin, ampicillin, cephalothin, chloramphenicol and penicillin. 9.72% (53/648) of the colonies were resistant to three antibiotics: amoxicillin, ampicillin and cephalothin. 2.62% (17/648) of colonies were resistant only to penciline, 2.31% (15/648) of colonies were resistant only to tetracycline, 1.08% (7/648) of colonies were resistant only to streptomycin , 1.08% (7/648) of colonies were resistant only to neomycin, 0.77% (5/648) of colonies were resistant only to enrofloxacin, 0.77% (5/648) of colonies were resistant only to gentamicin. 0.61% (4/648) of colonies were resistant only to ampicillin. 0.31% (2/648) of the colonies were resistant only to amoxicillin. 0.31% (2/648) of the colonies were resistant only to ceftiofur, 0.15% (1/648) of the colonies were resistant only to enrofloxacin, 0.15% (1/648) of the colonies were resistant only to chloramphenicol and 10.03% (65/648) of colonies were susceptible to all antibiotics tested.

Conclusions: These results indicate that multidrugs resistance to antibiotics is one great problem in Brazil and was caused by the prolonged and indiscriminate use of the same active principle, for this reason, more studies must to be made with the aim to describe a picture of the situation in Brazilian dairy farms.

Keywords: Production, bovine, cattle, hygiene, bacteria.

【MEMO】

【MEMO】

31

WBC

