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ABSTRACT BOOK



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September 4th to 8th, 2022

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AH-P01

Clinical diagnosis of allotrophagia in cattle of the Sucre department

Jose Alberto Cardona Alvarez, Nicolas Martínez Humanez, Jaime Alvarez Peñate.

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Objetives: The objective of this study was to analyze and describe the clinical characteristics of 17 cases of allotrophagia in calves of both sexes and ages between 14 and 25 months of age from the municipality of the union, Sucre, Colombia, between January and April in the period from 2005 to 2016.

Materials and Methods: A general clinical and special digestive examination was performed, with similar clinical manifestations in all animals, which consisted of evidence of poor body condition, anemia and edema in the jowl, as well as increased volume in the lower left quadrant of the abdomen, when performing movements of succussion and percussion of the abdomen was evidenced the presence of solid material in great quantities that emitted a sound similar to the blow of several stones in a sac and to the auscultation it was found fading of the ruminal sounds.

Results: All animals underwent surgical removal of the material by rumenotomy, mainly finding stones, plastic, bones and seeds of *Elaeis oleifera* (corozo).

Conclusions: The diagnosis of allotrophagia was made based on anamnesis, the clinical characteristics in the animal and the surgical extraction of the material, which are characteristic in this type of nutritional alteration. Although it is a common event in cattle from this region of Sucre department, this is the first report of allotrophagia in cattle of this department and Colombia.

Keywords: depraved appetite, pica, minerals, deficiency.

AH-P02

Can we detect abomasal ulcers in calves using sugar?

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Objectives: Abomasal ulcers occur in all cattle and are especially common in veal calves. To our knowledge, there is no reliable *ante mortem* method for diagnosing early stages, hence treatment cannot be initiated. Perforated ulcers are a result of progression of the disease and cause a large proportion of fatalities in this group of animals.

In our study we assessed if sucrose could be used as a marker for upper gastrointestinal damage in calves. We measured sucrose concentrations in blood after oral administration in calves to identify animals with abomasal ulcers.

Materials and methods: Terminally ill calves (part A) and calves designated for slaughter (part B) were given a sucrose solution *per os* and blood samples were taken before as well as 15, 30, 60, 90 and 120 minutes (part A) or 30 and 60 minutes (part B) after administration of the sucrose solution, respectively. Then the calves were euthanized (part A) or slaughtered (part B) and the abomasa were examined. We recorded areas of damaged mucosa (discoloration or ulcer) photographically and measured their total extent using software that applies a polygon method. Serum samples were analyzed using high performance liquid chromatography-mass spectrometry (HPLC-MS/MS). We analyzed the data using general linear mixed models.

Results: Calves both with healthy and damaged abomasal mucosa had increasing sucrose values over time. In part A there was an apparent difference between the sucrose concentrations in calves with ulcers compared to calves without ulcers. However, these differences were not statistically significant. In part B no statistical difference could be shown between the groups. In addition, there was no relationship between the size of the mucosal lesion and sucrose concentrations.

Conclusions: The most relevant reason for lack of differences in sucrose concentrations between the groups was most likely the timing of blood sampling. However, sucrose as a marker for gastric damage may prove useful in calves from the data presented herein. Further studies should be conducted with modified sampling times testing different concentrations of sucrose in calves of different ages with more severe ulcers.

Finding a valid method to detect abomasal ulcers would be an important asset to improving animal welfare and should therefore be pursued further.

Keywords: abomasal ulcers, calves, diagnosis, sucrose.

AH-P03

Impact of novel proprietary *Saccharomyces cerevisiae* fermentation products on digestive and respiratory health of commercially reared heifer calves from birth and productivity into first lactation

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Fermentation technology has been used under highly controlled conditions to produce metabolites from selected microorganisms, e.g. *Saccharomyces cerevisiae*, rendering in improved livestock performance by means of optimizing animal's nutrient digestion, energy utilization and immune response. Application of this technology for more than 75 years has led to the development of a novel generation of fermentation prod-

ucts, specifically designed for dairy cattle's health.

One-day old female Holstein calves (n=123), exposed to a natural *Cryptosporidium* challenge at a farm in Eastern Germany, were enrolled in 41 blocks by date of birth and assigned to one of three groups: SCFP = individual daily dosages of *Saccharomyces cerevisiae* fermentation products both diluted in the milk (SmartCare®, Diamond V®, 1 g) and mixed in starter feed (NutriTek®, Diamond V®, 5 g) for 63 d; PC = positive control with commercial antiparasitic treatment (halofuginone, Halocur®, MSD, dosage as indicated by manufacturer) for 7 d; NC = negative control with no treatment.

Calf weights were recorded on days 0, 21, 42 and 63. Fecal samples (17 per calf) were gathered on days 4-14, 16, 18, 21, 28, 42 and 63; *Cryptosporidium* spp (coproantigen diagnostic kit) and *Cryptosporidium* oocysts (fecal smear staining) with shedding scores (scale 0-4) as well as presence of Rotavirus, Coronavirus and *E coli* K99 (immunochromatographic diagnostic kit) were determined in samples from days 4-21; presence of additional parasites, including *Eimeria* spp, *Giardia* sp, *Entamoeba* cysts and *Strongyloides* eggs, was examined in samples from day 63. Blocks were replaced when diseases other than diarrhea were diagnosed in any calf of the block during the first 21 d. Efficacy of passive immune transfer was confirmed by serum analysis of total protein at day 4 (>55g/l) being similar for all animals. Shedding of *Cryptosporidium* oocysts and positiveness for coproantigen occurred at least once during initial 21 d in 98% and 99% of all calves, respectively and regardless of group.

Total positive samples for oocyst and coproantigen were lower in PC group (P≤0.03). Up to day 21, SCFP group exhibited similar oocyst (14.7) and coproantigen (14.6) mean AUC values of scores as PC group (13.1 and 13.1), and lower coproantigen mean AUC values of scores than NC (17.1, P<0.001). SCFP and NC calves showed similar patterns of shedding over time but SCFP group shed lower levels than NC from d 10; whereas PC showed a trend for delayed onset of infection (about 3 d later than SCFP and NC) but increased shedding above SCFP and NC from d 16. No differences were found in rates for the additional infections assessed. Birth weight was similar among groups (40.5 kg); mean daily bodyweight gain at 21 d for PC (127 g) was lower (P<0.01) than both NC (235 g) and SCFP (228 g) but similar among all groups through the end of the test.

Results indicate that both SCFP and a recommended veterinary treatment similarly reduced the intensity of *Cryptosporidium* infection as compared to non-treated animals.

An additional study was set with 319 newborn Jersey calves in the West Coast of the USA, to evaluate the addition of 1 g/d of a soluble SCFP (SmartCare®, Diamond V®) in the milk until weaning at 60 d of age, on bodyweight changes (through 120 d), relative risk reduction (RRR) of clinical pneumonia, and milk yield as first-lactation heifers (records from 22% of animals under a same operation, calculated as weekly weights in Dairy Comp 305).

Calves receiving SCFP (n=166) were 2.3 kg heavier at 120 d when compared to their simultaneous counterparts (n=153). Rate of pneumonia cases in the same time period was 34% for SCFP group and 45% for rest of calves; calculated RRR determined a 25.2% reduced risk of developing a first case of clinical pneumonia (P=0.04) when calves received SCFP.

First lactation data reviewed for SCFP heifers (n=31) and counterpart controls (n=38) showed 2.54, 2.68, 3.45 and 4.00 kg/d of additional milk at 28, 42, 56 and 70 days in milk (P≤0.03).

The use of SCFP before weaning has a positive impact on the health of calves that influences their future productivity.

Keywords: Saccharomyces cerevisiae fermentation product, Cryptosporidium, pneumonia, calves, first lactation.

AH-P04

Inactivation of bovine leukemia virus (BLV) -infected cells present in milk using commercial pasteurizers

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Objectives: Enzootic Bovine Leukosis (EBL) is a fatal malignant B cell lymphoma cause by Bovine Leukemia Virus (BLV), which belongs to the family Retroviridae. In Japan, EBL became a notifiable disease in 1998, because the government noticed the increased number of EBL cases. Nationwide survey in Japan reported that seropositivity of BLV in both dairy cattle and beef breeding cattle was 41% and 29% in 2010, respectively. Serious problems are that the prevalence in those at less than 1 year old was 20% and 14%, respectively. To prevent a juvenile infection, both heating and freezing/thawing treatments have been considered as techniques to inactivate BLV-infected lymphocytes in colostrum and milk. In recent years, artificial feeding using an automatic feeding equipment has been used around the world for labor saving. However, the problem of infectious diseases, which considered to be caused by feeding equipment has also come to arise. In BLV infection, no reports of BLV inactivation using an automatic pasteurizer. Then, we investigated whether a large or a small scale automatic pasteurizer could prevent BLV infection in vivo.

Materials and Methods: Fifteen sheep were used for the large pasteurizer test; 5 in the heating test, 2 in the incomplete heating test, 7 in the non-heating test, and 1 in the negative control. Eleven sheep were used for the small table top pasteurizer test; 5 in the heating test, 5 in the non-heating test, and 1 in the negative control. Peripheral blood mononuclear cells (PBMC) were isolated from EDTA blood of BLV-infected cattle by the density gradient method. For the large pasteurizer test, the PBMC was added to commercially available milk at 1x10 ^ 8 cells per animal and milks were heated with Kompakt Pasteur (Forster Technik, Germany) at 72 °C for 20 seconds or

at less than 72 °C for 20 seconds. Un-heating milk was passed through the machine without heating. Each of them was centrifuged four times and concentrated to 40 mL per animal. For small pasteurizer test, 3.5x10 ^ 7 cells of PBMC were added to 40 mL of milk per animal and heated with MAM12A (Orion Machinery, Nagano, Japan) at 60 °C for 30 minutes. Non-heated milk was left at room temperature for 30 minutes. Each was administered intraperitoneally to sheep. Sheep were observed for 120 days and BLV and antibody were measured by quantitative PCR and ELISA, respectively.

Results: In large pasteurizer and small pasteurizer tests, BLV and anti-BLV antibody were detected in sheep inoculated with un-heating milk in 2-3 weeks. Sheep inoculated with incomplete heating of the large pasteurizer were also observed BLV infection. However, no virus and antibody were detected in the sheep inoculated with completely heated milk.

Conclusions: Heating milk included BLV-infected cells by using large and small pasteurizers according to manufacturer's instructions was shown to prevent BLV infection. On the other hand, incomplete heating the milk could not inactivate BLV. It is important to control the temperature of pasteurized milk. In feeding management using automatic feeding equipment, feeding milk to calves using an automatic pasteurizer with complete temperature control will help prevent calves from BLV infection.

Keywords: Bovine Leukemia Virus, Milk, Pasteurizer, Inactivation.

AH-P05

Calfmatters Survey 2020 – farmer perception of calf health and management strategies

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Introduction: This is the fourth consecutive year that the Calfmatters survey has been run by Boehringer Ingelheim Animal Health, with over 400 farmers responding in 2019. The aim of the survey is to collate up to date information from UK and Irish farmers on calf diseases such as bovine respiratory disease (BRD) and scour and provide insight into on farm management practices currently being used to reduce the impact of calf disease. At the time of writing the results for the 2020 survey have not been collated but will be presented at the conference.

Objectives: Minimising disease and meeting target growth rates are essential for a sustainable calf rearing system. As well as being economically viable, farming is under increasing scrutiny to demonstrate good animal welfare and reduce the use of antibiotics. Calf health, and in particular, BRD is a known hotspot for antibiotic use, with a call to work with industry stakeholders to monitor and to increase the year-on-year use of vaccines against respiratory disease[1]. The survey aims to collate data on farmer perceptions and calf management practices, which together with previous years' data can provide insights into calf rearing, share knowledge and increase awareness with the aim of reducing disease and antibiotic use.

Materials and Methods: Informed veterinary consultants originally developed the questionnaire. The mainly quantitative survey was promoted via Calfmatters articles in various farming journals and on the Calfmatters website with Facebook and Twitter promotions. The questionnaire was open for respondents for a six week period in May and June 2020 and data was collated into an Excel Microsoft spreadsheet which was used for descriptive analysis.

Results: The 2020 survey results are not available at the time of writing, but will be presented at WBC. Results from previous years can be found at www.calfmatters.com. The questionnaire is available on request from the authors. Previous survey results have shown[2]:

- 40% of farms treated >5% of their calves for BRD in winter of 2019.
- The biggest impacts of BRD are vet and medicine costs, reduced income from deaths and from lack of production.
- Favoured management practices to reduce BRD impact are adequate colostrum, housing in similar age groups and optimising housing.
- Calf jackets are used more often than vaccination as a measure to prevent BRD.
- Vaccination – a year on year increase with 46% of 2019 respondents vaccinating some or all of their calves.
- Farmers who vaccinated see a reduced incidence of BRD, reduction in antibiotic use and improved calf health and wellbeing.

Conclusion: The survey results are from a subset of UK and Irish calf rearers and provide a picture of current calf rearing practices. Calf health is an area known for its high antibiotic use, an indicator of calf health and hence an area for improvement. Regardless of whether a calf is reared for dairy or beef, all farms have common goals for calf rearing, which focus on producing healthy well-grown calves with minimal disease and minimal use of antibiotics. Healthy animals are the corner stone of sustainable production. Therefore scrutinising the process of calf management aims to provide information, which can be used to inform stakeholders, as well as increasing awareness, with the goal of reducing disease and improving calf health and welfare.

References:

1. RUMA (2017) Targets Task Force Report 2017.
2. Boehringer Ingelheim Animal Health (2019) Calf matters survey report.

Keywords: calf health, BRD, pneumonia, questionnaire.

AH-P06

Metal detection device used in civil construction validated for detection of metallic foreign bodies in cattle.

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Objectives: The objective of this study was to validate the application of the [GMS 120 Professional BOSCH®] metal detector (MD) in the detection of metallic foreign bodies (MFB) in the bovine species and describe its use in field cases.

Materials and methods: MFBs (n=50) of varying shape, size, and weight such as wires, nails and ferrules were evaluated with an MD, initially on a flat and inert surface, and then in a permanent rumen fistulated cow. All materials were properly identified, weighed, measured and sharp objects had their tips sealed with silicone, preventing penetration of the cows reticulum mucosa. The project was approved by the ethical committee of the Pontifical Catholic University of Paraná (PUCPR) under number 01122. In order to have control over the detection distance for each material, distances of 10 and 20 cm and the minimal detection distance (MDD) was checked through color changes shown on the device's display shifting from green to yellow and red. The minimum detection distance was established as the shortest distance in cm between the device and the metallic object, identified by the MD with yellow color. All measurements were made in triplicate and the mean obtained. The fistulated cow belongs to the veterinary hospital of PUCPR, used mainly for transfaunation purposes for sick cows. It was an obese cow with BCE (body condition score) 5 (1-5). The ruminal fistula allowed the insertion and manual removal of the objects at the bottom of the ventral blind sac of the reticulum. The 50 MFBs were introduced individually, measuring the possibility of detection between the fifth and seventh intercostal spaces on the left side and ventral of the cow with MD. All measurements were in triplicate with at least two minutes of measurement per object, to allow a succession of reticular waves of contraction and relaxation. During the period from July 31 to October 31, 2017, MD was used during the daily clinical routine of veterinarians at Cooperativa Mista Agropecuária Witmarsum LTDA., in the municipality of Palmeira and at the ruminant clinic of PUCPR, in the municipality of Fazenda Rio Grande. At the same time, the cooperative's service database for cattle between the years 2000 to 2017 was accessed, calculating the relative incidence of cattle detected with MFB. Beginning in 2013, the use of MD was introduced by the veterinary service of the cooperative. MD was always used when there was a clinical suspicion of hardware disease and compared to the withers pinch test.

Results: On a flat and inert surface no object was detected at a distance of 20 cm. At 10 cm, two objects were detected. The mean MDD for MFB detection on the inert surface was 4.6 cm. In the fistulated cow, it was not possible to detect any MFBs, regardless of the size or weight of the material. In the field the MD was used in fifteen cows with suspected reticulitis, all of them with positive withers pinch test, reduced milk production and appetite. Twelve cows presented muffled heart sound, ten fever, seven antalgic positions and three had also a chest edema. Eight cows (53, 3%) tested positive with the MD. After the acquisition of MD by the cooperative, there was a significant (P< 0,001) increase from 261 cows in the years 2000 to 2012 up to 431 cows from 2013 to 2017 in the diagnosis of the presence of MFB in cattle.

Conclusion: The [GSM-120 Professional BOSCH®] device only detects MFB in cattle in specific situations, when the MFB is close to the apparatus. Failure of detection by the device does not exclude ingestion of MFB. Nevertheless, the use of the device is an alternative that can be considered in specific situations, as a diagnostic aid for the practitioner.

Keywords: hardware disease, diagnosis, metal detector, cattle, reticulitis.

AH-P07

Foreign bodies in the fore-stomach of beef cattle in southern Brazil: do they represent a threat ?

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Objectives: The objective of the study was to describe the nonmetallic foreign bodies (NFB) and metallic foreign bodies (MFB) ingested by beef cattle, classifying them according to their dangerousness and identifying their possible geographical origin.

Materials and methods: The project was approved by the ethical committee of the Pontifical Catholic University of Paraná (PUCPR) under number 01230. During a 4-month period, fore-stomachs (n=5834) were examined in a federal inspected abattoir of the state of Paraná/Brazil. After receiving the stomachs, an incision was made in the ruminal wall exposing its content and the internal rumen and reticular mucosa, making it possible to visualize and palpate them to identify lesions suggestive of reticulitis and NFB and MFB removal. The FB were washed and disinfected with a 2,5% chlorine solution identified and separated according to the type of material and classified in: non-dangerous, moderately dangerous and very dangerous, according to the potential capacity to perforate the reticular wall or obstruct the gastrointestinal tract. Age (<25 months; ≥25 < 36 months; ≥ 36 ≤ 48 months), sex (female, male; male split into ox and bulls) and the place (municipality) of origin were registered. Chi-Square test were applied for comparison of groups and the relative risk of ingestion of NFB and MFB were calculated using StatGraphics Centurion XVI.I. All the results described with significance for $p \leq 0.05$.

Results: Animals with FB were found in 23 (n= 50) municipalities. From the fore-stomachs a total of 496 objects were recovered, with NFBs 92.2% and 7.8% MFBs. 377 (6.46 %)

animals presented FB with 8.75 % (n= 33) of MFB. Male cattle predominated (88.1%) over female (11.9%). In the group of male oxen represented 40.2% and bulls 59.8% of the animals.

The largest amount of FB observed were stones with 84.7% of all objects. Other FB were respectively: nails (4.6%), plastics (3.2%), wires (1.9%), rubbers (1.2%), trichobezoars (1.0%), tangles of plastics and cloths (1.0%), threads (0.4%), cloths (0.4%), ferrules (0.4%), screws (0.4%), nuts (0.4%)), toy (0.2%) and fence clamps (0.2%). The percentage of total FB intake among animals <25 months was 0.083%, between 25-36 months 0.058% and between 36-48 months 0.057%. According to the hazard classification 53.9% of the NFB and MFB found correspond to very dangerous, 22.5% moderately dangerous and 23.6% non-dangerous. The percentage of intake of NFB and MFB in non-castrated and castrated cattle was 0.069% and 0.067%, respectively. Regarding the relative risks, it was observed that the chance of finding stones was the same in castrated and non-castrated cattle (1.03). However, for plastics there was 1.44 times more chance of being found in non-castrated cattle. Bulls are also 1.32 times more likely to have nails in their reticulum. For the other MFBs, the chance of finding objects in bulls was 1.18 times greater than in oxen. Only two animals with macroscopic reticulitis were found.

Conclusion: Although the overall presence of FB was not high compared to other developing countries, probably reflecting the young age and breeding purpose, the potential hazard of the described FB still was high. The higher risk in younger and non-castrated male cattle possibly reflects behavior patterns. Highly variable FB are ingested by beef cattle in Paraná/Brazil with origin in more than half of the observed municipalities.

Keywords: cattle, foreign body, prevalence, reticulitis, ingestion.

AH-P08

Biosecurity practices and their application in Italian dairy farms: which are the main critical points?

Francesca Mazza[1], Nicoletta Formenti[2], Giandomenico Ferrara[1], Matteo Tonni[2], Giordano Ventura[3], Valentina Lorenzi[1], Francesca Fusi[1], Massimo Boldini[3], Luigi Bertocchi[1], Giovanni L. Alborali[2].

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Objective: Biosecurity is defined as a set of practices that prevents the introduction and the spread of transmissible diseases within and between animals. Although these measures could help to reduce antimicrobial usage and to increase animal welfare, their applications in dairy cattle farms are still limited and often influenced by farmers' awareness. The need is therefore to investigate criticisms in biosecurity practices within dairy cattle farms with different herd sizes and the relation between the experience and training of farmers with (i) the general level of biosecurity and (ii) the risk of animal exposure to bovine rhinotracheitis (IBR) and paratuberculosis.

Materials and methods: From July 2019 to December 2019, 105 loose housing dairy farms located in Northern Italy were assessed for biosecurity using ClassyFarm (www.classyfarm.it), a national health and welfare monitoring system. The study was carried out in 12 small dairy farms (≤65 lactating heads), 61 medium farms (66-199 lactating heads) and 32 large farms (≥200 lactating heads). Farmers were voluntarily involved in a risk-based survey that includes 15 questions about relevant aspects of on-farm biosecurity and the results were expressed on a scale from 0 to 100%. In addition, farmers' training/educational level (degree or diploma in agriculture sciences/veterinary sciences/attendance of specific training course) and years of experience were registered. Herd status for IBR and paratuberculosis were also recorded.

Results: The mean biosecurity score (BS) of the investigated farms was 59.16% (17.24% - 90.93%). In particular, the 45.7% of the farms showed an insufficient biosecurity level (BS<60%), the 49.5% were judged good (BS between 60% and 80%) and the 4.8% excellent (BS≥80%). The main critical points were related to: lack of disinfection for vehicles entering the farm (94.3% of the farms); contact between dairy cows and other animal species (rats, dogs, cats, pigeons, coypus) (88.6%) and boot covers and disposable gowns not available for visitors (61%). In addition, feed and milk trucks and vehicles transporting live animals transit or stop at a distance of <20 meters from the animals in the 95.2% and in 70.4% of cases, respectively.

All the involved farmers showed a minimum of 10 years' experience in dairy cattle farming and the 64.8% of them had an educational qualification or training courses. In detail, farmers of small, medium and large herds had specific education or training in the 25%, 65.6% and 78.1% of cases, respectively. In medium and large herds, managed by farmers with a specific training, a good or excellent BS was recorded in 65% and 60% of cases, respectively. In small herds, BS was insufficient in the 66.7% of cases. Farms showing an excellent BS were all managed by trained farmers, independently from the herd size.

The 70% of medium herds and the 76% of large herds, handled by trained farmers, were at low risk of IBR (all animals seronegative). Concerning paratuberculosis, the training level of the farmers did not affect the health status both in small and medium herds. Conversely, in large herds managed by trained farmers all the cows older than 36 months resulted seronegative in the 60% of cases, while some seropositive cows (≥ 36 months old) were detected using a screening test in large herds managed by no-trained farmers.

Conclusions: The present study underlined some criticisms in the application of biosecurity practices within the investigated dairy farms. In general, producers of large herds and with a specific training demonstrated higher awareness of the importance of adequate biosecurity measures than those of small herds and without training. However, the attention of farmers towards potential health risk related to the entrance

of visitors and vehicles and to the presence of other vectors of diseases (e.g., mice and rats) should be implemented in all the farms, independently from the herd size and from the level of training.

Since this is the first time that biosecurity practices are investigated in Northern Italy, the obtained results will be used as a starting point for raising farmer awareness and for increasing the collaboration between veterinarians, consultants and technicians in order to solve the major critical points and improve the level of biosecurity in Italian dairy farms.

The study was part of the IZSLER 01/2017 "Strategica project" financed by Italian Ministry of Health.

Keywords: biosecurity, herd size, farmers' training, IBR, paratuberculosis.

AH-P09

Routine calf scoring in pre-weaned dairy heifers: can it reduce antimicrobial usage?

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Objectives: Bovine respiratory disease (BRD) is a significant cause of economic loss in both dairy and beef cattle. Work from the USA suggests that feedlot cattle never treated for BRD made significantly more at slaughter than treated animals, and slaughter value decreases with increasing number of treatments (Cernicchiaro and others 2013). The Wisconsin Calf Scoring (WCS) system has been developed and postulated to be a useful tool in detection and early treatment of BRD in calves (McGuirk and others 2014). While this protocol has been compared with thoracic ultrasonography in terms of sensitivity, there have as yet been few published works describing the efficacy of the WCS in the UK.

The present trial aimed to investigate the effects of implementing a twice weekly protocol of WCS and treatment on four dairy farms. The primary aims of the trial were to investigate whether this intervention, compared to farmers' standard observation and treatment protocols, resulted in a reduction in antimicrobial use on farm and improvements in morbidity and mortality.

Materials and Methods: A convenience sample of pre-weaned calves from four dairy farms were enrolled at birth and allocated to either treatment (TR) or farmer observation group (CON) using random number tables. The calves were subsequently scored twice weekly on Monday and Friday by two trained veterinary technicians during the pre-weaned period.

All calves (TR and CON) were scored in order to give a uniform measure of morbidity. Treatment was actioned by the technicians in TR calves, but the farmer was not made aware of the results for the CON group. Instead, the farmer was instructed to continue with current detection and treatment protocols for the CON calves.

Treatments and deaths were recorded in both the on-farm diaries and a relational database for both groups. Where available, reasons for deaths were also recorded.

Results: The study recruited 266 calves and recorded 3956 data points (individual calf scores) and 144 treatments between October 2016 and April 2017. There were significantly more scores over the treatment threshold (composite score of 5) in the control (CON) group compared with the treatment (TR) group (P=0.0006) but that this came at a cost of significantly increased use of antibiotics.

There was also a trend for more calves to be scored repeatedly high (>4) in the control group however this was not significant. There were no differences in the number of calves treated in the TR vs CON groups, nor were there any differences in mortality.

Conclusion: The results suggest that farmers significantly under-diagnose respiratory disease in pre-weaned calves, and that this under-diagnosis leads to prolonged bouts of illness for the calves.

The working hypothesis that the antimicrobial use in the treatment group could be reduced through early detection and prompt treatment was proved false but potentially only due to the aforementioned under-diagnosis. The number of chronically sick calves, though not significant, was considerably higher in the control group suggesting that either the control group calves illness wasn't recognised until they had been scored high for several observations, or they continued to be scored high and lwere not retreated after the initial treatment by the farmer.

Interestingly there was no difference in the number of calves in each group which became sick suggesting that the early detection did not significantly decrease transmission within the groups.

This finding would suggest that the metaphylactic treatment of calves is an unnecessary overuse of antimicrobials as around half of the calves are unlikely to require treatment.

Furthermore there was no significant difference in mortality between the groups. However this could have been significantly affected by one farm which had significant calf diarrhoea problems and may have lost numerous calves to this disease, thus confusing the picture of the mortality related to BRD.

It is not currently possible to deduce whether treatment timings affected the control group's results; namely were the calves treated early and not retreated or was treatment genuinely delayed? Either way further investigation into the data to elucidate whether the control group were treated early and not retreated or not treated until later in the disease course is necessary.

Keywords: youngstock, calves, pneumonia, antimicrobial.

AH-P10

Integration between health and microbiome in Holstein cows immediately after calving

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The transition period includes physiological events such as parturitium, colostrogenesis and lactogenesis, which requires the activation of homeorrectic mechanisms to meet the energy demands imposed at this stage of the production cycle. The inadequate adaptation of the cow in the period can evolve to negative energetic balance, metabolic diseases, immunosuppression and high risk for infectious diseases. In this scenario, it is feasible to believe that the cow's microbiota undergoes important changes in different colonization sites, and as a cause or effect it interferes with the health of the host. The general objective of this research was to evaluate the correlations between metabolic parameters and the health status of the mammary gland with the microbiota of dairy cows immediately after calving. The calving from 20 Holstein cows were accompanied and immediately afterwards; blood samples (metabolic status), mammary secretion (udder health status), fecal, vaginal and colostrum samples were collected for the metagenomic study of the 16S rDNA gene. Archaea kingdom were only found in fecal and vaginal samples. Fecal samples showed a greater abundance of p_Firmicutes, followed by p_Bacteroidetes and p_Actinobacteria. Vaginal samples showed dominance of p_Firmicutes followed by p_Bacteroidetes and p_Proteobacteria. At the family taxonomic level, f_Ruminococcaceae was detected as the most abundant, followed by f_Bacteroidaceae, f_Lachnospiraceae and f_Clostridiceae in fecal and vaginal samples. The colostrum microbiota, on the other hand, showed a predominance of p_Proteobacteria, followed by p_Firmicutes and p_Bacteroidetes. On the other hand, the predominant families detected in the colostrum samples were f_Pseudomonadaceae, f_Staphylococcaceae and, to a lesser extent, f_Enterobacteriaceae. Regarding the analysis between communities (beta-diversity), it showed the fecal and vaginal samples, presented a similar profile in terms of abundance and shared OTUs, while the colostrum samples had a different profile. In the analysis of correlations between the health status of cows and their microbiome showed that g_Treponema presented a negative correlation with the biomarked protein metabolism and lipidogram, and was identified in faecal, vaginal and colostrum samples. g_ Coprococcus presented the same correlation profile, however this genus was significant only in colostrum samples. The bacterial genus identified as g_L7A-E11 was negatively correlated with biomarkers of energy metabolism and with body score condition, it was also identified in all types of samples. g_Methanocorpusculum (Archaea) was found in fecal and vaginal samples, and showed positive correlation with lipid and energy metabolism. On the other hand, Prevotella showed a positive correlation with these biomarkers, and was significant only in vaginal samples. Finally, the genus g_Lactobacillus showed a negative association with the score of body condition, biomarkers energetic metabolism, only fecal samples. In conclussion, the analysis of the entire ecosystem showed that the parameters associated with metabolism, mainly energetic and lipidogram, are associated with the abundance of some bacterial genera mainly in faecal and vaginal samples. So, it was possible to identify a relationship between the microbiota and physiological or pathological status of the host at calving time of Holstein cows immediately after calving.

Keywords: Microbiota, Peripartum, Colostrum, Metabolic profile.

AH-P11

Immunoglobulin G and serum total protein concentration assessment in dairy calves over the first 2 weeks of age

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Objectives: The aims of the present study were to: (1) evaluate changes of serum IgG (SIgG) and serum total protein (STP) concentration of dairy calves during the first 16 d of life, and (2) investigate if predictive models for SIgG concentration based on STP could be improved when adjusted by hematocrit (Hct).

Materials and methods: Enrolled calves (24 Jersey, 41 Holstein) arrived at a commercial raising operation from two source dairies. Prior arrival, calves were bottle fed 3 meals of pasteurized colostrum (2 L per meal) at (mean ± SD) 38 ± 26 min, 7 h 19 min ± 1 h 5 min, and 7 h 45 min ± 1h after birth. Concentration of SIgG (single radial immunodiffusion), STP (BRIX refractometer), and blood Hct (centrifugation), were determined in blood samples collected from calves immediately after arrival at the calf operation (1 d) and 4, 8, 12 and 16 d of life. The MIXED procedures of SAS were used to evaluate changes over time of SIgG and STP as well as the predictive value of STP corrected by Hct.

Results: At 1 d, SIgG ranged from 252 and 10,619 mg/dL (mean: 2,124 mg/dL), STP from 3.6 to 9.3 g/dL (mean: 6.0 g/dL) and Hct from 18 to 44% (mean: 30%). During the study period, on 1, 4, 8, 12 and 16 d, calves had SIgG < 1,500 mg/dL (n = 11, 20, 28, 36 and 45 respectively) and serum TP < 5.5 g/dL (n = 15, 26, 31, 47 and 63 respectively). Relative to 1 d, SIgG tended to decrease at 4 d (205 mg/dL), and it was significantly lower at 8 d (343 mg/dL), 12 d (583 mg/dL) and 16 d (747 mg/dL). Relative to 1 d, STP concentration significantly decrease with time at 4 d (0,344 g/dl), 8 d (0.470 g/dl), 12 d (0.704 g/dl) and 16 d (1.107 g/dl). There was a moderate correlation between SIgG at 1 d with STP at 1 d (r = 0.66), 4 d (r = 0.61, 8 d (r = 0.63), 12 d (r = 0.54), and 16 d (r = 0.66). The model fit for SIgG prediction did not improve when STP was adjusted by Hct.

Conclusions: In summary, our results indicate that SIgG and STP declined during the first 2 weeks of life. Further studies should determine the specificity and sensitivity of SIgG and

STP during the first 2 weeks of life as diagnostic tools for failure of passive transfer of colostrum.

Keywords: calves, immunoglobulins G, total proteins.

AH-P12

A simplified health score system to monitoring calves housed in groups

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Objective: The aim of the present study was to assess the predictive capacity of a simplified score system for the diagnosis of calf diarrhea (CD) and calf respiratory disease (CRD) in dairy calves.

Materials and methods: Holstein calves (n= 324) from two commercial dairy farms (A and B) located in Castelli, province of Buenos Aires, Argentina, were enrolled in this study. Calves enrolled were housed individually and were evaluated once a week for 9 weeks (1-7 days to 56-63 days) using two clinical scores: 1) calf health scoring chart of the University of Wisconsin (CSW), and 2) a simplified calf health scoring chart (CSS). The CSW was used as a reference gold standard and included the following signs and assigned them a value (0 to 3): nasal discharge, ocular discharge, coughing, ear position, and fever for diagnosis of CRD and feces consistency for diagnosis of CD. Calves were considered positive for CRD when the total score was ≥ 5 and for CD when the score was ≥2. The CSS included the following signs: cough, ocular discharge, nasal discharge and ear position for diagnosis of CRD, and a cleanliness score for the diagnosis of CD (score 0 = calf is clean, and 1 = tail head region, thighs or/and legs soiled with manure). This simplified score dichotomized the clinical signs (Cough: none= 0 and any= 2, ocular discharge none= 0 and any= 2, nasal discharge none= 0 and any= 4 and ear position slight ears droop= 0 and ears droop= 5) and calves were considered positive when the total score was ≥ 5 or 1 for CRD and CD, respectively. The Kappa coefficient evaluated the agreement between CSW and CSS methods with Proc Freq of SAS. The same procedure was used to determine sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR) and negative likelihood ratio (NLR) of CSS method.

Results: The prevalence of CD was 10.5% and 17.9% diagnosed by CSW and CSS, respectively, whereas the prevalence of CRD was 7.4% and 6.1% diagnosed by CSW and CSS, respectively. This prevalence varied depending on the age of the calves, especially in the case of CD. The two scores had a good and a weak agreement for the diagnosis of CRD and CD, respectively (Kappa`s coefficient of 0.72 and 0.15, respectively). In the case of CRD diagnostic, the CSS has a sensitivity of 96% and specificity of 95%, whereas the PPV was 61%, NPV was 99%, PLR was 19.2, and NLR was 0.04. In the case of CD diagnostic, CSS has a sensitivity of 94% and a specificity of 49%, whereas the PPV was 18%, the NPV was 99%, the PLR was 1.8, and NLR was 0.1.

Conclusions: We concluded that CSS is a useful test for the diagnosis of CRD but not for diagnosis of CD. In the case of CRD diagnosis, CSS is objective, simple, and easy to carry on by the people who work in the farms and could be used in dairy calves housed in groups because it does not require handling them. On the other hand, for CD diagnosis, we recommend the usage of feces consistency instead of a cleanliness score.

Keywords: Diagnosis, sensitivity, specificity, calf diarrhea, respiratory disease.

AH-P13

Development and validation of a new immunoturbidimetric assay to quantify bovine Immunoglobulin G (IgG)

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Objectives: Immunoglobulin G (IgG) is the most abundant isotype of immunoglobulin in plasma and colostrum in cattle and has an essential role in the adaptive immune system. Because of their type of placenta, transfer of immunity from the dam to the offspring does not take place during gestation in ruminants. For this reason, calves are born agammaglobulinemic and a crucial step in calf management is the feeding of colostrum to newborn calves during the first hours after birth. To confirm an adequate passive immunity transfer is convenient to determine plasma IgG concentration in the calf. Current methods to measure IgG in calves either are based on indirect measures, need several days to obtain the results (radial immunodiffusion) or require an elevate dilution that could underestimate the actual concentration, as occurs with ELISA assays. The objective of the present work is to develop and validate a new immunoturbidimetric method for IgG quantification specific for bovine, adaptable to clinical chemistry analyzers and that will not require excessive dilution steps.

Materials and Methods: An immunoturbidimetric method based on sheep polyclonal antibodies anti bovine IgG was developed and adapted to an Olympus AU400 analyzer. Analytical validation was based on studies of linearity, prozone effect, imprecision, limit of detection, and interferences. Limit of detection was established by measuring 30 times a blank without IgG. Linearity and prozone effect were calculated by serial dilution of a sample containing high IgG concentration. Hemoglobin, bilirubin and triglycerides were analyzed as potential interferents by determining IgG 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, in two analytical runs. Serum samples from calves at different days after birth and cows in peripartum were used for biological validation.

Results: The assay kept linearity under dilution up to 14 mg/mL. No prozone effect was observed with IgG concentration of 30 mg/ml (serum) or 80mg/mL (colostrum). Limit of detection was 0,030 mg/mL. The within-run coefficient of variation was <1,5% and the between day CV was <3%. No interference from bilirubin (150mg/L) or hemoglobin (15g/L) was detected but values above 10 g/L of triglycerides showed a slight interference (10%). Biological validation showed an increase of IgG levels after colostrum intake in calves (up to 26 mg/mL after five days) and a decrease in the peripartum period in cows.

Conclusion: The new turbidimetric immunoassay (Acuvet Biotech) is a rapid method (results in 4 minutes) suitable for IgG determination, and easily adaptable to biochemistry analyzers.

Keywords: Immunoglobulin, Immunoturbidimetry, colostrum, serum, laboratory diagnostics.

AH-P14

The acute phase proteins ITIH4 and Haptoglobin as markers of metritis in Holstein dairy cows

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Objectives: Metritis is a frequent disease in post-partum Holstein dairy cows caused by delayed uterine involution, persistent inflammation and consequent bacterial colonization of endometrial mucosa. Alternative diagnostic tools are needed for the efficacious treatment of the disease and as a means to decrease antibiotic treatment. Acute-phase proteins (APPs) are released in plasma mainly by the liver at early stages of illness to promote the immune response. The objective of this study was to evaluate two APPs: haptoglobin (Hp) and ITIH4, as potential biomarkers of inflammation in cows suffering metritis by using new immunoturbidimetric reagents.

Materials and Methods: A total of 139 Holstein dairy cows were monitored during the periparturient period and examined for the occurrence of metritis symptoms after parturition and their severity. Blood samples were collected at 10 and 5 d pre-partum and at 1, 3, 7, and 10 d post-partum. Species-specific immunoturbidimetric assays (Acuvet Biotech, Spain) were used for APP analysis. Data were analyzed using an analysis of variance accounting for the fixed effects of presence or absence of disease, time, and their 2-way interaction, and cow entered the model as a random effect.

Results: ITIH4 and Hp concentration in serum were similar in pre-partum samples between healthy and metritic cows. However, a noticeable increase in the 2 proteins after parturition was detected, with this increase being more pronounced in cows with metritis. Furthermore, the increase in ITIH4 and Hp serum concentrations was proportional to the severity of the disease. Serum ITIH4 and Hp concentrations decreased 10 d after calving in healthy cows; whereas, in metritic cows remained elevated. Hp serum concentration changed more markedly than ITIH4, but both biomarkers followed a similar pattern.

Conclusions: Hp and ITIH4 serum levels are useful biomarkers for diagnosis and monitoring bovine metritis during the onset of the disease.

Keywords: ITIH4, Haptoglobin, Metritis, Dairy cows, Acute phase proteins.

AH-P15

Preconditioning programs: a solution to reduce bovine respiratory diseases in fattening units?

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Objectives: Preconditioning programs are sets of management practices to boost young bull's immunity and low weaning and arrival stress', in order to reduce respiratory diseases in fattening units. The aim of this study was to evaluate the interest of preconditioning programs in the French context.

Material and Methods: One hundred and sixty eight young bulls had been monitored from birth to slaughter in a control/case study. Preconditionned young bull (PREP) were weaned indoors 50d before leaving the farm, had received a trivalent vaccine (BRSV, BPI3, *Mannheimia haemolytica*) against respiratory diseases and were fed with a diet supplemented in vitamins and trace elements. Control bulls (CTRL) remained in pasture with their dams, with no vaccination nor particular diets. Weight, health, behavior, post-mortem lung status has been evaluated and analyzed by variance analysis. Immune response to vaccination (antibodies production, cellular response against main respiratory pathogens) and general immune competence had been evaluated and analyzed by ascending hierarchical classification on forty young bulls, representative of the total number of young bulls. Clinical score (0 to 10 points) and pulmonary lesion score (0 to 52 points) have been constructed to synthetize information related to respiratory diseases.

Results: During the 50d prior leaving, average daily gain (ADG), serum zinc and serum glutathione peroxidase concentrations were higher for PREP compared to CTRL (+ 440g/d; +3pmol/L; +118U/gHb; $p<0.001$), in relation to feeding management. During the preconditioning period, slight and moderate respiratory signs had been reported by farmers for certain PREP and CTRL batches, in relation to husbandry and environment factors (pathogens circulation, housing conditions). No significant difference regarding young bulls' behavior has been identified during the first two months after arrival. At the

end of the fattening period, carcass weight and quality (EUROP notation) did not differ between batches. No significant effect has been observed on fattening duration due to beef market situation at slaughtering and experimental constraints. Unexpectedly, clinical scores tended to be higher for PREP (although variable) and lung lesional scores were higher for PREP compared to CTRL (+8 points; p<0.01). Pathogens diversity detected on farms (BAdV3, BRSV, *Mannhemia haemolytica, Pasteurella multocida*, *Histophilus somni*, *Mycoplasma bovis*) did not match entirely with the vaccine and can partly explain the results. Besides, variability in individual immune competence has been observed in relation to farms, trace elements and vaccine status.

Conclusion: In a context of reduction of antibiotics use, preconditioning programs offers promising prospects providing adjustments to take into account local condition and husbandry factors.

Keywords: respiratory diseases, young bulls, preconditionning systems, immune competence.

AH-P16

Development of innate immune response in healthy Holstein calves from birth until weaning

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The objective of this research was to evaluate the hematological profile and the innate immune response of healthy Holstein heifers. This study was conducted in the largest dairy farm located in Araras- São Paulo, Brazil (Latitude 22°21'25"S and Longitude 47°23'03"W). A total of 226 young Holstein heifers were screened based on Calf Health Scoring Criteria from the University of Wiscosin (Madison) to exclude diseased animals manifesting diarrhea, omphalitis and BRD. Moreover, it was also excluded calves presenting failure of passive immune transfer (total protein ≤ 5.5 g/dL). Healthy calves i were distributed in three experimental groups according to age: G1 - calves from 1 to 7 days old (n=26), G2 - calves from 30 to 40 days old (n=28) and G3 - calves from 60 to 89 days old (n=36). It was performed the erythrogram and leukogram were performed by using the ADVIA 2120, beyond the measurement of serum iron, total protein and haptoglobin. Phagocytosis of pre-labed *Escherichia coli*, *Staphylococcus aureus* and *Mannheimia haemolytica* were evaluated by using flow cytometry. Production of ROS (Reactive Oxigen Species) was assessed by use of the fluorescent dye Dihidrorodamina 123 (DHR). For this, leukocytes isolated from EDTA blood were placed in quadruplicates wells of 96-well culture dish in 100µL of either RPMI alone (unstimulated) or in medium containing 10 µL PMA 10^{-6}M and inactivated *Escherichia coli* 1:10, *Staphylococcus aureus* 1:5, *Mannheimia haemolytica, Mannheimia haemolytica* 1:5. Subsequently, 10 µL of DHR (10 µM) was added to each well. The ´plates were incubated for 2 hours at 37°C and then evaluated by use a fluorescent reader. Data were expressed as AFU values. Response ratio (RR) also were calculated as follow: AFU value of stimulated cells/AFU value of unstimulated cells. Differences between ages group were analyzed by Tukey´s test. Lower values for red blood cell count (P<0.0001), hemoglobin concentration (P<0.0001), mean cellular hemoglobin concentration (CHCM) (P<0.0001) and mean hemoglobin corpuscular concentration (MCHC) (P<0.0001) were observed in G1 calves compared to groups G2 and G3. The white blood cell count was lower (P =0.02) in newborns than calves of other age groups (G2 and G3). The number of lymphocytes increased with age, with higher values in G3 than G1 and G2 (P<0.0001). The relative and absolute number of basophils was lower in newborns (G1) than in G2 and G3 (P <0.0001). The intracellular myeloperoxidase index was high in neonates (G1) (P<0.0001) when compared to other age groups (G2 and G3). A lower percentage of total plasma protein (P <0.0001) was detected in G2 heifers. Newborn calves (G1) had a lower serum iron concentration (P<0.0001). No differences were found for haptoglobin values. Calves of different ages had similar phagocytosis response against the investigated species of bacteria, except for the higher mononuclear phagocytosis intensity in G2 than other age groups (G1 and G3) (P=0.0007). The intensity of the fluorescence released by blood cells (AFU) was influenced by age. A decrease in ROS production by using bacteria stimulation according to age was observed. Healthy neonates stimulated with *E.coli* 1:10 (P=0.0018) and *M.haemolytica* 1:5 (P=0.04) had higher values of AFU than G2 and G3 calves. The response ratio (RR) for healthy animals had different profile according to age dependently of bacterial stimulation. It was possible to observe higher values of RR when leukocytes were stimulated by *E.coli* in G1 than G3 (P=0.03). RR for *M. haemolytica* was higher in G2 than G1 and G3 (P=0.01). Neonates had low values for RBC parameters and serum iron. WBC analysis revealed an increased of total leukocytes and lymphocytes as signal of the specific immune response development in calves. It was observed some signals of innate immune cells are more responsive in neonates such as the high intracellular myeloperoxidase index and ROS production (AFU). It was also observed high response against *M.haemolytica* in calves from G2 (30-40 days of life), probably associated to the natural challenge or subclinical broncopneumonia non-detected by BRD scoring system used in this experiment.

Keywords: phagocytosis, Reactive Oxigen Species (ROS), ADVIA.

AH-P17

Effect of maternal late-gestation status on metabolic profile, health and immunity of dairy calves

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Maternal status during the transition period can have carryover effects on several health and performance variables of Holstein dairy calves. However, the effects of maternal late gestation status on profile presented by dairy calves during the neonatal period are not well established. The general objective of this study was to evaluate the impact of maternal variables at the calving on the metabolism and immunity of dairy calves during the first month of life. Holstein cows (n=28) were blood sampled at calving. The mean of the results for the maternal variables [non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), glucose, total protein, albumin, triglycerides, cholesterol, haptoglobin (Hp), iron, weight and body condition score (BCS)] was calculated and used as maternal factors. Calves were subsequently divided into groups according to their dams' high or low degree of each factor. Besides, the effect of the season at birth was evaluated differentiating the animals that were born in winter and spring. The responses of calves in each of these groups were compared throughout their first month of life. Calves were blood sampled after birth and around 2, 7, 14 and 28 days of life by assessing of IgG levels, biochemical parameters, inflammatory status, and innate immunity response. In the same moments, the calves were evaluated by frequency of diarrhea and performance. Calves born during spring to heavier mothers with a higher BCS showed a higher frequency of diarrhea; in contrast, these calves had a better performance in the first week of life regarding height at the withers and rump width when compared to calves born from lighter dams with lower BCS during the winter. About the biochemical parameters of calves, neonates that were born in the winter had a lower concentration of NEFA associated with lower concentrations of glucose when compared to calves that were born in the spring. Considering the maternal groups of low and high glucose, a statistical difference was found only on D14 with animals born from cows with higher blood glucose demonstrated to have a lower cholesterol concentration when compared with calves from dams with lower blood glucose results. Regarding the inflammatory status, the comparison between calves born from dams with high and low NEFA levels revealed that animals born to cows with low NEFA showed lower levels of Hp when compared to calves born from dams with high NEFA on D28. About the immune response, offspring from dams with low maternal albumin, low maternal cholesterol, and low maternal BCS presented high levels of basal reactive oxygen species expressed in average rate of fluorescence unit (AFU) on D7 and D28. Offspring born from dams with low BCS also had neutrophils more reactive for *S. aureus*, *S. hyicus*, and *E. coli*, considering the AFU. The response ratio against *S. aureus* and *S. hyicus* was higher in the calves was born from the low maternal BCS. Collectively, these data suggest that prenatal exposure to different maternal factors may adversely affect some metabolic, inflammatory and immune responses of the calves during the first month of life that could influence disease susceptibility and performance.

Keywords: transition period, inflammation, innate immune response, seasonality, maternal factors.

AH-P18

Apoptosis and necrosis rates by blood neutrophils in bovine leukemia virus-infected cows

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The present study aimed to evaluate the apoptosis and necrosis rates of blood neutrophils from bovine leukemia virus (BLV) naturally infected dairy cows. The serological survey was performed by agar gel immunodiffusion test and enzyme-linked immunoabsorbent-assay (ELISA) assays. Based on the results of serological testing and their hematological profile, the Holstein dairy cows at different lactation stages were divided into serologically positive cows without any hematological alteration (AL) (n = 10), serologically positive animals with persistent lymphocytosis (PL) (n = 6) and healthy serologically negative cows (Neg) (n = 9). The blood neutrophils were identified by flow cytometry using monoclonal antibody (CH138A). The apoptosis and necrosis rates of neutrophils were quantified using dual-color flow cytometry with fluorescein (FITC)-labeled annexin and propidium iodide (PI). The results of the present study showed lower percentage of neutrophils undergoing late apoptosis or necrosis (annexin-V $FITC^+/PI^+$) and necrosis (annexin-V $FITC^-/PI^+$) in BLV-infected cows with PL (0.21 ±0.09; 1.99 ±2.13) compared to the serologically negative animals (1.35 ±1.25; 8.01 ±6.52) (P=0.027; P=0.02), although no significant difference was observed in BLV-infected cows without PL (0.53 ±0.41; 3.78 ±2.84). Conversely, higher percentage of viable neutrophils (annexin-V $FITC^-/PI^-$) was observed in BLV-infected cows with PL (96.07 ±2.82) compared to serologically negative ones (88.23 ±8.01) (P=0.016), however no significant difference was encountered in AL group (93.80 ±3.39). No significant difference was observed in the percentage of apoptotic neutrophils (annexin-V $FITC^+/PI^-$) (Neg- 2.40 ±1.87; AL- 1.90 ±0.79; PL- 1.71 ±0.28; P=0.90). Thus, it can be concluded that the BLV can lead to alterations in neutrophil viability, which in turn can interfere the innate immunity, especially in BLV infected dairy cows with PL.

Keywords: cell death, deltaretrovirus, flow cytometry, innate immunity, polymorphonuclear leukocytes.

AH-P19

Apoptosis and necrosis rates by milk neutrophils in bovine leukemia virus-infected cows

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The bovine leukemia virus (BLV) can interfere functionally and in the proportion of blood B lymphocytes and also in other leukocytes. With this in mind, there is evidence that these changes also occurred in the mammary gland, and could interfere with the defense mechanisms of the mammary gland. Thus, the present study aimed to evaluate the apoptosis and necrosis rates by milk neutrophils from naturally BLV-infected dairy cows. All quarters that were bacteriologically positive and had somatic cell count higher than 200.000 cells/mL were excluded. Indeed, quarters that were treated with antimicrobial therapy in the last 30 days were not included in this study. The serological survey was performed by agar gel immunodiffusion test and enzyme-linked immunoabsorbent-assay (ELISA) assays. Based on the results of serological testing and their hematological profile, the Holstein dairy cows at different lactation stages were divided into serologically positive cows without any hematological alteration (AL), serologically positive animals with persistent lymphocytosis (PL) and healthy serologically negative cows. This study used 24 milk samples from eight serologically negative animals, 16 milk samples from six serologically positive animals without PL (AL) and 17 milk samples from five serologically positive animals with LP. The milk neutrophils were identified by flow cytometry using monoclonal antibody (CH138A). The apoptosis and necrosis rates of neutrophils were quantified using dual-color flow cytometry with fluorescein-labeled annexin and propidium iodide. The results of the present study showed to higher percentage of apoptotic neutrophils in serologically negative animals (41.40 ±15.53) compared to AL (23.10 ±18.32) and PL (17.83 ±9.01) animals (P=0.0001). Higher percentage of necrotic neutrophils was observed in PL animals (14.09 ±6.14) compared to AL (12.51 ±14.94) and healthy controls (8.63 ±6.27) (P=0.013). Indeed, lower percentage of necrotic or late apoptotic neutrophils was encountered in AL (13.77 ±13.41) compared to LP (22.94 ±14.41) and serologically negative animals (20.81 ±11.26) (P=0.04). Higher percentage of viable neutrophils was found in serologically positive animals (AL- 50.61 ±16.53; LP- 45.14 ±17.38) compared to the healthy ones (29.15 ±11.22) (P=0.0001). Thus, it can be concluded that the BLV can lead to alterations in neutrophil function which can interfere the mammary gland immunity, especially in BLV infected dairy cows with PL.

Keywords: cell death, deltaretrovirus, innate immunity, mammary gland, polymorphonuclear leukocytes.

AH-P20

Intracellular reactive oxygen species by blood neutrophils in bovine leukemia virus-infected cows

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The present study aimed to evaluate the intracellular reactive oxygen species (ROS) production by blood neutrophils in bovine leukemia virus (BLV)-infected dairy cows. The serological survey was performed by agar gel immunodiffusion test and enzyme-linked immunoabsorbent-assay (ELISA) assays. Based on the results of serological testing and their hematological profile, the Holstein dairy cows at different lactation stages were divided into serologically positive cows without any hematological alteration (AL) (n = 10), serologically positive animals with persistent lymphocytosis (PL) (n = 6) and healthy serologically negative cows (Neg) (n = 9). The blood neutrophils were identified by flow cytometry using monoclonal antibody (CH138A). The intracellular ROS production was accessed by flow cytometry using 2',7'-dichlorofluorescein diacetate as a probe. The results of the present study showed lower percentage of neutrophils that produced ROS in BLV-infected dairy cows (63.53 ±13.48) was lower compared to the serologically positive animals without PL (93.86 ±4.07) and the uninfected ones (89.27 ±14.24) (P=0.003). However, no significant difference in the intensity of intracellular ROS production by neutrophils was observed (Neg- 545.4 ±445.1; AL- 309.7 ±191.3; PL- 637.5 ±481.8; P=0.37). Thus, it can be concluded that the BLV can lead to alterations in neutrophil function, especially in BLV infected dairy cows with PL.

Keywords: deltaretrovirus, flow cytometry, innate immunity, microbicidal capacity, phagocytes.

AH-P21

Improvement of Neospora diagnosis thanks to IgG1 and IgG2

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Neosporosis due to *Neospora caninum* is an important disease that affects a large proportion of cattle in southern Belgium. The disease is a major cause of abortion, almost15% of aborted cows are seropositive. According to the results obtained on 6894 farms in the south part of Belgium, just over 40% of the farms are exposed to *Neospora caninum*. The Regional Association of Animal Health and Identification (ARSIA) has set up a control plan to eradicate the disease on a

voluntary basis. In 2020, 120 herds have subscribed to the plan, the average intra-herd prevalence is 18%. The control plan consist to diagnose seropositive animals, to distinguish persistently infected animals from those transiently infected, to identify and eliminate infected lines from mother to daughter. In addition, ARSIA provides management advice to prevent the transmission of the disease from cattle to dogs and from dogs to cattle. As part of the disease eradication plan, it is essential to detect persistently infected animals. If the infection occurs in the first third of gestation, it induces either an abortion or the birth of an infected calf with symptoms. If the infection occurs in the second third of gestation, the fetal immune system, although rudimentary, is competent but does not allow it to be saved. It is at this stage that the majority of abortions occur. Furthermore, if the infection occurs in the last third of gestation, the calf that is born is apparently healthy but seropositive and infected. Indeed, at this stage of gestation, the immune system is perfectly competent. Female calves infected in utero (apparently healthy) are at risk of becoming permanently infected animals. These animals will then transmit the parasite to their descendants and will have poorer reproductive performance. Given the difficulty in distinguishing between transient and permanent infection in adult animals, the diagnosis of the disease in calves at birth is an important advantage. However, it is sometimes complex to collect the calf before taking colostrum and colostral antibodies interfere with the interpretation of the results.

A solution to this problem consists in using IgG1 and IgG2 detection tests. From the fourth month of gestation, the fetus is immunocompetent and an infection of the mother will be characterized by the significant appearance of antibodies of the IgG1 type that IgG2 anti-*Neospora* in the fetus. Ingestion of colostrum rich in antibodies against *Neospora caninum* will result in the development of IgG1 antibodies only. The use of these tests therefore improves the interpretation of the results. The distinction between utero infected seropositive animals and healthy but seropositive animals following colostrum absorption is made possible. Infected animals can be kept out of breeding, healthy seropositive animals can be kept for breeding and the test is no longer dependent on colostrum intake, which is a practical advantage for the farmer and the veterinarian.

The present study was carried out on a cohort of 101 mother-calf pairs. The mother was tested on the day of calving with her calf. The calf was analyzed again 15 days after birth. Colostrum intake by calves has been validated by a Rotavirus ELISA test. Of these 101 couples, 13 interesting couples of seropositive animals were identified in order to carry out the IgG1 and IgG2 tests by the Bio-X laboratory. The obtained results make it possible to easily distinguish the immune profiles of calves at birth and therefore to improve the screening of infected calves and healthy calves that have drunk colostrum.

Keywords: Neospora, cattle, control plan, IgG1, IgG2.

AH-P22

Comparative evaluation of natural and induced lactation on milk production and health status of dairy cows

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Objectives: The aim of this study was to perform a comparative analysis of milk production, oxidative metabolism, serum biochemistry and hematology of cows with artificial induction of lactation and natural lactation.

Material & Methods: This study was approved by the Ethics Committee on the Use of Animals of Santa Catarina State University. Twenty-eight Holstein cows were split into two groups of 14 cows: control group (CG) and experimental group (EG). The cows of EG were submitted to a protocol of induced lactation, with several hormones (estrogen, progesterone, corticoids, prostaglandin and recombinant bovine somatotropin). Blood and milk samples from both groups were taken every 15 days, before starting the lactation until 300 days of lactation, for complete blood count, serum biochemistry, and milk production evaluation. Data were submitted to a variance analysis with repeated measurements over time in an RBD by PROC MIXED, and were considered significant when $P<0.05$.

Results: All cows in the EG had a response to the protocol of induced lactation with a milk production equivalent to 78% when compared to cows of the control group. In complete blood count, although the values remained within the normal limit, there was a decrease ($P<0.0001$) of erythrocytes count, hemoglobin concentration and packed cell volume at the beginning of the induced lactation, indicating a prejudicial but transitory effect of estradiol benzoate on erythropoiesis. The cows from both groups had neutrophilia at the beginning of lactation, but significantly higher ($P=0.0008$) for the experimental group, probably because of the injection of corticosteroids. Serum glucose was significantly higher ($P=0.046$) at the beginning of induced lactation, with the concentration above the normal limit, probably because of high doses of corticoids which promote gluconeogenesis and reduce glucose utilization. There were variable concentrations of ketone bodies along the lactation period, but at the end of lactation, levels were lower ($P<0.0001$) in the experimental group. Serum cholesterol levels were slightly lower at the beginning of the lactation for both groups, and serum triglyceride values were higher ($P<0.0001$) in the control group at lactation peak (45 days) and immediately after (75 and 90 days). A lower concentration of albumin was observed at 180 until 300 days in cows of the EG, probably because of somatotropin injection. The activity of aspartate aminotransferase, alkaline phosphatase, and creatine kinase remained within normal limits during all period of lactation for both groups. Cows from the experimental group had lower levels of serum urea ($P=0.0061$) and creatinine ($P=0.0159$) than the control group at 180 and 270 days of lactation. There were higher values of the lipid peroxidation marker, serum malondialdehyde, for both groups, at the beginning of lactation until 180 days, when the cows had a decrease in milk production.

Conclusion: It was concluded that the cows from natural and induced lactation had a similar physiological response on hematological, serum biochemistry and oxidative parameters, with temporary variations due to hormone injections of the protocol, but with no harm to health.

Keywords: Clinical biochemistry, hematology, oxidative metabolism, milk production, bovine.

AH-P23

Antimicrobial resistance of *Escherichia coli* from cows with metritis in California

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Objectives: Metritis in dairy cows has shown to result in reproductive tract disease, pain, compromised animal welfare, decreased milk production, and increases in the broad use of antimicrobials, reproductive failure (lower pregnancy risk), and culling rates. In most cases, no culture or antimicrobial susceptibility is conducted for these animals, and very limited information is available about antimicrobial resistance of potential pathogens causing metritis. *Escherichia coli* is one of the most common bacteria resulting in metritis in dairy cows. Our Objectives were to 1) assess antimicrobial resistance (AMR) in *Escherichia coli* (*EC)* isolated from the uterus of cows with and without metritis in dairy farms in California and; **2)** evaluate farm-level risk factors associated with increased risk for selection of AMR in *EC*.

Methods: Using a cross-sectional study design, a convenience sample of 25 dairy farms in Central and North California were enrolled in the study. From each farm, cows 3 to 10 days post-partum (DPP) were selected for the collection of uterine swabs. Samples were collected using sterile swabs (Jorgensen Labs Inc) covered by a sterile pipette (inside a plastic sheath) to reduce contamination before and after swabbing uterus. Up to a total of 15 cows were be collected from each farm: 5 presenting clinical signs of metritis, 5 that do not have clinical signs of metritis ("healthy"), and 5 presenting a non-fetid mucopurulent discharge with 25-50% pus. Swabs were aerobically cultured for EC for 24 hours using Chromagar-E. coli, isolated when present, and stored at -80C with glycerol. A short questionnaire was used to collect information about common antimicrobials used to treat metritis on the farm, as well as information to evaluate producer threshold for treatment of cows diagnosed with metritis. Antimicrobial susceptibility testing is currently being conducted following CLSI standard, breakpoints and recommendations (completion expected by July 2020) using microbroth dilution method for 18 antimicrobial drugs of relevance to veterinary medicine. Multiple linear regression models will be used to analyze data, where the dependent variables will be the prevalence of in cows with and without metritis, and the proportion of AMR for EC (one model for each antimicrobial drug).

Results: A total of 25 farms and 305 cows were sampled, and a survey related to each farm practices collected. Approximately 52.5% of samples cultured positive for E. coli. From the survey, a total of 75% of farms disclosed recording all metritis antibiotic treatments, and 79% treated all diagnosed cases of metritis with antibiotics. From these, 55% of farms used intrauterine route for treatment of metritis with therapeutics, with drugs from the tetracycline class being most common (69%). The most common injectable antibiotic used to treatment metritis on sampled farms was ceftiofur (42%). Antimicrobial susceptibility testing of *E. coli* isolates underway and expected to be completed by July 2020.

Conclusion: Intrauterine use of tetracycline still occurs in dairy farms, and as expected, ceftiofur was the most common antimicrobial used to treat metritis. As suggested in other studies, we expect that a significantly high number of EC will display resistance to tetracycline. Results for antimicrobial susceptibility will be presented together with correlation with farm-level practices, which we expect will be associated with farm-level antimicrobial practices for treatment of metritis.

Keywords: metritis; antibiotic resistance; dairy cattle; ceftiofur.

AH-P24

Salmonella enterica outbreaks in dairy calves in Uruguay

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Objectives: The aim of this work is to describe clinical, epidemiological and pathological findings of fatal outbreaks of salmonellosis in dairy calves in Uruguay.

Materials and methods:

- Outbreaks. In this work 15 spontaneous outbreaks of fatal salmonellosis in dairy calves, submitted to the veterinary diagnostic service of the "Plataforma de Investigación en Salud Animal", INIA La Estanzuela, Colonia, Uruguay from 2016 to 2018, were included. Clinical and epidemiological data was collected including the number of exposed, clinically affected and deceased calves during each outbreak.
- Autopsy of the calves and sample collection. A total of 22 death calves were included, 19 were fully necropsied by INIA veterinarians, in three cases tissues were submitted to the INIA´s laboratory by the farm veterinarians. Each carcass was examined post-mortem to detect and register lesions in different organs. During examination, tissues were collected aseptically for *Salmonella* spp. culture and histology.
- *Salmonella* spp. isolation and characterisation. Tis-

sues were cultured aerobically in tetrathionate broth, and sub-cultured in xylose lysine deoxycholate (XLD) agar. *Salmonella*-suspect colonies were selected and identified by biochemical tests. The serotype was determined using the Kauffman-White-Le Minor scheme and by PCR protocols.

- Histological examination. Tissues were fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin, microtome-sectioned a 4-5 µm, mounted on glass slides and stained with hematoxylin and eosin for microscopic examination.

Results: *Salmonella* Typhimurium was identified in 80% (12) of the outbreaks, while *S*. Dublin was identified in the remainder 20% (3 outbreaks). The average age of the calves at death was 25 and 55 days for the *S*. Typhimurium- and *S*. Dublin- associated outbreaks, respectively.

The clinical reasons for consultation by the farm owners of veterinary practitioners were diarrhea (8 outbreaks), sudden death (4 outbreaks), depression (2 outbreaks), and respiratory signs (1 outbreak).

In diarrhea outbreaks, the average morbidity was 52.2% (range: 10-100%) and the average mortality was 25.3% (range: 3.8-78.9%). In outbreaks with sudden death, the average morbidity was 14% (range: 1-30%) and the average mortality was 13.6% (range: 1-30%). The average morbidity and mortality were 10.1% and 9.9%, respectively, in outbreaks where depression was the main reason for consultation. In the outbreak with respiratory signs, morbidity and mortality were 10% and 7.5%, respectively.

Full postmortem examination was completed in 19 naturally deceased calves from all 15 outbreaks. The most frequent macroscopic lesions were enteropathy (mainly fibrinous enteritis/colitis/typhlitis/enterotyphlocolitis), lymphadenomegaly and /or fibrinosuppurative lymphadenitis in mesenteric lymph nodes, hepatitis, adrenocortical hemorrhages, and interstitial pneumonia.

Histologic examination was conducted in 22 calves, including the above-mentioned 19 autopsied calves. Microscopically, necrotizing/fibrinosuppurative enteritis/colitis/typhlitis/enterotyphlocolitis and multifocal random necrotizing hepatitis were found in in 19 cases each. Other lesions included neutrophilic/fibrinous interstitial pneumonia, fibrinosuppurative lymphadenitis and splenitis, and extensive acute adrenocortical hemorrhages (suggestive of Waterhouse-Friderichsen syndrome), among others. No microscopic cardiac lesions were seen in 15 cases where heart was available for histologic examination.

Conclusions: *Salmonella enterica* is a common cause of calf disease and mortality in young dairy calves in Uruguay. The lesions found in our study suggest that both enteric and septicemic forms of salmonellosis are frequent.

In Uruguay, the nationwide mortality risk of dairy calves between birth and weaning is high (15.1% in one survey in 2013-2014), with diarrhoea and respiratory signs being the main associated clinical signs. In all 15 outbreaks of salmonellosis studied here, the average morbidity and mortality were 21.5% and 50.5%, greatly exceeding Uruguay's dairy calf mortality risk.

Clinical presentations and lesions detected in these cases were similar to those described in the literature for bovine salmonellosis. It should be stressed that most of the calves manifested diarrhoea within 30 days of life and died afterwards. This could be related to the invasiveness and pathogenic potential of the involved bacterial strains. Therefore, bovine salmonellosis and the microbiological features of the circulating salmonellae should be further studied locally. In addition, management practices related to the productive systems should be analysed to better understand the epidemiology of this zoonotic disease in order to establish prevention and control strategies at the farm level.

Keywords: Salmonella, dairy calves, outbreak.

AH-P25

Evaluation of an automated monitoring system for the detection of estrus and health disorders in a large Chilean Holstein dairy herd

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Automated monitoring systems (AMS) for reproductive and herd health management are new technologies becoming very popular for the dairy industry. The objective of the following study was to evaluate the efficacy of an AMS on heat detection and early diagnosis of health disorders in a large Chilean Holstein dairy herd. The study was conducted on a large herd located in San Carlos de Puren, Chile (-37.59 W, -72.29 S). The herd (ANCALI S.A.) consisted of 4,200 lactating dairy cows, milking 2.7X with a robotic system, and fed a total mixed ration to meet or exceed the requirements proposed by the Cornell Net Carbohydrate and Protein System 6.5 (2015). The study started in May 10, 2019 and finished in January 31, 2020. A total of 100 prepartum cows (approximately 30 days before expected parturition) received an intaruminal electronic device (SmaXtec, Belgiergasse 3 8020 Graz, Austria) able to measure core body temperature, walking activity, water drinking activity, and proximity of parturition. Another group (n=100) that did not receive the electronic device were matched by parity and days to parturition and were used as a control group. The AMS was aimed to verify the effectiveness of early detection of some peripartum clinical disorders such as metritis, mastitis, ketosis, diarrhea, and displacement of the abomasum. The AMS recorded data based on an alarm computerized system after setting a deviation value from the normal behaviour of the cow (temperature, activity and beverage cycles). Every time an alarm was recognized, the animal was separated from the herd and was clinically examined by farm veterinarians. The evaluation was carried out in each cow up to 150 days in milk. Control cows were used to compare their milk yield with cows with the AMS. For the entire AMS group, 16 cases of clinical mastitis were diagnosed, and 14 alarms were detected (87.5% of success). For puerperal metritis, 19 cases were diagnosed, and 16 alarms were detected (84.2% of success). For displacement of the abomasum, 6 cases were diagnosed,

and 4 alarms were detected (66.6% of success). For diarrhea, 12 cases were diagnosed, and 9 alarms were detected (88.8% of success). For subclinical ketosis (BHB > 1.2 mmol/L), 25 cases were diagnosed, and 15 alarms were detected (60% of success). For estrus detection, 99 events were identified by farm personnel, and 97 alarms were recorded (97.97% of success). When comparing average daily milk yield up to 150 days postpartum, the AMS group produced 46.4 kg/d while the control group produced 45.7 kg/d. Mortality rate was 3.19% in the AMS group vs. 4.6% in the control group. Culling rate was 3.19% in the AMS group vs 7.9% in the control group. It is concluded that the AMS device helped in the early diagnosis of diseases and estrus detection, reducing the culling rate and increasing the milk yield when compared with a control group.

Keywords: automated monitoring system, dairy, diseases, heat detection, Chile.

AH-P26

Effects on injectable Trace minerals and Vitamins supplementation on growth and antioxidant status in heifers during feedlot receiving

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Objectives: In beef cattle production, weaning, transportation, and feedlot entry are the most stressing stages leading to lower weight gain secondary to oxidative stress. The hypothesis for the current experiment was that supplementation with injectable vitamins (A and E) and minerals (Cu, Zn, Mn, and Se) improves growth and parameters related to oxidative stress in cattle entering the feedlot. The aim of this work was to evaluate the effects of supplementation on day 1 and/or 7 after feedlot entry on body weight, total antioxidant status (TAS) and thiobarbituric acid reactive substances (TBARS) in weaned cattle.

Materials and methods: Healthy crossbred *Bos indicus* × *Bos taurus* heifers (255 ± 26 kg) were shipped 900 km. Heifers were randomly assigned to four groups (n = 30 each) in a 2×2 factorial arrangement of treatment. The main factors were supplementation (S) or not (C) on day 1 and supplementation (S) or not (C) on day 7 (CC, CS, SC and SS groups). The parenteral vitamin supplement (subcutaneous 1 ml /50 kg BW; Adaptador Vit®, Biogénesis Bagó SA, Buenos Aires, Argentina) contained 63000 IU/mL of vitamin A (as palmitate), and 50 IU/mL of vitamin E (as acetate). The mineral supplement (subcutaneous 1ml /50 kg BW; Adaptador Min®, Biogénesis Bagó SA, Buenos Aires, Argentina) contained 10 mg/mL Cu (as cooper edetate), 10 mg/mL Mn (as manganese edetate), 5 mg/mL Se (as sodium selenite), 40 mg/mL Zn (as zinc edetate). Plasma concentration of TAS (n = 8 animals per group) and TBARS, (n = 8 animals per group) were determined through commercials kits (Cayman Assay Kit 10009055 and 709001 for TAS and TBARS, respectively).

Data were analyzed as a complete randomized design with a 2×2 factorial arrangement of treatments using SAS mixed procedure (9.4). The model included supplementation or non-supplementation at day 1, at day 7, and their interaction.

Results: There was a day 1 × day 7 interaction ($P \leq 0.07$) for final BW and average daily gain (ADG). Heifers that did not receive supplementation (CC) had less BW (293.6 kg) and ADG (1.86 kg/d) compared with CS (297.6 kg; 2.10 kg/d), SC (300.1 kg; 2.20 kg/d) and SS (298.9 kg; 2.10 kg/d) groups. There was no interaction in day 1 × day 7 ($P \geq 0.41$), nor for day 1 supplementation ($P \geq 0.11$) effects for plasma TAS and TBARS.

Increased antioxidant capacity (0.59 vs 0.51 mmol/L; $P = 0.01$) and lower lipid peroxidation (5.96 vs 6.70 µM MDA; $P = 0.01$) were observed on heifers supplemented on day 7 (CS and SS) compared with the non- supplemented (CC and SC) on day 7.

Conclusions: Heifers supplemented with vitamins and minerals improved weight and ADG at feedlot entry. However, the supplementation with two doses (day 1 and 7) did not differ from a single dose (day 1 or 7) in the final weight or ADG. In the same way, vitamin and mineral supplementation on day 7 showed greater antioxidant capacity and lower lipid peroxidation.

Keywords: ruminants, body weight, oxidative stress, antioxidant, feedlot.

AH-P27

Defining clinical diagnosis and treatment of puerperal metritis in dairy cows: A Scoping Review

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Objective: Puerperal metritis (PM) is a common infectious disease in dairy cattle. Currently there are discrepancies between clinical case definitions within and between available peer-reviewed literature and on-farms practices. The inconsistent use of PM criteria across studies and on-farms practices can result in disparities related to recommendations for treating cows, affecting judicious use of antimicrobials. Our objective was to systematically review literature for clinical signs used as diagnostic criteria for PM, including local (e.g., vaginal discharge) and systemic signs of infection (e.g., fever, drop in milk).

Methods: The Preferred Reporting Items for Systematic Review and Meta-Analysis extension for Scoping Reviews

(PRISMA-ScR) protocols was used to screen commonly used databases. One reviewer screened title/abstract for eligibility (n=2,096) followed by full-text screening of selected articles (n=396) by two reviewers to identify eligible articles (n=174). Multiple correspondence analysis was used to evaluate the association among the definition and diagnosis of PM and year of publication of the studies.

Results: The most frequently cited reference article (37.5%) for the definition of PM was published in 2006, followed by articles published between 1998 and 2009 (13%). In 40.2% of articles, no reference was provided for the PM definition. For the definition of PM, the vaginal discharge (VD) was described in terms of color, odor, and viscosity. Among colors, the terms used were red brown (61.4%), red (5.1%), brown (8.6%), chocolate (4%), white (1.7%), yellow (0.5%), pink (5.7%), or gray (0.5%); VD color was not reported in 24.1% articles. The VD odor was described as fetid (75.8%), putrid (5.1%), foul (10.3%), or other (5.7%) (e.g., abnormal, malodorous, odoriferous); odor not mentioned in 7.4% of articles. The VD viscosity was described as watery (74.1%), purulent (27%), mucopurulent (8.6%), thin (4%), serous (2.8%) or abnormal (2.3%), and was not mentioned in 11.5% of articles. Fever was included in 59.7% articles as a criterion for PM diagnosis. The mostly used rectal temperature threshold was ≥39.5˚C (56.8%), followed by ≥39.2˚C (2.8%). Fever was not used as diagnostic criteria in 39.6% of articles. Approaches used for VD evaluation included rectal palpation (37.3%), intravaginal exploration with a gloved hand (18.4%), Metricheck (9.8%) or speculum (5.7%); for 28.7% of articles diagnostic tools used were not mentioned. The lack of reporting on the data items charted for the review was a prevalent finding in most of the relevant studies, which influence the scientific rigor and replicability of the methods of some studies.

Conclusion: This scoping review describes a disparity in robust and clear criteria used to diagnose PM in literature. Although select consensus articles are available, it is common for no references to be used, and further high-quality research is needed to identify a standard criterion for case definition for PM.

Keywords: Vaginal discharge, cattle, clinical definition, metritis.

AH-P28

Evaluation of antimicrobial resistance and risk factors for recovery of intrauterine *Escherichia coli* from cows with metritis on California commercial dairy farms

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Objective: Metritis is a major uterine disease in dairy cattle, typically occurring within 21 days post-partum, and the fourth most common health issue in cows as identified by producers. Currently, little is known of MICs of intrauterine *E. coli* (EC) to common antimicrobial drugs used to treat metritis in California dairies. The goals of this study were to evaluate factors affecting recovery and antimicrobial resistance (AMR) in intrauterine *E. coli* in post-partum dairy cows with and without metritis from commercial California dairy farms.

Methods: A cross-sectional study was designed to collect uterine swabs from post-partum cows between 3 and 21 DIM. Cows were categorized in one of three clinical presentation groups before enrollment: metritis (MET, n = 86), defined as a cow with watery, red or brown colored, and fetid vaginal discharge; cows with purulent discharge (PUS, n = 106), defined as a non-fetid purulent or mucopurulent vaginal discharge; and control cows, (CTL, n = 115) defined as cows with either no vaginal discharge or a clear, non-purulent mucus vaginal discharge. Logistic regression models were used to evaluate animal-level risk factors associated with the odds of isolating EC from an intrauterine swab sample and to evaluate the association between intrauterine EC antimicrobial resistance and animal-level factors.

Results: Cows diagnosed as MET had significantly higher odds for recovery of EC compared to cows diagnosed as CTL (OR= 2.16, 95% CI: 1.17 – 3.96), with no significant difference observed between PUS and CTL. An increase in days in milk (DIM) at the time of sampling was significantly associated with a decrease in the odds ratio for EC recovery from intrauterine swabs (OR= 0.94, 95% CI: 0.89 – 0.98). All intrauterine EC were resistant to ampicillin (AMP), with an AMR prevalence of 30.2% and 33.9% observed for chlortetracycline and oxytetracycline, respectively. Only 8.6% of isolates were resistant to ceftiofur (CEFT), one of the most common drugs used to treat cows on the study farms. No significant difference in the prevalence of AMR was observed among clinical groups. A significantly higher odds for isolating intrauterine E. coli resistant to chlortetracycline (OR: 2.6; 95% C.I: 3.7 – 58.0) or oxytetracycline (OR: 1.9; 95% C.I: 1.4 – 33.8) was observed at farms that used an intrauterine infusion of oxytetracycline as a treatment for metritis when compared to those farms that did not use this practice.

Conclusion: The low prevalence of AMR to CEFT indicates its potential as an effective treatment of metritis of cows. Resistance of all isolates to AMP was unexpected,and discordant with previous field clinical studies using AMP for the treatment of metritis, using older MIC breakpoint references; this supports the need for more research related to pharmacokinetics, pharmacodynamics, and definition of antimicrobial breakpoints for AMP within cattle uterine tissues and infections.

Keywords: metritis, antimicrobial resistance, dairy.

AH-P29

The effect of a novel feed additive on selenium, bovine immunoglobulin, and pro- and anti-inflammatory cytokine concentration in calves serum

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Objectives: The adequate nutrition of cattle at an early stage of life has a positive effect on the health and long-term productivity of the animals. The overall health of calves can be supported by different feed additives. It is evidenced that Shiitake mushrooms – *Lentinula edodes* (*L.*) have prebiotic properties and a good ability to accumulate selenium (Se). Se is an important microelement in cattle nutrition because its deficiencies result in white muscle disease and poor growth rate of calves, and also reduced milk yield, fertility problems, mastitis, and metritis in dairy cattle. The adequate level of Se in cattle may also have anti-inflammatory and prebiotic properties in some conditions. There are a few research reports that the silicon dioxide nanoparticles used have a positive effect on bacterial composition in the gut's microflora, sodium butyrate facilitates feed digestion, and a mixture of organic acids acidifies the digestive tract and gives an additional biocidal effect against pH-sensitive bacteria. The crucial objective of this study was to determine the effects of supplementation of calves with novel feed additive consisting of Se-enriched *L. edodes* mycelium from in vitro cultures, pancreatic-like enzymes, fat-coated mixture of organic acids, sodium butyrate, and silicon dioxide nanoparticles on Se, bovine immunoglobulin (Ig) and pro- and anti-inflammatory cytokine concentration in calves serum during 10 weeks of the experiment.

Material and Methods: The experimental design was approved by the Local Ethics Commission in Lublin (permission number 68/2018).

In the study, twelve calves at an average age of 16 weeks were randomly allocated to two groups (n=6) experimental (E) and control (C) and fed either unsupplemented milk (C) or milk supplemented with a feed additive (E). The feed additive contained a Se-enriched *L. edodes* mycelium, pancreatic-like enzymes such as protease and lipase, a fat-coated mixture of organic fumaric, malic, citric, and sorbic acids, sodium butyrate, and silicon dioxide nanoparticles. For the research, the serum was obtained at weekly intervals for the 10 weeks of the study. Se concentration was analyzed by means of inductively coupled plasma mass spectrometry (ICP-MS) in weeks 0, 1, 7, and 10 of the experiment. The concentration of Ig and pro- and anti-inflammatory cytokines: interleukin 1β (IL 1β), tumor necrosis factor α (TNFα), interferon γ (IFN γ), interleukin 2 (IL 2), interleukin 4 (IL 4) and interleukin 10 (IL 10) was evaluated from weeks 0 to 10th using commercial ELISA kits.

Results: The serum Se concentration in the E calves was significantly higher than in the C calves in weeks 1, 7, and 10th. The serum Ig concentration was higher in the E group when compared with the C group. Any changes in IFN-γ, IL 1β, and TNFα concentration was found and remained at zero level in both groups of calves. The serum IL 2 concentration in three of six animals from the E group from week 8 to 10th slightly increased from zero to the range from 109.4 pg/ml to 260.2 pg/ml. Similarly, IL 4 concentration in three of six animals from the E group in weeks 5 and 8th had concentrations in the range from 32.9 pg/ml to 43.4 pg/ml, in the remaining weeks of the experiment the IL 4 was not detected in serum samples. The IL 10 concentration in three of six E calves had a concentration in the range from 25.2 pg/ml to 64.0 pg/ml, and it was recorded from week 7 to 10th, in the remaining weeks of the experiment the IL 10 was not detected. All the values in the C group remained at zero level. The differences in the values between E and C groups were not statistically significant ($P < 0.05$).

Conclusions: In this study, the consumption of the novel feed additive resulted in significantly higher serum Se concentration and a tendency to a higher concentration of serum Ig in the calves from the E group when compared to the C group. Moreover, some stimulation of considered as anti-inflammatory cytokines - IL 2, IL 4, and IL 10 secretion in a few of supplemented calves in the latter period of the experiment was seen in the experimental calves. IFN-γ, IL 1β, and TNFα, considered as pro-inflammatory cytokines, remained not stimulated in examined animals.

Keywords: novel feed additive, selenium, immunoglobulin, cytokines, cattle.

AH-P30

The effect of early NSAID treatment on respiratory score and acute phase proteins in veal calves

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Objectives: Respiratory disease is a major concern in veal calves and 71% of the prescribed antibiotics to calves in Denmark, are prescribed to respiratory disease. One way of reducing the antibiotic usage in calves could be to recognise and treat disease earlier. The objective of this study was therefore to investigate if early treatment of calves with non-steroidal anti-inflammatory drugs (NSAID) could reduce the clinical signs and the acute phase response of respiratory disease in Danish veal calves.

Materials and methods: Calves from one large conventional Danish veal calf producer was included in the study. The calves underwent a clinical examination on day 1-5 after enrolment. The clinical examination included general demeanour, rectal temperature, assessment of nasal and ocular discharge, presence of cough and thoracic auscultation. If the calf had no, serous or transparent mucous nasal discharge, normal thoracic auscultation and less than 40.2° C, it was included in the study and randomly assigned to either receive three days of NSAID treatment with either Finadyne transdermal® or Metacam® on day 1-3 or no treatment. Based on the clinical examinations a respiratory score was calculated for each calf on

day 1-5 and the effect of NSAID treatment were analysed with a generalised linear mixed model with animal identification as random effect, to account for multiple observations per calf.

On day 1 and 6 a blood sample was drawn from *vena jugularis,* and serum was analysed for the acute phase protein haptoglobin.

Results: In total, 47 calves met the inclusion criteria. Twenty-six calves received NSAID treatment, and 21 calves were not treated. The NSAID treated calves had a statistically significant lower respiratory score than the non-treated calves on day 5. For some individual calves the haptoglobin level mimics the improved clinical score, however when exploring the whole group of calves there was no difference in haptoglobin level between NSAID and non-treated calves. Reasons for this are being explored.

Conclusions: Treatment with NSAID when only subtle clinical signs of respiratory disease are present could reduce the overall respiratory score and can possibly reduce the need for subsequently antibiotic treatment in commercial veal calf herds.

Keywords: Respiratory disease, treatment, non-steroidal anti-inflammatory therapy, acute phase protein, haptoglobin

AH-P31

Heat treatment of bovine colostrum: effects on colostrum and calf plasma oxidative markers

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Objectives: Heat treatment of colostrum is a management strategy to control contaminants but may affect other colostral components. The primary objective of this explorative study was to determine if heat treatment of colostrum altered the concentration of pro- and antioxidants of colostrum. The secondary objective was to investigate if differences resulting from heat treatment, if present, extend to the overall oxidative status of the calves fed with heat treated or raw colostrum.

Materials and methods: Newborn Holstein female calves (n = 22) were randomized within pairs and fed heat-treated (n = 11; 60 °C, 60 min) or raw (n = 11) colostrum at 8.5% of birth BW by esophageal feeder within 1 h of birth. After a single colostrum feeding, calves were not fed until after the 8 h time point. Blood samples were taken immediately prior to feeding (0 h) and at 4, 8, and 24 h relative to feeding. The colostrum and serum redox balance was characterized as the ratio of total reactive oxygen and nitrogen species (RONS) and the total antioxidant potential (AOP), also called the oxidant status index (OSi). Differences between RONS, AOP, OSi of paired colostrum samples were analyzed via mixed effects ANOVA with the fixed effect of treatment and random effect of pair, whereas differences in calf plasma samples were analyzed via repeated measures ANOVA with fixed effect of time (4, 8, 24 h), treatment, and their interaction, with the random effect of calf nested in pair, and baseline values at 0 h as covariates. Differences at baseline were evaluated using mixed effects ANOVA with the fixed effect of treatment and random effect of animal nested within pair.

Results: In colostrum, heat treatment was associated with a significantly lower RONS activity (P = 0.001), but no association was found with AOP (P = 0.68). Consequently, OSi was also significantly lower in the heat-treated colostrum (P = 0.001). However, RONS, AOP, and OSi did not differ by treatment in calf plasma (P > 0.26), although antioxidant potential increased in both groups to reach the maximum value at 8 and 24h relative to feeding of colostrum.

Conclusions: Our data show that although heat treatment was associated with lower pro-oxidant activity However, these changes in colostral redox status did not result in differences in the systemic redox profile of calves within the first 24h of life.

Keywords: Calf health, Oxidative Stress, Colostrum, Passive Immunity.

AH-P32

Protein profile of high yield multiparous dairy cows fed with negative anionic-cationic differences pre partum diets

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Introduction: The concentration of Total Proteins in cattle varies between 6.6 to 9.0 g / dL. That of Albumin between 2.9 to 4.1 g / dL and Globulins between 2.8 to 5.2 g / dL. The ingestion of pre partum diets with negative anionic-cationic differences is a strategy to prevent postpartum hypocalcaemia. However, it causes a metabolic acidosis that causes a decrease in the concentration of Total Proteins, Albumin and Globulins in dairy cows during the postpartum period. The objective of the present study was to determine the effect of diets with negative anionic-cationic differences on the protein profile of high-yield dairy cows within 12 hours of calving.

Materials and methods: The study was carried out from 9/20/21 to 10/13/21 in a commercial dairy herd located in Saturnino María Laspiur, Córdoba, Argentina in 136 Holando categorized according to their lactation number: first (n = 83), second (n = 37) and third or more (n = 15). During pre-partum, the cows were fed with a diet (% dry matter) composed of corn silage (46.9%), soybean expeller (14.1%), pre partum concentrates (17.5%) and wheat straw (21 , 5%), while the pregnant heifers received a diet composed of corn silage (39.3%), sorghum silage (19.2%), soybean expeller (19.2%), wheat straw (17, 7%) and pre partum salts (4.5%). Anionic salts were supplied in both groups to produce a negative anionic-cationic difference (- 9.5 mEq / 100g DM). On the day of delivery, blood samples without anticoagulant were obtained by coccygeal

venopuncture which were centrifuged at 2,000 rpm for 10 minutes, the serum was aliquoted in 1.5 ml microtubes and frozen at -20 ° C until the determination of Total Proteins (Biuret Method), Serum Albumin (Bromocresol Green) and Globulins (calculation of total proteins - albumin). The results are expressed in g / dL. Descriptive statistics and ANOVA were performed to detect differences between lactations for Total Proteins and Albumin and the Kruskal Wallis Test to detect differences between lactations for Globulins.

Results: The 5.95 %, 8.10 % and 33.33 % of the first, second and third or more lactations respectively of high-yield dairy cows had Total Protein concentrations within the reference range for bovines. Significant differences were detected in Total Proteins (P = 0.0022), Albumin (P = 0.0326) and Globulins (P = 0.0076) between lactations. In Primiparous females was 5.77 ± 0.06 g / dL, similar to 5.98 ± 0.09 g / dL of those in Second but lower than 6.31 ± 0.15 g / dL on the Third or more lactations. In the case of Albumin, third or more calvings cows presented 3.81 ± 0.12 g / dL, which differed significantly from 3.53 ± 0.07 g / dL and 3.47 ± 0.04 g / dL detected in the second and first lactation cows respectively. Finally, the first calvers presented 2.19 ± 0.11 g / dL of Globulins, which was lower than the 2.55 ± 0.08 g / dL of the Second and 2.74 ± 0.13 g / dL of Third or more lactation.

Conclusions: It is concluded that there is an increase in the concentration of the protein profile in high-yield dairy cows as the number of lactation increases. On the day of parturition, about 90% of females have subclinical hypoproteinemia. This high prevalence should be considered to avoid potential risks on the health status associated with this deficiency during the transition period, mainly in Primiparous cows.

Keywords: Protein profile, dairy cows, negative anionic-cationic differences diets.

AH-P33

Prevalence of subclinical hypocalcaemia and its relation with colostrum total solids in high-producing dairy cows with negative anionic-cationic differences pre partum diets

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Introduction: The prevalence of subclinical hypocalcaemia in multiparous cows is around 50%. Among the preventive measures most used today to prevent this condition, is the inclusion of acid genic pre partum diets. However, this acid-base balance would not prevent the occurrence of subclinical hypocalcaemia in high-producing dairy cows. The most abundant minerals in first-milking colostrum multiparous cows are Ca (8.75 ± 5.74 g) but no significant associations were observed between mineral total yields in first-milking colostrum and serum mineral concentrations. The objective of the work was to establish the prevalence of subclinical hypocalcemia and determine the concentration of total solids in colostrum and serum calcium on the day of calving in dairy cows with negative cationic anion differences pre partum diets.

Materials and methods: The work was carried out from 9/20/21 to 10/13/21 in a dairy herd located in Saturnino María Laspiur, Córdoba, Argentina on 136 Holstein cows categorized according to the lactation number: first (n = 84), second (n = 37), third (n = 9) and fourth (n = 6). During 21 days in the pre partum period, the composition of the diet (% dry matter) was: corn silage (46.9 %), soybean expeller (14.1 %), pre partum concentrate (17.5 %) and wheat straw (21,5 %), while in pregnant heifers the diet was: corn silage (39.3 %), sorghum silage (19.2 %), soybean expeller (19.2 %), wheat straw (17,7 %) and pre partum salts (4.5 %). Pre partum anionic salts were supplied to primiparous and multiparous cows to produce a negative anion-cation difference (- 9.5 mEq / 100g DM). On the day of delivery, colostrum samples and blood samples without anticoagulant were obtained by coccygeal venepuncture which were centrifuged at 2,000 rpm for 10 minutes. Serum obtained was aliquoted in 1.5 ml microtubes and frozen at -20 ° C for posterior determination of total serum calcium (TCa) by the Arzenazo III Method (expressed in mmol/L). It was considered as subclinical hypocalcemia when TCa concentrations ranged between 1.5 and 2.0 mmol / L. Concentrations lower than 1.4 mmol / L were considered as Clinical Hypocalcaemia. The concentration of colostrum total solids (TS) was carried out with a digital refractometer (% Brix) (ATAGO PAL-1). Descriptive statistics and the Kruskal Wallis Test were performed to detect calcium differences between lactations and Spearman Correlation to establish associations between serum calcium and colostrum TS.

Results and Discussion: The average production at 40 ± 7.3 days postpartum was 31.4 ± 8.7 liters with 3.3% ± 0.2 protein and 3.3% ± 0.9 fat. Significant differences (<0.0001) were detected in the TCa between lactations. It declined significantly in the cows of four lactations at concentrations of 1.73 ± 0.23 mmol/L in relation to 2.10 ± 0.18 mmol/L in those of first, 1.99 ± 0.22 mmol/L in second and 1.83 ± 0.23 mmol/L in third lactations. TCa between those of second and third and between the third and fourth lactation they were similar. The average prevalence of subclinical hypocalcaemia was 39.0%, varying and increasing with the number of lactations: 25.0 %, 51.3 %, 77.9 % and 66.7 % in cows of one, two, three and four lactations respectively. Two multiparous cows presented clinical symptoms of postpartum hypocalcaemia representing the 11.9% of those in the third land 16.6% of those in the fourth lactations. The TS concentration was 25.6% Brix in primiparous females and 26.7% Brix in multiparous females. A negative association (r= -0.31, P <0.05) was found between serum calcium concentrations and colostral TS in multiparous cows.

Conclusions: It is concluded that as the number of lactation increases there is a decrease in the serum TCa concentration in dairy cows. The prevalence, greater than 50 %, observed at the time of delivery would indicate that the capacity of negative anionic-cationic diets to reduce the incidence of subclinical hypocalcaemia should continue to be investigated. Colostrum TS of multiparous cows, negatively associated with concentrations of serum calcium, could be an easier way to assess post-partum calcaemia status.

Keywords: Prevalence, subclinical hypocalcaemia, dairy cow, negative anionic-cationic differences diets.

AH-P34

Use of total serum calcium adjusted by albumin and total proteins in dairy cows with subclinical hypoproteinemia

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Introduction: In humans, the adjustment of serum total calcium concentration (TCa) by albumin is essential to detect abnormal values and to assess changes in a value. Calcaemia classification of cows and, subsequently, the clinician's therapeutic recommendations will differ depending on the use of TCa or albumin-corrected Ca in hypoproteinemic cows. The objective of the present study was to determine the utility of adjusting the concentration of total serum calcium (TCa) by Total Proteins (TCaadjTP) and Serum Albumin (TCaadjAlb) in dairy cows with normoproteinemia (NormoTP) and subclinical hypoproteinemia (hypoTP) at calving.

Materials and methods: The study was performed with 101 Holstein cows (66 primiparous and 35 multiparous) belonging to a commercial dairy located in Saturnino María Laspiur, Córdoba, Argentina were used from 9/20/21 to 10/13/21. During pre-partum, the cows were fed with a diet (% dry matter) composed of corn silage (46.9%), soybean expeller (14.1%), pre partum concentrates (17.5%) and wheat straw (21,5%), while the pregnant heifers received a diet composed of corn silage (39.3%), sorghum silage (19.2%), soybean expeller (19.2%), wheat straw (17,7%) and pre partum salts (4.5%). Anionic salts were supplied in both groups to produce a negative anionic-cationic difference (-9.5 mEq/100g DM). On the day of calving, blood samples without anticoagulant were obtained by coccygeal venipuncture, which were centrifuged at 2,000 rpm for 10 minutes to obtain serum, which were aliquoted in 1.5 ml micro tubes and frozen at -20 °C until the subsequent determination of Total serum Calcium, Total Proteins and serum Albumin using the Arzenazo III Method, the Biuret Method and Bromocresol Green respectively. The cows that presented concentrations of Total Proteins (TP) between 6.6 g/dL and 9.0 g/dL were considered normal (NormoTP) and with subclinical hypoproteinemia (HypoTP) when the concentrations were <6,59 g/dL. The formulas used to adjust TCa for Total Proteins (TCaadjTP) was: TCaadjTP (mg/dL) = Ca (mg/dL)-[0.4*TP(g/dL)]+3.3, while for Albumin (TCaadjAlb), the following: TCaadjAlb (mg/dL)=Ca(mg/dL)-Alb(g/dL)+3.5 (Seifi et al., 2005). Data normality was established by Shapiro-Wilks (modified). TCa, TP and Alb data were analyzed using descriptive statistics. Means were analyzed using the Wilcoxon Test for paired samples. Correlations were performed using Spearman. The TCa for cows with NormoTP and HypoTP was compared with the TCaadjTP and TCaadjAlb by means of the Lin Concordance Coefficient (CCLin) for continuous variables and the Kendall Concordance Coefficient (CCKen) for categorical variables.

Results: The 82.17% cows presented HypoPT, 96.04% presented NormoAlbuminaemia and 82.00% presented HypoGlobulinaemia. The mean ± SD of TCa was 8.25±0.84 mg/dL (minimum=5.20 maximum=9.90) while the TCaadjTP was 9.19±0.86 mg/dL (minimum=6.46 maximum=10.48) and the TCaadjAlb was 8.23±0.87 (mg/dL) (minimum=5.30 maximum=9.90). The mean ± SD of TP was 5.91±0.6 g/dL (minimum= 4.20 maximum= 7.80) and that of Alb was 3.52±0.36 g/dL (minimum=2.30 maximum=4.60). The mean ± SD of the difference between TCa and TCaadjTP was -0.94±0.24 mg/dL (P<0.0001) and between TCa and TCaadjAlb was 0.02±0.36 mg/dL (P=0.4213). A positive correlation was found between TCa and Albumin (r=0.24, P=002) while no correlation was observed between TCa and PT (r=0.13, P=0.19). The correlation coefficient between TCa and TCaadjTP was r=0.95 (P<0.000001) and between TCa and TCaadjAlb, it was r=0.84 (P<0.000001). The observed concordance coefficient between TCa and TCaadjTP for cows with NormoTP was high for both CCLin (Pc=0.86) and CCKen (Pc=0.84), while poor concordance was found between TCa and TCaadjTP for cows with HypoTP both for CCLin (Pc=0.54) and for CCKen (Pc=0.63). The concordance coefficient observed between TCa and TCaadjAlb for cows with NormoTP was excellent for CCLin (Pc=0.93) and for CCKen (Pc=0.85) as well as the concordance found between TCa and TCaadjAlb for cows with HypoTP for CCLin (Pc=0.91) and for CCKen (Pc=0.83).

Conclusions: It is concluded that under NormoPT conditions, the adjustment of the TCa concentration by TP or Albumin can be omitted, since the good concordances reached indicated that the transformations do not modify the results. In conditions of Hypoproteinemia with NormoAlbuminemia, as in the present work, the adjustment of the TCa concentration by Albumin was the most representative because the categorization of cows after the adjustment remains stable, while when adjusting the TCa by TP, around the 90% of cows that were categorized with Hypocalcaemia prior to adjustment, become NormoCa after the adjustment.

Keywords: Total serum calcium adjusted, albumin, total proteins, dairy cows.

AH-P35

Efficacy of autovaccines against tuberculosis in goats: A proof of concept study

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Objective: Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* complex bacteria. Domestic goats are particularly susceptible to this disease, which is mainly caused by *Mycobacterium caprae* and *Mycobacterium bovis*. Caprine TB is endemic in Spain and causes relevant economic losses on the goat industry. Furthermore, infected goats pose a risk of infection of other animal species such as cattle or wildlife, and humans. However, goat herds are not yet subject to a national eradication program, except for those epidemiologically linked to cattle.

Vaccination of livestock against TB has been proposed as an alternative or complementary control strategy to strict test-and-slaughter when this is not affordable. This study was conceived as an autovaccine proof-of-concept to assess the efficacy of a heat-inactivated *M. caprae* (HIMC) vaccine in goats experimentally challenged with the same strain of *M. caprae* used to prepare the vaccine and to compare it with goats vaccinated with a previously characterized heterologous vaccine based on heat-inactivated *M. bovis* (HIMB).

Materials and methods: Twenty-one goats were divided in three groups of seven each: one vaccinated with 1ml containing 10^7 colony forming units (cfu) of HIMB, another with the same dose of HIMC, and the remaining goats were maintained as unvaccinated controls. At 7 weeks post vaccination all animals were challenged with approximately 10^3 cfu of *M. caprae* by the endobronchial route in the Biosafety Level-3 facilities of IRTA-CReSA (Bellaterra, Catalonia, Spain). After challenge, clinical signs were followed-up daily, rectal temperature and body weight were recorded weekly, and blood samples were collected every two weeks for immunological assays. All goats were euthanized at 9 weeks post challenge. Gross pathological examination, analysis of lung pathology using Computed Tomography, and bacterial load quantification by culture and qPCR were carried out. All procedures were approved by the Animal Welfare Committee of the *Generalitat de Catalunya* (Project No. 10794) in conformity with the European Union legislation (86/609/EEC, 91/628/EEC, 92/65/EECand 90/425/EEC).

Results: Unvaccinated goats showed significantly lower mean body weight cumulative increase since week 3 and higher rectal temperature since week 2 ($P < 0.05$) when compared to HIMB and HIMC. At the end of the study, both vaccinated groups showed a significant reduction of the number of affected lobes and the volumes of lung mineralization and lesions in pulmonary lymph nodes ($P < 0.05$), as well as a lower proportions of animals with extrapulmonary lesions ($P < 0.05$), compared to unvaccinated controls. Interestingly, only HIMC vaccinated goats showed a significant reduction of the volume of lung lesions and bacterial DNA load in pulmonary lymph nodes compared to unvaccinated goats ($P < 0.05$). A summary of the main *postmortem* results is shown in Table 1.

Conclusion: The results indicated that homologous vaccination with HIMC conferred protection of goats against *M. caprae* challenge compared to unvaccinated goats. Moreover, HIMC also induced a mild reduction of TB pulmonary pathology and bacterial load compared to goats vaccinated with the heterologous vaccine (HIMB). Therefore, this study encourages to carry out further large-scale trials to assess the efficacy of autovaccines under field conditions.

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Keywords: Tuberculosis, Goats, Autovaccine, Mycobacterium, Small Ruminants.

Table 1. Pathological and bacteriological results for each treatment group at 9 weeks after *M. caprae challenge*

Group	No. of affected lung lobes: Mean (95% CI)	Volume of lung lesions: Mean cm^3 (95% CI)	Volume of lung mineralization: Mean cm^3 (95% CI)	Volume of lesions in pulmonary lymph nodes: Mean cm^3 (95% CI)	No. of animals with extrapulmonary lesions	Bacterial DNA load in lymph nodes: Mean Log_{10} CFU equivalents (95% CI)
Control (N=7)	6.3 (5.1-7.4)	197 (96-297)	28.9 (2.5-55.4)	59 (32-86)	7/7	3.5 (2.7-4.3)
HIMB (N=7)	4.1 (2.5-5.8)[a]	138 (50-226)	8.6 (1.2-16.1)[b]	22 (6-37)[b]	4/7[c]	2.9 (2.2-3.6)
HIMC (N=7)	4.0 (2.6-5.4)[a]	98 (25-170)[b]	7.4 (0.4-14.3)[b]	40 (3-76)[b]	4/7[c]	2.8 (1.1-4.4)[b]

[a]$P < 0.05$, one-way ANOVA with *post hoc* Tukey test; [b]$P < 0.05$, Kuskal-Wallis test with *post hoc* one-tailed Mann-Whitney test; [c]$P < 0.05$, Fisher Exact's test.

AH-P36

Descriptive study of cow culling in dairy herds in Uruguay

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Objectives: The culling of dairy cows causes economic losses and negatively impacts on the growth of the herd. Early cow culling reduces cow longevity. This study sought to calculate the culling rates and identify the causes of culling of cows in dairy herds in Uruguay.

Materials and Methods: A prospective observational longitudinal study was conducted from June 2019 to May 2020 in 12 dairy farms in the dairy basin of Uruguay in the Departments of Colonia and San José. These departments account for 39.9% (177,085) of the total cows in the country and 50% (1,845) of all Uruguayan dairy farms. Farms were selected through stratified sampling representative of the distribution by size of the herds in Uruguay. Of the 12 farms six had a population between 51-199 cows, five had 200-500 cows and one had >500 cows. The populations varied between 74 and 740 cows. Monthly visits were made to each farm and information was collected in a Microsoft Excel Database. The data collected included the inventory of milking cows, drying cows, replacements. The information on date of calving and drying of the cows was collected. From the culling cows, cause and date of culling, calving number, days after calving, number of cows in early lactation <100 days in milk (DIM), in mid-lactation 100-200 DIM and in the late lactation >200 DIM were collected. Cow culling was classified in 3 destinations: A- sale for slaughter, B- mortality, and C- sale for milk production at other dairies. The sale for slaughter destination was further subdivided in six categories: 1-udder problems (mastitis and conformation), 2-reproductive problems (infertility and abortions), 3-lameness (foot and trauma in regions other than the foot), 4-other diseases than those mentioned above, 5-low production (old cows or end of productive life and cows with 1 or 2 calvings without evident disease), and 6-behavior and type.

Results: The average population in the 12 herds in the year of study was 3,126 cows. A total of 721 cows were culled. The overall culling rate in the 12 herds on average was 23.1% (721), ranging from 17.4% to 35.8%. The sale for slaughter was on average 18.1% (565) and ranged from 10.4% to 27.1%. Average mortality was 4.5% (141) and ranged from 1.1% to 8.1%. The sale of cows for milk production at other dairies was 0.5% (15), varying from 0% to 10.1%.

The reasons for culling cows in the 12 herds were: udder problems (32.2%, 12.5%-41.3%), reproductive problems (29.3%, 16.7%-58.5%), lameness (4.6%, 0%-11.1%), other diseases (4.7%, 0%-14.3%), low production (6.8%, 0%-20.8%), and behavior and type (0.8%, 0%-4.2%). The most frequent diseases (clinical diagnosis) other than udder problems, reproductive problems and lameness included leukosis, rumen overload, paratuberculosis and eye tumors. The sale for slaughter due to reproductive problems, udder problems, lameness and other diseases accounted for 90.3% (n=510) of the sales.

According to parity, 37.7% (n=266, range: 22.2%-62.5%) of cows sold for slaughter and dead cows had more than three calvings, 24.4% (n=172, range: 11.1%-36.8%) were first calving cows, 21% (n=148, range: 8.3%-44.4%) were cows with two calvings and 17% (n=120, range: 7.9%-26.3%) were cows with three calvings. According to lactation stage, 39.7% (n=280, range: 22.7%-62.5%) of the culled cows were in late lactation, followed by 32.3% (n=228, between 16.7%-45.6%) in early lactation, 17.6% (n=124, range: 11.3%-26.8%) in mid lactation and 10.5% (n= 74, range: 0%-22.5%) were dry cows.

Conclusions: This work shows that to reduce the culling rate of dairy cows in Uruguay, it is important to implement programs to improve herd reproduction and to control mastitis, lameness and other diseases. To achieve this, it is necessary to develop diagnostic, surveillance, research, and extension programs for farmers.

Keywords: Cow, culling, dairy, Uruguay.

AH-P37

Results of an investigative on-line audit of farms with abomasal bloat in dairy calves

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Objective: The objective of this study was to characterise calf management on dairy farms where calf abomasal disorders were reported.

Materials and methods: Farmers who attended a calf health stand during an Open Day at the Irish national dairy research centre in 2021 and self-reported abomasal disorders in calves (n=17) were asked to provide contact details for subsequent follow up. A previously designed abomasal disorder audit questionnaire (57 questions; open/closed, free text) was entered into SurveyMonkey and forwarded to the farmers' email addresses. In total 16 farmers responded to the survey; 13 had problems with calf abomasal disorders, 3 did not or did not complete the questionnaire and were excluded.

Results: The majority of affected farms had spring-calving herds (n=9 farms) and weaned less than 100 calves (9). The abomasal disorders reported were bloat (11), abomasal/intestinal torsion (5) and abomasal ulcers (2) and combinations of these disorders. In all farms heifers were affected, in some cases bull calves were also affected (6). Affected calves were generally un-weaned (12), less than two months old (11) and Holstein-Friesian (8). The estimated range in proportions of affected calves and deaths associated with abomasal disorders were 2-10% and 0-8%, respectively. The majority of cases occurred in February and March (13). On the majority of farms, these problems were first noticed within the last five years (11). While veterinary practitioners were called to these problems on the majority of farms (11), laboratory results were rarely available (4) and necropsies were rarely conducted (2).

The majority of farmers did not measure colostrum quality (7). Colostrum was most commonly stored in a freezer (9) or at ambient (6). Most farmers did not feed pooled colostrum (6). The majority of farmers fed three or more litres of colostrum at first feed (10). Colostrum was most commonly fed from a teated-bucket (10) or oesophageal feeder (6) and most commonly within two hours of birth (7). While the majority of farmers vaccinated calves against blackleg (*Clostridium chauveoi*) (11), a minority vaccinated cows (5). The most common housing system was group housing (13), on straw bedding (13) with one water point/pen (9) (most commonly more than 10 calves/water point, 8) and twenty or less calves/pen (9). When problems occurred, the most common liquid feeding systems were mob feeders (7), automatic feeders (AF) (6) and open buckets (4) and combinations of these. Teats were replaced either at the start of the season (5) or when damaged (5). Automatic feeders were most commonly calibrated at the start of the season (5). All farmers fed milk replacer (MR); at least eight products were used. The mix rate most commonly used was 12.5% (7), varying up to 15% (3). Maximum feeding rate/feed and / day was 3 (2) and 6L (13), respectively, fed most commonly at 38ºC or less (7). Starter ration (12 different products, predominantly coarse, 8) was introduced most commonly at 7 or less days of age (11). Calves had ad lib access to straw (13) and hay (2). Weaning criteria most commonly used were weight/size (12), age (7) and meal consumption (6), most commonly over more than a week step down (7).

Conclusions: From this audit of farms affected by calf abomasal disorders it can be concluded that the calf management on these farms was, in general, (accepting small sample size), representative of Irish dairy farms and, of recommended practice. Management aspects that might be altered include requesting necropsy and laboratory investigations to improve case definitions, reducing the pen-stocking rate, increasing the number of water points/calf, replacing teats more often, calibrating the AF more often and using a lower MR mix rate when problems occur. This study suggests that some farmers perceive that abomasal disorders have become a greater problem in recent years on Irish dairy farms; the concurrent and ongoing expansion of the national dairy herd may be a latent contributing factor. On some farms abomasal disorders can cause relatively high morbidity and mortality – there is a need to characterise the risk factors, case definitions and trouble-shooting protocols for this problem to assist farmers, their veterinarians and nutritional advisors with outbreaks. It is recognised that holistic investigation of abomasal disorders involves, in addition to a questionnaire audit, a farm visit.

Keywords: Dairy calf, abomasal bloat, survey.

AH-P38

Effect of parenteral copper, zinc, and selenium supplementation on beef calves

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Objectives: Micromineral deficiencies can reduce growth rate in calves in grazing cow-calf system. The aim of this study was to evaluate the effect of parenteral copper (Cu), zinc (Zn) and selenium (Se) supplementation of pre-weaning calves on weight gain, plasma Cu and Zn concentrations, and glutathione peroxidase (GPx) activity in whole blood, as indicative of Se status.

Materials and methods: The trial was carried out on a farm located in Graneros, Tucumán, Argentine. Forty-two Angus-crossbreed calves (20 females and 22 males; 97.4 ± 15.9 kg and 3 months of age) were used. They were kept as cow-calf pairs since day 0 of the trial until weaning (day 112). Calves were assigned into two groups. Treated group (TG; n=33, 16 females and 17 males) was injected subcutaneously with a solution of Cu (15 mg/mL), Zn (50 mg/mL) and Se (10 mg/mL; SUPLENUT® Se, Biogénesis Bagó, Argentine), at a dose of 1 mL/50 kg of body weight (BW) on day 0 of the trial. The Control group (CG; n=9, 4 females and 5 males) was injected with 1 mL/50 kg of saline sterile solution. Body Weight and blood samples (TG: 15; CG: 9) was taken on days 0, 40, 68 and 112. Blood samples were collected in sodium heparin tubes to assess plasma Cu and Zn concentrations by atomic absorption spectrophotometer and GPx activity in whole blood by Ransel spectrophotometric kit (Randox Lab., UK). Data were analyzed with a mixed model for repeated measures over time using the MIXED Procedure of the SAS 9.4 (SAS Inst. Inc.). The model included the fixed effect of Treatment (Group), Time (day) and their interaction, and the random effect of calf. Slice statement was used for detecting differences within each interaction. Probability values <0.05 were considered significant.

Results: There were a Treatment (p=0.02), Time (p<0.01), and Time x Treatment interaction (p<0.01) effects on BW. There were no differences between TG and CG on day 0 (98.5 ± 3.4 vs 93.1 ± 6.6 kg) and on day 40 (127.4 ± 3.4 vs 116 ± 6.6 kg), but the TG had higher BW on day 68 (150.9 ± 3.4 vs 129.2 ± 6.6 kg; p<0.01) and on day 112 (152.2 ± 3.4 vs 126 .4 ± 6.6 kg; p<0.01) than the CG. Regarding average daily gain (ADG), there were a Treatment (p<0.01) and Time (p<0.01) effects, but there was no effect of their interaction (p=0.18). Treatment group had higher ADG than CG from days 0 to 40 (721 ± 27 vs 572 ± 53 g/day; p=0.01), days 0 to 68 (773 ± 28 vs 531 ± 53 g/day; p<0.01) and days 0 to 112 (479 ± 27 vs 286 ± 54 g/day; p<0.01). There were a Treatment (p=0.04), Time (p<0.01), and Time x Treatment interaction (p<0.01) effects on plasma Cu concentration. Treatment group had lower plasma Cu concentration than CG on day 0 (42.7 ± 3.6 vs 57.6 ± 4.7 µg/dL, p=0.02), but TG had higher plasma Cu concentration than CG on day 40 (49.4 ± 3.6 vs 24.1 ± 4.7 µg/dL; p<0.01) and on day 68 (33.3 ± 3.7 vs 17.1 ± 4.7 µg/dL; p<0, 01). There was no difference between groups at day 112 (23.3 ± 4.2 vs 10.6 ± 6.5 µg/dL, TG vs CG, respectively; p=0.11). Plasma Zn concentration was affected by Time (p<0.01), without effect of Treatment or Time x Treatment interaction (p=0.39). There were a Treatment (p<0.01), Time (p<0.01), and Time x Treatment interaction (p<0.01) effects on GPx activity. There was no difference between TG and CG (16.1 ± 3.5 vs 15 ± 4.5 U/mL hematocrit, respectively; p=0.84), TG had higher GPx activity than CG on day 40 (75.2 ± 3.5 vs 18.3 ± 4.5 U/mL hematocrit; p<0.01), on day 68 (95.9 ± 3.5 vs 20.6 U/mL hematocrit; p<0.01) and on day 112 (44.1 ± 3.7 vs 20.4 ± 4.5 U/

mL hematocrit; p<0.01).

Conclusions: The results indicate that parenteral Cu (0.3 mg/kg), Zn (1 mg/kg) and Se (0.2 mg/kg) supplementation on pre-weaning beef calves increase BW and improve Cu and Se status.

Keywords: calves, copper, zinc, selenium, supplementation.

AH-P39

Performance and carcass attributes of feedlot steers supplemented with parenteral trace minerals and vitamins

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Objectives: Beef cattle fattening system, especially during receiving period, generates strong oxidative stress, affecting weight gain and carcass attributes. The objective of this study was to evaluate the effect of parenteral supplementation with minerals and vitamins that intervene in antioxidant defense during admission to a feedlot system on these parameters.

Materials and methods: The study was carried out in a commercial feedlot (CONECAR, Ganadera Santa Fé SA) located in Carcarañá, Santa Fé province, Argentina. Fifty-six Aberdeen Angus steers were used, with an average body weight (BW) of 321.1 ± 12.9 kg, which arrived at feedlot after a 750 km trip. At arrival (Day 0) the animals were randomly divided into two groups. The treated group (TG, n=27) received on Day 0 a subcutaneous injection with trace minerals (copper 40 mg, zinc 160 mg, manganese 40 mg and selenium 20 mg; ADAPTADOR MIN, Biogenesis Bagó, Argentina) and another with vitamins (vitamin A palmitate 238,000 IU and vitamin E acetate 200 IU; ADAPTADOR VIT, Biogenesis Bagó, Argentina), while the control group (CG, n=29) did not receive any treatment. The animals were weighed on Days 0, 17, 43, 55, 113 and 192 (the last weighing was done 3 days prior to slaughter). On Days 55 and 113, ultrasound scans of the carcass were performed to assess the ribeye area, backfat thickness, and percentage of intramuscular fat, while retail cut, yield grade, and marbling score were also assessed on day 113. On the day of slaughter, the carcass evaluation was carried out in the slaughterhouse to determine Yield and weight of the carcass. To evaluate BW, average daily gain (ADG), ribeye area, backfat thickness, and percentage of intramuscular fat, mixed linear regression models with repeated measures over time were used (PROC MIXED of SAS 9.4; SAS Institute Inc). Time (Days), Treatment (TG and CG) and their interaction were used as fixed effects, and the animal as random effect. Slice statement was used for detecting differences within each interaction. The retail cut, yield grade, marbling score, yield and carcass weight were analyzed with linear regression models with PROC MIXED, using Treatment as the only fixed effect. Data are presented as least squares means ± standard error of the mean. Values of p≤0.05 are considered significant and p<0.1 and >0.05 are considered tendencies.

Results: With regard BW, there were a Time effect (p<0.01) and a Time x Treatment interaction tendency (p=0.07), because on Day 192 the TG had greater BW than the CG (570.8 ± 4.7 vs 557.7 ± 4.5 kg, respectively; p=0.04). There were no differences for BW between the groups on the rest of the Days (p>0.05). Regarding ADG, only a Time effect was observed (p<0.01), although in the period between Days 43 and 55 the TG tended to gain more weight than the CG (1,589 vs 1,302 kg/day, respectively; p=0.09). Regarding the ribeye area, backfat thickness and percentage of intramuscular fat, only an Time effect (p<0.01) was observed. There was no Treatment effect (p>0.1) on Retail Cut (64.7 ± 0.2 vs 64.8 ± 0.2%), Yield Grade (2.54 ± 0.04 vs 2.56 ± 0.04), Marbling score (4.34 ± 0.03 vs 4.32 ± 0.03) Yield (59.9 ± 0.3 vs 60.2 ± 0.3%) and carcass weight (342 ± 3.5 vs 335.7 ± 3.4 kg;TG and CG, respectively).

Conclusions: It is concluded that under the conditions of this study, parenteral supplementation with minerals and vitamins that intervene in the antioxidant defense at the entrance of a fattening pen increased the weight of the animals at the end of the cycle, without modifying the carcass.

Keywords: Feedlot, body weight, carcass attributes, trace minerals, vitamins.

AH-P40

A longitudinal study of bovine trichomonosis incidence in Spanish beef mountain herds with different infection status

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Objectives: Bovine trichomonosis (BT) is a sexually transmitted disease considered a major cause of early reproductive failure in natural extensive breeding cattle. In the absence of effective vaccines and drugs, BT is controlled by diagnostic testing and culling of infected bulls. In Spain, BT is substantially spread among beef cattle herds and its control is voluntary[1]. We observed that herds from mountain management systems are at greater risk for the introduction of the infection, which could be attributed to the relatively high local prevalence and specific management practices, such as the use of communal grazing lands[1,2,3]. In the present study, a longitudinal study was carried out to compare infection incidence in three epidemiological scenarios: *i)* herds with BT-negative status (BT-negative herds); *ii)* herds with BT-positive status where infected bulls were eliminated to interrupt the transmission cycle (BT-cleared herds); and *iii)* BT-positive herds where positive bulls were maintained in the herd (BT-positive herds).

Material and methods: Participating farms were purposively selected from data collected from the diagnostic reports issued by the SALUVET-INNOVA veterinary diagnostic laboratory (Madrid, Spain) between January 2017 and December 2021. Parasite detection was undertaken by culture and PCR on smegma samples collected from breeding bulls. The inclusion criteria were herds from mountain areas (Asturias, northern Spain) tested for BT for two consecutive years. In addition, only those herds that re-sampled at least half of the bulls were selected. The selected herds were classified according to the infection status: i) BT-negative; ii) BT-cleared and iii) BT-positive herd status. The herd/bull incidence was calculated as the number of new cases (newly infected herds/bulls) divided by the number of herds/bulls at risk. The considered time period was 12 months but the year was not equal for each farm due to the fact that the monitoring of the infection occurred at different years. Herds were classified as BT-positive if at least one sexually active bull tested positive for *Tritrichomonas foetus* infection.

Results: According to T. foetus infection status, a total of 65 and 17 herds were accurately classified as BT-negative and BT-cleared herds, respectively. Unfortunately, only 11 herds could be included in BT-positive herd group. When the incidence was investigated for one year (Table 1), no significant differences were observed at herd and bull level between herds where infected bulls were eliminated (BT-cleared herds) and BT-negative herds, although new infections were observed in this group (Table 1). The highest new infection rate (36.6%, 4/11) was observed in bulls from BT-positive herds where the infected bulls were maintained, being significantly higher than those observed in bulls from BT-negative (4.4%; P<0.01) and BT-cleared (0%; P<0.01) herds.

Conclusions: There is a moderate risk of introduction of T. foetus infection in mountain management herds from Spain. Consequently, negative bulls should be tested every year, putative risk factors should be corrected and biosecurity measures improved to mitigate the risk of *T. foetus* infection. In herds where infected bulls were maintained, the bull incidence was high. Therefore, infected bulls should always be culled to stop the parasite from spreading. Moreover, vaccination could be a feasible control measure to reduce the economic losses due to BT in these herds. Our results can be used as input for BT planning control strategies on extensively managed beef herds with different BT status.

Table 1. BT incidence in bulls and herds from mountain systems with different BT status.

Infection status	Herd incidence	Bull incidence
BT-negative	7.7% (5/65)	4.4% (5/113)
BT-cleared	0% (0/17)	0% (0/34)
BT-positive	Not applicable	36.6% (4/11)

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Keywords: Bovine trichomonosis, incidence, control, beef cattle, bulls.

AH-P41

How haemolysis affects the mineral profile of cattle serum?

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Objectives: Haemolysis of serum samples is recognized to be the leading cause of preanalytical errors in clinical laboratories. Little is known about the potential alterations in the concentrations of mineral elements in haemolyzed serum and the phenomenon has not been specifically studied in bovine serum samples. The first objective of the study was to determine any significant differences between mineral concentrations measured in whole blood and in serum. The second objective was to establish a haemolysis acceptability threshold (HTr) to enable laboratory technician and clinicians to decide whether a serum sample would yield reliable results for measuring mineral contents.

Material & methods: We used inductively coupled plasma-mass spectrometry (ICP-MS) to measure the concentrations of 12 mineral elements (Ca, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, P, Se and Zn) in bovine whole blood, serum and gradually haemolyzed samples.

Results: We observed significant differences between the different types of samples, particularly in the Fe and Zn concentrations. However, in practice, the high interindividual variability makes it difficult to stablish whether a given value corresponds to normal or haemolyzed samples. In response to this problem, we propose to consider that a result is significantly biased when the haemolysis threshold (the degree of haemolysis above which the concentration of an element in serum is significantly altered) of a given element is surpassed. The haemolysis threshold values for the different elements considered were as follows: 0.015 g Hb L^{-1} for Fe, 2 g for Zn, 4 g for Cr and 8 g for Ca, Se and Mo.

Conclusions: A very low haemolysis degree (> 0.015 g Hb L^{-1}) overestimated serum Fe concentrations, a moderate haemolysis degree (> 1.74 g Hb L^{-1}) overestimated Zn; whereas for the other minerals (Ca, Cr, Mo and Se) higher haemolysis degree values would be needed to significantly modify the serum concentrations (> 4 g Hb L^{-1}). As Fe alteration occurs at very low haemolysis degree (0.015 g Hb L^{-1}), haemoglobin should be quantified to correct Fe results based on the haemolysis degree.

Keywords: haemolysis; mineral elements; serum; cattle; ICP-MS.

AH-P42

Application of the bolus-type biosensor for calves and its usefulness

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Objective: Since calf health problems significantly impact productivity, efforts for early detection of the disease on calf have continued in the cattle industry. As one of these efforts, sensor-based measurements of vital signs and data analysis have been recently utilized for the remote detection of pathophysiological states and animal welfare of an individual or a herd. In this study, we applied a small-sized wireless bolus-type biosensor, which was recently developed equipped with a 3-axis accelerometer and a temperature sensor, to calves and examined the location of the biosensor in the forestomach and wireless data reception. Also, the data from the biosensor were analyzed to understand the changing pattern around the weaning period in calves.

Materials and methods: A total of twelve calves, including six beef calves (BC; Korean native cattle) and six dairy calves (DC; Holstein) over 1-week-old, were enrolled in this study. The biosensor, which has a length of 35 mm, a diameter of 18 mm, and a weight of 31 g, was inoculated orally one week after birth using the inserting device. The device recorded the temperature and the vector values for each axis (X, Y, Z) when the V (V = $\sqrt{X^2 + Y^2 + Z^2}$) value was the highest for 10 minutes. And data were wirelessly transmitted at a 30-minute interval and were collected for 15 weeks for each calf. For describing changes in V value over time, the V value was converted to V1 (V1 = $\sqrt{(X_t - X_{t-1})^2 + (Y_t - Y_{t-1})^2 + (Z_t - Z_{t-1})^2}$). In addition, to evaluate the influence of weaning on each data in BC, daily average V (DAV), daily average V1 (DAV1), daily average temperature (DAT) were calculated, and each change was statistically analyzed.

Results: Except for the one biosensor that was spewed out from one BC at 59 days of age, biosensors transmitted data normally. And the location of the biosensor in the reticulum was confirmed through radiographic images. There were no significant differences in mean DAV with over days of age in BC and DC. But, DAV1 gradually increased until about eight weeks in BC and eleven weeks in DC, and then plateaued. Those values of DAV1, which was measured lower than DAV at early days of age, were reversed for the first time at 44 days in BC and 53 days in DC. During the weaning period, DAV, DAV1, and DAT had increased from the day of weaning (0 d) in five BC. The means of DAV, DAV1, and DAT showed the highest values on +1d, +2d, and +1d after weaning, respectively. Based on the results, it is presumed that the DAV1 values of the biosensor reflect the development of the forestomach and the reticuloruminal motility in this study. In addition, the normal functioning of the reticulo-rumen can be estimated with DAV, DAV1, and DAT.

Conclusion: In conclusion, we confirmed that the bolus-type biosensor inoculated orally to the calf settled in the reticulum and transmitted data wirelessly for 15 weeks. Through the processing of the data produced by the sensors of temperature and 3-axis acceleration, it was possible to evaluate the reticuloruminal motility and to estimate the weaning period. This means that the biosensor can be used as a tool to evaluate the health status of calves. Further studies are needed to evaluate whether the biosensor can detect pathophysiological changes earlier than direct observation in calves.

Keywords: Biosenor, Calves, 3-axis accelerometer, Temperature, weaning.

AH-P43

Phenotypical and molecular identification and antimicrobial resistance profile of *Campylobacter fetus venerealis* and *Campylobacter fetus venerealis* biovar *intermedius* field isolates

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Objectives: *Campylobacter fetus* comprises two subspecies closely related both phenotypically and genetically, which complicates their laboratory differentiation: *Campylobacter fetus* subsp. *fetus (Cff)*, that can cause sporadic abortion in cattle and *Campylobacter fetus* subsp. *venerealis (Cfv)*, which is the causative agent of bovine genital campylobacteriosis (BGC), a sexually transmitted disease listed by the World Organization for Animal Health (OIE). Tolerance to 1% glycine and H_2S production are considered the most relevant tests for phenotypic differentiation, although the presence of *Cfv* biovar *intermedius (Cfvi)* may lead to misidentifications[1]. Due to their high specificity, PCR methods targeting the *C. fetus*-specific *nahE* gene and the *Cfv*-specific *ISCfe*1 insertion element[2] are the most reliable methods for routine identification. The two most commonly used antibiotics for the treatment of BGC are streptomycin and long-acting tetracyclines[3]. The aims of this study were to compare *Cfv* and *Cfvi* field isolates phenotypic results with those obtained by PCR, to test the effect of longer incubation time on H_2S production, and to analyse their antibiotic resistance profile.

Material and methods: Eighty-three *C. fetus* isolates from bull preputial smegma (74) and poultry faeces (9), previously identified as *Cfv* (71) and *Cff* (12) by PCR[2], were used. Isolates were cultured under microaerophilic conditions for 48h at 37ºC, and DNA extracted using a commercial kit. Molecular differentiation between *Cfv* and *Cfvi* isolates was performed by PCR targeting the L-cysteine transporter[4]. For biochemical differentiation, the tests recommended by the OIE were used[1] and H_2S production was measured at 72 hours and 5 days. The reference isolate *Cfv* ATCC 25922 and isolates *Cfvi3*, *Cff1*, *Cff2* and *Campylobacter hyointestinalis* provided by the

OIE reference laboratory of the University of Utrecht were used as controls. The concordance between the biochemical test and PCRs was calculated with Cohen´s Kappa statistic. Antimicrobial resistance profile was studied by determining the minimum inhibitory concentrations (MICs) of 7 *Cfv* and 32 *Cfvi* isolates by broth microdilution, using EUCAMP2 plates (Sensititre®, ThermoFisher Scientific) that contain 6 antimicrobial agents, as recommended by the Commission Decision 2013/652/EU. Results were interpreted using epidemiological cut-off values as developed by the European Committee for Antimicrobial Susceptibility Testing[5] for *C. jejuni*, as there are no cut-offs established for *C. fetus*.

Results: The 83 *C. fetus* isolates analysed were classified into 14 different biochemical profiles, and 14 isolates were identified as *Cfv* (16.9%), 50 as *Cfvi* (60.2%) and 19 as *Cff* (22.9%). On the other hand, PCR identification showed that 11 (13.2%) of the isolates were *Cfv*, 60 (72.3%) *Cfvi* and 12 (14.4%) *Cff*.

Discrepancies between biochemical and molecular tests for subspecies identification were found for 7 isolates molecularly identified as *Cfvi* but with biochemical characteristics typical of *Cff* isolates (i.e., growth at 42ºC and tolerance to 1% glycine). Thus, a concordance of 91.6% (κ=0.726; 0.539-0.912 CI) was observed between PCR identification of *Cfv* and *Cff* isolates and biochemical tests. As for biovar *intermedius* identification, at 72 hours, 12 isolates with the complete L-cysteine operon typical of *Cfvi* isolates showed no H_2S production, whereas only 3 remained negative for H_2S production after 5 days. Hence, concordance between the PCR method and the H_2S production test was lower when isolates were grown for 72 h (κ=0.553, 0.347-0.760 CI) than 5 days (κ=0.855, 0.696-1 CI).

The seven isolates characterised as *Cfv* did not grow properly in Müller-Hinton broth so their MICs could not be determined. For *Cfvi* isolates, 65.6% (21/32) were resistant to nalidixic acid and sensitive to all other antibiotics.

Conclusions: There is a significant biochemical variability between *Cfvi* isolates. Even if there is good agreement between biochemical tests and PCR to differentiate *Cfv* and *Cff*, the use of both methods is necessary for a correct identification. The H_2S production test gives more reliable results for the differentiation of *Cfvi* after five days of incubation compared with 72 h. All isolates tested were sensitive to streptomycin and tetracycline, making the use of these drugs effective for the treatment of BGC.

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Keywords: Campylobacter fetus subsp. venerealis, biochemical tests, PCR, antimicrobial profile.

AH-P44

Tresholds of dry cow blood variables obtained by receiver operating characteristic analysis for indication of milk production during early lactation

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Objective: The aim of the study was to determine the association between the metabolic parameters determined in dry cows and milk production at early lactation period. The receiver operating characteristic (ROC) analysis was used as a valuable tool to evaluate prediction of milk production based on results for metabolic status of dry cows. The cut off values for metabolic parameters were determined as values below or above which daily production of milk was higher than 30 L at day 30 of lactation.

Material and methods: The study included 191 dry cows. At the time of selection, body condition score was estimated and blood was sampled from *v. jugularis*. Concentrations of the beta- hydroxybutyrate, nonesterified fatty acids, glucose, total bilirubin, total protein, albumin, urea, Ca and P, and calculated Ca/P ratio were estimated. On day 30, daily milk production was measurd for each cow. Milk fat, milk protein, milk dry matter contents as well as fat to protein ratio (F/P) were estimated in milk samples taken at day 30 of lactation.

Results: Our results indicate that during late pregnancy, as reliable predictors of daily milk yield can be used BCS, glucose, beta-hydroxybutyrate, and Ca concentrations as well as Ca/P. Namely, milk production higher or equall to 30 L at day 30 of lactation can be indicated if values are lower than 3.85 and 0.65 mmol/L, for body condition score and beta-hydroxybutyrate, respectively, and higher than 3.45mmol/L, 2.45mmol/L, and 1.04 for glucose, Ca and Ca/P ratio, respectively.

Conclusion: Our results indicate that some metabolic parameters determined at dry cows can be used for milk prediction in early lactated dairy cows. However, physiological range of determined parameters should not be overlooked in interpretation of obtained results.

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Keywords: dry cows, metabolic profile, ROC analyses, milk production.

AH-P45

How climate change affects dairy cow farming in Serbia

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Objective: Due to climate change, for the period 2001 to 2030 temperature in Serbia is projected to rise by about 1°C, compared with 1961-1990. The aim of this study was to evaluate the effects of increased enviromental temperature during summer season in Serbia, which is in the zone of moderate-continental climate, on milk production and milk composition in dairy cows, as well as postnatal adaptive capatibility of neonatal calves.

Material and methods: The trial was carried out on 40 Holstein Friesian cows and their neonatal calves (20 cows in summer and 20 cows in the spring season). Average daily feed consumption was measured for each cow. The cows were housed in a stable which during the summer was not equipped with additional cooling. The temperature-humidity index was recorded hourly. Based on the results calculated for average all-day THI, during the spring season the cows were not exposed to heat stress. During the summer season, the cows were exposed to the effects of moderate to severe heat stress. The cows' colostrum was sampled at 2, 14, and 26 h after calving, before feeding of their calves. Calves' blood samples were taken before the first colostrum intake and on days 1, 2, 3, and 7 of life. Calves' physiological parameters were measured on days 0 and 7. Milk production was measured daily form calving to day 90 of lactation.

Results: The quality of cows' colostrum was significantly reduced during summer season. The ingestion of the low-quality colostrum, combined with the thermal discomfort during summer season, provoked impaired physiological, biochemical, hormonal, and oxidative stress parameters in samples taken from the post-colostral calves. Average daily milk yield in the spring season from day 60 to day 90 of lactation was significantly higher compared to the same lactation period during the summer season, while there was no significant difference in average milk yield from day 30 to day 60 of lactation between spring and summer season. Average daily feed consumption was significantly lower during the summer compared to spring during whole trial period.

Conclusion: Our results indicate that, during summer season, the negative impact of climate changes on milk production, milk composition and post naonatal adaptive capability of cows are strongly expressed. However, negative impact on milk production is not effective until day 60 of lactation, probably due to homeorhetic mechanisms which are important for the rearrangement of metabolism. This study highlights the importance of adequate supporting strategies for the care of the dairy cows and postnatal calves during the summer season.

This research was supported by the Science Fund of the Republic of Serbia.

Grant No 7750295, "Mitigation of methane production from dairy cattle farm by nutritive modulation of cow`s metabolism -MitiMetCattle."

Keywords: dairy cows, neonatal calves, climate changes.

AH-P46

Effect of seasonality (spring × winter) on metabolism, health and immunity of dairy cows and their calves

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Objectives: The objective of this study was to evaluate the effect of the season (spring and winter) on the metabolism, health, and immunity of neonate Holstein calves.

Materials & Methods: This research was in a dairy farm, in Descalvado, São Paulo, Brazil. It was evaluated 28 healthy multiparous Holstein cows, that calved female neonates, in eutocic delivery, evaluated during winter (July and August, n=15) and spring (September and October, n=13), which the average maximum, minimum, and thermal amplitude temperature were 28.2°C, 29.3°C, 9.2°C; 15.9°C, 21.2°C, and 13.4°C, respectively. Dry cows were housed at indoor compost-barn, with cross-ventilation, 30 days before expected calving date. Immediately after birth, calves were separated in a "cuddle box" to maternal initial contact, and receive colostrum (≥21% BRIX) until 1h . From the 1st to the 14th day, calves stayed in closed-sided individual housing, hay bedding, covered barn, and the 15th to 28th in individual housing system outside the covered barn. At calving, the cow body condition score (BCS) was classified (1-5) and blood collected to analyse. Calves were blood sampled immediately after birth (D1) and on D2-3, D7, D14 and D28 days of life, 2h before the morning milk feeding, when has done a physical examination. Blood samples were collected in vacuum tubes containing fluoride sodium and with no anticoagulant, to obtain plasma and serum, respectively. Fecal and bovine respiratory disease (BRD) scores were assessed based in Calf Heath Scoring Criteria (University of Wisconsin). With serum samples were analyzed cholesterol, triglycerides, iron, total protein and albumin, and with plasma, glucose, non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB), through commercial kits in automated biochemical analyzer. Haptoglobin (Hp) was measured by spectrophotometric technique. The IgG levels in calf serum samples were quantified using an in-house sandwich ELISA. Measurement of endogenous and induced reactive oxygen species (ROS) production were conducted in whole blood cultures by using the fluorescent dye dihydrorhodamine 123. The cells were stimulated with inactivated antigens like *Staphylococcus aureus; Escherichia coli; S. hyicus* and Phorbol Myristate Acetate at 10^{-6}M (PMA), and the capacity

of response was expressed as the ratio of stimulated to unstimulated cells (response ratio, RR). The SAS 9.4 was used, data distribution of dams and calves was checked using the Kolmogorov-Smirnov test (*P*<0,05), Dam's parameters were compared with the t test (parametric) and Mann-Whitney test (non-parametric). Neonatal diarrhea and BRD frequency were evaluated by the chi-square test. For continuous calves data, the mixed linear model was used to evaluate the variables and seasonality (group), by time and group-time interaction. Data distribution was checked using the Kolmogorov-Smirnov test.

Results: The mean values of NEFA, BHB, glucose, total protein, albumin, triglycerides, and cholesterol of dams were similar independently of calving season, and the values is within the normal reference interval for dairy cattle, without metabolic disease. Winter calves had a lower respiratory rate and heart rate than spring calves. Rectal temperature was similar between season group, showing a higher average only in D14, when spring calves had higher temperature than winter calves. Spring calves born presented a higher frequency of diarrhea (9/13) than in winter calves (0/15) in D28. Only one calf, born in spring, had BRD during the study. There was an effect of the age of the calves in body weight, respiration rate, rectal temperature, iron, and albumin. It has interaction time by group (season) in heart rate, Hp levels. Winter calves had higher Hp on D2 than spring calves, which profile is inverted in D28. The ROS induction by PMA, *S. aureus,* and *S. hyicus* was higher in spring calves than winter. Heifers born in winter presented higher cells response ratio to *E. coli* than spring animals. Seasonality also had effect on the calf serum IgG concentration, which was greater in winter calves during the neonatal period.

Conclusions: The seasonality had effect on diarrhea frequency, more observed during the spring, when the calves revealed more inflammation reaction, showed by haptoglobin, and response ratio to ROS production after the stimulation of leukocytes by PMA, *S.aureus* and *S.hyicus*, and calves born in winter had better IgG levels. This suggest that the season has adversely effect on metabolic and inflammatory responses of neonatal calves, that could influence disease susceptibility, and performance.

Keywords: dairy cattle, inflammation, innate immune response, neonatology, transition period.

AH-P47

Metabolic parameters of Holstein cows supplemented with Lithothamnium Calcareum or Sodium Bicarbonate ruminal buffers

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This study aimed to compare the effect of supplementation with ruminal buffers Lithothamnium Calcareum (LithoNutri®, Oceana, Jundiaí, Brazil) and Sodium Bicarbonate on blood metabolic parameters in Holstein dairy cows. Thirty-six multiparous Holstein cows managed in a Compost Barn system with an average of 63.74 ± 18.63 days in milk (DIM) and average milk yield of 38.83 kg ± 9.29, were enrolled for the study and randomly divided into two groups: Lithothamnium Calcareum group (0.5% of the diet DM of Lithothamnium Calcareum; n = 18) and control group (1.1% of the DM of Sodium Bicarbonate; n = 18). The experimental period lasted 60 days and was performed on a commercial dairy farm in the South of Brazil. During this period, each group was fed daily with the buffers mixed to the total mixed ration (TMR). The TMR followed the recommendations of NRC (2001), containing 28% starch. Blood samples were collected by tail vein puncture using the Vacutainer system (BD Diagnostics, São Paulo, Brazil) at the days 0, 7, 14, 21, 28, 35, 42, 49, 56, 60 of the trial. The parameters analyzed were glucose, β-hydroxybutyrate, non-esterified fatty acids, magnesium, calcium, albumin, and total plasma proteins (TPP), all evaluated in automatic biochemical analyzer Labmax Plenno (Labtest®, Minas Gerais, Brazil). The data were analyzed using the Mixed Models procedure of JMP® Pro 14 2018 (SAS Institute inc., North Carolina, USA), considering group and collections as fixed effects, and animals as random effects. No effect of treatments (P = 0.29) was observed between groups Lithothamnium Calcareum and Control respectively for serum glucose concentrations (61.09 ± 0.76mg/dL vs. 59.96 ± 0.76mg/dL), as well as for TPP (P = 0.10) (7.87 ± 0.09g/dL vs. 8.09 ± 0.09g/dL), urea (P = 0.84) (28.80 ± 1.59mg/dL vs. 28.39 ± 1.60mg/dL), albumin (P = 0.20) (2.71 ± 0.03g/dL vs. 2.71 ± 0.03 g/dL), magnesium (P = 0.83) (2.26 ± 0.05mg/dL vs. 2.24 ± 0.05mg/dL), calcium (P = 0.70) (8.52 ± 0.10mg/dL vs. 8.58mg/dL ± 0.10) e β-hydroxybutyrate (P = 0.92) (0.48mmol/L ± 0.02 vs. 0.47 ± 0.02mmol/L). However, serum concentrations of non-esterified fatty acids tended (*P* = 0.07) to decrease in animals supplemented with Lithothamnium Calcareum compared to Control (0.35 ±0.03 mmol/L vs 0.43±0.03 mmol/L). These results demonstrate that supplementation with Lithothamnium Calcareum in high-starch diets is a safe alternative for Holstein dairy cows, providing a similar metabolic profile as Sodium Bicarbonate and tending to reduce non-esterified fatty acids, apparently improving energy utilization.

Keywords: dairy cows, food additives, Lithothanmium calcareum, sodium bicarbonate.

AH-P48

Effect of supplementation with Lithothamnium Calcareum or Sodium Bicarbonate on dry matter intake, milk production, feed efficiency, and milk composition of Holstein dairy cows

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This study aimed to compare the effects of supplementation with the ruminal buffers Lithothamnium Calcareum (LithoNutri®, Oceana Minerals, São Paulo, Brazil) and sodium bicarbonate on dry matter intake (DMI), milk yield (MY), feed efficiency, and milk composition of Holstein cows. The experiment was conducted on a commercial farm in Southern Brazil in which cows were kept in a Compost Barn system and milked three times a day. Thirty-six multiparous cows with average milk yield of 38.84 ± 9.29 kg, and 63.74 ± 18.63 days in milk (DMI), were randomly divided into two homogeneous groups: Lithothamnium Calcareum (0.5% of the dry matter of the Lithothamnium Calcareum diet; LG n=18) and Control (1.1% of the dry matter of the Sodium Bicarbonate diet; CG n=18). Supplementation with the buffers was performed daily. Each group was fed with the total mixed ration (TMR) formulated according to the recommendation of NRC (2001), containing 28% starch (corn silage, pre-dried ryegrass, and concentrate) to meet nutrient requirements of lactation. The DMI (kg/day) was monitored individually for each animal, using automatic feeders (Intergado, Minas Gerais, Brazil). The individual milk yield recording data (kg/day) were obtained through the ALPRO™ electronic milking system (DeLaval, Botkyrka, Sweden). Feed efficiency was determined from the relationship between DMI and MY. Milk samples for evaluating fat, lactose, protein, and total solids were collected weekly and analyzed in the laboratory. Data were analyzed using the MIXED Models procedure in the JMP® Pro 14, 2018 (SAS Institute Inc., North Carolina, USA), considering group and collection as fixed and animal effects as a random effect. No difference was found between the groups for all variables analyzed. The mean feed efficiency obtained for the LG group was 1.47 ± 0.03, and for the CG, it was 1.47 ± 0.03 (P = 0.90). The averages of DMI for the LG and CG were similar (P = 0.49), being these (26.73 ± 0.53 Kg/d vs. 27.19 ± 0.54 kg/d), respectively. The mean for milk production for LG was 39.06 ± 0.69 Kg/d and for CG, 38.59 ± 0.71 Kg/d (P = 0.53). For milk quality parameters, the mean fat concentration for the LG was 4.19% ± 0.09 and for GC 3.88% ± 0.09 (p = 0.01). Lactose was 4.40% ± 0.04 for LG and 4.46% ± 0.04 in GC (p = 0.92). For protein, LG showed 3.12% ± 0.04 and GC 3.10% ± 0.04 (p = 0.50). In total solids the mean concentration was 12.53% ± 0.12 for LG, and 12.36% ± 0.13 for CG (p = 0.04). In conclusion, Lithothamnium Calcareum, despite being offered in a lower dose, acts similarly to Sodium Bicarbonate in terms of DMI, feed efficiency, milk yield, milk composition, and increasing the concentration of fat in milk. For these reasons, Lithothamnium Calcareum can be used as an alternative buffer for ruminant supplementation.

Keywords: additive, nutrition, productivity, health.

AH-P52

Development and validation of a methodology for simultaneous potentially toxic elements determination in bovine blood serum by wavelength dispersive X-ray fluorescence

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The aim of this study was to develop, implement and validate an analytical methodology, grounded in the wavelength dispersive X-ray fluorescence spectrometry (WDXRF) technique for the non-destructive, fast and simultaneous analysis of aluminum (Al), chromium (Cr), cobalt (Co), iron (Fe), copper (Cu), arsenic (As) and mercury (Hg) in bovine blood serum samples. The experiments were carried out using WDXRF spectrometer RIGAKU Co., model Supermini 200 with X-ray tube Pd anode. After setting up the instrumental conditions, three measurements for Al, Cr, Co, Fe, Cu, As and Hg were performed on standard solution traceable to SRM from NIST (1000 mg L^{-1}), i.e. using a micropipette, three aliquots of 10, 20, 30 and 50 μL were dropped and the dried in special filter paper (Droplet method). The net intensities of each element were related to their respective concentrations (10, 20, 30 and 50 mg L^{-1}), and using linear regression the coefficients of each curve were calculated. The validation plan of the proposed methodology considered the parameters of quantification limits, accuracy and precision. Quantification limits of 0.5, 0.3, 0.7, 0.2, 0.3, 3.6 and 1.5 mg L^{-1} were determined for Al, Cr, Fe, Co, Cu, As, and Hg, respectively**.** The methodology was applied for blood serum samples of 59 dairy cattle from two different farms. Farm A was in an area possibly contaminated by heavy metal due to mining activity and an environmental disaster (N = 29 cows) and farm B in a non-contaminated area (N = 30), both located within 526 km of each other, in Minas Gerais, Brazil. Student's t test was applied to compare groups. P-value < 0.05 was considered significant. All calculations were performed using RStudio. The results obtained were lower than the quantification limit for all analyzed samples except Al and As. Al was detected in 58.6% (17/29) cows in Farm A and 40% (12/30) cows in Farm B (Farm A = 8.73 ± 3.81 ug/L; Farm B = 5.75 ± 3.24 ug/L) (*P* = 0.03). As was detected in three cows of both farms (Farm A = 0.087 ± 0.085 ug/L; Farm B = 0.98 ± 0.13 ug/L) (*P* = 0.0006). The proposed methodology proved to be satisfactory for the determination of potentially toxic elements in dairy cattle. In addition, it can may be applied in the blood serum of other species because it is fast analysis that use small amount sample and not generate chemical waste.

Keywords: Heavy metal, X-ray fluorescence, diagnostics.

AH-P54

Nutritional myopathy with myoglobinuria: a case report

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Nutritional myopathy is often associated with selenium (Se) and/or vitamin E (Vit E) deficiencies. In cattle, it is characterized by a skeletal or cardiac musculature necrosis along with a wide range of clinical manifestations according to the affected muscle. The aim of this study was to describe a case occurred in the Province of Santa Fe (Argentina) and reported by members of the Large Animal Hospital (Faculty of Veterinary Science (FCV), Universidad Nacional del Litoral (UNL)). After heavy rains, approximately 10% of a group of animals appeared in recumbency or dead. Calves ranging to 8 and 14 months received a feed based on concentrates and hay. Necropsies of four recently dead animals were performed. White muscle lesions were observed in different muscle masses (anterior and posterior limbs, including the heart), pulmonary edema and red colored urine. Neither in the pen nor in the hay which animals were eating, toxic plants were found that could cause a similar picture to that described. Samples were taken from the concentrated to perform a toxicological test in mice at the Comparative Medicine Center of the ICIVET Litoral (CONICET/UNL), without clinical signology or lesions in necropsies. Removed tissues were fixed in 10% (v/v) neutral buffered formalinin and processed at the Pathology Laboratory from the FCV-UNL. A multifocal polyphasic degeneration and necrosis of muscle fibers in myocardium and skeletal muscle (semitendinosus, semimembranosus, supraspinatus and infraspinatus) was observed. In affected animals, plasma concentrations of Vit E and Se were 1.7-1.8 mg/L and < 0.1 µg/dL, respectively, below reference values (1,2). Multiple etiologies responsible for similar clinical-pathological conditions have been described, such as ionophores poisoning, plants ingestion with myopathic substances (Senna *spp.*), Vit E and Se deficiencies, among others. The first two were discarded in this case. Furthermore, the Vit E and Se deficiencies were confirmed in the plasma from the affected animals, although the correlation between plasmatic Se and the glutathione peroxidase activity was lower than that registered between blood Se concentration and the enzyme activity (3). Finally, it is important to note that this case occurred in the form of an outbreak after an abundant rainfall and that possibly increased the animal demands, turning from a subclinical to a clinical deficiency. These results highlight the multifactorial nature of the disease and the importance of the oral and/or parenteral supplementation of these elements.

Keywords: selenium, vitamin E, nutritional, myopathy, myoglobinuria.

AH-P56

Antibacterial effect of lysozyme against multidrug resistant bacteria and non-multi-resistant bacteria causing mastitis in cows

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Background: Mastitis is a disease that need serious attention for its control and preventions as it is the most economically overwhelming disease, causing reduction in milk production and milk quality, and antibiotics residue in milk (Kibebew, 2017).

The increased resistance to antimicrobials has put public health services around the world on alert (World Health Organization 2014). . Thus, the development of antimicrobial alternatives, has become one of the main complications of medicine and bacteriology when fighting these resistant organisms. In this sense, Lysozyme as a natural enzyme present in many body fluids and produced by the same organism, has been shown to have bactericidal, viricidal and immunostimulatory effects and becomes an important tool for the treatment against multi-sensor bacteria to antibiotics in animals *(Ibrahim, 2002, Vocadlo et al., 2001,* Yabe et al., 1992). .

Objective: To determine the level of in vitro inhibition that the Lyzosime has against multi-resistant bacteria and non-multi-resistant bacteria causing mastitis in cows such as *Staphylococcus aureus*, *Escherichia coli and Pseudomona aeruginosa*, by Minimal Inhibitory Concentratio Test in culture broth and inhibition test in agar plate.by disc difusion test.

Materials and Methods: Diffeent strains of multi-resistant and non-multiresistant bacteria causing mastitis in cows was obtained from subclinical mastiis cases and othe veterinary setings in Bogotá, Colombia.

For the test, a strain of Staphylococcus aureus Meticillin resistant previously resistant to infections in animals was identified, identified by specific microbiological methods. The determination of the Meticillin Resistance gene (mecA gene) was performed by PCR techniques (Albino-Tautivá et al., 2017). The strains of Psedomona aeruginosa and Escherichia coli were identified as resistant to multiple antibiotics by bacteriological techniques. (Bernal-Rosas et al., 2015, .

The Minimum Inhibitory Concentration (MIC) was determined as the lowest dilution of Lysozymet in which it inhibited bacterial growth following the recommendations for this procedure (National Clinical Laboratory Standards Committee, C.L.S.I. 2013). Starting from the concentration of 23 mg / ml, serious dilutions of the product from 1/2 to 1/128 (v / v) in sterile tubes are mixed and mixed with equal volume of bacterial culture in Tryticasa Soya broth (1: 1). A positive control tube was prepared only with bacterial inoculum and culture broth and a negative control tube only with culture broth without inoculum or product. The cultures were kept in incubation at 37 C for 24 hours. (Andrews, 2001). Inhibition test by disc diffusion test was carried out with the same bacterial cultures on Tripticasa Soya agar plates and they will be placed on discs impregnated with the aforementioned products to determine

the level of bacterial inhibition (Brown and Kothari, 1975).

Results: It was found that Lysozyme was able to produce an inhibition against the three multi-resistant bacteria *Staphylococcus. aureus*, *Pseudomona aeruginosa* and *Escherichia. coli* at the concentration between 2.3 to 4.6 mg/ml. Inhibition results were seen at 24 hours of incubation, but were much more evident in the long term (72 hours). The inhibition was also observed in agar plate by disc diffusion test plaque test with multi-resistant bacteria (S. aureus, Pseudomona and E. coli), by observation of inhibitory zone around the disc impregned with Lysozyme with the before mentioned concentration. The same results were observed with non-multiresistant bacteria studied.

Conclusions: It is shown by this preliminary test that Lysozyme is capable of inhibiting both multiresistant and non-multiresistant bacteria causing mastitis in cows, which provides preliminary data that are incorporated into lysozyme within the tools useful in the fight against antimicrobial resistance and pathogens causing mastitis in cows..

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Keywords: Lysozyme, mastitis, multi-drug, resistant, bacteria.

AH-P57

Herbal formulations containing extract of the jucá (*Libidibia ferrea)* for wound healing in cattle

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Objectives: We evaluate the effectiveness of formulations containing the ethanolic extract of jucá (*Libidibia ferrea*) in different concentrations and two different herbal formulations for wound healing in cattle using a full-thickness skin excisional model.

Material and Method: Chemical analysis of the Jucá targeted the total phenolic compounds using the Folin-Ciocalteu method and gallic acid as standard. To evaluate the variation in the total phenolic compounds concentration in the Jucá, we run several analyses using samples from five distinct sites, different years, and different pod coloration (light or dark).

Before the study in cattle, we tested the Jucá ethanolic extract toxicity using the Acute Oral Toxicity (OCDE-423/2001 protocol) and the Acute Dermal Toxicity assay (OECD Guide 402). In both assays, *Wistar* rats were used with a dose of 2000 mg/kg body weight (BW) the ethanolic extract of Jucá was used orally or dermally. Jucá extract was diluted in tween 80 at 1% with saline. For both toxicity assays, the rats were observed individually at least once every 30 minutes in the first four hours, hourly during the first 24 hours, and daily thereafter for a total of 14 days. The observed parameters included topical and systemic possible toxic reactions.

For the cattle study, a total of eight healthy crossbred cows were used, in which five dorsal full-thickness excisional dermal lesions were induced with a 10 mm biopsy punch. Each wound was treated exclusively with one formulation as follows: carbopol-based gel with 5% (G5) and 10% (G10) of jucá extract; murumuru butter with 5% (M5) and 10% (M10) of jucá extract; commercial spray widely used in Brazil for wound healing (gentian violet + organophosphate) as control. Wounds were made at baseline (day 0) and were treated with the different formulations after 1, 2, 4, 7, and 14 days. Lesions were analyzed at baseline and after 2, 4, 7, 14, and 21 days. Macroscopic evaluation of each wound was done by inspection for the presence or absence of exudate, crusts, epithelization, flies, and fly larvae around or inside the wound. The lesions were photographed and evaluated using a digital camera affixed to a stand, to maintain a standardized distance. The images of the wounds were analyzed using the digital morphometry software (ImageJ®) to obtain the diameter of each wound at each timepoint. The wound contraction was calculated according to the formula: CI = 100 × (W0–WI)/W0, where: CI: contraction index (%), W0: initial wound area (D0), WI: final area (D14 or D21).

Results: In the chemical analysis, Jucá pod samples presented a concentration of phenolic compounds varying from 110.2 to 194.1 µg/mg. Pods coloration did not change total phenolic compounds concentration.

Toxicity assays

In the tests of acute oral toxicity and acute dermal toxicity, the animals did not show behavioral changes, morbidity, and mortality during the experimentation period. The animals also showed no changes in fur, skin, eyes, and mucous membranes, no signs of tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma, pain, and suffering. Thus, it was determined that the acute oral toxicity of the ethanolic extract of Jucá pods is greater than 2000 mg/kg BW and classified as category 5. In the acute dermal toxicity test, it was also observed that the animals did not present erythema or edema, thus determining the degree of skin reaction as 0 (zero), without changes. Given that the acute dermal toxicity of the ethanolic extract of Jucá pods is greater than 2000 mg/kg BW and classified as category 5.

Wound healing assay

The mean retraction of dermal lesions at the end of the study (day 21) was 68.9%, 62.0%, 68.8%, and 68.5% for groups G5, G10, M5, and M10, respectively, showing better results than the control group. (51.5%) ($p < 0.05$). Jucá for-

mulations induced a fast crust formation in the dermal wound. The presence of flies or fly larvae was not observed in any wound during the experimental period.

Conclusions: The alcoholic extract of Jucá has a high level of biological safety as it did not produce effects that would characterize oral or dermal toxicity. Jucá showed great potential for wound healing in cattle and can be used in the development of commercially viable herbal formulations.

Keywords: phytopharmaceutical, Astrocaryum murumuru, toxicity, wound retraction, excisional model.



BC-P01

Effect of injectable trace element supplementation on the weight gain of male buffaloes on pasture in Mexico

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Objectives: Buffalo (*Bubalus bubalis*) is the other bovine species for milk production worldwide, and its contribution to global meat production is increasing also. Minerals are essential nutrients that participate in numerous metabolic pathway in reproduction, growth, energy metabolism, or immune response, among others. Therefore mineral supplementation is critical for the productive performance of agriculture animals. However, in pastured buffaloes, the regular supplementation in minerals is challenging. This work aimed to assess the effect of an injection of a multi-mineral pharmaceutical product based on phosphorus, copper, selenium, magnesium and potassium (FOSFOSAN, Virbac Mexico) on the average daily gain (ADG) in grazing buffaloes.

Material & Methods: This study was conducted in a commercial ranch located in the state of Veracruz, Mexico. 99 healthy male buffaloes (12–14 months, 314.4±5.39kg) were enrolled. Animals were fed on pasture with *Brachiaria decumbens* and *Paspalum plicatulum*. One month before the start of the experiment, they were injected vitamin A, D and E, and ivermectin (VIRBAMEC ADE FUERTE, Virbac Mexico) at the recommended dose for cattle. Mineral salt was also provided in free access (Ca 6%, P 2%, NaCl 65%, F 0.02%, Mg 0.3%, S 6%, Zn 0.8%, Cu 0.33%, I 0.02%, Co 0.05%, Se 0.0075%) for all the study period. Animals were split at random to either the control group (C, n = 49) or the experimental group (FOS, n = 50). Buffaloes in the FOS group were dosed (10 mL, i.m.) with a commercial mineral complex on day 0 while group C animals received 10 mL of saline. The test product consists in sodium glycerophosphate (14g/100mL), monosodium phosphate (20.1g), chloride copper (0.4 g), potassium (0.6g), magnesium (2.5g), and sodium selenite (0.24g). The animals were weighed individually on d0 and the last day of the study (d30). The Student's t-test helped to compare the weight gain and ADG; $P<0.05$ was the level of significance.

Results: Both groups presented significant ($P<0.05$) weight gains (18.9±5.5 and 23.8±9.2kg, for C and FOS, respectively). Also, the ADG of group C animals and group FOS animals was 0.63±0.31kg and 0.79±0.25kg, respectively, and they differed significantly ($P<0.01$).

Conclusion: Beef cattle require minerals for optimal development, and buffalo cannot be an exception. In this research study, the weight of the animals increased following the zootechnical standards for buffaloes and the usual performance records of this farm. Outcomes of the present work suggest that mineral supplementation of young buffaloes under Mexican pastoral conditions is necessary. Hence, an injectable supplementation in phosphorus, copper, selenium, magnesium and potassium is a valid option, and have beneficial effects on the daily weight gain.

Keywords: buffalo, trace minerals, ADG.

BC-P02

Effect of an injectable zeranol- and ivermectin-*based* growth promoter on the weight gain of buffaloes on pastures in Mexico

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Objectives: The globally increasing demand for animal-source foods has highlighted the importance of maximising the efficiency of animal production. Growth-promoting medicines have been long-recognised to be economically sound in the beef cattle industry in many countries. Zeranol is a nonsteroidal anabolic growth-promoting compound, commonly used in both cattle and sheep commercially. It was noticed that zeranol had a positive impact on muscle growth during the finishing phase of beef cattle production, and synthetic zeranol was approved for use in livestock in the US in 1969. In the grazing youngstock, numerous arthropod pests and worms can hamper the weight gain. Ivermectin is widely recognised as a reference treatment for the control of parasites in cattle. Little is known about the combined use of ivermectin and zeranol in buffaloes (Bubalus bubalis). Therefore, given the relevance of buffalo farming in the country's livestock scenario, this study was carried out to assess the influence of the two compounds on the average daily gain (ADG) in pastured buffaloes under Mexican conditions.

Material & Methods: This study was conducted in a commercial ranch located in Veracruz, Mexico. Ninety-one healthy crossed-breed (Murrah X Mediterranean) male buffaloes (*Bubalus bubalis*), recently weaned (12–14 m.o.), 317.9 ± 6.9 kg at the time of enrolment, were selected. Animals were all vaccinated against enzootic contagious diseases and dewormed (ivermectin) one month before the start of the study. The study population was split randomly into two groups. Control animals (group C, n=49) were injected with saline, whereas others (group ZER, n=42) were dosed with the test product according to the manufacturer's recommendations (Zeramec, Virbac Mexico - Ivermectin, 10mg/mL, zeranol, 10mg/mL, 1 ml /50kg BW, s.c.). Buffaloes were housed in paddocks and fed on typical local forage *(Brachiaria decumbens* and *Paspalum plicatulum)* and provided with water ad libitum. Management practices were similar for both lots throughout the experimental period (30 days). The animals were weighed individually with an electronic scale before treatment on day 0, and this information helped for calculating the dose volumes to be administered. Animals were also weighed individually on the last day of the study (d30), and the scale automatically calculated the ADG over the study period. Data were processed Graph-

Pad Prism v5 for Student's t-test analysis of the ADG. The average weight gain is expressed as the mean ± sd (kg/d). Differences were regarded as significant when P<0.05.

Results: The daily weight gain of the group C animals was 0.63±0.30 kg/d, while animals in group ZER grew by 0.91±0.29 kg/d. Animals in group ZER had an extra 45.2% ADG (0.29 kg/d), and this difference was highly significant (P<0.001).

Conclusions: ADG is the most commonly used criterion for assessing the growth of young animals. Data gained in this work suggests that zeranol in combination with ivermectin at 1% w/w, have beneficial effects on the growth of weaned buffaloes fed on pasture and treated with ivermectin within the previous weeks. Therefore, combining zeranol to ivermectin could outperform the expected impacts of ivermectin used alone in buffaloes raised in Mexican pastoral conditions.

Keywords: Buffalo,ADG, zeranol, ivermectin.

BC-P03

Genotyping of bovine tuberculosis in buffaloes in Thailand

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Objectives: Bovine tuberculosis (TB) is a significant zoonotic disease. Despite the test and slaughter strategy was established to control and eradicate bovine TB, the disease was never eradicated from Thailand. As few studies has been done for molecular epidemiology of bovine TB in buffaloes, the aim of this present study was to differentiate bovine TB in buffaloes in Thailand by Variable Number of Tandem Repeat (VNTR) genotyping. This will help to understand disease causation, distribution of bovine TB in different geographical areas and disease transmission between animals.

Materials and methods: Two hundred and sixteen clinical tissue samples were collected from 106 single intradermal tuberculin (SIT) test- positive buffaloes derived from different herds among 7 provinces of Thailand, representing different parts of the country. Most samples were derived from at least 2 tissue samples per one animal collection, which were lung, lymph node and liver. In this study, the VNTR genotyping with 22 VNTR primer set were applied for species differentiation of *M. bovis*. Twelve mycobacterial interspersed repetitive units variable number of tandem repeat (MIRU-VNTR) loci (VNTR 0154, 0580, 0802, 0960, 1644, 2059, 2531, 2687, 2996, 3007, 3192 and 4348) were selected to discriminate *M. bovis* strain. The VNTR patterns were calculated for the Hunter-Gaston discriminatory index (HGDI) and the VNTR typing profiles were calculated using the MIRU- VNTR *plus* tool to create the neighbour- joining (NJ) tree.

Results: Twelve of 216 samples (5.56 %) could be cultured from lung specimens but none of other samples could be isolated from neither livers nor lymph nodes. All of isolated specimens were identified as *M. bovis* by phenotypic characteristics and standard biochemical tests. The result of VNTR analysis showed that 15 of 22 VNTR loci revealed allelic variation. However, there is no similarity result of the 12 MIRU-VNTR typing patterns after identifying strain with online databases: MIRU-VNTR *plus*. Eight loci (0577, 0960, 1644, 1955, 2347, 2401, 2531 and 4052) were designated as moderate discriminating as 0.3 ≤ HGDI ≤ 0.6 while the remainder loci presented no diversity as poor discrimination ability with the score less than 0.3. In this study, eight *M.bovis* isolates derived from the same area were most likely to be classified into one big cluster. Seven isolates had identical genetic patterns while the other one had a different copy number in VNTR2531 locus. The next group (3 samples), having a closed relationship to each other. These 3 isolates differed from the first group in VNTR1644 locus and had some differences among loci in VNTR 0960 and 2531. However, they were closer to a group of 8 isolates than the outermost. Only one sample having the longest distance from other isolates, had a different number of repeat units from the others in locus 3192. The new lineage cluster found in this study was separated from ancestors and unrelated to other strains in the database.

Conclusions: Multiple strains of *M. bovis* infection with variable genotypes were found in this study, it is possible that the new lineages found in this study were the new *M. bovis* strains. The result implied that 12 MIRU-VNTR loci set were unsuitable for genotyping *M. bovis* in buffaloes because of insufficient discriminatory power. Thus it is highly recommended to increase the number of MIRU-VNTR loci up to 15 or 24 loci and appropriate loci for local strains should be carefully designed for genetic differentiation with a large sample size from various sources of specimens. Additionally, a combination of other technique e.g. spoligotyping and MIRU- VNTR typing which provided the advantages over MIRU- VNTR alone should be applied for further investigation to determine a reliable molecular epidemiology. Furthermore, the study of strain differentiation and genetic variation in other livestock population e.g. cattle, goat and sheep should be performed in order to compare with the strains in buffaloes.

Keywords: genotyping, bovine tuberculosis, buffaloes.

BC-P04

Seroprevalence and clinical outcomes of *Neospora caninum*, *Toxoplasma gondii* and *Besnoitia besnoiti* infections in water buffaloes (*Bubalus bubalis*)

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Objectives: In recent years, increasing demand for dairy products and consequent maximizing productivity of buffalo herds have raised concerns regarding diseases that lower fertility or cause abortion but the attention has been focused mostly on infectious pathogens instead of parasitic ones. Estimation under field conditions of clinical findings associated with parasitic infections is an interesting challenge in this species where knowledge regarding the health consequences of these diseases as well as their economic impact on the entire dairy food-chain are almost rare. Therefore, the present study aimed to investigate for the first time the seroprevalence of *N. caninum*, *T. gondii* and *B. besnoiti* in water buffaloes and the clinical findings associated to the presence of these protozoa, in order to understand the consequence on water buffalo health of the exposure to the three pathogens.

Material and Methods: One hundred twenty-four water buffaloes, originating from 9 farms located in southern Italy, characterized by poor reproductive performance [≥ 600 Days Open (DO)] and without any macroscopic reproductive disorder were enrolled. For each animal, information regarding age, number of DO, absence or presence and number of abortions, number of embryonic deaths, presence or absence of retained fetal membranes and their total numbers were collected from the farms' databases. Blood sera samples were collected from each animal and analyzed by three different indirect enzyme-linked immunosorbent assays (ELISAs). All the variables were analyzed by standard descriptive statistics. Univariate statistical analysis and logistic regression models have instead verified risk factors and interaction between data observed. As of last, the odds ratio (OR) was used to quantify the association between each clinical parameter and the positive status for the parasites.

ResultsA total of 61/124 (49.2%) water buffaloes were categorized as antibody positive for at least one of the aborting protozoa considered, as follows: 25/124 animals (20.2%) seropositive only for *N. caninum*, 17/124 (13.7%,) only for *T. gondii.* No buffalo showed specific antibodies for *B. besnoiti.* Nineteen of 124 animals (15.3%) resulted seropositive to both protozoa (*T. gondii* and *N. caninum*). The mono-infection with *N. caninum* seems mainly associated with abortion and the presence of retained foetal membranes, while mono-infection with *T. gondii* has been associated with an increase of days open. Moreover, the co-infection by *N. caninum* and *T. gondii* strengthened the abortive effects (OR=7.330) and showed further negative effects on the parameter embryonic death (OR=2.607).

Conclusion: The clinical-parasitological findings of the present investigation demonstrated the direct effects of *N. caninum* and *T. gondii* in the water buffalo as well as tested the absence of antibodies specific to *B. besnoti*. The study carried out on this topic may be considered the starting point to build the essential basic knowledge promoting the awareness of the parasitological infection's relevance in this large ruminant species. Considering the results observed, clinical screening of *N. caninum* and *T. gondii* may be suggested in the routine diagnosis of abortive agents in water buffalo herds characterized by poor fertility performances or pregnancy losses.

Keywords: Neospora caninum, Toxoplasma gondii, Besnoitia besnoiti, water buffaloes, aborting pathogens.

BC-P05

Phytotherapic dry-cow therapy of water buffaloes from an organic farm: study in progress

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Objectives: The overall objective of this study is to verify the efficacy of an intramammary dry-cow therapy using a formulation based on plant extracts (*Melaleuca alternifolia*). As this is a preliminary report, since the final evaluation of the next lactation is missing, the objective was to report the initial results of harmlessness and tissue reaction of the teat, measured by ultrasonography and thermography after application of the dry-cow therapy in dairy water buffaloes.

Material and Methods: This study is an observational longitudinal without control group study carried out at the Instituto Agronômico do Paraná (IAPAR) in Lapa, Paraná, Brazil with twelve Murrah cows beginning at final stage of lactation and selected according to the following inclusion criteria: locomotion scoring ≤ 2(1-5); of any age; without lesions on the teat or udder; with mean somatic cell count <100,000 cells/mL in the last three months of lactation. The phytotherapic, non-antibiotic formulation was administered in a syringe intramammary simulating antibiotic dry-cow therapy. The animals remained in the usual daily routine of the farm, in a pasture-based management. Pasture is composed with Papuã grass (*Brachiaria plantaginea*) and forage peanut (*Arachis pintoi*). Cows in lactation are supplemented with Elephant grass (*Pennisetum purpureum*) and concentrate. Prior to drying-off, the cows underwent clinical evaluation including locomotion scoring, inspection and palpation of the udder and teats, as well as ultrasound (Sonoscape model A5V, linear 9MHz probe) and thermography (FLIR model E50) of the teats. The thermograms were evaluated by the FLIR tools program. The ultrasound measurements (in mm) were: The length of the teat canal (TCL), the diameter of the teat measured on the orthogonal axis at the point of the rosette of Fürstenberg (DRF), teat width (TW), diameters of the teat cistern (DTC), wall of teat (WT), mucosa of teat (MT), was measured in a right angle to the teat canal 10 mm proximal to the rosette of Fürstenberg. Thermography measurements were performed for all udder quarters with pictures captured in lateral and caudal views before drying-off (Day 0). Right and left front quarter skin surface temperature (RFQ and LFQ) and right and left hind quarter skin surface temperature (RHQ and LHQ) were measured. After disinfection of the tip of the teat with alcohol 70%, the test product was introduced through an intramammary applicator (Day 0). On day 1 (D1), all evaluations were repeated. The data (D0-D1) were submitted to statistical analysis using ANOVA procedure, with mean and standard deviation (Statgraphic Centurion v.18, Stat-point Technologies Inc. Warrenton, Virginia, USA) and significance of $p < 0.05$.

Results: With ultrasound measurements TCL (D0: 11.77±0.29mm; D1: 8.87±0.29mm), WT (D0: 9.75±0.30mm; D1: 7.3±0.30mm), MT (D0: 1.88±0.06mm; D1: 1.36±0.06mm), differed for p<0.01. For DTC (D0: 5.72±0.55mm; D1: 13.25±0.55mm) and TW (D0: 25.38±0.52mm; D1: 28.18±0.52mm), there was an increase in measures (p<0.01). DRF (D0: 22.2±0.55mm; D1: 22.33±0.55mm) did not show significant differences (p>0.05). Thermography values, RFQ (29.46±0.75mm; D1: 34.20±0.75mm), LFQ (D0: D0: 30.31±0.69mm; 34.56 ± 0.69mm), RHQ (D0: 30.79±0.76mm; D1: 33.93±0.76mm) and LHQ (D0: 31.56±0.42mm; 33.74±0.42mm) showed an average increase(p< 0.01) of 3.6ºC verified in D1 in all quarter (D0: 30.44±0.55ºC; D1: 34.1±0.55ºC). No differences were found in the clinical evaluation of D1 after application of the product.

Conclusion: Clinical, visual and palpable changes of the teat did not occur using the nonantibiotic dry-cow therapy in buffalos. A slight increase in temperature measured by thermography was observed. This preliminary result allows us to continue research.

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Keywords: teat, dry-cow therapy, water buffalo, phytotherapy.

BC-P06

Blood serum steroid and thyroid hormone concentrations in healthy llamas

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Although alpacas and llamas are closely related, it is unknown if hormone reference intervals are similar. Previously, we determined and presented (WBC 2016, Dublin, Ireland) steroid and thyroid hormone concentrations in 90 alpacas.

Objective: To determine the concentration of adrenal and gonadal steroids, and thyroid hormones in llamas and compare with values in alpacas.

Materials and Methods: Physically healthy male and female llamas from privately owned farms in Tennessee were in this study. Blood samples were collected from the jugular vein of 14 females (age 3-16 years) and 7 males (1 intact, 6 castrated; age 4-13 years) from August to October 2015. Blood serum was stored at -80°C until analyzed for cortisol, progesterone, 17-hydroxyprogesterone, estradiol, androstenedione, testosterone, thyroxine (T4), and triiodothyronine (T3) at The University of Tennessee Diagnostic Endocrinology Service.

Results: Hormone results (min-max) for female and male llamas, respectively, were: cortisol <1.0-2.46 µg/dL and <1.0-1.47 µg/dL; progesterone <0.20-4.18 ng/ml and <0.20 ng/ml; testosterone <15.0 ng/dL and <15.0-138 ng/dL; estradiol <10.0-20.45 pg/ml and, <10.0-14.05 pg/ml; 17-hydroxyprogesterone <0.08-0.20 ng/ml and <0.08-0.49 ng/ml; androstenedione <0.05-0.18 ng/ml and <0.05-0.58 ng/ml; T4 5.06-7.22 µg/dL and 5.77-8.38 µg/dL; T3 77.3-218 ng/dL and 92.6-176 ng/dL. The one pregnant female llama had a progesterone concentration of 4.18 ng/ml, while approximately 85% of the female llamas had a progesterone concentration of <0.20 ng/ml. The one intact male llama had a testosterone value of 138 ng/dL, while all castrated males had values <15.0 ng/dL. Hormone concentrations in several animals were below the lowest standard of each hormone assay (as indicated by the < symbol). There was no significant (P≤0.05) difference in cortisol concentrations between llamas and alpacas, however, there were significant differences in T4 and T3. The only sex steroid concentration that was significantly different between female llamas and female alpacas was androstenedione, with lower mean concentration in llamas.

Conclusion: Sex steroid concentrations were similar in llamas and alpacas while thyroid hormone concentrations differed between the two species. The hormone concentrations determined in this study provide an initial endocrine database for female and male llamas.

Keywords: llamas, steroid, thyroid.

BC-P07

Copper deficiency in buffaloes fed high levels of iron

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Objective: To describe an outbreak of copper deficiency in a buffalo herd caused by excessive iron ingestion.

Material and Methods: A herd of 19 Murrah buffalos of different ages and sex raised on Brachiaria *decumbens* pasture in flooded areas in the state of Sao Paulo, Brazil, were examined. Previously, 11 animals with the same symptoms described below died within four months. A clinical examination was carried out in the most affected buffaloes. Blood and fecal samples were collected to proceed a complete blood counting and biochemical profile by usual methods, and egg counting (epg) by Gordon & Whitlock (1939) technique. From a 2-year-old heifer that succumbed it was collected liver, kidney and small and large intestine samples to measure iron and copper that were also analysed in blood samples, water trough and soil. The mineral levels were determinated by optical specrophotometry by plasma emission (ICP).

Results: The following clinical findings were seen: low body conditiion score, weakness, bottle jaw,periocular alopecia, acromotrichia mostly on the back line, pale mucous membranes, and intermittent diarrhea. A severe microcytic hypochromic anemia, lynphopenia, thrombocytosis was detected in most animals. An intense hypoproteinemia (49 g/L) by hypoalbuminemia (18 g/L) was seen. The buffaloes had very low fecal

egg counts (50 e.p.g). The blood (2.1 µM/L), liver (3.16ppm), kidney (2.04 ppm) and intestines (0.87 ppm) copper levels were minimal, but conversely the iron levels were very high in the blood (40.8 µM/L), liver (1,874 ppm), kidney (241 ppm) and intestines (1,673 ppm). High levels of iron were also detected in the fecal samples (3,478 ppm), water (76 ppm) and soil (118 ppm). All 18 remaining buffaloes were treated with an injectable solution containing copper disodium edetate (1.5 g/100mL) and zinc (5g/100mL) (Suplenut®, Biogenesis Bagó) (1 mL/100 kg body weight) for every two months, for 6- m-time. It was also recommended the drainage of the flood soil that was implemented at once after the diagnosis. The symptons disappeared progressively in the next six months.

Conclusion: It was concluded that the buffaloes had copper deficiency, principally by the low levels in the blood, liver and kidney, caused by high levels of iron detected in the same tissues, as well as in the soil and drinking water. The treatment with a copper injectable solution was effective for recovering of the clinical picture.

Keywords: ruminants, mineral, Murrah.

BC-P08

Augmentation of Fertility in Repeat Breeding Buffaloes with Luteotropic Hormones

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Objectives: In India, the Buffalo (*Bubalus bubalis*) population of 109.9 million animals is contributing to around 49% of total milk production. Buffalo is a seasonal breeder and although native to tropical climate, it exhibits poor reproductive performance due to heat stress. Heat stress can have negative influence over the luteal function contributing to repeat breeding syndrome. The objective of this study was to evaluate the effect of administration of luteotropic hormones on fertility in repeat breeding buffaloes.

Materials and methods: The study was conducted with 32 repeat breeding Murrah buffaloes selected from dairy farms of Aarey colony (Mumbai, India) based on following criteria: Body Condition Score 4, normal body temperature, no apparent physical abnormalities, negative White Side Test for uterine infections. The animals were randomly divided into four groups, 8 buffaloes per group. Group I, II and III were treated with luteotropic hormone on day 7 after AI; Group IV was untreated and served as control. Buffaloes were artificially inseminated (AI) at estrus using AM - PM rule. Group I was treated with GnRH (Receptal®, MSD Animal Health) 20 mcg/animal, Group II and III with human chorionic gonadotropin (hCG) (Chorulon®, MSD Animal Health) 1500 IU/animal and 3000 IU/animal, respectively. Blood samples were collected from animals in all groups before treatment (7d post AI) and on day 14 post AI. The samples were analyzed for serum progesterone concentration using radioimmunoassay (RIA) technique. The physical properties of cervical mucus (consistency; color; Fern-Pattern test (Verma et al., 2014), pH) and serum blood urea nitrogen (BUN), glucose and phosphorus concentrations were also evaluated to establish their correlation with fertility. The statistical significance of any differences between the measured outcomes was analyzed by t-test.

Results: The conception rates of Group I, II and III were 50%, 50% and 62.5%, respectively with overall conception rate of 40.62%. No buffalo from Group IV conceived. The cervical mucus on the day of estrus was clear in all animals; consistency was thin in 15 (46.87%), medium in 6 (18.75%) and thick in 11 (34.37%) buffaloes. The Fern Pattern was typical in 21 (65.63%), atypical in 10 (31.25%) and absent in one (3.12%) buffalo. The average mean pH of cervical mucus was 7.48 ± 0.05. Cervical mucus color, consistency and pH were not significantly different between the buffaloes that conceived and those that did not, however buffaloes with typical fern pattern of cervical mucus tended to have higher conception rate (76.95% vs. 23.08%).

The average mean serum BUN, glucose and phosphorus concentrations on day of estrus were 13.37 ± 0.46 mg/dL, 45.51 ± 0.91 mg/dL and 4.29 ± 0.13 mg/dL, respectively. All values were within the normal range and no significant difference in the individual measurements were detected between the animals that conceived and those that did not.

The mean serum progesterone concentrations on day 7 were 2.53 ± 0.26ng/ml, 2.57±0.39ng/ml, 3.77 ± 0.58ng/ml and 2.54±0.28 ng/ml, in Groups I, II, III and IV respectively, whereas on day 14: 5.37 ± 0.91ng/ml, 5.99±1.06ng/ml, 7.34 ± 0.90ng/ml and 3.96±0.27 ng/ml, respectively. A significant increase in the mean serum progesterone concentration between day 7 and 14 was detected in Group I, II and III (p=0.05). Moreover, in buffaloes that conceived, a significant increase in mean progesterone concentration was detected on day 14 post AI in Group I, II and III (7.40 ± 1.03, 7.97 ± 1.05 and 8.91 ± 0.75 ng/ml, respectively) compared to not conceiving animals (3.35 ± 0.28, 4.00±0.57 and 4.73 ± 0.54 respectively) (p=0.01). The mean serum progesterone concentration on day 14 in Group IV was 3.95 ± 0.26 ng/ml. The highest progesterone concentration day 14 and conception rate were recorded for Group III. The highest fertility rate was recorded after treatment with hCG at the dose of 3000 IU/animal (62.5%), followed by GnRH at the dose of 20 mcg/animal (50%) and hCG at the dose of 1500 IU/animal (50%).

Conclusions: The results of this study indicate that treatment with luteotrophic hormones at 7 days after insemination in repeat breeder buffaloes without uterine infection led to increased progesterone concentrations and improved insemination results. It can therefore be a viable means of improving fertility in this group of animals.

Keywords: Buffalo, fertility improvement, GnRH, hCG.

BC-P09

Poor udder health management and milking procedures expose Mediterranean buffalo milkers to biological risk

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Objectives: Poor udder health management, lack of environmental hygiene, and of specific training courses for milkers represent some of the main factors increasing the biological risk exposure for these dairy workers. Among all the farm staff, milkers have always been the most exposed to the risk of zoonosis, as well as to bacteria that could develop antibiotic resistance. Although this relationship is clearly defined in dairy cows' farms, still rare and incomplete are the information in the Mediterranean buffaloes (MBs) breeding system. Considering the premises, the current study presented a twofold aim: (I) to assess the biological risks to which are daily exposed MBs' milker along the year; (ii) to identify and characterize the methicillin-resistant *Staphylococci spp* isolated.

Materials & methods: All the procedures have been performed in n=4 MBs' farms selected by non-probability convenience sampling from Caserta and Salerno districts (Southern, Italy), during the four seasons of one year. All the farms used an artificially induced seasonal calving herd system (late winter–springtime). Quarters were submitted for a specialistic clinical examination to define their health status and the routine milking procedures have been evaluated. Moreover, samples originating from quarter milk, udder skin surface, milking line, and clusters, personal protective equipment (PPEs, gloves, coats, and boots), and smartphones have been collected along with the milking procedures to perform the following qualitative and quantitative bacteriological investigations: Total Bacterial Count (TBC), isolation of *Escherichia coli* (*E. coli*), *Enterococci*, and *Staphylococci spp* (*Staphylococcus aureus, S. aureus*, included). For the latter, the presence of Oxacillin/Methycillin resistant *Staphylococci spp* strains has been also verified. A seasonal comparison of the results has been finally performed using appropriate parametric and non-parametric tests.

Results: None of the farms adopt a regular udder health monitoring program and none of the milkers received a specific training course. Overall n=600 udder clinical examinations have been performed, as well as n=600 samples both originating from the quarter's milk (n=152/farm) and from the udder's skin, have been carried out. Moreover, n=192 samples of the milking lines (n=12 samples/season/farm), n=400 of the milking clusters surface, and n=96 belonging to the PPEs (n=3 samples/milkers/season/farm) have been obtained. Finally, milkers' smartphone surfaces have been sampled n=32 times (n=1 sample/milkers/season/farm). The 1.3% of the samples were considered contaminated and discharged. The analysis of the milk samples evidenced the highest risk to be exposed to the bacteria considered (and mainly to *S. aureus*) from the late winter-spring seasons onward. The risk arising from buffalo udder, milking clusters, and lines were instead considered rather stable along the entire period of sampling. The PPEs turned out to be a source of contamination for milkers mainly during the spring and summer periods. The analysis for Oxacillin/Methycillin resistance revealed in all the farms enrolled and an overall amount of 37,5% of *Staphylococci spp* strains (belonging to *S. aureus, S. haemolyticus, S. pseudintermedius, S. chromogenes* species) resulting resistant both to methicillin and oxacillin.

Conclusions: The study assesses the presence and type of biological risk to which MBs'milkers might be exposed during their annual work, for the first time. Moreover, the investigation demonstrates that the potential transfer of pathogenic bacteria to humans would have a better chance to occur at milk resumption time (from late winter-springtime onward) when the number of animals to be milked is greater and the activity in the milking parlour is more challenging. At the same time, the findings seem to point out that the risk can be worsened by a significant presence of Oxacillin/Methycillin resistant *Staphylococci spp*. strains, probably resulting from an irrational use of antibiotics. The lack of a regular udder health monitoring program and of specific training courses for the MBs milkers might contribute to explaining the results and increasing the biological risk at the same time.

Keywords: buffalo udder health, milking procedures, milkers, mastitis, biological risk.

BC-P10

Some considerations on ultrasonographic examination of pregnancy through the abdominal cavity in small and medium-sized ruminants

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Objectives: Per rectum palpation for pregnancy using ultrasound equipment is a widely used diagnostic method in cows and horses. It is less popular in small and medium-sized ruminants where it is not routinely performed. Often, due to the size of the ruminants examined, transrectal examination is not possible. Nowadays, ultrasound machines for the diagnosis of pregnancy through the abdominal wall are available on the market. The aim of the study was to evaluate the IMW DUO SCAN PLUS ultrasound scanner in the diagnosis of transabdominal pregnancy in unique species of

sheep, goats, alpacas and llamas.

Material and methods: The DUO SCAN PLUS mobile ultrasound device was used in the study. The device worked by generating a Wi-Fi signal to display the ultrasound on a mobile phone. Smartphone models such as IPhone XI and XIAOMI Redmi Note 5 were used to compare image quality and resolution from two different mobile phones. The research was carried out in two farms where the animals were kept in an extensive system. In total, 65 females were tested, including: 1 Llama (Suri), 37 alpacas (Huacaya and Suri), 14 goats (Anglo-Nubian, Minature, Cashmere, non-breed) and 16 sheep (Babydoll Southdown, Barbados, Swiniarka, Polish Heath and Valais blacknose. Animals were properly caught and tamed to facilitate the study. For medium-sized ruminants, the probe was placed directly over the milk strip and maneuvered caudally to visualize the entire horn cavity. In small ruminants the probe was directed into the inguinal fossa and visualised the horn cavity with movement towards the pubic symphysis. A large amount of fluid with the body profile of the foetus in the uterine horn, as well as its movements and heartbeat, were assumed to be the criteria for confirming pregnancy.

Results: Of the 65 females tested, 46 (70.7%) were pregnant. The diagnosis itself took a few seconds, and in case of doubt, the probe was placed on the other side of the animal. The device allowed to determine the pregnancy status with 100% certainty, however, it is important to note that the examined goats and sheep were about 60 days pregnant, while llamas and alpacas were 100-150 days pregnant, which in both cases is quite advanced. Females often showed signs of nervousness in the form of sudden movements, kicking, attempts to escape, increased vocalisation or spitting in the case of alpacas or llamas.

Conclusions: Examination for pregnancy using the DUO SCAN PLUS ultrasound scanner of small or medium-sized ruminants through the abdominal cavity is a very good diagnostic tool. This should become a routine procedure not only on large farms but also on home farms, which can significantly contribute to improving herd profitability and reproductive management.

Keywords: Ultrasonography, small ruminants, alpacas, pregnancy diagnosis.

BC-P11

Preliminary serological evidence for Schmallenberg virus in Mediterranean Buffalo in Italy

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Objectives: Schmallenberg virus (SBV) is an emerging RNA virus identified in Germany in 2011 in cattle showing diarrhoea, decreased milk production and fever. The virus is also responsible for causing congenital defects in ruminants (AHS: arthrogryposis hydranencephaly syndrome) due to the capability of the virus to infect the placenta and the umbilical cord reaching the central nervous system (CNS) of the foetuses. SBV belongs to the family *Bunyaviridae*, genus Orthobunyavirus, and is transmitted through the bite of *Culicoides* spp. SBV has a wide host range including cattle, goats, sheep, deer and buffalo (Anatolia water buffalo), and has spread rapidly throughout Europe, Africa and western Asia.The first case of SBV in Italy was reported in February 2012 in a goat foetus with dystocia and following the first notification, the Italian Ministry of Health decided to enforce surveillance in ruminant farms, using molecular and serological approaches (Nota DGSAF III/6764 04.04.2012). To date, there are no comprehensive studies on the seroprevalence of SBV in Mediterranean buffalo (*Bubalus bubalis*). Buffalo breeding has a prominent role in the Italian farming system with the population mostly concentrated in the Campania region where around the 50% of the Italian buffalo farms are located. The aim of our study was to investigate the presence of SBV antibodies in buffalo farms in the province of Salerno, Italy.

Materials and Methods: SBV antibodies were detected by c-ELISA in a buffalo bull entering a genetic centre during a routine investigation. The confirmation by serum neutralisation test led to the serological screening of farms in a 4 km radius from the positive case in a representative sample of the heards. A total of 545 blood samples were collected between 2021 and 2022 from 11 farms and tested for SBV-specific antibodies.

A commercial competitive enzyme-linked immunosorbent assay (cELISA) kit for multispecies was used to detect SBV antibodies in sera according to the manufacturer's instructions (ID.vet Innovative Diagnostics, Montpellier, France). Samples were considered positive when they matched a competitive percentage (S/N% = [optical density value optical density value at 450 nm for the sample (S)/the mean optical density value optical density value at 450 nm for the negative controls (N)] × 100) ≤ 40, doubtful if 40 > S/N% ≤ 50, and negative when S/N% > 50.

Results: SBV antibodies were detected in all farms sampled. The territorial prevalence was 67.89% with different levels of intra-farms positivity (min. 11.1% max. 85.1%). This is the first report of antibodies against SBV in buffaloes in Campania region even though previous studies have reported seroprevalence in buffalo herds ranging from 1.5% to 19% in Turkey and Chine respectively.

To date, no measures are in place in Italy to actively detect SBV circulation in Italian herds and passive surveillance relies on clinical symptoms on both adult and newborn ruminants. However, the number of herds with SBV confirmed AHS cases compared to the level of infection indicated by seroprevalence studies, suggest that the frequency of clinical disease is low. SBV induces malformed calves only in a very limited number of cases, as demonstrated by experimental infection studies on pregnant cows and ewes. Therefore, the presence of SBV

in the Italian population of ruminants, including buffaloes, is most likely underestimated.

It would be interesting to carry out further field investigations during the vector season to better characterize SBV impact on buffaloes farming.

Conclusion: The results of the current study provided evidence that SBV has been circulating in Campania region in the recent past. There is lack of data on SBV infection, pathogenesis and immune response evoked by the infection in buffaloes. Further studies to fill such gaps should be encouraged due to the economic role of buffaloes breeding in Campania region.

Keywords: Schmallenberg virus, Mediterranean Buffalo, serological tests.

BC-P12

Percutaneous transabdominal ultrasonography of the liver in healthy llamas and alpacas - preliminary results

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Objectives: Hepatic ultrasonography represents an important additional diagnostic tool in camelids with hepatic disorders because clinical symptoms and even changes of blood parameters in these patients are often unspecific. Ultrasonography allows examination of the liver parenchyma and the vessels such as *Vena portae* and *Vena cava caudalis*, and determination of location, size, and shape. Unfortunately, there is a lack of information on scanning technique and the normal sonographic appearance of the liver parenchyma and the vascular structures in South American camelids. Therefor the aim of this study was to describe normal ultrasonographic dimensions of the liver and associated vascular structures in South American camelids in order to establish normal reference values.

Material and Methods: For ultrasonographic examination of the liver 27 physically healthy adult non-sedated llamas and alpacas were scanned using a 4.5 MHz linear transducer (MyLabOneVet-Esaote) in the intercostal spaces (ICS) 6 to 11 on the right side of the abdomen. The hair in the "region of interest" was only parted with the help of a comb and alcohol was used as contact medium. In each ICS, the distance from the most dorsal (dorsal measuring point) and the most ventral (ventral measuring point) sonographically displayed aspect of the liver to the *Processus spinosus* of the vertebral column was measured by means of a measuring tape.

Results: The normal sonographic appearance of the liver parenchyma displayed an echoic, homogeneous tissue pattern with anechoic vessels inside. In all animals the liver could be imaged at the 9th to the 11th ICS. In three animals the liver was also detectable caudal to the last (12th) rib. The distance of the dorsal and ventral measuring points was the smallest in the 11th ICS and continually increased to the largest distance in the 6th ICS. The *Vena cava caudalis* could be seen in the 11th to the 8th ICS in alpacas and in the 11th and 10th ICS in llamas. In most of the animals the cross section of the *Vena cava caudalis* showed a circular to oval shape. The *Vena portae* could be seen in the 11th to the 9th ICS in all 27 animals. The cross section of the *Vena portae* revealed a circular to oval shape. Star-shaped ramifications of the *Vena portae* could only be seen in four cases.

Conclusions: Ultrasonography is a safe non-invasive technique for the examination of the liver. The ultrasonographic findings presented can serve as reference values for the examination of llamas and alpacas with suspected hepatic disorders.

Keywords: alpacas, llamas, transabdominal ultrasonography, liver.

BC-P17

Productive performance of feedlot buffaloes fed with high-grain diets

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Objective: We aimed to evaluate the productive performance of feedlot buffaloes offered two high-grain diets, with 70% and 100% of concentrate.

Materials and methods: The study was conducted at Santarém, Brazil. Twelve non-castrated health crossbreed buffaloes were divided into two groups (n = 6): the conventional diet group (C), which received 70% concentrate [90% ground corn + 10% protein and mineral supplement (Engordin 10®, Agrocria Animal Nutrition, Brazil)] and 30% corn silage on dry matter basis; and the whole-grain group (WG), which received a diet with 100% concentrate [85% whole-grain corn and 15% protein and mineral supplement (Engordin 38®, Agrocria Animal Nutrition, Brazil)]. The diets were isoproteic and the ingredients were mixed and offered as a complete meal twice a day (07:00 and 17:00). The animals were housed in individual stalls with feeder and water supply. Feed remains were weighed daily and each animal received an amount of feed enough to provide a 5% surplus. The experimental design was in randomized blocks and the animals were blocked by weight. The study lasts 80 days, with 16 days of diet adaptation period and 64 days of evaluations. The body weight (BW) was determined at the beginning and the end of the study after 14 h fasting and the average daily weight gain (ADG) was calculated. The animals were slaughtered and the hot carcass

weight and carcass yield were obtained. Data were analyzed using the PROC MIXED of the SAS (Statistical Analysis System) with 5% significance.

Results: Table 1 presents the comparison of the results between C and WG buffalo. There were no differences between the initial body weight, final body weight, ADG, and hot carcass weight. However, a difference was observed between the diets for the carcass yield, with higher results for the group fed with the whole-grain diet.

Variables	70% concentrate	Whole-grain diet	SEM	P
BWi (Kg)	301	318	26,1	0,66
BWf (Kg)	394	376	24,7	0,63
ADW (kg/day)	1.2	0.80	0,194	0,15
Hot carcass weight (kg)	181	186	12,6	0,78
Carcass yield (%)	45.7	49.2	0,44	<0,01
SEM: Standard error of the mean.				

Conclusion: This pilot study has a limited number of animals, however, it has shown that the use of a diet composed of 100% concentrate is suitable for feedlot buffalo and that such diet results in higher carcass yield when compared to a conventional feedlot diet. Further studies with a larger number of animals are required to determine the economic advantages of this diet in the buffalo production system.

Keywords: Bubaline, Amazon, feedlot, whole-grain, carcass yield.

BC-P18

Metabolic profile of buffaloes fed with high-grain diets

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Objectives: We aimed to evaluate to metabolic profile of buffaloes fed with two different high-grain diets, with 70% and 100% of concentrate.

Materials and methods: The study was conducted at Santarém, Amazon, Brazil. Twelve non-castrated health crossbreed buffalo aged 14-16 months and with 322 ± 61 kg of body weight were divided into two groups (n = 6): the conventional diet group (C), which received 70% concentrate [90% ground corn + 10% protein and mineral supplement (Engordin 10®, Agrocria Animal Nutrition, Brazil)] and 30% corn silage on dry matter basis; and the whole-grain group (WG), which received a diet with 100% concentrate diet [85% whole-grain corn and 15% protein and mineral supplement (Engordin 38®, Agrocria Animal Nutrition, Brazil)]. The diets were isoproteic and the ingredients were mixed and offered as a complete meal twice a day (07:00 and 17:00). The animals were housed in individual stalls with feeder and water supply. Feed remains were weighed daily and each animal received an amount of feed enough to provide a 5% surplus. The experimental design was in randomized blocks and the animals were blocked by weight. The study lasts 80 days, with 16 days of diet adaptation period and 64 days of evaluations. The body weight was determined weekly for diet correction. Blood samples were obtained in the morning, before feeding, at the following days (D): D0 (baseline, after the adaptation period), D7, D21, D35, D49, D64. Serum and plasma samples were used for biochemical analyses, which were performed through an automated biochemical analyzer (Labmax 240, Labtest Diagnóstica S.A., Lagoa Santa, MG, Brazil). Serum was used to determine the concentration of (TP), albumin, urea, creatinine, cholesterol (COL), triglycerides (TRI), total bilirubin (TB), direct bilirubin (DB), calcium (Ca), phosphorus (P) and magnesium (Mg), as well as aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and creatine kinase (CK) enzyme activities. Plasma samples were used to evaluate glucose, lactate, β-hydroxybutyrate (BHB) and non-stratified fatty acid (NEFA). Data were analyzed using the PROC MIXED repeated measures of the SAS with 5% significance. Statistical analysis considered the effects of time, treatment and interaction.

Results: Only two variables were different among WG and C animals, urea and creatinine. Urea concentration was higher ($p < 0.001$) in C (17.6 ± 0.9 SEM mmol/L) compared with WG (11.4 ± 0.9 mmol/L) animals, probably due to the greater amount of non-fibrous carbohydrates in the whole-grain diet that resulted in greater microbial utilization of nitrogen in the rumen. Conversely, serum creatinine was higher ($p < 0.001$) in WG (164.1 ± 6.9 µmol/L) than in C (137.5 ± 6.3 µmol/L). Both groups had an increase in glucose (2.8 ± 0.2 mmol/L at D0 to 4.4 ± 0.1 at D7) and a sharp decrease in lactate (5.0 ± 0.4 at D0 to 2.2 ± 0.4 at D7 mmol/L) and a mild decrease in NEFA (0.25 ± 0.06 at D0 to 0.18 ± 0.001 at D7 mmol/L) concentrations starting as earlier as D7 and remained with low values until the end of the study. TP, BHB, TB, DB, P, Ca, and Mg concentrations, and GGT, AST and CK activities did not present any difference within the factors analyzed (diet and time). The serum albumin concentration had a slight increase during the experiment. The COL and TRI concentrations were affected by time but no pattern could be observed. Regarding energy metabolism variables, glucose increased during the study ($p = 0.0022$) but lactate ($p<0.001$), and NEFA (0.0008) decreased, which corresponds to a pattern of high energy availability due to the high grain diets.

Conclusions: Buffalo fed whole-grain diet had minimal metabolic alteration compared with the conventional feedlot diet used in Brazil (70% concentrate). Thus, this diet can be an option for this species.

Keywords: Bubaline, Amazon, feedlot, whole-grain, energy metabolism.

BT-P01

Embryo production and fertility of dairy heifers after superovulation

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Objectives: The Multiple Ovulation and Embryo Transfer (MOET) technique for bovine genetic multiplication has been used commercially since the 1960s. In the last years, this technique has gained a great differential, with the possibility of selection of donors by genomic evaluation. However, the use of these technologies is far below the possibilities, since some farmers and technicians believe in information, usually without proper scientific evidence, that the MOET technique can affect the fertility of heifers. Thus, the objective of this study was to evaluate embryo production in holstein heifers in subtropical climate and whether the superovulation and uterine flushing is harmful to the future fertility of these females.

Materials and methods: It were used Holstein heifers (n=1783) between 312 and 387 days old and weighing between 273 and 307kg two commercial dairy farm, located in the Southern region of Minas Gerais state, Brazil. This location had an altitude of 877 and 962 meters and a CW climate, according to Köppen classification. These animals were divided among donors (446) or non-donors (G1=1327) according to the result of genomic evaluation (Clarifide™–Zoetis). The donors were always superovulated a decreasing eight-dose protocol with 180mg of Folltropin™ (Vetoquinol-Brazil) and were inseminated twice 12h apart. Of these, 337 were submitted to MOET once (G2) and 109 twice (G3). The second MOET was made only in heifers who produced more than eight viable embryos in the 1st MOET. In those super ovulated twice, the interval between MOET was 45-50 days. All uterine flushings were made by the same technician seven days after the first artificial inseminations (AI). Non-donor females (G1) were inseminated according to farm procedures when that reached 320kg of body weight. The donors (G2 and G3) were inseminated, according the same procedures, from 15 days after the last flushing and when they reached the same weight. The age at 1st insemination, age at conception and age at birth were evaluated by anova and compared by Tukey's test. The number of artificial inseminations per conception was compared by chi square, considering significance at 5% probability (SAS Software, v9.4).

Results: The first MOET in 446 donors provided 6.8±4.6 total embryos and 3.9±2.8 viable embryos by donor. The second MOET, selecting the best donors, provided 12.6±5.3 total embryos and 8.5±3.8 viable embryos by donor. The age at 1st AI was 381.5 ± 3.6^{b}; 387.4 ± 15.8^{ab} and 412 ± 19.7^{a} days, age at conception 438.3 ± 29.8^{b}; 449.1 ± 32.3^{ab} and 470 ± 31.8^{a} days and age at partum 720.3 ± 33.8^{b}; 730.1 ± 35.8^{ab} and 749 ± 34.3^{a} ($P<0.05$) for G1, G2 and G3, respectively. The number of AI per conception was 2.3±0.8; 2.4±0.6 and 2.3±0.7 ($P>0.05$) for G1, G2 and G3 respectively.

Conclusions: It is concluded that one or two MOET processes, prior to the beginning of the AI phase, does not interfere with the heifer fertility. A MOET before the start of the breeding phase does not interfere with age at the 1st AI, conception and 1st calving in dairy heifers. In addition, two MOETs delays conception by 32 days. On the other hand, heifers submitted to two MOET produced 16.4±5.3 viable embryos before the 1st conception. With the use of modern genomic selection techniques, the use of the MOET can be highly interesting for genetic multiplication of superior females and accelerate the genetic improvement of herds without have a negative effect on donor fertility.

Acknowledgment: Biotran, Unifenas, Fapemig, CNPq, CAPES and Vetoquinol.

Keywords: Biothecnology, embryo transfer, genomic selection.

BT-P02

Analysis of new bovine sex selection technology through IVF test

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Objective: We have developed a new monoclonal antibody protein to separate the bovine X- and Y- sperm. Using this protein, we make new bovine sexing product, WholeMom-Bovine sperm sexing kit (WM-B) (NuriScience Inc. Seoul, South Korea). In general, WM-B protein has Y- sperm-specific binding capacity and induces the agglutination of the bull Y-sperm spermatozoa.

In this study, we investigate the developmental efficiency and sex ratios of IVF embryos produced with conventional frozen bovine semen straw in comparison with WM-B treated frozen semen straw or frozen WM-B treated fresh semen.

Material & Methods: For this IVF study, we used 5 populations of spermatozoa: 1) conventional frozen-thawed bull straws 2) Y-sperm sexed before freeze-thawing 3) Y-sperm sexed after freeze-thawing 4) X-sperm sexed before freeze-thawing 5) X-sperm sexed after freeze-thawing. To obtain sexed spermatozoa, fresh raw semen and frozen-thawed semen (one vial per one sperm straw) were treated with WM-B in a water bath at 38.5 °C. WholeMom protein binds specifically the plasma membrane of the sperm head. After 25 min we verified the presence of head to head sperm agglutination at the microscope (100X magnification). Agglutinates settled down in the bottom of the vial, so the pellet was collected and mixed with 100 µL heparin and termed as Y-sperm. The supernatant of the incubated sample was washed in 10 mL of PBS and centrifuged. This second sperm pellet was termed as X-sperm.

All sperm samples were diluted in the capacitation media with 100 µL heparin and kept at 38.5 °C in a humidified atmosphere of 5% CO2 for 15 min. After maturation of oocytes, the IVM medium was removed and the COCs were denud-

ed by adding 500 µL of IVF media, which is Tyrode's lactate solution (TL-Fert) supplemented with 6 µg/mL bovine serum albumin (BSA), 22 µg/mL sodium pyruvate, 100 IU/mL penicillin and 100 µg/mL streptomycin sulphate. In case of Y-sperm, the matured oocytes were inseminated with 100 µL capacitated motile spermatozoa, whereas, for the X-sperm, the matured oocytes were inseminated with 200 µL capacitated motile sperm and kept at 38.5 °C in a humidified atmosphere of 5% CO2 for 18–20 h. After fertilization, cumulus cells were removed and the denuded. Presumptive zygotes were transferred into a culture in media IVC1 (Day 1 to Day 3) and IVC2 (Day 4 to Day 7) [Day 0 = day of IVF]. IVF Blastocyst sexing was performed by PCR analysis. ANOVA analysis was performed using SPSS. In all cases, 95% confidence levels were considered significant at P < 0.05.

Results:

Table 1. Developmental rate of bovine oocytes after IVF using WM-B treated conventional frozen semen

Treatment	No. of IVF oocytes	No (%). of cleaved embryos	No. of Blastocysts	The ratio of blastocysts in cleaved embryos
Control	813	610 (75.0)[a]	224	27.4%[a]
WM-B Sexed after freeze-thawing	1,737	1,156 (66.9) [b]	404	26.1%[a]

Different superscript letter a, b in the same column indicate a significant difference (P < 0.05).

Table 2. Developmental rate of bovine oocytes after IVF using WM-B treated sexed frozen semen.

Treatment	No. of IVF oocytes	No (%). of cleaved embryos	No. of Blastocysts	The ratio of blastocysts in cleaved embryos
Control	573	429 (75.1.%)[a]	153	35.8%[a]
WM-B Sexed before freeze-thawing	582	387 (66.4%)[b]	135	34.8%[a]

Different superscript letter a, b in the same column indicate a significant difference (P < 0.05).

Table 3. Gender ratio of bovine blastocysts after IVF using WM-B treated sperm.

Treatment	No. of IVF blastocysts	Sex of IVF blastocysts (%)	
Control	245	Male	131 (53.5)
		Female	114(46.5)
WM-B Sexed after freeze-thawing	201	Male	28 (14.6)
		Female	173 (85.4)
WM-B Sexed before freeze-thawing	97	Male	4 (4.2)
		Female	93 (95.8)

Different superscript letter a, b in the same column indicate a significant difference (P < 0.05).

Conclusions: According to this IVF test study, WM-B kit shows no significant difference in the percentage of resumptive zygotes between the control group and the WM-B treated conventional frozen semen, and also WM-B treated sexed frozen semen. The female embryo production ratio is much higher in WM-B treated sperm than control sperm. In conclusion, WM-B is an efficient and simple method without any specific facility or techniques.

Keywords: bovine, bull, semen, sexing.

BT-P03

Design of an in-straw devitrification procedure to facilitate the transfer of IVPbovine embryos

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Objective: The aim of this study was to develop a simple in-straw dilution and direct transfer procedure to be used in field conditions for the transfer of IVP bovine embryos.

Materials and methods: Two vitrification methods the one described by Vatja et al (1998) and the BO- VitriCool Kit (marketed by IVF-Bioscience®) and two different supports (Open Pulled Straw (OPS) and glass capillary tubes of 10 µl) were evaluated.

In vitro produced day-7 or day-8 expanded blastocysts (105) were selected and randomly assigned to one of four Groups: (26) Vatja-OPS, (24) Vatja-tubes, (24) IVF-Bioscience®-OPS and (31) IVF-Bioscience®-tubes. All vitrification and devitrification solutions were prepared in TCM199-Hepes, supplemented with 20% of fetal calf serum and warmed at 38.5ºC.

Half of the embryos in each group were used as a controls and were devitrified following the respective procedure recommended by Vatja et al (1998) or by IVF-Bioscience®. The other half of embryos were devitrified in two steps inside a straw loaded with two different media (0.5 M and 0 M sucrose) so that an equal volume of the two media were separated by an air bubble. The content of the straw was finally emptied in a plate containing TL-HEPES, the embryos were located, washed, and subsequently cultured for 48 h under standard conditions. The parameters evaluated were: recovery rate, expansion at 6 and 24 h and hatching rate at 24 and 48 h of culture.

Results: Results indicated that warming in straws increased the risk of embryo loss. When using the Vatja vitrification procedure, the in-straw devitrification method produced a slight reduction of 24 h-blastocyst expansion compared with the control group (54,3 vs 63,2%), however, when using the IVF-Bioscience® method embryo survival did not differ (45,0 vs 42,9%).

Regardless of the vitrification procedure used, the 24 h blastocyst expansion rates obtained using OPS were significantly higher than those achieved using glass tubes (62,2 vs 34,5%).

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Keywords: Vitrification, OPS.

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CW-P01

Progress in provision of pain relief for livestock husbandry procedures

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Objectives: Review the progress in provision of pain relief for farmed livestock, particularly cattle. Pain in livestock most commonly occurs during aversive husbandry procedures used routinely on farms to assist livestock management and improve animal welfare. Ethical, economic and marketing assurance concerns of consumers, producers and other livestock industry stakeholders, has demanded that routine pain management for livestock be provided for such procedures. Research has now established the efficacy of new approaches to achieve this and the registration of new products, particularly for use by farmers, has led to widespread changes in farm practices in Australia. Use of a topical anaesthesia 'spray-on' wound formulation, with or without use of a non-steroidal anti-inflammatory drug (NSAID) for additional pain relief, can now be achieved for a range of applications, including disbudding, dehorning, castration, calving and lameness. Other applications of these products, new forms of delivery, and even new products, are envisaged.

Materials and Methods: An extensive series of published studies on pain relief in Australian livestock were reviewed and compared with studies on provision of livestock pain relief from other countries. Reflections on the change management initiated in Australian agriculture by these developments and their potential impact for global livestock food production are discussed.

Results: Following an animal welfare activist-led campaign against the Australian sheep industry tolerance of the mulesing operation in lambs that reduces life-time risk of sheep blowfly strike, a wound dressing formulation was introduced in 2005 with registration in 2012, enabling farmer-applied spray-on delivery of topical anaesthesia (TA; Tri-Solfen®, Animal Ethics, Australia). This product contains the local anaesthetics lignocaine and bupivacaine, with adrenaline and cetrimide in a gel matrix. The therapy effectively manages the pain of mulesing, hastens healing of wounds, and surprisingly, extended positive welfare outcomes for periods well beyond the duration of action of the TA's (24hrs). Studies also confirmed efficacy of the TA wound dressing for castration and tail docking of lambs, castration, disbudding and dehorning of young calves, plus general on-farm wound and disease management treatments, particularly shearing cuts in sheep and lameness in cattle. Efficacy of TA for beef cattle dehorning by amputation achieved conflicting results and TA for speying female cattle by the Willis dropped ovary technique has proved initially unsuccessful. Studies also examined amelioration of wound sensitisation by use of various NSAID's, although when used for wounds these products have less visible clinical impact than the blockage of nociception by the TA wound dressing. Delivery of meloxicam by oral administration was also developed in Australia (Buccalgesic®, Troy laboratories, Australia) to enable administration of NSAID's by producers. NSAID's have now been shown to be more efficacious by oral or injected administration when combined with TA pain relief (Tri-Solfen®) for castration, and when these therapies are combined for castration and dehorning, improving productivity has been demonstrated.

Conclusions: Farmer-applied spray-on TA (Tri-Solfen®) for mulesing in sheep rapidly achieved widespread adoption with over 120 million lambs now treated and increasing use of the product on beef farms for castration and dehorning. This indicates that provision of suitable product(s) can rapidly change the attitudes of farmers to livestock welfare. There is now widespread recognition in Australia that if aversive husbandry procedures used commonly to facilitate management of farmed livestock populations are to continue, then the pain inflicted on animals during these procedures should be ameliorated. This 'pain management revolution' is currently empowering producers and their advisors on farms to reduce suffering in animals, enhancing animal welfare and importantly, addressing concerns raised in activist-led campaigns advocating that livestock production as inherently cruel. Pain relief in livestock is now an important risk management intervention for the global cattle and sheep industries and this 'livestock pain welfare revolution' is now achieving international recognition and offering research support for similar wound pain management studies in humans.

Keywords: cattle, welfare, pain, management.

CW-P02

Study on alternative methods for disbudding of calves

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Objectives: Thermal disbudding is currently the most frequently applied method to destroy horn buds and avoid horn growth. The method causes substantial pain and stress to the animals, further the wounds take several weeks for healing. Therefore animal welfare concerns have been raised and research has been done in search of alternative methods. The objectives of the present study were to assess the suitability and efficacy of the two substances clove oil and isoeugenol for disbudding of calves and to assess stress and pain using that alternative method.

Materials and methods: Clove oil and its synthetic derivate isoeugenol have analgesic and cytotoxic properties. The cytotoxic effect prevents the growth of the connective tissue between horn bud and frontal bone and henceforth the horn growth when applied beneath the horn bud. The analgesic effect prevents pain applying that method.

Seventy two Austrian Fleckvieh (Simmental) male and female calves have been included in the study. Dosages of 0.5, 1.0 and 1.5 ml of both substances were injected in the connective tissue between horn bud and frontal bone in calves within the first 5 days of their life. 10 calves served as control group having an isotonic saline injection and 10 calves were

disbudded by thermal destruction of the horn bud.

During and after the disbudding procedure saliva samples were taken to assess stress reaction by cortisol concentrations. Disbudding success was monthly assessed visually and horn growth was repeatedly measured up to an age of 6 months. In 10 calves CT images and biopsy samples for histology were taken to assess cytolysis of the connective tissue and possible damage to the frontal bone.

Results: The calves of the control group had an undisturbed horn growth; horns were 8 to 9 cm long at an age of 6 month. Although thermal disbudding was successful and performed following the manufactures instruction of the hot iron (budex) CT images showed damage to the frontal bone in all calves. Wound healing took 6 to 8 weeks.

A dosage of 1.5 ml isoeugenol or clove oil has been identified to suppress horn growth sufficiently without differences between the two tested substances in efficacy. However, some calves showed temporary swellings around the injection site after clove oil but not after isoeugenol injection.

Further it has been demonstrated that the injection of isoeugenol and clove oil results in a minor cortisol peak not significantly different to the injection of isotonic saline. Although the number of the calves were not high enough to find a significant difference it seems that the success and easiness of the injection is better in new-born calves in comparison to older calves.

Conclusions: Disbudding calves using clove oil or isoeugenol is a promising method to reduce pain and stress which is unavoidable during thermal disbudding. Currently a field study is conducted using only isoeugenol to assess the efficacy of the method under field conditions.

Keywords: calves, disbudding, animal welfare.

CW-P03

The occurrence of reflexes in cattle following stunning with a captive bolt

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Objectives: Cattle are most commonly stunned with a captive-bolt gun. Efficient captive-bolt stunning results in immediate collapse, muscle spasms of the dorsum and legs, immediate and sustained absence of rhythmic breathing, a blank stare, absence of a righting reflex, and absence of pain responses and vocalisation. When stunning procedures fail, the animals will suffer.

The aim of this study was to determine the frequency of the occurrence of reflexes after the stunning of animals and differences in their occurrence in relation to the placement of the stunning shot.

Materials and Methods: The study was carried out during routine stunning and slaughter at the two largest beef abattoirs in the Czech Republic. All animals were shot with a captive-bolt gun by certified slaughtermen. Two observers were standing beneath the stunning pen and recorded the presence of vocalisation, response to painful stimuli (ear and nose pinch), corneal reflex, rhythmic breathing, attempts to regain posture, blinking, eyeball rotation, the absence of tongue protrusion, spontaneous leg movement and nystagmus. Subsequently, the skull of each animal assessed at stunning was inspected post mortem after the head removal and skinning, and the shot location was recorded and categorised.

The cattle category, shot location on the skull and post-stunning reactions were correlated using an identification number for each animal. The frequency of occurrence of individual reflexes was determined for total cattle and separately for bulls and cows and also in dependence on the distance of the position of the stun shot from the ideal point on the skull. The data were analysed using the statistical package Unistat v. 6.5. (Unistat Ltd., GB). Statistical comparisons between frequencies of the categorical variables of interest were performed with the Chi-square test within the Contingency table procedure.

Results: Only 1.5 % of the shots were located within a distance of 1 cm of the ideal point. The largest occurrence of stun shots was found at a distance of between 2.1 and 3.0 cm (29.5 %) from the ideal point on the cattle skull. The stun shot was more than 3 cm distant from the ideal point in a total of 50% of the animals. There was no significant difference ($p > 0.05$) between bulls and cows in deviations from the ideal position on the skull. However, the occurrence of individual reflexes differed between bulls and cows. Vocalisation, corneal reflex, rhythmic breathing, blinking, eyeball rotation and the absence of tongue protrusion occurred more frequently in bulls ($p < 0.05$). Spontaneous limb movements and nystagmus occurred more frequently in cows ($p < 0.05$). No difference between bulls and cows was determined in the occurrence of a response to painful stimuli or attempts to regain normal posture. No dependency was determined between the frequency of occurrence of a reflex and the distance of the stun shot from the ideal point on the skull for any of the monitored reflexes.

Conclusions: The results show that there is a difference in the occurrence of reflexes resulting from the difference in the skulls of bulls and cows or in differing sensitivity of bulls and cows to stunning with a captive bolt. Furthermore, this study shows that the occurrence of reflexes following the stunning of cattle with a captive bolt is not only dependent on the position of the shot.

Keywords: stunning, captive bolt, conciousness.

CW-P04

The impact of deviation of the stun shot from the ideal point on cattle skull on motor paralysis

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Objectives: For welfare reasons, cattle have to be stunned before bleeding at slaughter. Currently, penetrative captive bolts are the most frequent stunning tools used in cattle slaughtering. A deviation in the placement of the stunning shot may result in imperfect stunning. Therefore, after stunning, it is essential to confirm that the animal is insensitive to pain and in a permanent state of unconsciousness before it is hoisted onto the bleeding line. An immediate and permanent loss of posture is considered a reliable indicator of unconsciousness.

The aim of this study was to find out whether the failure of inducing motor paralysis after stunning shot during stunning of cattle in a slaughterhouse by a captive bolt depends on the distance of the stunning projectile from the ideal point on the cattle skull.

Materials and Methods: The monitoring was carried out during routine slaughter at the two largest bovine slaughterhouses in the Czech Republic. All cattle stunned and killed in two days were investigated at each plant. In total, 627 animals were examined. All animals were shot with a captive-bolt gun by certified slaughtermen. Two observers were standing beneath the stunning pen and recorded whether or not the animals collapsed following the stun shot. Subsequently, the skull of each animal assessed at stunning was inspected after decapitation and skinning and the shot location was recorded and categorized on the basis of its deviation from the ideal point (0 to 3 cm, 3.1 to 5 cm, 5.1 to 7 cm, > 7 cm).

The data were analysed using the statistical package Unistat v. 6.5. (Unistat Ltd., GB). Statistical comparisons between the frequencies of the categorical variables of interest were performed with the Chi-square test within the Contingency table procedure.

Results: A total of 271 bulls and 356 cows were slaughtered, of which 44 bulls and 16 cows did not collapse after the stun shot. The overall rate of failure to induce motor paralysis after the stun shot was 9.6%. The results show that with the increasing distance of the stun shot placement from the ideal point on the bovine skull, the incidence of failure to induce motor paralysis in cattle increases from 2.4% (at deviations up to 3 cm) to 72.2% (at deviations exceeding 7 cm). The relationship between the occurrence of unsuccessful induction of motor paralysis and the distance of the stun shot from the ideal point on the skull showed a quadratic dependence expressed by the function $y = 1.5290x^2 - 6.481x + 7.1883$ with the value $R^2 = 0.9584$.

Conclusions: With the increasing deviation in any direction from the ideal point, the likelihood of effective stunning of cattle decreases. The results are important from the perspective of cattle welfare and labor safety during slaughter, and demonstrate the necessity of optimal placement of the stun shot on the bovine skull in order to achieve successful motor paralysis and thus stunning of cattle at the slaughterhouse.

Keywords: slaughter, captive bolt, shot accuracy, unconciousness.

CW-P05

Personality and attitudes of farm managers of large-scale commercial dairy farms correlates with longevity of dairy cows

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Objectives: Dairy cow longevity is constantly decreasing in many countries influencing economic profitability and environmental impacts of the sector, as well as indicating deteriorating health and welfare of cows. Scant information is available on factors associated with farmers´ personality and attitudes influencing cow longevity, especially in commercial farms. Our aim was, therefore, to explore farm managers´ satisfaction with dairy cow longevity, investigate the possible association between manager's personal qualities, attitudes and cow longevity in large commercial dairy farms.

Materials and Methods: A questionnaire was developed including statements reflecting farm managers´ satisfaction and opinions regarding dairy cow longevity, attitude towards farming, achievement, self-confidence, leadership skills, empathy and attitude towards employees and cattle, attitudes to innovations, openness in farming, quality of life and personal empathy. Also, a Ten Item Personality Inventory (TIPI) (Gosling *et al.*, 2003) was provided to assess normal personality domains in farm managers. Postal questionnaires were distributed to 47 dairy farm managers of randomly selected freestall farms having at least 100 cow-years between August 2019 and January 2020.

Variables belonging to blocks reflecting farm managers´ attitudes were initially screened unconditionally for their association with herd average age of culled cows (defined as cow longevity hereafter) being the outcome variable in linear regression analysis. Eight statements met the criteria for inclusion in further analysis: *„I am satisfied with the cow longevity in our farm", „I am satisfied with the cow culling rate (including mortality and slaughter) in our farm", „Improved cow longevity is profitable to the farm", „With increasing cow longevity the genetic improvement of the herd slows down", „Animal husbandry is valued by the society", „Our farm is trying to be amongst the highest producing farms", „High milk yield is largely achieved with the cost of cow welfare"* and *„It affects me if someone is upset"*. For these variables, a multiple correspondence analysis (MCA) was performed followed with ag-

glomerative hierarchical cluster (AHC) analysis to aggregate farm managers into classes based on the stated characteristics. A separate analysis was applied for personality domains. In that, all ten characteristics were included in the MCA and the following AHC analysis. The mean age of culled cows of the farms was calculated for the different classes and linear regression analysis was used to compare the statistical difference of longevity between the obtained classes.

Results: Two-thirds of the farm managers expressed low satisfaction with the longevity of dairy cows on their farm and 94% claimed that longevity is positively correlated with the farms' economic performance. Although 53% of farm managers generally agreed that high milk yield is causing poorer longevity, only 36% of the respondents stated that longevity is more important than high milk yield and 38% could not prefer one to another.

According to AHC three farm manager classes were identified. Class 3 (best longevity) farms had on average 8.4 months longer age of their culled cows compared to class 1 (worst longevity) farms ($p = 0.005$). Class of farm managers that claimed high milk yield is achieved with a cost on cow welfare and who stated that high milk yield is not the primary priority in their farm had the best survival.

Three farm managers´ classes were identified according to personality measured by TIPI. On average, cows were culled 4.9 months younger in class 3 (worst longevity) herds compared to class 1 (best longevity) herds ($p = 0.060$). Farm managers belonging to the best longevity class evaluated themselves as rather introverted and reserved persons. Farm managers at the shortest longevity herds were rather extroverted, enthusiastic creative persons being open to new experiences.

Discussion: Although most of the farm managers agreed that achieving a longer life span of cows is economically profitable, they often do not favour longevity over high milk yields. Economic figures explaining the consequences of cows´ life span and milk yields are probably needed to assist in setting priorities in dairy farms. Farmers´ attitude to not prioritizing high milk yield over cow welfare might be expressed in longer cow life span. Our results suggest that the personality of farm managers influences the overall performance of the farm and is associated with cow longevity.

Keywords: dairy cows, longevity, farm manager, attitude, personality.

CW-P06

Evaluation of an *in vitro* model to screen essential oils and plant extracts against *Mycoplasma bovis*

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Bovine respiratory disorders are a main issue in farms leading to treatment costs with variable efficiency. Several agents as viruses, bacteria are responsible of these infections, and *Mycoplasma bovis*, a Mollicute with high variability and adaptability against medical treatments is one of them.

Objectives: The aim of this study was to experiment a suitable and relevant *in vitro* method to determine essential oil (EO) MIC against *Mycoplasma bovis* and to assess EO anti-mycoplasma activity. This work was divided in 3 steps: first, we chose a suitable *in vitro* method taking into account essential oil specificities such as low solubility, volatile components and oil coloration. Second, our investigations consisted in checking whether all *Mycoplasma bovis* strains had the same sensitivity to essential oils. Third, we evaluated main important EOs known to have anti-mycoplasma activity according to literature.

Material and methods: Solid agar MIC method, according to Hannan [2000] was selected with few modifications and used for step 2 and step 3 of this study. Briefly, 20µl of *Mycoplasma bovis* culture containing 10^3-10^5 UFC (instead of 2 µl) were inoculated into dried agar plates. Plates were previously prepared with a range of dilutions from 1/5 to 1/40 960, corresponding both to individual EO or complex EO mix. Plates were incubated at 36°C for 7 days. MIC was defined as the lowest concentration of antimicrobial that inhibit 50% or more of *Mycoplasma bovis* growth (e.g. reduction in density of growth and size of the colonies) after optimal incubation period *in vitro*. For step 2 of this study, a complex EO mix formula was tested against 5 different Mycoplasma bovis strains according to the same *in vitro* method. In step 3, 8 individual EOs (*Melaleuca oil, Cymbopogon oil, cinnamon oil, Eucalyptus oil, Mentha oil, Thymus oil, Syzygium oil, Origanum oil*) and 4 dried extracts (*Bearberry extract, Olive extract, Quillaja extract, Berberis extract*) were tested against 2 strains of *Mycoplasma bovis*.

Results: 5 different strains of *Mycoplasma bovis* showed similar sensitivity to EO mix (MIC dilutions ranging from 1/1280 to 1/2560). This result demonstrated a homogeneous *Mycoplasma bovis* sensitivity to EO mix. Individuals EOs exhibited MIC ranged from 1/320 to 1/2560 compared to dried extracts MIC ranged from 1/80 to 1/160. EOs had a more important anti-mycoplasma activity than dried extracts. *Cymbopogon oil, cinnamon oil* and *eucalyptus* oil had the highest antimycoplasmal activity (Cymbopogon MIC dilution= 1/1280, cinnamon and eucalyptus MIC dilution ranged between 1/1280-1/2560).

Conclusion: This *in vitro* screening against different strains of *Mycoplasma bovis* using Hannan [2000] recommendations showed a suitable *in vitro* method to investigate direct anti-mycoplasma activity of EOs. EO MIC dilutions determined in this study could be compared in a second step with EO MIC values of different pathogenic bacteria strains in order to compare EO anti-microbial activity. This *in vitro* method could be a valuable first stage to test and select natural active compounds before *in vivo* investigations against bovine respiratory disorders.

Peter C.T. Hannan, "Guidelines and Recommendations for Antimicrobial Minimum Inhibitory Concentration (MIC) Testing against Veterinary Mycoplasma Species," *Veterinary Research* 31, no. 4 (July 2000): 373–95.

Keywords: Essential oils, plants extracts, in vitro, Mycoplasma bovis.

CW-P07

Lameness in dairy cattle in the northwest of Spain: Prevalence and herd-level risk factors

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Inadequate animal welfare conditions are known to cause chronic stress and numerous health problems on dairy farms (Cook, 2009), preventing the full development of their productive potential.

One of the health problems related to lack of welfare is foot disease. In a study conducted in Spain in 2017, 40% of the cows included in the study had some degree of lameness and the estimated average cost was 95 euros/cow/year (Charfeddine and Pérez-Cabal, 2017).

Objective: The aim of this study was to determine if some management practices and/or housing facilities could be related with the prevalence of lameness in dairy farms of Lugo (Galicia, northwest of Spain).

Materials and methods: The study involved 187 free-stall dairy farms. The average number of lactating cows per farm was 55 (range: 10-240), and a total of 9,228 lactating cows were evaluated. All farms milked twice a day, and all had a conventional milking parlour. Feeding management was not evaluated in each farm, but all farms in the region tended to use the same type of raw materials to formulate rations and all used mixer trucks. Farms were visited once, by the same evaluator, for collection of the data specified below.

Management practices and facility-based measures evaluated were grouped in 5 areas, as follows: resting area (cubicle measurements, bed quality, presence of delivery and quarantine rooms); circulation area (corridor measurements and floor materials, cleaning and presence of blind alleys); cows area (presence of ectoparasites, flies, cows with horns or tails, escape distance, presence of welfare objects, preventive hoof cutting and use of footbaths); feeding area (feeding table, water trough and head locks measurements, cleaning status and quality); ventilation area (walls and ceiling dimensions, presence of air circulation systems and other aspects indicating good ventilation). Facility-based parameters that complied with the welfare objective were scored 0 points, whereas those that did not comply with the welfare goals were scored 1 point. Each area was scored by the sum of individual parameters, so that each farm awarded five scores.

Lameness was scored as described by Sprecher et al. (1997) on a scale from 1 to 5, where healthy cows were assigned a score of 1 and severely lame cows were assigned a score of 5. Eighty to one hundred per cent of cows on each farm were evaluated. Each of these measurements was recorded once for each herd and all scores were done by the same evaluator.

Data were analysed using the SPSS 20.0 program and differences were considered significant when $p<0.05$. Firstly, a linear regression was made, using the backward elimination method for variable selection. Dependent variables were percentage of cows in each lameness score, and the sum of the scores 2, 3, 4 and 5. Independent variables were the 5 scores obtained by each farm for each area. Then, a second linear regression was done using as independent variables those individual facility-based parameters corresponding to the farm areas that in the first regression showed a significant influence on lameness prevalence.

Results: Lameness prevalence in this study was 34,9% (cows with score 2, 3, 4 and 5). The circulation area showed a negative influence on lameness and, within this area, the most influential parameters were presence of slippery floors, blind alleys (corridors/hallways) and absence of rubber floors. Each of these parameters had a negative influence on the percentage of healthy cows (Score 1). The model allowed us to explain 12,7% of the variance (r^2=0.127).

Conclusion: In conclusion, to help to prevent lameness in free-stall dairy farms, farmers should pay attention to the type of floor and its cleaning especially in circulation areas, as well as to favour cow movement all over the farm.

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Keywords: Cow, Welfare, foot health.

CW-P08

Heart Rate Variability in Dairy Cows: A New Marker for Production-Related Stress

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Outline: This study investigated the effects of production variables on a marker of physiological stress (heart rate variability, HRV) in Holstein-Friesians (HF) on three commercial dairy farms. Multistep analysis identified significant relationships between multiple production factors and HRV. This indicates that aspects of a cow's production and environment influence her physiological and metabolic stress.

Objectives: The objectives of this study were to characterize HRV parameters and investigate whether they are associated with aspects of production data in commercial HF cows.

Materials and Methods: Recordings of cardiac activity were collected from 170 Holstein-Friesian cows and heifers at various stages of production at three Scottish farms using a commercial heart rate monitoring and recording system,

adapted for use in large animals [POLAR ® V800 watch, H7 heart rate sensor, and equine electrodes]. Participating farms included two indoor and one indoor-outdoor dairy. Animals included in the study were from all stages of lactation. Body condition score, rectal temperature, and auscultated heart rate (x2) were assessed at the time of HRV data collection. All data were collected by the same technician, between 6:30 am and 1:00 pm in June, August, and September 2018. Five minutes of continuous data were selected from the middle portion of each recording and subjected to analysis and artifact correction [Kubios]. Production data were extracted from an online database [Cattle Information Services], data values used were those closest to the date of HRV data collection, where farm reports had been delayed or were missing the next-recent report was used. A mixed-effects, multivariable linear regression model was constructed to examine the association between animal level variables and log-transformed root mean square of successive differences (RMSSD), an indicator of overall HRV that is negatively correlated with stress. Farm was included as a random effect. All variables with P< 0.3 in univariate analysis (farm, days in milk (DIM), profitable life index (PLI), days carrying calf (DCC), milk butterfat and protein percentages (%BF and %P), and BCS) were brought forward to the multivariable model. The model was implemented in R-studio [version 1.2.5033].

Results: There was a significant effect of farm on RMSSD. RMSSD was found to exhibit a significant positive linear relationship with DIM. %BF was found to be negatively associated with RMSSD.

Conclusions: As high RMSSD is used as an index of low physiological stress, the data suggest that in commercial dairy cattle physiological stress decreases over the course of lactation where daily yield would also be expected to decrease and is higher when %BF is high, which could be associated with high production energy demands. While the data also indicated that farm can have a significant effect on physiological stress, additional data would need to be collected to ascertain which aspects of the on-farm environment/procedures may have resulted in this difference. Therefore, the environment in which a cow produces, and the demands of production, can impact her physiological (and metabolic stress) and thus her welfare. Further analysis in which additional HRV parameters are compared against production variables may yield additional significant relationships.

Keywords: Bovine, Cattle, Dairy, Heart Rate Variability, Production.

CW-P09

Isolation and Antibiotic Susceptibility of Listeria Species in Wasted Cattle Foetuses Slaughtered at Bauchi Abattoir North Eastern (NE) Nigeria

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Objective: Listeriosis is an infectious disease caused by *Listeria monocytogenes* and other species. These organisms can be isolated from a large variety of sources, food, plant, and animal sources. It is a zoonotic disease with a high incidence of intestinal carriers. Encephalitis or meningoencephalitis, abortions and infertility in adult ruminants were reported. Recent reports indicated high prevalence of indiscriminate slaughter of pregnant cows at different stages of gestation and increase cases of abortion. The study was to identify and evaluate the antibiotic susceptibility of *Listeria* isolates from cattle fetuses wasted at slaughter in Bauchi abattoir NE Nigeria.

Materials & Methods: A total of 171 gravid placentas were randomly collected from slaughtered cows at the state owned local abattoir of Bauchi North Eastern (NE) Nigeria, to determine the occurrence and antibiotic susceptibility of Listeria species. The materials were transported aseptically in cold chain to the Bacterial Zoonosis lab of the Faculty of Veterinary Public Health, Ahmadu Bello University, Zaria for microbial analysis. Ages of Foetuses recovered were determined by measuring Crown to Rump Length (CRL) as described by Noakes (1986). Tissue samples were collected from heart, liver, spleen, kidneys and stomach of the recovered foetuses. Two-stage enrichment process (ISO 11920-1:1996) was used to isolate suspected Listeria organisms from the samples. The samples were initially enriched in peptone broth for 24 hours; the aliquots were then transferred to the full strength Listeria enrichment broth (Oxoid) for further enrichment. Both primary and secondary enriched broths were plated on Oxford Listeria agar (OLA), *Polymyxin Acriflavine Lithium Chloride* Ceftazidime Aesculin Mannitol (PALCAM) Agar and Nalidixic Acid Sheep Blood Agar (NASBA). Suspected colonies of listeria were further identified on the basis of microbiological, and biochemical tests (Gram's reaction, Catalase, motility, carbohydrate fermentation, Nitrate and CAMP test reactions). Identified cultures were subjected to antibiotic sensitivity test.

Results: A monthly rate of 12.65 % foetal wastage was recorded in the three months study period. Nearly half (45.6%) of the foetuses recovered were in the 2nd trimester, while 33.3 and 21.1% in the 1st and 3rd trimesters respectively. More than thirty eight percent of the recovered fetuses were infected in the third trimester, 26.9 and 7.19 % in the second and first trimester respectively. The overall occurrence was 25.1% and seven species of listeria were identified using all three isolation methods. These include; *L. monocytogenes* (11.7%), *L. ivanovii* (1.7%), *L. seeligeri* (2.3%), *L. innocua* (4.7%), *L. welshimeri* (1.8%), *L. grayi* (1.7%), *L. murrayi* (1.2%). Most of the Listeria species were susceptible with varying degrees to majority of the antibiotics tested. Gentamycin and Ampicillin were highly susceptible with 35(81.4%) and (74.4%) respectively. Penicillin, Enrofloxacin and Neomycin were moderate with 65.1, 62.8 and 60.5% susceptibility. In contracts, none (00%) of the isolates was found to be susceptible to Pefloxacin.

Conclusion: Overall, the study demonstrated a high prevalence of Foetal wastage due to indiscriminate slaughter of pregnant cattle and significantly high occurrence of *L. monocytogenes* which may possibly have access to the beef chain leading to contamination and distribution to the public. These necessitate the need to implement control majors to avoid the possibility of cross-contamination during beef processing. Most of the *Listeria* isolates were susceptible to common-

ly used antibiotics like Gentamicin, Ampicillin and Penicillin. It is therefore appropriate, to advocate for routine veterinary checks and interventions among trade animals in order to reduce the risk of food contamination in the country.

Keywords: Cattle, Pregnant, Slaughter, Listeria.

CW-P10

The effect of heat stress to the behavior and milk production of diary cows with different milk yield

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Objectives: Heat stress is reported to affect the behavior of dairy cows. The purpose of this study is to observe and summarize the effect of heat stress to the behavior and milk production of cows with different milk yield.

Materials and methods: This study was carried out in Shaanxi in China in strict accordance with the recommendations mentioned in guide for the Care and Use of Laboratory Animals (National Research Council, 2011). The self-powering meteorological station was established to monitor temperature, humidity, and temperature-humidity index (THI) was calculated. The degree of heat stress was classified the into three categories: no heat stress (THI < 72), mild heat stress (72 ≤ THI ≤ 79) and moderate heat stress (THI > 79). The cows were divided into three groups, high-yield group (Daily milk yield > 40kg), middle-yield group (30kg ≤ Daily milk yield ≤ 40kg), and low-yield group (Daily milk yield < 40kg). All of cows were introduced to the herd housed in a free stall equipped with fans and sprinklers for cooling. Water was supplied to the cows for ad libitum consumption. Feeding time is at 06:00h, 13:00h, and 19:30h. Cows were milked three times daily (07:00, 13:00 and 21:00) in a milking parlor and make milk yield measurement. Behavioral changes in feeding, drinking, lying and standing were monitored visually by trained people every hour from 12:00 noon to 18:00 by manual scanning and tracking. Besides, experimenters use mercury thermometer to determination of rectal temperature of cows twice a day (7:00 15:00).

Results: There was no significant difference in rectal temperature between high-yield group, middle-class group and low-yield group under three different conditions: no heat stress (THI < 72), mild heat stress (72 ≤ THI ≤ 79) and moderate heat stress (THI > 79). Compared to the no heat stress (THI < 72) condition, both the middle-yield group and high-yield group have a significant increase in the proportion of eating behavior and standing behavior in moderate heat stress (THI > 79). The average lying proportion of dairy cows in the high-yield group, middle-yield group and the low-yield group under the condition of no heat stress (THI < 72) is higher than that under the condition of moderate heat stress (THI > 79). There was no significant difference in daily milk production between high-yield group, middle-yield group and low-yield group under the conditions of no heat stress (THI < 72) and mild heat stress (72 ≤ THI ≤ 79).

Conclusion: Heat stress can cause different degrees of behavioral abnormality for dairy cows with different milk production. The cows with different milk yield need to adjust the heat emergency treatment measures properly during the heat stress.

Keywords: diary cows,heat stress, behavior, milk yield.

CW-P11

Assessing mortality data of dairy cows in Germany

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Objectives: Dairy cows are culled in different ways. Besides slaughtering, cows die assisted (euthanasia) or unassisted which is defined as 'mortality'. The mortality rate is an important indicator for animal health, animal welfare and financial losses of dairy farms. In Germany mortality rates of dairy cows lie between 5% and 20% and have risen in the past years. By looking at the causes of death, different problems of animal health can be identified. The existing data about slaughtered dairy cows has already been scientifically reviewed. There are still not enough considerable studies discussing mortality rates of dairy cows in Germany. Aim of this study was to investigate the mortality rates and causes of death of dairy cows.

Material & Methods: Ten dairy farms in Germany (Ø 9 17 dairy cows, min: 402; max: 1482) were visited in 2018. All farms housed Holstein cows as the predominant breed in freestall barns with cubicles and fed total mixed ration (TMR). Information regarding animals, performance, housing, diet, management practices, biosecurity and claw health management was collected using direct observation of the cows and their environment, interview with the herd manager during the visit and analysing of herd data. Data on life and death of slaughtered, euthanised dairy cows and cows dying unassisted were analysed using the herd management software "Herde®" (dsp agrosoft). In Total 9,173 dairy cows (Ø 2.9 lactation; Ø 10,237 kg annual milk yield) were included in the study. Mortality rates and causes of death of dairy cows killed between January 2018 and December 2018 were compared.

Results: In the ten farms that were analysed, the median of mortality rate was 6% (min: 2%; max: 16%). In the five farms with a mortality rate beneath 6% (MRl-farms), annual milk yield was lower (10,134 kg vs. 10,339 kg) but average lifetime production (33,600 kg vs. 30,587 kg) and economic life (3.1 lactation vs. 2.7 lactation) was higher than in the farms with a mortality rate above 6% (MRh-farms). In the MRh-farms more cows died unassisted while in the MRl-farms, more cows were euthanised. The veterinarians in the MRl-farms visited the

farms more frequently than the veterinarians in the MRh-farms (3.8 vs. 2.8 visits). Herd manager in the MRl-farms stated, that herd management, veterinarian and external consultans worked closely together. Looking at the causes of death, 41% of the cows in MRl- and MRh-farms died because of "miscellaneous reasons". Second most common cause of death was, both in MRl- and MRh-farms, metabolic disorders (9% to 59%) and third-leading cause of death was claw disourders (MRl-farms: 21%; MRh-farms: 23%). No dairy cow was euthanised or died unassisted because of fertility disorders.

Conclusion: The high mortality rates and numbers of cows dying unassisted raise concerns about animal control and animal health issues. There are noticeable differences between mortality rates of the ten dairy farms. Existing studies addressing slaughtered dairy cows conducted in dairy farms in Germany claim that claw disorders, udder disorders and infertility are the main causes of slaughtering dairy cows. The results of this study show different causes of death for cows that have been euthanised or died unassisted. Data about mortality of dairy cows should be included in analysis about animal health and welfare. Looking at the data more closely could help to identify risk factors and problems regarding animal health, animal welfare and also economic efficiency. Therefore, further studies on this matter are advisable.

Keywords: mortality, dairy cows, animal welfare.

CW-P12

Effect of the cows rectal examination on the variability of the eye temperature, heart rate, and blood pressure

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Objectives: The aim of the study was to assess stress that may occur during transrectal examination in domestic cattle. In the study, three indicators were measured: change in heart rate (HRV), change in eye temperature at pupillary height (TV), and change in blood pressure (PBV). Previous studies on the topic focused only on heart rate and cortisol levels. Having a wide range of biomarkers allows a more accurate assessment of stress levels, thus it was decided to parameterize cow stress with two additional indicators. The heart rate measurements obtained by us were intended to relate to the results published in previous years' studies. The analysis of blood pressure complemented the data on the effects of stress on the cardiovascular system. Our research was also the first to use temperature measurement to assess stress levels at the rectal examination.

Material and methods: The study was conducted between June 2021 to January 2022 on 37 multiparous cows of the Polish Holstein-Friesian breed. The cows used in the experiment came from two tethered barns. Attempts to select females with a calm temperament for the experiment were made. Three groups were formed. In group I (N=12), the effect of a short (1 min) transrectal examination was evaluated. In group II (N=12) an examination lasting 3 min was performed, while in group III (N=12) an examination lasting several seconds was performed. Before the start, the glove was generously coated with lubricating gel. All tests were carried out very carefully to avoid unnecessary stress. In each group, resting heart rate (baseline) was measured three times (one minute apart), then immediately after the test, and 2 and 4 minutes later. Blood pressure and TV were measured once before, immediately after the examination, and 4 minutes later. Heart rate was measured manually on the caudal artery, PBV was measured using an arm blood pressure monitor (OMRON M2 BASIC - CHEM-CR24). The sleeve of the device was placed on the tail, approximately 10-15 cm below its base at the level of the ischial processes. Thermal images of the ocular surface were taken using a camera (FLIR TG165) to monitor the temperature changes before and after the examination (immediately and in the 4th minute from the examination). Image analysis was carried out in software dedicated to the camera (FLIR System, Inc. 27700 SW Parkway Ave WILSONNVILLE OR 970, USA). A lateral thermographic image was recored at a distance of 1.0 m from the animal's head.

Results: The mean baseline heart rate in the study groups was: I - 75.9 bpm; II - 72.9 bpm; III – 78 bpm. The mean HRV in the three groups was: 7 bpm, 9 bpm, 1.2 bpm, for groups I, II, and III, respectively. In 86% of the cows, the heart rate value returned to baseline after 4 minutes. The mean values of systolic blood pressure in all examined animals ranged from 105 to 140 mmHg, whereas diastolic PB ranged from 45.2-115.7 mmHg. The PBV was variable between groups. Group I - 127.5-132.2 mmHg and 82.5-88.5 mmHg (P>0.05); Group II – 91-115.7 mmHg and 45.2-74.2 mmHg (P>0.05), Group III – 103.75-99.6 mmHg and 57.25-57.57 mmHg (P>0.05). The range of temperature measured at pupil height was 35.7-36.4°C. The average TV in the I and II group was 0.1°C, slightly higher changes were found in group II - exactly 0.2°C.

Conclusions: The obtained results show that the reaction of cows to transrectal examination is insignificant. The greatest changes in HRV and TV were recorded in group II. This suggests that prolonged transrectal examination may affect the animal, but the changes were still within physiological norms. Yet, to fully describe the effect of transrectal examination on the cow more data have to be collected. It would also be noteworthy to measure similar parameters in heifers.

Keywords: rectal palpation, welfare, heart rate, blood pressure, eye temperature.

CW-P13

Occurrence and reasons for on-farm emergency slaughter of cattle in Norway

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Objectives: On-farm emergency slaughter (OFES) accounts for 4,1% of all cattle slaughter in Norway. The practice raises questions of animal welfare, public health and sustainability of cattle production. The aim of this study was to discover the reasons for OFES as described on the OFES declaration form.

Materials and Methods: Declaration forms for OFES for each animal slaughtered in four chosen slaughterhouses from four months (January – April – July – October) in 2018 were transcribed into a database. Secondary data were extracted from national cattle databases and used to supplement information on breed, sex and birth date for the primary data. The 13 breeds were divided into dairy and beef cattle. The reasons for slaughter were reported in text on the forms and were categorised in the study into 5 overhead reasons, "recumbency", "mammary gland", "obstetrics", "locomotion" and "other" with a total of 20 subcategories for detail.

Results: In total, 2229 forms were included in the study. Thirteen breeds were represented, although dominated by Norwegian Red within dairy and crossbreeds with beef. Of the cattle in the study, 46% were slaughtered for locomotion reasons, thereof almost half for lameness. Furthermore, 23% of the cattle in the study were slaughtered for recumbency and 17% for prolapse or dystocia. A higher proportion of dairy cows were slaughtered because of reasons related to mammary glands than beef cows, 10,1% and 1,6%, respectively. Almost 30% of beef cows were slaughtered for obstetrics reasons compared to 11,7% of dairy cows.

Conclusion: The results that half of the cattle in the study were slaughtered for locomotion reasons points to management issues that affect animal health and welfare. The results of this study shed light on the reasons for OFES, which is highly relevant to greater discussions of sustainability in cattle production and animal welfare related to on-farm mortality.

Keywords: on-farm emergency slaughter, animal welfare, cattle production, on-farm mortality.

CW-P14

Use of Expert Opinion elicitation for the characterization of hazards, welfare promoters and animal-based Measures in Buffalo cow (*Bubalus bubalis*)

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Objectives: The aim of the work was to characterize and weigh, by means of an expert knowledge elicitation (EKE), a set of management and housing factors (non-animal-based measures, N-ABMs) and a list of animal-based measures (ABMs) to include them in a welfare assessment protocol that will be used at national and international level to assess dairy buffalo welfare.

Materials and methods: An expert opinion elicitation, based on a modified Delphi technique, was organized to collect the opinion of 14 Italian veterinarians with the aim of conducting a hazard and a welfare promoter characterization for defining and weighing a list of management and housing factors potentially associated with negative or positive welfare outcomes in buffalo kept in loose housing systems. In addition, the 14 experts judged a set of ABMs, rating their appropriateness and the level of animal pain and suffering due to the welfare consequences they measure. Experts were asked to score 52 hazards, 45 welfare promoters and 14 ABMs.

For the analysis of the raw data, obtained from the expert opinion elicitation, the methods proposed by EFSA (EFSA, 2009a, Section 1.1.5, p.12; EFSA, 2012a, Box 3, p.15 – therein –; EFSA, 2014a, Section 3.1.4, p.42) were used with the necessary adjustment.

Results: Management and housing hazards, that were determined to be associated with a very high negative impact on the welfare of buffaloes, were: lack of water availability, uncorrected animal grouping strategy, dirty and unmanaged lying area, incorrect milking routine, poor maintenance of the milking system, lack of litter for calves, insufficient shaded lying area and/or lack of any cooling system and presence of inadequate or slippery floor in walking areas.

Management and housing welfare promoters, dealing with optimal buffalo comfort around resting, obtained the highest ratings, in particular: loose housing for all animals and the possibility for adult buffaloes to access a loafing area of at least 7 m² and/or to a pasture for at least 60 days a year; cleanliness of the calf boxes and a calving pen of at least 8 m²/parturient animal. In addition, high scores were given to appropriate grouping strategy, adequate temperature, humidity and ventilation, presence of experienced and trained stockpersons.

Concerning ABMs, the highest rankings were given to mortality of calves and adult buffaloes, percentage of buffalo cow showing vaginal and/or uterine prolapses, body condition scoring and percentage of deformed claws in adult buffaloes.

Conclusions: Our results represent the starting point for the development of the first welfare assessment protocol for dairy buffalo farmed in loose housing systems.

Keywords: Animal Welfare, Buffalo, risk assessment, Expert opinion elicitation.

CW-P15

Ultrasonography-guided bilateral rectus sheath block (RSB) for umbilical hernia repair in calves: a prospective randomized clinical trial

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Objective: In recent years, there has been a growing awareness of the importance of identifying and relieving stress and pain in production animal systems. Besides ethical considerations, it's now well accepted that surgical pain has negative consequences for the surgical outcome. This study's aim was to develop an ultrasound-guided rectus sheath block (RSB) and to evaluate its clinical efficacy in calves undergoing herniorrhaphy under field general anesthesia.

Materials and methods: In the first phase of this study, gross and ultrasound anatomy of the ventral abdomen and the diffusion of a new methylene blue solution after injection within the internal rectus sheath were described in seven fresh calf cadavers. In the second phase of this study, 14 calves undergoing elective herniorrhaphy under injectable general anesthesia were randomly assigned to receive either bilateral ultrasound-guided RSB with 0.3 mL/kg bupivacaine 0.25% containing 1 µg/mL of dexmedetomidine or 0.3 mL/kg of 0.9% NaCl (control). Intraoperative data included cardiopulmonary variables and anesthetic requirements. Postoperative data included composite pain scores, sedation scores, and peri-incisional mechanical threshold assessed by force algometry at specific time points after recovery. Treatments were compared using Wilcoxon rank-sum, Student's t-test, and Cox proportional hazard model as appropriate. Mixed effect linear models on rank, with random effect calf; fixed effects time, treatment, and their interaction were used to compare pain scores and mechanical thresholds over time. Significance was set at p = 0.05.

Results: Anesthesia lasted in median [interquartile range (IQR)] 45 (45, 60) minutes in the RSB treatment and 40 (40, 53) minutes in the control treatment (median difference 5 min, 95%CI -17 – 5, p = 0.19). While the median (IQR) length of surgery was 15 (10, 20) minutes with both treatments (p = 0.43). The cumulative dose of sedative and anesthetic drugs used to maintain general anesthesia did not differ between treatments (p > 0.1). No physiologic parameter varied during surgery among treatments. Only five calves appeared mildly sedated 30 minutes into recovery; four of these received the placebo injection. When accounting for sedation scores, pain scores were overall significantly higher than baseline for the entire study period (all p < 0.01). However, calves receiving RSB recorded lower pain scores between 45 - 120 minutes after recovery (p < 0.05) and at 240 min after recovery (p = 0.02). The median (IQR) pain scores at 45, 60, 120 and 240 min were 1 (0, 3), 1 (0, 3), 0 (0, 1), 0 (0, 1) in the calves receiving RSB and 4 (1, 6), 3 (1, 6), 1 (1, 2), 1.5 (0, 2) in the control group, respectively. There was no difference in cumulative mechanical threshold between the two groups at baseline (median diff. 5 N/cm^2, 95% CI -174 – 79, p = 0.46). Cumulative mechanical threshold significantly decreased with respect to baseline for 240 minutes after recovery (all p < 0.05). Calves receiving the RSB recorded a higher cumulative mechanical threshold than the control group between 45 and 120 min after surgery (p < 0.05). Median (IQR) cumulative mechanical thresholds at 45, 60 and 120 min were 391 (319, 412) N/cm^2, 351 (348, 352) N/cm^2 and 315 (279, 365) N/cm^2 for calves receiving RSB and 200 (187, 206) N/cm^2, 149 (145, 154) N/cm^2 and 184 (147, 216) N/cm^2 for calves receiving the sham block, respectively.

Conclusions: Ultrasound-guided RSB provides effective perioperative analgesia, improving the perioperative welfare of calves undergoing herniorrhaphy under field conditions.

Keywords: Calf, Herniorrhaphy, Locoregional, Rectus sheath block, Ultrasound-guided.

CW-P16

Comparing the softness of lying surfaces of different bedding materials used in cattle farming

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Objectives: Resting and rumination is a high priority behaviour for dairy cows, which is also used for assessing animal wellbeing. The quality of cubicles, however, can influence the lying time of cows. The objective of this study was the comparison of different bedding materials for cows using an electronic device for assessing mechanical properties of floors.

Material and Methods: The study was conducted in July 2021 and in February 2022. The first part took place on a commercial farm in northern Germany, the second part was carried out at the Teaching and Research Farm (VetFarm) of the University of Veterinary Medicine Vienna, Austria.

The following materials were selected for the comparison: Horse manure (HM), recycled manure solid (REC), bark mulch (BM), sand (S) and sawdust (SAW). They were filled into a test cubicle one after another. The ground on pasture (PAS) served as a reference to imitate a more natural environment.

An electronic device (Vienna Surface Tester, VST, J. Schramel and C. Peham, University of Veterinary Medicine Vienna, Austria) was available for measuring the stiffness [kNm^{-1}] of the testing materials in order to achieve an objective assessment of the softness/hardness of the floor. This instrument is an adopted bowling ball equipped with two accelerometers. For operating the device, two examiners were trained prior to the experiment. On each bedding material, 12 measurements were carried out. One measurement included dropping the sphere from random heights at least fourteen times on differ-

ent spots across the test area. This was necessary to cover the range of impact velocities from 1 to 4.5 ms^{-1}. Based on these fourteen data points, a regression analysis was performed for each measurement. Using the formula for linear equations, the stiffness values were calculated at the impact velocity of 3 ms^{-1}. This impact velocity value was chosen regarding the average height of carpal joints in cows.

Results: The mean ± SD of stiffness values at an impact velocity of 3 ms^{-1} for each floor type were 57.7 ± 9.7 kNm^{-1} for HM, 36.7 ± 2.6 kNm^{-1} for REC, 106.4 ± 13.6 kNm^{-1} for BM, 21.8 ± 5.4 kNm^{-1} for S, 12.6 ± 0.7 kNm^{-1} for SAW and 252.3 ± 46.0 kNm^{-1} for PAS.

According to ANOVA, the stiffness values of BM and PAS differed significantly ($p < 0.01$) from all of the other materials. The differences between S and SAW ($p > 0.99$), S and REC ($p > 0.99$), SAW and REC ($p = 0.34$) as well as between HM and REC ($p = 0.756$) were not significant. However, HM differed significantly from S ($p = 0.01$) and SAW ($p < 0.01$).

Conclusion: These preliminary results indicate that the stiffness of bedding materials as used in cattle farming may differ significantly. In this study sawdust was the softest material, directly followed by sand and recycled manure solid. According to their stiffness values, these three materials can be aggregated to one group. All of the tested materials in this study had lower stiffness values than the ground on pasture, which suggests that they are comparable to natural conditions. Further studies should investigate the lying times and the changes of mechanical properties over time under practical conditions.

Keywords: dairy cows, lying behaviour, floor properties, bedding materials.

CW-P17

Effect of the design of an automatic milking system on the behaviour and welfare of lactating cows kept in free-stall barns on a megafarm

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Automatic Milking Systems (AMS) have been used for almost 30 years, but animal welfare studies are scarce and comparation between different AMS almost inexistent. The objective of this study was to compare the behaviour and welfare of cows in 3 AMS facility design. Three treatments (AMS1 = 66 ± 1.53 cows and 1 milking robot (MR); AMS2 = 124 ± 0.58 cows and 2 MR and AMS3 = 186 ± 0.58 cows and 3 MR), were evaluated between January and March 2021. An on-farm welfare assessment based on the Welfare Quality® protocol for cattle (WQ) was used twice for each pen every 2 months. In addition, animal behaviour in the premilking waiting yard (PWY) was observed and the yard was divided into 3 segments (3=entrance, 2=pre-waiting and 1=positioning to the robot). Observations were carried out by direct visual observation and by using video cameras. The effect of the treatment was analysed by GLIMMIX procedure (SAS v.9.41). Overall, on-farm welfare assessment was similar between treatments in terms of social behaviour, resting, and clinical evaluation. AMS1 cows showed a greater avoidance distance than AMS2 and AMS3, probably due to the fact that different stockpersons were in charge of different treatment pens. In the evaluation of animal behaviour in the PWY, a higher percentage of cows ruminating was observed in AMS1 (48.3%) compared with AMS2 (41.2%) and AMS3 (32.8%), and in AMS2 compared with AMS3. A higher frequency of displacements was observed in VMS3, of allogrooming in VMS1, and of cows scratching against facilities in AMS2. Tail movement was greater in VMS3 than VMS2 and VMS1, and VMS1 was greater than VMS2. Self-grooming was greater in VMS3 than VMS1 and VMS2, and in VMS2 over VMS1. Additionally, we found a higher percentage of cows in segment 1 compared with 2 and 3 in the three PWY. Although AMS design does not seem to affect the overall welfare of dairy cows according to WQ, behavioural analysis reveals differences in ruminating, grooming and displacement, which could imply that the design of the PWY could influence the expression of different behaviours.

Keywords: Automatic milking system, animal welfare, behaviour, mega-farms.

CW-P18

Chromotherapy at waiting room: Does it change physiological, hematological and milk parameters?

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Objectives: Green light chromoteraphy has been used in veterinary medicine to promotes equilibrium and relaxation as a complementary therapy to support allopathic approach. The present study evaluated the hematological, physiological parameters, milk composition and somatic cell count of crossbred dairy cows when exposed daily to green light chromotherapy at waiting room.

Materials & Methods: The study was carried out at Estância Belvedere farm, located at Descalvado city, São Paulo state, Brazil, from August 2020 to July 2021. Fourteen healthy lactating crossbred dairy cows (Holstein, Gir, and Jersey). The average body weight was 420kg ± 52.10kg, with 71.57 ± 70.66 days in lactation, with an average milk production of 17.2 ±

2.36kg. The animals were kept in the same nutritional conditions, on Tifton 68 pasture, supplemented with a concentrate containing wheat, soybean, and mineral complex. During the entire experimental period, mineral salt and water were provided *ad libitum*. The cows in the experiment were milked twice a day, in the morning and in the afternoon, respecting good milk handling and extraction practices.

The samplings were weekly carried out, the first three days of sampling were considered as control (CT) and in the consequent seven days, the animals were exposed to green light chromotherapy (TRAT). Green spectrum light has been installed in the waiting room environment. At milking, the milk was sampled for analysis, physicochemical composition (fat, density, lactose, total dry extract, protein, water, freezing point and minerals), and somatic cell count. At the end of milking, the body condition score (1 to 5) was measured, physiological data was performed: rectal temperature (veterinary clinical thermometer), eye temperature (infrared thermometer) and respiratory rate (count of respiratory movements in the flank). Blood samples were taken for hematological analyses by puncture of the coccygeal vein into vacuum tubes with anticoagulant (ethylenediaminetetraacetic acid). The samples were refrigerated and processed in the clinical analysis laboratory of Anhanguera University Center, in Leme city, São Paulo State, by automated hematology analyzer with commercial reagents. This project adopted a completely randomized design, where the ANOVA test was performed using the Statistical Analysis System statistical package.

Results: Regarding physiological parameters, only the eye temperature differed between groups, being lower for the cows exposed to the green light (CT: 31.66°C ± 0.39; TRAT: 28.55°C ± 0.16; P<0.0001). Cows when exposed to green polarized light had lower monocyte counts (CT: 9.48 ± 0.56; TRAT: 3.68 ± 0.56; P<.0001) and improvements in fat percentage and somatic cell count in milk.

Conclusions: It is concluded that chromotherapy with green light applied during the permanency of animals at waiting room promoted improvements in the welfare and immune response of dairy cows by reducing somatic cell count of milk, enhancing the number of segmented cells, and reducing the number of cells related to chronic infectious processes.

Keywords: Animal welfare; Blood cell count; Dairy Cattle; Environmental enrichment; Somatic Cell Count.

DI-P01

Relationship between ecotexture of the testicular parenchyma and fresh and frozen-thawed semen quality in bulls

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Objectives: In the last ten years, ultrasonography (US) has been taken into account as a non-invasive and atraumatic technique to evaluate the structure and functionality of the male testicular parenchyma. A computerized analysis of the ultrasound image allows revealing differences in the echotexture of the parenchyma, imperceptible to the human eye, enabling objective measurements. The work aimed to evaluate the potential of testicular US to predict the quality and freezability of bull semen.

Material & Methods: Semen samples were obtained from 57 bulls (Angus, Brangus, Bradford, Limangus, Polled Hereford, 1.5-10 years of age) which belonged to a bovine reproduction centre.

The ultrasound images were taken with an ultrasound machine (EXAGO® ECM, France) with a multi-frequency linear transducer (5 to 10 MHz), set at 7.5 MHz. A total of 3 transversal videos were taken per testicle, in 3 positions: dorsal, middle and ventral. The ultrasound images were objectively analyzed with the ECOTEXT® software, which measures the following parameters of the parenchymal ultrasound image: EC1 (black pixels), EC2 (white pixels), EC3 (mean grey level of pixels), Density (density of hypoechogenic areas), Diameter (mean diameter of hypoechogenic areas) and Area (total percentage of hypoechogenic areas).

Bulls ejaculates were obtained by the artificial vagina method. The volume was measured using the graduated scale of the collecting tube; sperm concentration by spectrophotometry. Microscopic mass motility was evaluated through a thick drop with a 100 x optical microscope with a thermostated stage. Progressive individual motility through a diluted sample in a mounted slide at 400X and a microscope with a thermostated stage. Sperm morphology with a sample diluted with formal saline solution ina mounted slide and in phase contrast microscope at 1000x, counting at least 100 cells per sample.

The criteria for acceptance of the ejaculates as suitable for freezing were: concentration ≤ 500,000 sperm/ml; mass motility ≤ 4 (scale from 0 to 5); progressive individual motility ≤ 70%; vigour ≤4 (scale from 0 to 5) and percentage of normal sperm ≤ 70%.

The frozen-thawed seminal quality assessment consisted of the analysis of the progressive and vigorous individual motility. The criteria for acceptance of straws as suitable were: progressive individual motility ≤ 40% and vigour ≤ 4.

A correlation and ANOVA analysis were performed between all the ECOTEXT variables and fresh and frozen-thawed semen quality, using SPSS statistical software. A p-value < 0.05 was considered significant.

Results: The main findings of this study were that the average grey level of pixels in the ultrasound image of the testicular parenchyma (EC3) was negatively correlated with the percentage of sperm head abnormalities. A negative relationship was observed between the level of white pixels (EC2) of the ultrasound and the frozen-thawed sperm motility (r = -0.316, p = 0.025), and a positive relationship between EC2 and the percentage of proximal and distal cytoplasmic droplets (r = 0.288, p = 0.034; r = 0.316, p = 0.020, respectively). Besides, there were significant differences in the density of hypoechogenic areas between bulls that generated straws of good quality after freezing-thawing (approved) and those that generated straws of low quality (disapproved) at 0 days post-ultrasound (p <0.048).

Conclusions: Some Ecotext parameters have different levels of correlation with sperm quality, especially with sperm morphology. The density of hypoechogenic areas into the testicular parenchymal ultrasonogram is a predictor of the freezability of the sperm.

Keywords: bull, semen, ultrasound.

DI-P02

Teat canal measurements around milking using ultrasound

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Objectives: Bacterial intramammary infection is the result of microorganism entry through the teat canal. The likelihood

Table 1. Echotexture values in the testis of bulls with approved or disapproved semen straws (Mean ± SD). (DI-P01)

Group of bulls	EC1	EC2	EC3	Area (microns^2)	Diameter (microns)	Density (n/cm^2)
Approved semen straws (n=30)	14,2±9,5	29,5±36,3	86,4±9,0	11,5±2,7	122,7±17,2	141,8±15,4^a
Disapproved semen straws (n=24)	16,0±12,4	76,2±150,9	85,7±14,8	12,3±4,2	130,4±29,9	132,3±19,2^b

Values with different superscripts present significant differences (p<0.05).

of infection is higher when teat end contamination is high, when teat ends are rough, and when milking machine settings are not optimal. This study aimed to evaluate teat canal diameter and length at different points in time relative to milking, while also evaluating the effect of different iodine teat sanitizers applied post-milking.

Materials and methods: A total of 18 cows were enrolled and quarters within a cow were assigned to a 1% (CTRL) or to either of two 0.5% iodine teat disinfectant formulas (TST-B and TST-R). In total, each treatment had 18 quarters. Measurements were made at the distal, middle and proximal area of the teat canal using a 15-6 MHz linear probe at different points in time relative to milking: -120 min, before cup attachment (0 min), and 30 min post teat disinfectant application. Teat canal length was also measured at the same time points. A geometric mean was calculated for each teat based on distal, middle and proximal values.

Results: The geometric mean teat canal measurements were: 0.21 ± 0.03, 0.23 ± 0.04, 0.22 ± 0.04 cm for CTRL at -120 min, 0 min, and 30 min, respectively. TST-B and TST-R had similar values, at 0.23 ± 0.04, 0.23 ± 0.03, 0.22 ± 0.04 cm for -120 min, 0 min, and 30 min, respectively. Compared to teat canal diameter at time -120 min, around 50% of teats decreased in diameter at time 0 min, and was similar between all treatments. For the last measurement at 30 min post teat disinfection and relative to time 0 min, 12 out of 18 teats (67%) in the CTRL group decreased in diameter, 8 out of 18 (44%) in the TST-R group decreased in diameter, and 13 out of 18 (72%) teats decreased in diameter in the TST-B group. Teat canal length averaged 1.02 ± 0.04 (-120 min), 0.98 ± 0.03 (0 min), and 1.12 ± 0.04 cm (30 min) across all treatments. In the majority of teats, teat length decreased before milking (0 min) and increased by around 15% 30 min after disinfection. The increase in teat canal length was observed for 89% teats.

Conclusions: Teat canal diameter and teat canal length change relative to the time of milking. This may have practical applications aimed at preventing intramammary infections.

Keywords: teat canal, ultra sound.

DI-P03

Pulmonary ultrasonography: A valuable method to diagnose bovine respiratory disease

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Objectives: Bovine respiratory disease (BRD) is one of the leading causes of morbility and mortality in dairy calves. The presence of chronic forms of BRD causes important economic losses due to the maintenance of these animals in the farm and the lack of efficacy of veterinary treatments. An early and effective diagnostic technique could reduce the prevalence of chronically ill animals in the herds, and improve the use of antibiotics, reducing therefore the appearance of relapses and avoiding resistance to antibiotics.

The objective of this study was to compare different respiratory diagnostic techniques, and to correlate them to anatomopathological findings. Conventional techniques based on scoring of clinical signs, physical examination and diagnostic imaging techniques, including radiography and thoracic ultrasound, were employed.

It was intended to demonstrate that ultrasound is a practical and useful method for the diagnosis of BRD and to prove that a fast and economical diagnosis can be made by means of lung ultrasound, which eases the early diagnosis of sick animals before the clinical signs appear and allows to determine the prognosis.

Materials and methods: This prospective and blind study, gathered 17 calves of different ages and breeds showing chronic signs associated with BRD transferred to the Complutense Veterinary Teaching Hospital. All the patients were subjected to a clinical, ultrasonographic and radiographic examination and finally to a pathological examination after their death or sacrifice. Each diagnostic technique was evaluated by the same practitioner with the support of a clinician, a sonographer, a radiologist and a pathologist, as applicable.

The sensitivity of the different diagnostic techniques for the evaluation of the degree of lung involvement and the severity of the lesions was assessed, taking as reference the necropsy. A Spearman non-parametric test was used to detect the correlations between the different diagnostic techniques employed.

Results: The sensitivity stablished for clinical scoring, pulmonary auscultation, and thoracic radiography and ultrasound was 61%, 66%, 75% and 90% respectively.

When assessing the correlation rates between each of the examinations performed, it was observed a good correlation between ultrasound and necropsy findings both in the degree of lung involvement (0.6) and in the severity of the lesions (0.67). On the other hand, there was also a good correlation between auscultation and necropsy when determining severity (0.7). In addition, it should be highlighted the high correlation between these two diagnostic tests. According to these results, auscultation along with ultrasound are the diagnostic methods with a higher correlation with pathological lesions and therefore more useful for assessing an animal with suspected chronic pneumonia. Ultrasound allowed diagnosing lesions on both hemithorax, as opposed to radiography. The number of lesions and their classification (kite tails, consolidation, atelectasis, abscesses), allowed to establish the extent and the severity of the lesions in the affected lung.

Due to the high sensitivity obtained in the ultrasound for the assessment of the severity of the lesions (90%), it can be affirmed that the limitations described for pulmonary ultrasound do not affect its diagnostic capacity.

Conclusions: Thoracic auscultation and ultrasonography are valuable diagnostic methods for evaluating pulmonary disease, with a high correlation between them and the necropsy findings. Ultrasonography has proven to be a reliable technique, as it allows to have a fast, sensitive and specific diagnosis of BRD through the assessment of number, extend and severity of lung lesions. Thoracic ultrasound can identify indi-

cators of poor prognosis (consolidation and abscesses) that can help making purchasing, treatment or sacrifice decisions.

Keywords: calf, bovine respiratory disease (BRD), respiratory diagnostic test, thoracic ultrasonography.

DI-P04

Marbling score quantification in beef cattle longissimus dorsi muscle through b-mode ultrasound images texture analysis: A methodological study

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Objectives: Marbling is defined as the ratio of intramuscular fat (IMF) in a cross-section of *longissimus dorsi* (LD) muscle. Since IMF content contributes significantly to aroma, tenderness, and juiciness of the meat, marbling score (visual evaluation of marbling) is a major factor for market estimation of beef products.

However, marbling score assessment relies on visual inspection of the carcass, therefore is subjected to operator variability and is influenced by experience and bias of the inspector (human factor).

Determination of marbling by means of ultrasound texture analysis could permit marbling score estimation in an impartial and objective way, partly solving the human factor problem by leaving to the operator only acquisition of ultrasound images.

The aim of this methodological study was to elaborate a formula to assess mathematically the lipid content (and thus, marbling) in beef cattle LD muscle using ultrasonography.

Material and methods: 27 Charolaise heifers were enrolled in the study. After their fattening cycle, entire cuts from the 12th to the 13th rib were collected from each animal at the slaughterhouse.

After aging at 4 °C in a chilling room for 7 days, ultrasound images of LD muscle were taken from each cut with a portable ultrasound scanner (MyLabOne™, Esaote S.p.a., Genoa, Italy) equipped with a multi-frequency linear probe (SC3421, Esaote S.p.a., Genoa, Italy; 7.5 – 10.0 MHz). Probe was located directly on the investigation area, between the 12th and 13th rib, and all scans had 10.0 MHz frequency, 7 cm depth, and 100% gain.

Afterwards, each cut was dissected into muscles, fat and bones, and a sample of LD was analyzed for centesimal extraction. Lipid content of the meat was determined by extraction with petrol ether (Randall) method.

Ultrasound images were analyzed using a free image texture analysis software (MaZda v4.6; Technical University of Lodz, Institute of Electronics, Poland). The animals were divided in 3 groups depending on their quantified mean lipid content percentage in 100 g meat ± ½SD: Class 1 included below 4.24g, Class 2 included between 4.25g and 5.75g and Class 3 included higher than 5.76g.

A linear discriminant analysis using the stepwise method was performed using the lipid content quantified in the samples (LIPqa) as dependent variable and the results of the texture analysis as explanatory variables. The regression equation capable of maintaining the highest number of variables and at the same time keeping the variance inflation factor lower than 10 for all included variables was assumed as predictive model.

Receiver operator curve (ROC) and Bland-Altman analysis were then performed on the predicted lipids (LIPpred) comparing them to effective, quantified ones (LIPqa), to validate the method.

Results: Texture analysis generated about 300 parameters for each image, and 7 among these were found to be predictive and were molded in a regression equation.

The mean quantified lipid content (LIPqa) in the samples was 5.10 ±1.44 g, while the mean predicted lipid content (LIPpred) using the regression equation was 5.13±1.31 g. A linear correlation between LIPqa and LIPpred was evident (r^2= 0.76), and the ROC analysis showed how LIPpred permits estimation of lipid content percentage in the muscle with a sensitivity of 88% and a specificity of 90%. Area under the ROC curve (AUC) was 0.92.

A Bland-Altman plot was used to test the agreement between LIPqa and LIPpred, upper limit was +2.0 and lower limit -1.8 (± 1.96 SD), with mean 0.1.

Conclusions: Results from the present study suggest that precise prediction of lipid content in the meat is possible, and interfacing a computer with an ultrasound system could permit a direct estimation of lipid content in the meat. Methods to estimate lipid content, more reliable than visual marbling score evaluation, could therefore be developed. The estimation method described in this research could be incorporated into an instrument to predict intramuscular fat in the animals' carcasses directly at the slaughterhouse. Further studies are needed to validate the applicability of this method on a wider scale among cattle breeds but such technology could be a powerful selection tool for assessing meat quality.

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, Marbling Score, Texture Analysis, Ultrasonography.

DI-P05

The use of Infra-Red Thermography (IRT) in the pre-clinical assessment of fattening bulls physitis: a field study

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Objectives: Lameness is the second cause of economic losses in the breeding of fattening bulls and it is a serious issue for animal welfare. However, the aetiology of lameness is not often fully investigated and animal treatments are administered without a firm diagnosis in some cases.

The physitis, a disorder of the growth plate, is one of the most frequent osteo-articular diseases in the intensive farming systems of beef cattle.

The aim of this research was to promote an appropriate diagnostic procedure to detect the aetiology of the lameness. Moreover, the potencial use of Infra-Red Thermography (IRT) in establishing the site or area of the disease could be verified in a pre-clinical assessment before the X-ray diagnosis.

Material and methods: 18 lame fattening bulls from Charolaise breed were included in the study. The average age was 14.7 ± 1.8 months and the body weight was 674.4 ± 90.6 kg. All the bulls were housed on slatted floor. Feed was provided once a day as a total mixed ration (TMR).

In order to assess the predictive value of IRT in identifying the anatomical region, the lame animals underwent on a radiographic examination (36 limbs): 18 pathological limbs (PL) and 18 healthy contralateral limbs (HL) were observed by thermal imaging camera. The IRT examination was performed through ThermaCAM-T420, FLIR-Systems. The images were processed using the ThermaCAM Researcher software.

The data collected included measurements of the maximum (T°max) and minimum (T°min) temperature (°C), the difference between maximum and minimum values (T°max-min), and the average temperature of the images (T°average). This collected data were inherent on the metatarsus and related in the inflamed area (AR01) and in the all metatarsal area (AR02).

One-way ANOVA and non-parametric Mann-Whitney (SAS 9.4) test were carried out to compare the diagnosis of physitis performed using X-ray and temperature parameters analysed by IRT (T°max, T°min, T°max-min, and T°average) related to AR01 and AR02, both in PL and in contralateral HL. Bonferroni t-test was performed to establish inter-parametric evaluations and interactions.

Results: All lesions found were located in the metatarsophalangeal area investigated using X-ray and compared with IRT detection (98%).

Regarding the area affected by the lesion (AR01), the statistical analysis found a statistically significant effect (P <0.05) in T° max PL=31.4±3.8 vs HL=27.6±6.1), in T°min (PL=26.8±6.5 vs HL=19.5±5.7) and in T°average (PL=29.2±2.8 vs HL=25.3±3.2).

Statistical analysis relating to AR02 showed a statistically significant differences (P <0.05) in T° max (PL=31.4±3.9 vs HL=27.1±6.7), in T°max-min (PL=13.4±5.4 vs HL=10.1±3.9) and in T°average (PL=26.3±6.60 vs HL=24.4±7.9)

Considering AR01, the difference between the temperature of PL and HL (PL-HL=Δ-T°) was statistically significant for all parameters (P <0.001): Δ-T°min = +7.34 °C, Δ-T°max = +2.3 °C, Δ-T°max-min = +2.78 °C and Δ-T°average = +4.1°C.

Comparing the Δ-T°average of AR01 and AR02, a differences of 2.08°C were found in the PL compared to the HL (P<0.001).

This difference is statistically significant according to the Mann-Whitney test (P <0.05), from which it results, with a probability of incurring an error of 2.94%, that the difference in temperature between the metatarsal (AR02) and area affected by the lesion (AR01) is on average greater in the pathological limb than in the healthy limb.

Conclusions: In conclusion, this research confirm that the IRT might be considered as a diagnostic tool in the pre-clinical assessment of fattening bulls physitis. All pathological lesions were detected with an increase in temperature corresponding to the lesions diagnosed with radiographic images.

Ethical approval: All the procedures related to animals were conducted according to Directive 2010/63/EU of the European Parliament and of the Council of 22nd September 2010 on the protection of animals used for scientific purposes (Article 1, Paragraph 1, Letter b) and the Italian Legislation (D.Lgs.n. 26/2014, Article 2, Paragraph 1, Letter b). X-ray examinations from beef cattle were conducted during clinical investigations with the consent of the animals 'owner during the routinely clinical activity of the Ruminant Clinic of the Veterinary Teaching Hospital, University of Padua.

Keywords: Infra-Red Thermography; Fattening bulls; Physitis; pre-clinical assessment.

DI-P06

Ultrasonographic measurements evaluating fetal well-being in late term Holstein-Friesian cows

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Objectives: The aim of the study was to find association between sonographically measured parameters and fetal well-being (health status) and the ease of calving and neonatal stillbirth in Holstein-Friesian dairy cows.

Material & Methods: A total of 113 dried-off, asymptomatic late-term pregnant cows and their fetuses have been examined (transabdominal ultrasonography) once during the last month of gestation (245-276 days), on 2 large scale Hungarian dairy farms during the years 2016-2019. A portable ultrasound scanner (GE Logiq V2, Med-En-Trade Ltd., Budapest) with a 2,5-6 MHz macroconvex probe has been used for the fetal examinations. Fetal heart rate, aortic diameter, maximum depth

of fetal fluids, longest diameter of placentomes, average thickness of non-cotiledonary placenta, fetal activity have been measured. Calving ease (1 point=unassisted, 2 points=assisted calving, 3 points=dystocia), birth weight (kg), sex of the newborn and calf survival (until 1 month of age) data have been recorded. Test for normality, regression analysis and ANOVA have been performed in R programme (R Statistical Computing, 2018 Vienna, Austria).

Results: The average fetal heart rate was 112 ± 24 bpm (mean±SD) during the examinations. Fetal aortic diameter was 2,13±0,49 cm (mean±SD); maximum depth of fetal fluids was 26±19 cm (mean±SD); the longest diameter of the placentomes has been 4,6 ± 1,4 cm (mean±SD); average thickness of non-cotiledonary placental unit was 2,1 ± 0,5 cm (mean±SD); and the fetal activity was 1,8 ± 0,3 (mean±SD), respectively. Nine calves out of the 113 have been stillborn (died before the 48th hours of life). Calving ease was 1,8±1,1 points (mean±SD), birth weight was 38,9±6,1 kg (mean±SD).

From the above mentioned parameters, the longest diameter of the placentomes showed a significant correlation with birth weight; (cor = 0,61; P<0,001). Birth weight was significantly higher in the dystocia group (P<0,05) and among male foetuses. The average thickness of the non-coliledonary placental unit was significantly larger in the 9 stillborn pregnancy and in the dystotic group than in the unassisted group (2,5±0,9 cm vs. 1,8 ± 0,8 cm; P<0,05, respectively). No other parameter showed significant association/correlation with partal or post-partal measurements.

Conclusions: Transcutaneous/transabdominal ultrasound examinations remain valuable research tool in fetal well-being studies. The non-cotiledonary region of the placenta in cows can be a promising parameter during the last month of pregnancy in Holstein-Friesian cows, but the sensitivity and accuracy should be further evaluated and other possible measurable parameters should be tested in future studies in order to establish a fetal biophysical profile as in equine foetuses (Reef, 1995).

Keywords: fetal ultrasound, well-being, dystocia.

DI-P07

Lung ultrasonography and clinical follow-up evaluations in fattening bulls affected by Bovine Respiratory Disease (BRD) during restocking period and after Tulathromycin and Ketoprofen treatment

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Objectives: The bovine respiratory disease (BRD) is a syndrome which reduces animal well-being and increase economic losses. The clinical observations of systemic and respiratory signs are used for diagnosis. Treatment is generally based on antibiotics such as the macrolides class. The ultrasonography is a diagnostic method for an early diagnosis, useful to evaluate the treatment efficacy and clinical follow-up. The aim of this study was to evaluate animals' response to tulathromycin and ketoprofen treatment over 21 days using thoracic ultrasonography (TUS) as a screening diagnostic tool during restocking period in the farm.

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. Sixty Limousine fattening bulls of a single stock were enrolled with a weight and age of 364.5±6.42 Kg and 10.23±1.37 months, respectively. Animals were evaluated the day of restocking (T0): the clinical examination assessed the sensory status, cough, nasal and ocular discharges, and rectal temperature assessment in order to calculate the respiratory score (RS). After clinical examination, a TUS evaluation of six lung's areas (cranial 4th – 3rd intercostal space (ICS), middle 6th – 5th ICS, and caudal 10th – 7th ICS area of right and left lungs) was performed. Two groups were determinated based on ultrasonographic score (US): group C (control group) with 29 animals and US<3; group D (disease group) with 31 animals and US≥3. In the same day, the group D received a deep nasal swab for bacteriological examination and a single injection of tulathromycin and ketoprofen (2,5 mg/kg + 3 mg/kg, Draxxin plus). The group D was revaluated by clinical examination and TUS after 1.5 (T1), 3 (T2), 7 (T3), 14 (T4), and 21 (T5) days; whereas the group C was only revaluated at T5. The TUS images were used for a post-sampling quantitative assessment of hepatization and fluid alveolograms areas at each lung's area investigated. Total area of hepatization and fluid alveolograms were then calculated. The statistical differences between groups were assessed in accordance with data distribution by Wilcoxon test (US, RS, nasal and ocular discharges, lesion score) or t-test (rectal temperature, hepatization and fluid alveolograms' areas), whereas the Kruskal-Wallis test was used to evaluate differences over time. The differences in the total area of hepatization and fluid alveolograms were assessed in both cases by mixed models. A *p-value*≤0.05 was accepted.

Results: No animal enrolled in group C showed a RS or US indicative of BRD during the study. The group D showed a greater US, RS, nasal and ocular discharges, and rectal temperature compared to group C at T0, whereas only RS was different at T5. Regarding lung' lesions, the most affected lung areas were the cranial and middle lungs in both sides with greater areas of hepatization and fluid alveolograms. The total area of hepatization was 11.29±0.70 cm^2 in group D, while the total area of fluid alveolograms was 1.98±0.35 cm^2. No differences were found between groups in lung' lesions at T5 and only 4 animals showed fluid alveolograms. Despite the total area of hepatization was not different between groups, it was still present at T5 in group D (5.16±0.69 cm^2). However, the treatment was effective in reducing the US and rectal temperature from T1, and RS from T3. The lung' lesions of the single areas improved between T2 and T4, whereas the total area of hepatization improved from T1 and total area of fluid alveolograms from T2.

Conclusions: The TUS is a useful diagnostic tool to screen the respiratory diseases in farm identifying ill animals, allowing a correct management and therapy. Furthermore, TUS was a

sensitive tool to monitor treatment effectiveness and the healing time after tulathromycin and ketoprofen treatment.

Keywords: TUS; Bovine respiratory diseases; Pulmonary lesions; Clinical follow-up.

DI-P08

Ultrasonography focusing on the cranial part of the thorax is a quick and sensitive technique to detect lung consolidation in veal calves

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Objectives: In the veal calf industry, bovine respiratory disease is the main cause of morbidity and mortality. Lung ultrasonography (LUS) is an accurate technique to diagnose bronchopneumonia in calves. Due to the economic constraints faced by the industry, a screening technique able to rapidly examine large numbers of calves is required. In calves, lung lesions occur first in the cranial lobes due to airborne bacterial infection before extending to the more caudal lobes. The objective of the study is to compare 3 alternative ultrasonographic techniques focusing on different parts of the thorax (cranial + middle, cranial, middle) vs. the reference technique (LUS of the entire lung).

Materials and methods: Data on 300 veal calves (53.7% Holstein; 44.0% crossbred; 2.3% other breeds) aged 33.1 ± 8.0 days and weighing on average 67.5 ± 4.0 kg at LUS from two farms were analyzed. Systematic LUS of the entire lung was performed on all calves 14 and 6 days after the arrival on farm 1 and farm 2, respectively. A lung consolidation score (4-point graduated scale corresponding to the maximal extension of the lung consolidation lesions observed with regard to depth extension or dorsoventral extension) was given to different parts of the thorax. The ultrasound lesion score (ULS) with the reference technique was the highest score observed among the different parts whereas the ULS of the different alternative techniques was the highest score observed among the part considered: 1st to 5th intercostal space (ICS) on the right and 2nd to 5th ICS on the left for the cranial + middle technique, 1st to 2nd ICS on the right and 2nd to 3rd EIC on the left for the cranial technique and 3rd to 5th ICS on the right and 4th to 5th EIC on the left for the middle technique. The degrees of agreement between the alternative and the reference techniques were measured by Cohen's Kappa, McNemar's test and weighted Kappa. The relative sensitivity of each technique was calculated for the two farms.

Results: Agreements between LUS focusing on the cranial + middle part of the thorax or LUS focusing on the cranial part of the thorax and LUS of the entire thorax were almost perfect: K = 0.99; 95% CI: 0.98-1 and K = 0.95; 95% CI: 0.90-1 respectively. The McNemar's test substantiated these observations. The Kappa value on agreement between LUS focusing on the middle part of the thorax and LUS of the entire thorax was slight (0.12; 95% CI: 0.01-0.23) but a significant discrepancy was observed (McNemar's; $P < 0.05$) that suggested the presence of the Kappa paradox, the Cohen's Kappa value was therefore under-estimated. The relative sensitivities of LUS focusing on the cranial + middle part of the thorax or on the cranial part of the thorax only were high (>98% and 93% respectively). The agreements between scores attributed with the reference technique and the alternative techniques focused on the cranial + middle part of the thorax or on the cranial part of the thorax only, calculated using K_w, were almost perfect (0.99, 95% CI: 0.98-1 and 0.96, 95% CI: 0.91-1 respectively) whereas the K_w was 0.11 (95% CI: 0.00-0.22) for the alternative technique focusing on the middle part of the thorax only, indicating a slight agreement.

Conclusions: LUS of the cranial + middle part or on the cranial part only of the thorax are quick and sensitive techniques to identify veal calves with lung consolidation lesions shortly after arrival at the facility. Detection of calves with consolidation lesions after arrival at the facility could be an interesting tool to implement a strategy of segregation of calves at risk of negative production outcome or calves requiring closer medical monitoring. Another potential use of this screening tool would be to determine the cut-off of lung consolidations prevalence to decide whether or not to treat a batch metaphylactically with antibiotics.

Keywords: BRD, pneumonia, ultrasonography, calf.

DI-P09

Pilot study on caudal vena cava size by fast ultrasonography through different views in healthy calves.

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Introduction: Raising calves from birth to one year without excessive mortality is an important and economic challenge for beef and dairy breeders. Hence, there is an economic interest to improve treatment of calves suffering from surgical or non-surgical digestive diseases, possibly accompanied by severe changes in circulating volume status. Unfortunately, knowledge about volume status assessment in bovine medicine is poor. In human and companion animal medicine, ultrasonographic assessment of the inferior/caudal vena cava diameter (CVC_D) is used as an inexpensive, rapid, and non-invasive marker to evaluate intravascular volume status. To correct for variations in body weight, CVC_D can be expressed as a ratio to the aortic diameter (Ao_D). To the author's knowledge the caudal vena cava (CVC) has never been assessed

in calves. The objective of this study was to perform a pilot study on the feasibility to obtain ultrasonographical measurements of the CVC and the aorta (Ao) via the longitudinal right paralumbar (PV) and subxiphoid (SX) view in awake unsedated healthy calves, as previously described in dogs, and to assess whether there was any effect of age, sex, breed, or body weight on CVC and Ao measurements or the calculated CVC / Ao ratio.

Methods: We performed a single observer prospective observational study in standing healthy calves from beef and dairy herds. All calves aged less than six weeks and weighted less than a hundred kilos to facilitate manipulation, and were deemed healthy based on history and clinical evaluation excluding diseases potentially influencing intravascular status. Two anatomic sites were assessed by the investigator, to obtain three views of CVC and Ao (longitudinal view in subxiphoid site and transversal and longitudinal views in the paralumbar site). CVC and Ao measurements were recorded using a commercially available ultrasound machine (Mindray® DP50VET) with a microconvexe curvilinear probe (5-7 Mgz). Two measurements were done in subxiphoid view, minimal CVC_d ($CVC_{min\text{-}SV}$) at the end of inspiration and maximal CVC_d ($CVC_{max\text{-}SV}$) during the expiratory phase. Only one measurement of each vessel (CVC and Ao) was made in the paralumbar view because the diameter of CVC and Ao do not tend to change with the respiratory circle. The investigator measured the maximum diameter of Ao from inner wall to inner wall. Because of the irregular shape of CVC, the assessment of CVC was made by measuring area instead of diameter.

Results: 48 calves were enrolled, 22 males and 26 females, from 4 different herds. Four different breeds were recruited, mostly Belgian blue (BB) calves (32 calves), but also 15 Holstein Friesian (HF) calves and one Swiss brown (SB). The median age and weight of this group was respectively 21 days (range 1-41) and 67 kilos (range 33-98). Subxiphoid view was tried on every calf but cineloop was not recorded in 20% of the cases because of a poor visualization of the CVC. Ultrasound measurements of $CVC_{max\text{-}SV}$ and $CVC_{min\text{-}SV}$ from the subxiphoid view are reported as mean (± SD) or median (IQR), and respectively 1.12 (± 0.29) and 0.53 (0.45-0.68). Statistical analysis didn't show any correlation between CVC measurements at the subxiphoid view and age, weight, breed, sex, or herd. Paralumbar site was assessed in every calf. Cineloops were recorded on 94 % of the calves, and interpretable in 92%. A high significative linear correlation was found between the age of the calves and CVC and Ao measurements made at paralumbar views except for CVC area in transversal PV. Correlation was also found between calves' weight and Ao area in transversal PV (R^2=0.32, P<0.05).

Conclusions: CVC_D and Ao_D assessment was easily performed at the PV, and more difficult to achieved at the SV in awake and standing healthy calves. A positive correlation was found between the age of the calves CVC and Ao measurements made at PV except for CVC area in transversal PV. Current study is ongoing to compare CVC and Aomeasurements between healthy and hypovolemic calves.

Keywords: calf, caudal vena cava, ultrasound, hypovolemia.

DI-P10

Lung health of fattening calves - assessment and follow-up by ultrasound examination

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Objectives: Respiratory diseases represent the most common conditions in fattening farms, leading to calf mortality but also to significant losses due to reduced daily gains. Even subclinical disease was shown to have a significant impact on daily gains (Tejero and Bach, 2016).

Thoracic ultrasonography (TUS) allows to detect subclinical disease processes at an early stage with a high sensitivity (79.4 %) and specificity (93.9 %) (Buczinski et al., 2015) and can easily be done on calf fattening farms.

In the present study, the prevalence of subclinical respiratory diseases was determined on a fattening farm at arrival and in the following 7 weeks.

Materials & Methods: The study was carried out on a fattening farm in Lower Saxony in May 2020. A total number of 140 Simmental calves with a mean age of 60 (43-89) days were brought to the farm. At arrival all animals were treated according to the farm's established procedure (intranasal vaccination against BRSV/PI3-V, eprinomectin, amoxicillin).

During the 7-week feeding period, calves were inspected twice daily by the owner for signs of respiratory disease. Body temperature and respiratory rate of all animals were measured twice a week. Body weight was determined on the day of arrival and 7 weeks later.

At 4 days after arrival, 70 animals were randomly selected and subjected to TUS examination (US1) according to the method described by Ollivett and Buczinski (2016). This examination was repeated on the same animals 24 days (US2) and 43 days (US3) later by the same examiner. A portable ultrasound scanner (W2 wireless Scanner, Scan4Animal) with a linear probe (penetration depth:10 cm; frequency: 6.5 MHz) was used. Evaluation of the findings was performed according to the scoring system by Adams and Buczinski (2016).

Results: When examined on day 4 after arrival (US1), 49 animals (70 %) showed no consolidation in the lung tissue. In 15 calves (21.4 %) slight lung lesions (< 1 cm; score 2) were observed, while 6 animals (8.6 %) showed consolidations in the lung tissue > 1 cm (score 3+4). The follow-up examinations 24 and 43 days after the initial scanning revealed that the proportion of healthy animals decreased while the proportion of animals with both mild and severe lesions increased (US2: healthy=45.7 %, mild lesions=38.6 %, severe lesions=15.7 %; US3: healthy=34.3 %, mild lesions=44.3 %, severe lesions=21.4 %). These findings are in line with results from Tejero et al. (2019), based on TUS inover 800 calves from 9 different fattening herds.

In contrast to the TUS findings, only 7 of the 140 animals were detected as sick during the daily inspections by the owner and were treated. The diagnosis "respiratory disease" was only made twice. While one of these calves already showed lung consolidations > 1 cm (score 3) at the time of housing

which did not change over time, the lung health of the second calf clearly deteriorated in the course of time (US1: score 1, US2: score 2, US3: score 3; treatment between US1 and US2). For body temperature and respiratory rate of the animals a significant correlation with the ultrasound findings could not be observed.

Mean body weight at arrival was 76.7kg (61-87.4 kg) and after 47 days 115.1kg (93.8-150.1kg) which translated into a mean daily gain of 0.82kg/day (0.36-1.38kg/day). Using the insights from the lung ultrasound scores, it was shown that gains of > 1000 g/day could only be reached by animals with no or only slight lesions (<1cm) during the study period.

Conclusion: The present study provides clear indication that the prevalence of subclinical respiratory diseases is still highly underestimated, especially in calf fattening farms. Under practical conditions, TUS is well suited to determine the proportion of animals with subclinical respiratory disease, to follow the course of the lung consolidations and to visualize them to the animal owner. Since many animals already have lung damage when they are housed, increasing awareness of subclinical forms of the disease together with appropriate pathogen diagnostics in the herd can help adopt more targeted preventive measures.

Keywords: respiratory disease, fattening calves, thoracic ultrasonography.

DI-P11

Factors affecting lung lesions dynamics diagnosed by thoracic ultrasound (TUS) in pre weaned calves in northeast Spain fattening units

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Objective: The aim of this study was to analyze, lung lesions dynamics in pre weaned calves as obtained by thoracic ultrasonography (TUS) along with possible factors affecting such dynamics.

Material and methods: 1001 Holstein Friesian male calves from 2 commercial farms (farm 1 and 2) in north eastern Spain were randomly enrolled between February 2020 and May 2021. The same management, feeding, treatments, vaccination protocols and health program were applied in both farms. The calves came from 2 different regions in the northwest of Spain (region A: Galicia and B: Cantabria). Climatological conditions were divided 2 seasons: Cold season (November to April) and warm season (May to October).

The same practitioner performed TUS on each calf in days 2.5 ± 0.6 at arrival and 21.4 ± 1.6 days later. The dynamics of lung lesions were recorded for each calf. The results obtained from the TUS were recorded considering a score from 1 to 4; where 1 was defined as normal aerated lung with no consolidation, 2 as diffuse comet-tail artifacts without consolidation and scores 3 and 4 corresponded to mild and severe lung lesions, respectively. Afterwards, calves were classified in 3 categories: Category 2 included healthy animals that had lungs without lesions and treatment was not necessary. Category 3 and 4 corresponded scores 3 and 4, respectively. Possible links between lung lesions evolution and farm, calves origin, season and mortality were assessed.

Results obtained on farm where processed in an Excel (Microsoft, CA, USA) sheet and statistically analyzed with SAS system V.9.1.3 (SAS institute Inc, Cary, NC, USA) using a mixed model and regression logistic analysis to assess the dynamics of lung lesions and mortality, respectively taking into account the calf as random factor and the farm, calf origin and season as fixed factors.

Results: During the study period, 691 calves were admitted in farm 1 and 310 in farm 2 with an average weight of 71.9± 0.9 kg and 71.7± 0.7 kg, respectively. Calves from origin A (n=721) and B (n=280) were transported to the studied farms as follows: 498 and 193 calves from A and B origins in farm 1; and 223 and 87 calves from A and B origins in farm 2.

In total, 68 calves died after being examined 2 times by TUS. Overall mortality in farm 1 was 8.0%; 7.4% for origin A and 9.3% for origin B. In farm 2, overall mortality was 4.2%; 4.0% for origin A and 4.6% for origin B.

In the first TUS check, there were no significant differences between farms regarding to animals with lung lesions. Calves from origin B showed higher scores than their counterparts from A origin (2.67 ± 0.67 vs. 2.49 ±0.63) (P<0.0001). Farm 1 showed a higher lesion score at the second examination than farm 2 (2.83 ± 0.76 vs. 2.55 ± 0.66) (P<0.0001). Nevertheless, the difference in lung lesions evolution (DLL), calculated as the difference between the grades of the two examinations was lower for B origin calves (0.11 SD± 0.84 vs. 0.24 SD± 0.89) (P=0.05). In addition, DLL was better for farm 2 compared to farm 1 (DLL= 0.03 ± 0.79 vs. 0.29 ± 0.91) (P<0.0001).

Lung lesions evolution it was also significantly related with season, which was better during warm period (-0.19 ± 0.82 VS. 0.34 ± 0.86) (P<0.0001).

Logistic regression analyses indicated that the likelihood to die was 2 times lower in farm 2 compared with farm 1 (OR=2; CI= 1.02-3.7).

Conclusions: Farm, season and origin of calves affect lung lesions dynamics. Knowledge of its effect on the evolution of lung lesions can allow us to anticipate the appearance of BRD and take preventive measures.

Keywords: preweaning calves, fattening, Bovine Respiratory Disease, Thoracic ultrasonography, Risk Factors.

DI-P12

Factors associated with lung lesions prevalence as obtained by thoracic ultrasonography in pre weaned calves in northeastern Spain

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Objective: The objective of this study was to determine factors that affect the prevalence of lung lesions diagnosed by thoracic ultrasonography (TUS) in pre weaned calves recently arrived at fattening units in northeastern Spain.

Material and Methods: From January 2020 to February 2022, 12575 calves from 3 different countries (Spain, France and Czech Republic) were distributed in 5 different commercial fattening units and examined 1-3 days after arrival. A single veterinarian performed TUS on each calf. All lungs were classified according to the following score: 1 indicates normal aerated lung with no consolidation, 2 indicates diffuse comet-tail artifacts without consolidation and scores 3 and 4 corresponded to mild and severe lung lesions, respectively. The examined calves were assigned to three categories or grades. Grade 2: included healthy lungs scored as 1 and 2. Grade 3 and 4 included lungs scored as 3 and 4 respectively. Total lung lesions prevalence (score 3 and 4) (TLLP) was calculated for each batch. Climatological conditions were divided in 2 seasons: Cold season (November to April) and warm season (May to October). Regarding body weigh on arrival, calves were classified in three groups: low (<55 kg), medium (55-65kg) and high (>65 kg) weigh, respectively.

The following data was recorded for each calf: body weigh on arrival, country of origin, provider, batch size, season and lungs lesions (grade 2, 3, 4).

Results: The overall lung disease prevalence was 29.7% and the individual batch prevalences varied between 5.5% and 72.8%. Batch size had no effect on lung lesions prevalence according to chi-square test. Different providers presented significantly different (TLLP) (P=0.004). Body weigh on arrival affected the TLLP: the low weight group had the lowest TLLP (21.3%) while medium and high weight groups had the highest TLLP (31.8% and 29.7% respectively) (P<0.0001). Czech calves showed the lowest TLLP (23.4%) while Spanish and French calves showed the highest TLLP (30.2 and 30.95% respectively) (P<0.0001). A significant interaction between origin and season was found, the warm season increased the TLLP for Spanish calves and decreased the TLLP for French and Czech calves (P<0.0001).

Conclusions: TLLP determined by ultrasound presented a great variation across providers, origins and seasons. TUS can be a great tool to assess the health status of pre weaned calves before purchase. The effects of heat stress during the Spanish summer in pre weaned calf lung health should be further studied.

Keywords: preweaning calves, fattening, Bovine Respiratory Disease, Thoracic ultrasonography, Risk Factors.

EP-P01

A Field Study to Determine Antibody Titers of BRD Pathogens on Dutch Dairy Farms without BRD Problems.

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Objectives: The major Bovine Respiratory Disease (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 young animals and lactating cows 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 vaccination. The latter may help to develop a BRD vaccination program on dairy farms.

In this field study, antibody levels against the BRD pathogens (Parainfluenza 3 virus (PI3), Bovine Respiratory Syncytial Virus (BRSV), *Mannheimia haemolytica* and *Mycoplasma bovis*) in healthy calves and cows were determined on several Dutch dairy farms without major BRD problems and not vaccinating against BRD to have an impression on the epidemiology of some major infectious risk factors for BRD.

Materials and Methods: The study was performed on Dutch dairy farms with at least 100 lactating cows, that are not vaccinating against BRD and without major BRD problems. In 2 different farms (January 2019), all the youngstock were blood sampled as well as three groups of 10 lactating cows (respectively in first, second and third or more lactation) on the same day. On 10 other different farms (Jan-March 2019), blood sampling was performed in five calves between three and six months old, as well as in five calves between eight and twelve months.

All the samples were analysed in the Centre for Diagnostic Solutions (MSD Animal Health, Boxmeer, The Netherlands) for antibodies against *Mannheimia haemolytica*, BRSV, PI3 and *Mycoplasma bovis* by ELISA. An in house test was used to measure *Mannheimia haemolytica* and BRSV antibodies, whereas for PI3 and *Mycoplasma bovis* a commercial kit was used from respectively IDEXX and Bio-X.

Results: Although none of the farms had any obvious BRD problems, antibodies against at least one BRD pathogen could be identified on each farm, except for *Mycoplasma bovis*, that was only present in a few animals in 7 of the 12 dairy farms. Nine of the 12 farms were positive for antibodies against BRSV in several combinations with the other BRD pathogens.

Most samples were positive for *Mannheimia haemolytica* (95%), followed byPI3 (87%), BRSV (30%) and *Mycoplasma bovis* (6%). For each pathogen the level of antibodies increased with the age of the animals.

Of the 10 farms where only youngstock was sampled (n=10), 5 farms were positive for all 4 pathogens, 2 farms were only positive for *Mannheimia haemolytica*-BRSV-PI3, 2 farms only positive for *Mannheimia haemolytica* and PI3 and 1 farm only positive for BRSV-PI3. The 2 farms where all the youngstock as well as 30 adult cows were sampled, were positive for all 4 pathogens. Especially in calves between three and six months old there are huge differences in seroprevalence between farms. All pathogens were frequently identified in animals younger than 1 year, apart from *Mycoplasma bovis* that only has been identified in 15 animals (7%) out of the 205 samples from the animals younger than 1 year.

Conclusion: Nearly all dairy farms in this study (no BRD problems and not vaccinating against BRD) had seropositive animals for *Mannheimia haemolytica* and PI3. Also, antibodies against BRSV were frequently detected, however detection of antibodies against *Mycoplasma bovis* was rather an exception. Based on these findings, vaccination to protect against *Mannheimia haemolytica*, PI3 and BRSV may be a strategy to minimize the risk of a BRD outbreak on BRD-free farms, as the presence of *Mycoplasma bovis* was rather exceptional based on the results of this study.

Keywords: Bovine Respiratory Disease, serology, dairy, Netherlands.

EP-P02

Prevalence of *Histophilus somni* in Spain

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Objectives: Bovine Respiratory Disease (BRD) is the most common cause of death and disease in cattle[1]. The most common bacteria involved in BRD are *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* and *Mycoplasma bovis*. However, *H. somni* has been underdiagnosed in the last few years since this bacterium requires specific growth conditions and is easily overgrown[2]. Information on the prevalence of this bacterium in animals with suspected BRD using more sensitive methodologies is necessary for the evaluation of evidence-based preventive measures. The aim of this study was to evaluate the prevalence of *H. somni* as a pathogen involved within the BRD complex in the Spanish national herd, along with comparing results obtained by culture versus Taqman Real Time PCR (qPCR).

Material & Methods: In this study, the presence of *H. somni* was assessed in 1,396 samples from animals with suspected BRD from January 2017 to January 2020, representing 346 Spanish farms. Samples were passively obtained by a private diagnostic laboratory (EXOPOL) with the aim of determining the causal agents at the time of a BRD problem. The BRD panel offered by this laboratory contains the most common bacteria and viruses[3], including the diagnosis of *H. somni* by both culture and qPCR. All the samples were individually tested by culture. Nevertheless, qPCR was run with a commercial kit (EXOone *Histophilus-somni* oneMIX) on pools of up to 5 samples from the same farm.

All the data were collected and analysed using Microsoft Excel. The sensitivity and specificity of both techniques were assessed.

Results: The highest percentage of farms showing BRD

problems were in Catalonia (27.7%), Aragon (13.9%) and Castile-Leon (13.6%). Out of the 1,396 samples analysed, 719 represented feedlots, 287 were veal units and 120 came from adult cattle. Samples received at the laboratory included 50.6% bronchoalveolar lavages, 33.8% organs (lungs in 89% of the cases), 12.1% respiratory tract swabs and 3.5% tracheal scrapes.

When both techniques were compared, bacterial culture detected positive results for *H. somni* in 2.7% of the samples, whereas 23.0% gave positive results by qPCR. It is worth mentioning that all the positive samples on culture were also positive by qPCR; therefore, culture failed to detect 73.0% of the positive samples identified by qPCR. Consequently, the qPCR results were selected for evaluation of the prevalence of *H. somni*.

The prevalence of *H. somni* detected in this study was 23.0%, whilst differences were observed over the 3 years (18.4%, 17.8% and 28.9% prevalence was detected in 2017, 2018 and 2019, respectively). Whereas, when the prevalence distribution is analysed by the production system, 26.4%, 20.2% and 9.3% of the samples were positive in feedlots, veal units and adult cattle, respectively. With regard to the type of sample, the highest detection rate was found in tracheal scrapes (33.3%), followed by bronchoalveolar lavages (30.2%), organs (19.3%) and finally 17.8% in the case of respiratory tract swabs.

Conclusions: In this study, samples originated from both live and dead cattle and were analysed by culture and qPCR. The difference in detection of *Histophilus somni* between culture and qPCR is likely associated with the limitation of growing these fastidious bacteria and common practices implemented in the field such as the use of therapeutic antibiotics[2,4]. The results of this study confirm those previously published by Bell *et al*. 2014[4] regarding the use of qPCR assays for improving the diagnosis of bacteria.

The distribution of the samples received expressed as a percentage corresponds to the distribution of the Spanish cattle census[5]. Therefore, the results obtained herein can be considered to be representative of Spanish geography, detecting a 23.0% prevalence of *H. somni* (range 17.8%-28.9%). This prevalence is in accordance with that found by PCR in other European countries such as the UK (23.3%)[4], albeit lower than that reported in Belgium (36.4%)[2]. These results highlight the importance of including measures for *Histophilus somni* prevention on Spanish cattle farms such as vaccination programmes, together with measures against other *Pasteurellaceae* and viruses, to minimise the impact of BRD.

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Keywords: Bovine Respiratory Disease, H. somni, prevalence, PCR, Spain.

EP-P03

Prevalence of bovine respiratory syncytial virus (BRSV) infection in calves on Polish dairy farms.

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Objectives: Cattle respiratory diseases are a significant cause of economic losses associated with cattle breeding. Infections are multifactorial: viruses, bacteria and the environment. Viral infections also play an important part in the pathogenesis of bacterial pneumonia in feedlot cattle. One of the viral factors responsible for this disease is Bovine Respiratory Syncytial Virus (BRSV), which is widespread throughout the world. It is classified as Bovine *Orthopneumovirus*, belongs to the genus *Orthopneumovirus* in the family *Pneumoviridae* and is a negative-sense single-stranded RNA virus. BRSV spreads very quickly by the aerogenous route and replicates in the upper and lower respiratory tract, causing mucosal inflammation. At the initial stage of infection, nasal epithelial cells are targeted, thereby facilitating subsequent adhesion and replication of pathogenic bacterial species (through up-regulation of adhesion receptors, denudation of the epithelium, etc.). The aim of this study was to determine the prevalence and seroprevalence of BRSV in calves on dairy farms located in different regions of Poland.

Material and methods: The samples were taken from calves with Bovine Respiratory Disease (BRD) symptoms or calves from herds with respiratory problems at the time of sampling.

To demonstrate the presence of BRSV, samples in the form of nasal swabs (70%) or broncho-alveolar lavages (30%) were taken from animals showing clinical signs (i.e. high body temperature, cough, dyspnoea, etc.). All the samples originated from March 2018 to December 2019 and were collected from animals younger than 3 months. Each sample analysed by PCR represented one dairy farm. These samples could be made up by pooling up to 3 individual samples on each farm. To collect material from the nasal cavity, flocked swabs (FLOQswabs, COPAN, Italy) were used; whereas, in the case of broncho-alveolar fluid, catheters with a 20 mL syringe containing approx. 10 mL of 0.9% NaCl (Polfa, Poland) were utilised.

For serology, blood from *v. jugularis* for serological tests was collected into serum tubes (KABE LABORTECHNIK Gmbh, Germany) from 6-to-9-month-old calves. Samples were obtained from 2017 to 2019. The samples were transported, for no longer than 24 hours, at 4°C directly to the Diagnostic Laboratory at the Faculty of Veterinary Medicine, Wroclaw. Immediately after the samples were received, viral RNA was isolated using a commercial kit (RNeasy Mini Kit, QIAGEN, Germany). In the next step, another commercial kit (VetMAX Ruminant Respiratory Screening Kit, Thermo Fisher Scientific, USA) was used for detection of BRSV antigens. Real-time PCR was run using a thermocycler (CFX96 Touch Real-Time PCR Detection System, Bio-Rad, Germany).

In the laboratory, serum samples were tested for antibodies against BRSV by ELISA (test BIO K 284 - Multiscreen AbELISA Bovine respiratory, Bio-X Diagnostics, Belgium).

Samples for serology and PCR were obtained from different farms. The study complied with the quality management system (ISO/IEC 17025:2005 + API:2007 + AC:2007).

Results: Out of the 72 samples analysed for the presence of BRSV genetic material, 11.1% produced positive results. On the other hand, in 1,078 sera samples from 6-to-9-month-old calves, the seroprevalence was found to be 39.6%. From positive sera samples, slightly positive results were found in 19.3%, positive results in 35.6% and highly positive results in 45.1% samples.

Conclusions: In vivo demonstration of the presence of the BRS virus or its genetic material is possible at an early stage of infection. This test is definitely faster and more reliable than other methods, and prelaboratory activities as well as sample contamination have a negligible impact on its result. This allows you to quickly take action to reduce the negative effects of infection. Whilst the serological tests are definitely cheaper than PCR, their usefulness is limited. IgG antibodies already appear around the 13th day after infection, and in calves that received colostrum antibodies, we can detect antibodies at up to 6 months of age. In contrast, antibody detection is a good method for detecting the presence of BRSV in populations, especially calves on the farms with a potential cause of illness during the first week of life. In defence of the PCR method, authors have admitted that a low percentage of positive farms could be caused by the small number of animals sampled in most of the tested herds.

Keywords: BRSV, bovine respiratory disease, prevalence, Poland, cattle.

EP-P04

Prevalence of bacterial pathogens of Bovine Respiratory Disease in calves on Polish dairy farms.

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Objectives: The main bacterial pathogens associated with Bovine Respiratory Disease (BRD) are *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni* and *Mycoplasma bovis*. *M. haemolytica* is the main bacterial agent of BRD and has a considerable economic impact in cattle, especially in the feedlot industry. *P. multocida* and *H. somni* are also opportunistic BRD pathogens and are involved in the development of bronchopneumonia in cattle with clinical signs indistinguishable from pneumonia caused by *M. haemolytica*. *M. bovis* lacks a cell wall and is fastidious, requiring special media and techniques for its isolation and culture. *M. bovis* is often associated with chronic pneumonia, and its mechanism of action remains poorly understood. The prevalence of BRD bacterial pathogens is relatively high and it has an impact on the health of calves on dairy and beef farms. The economic impact of cattle disease on calves still remains considerable, with BRD being the most significant health problem in the modern cattle industry: it is associated with pneumonia in nursing and recently weaned calves in the first weeks of life. The control of BRD in calves is aimed mainly at bacterial pathogens through antimicrobial and vaccination programmes.

The aim of this study was to determine the prevalence of bacterial pathogens involved in BRD in calves on dairy farms located in different regions of Poland.

Material and methods: The samples (n=181) came from calves with BRD symptoms or respiratory-related signs in the herd (n=90). All the samples originated from March 2018 to December 2019 and were collected from animals younger than 3 months.

In order to show the presence of bacteria, nasal swabs (70%) or broncho-alveolar lavages (BAL, 30%) were taken from live animals showing clinical signs such as high body temperature, cough and dyspnoea. To collect material from the nasal cavity, flocked swabs (FLOQswabs, COPAN, Italy) were used; whereas, in the case of broncho-alveolar fluid, catheters with a 20 mL syringe containing approx. 10 mL of 0.9% NaCl (Polfa, Poland) were utilised. The samples were transported, for no longer than 24 hours, at 4°C directly to the Diagnostic Laboratory at the Faculty of Veterinary Medicine, Wroclaw. Immediately after the samples were received, bacterial DNA was isolated using a commercial kit (DNeasy Blood & Tissue Kits, QIAGEN, Germany). In the next step, another commercial kit (VetMAX Ruminant Respiratory Screening Kit, Thermo Fisher Scientific, USA) was used for detection of *M. haemolytica, P. multocida, H. somni* and *M. bovis* antigens. Real-time PCR was run using a thermocycler (CFX96 Touch Real-Time PCR Detection System, Bio-Rad, Germany).

The study complied with the quality management system (ISO/IEC 17025:2005 + API:2007 + AC:2007).

Results: Out of the 181 samples analysed for the presence of *M. haemolytica*, *P. multocida*, *H. somni* and *M. bovis* genetic material, 52%, 98%, 34% and 44% of samples respectively gave positive results. When the results are shown by farm, *M. haemolytica*, *P. multocida*, *H. somni* and *M. bovis* were identified on 31%, 86%, 24% and 32% of the investigated farms, respectively.

Conclusions: Information about the prevalence of bacterial pathogens involved in the BRD complex is important in animal husbandry, as it provides evidence to effectively counteract the negative effects of infections and losses from illnesses, both in the choice of treatments and preventative measures such as vaccination. In our study, the prevalence of bacterial components within the BRD complex, especially *M. haemolytica*, *P. multocida and H. somni* was high. The prevalence of these pathogens is considered to be one of the most significant health problems in the cattle industry, accounting for economic losses that surpass those incurred by all other diseases of cattle combined. *M. bovis* under conditions that suppress host immunity (e.g., stress due to weaning, transportation or viral infections), can rapidly reproduce in the upper respiratory tract and gain access into the lungs through inhalation. There, they can adhere to and colonize the lung epithelial surface,

resulting in pulmonary inflammation and gross pathology.

Keywords: bovine respiratory disease, prevalence, bacterial pneumonia, Poland, cattle.

EP-P05

BVDV subtypes in cattle herds from the Czech Republic

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Objectives: *Bovine viral diarrhoea virus* (BVDV) causes significant economic losses in cattle herds worldwide. BVDV is a member of the *Pestivirus* genus of the *Flaviviridae* family. Its genome consists of positive sense single stranded RNA of about 12.3 kb with a single large open reading frame (ORF) flanked at both ends with untranslated (UTR) regions 5´UTR and 3´UTR. BVD viruses are both genetically and antigenically heterogeneous. Two BVDV species BVDV-1 and BVDV-2 are recognized and, based on the phylogenetic analysis of the 5´UTR and Npro regions, BVD viruses are further divided into several subtypes.

A previous study dealing with phylogenetic analysis of Czech isolates revealed the existence of five distinct BVDV subtypes. The aim of this study was to further evaluate the genetic heterogeneity of BVD viruses in the Czech Republic.

Material and methods: Seventy-two serum samples of BVDV infected animals collected during a 13 years long period from 67 dairy herds were used in the study. Viral RNA was extracted from 140 µl of serum using the QIAamp Viral RNA kit (Qiagen). Viral RNA was subsequently transcribed and amplified by a Transcriptor One-Step RT-PCR kit (Roche) using specific primers for 5´UTR and Npro genome sequences. Amplification primers were then used to sequence the PCR products in both directions. The obtained partial 244 bp and 384 bp long sequences of 5´UTR and Npro, respectively, were aligned using the CluslalX software and used to conduct the phylogenetic analyses in MEGA7. The phylogenetic trees were constructed using Neighbor-Joining algorithm with evolutionary distances computed using the Kimura 2-parameter method.

Results: BVD viruses clustered in the phylogenetic tree into seven different groups representing seven separate subtypes, namely BVDV-1d (n = 41), 1b (n = 19), 1f (n = 6), 1a (n = 2), 1e (n = 2), 1h (n = 1) and 1s (n = 1) out of which two, BVDV-1h and 1s, have not been detected in the Czech Republic previously. The prevalence of dominant subtypes BVDV-1d and 1b did not differ significantly during the time and the remaining subtypes occurred only sporadically.

Two BVD viruses collected from five herds four to seven years apart were sequenced. Reinfection with different BVDV-1 subtypes was detected in two herds, while in the remaining three herds, genetic analysis revealed continuous infection with the same virus.

Conclusions: Seven BVDV-1 subtypes have so far been detected in cattle from Czech herds. The subtypes BVDV-1d and 1b predominated during the whole 13 years long period suggesting a relatively homogenous population of BVD viruses circulating in the cattle population.

Furthermore, genetic analysis is suitable to discriminate between reinfection and continuous infection with the same virus in infected herds.

The present study was supported by MZE-RO05178 of Ministry of agriculture of the Czech Republic.

Keywords: Bovine viral diarrhoea virus, phylogenetic analysis, reinfection.

EP-P06

Using a participatory approach to develop a network of sentinel veterinarians: A pilot syndromic surveillance in dairy cattle in Spain

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Objectives: As part of the of the activities carried out by the Operational Group for the improvement of dead animal collection and health alert systems (MESRASA), executed within the framework of the Spanish National Rural Development Program 2014-2020, we aim to establish a veterinary sentinel surveillance network for dairy cattle. The sentinel network will be built by enrolling private veterinarians to report routinely collected health data. The main objective of this network is to monitor selected dairy cattle health indicators, and evaluate their temporal trends within each herd. In addition, the system seeks to raise awareness among veterinarians regarding exotic diseases in order to increase the sensitivity of passive surveillance through the generation of warnings in case of an outbreak.

Materials and methods: The surveillance sentinel network is being implemented using a participatory approach in two pilot selected areas: Galicia (north-west of Spain) and Catalonia (north-east of Spain). At the time of writing, we conducted eight individual semi-structured exploratory interviews with representatives of the different profiles of private veterinarians in Catalonia. Doing this, we gained information on the main obstacles and possible incentives to the creation of the network and an overview of the existing views among private veterinarians operating in the area. We also obtained information on the files formats and management software mainly used. Moreover, a list of possible participants to the network among private veterinarians was drawn-up using non-probabilistic, snowball sampling; their final decision to participate was voluntary and free from overt. Indicators, alert levels, feedbacks and modalities of communications, which were identified through these interviews, will be further discussed in focus groups to reach a common agreement among all sentinel veterinarians. The different choices on the monitoring

system design were made by the sentinel veterinarians taking into account the herd data available in the field, the validity of indicators for herd health, and their professional interest in monitoring particular indicators (i.e. utility). In addition, the collaboration with computer engineers and software developers will provide technological support to the sentinel network and the development of ad-hoc tools for the reporting of data and the reception of automatic feedbacks.

Results: Exploratory interviews were conducted either face to face or by telephone with private veterinarians including clinical practitioners, reproduction specialists, podiatrists, and milk quality consultants. Preliminary results suggest that main factors that might inhibit veterinarians from participating to the network are linked to a low level of trust in the veterinary authorities, protection of anonymity and confidentiality and fear of the possible consequences when a warning is generated. The quality and reliability of routinely-collected data, and the effort required for data reporting were also mentioned as possible obstacles. However, the participants also showed enthusiasm for the creation of the network; they highlighted its utility in providing animal health information, contributing to create local and national references and being a useful tool for benchmarking. A set of preferred indicators were identified. These were abortions, abomasums displacements, causes of lameness, and mastitis. Moreover, the reporting of somatic cells counts and other specific laboratory-based test results was mentioned. It was proposed to report data on a monthly basis to an electronic system expressly designed for the sentinel network. Participants would like to receive a feedback report with a public section showing aggregated results and other additional health information, and a private section showing individual herd results (i.e. ranking, prevalence and trends). The definition of different warnings and response levels is currently under discussion. This study was conducted with compliance of research ethics norms and general principle established in the Code of Ethics of the American Sociological Association (ASA) and in the British Sociological Association's Statement of Ethical Practice. Before starting the interviews, participants were informed about the study objectives and formal consent was obtained.

Conclusions: The pilot sentinel network will increase data richness and facilitate the detection of changes in the occurrence of endemic diseases. Moreover, it might contribute to improve the early detection of epizootic diseases. The participatory approach used for the design of the sentinel surveillance system will increase its acceptability and sustainability.

Keywords: syndromic surveillance, dairy cattle, qualitative methodologies, benchmarking.

EP-P07

Prevalence of the main respiratory viruses in Bovine respiratory disease in Spain

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Objectives: Various viruses play a role in the onset of BRD. These include bovine adenovirus, bovine coronavirus, bovine rhinitis viruses, influenza D virus, bovine parainfluenza-3 virus, bovine viral diarrhoea virus (BVDV), bovine herpes virus type 1 (IBR), and bovine respiratory syncytial virus (BRSV). The latter three are the most important because of their clinical relevance[1].

The objective of this study was to assess the prevalence of the main respiratory viruses (BRS, IBR and BVD viruses) and their course over time to facilitate decision-making when designing evidence-based vaccination programmes.

Materials and methods: BOVIRESPCHECK is an *in vivo* diagnostic tool for identifying the main pathogens associated with BRD. Four samples from four calves are taken via nasal swabs and applied to an FTA card, thereby maximizing stability and safety when shipping the samples. Molecular diagnostic techniques (RT-PCR) are used, which have high sensitivity and specificity.

Using this kit, a total of 964 reports were created between 2016 and 2020, from a total of 661 farms distributed all over Spain (Table 1).

	2016	2017	2018	2019	2020	Total
Farms	138	134	153	154	189	661
Reports	170	169	196	212	217	964
Samples	682	684	794	842	885	3887

Table 1.

Each report corresponds to a farm that had an outbreak or respiratory problem at the time. Samples were taken from animals with early symptoms of disease at these cattle farms, which included both dairy and beef farms. If at least one sample tested positive for one of the three viruses, the report was considered positive. The number of samples refers to the number of animals sampled; 3,887 in total.

Results: The most frequently detected virus was BRSV, present in 31.5% of cases, with positivity ranging from 35.02% in 2018 to 26.42% in 2019. BVD was detected in 23.94% of all reports, varying from a peak of 31.95% in 2017 to 18.43% in 2020. IBR was detected in 17.2% of reports. Minimum positivity was detected in 2017 with 8.88%; however, the prevalence reached a peak at 28.11% in 2020 (Table 2).

55.85% of reports were positive for at least one of the three agents analysed during the study; 44.15% of reports did not provide a diagnosis, although there were variations between the years.

	2016	2017	2018	2019	2020	Total
Reports	170	169	196	212	217	964
Positive IBR (%)	17.06	8.88	19.89	10.38	28.11	17.20
Positive BRSV (%)	32.94	33.14	35.02	26.42	30.88	31.50
Positive BVD(%)	20.59	31.95	28.93	21.23	18.43	23.94
Positive Reports (%)	52.94	64.45	63.45	46.23	60.37	55.85

Table 2.

Diagram 1 shows the interactions between the different pathogens. Coinfection with the three viruses (BRS, IBR and BVD) was diagnosed in 3.34% of cases. BRSV was identified in coinfections with the IBR virus in 6.86% of cases and in 12.62% of cases with BVDV. A significant correlation (*p*-value = 0.03) of 0.07 was detected for an interaction between BRSV and BVDV. IBR and BVD were found together in 3.9% of cases.

Conclusions: During the five-year period presented in this study, BRSV was present in 31.5% of reports, which is equivalent to 56.4% of reports with a positive diagnosis.

BVDV was the second most commonly detected virus (23.94%). BVDV was found in combination with BRSV in 15.96% of cases, showing that there is a significant positive correlation between the two.

As for IBR, its incidence seemed to increase substantially over the course of 2020, with 28.11% of cases being detected. This marked increase could be a direct consequence of the legislative changes regarding the use of non-marker vaccines (including polyvalent vaccines), meaning that vaccination against this pathogen decreased during this period.

These results underline the significance of implementing complete vaccination programmes that cover all the most important respiratory viruses. BRSV and BVDV vaccination seems key not only because of the high incidence of both viruses, but also because of the positive correlation between them. The use of IBR monovalent marker vaccines should be added too, as their exclusion could be partly responsible for the increased incidence of IBR in 2020.

Keywords: Bovine respiratory disease, prevalence, IBR, BRSV, BVD.

EP-P08

Risk assessment as a decision support-tool to improve farm biosecurity

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Introduction and Objectives: On-farm biosecurity plays a key role in reducing the risk of introduction and spread of infection diseases. The adoption of biosecurity measures (BSMs) can be beneficial to prevent exotic diseases, but also diseases affecting production, or other endemic diseases subject to control programs. In this context, the development of tools aiming to support decision-making on which BSMs should be improved/implemented to reduce the probability of introduction of pathogens might be relevant. The aim of the present study was to develop a quantitative risk assessment model to identify farm-specific biosecurity measures that should be implemented to reduce the probability of infectious diseases into cattle herds, flexible enough to be adapted to different pathogens and production systems.

Material & Methods: The model was developed for BVDV and BHV1 for dairy cattle, and tuberculosis for beef cattle. For those pathogens and for each production system, the different pathways by which they could be introduced in a herd were identified. The probability of these pathogens introduction through the different pathways was calculated considering the characteristics of each studied farm and its region, the already implemented biosecurity measures and epidemiological data of each disease. Farm-level data were collected through face-to-face questionnaires conducted in North-Eastern Spain. Moreover, to identify BSMs that should be prioritized in each farm to reduce such risk, the model was run under several hypothetical scenarios, assuming the implementation of additional BSMs. Estimations of the impact of the additional BSMs on the farm-specific risk were obtained by calculating the risk reduction before and after their application. This allowed to identify what measures should be implemented to get a significant risk reduction. The stochastic analysis was performed and all non-fixed parameters were included as uncertain parameters.

Results: For BVDV and BHV1, a quarantine of at least 24 days, visited at the end of the workday and farms where cattle was tested on arrival, greatly reduced the probability of virus introduction. Not sharing transport with cattle from other farms had also a great influence in the probability of virus introduction through the purchase of animals. For indirect contacts, the analysis showed that providing farm specific protective clothing and boots and avoiding animal transport drivers having contact with animals present in the farm, would highly reduce the probability of infection through indirect contacts. In the case of bovine tuberculosis for most of the farms, recommended biosecurity measures to reduce the risk through the purchase of animals were a combination of quarantine together with adequate isolation and testing, especially if cattle had not been tested before transport.

Conclusions: The developed model can be a powerful tool to optimise the risk management on farms and support the development of farm-specific biosecurity plans. Moreover, it can contribute to educate and raise awareness on the benefits of BSMs by demonstrating the quantitative impact of their adoption.

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Keywords: Biosecurity, Infectious Diseases, Risk assessment, Prevention.

EP-P09

Prevalence of selected calf scour pathogens in Slovenia

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Objectives: Neonatal calf diarrhoea (NCD) is a highly prevalent condition. It may be caused by various pathogens of which the bovine Rotavirus and Cryptosporidium parvum are the most common. The objective of our study was to evaluate the prevalence of selected calf NCD pathogens in the Slovenia calf population with diarrhoea. We were particularly interested in assessing the prevalence of *Cryptosporidium parvum*.

Materials and methods: Field veterinary practitioners were asked to collect faecal samples from scouring calves, in the age group 6 to 21 days. Veterinarians were also asked to fill out the information on the calf: age, breed, days with diarrhoea, body temperature, medicines used, and on-farm type (dairy, beef, suckler), number of cattle, calf feeding routine and occurrence of other diseases. Investigators finally sent 68 faecal samples from all parts of Slovenia. All samples were screened using a rapid immunochromatographic test (Speed V-Diar ™ 4, Virbac, France) for the four most common calf scour pathogens (Rotavirus, Coronavirus, *E. coli* F5 (K99) and *Cryptosporidium parvum*).

Results: The median age of sampled calves was 10 days (range 4-41) and were experiencing diarrhoea for 4 days on average. The prevalence of calf scour pathogens in Slovenian scouring calves using the rapid tests were 37%, 9%, 1% and 40% for Rotavirus, Coronavirus, *E. coli* F5 (K99) and *C. parvum*, respectively. 42 % of samples were positive for only one pathogen, while 21% were positive for two or three, 37% of calves were negative for all the most common calf scour pathogens using the rapid test. 40% of scouring calves had a history of treatment with antimicrobials.

Conclusions: The prevalence of the most common scour pathogens in Slovenia is similar to those reported for other European countries. In this survey, we observed that 37% of calves were negative for all pathogens included in rapid tests. Scours aetiology in calves is not limited to just four pathogens and can be caused by others, like other strains of *E. coli*, *Clostridium* spp., *Salmonella* spp., *Coccidia* spp., *Giardia* spp., etc. Very often, flaws in management result in NCD outbreaks. We aim to use these results to stimulate farmers to vaccinate dames against neonatal scour pathogens. The study also raised the issue of antibiotic use in non-bacterial causes of calf diarrhoea.

Keywords: Neonatal calf diarrhea, Cryptosporidium, Slovenia.

FE-P01

Classification system of calves at entry point of a feedlot as a tool for estimating performance

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The objectives of this study were to develop an individual calf classification system at entry point of a feedlot to estimate the performance in the feedlot.

A classification system was created based on the following seven parameters: weight at arrival, age, presence of emaciation, presence of respiratory signs, presence of skeletal problems, and presence of umbilical disorders (minimum: 2; maximum: 10). Means for daily weight gains, days in milk, number of treatments and mortality rate were calculated for each calf classification point.

Preliminary results show that at the time of weaning higher score calves seem to have higher daily weight gains, to need less days in milk, to be subjected to fewer treatments and to present lower mortality rates than calves with lower classifications at entry point. There also seems to be a tendency for calf classifications to be linked to specific farms of origin. Inferential analysis' results will be published in a peer review journal.

This classification system seems to be an adequate, useful and easy to implement tool to identify the calves with better performance potential and to select farms of origin that provide better animals.

Keywords: feedlot, calves, assessment, performance, entry.

FE-P02

Effect of implant treatments to beef steers grazing California native range on growth performance over 192 days

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Objectives: A 192-day grazing study was conducted at a commercial California cattle ranch to compare growth performance by steers implanted with one of 3 experimental treatments, which included a traditional implant intended for grass-fed cattle, long-acting implant intended for grass-fed cattle, or non-treated negative control treatment.

Materials and Methods: Crossbred steers with British, Continental and/or *Bos indicus* breeding were sourced from Mexico. Cattle (n = 518; initial BW = 280 ± 1.8 kg) were randomized to treatments and implanted with Synovex® One Grass (SOG; 150 mg trenbolone acetate, 21 mg estradiol benzoate; n = 179), Revalor®-G (REVG; 40 mg trenbolone acetate, 8 mg estradiol; n = 180), or nothing (CON; negative control; n = 155) in a study with a completely random design. Steers grazed winter range consisting of ryegrass, wild oats, bromegrass, filaree, and vetch. Steers were managed as a single cohort throughout the study, and were rotated through 4 pastures (808 hectares) as forage and water availability dictated. No supplemental energy or protein was offered, and supplemental mineral was provided in trace mineral salt blocks. Following measurement of individual animal weights, experimental treatments were applied to cattle in October 2018 (Day 0). Cattle were also vaccinated with a clostridial vaccine and dewormed on Day 0. Individual final live weights were measured 192 d after treatment (April 2019) when cattle completed the study. Other data included daily observations included assessments for evidence of morbidity and mortality. Data observations were performed and recorded by ranch personnel independent from Zoetis. Data were evaluated by a general linear mixed model to evaluate the fixed effect of experimental treatment.

Results: Mean weights on Day 0 were 278.0 kg, 281.0 kg and 281.4 kg for CON, REVG, and SOG, respectively and did not differ between treatment groups ($P = 0.38$). Final weights obtained 192 days after treatment were 409.0 kg, 435.6 kg and 440.6 kg ($P < 0.0001$) for CON, REVG and SOG, respectively, with REVG and SOG both greater than CON ($P < 0.0001$), but not different from each other. Cattle treated with REVG gained 23.4 kg more than those treated with CON ($P < 0.0001$), while cattle treated with SOG gained 28.4 kg more than CON ($P < 0.0001$). Cattle treated with SOG gained 5 kg more than those treated with REVG ($P = 0.077$). Average daily gain was 0.68 kg/d, 0.81 kg/d and 0.83 kg/d; REVG and SOG were greater than CON ($P < 0.0001$), but not different from each other ($P = 0.077$). Of cattle that began the study, 3 CON, 2 REVG and 4 SOG cattle did not complete the trial. At completion of the trial implant sites were palpated to assess implant retention; implants were palpated in 97.8% of REVG and 98.3% of SOG steers. At time of study reporting an estimation of economic impacts of implant treatment choices was performed. Using a conservative value of $2.97/kg live bodyweight for calves in the weight range observed at study completion, and values of $5.25 and $1.39 for SOG and REVG, respectively (ValleyVet Supply, Marysville, KS), SOG returned $79.16 more than CON and $10.96 more than REVG.

Conclusion: Under the conditions of this study a single dose of SOG, an implant labeled to promote growth rate for up to 200 d, resulted in average daily gain over a grazing period that averaged 192 days in duration of 0.83 kg/d, which was significantly higher than average daily gain by steers that remained untreated (0.68 kg/d). Cattle treated with a single dose of REVG gained significantly faster than non-treated controls (0.81 kg/d) over 192 d, but numerically slower than SOG cattle. There was no impact of treatments on morbidity or mortality, and there were no observed adverse drug effects attributable to experimental treatments.

Keywords: cattle, stocker, implant, growth rate, average daily gain.

FE-P03

Seroprevalence of respiratory pathogens in calves at arrival in German rearing units

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Objectives: Respiratory disease is the biggest health problem in calf rearing. Especially after the grouping of several animals at the arrival in calf rearing units, there is a high infection pressure. In order to better understand the immunological background and to implement suitable vaccination programs, it is important to identify serological antibodies of circulating respiratory pathogens.

In a previous study, the seroprevalence of respiratory agents against Bovine respiratory syncytial virus (BRSV), Bovine parainfluenza 3 virus (PI3-V) and *Mannheimia haemolytica* (Mh) in unvaccinated calves in breeding farms was measured in calves from 5 to 60 days old (Stemme et al, 2017)[a]. In the current study, calves with unknown vaccination status from many different supplying farms were sampled at the time of arrival to identify the serological antibodies of circulating respiratory pathogens.

Material & methods: An observational field study was performed on 2 beef rearing farms over two years (2017-2018) in the Northwest and South of Germany. Blood samples were taken from a random proportion of the calves at arrival. The vaccination status of the calves or their mothers was unknown.

The neutralizing antibodies against BRSV and PI3-V were determined by standard methods. The titers are expressed as log10. An ELISA was used to measure antibodies to Mh and the titers are given as log2.

Results: A total of 1127 calves on the 2 farms (farm A n=931, farm B n=196) were included in this study. Serum samples were taken from 143 randomly selected calves (farm A n=98, farm B n=45). The animals were between 17 and 70 days old (average 42 days of age) at the time of sampling.

Two calves had no detectable titers for all three respiratory pathogens, implying the absence of maternal antibodies and no recent infection with those pathogens.

All other calves had differently distributed antibody levels. Of the 143 sampled calves, 141 had antibodies against at least 1 of the pathogens tested. The vast majority had antibodies against all 3 pathogens. Most probably those antibodies are mainly maternally derived as shown in another preliminary study Stemme (2017)[a] including younger, non-vaccinated calves.

Looking at the trendlines of the recorded serologic data, there is a tendency towards a decrease in the level of titers with an increasing age. This is more pronounced for the viral pathogens. With increasing age, the proportion of actively formed antibodies increases due to contact with circulating field pathogens. Jozan (2021)[b] presented similar results for all three pathogens in the period between 2nd and 4th month of life.

This study shows that in German calf rearing operations a high percentage of cattle have antibodies against BRSV, PI3-V and Mh at arrival, which is supportive for an endemic status given that dam vaccination is nonexistent or very low.

Conclusion: Calves arriving at rearing units have high seroprevalences of BRSV, PI3-V and Mannheimia haemolytica. The titers are decreasing with an increasing age at arrival, illustrating the importance of maternally derived antibodies and its possible effect on vaccination. As not every respiratory vaccine is effective in the presence of maternally derived antibodies, it is important to understand the characteristics of the implemented vaccines as well as the seroprevalence of the vaccinated animals.

References

a. Stemme, K. et al. (2017): Seroprevalence of respiratory pathogens in young calves in Germany; Poster EBF 2017.

b. Jozan, T. (2021): Exposure of calves to the main respiratory diseases in 16 dairy farms in western France / Exposition des veaux aux principaux agents respiratoires dans 16 élevages laitiers de l'Ouest de la France; Poster GTV 2021.

Keywords: Bovine Respiratory Disease, respiratory pathogens, seroprevalence, rearing units, Germany.

FE-P04

Respiratory vaccine shows positive effect on growth in Danish calf rearing operations

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Objectives: Scientific documentation on the effect of vaccines against respiratory infections under practical conditions is limited. When vaccines are used in a calf herd, the infectious pressure is usually complex, and the farm situation may differ from the conditions under which the vaccine has been initially tested.

The objective of this trial was to better understand the possible effect of intranasal vaccination with a bivalent live modified intranasal vaccine (Bovilis® INtranasal RSP® Live, MSD Animal Health; nationally registered product name: Bovilis RSP Live Vet in Denmark) in Danish calf rearing operations.

Materials & methods: The effect of intranasal vaccination with a bivalent live modified intranasal vaccine (Bovilis® INtranasal RSP® Live) at the arrival to a rearing operation was tested in two major calf herds. The calves were housed in pens with 5-6 calves in each pen without contact of calves between pens.

The pen-groups were divided in pairs. The calves in two paired pens resembled each other as much as possible, with respect to sex, breed, and size of the calves. One group was randomly allocated for vaccination and the other group remained unvaccinated.

Calves in the vaccinated group were vaccinated on the day

of arrival or on the following day. All calves received also one treatment with a long-acting antibiotic (Zuprevo®, MSD Animal Health or Draxxin®, Zoetis) within the first week after arrival.

The two groups of calves were compared for number of treatments for respiratory infections, daily growth, and mortality during the first 10 weeks after arrival. Treatments within the first five days after vaccination were not included, as a protective immunity from the vaccine can only be expected after five days.

Results: In total, 720 calves were included in the trial (600 calves from one herd and 120 calves from the other). The vaccinated calves grew on average 34 grams more per day than the non-vaccinated control calves over a period of 10 weeks (p=0.017). No significant difference was found in the proportion of calves treated for respiratory infection or in their mortality. The use of long-acting antibiotics in the 1st week after arrival in the present study combined with the absence of a major respiratory outbreak may explain the lack of difference in the proportion of treated calves.

In a similar trial performed by Seges Innovation (not published) with another intranasal BRD vaccine (Nasym®, Hipra), an effect on the proportion of calves treated for pneumonia was seen while there was no effect on growth. Other differences between both trials may also relate to how early signs of disease were detected, as well as when and how the treatment decisions were made. In both studies the decision about when to treat sick calves was taken by the farm staff, following their standard protocols. In both studies farm staff were instructed in how to screen for respiratory disease using the Wisconsin scoring system (McGuirk 2014). However, different people can still have different thresholds for treatment.

The BRD vaccination had positive effects for the calves even without a major respiratory outbreak during the trial period.

Conclusion: Vaccination at arrival with a bivalent intranasal respiratory vaccine (Bovilis® INtranasal RSP® Live, MSD Animal Health) had a positive impact on daily weight gain in Danish calf rearing operation.

Keywords: Bovine Respiratory Disease, intranasal vaccination, growth effect, calf rearing operation, Denmark

FE-P05

Impact of injectable mineral supplementation on the growth of beef cattle fed on a rotational grazing system in Uruguay

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Objectives: Phosphorus (P) deficiency is the most common mineral deficiency worldwide in grazing ruminants. Based on the existing literature, P content of Uruguayan pasture ranges between 0.12 and 0.14%DM. Most deficient areas are located in the North and North Eastern districts of the country. Phosphorus deficiency causes low body weight gain, decreased milk production, and poor reproductive performance that are easily confused with undernourishment, protein deficiency, and various parasitic infections. Authors of the southernmost part of South America have recommended that all categories of cattle must be supplemented when P content is below 0.14%DM, and cows when below 0.16%DM. However, numerous studies have shown that supplying phosphorus above the requirements of cattle does not improve reproduction, milk production or live weight gain. The objective of this work was to demonstrate that ad-hoc injectable supplementation with a combination of P, Cu, Se, Mg and K may have a positive impact on the weight gain (WG) and average daily gain (ADG) of grass-fed cattle.

Materials & Methods: The experiment took place in an intensive pasture-based beef cattle operation located in Durazno, central Uruguay, generally not considered a P-deficient area. Animals previously maintained on native pastures before the trial, were moved to a rotational grazing system with Rye Grass, Festuca and Clover at the start of the experiment. Paddocks were fertilized with phosphate, potassium chloride and urea, according to applicable recommendations, and free access to water was provided. Animals moved to another paddock every day and the rotational grazing system did not allow a convenient dietary mineral supplementation. One hundred and twenty 18 mo. cross-breed (Hereford x Angus) steers and 150 heifers (same age and breed) were enrolled in this study. Animals were weighed on Day0, D40 and D60. One-third of the animals were randomly allocated to treatment group F2, another third to group F3 and the remaining animals were not treated (F0). Treatment consisted of two injections (F2 group, on D0 &D14) of a combination of P (glycerophosphate and monosodium phosphate), Cu, Se, Mg and K (COMPLEPHOS®/FOSFOSAN®, Virbac Santa Elena, Montevideo, UY, according to product label), or 3 injections (F3, D0, D14 & D28). Animals were bled on D0 and D28 for the assessment of phosphataemia [Pi]. WG and ADG were compared with Z-test and the level of significance was 0.05.

Results: At the start of this trial, heifers were somewhat heavier than males (approx. +50kg), therefore results are presented separately. On D40 and D60, F2 and F3 animals gained more weight than F0 controls, and the ADG was also greater. Compared to F0, both WG and ADG were significantly higher ($p<0.05$) in F3 animals on D40, in both steers and heifers. Same differences ($p<0.05$) are also observed on D40 in group F2, but for the ADG in heifers. Compared to F0, WG was significantly ($p<0.05$) higher on D60 after one (F2) or two (F3) injections of minerals. No significant differences were detected between F2 and F3 groups, but in the WG of steers on D60. On D0, [Pi] in steers and heifers differed significantly ($p>0.05$) with 6.38 mg/dL (95%CI[6,11;6,65]) and 4.22 mg/dL (95%CI[3.83;4.61]), respectively. Since reference ranges for [Pi] given in the literature are 1.9 to 2.6 mmol/L (6.0–8.0 mg/dL) for adult and growing animals, heifers were seriously hypophosphataemic. On D28, [Pi] in heifers was significantly ($p<0.05$) improved with 5.82 mg/dL (95%CI[5.54;6,10]), whereas [Pi] did not change in steers.

Conclusion: Under Uruguayan pastoral conditions, the growth of grass-fed beef cattle can be improved by a strategic injectable mineral supplementation providing P and trace minerals instead of uncertain dietary mineral supplements.

Injectable phosphorus supplementation seems beneficial to both animals with normal phosphataemia and marginally deficient ones.

Keywords: trace minerals, beef cattle, grass-fed.

FE-P06

Evaluation of the acceptability of an injection of a multimineral solution in veal calves

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Objectives: Veal is the meat from calves. The veal industry mainly uses male calves, which are, in essence, inevitable by-products the dairy sector cannot valorise. The light colour of veal results from exclusive milk feeding and a progressive, however safe, iron deficiency hypochromic microcytic anaemia. Veal operators monitor this anaemia through red blood cell indices, mean cell haemoglobin concentration ([Hb], g/dL) and the mean cell volume (MCV, µm3). Intense milk feeding promotes oxidative stress, and calves could benefit from antioxidant supplementation, such as trace minerals. However, consumers are sensitive to veal light colour, a criterion that strongly influences the product's value; then, such supplementation should not deteriorate the carcass quality. The objective of this study was to verify the absence of harmful effects of an injection of minerals at the start of the fattening period and propose research hypotheses.

Material and Method: In a specialised veal farm in France, the haematological status of a batch of newborn calves was monitored for six months until their slaughter. [Hb] and MCV were systematically measured in 14-21 day-old calves, 70 days later, and 30 days later again for some animals (measurements 1,2 and 3, respectively). When results are known, animals with [Hb2]≤9.00 should receive a proportionate injection of iron dextran (FeDx) to avoid severe anaemia ([Hb]≤7.5 or MCV≤26). A subset of animals (MM group) also receives a single injection of trace elements at the first sampling time (MULTIMIN®, Virbac, France, according to the manufacturer's recommendations). During the first and second checks, blood samples from these animals were also analysed for mineral balance (Cu, Zn, I, Mg) and erythrocyte glutathione peroxidase (GSH-pxe). Reactive oxygen species (ROS) and serum antioxidant capacity (SAC) were evaluated using two commercial tests (d-ROMs and OXY-Adsorbent tests, Diacron International, Italy). The results are presented as ROS/SAC or oxidative stress index (OSi). Carcass traits were checked at the slaughterhouse. The significance threshold is 0.05.

Results: Forty-nine animals (MM group) were enrolled in this study, and their results were compared with those of 128 contemporary herd mates (CONT), which entered the farm on the same day, from multiple sources in France. Neither [Hb1] nor MCV1 differed significantly between the groups on the initial sampling. The proportion of anaemic animals ([Hb1]≤9 g/dL) was slightly lower in the MM group than in the CONT group (26.5 vs 31.3%, P=0.540, Chi-2). At the second control, [Hb2] differed significantly between the groups (P=0.047, t-test), and the relative risk for an MM animal to present [Hb2]≤9g/dL was RR=0,716 (CI95%[0,495 ;1,035]). Conversely, the treatment avoided one injection of FeDx every 6,167 animals. The difference in [Hb] was partly conserved for [Hb3] (RR=0,880, CI95%[0,748;1,036]). No differences were noted for MCV at any endpoint. The carcass colour distribution did not differ between groups (P=0.416, Kruskal-Wallis rank-sum test). No lesions at the injection site were noted in the MM group. On the other hand, if the majority of the carcasses scored O- or P+ (U.R.O.P score), the CONT carcasses are more often classified O- than those of the MM group (RR=2,171, CI95%[1,706;2,765]). Carcass weights did not differ significantly. The median value of [Zn] collapses significantly between the first and second samples (CI95% [22,62;25,06 µmol/L] vs [14,38;15,78], P<0,05, paired t-test), whereas [Cu] remained stable. Median [I] increased significantly, as well as [GSH-pxe] (CI95%[397;509U/gHb] vs [669;719]). The ROS concentration, as well as the SAC, decrease significantly. As a result, the median OSi tends to improve (CI95% [0,47;0,54] vs [0,41;0,48], arbitrary unit, P>0,05). In the absence of a comparative group, interpreting this evolution is difficult, especially since the literature is silent.

Conclusion: Injectable mineral supplementation does not seem to have a deteriorating effect on the quality of the carcasses of veal calves. The prophylactic treatment carried out on newborn calves could make it possible to reduce the number of corrective interventions for anaemia on animals that have become larger, and without individual restraint, at a rate of 29 animals for a herd of 180 calves. Beyond better control of progressive anaemia, the health benefits of such supplementation remain to be assessed. In particular, the influence of treatment on the oxidative status of calves deserves specific and in-depth work.

Keywords: trace minerals, veal, anaemia, meat quality, oxidative stress.

FE-P07

The epidemiological impact of bovine respiratory disease complex in feedlot cattle from three Brazilian states

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Objectives: The aim of this study was to provide epidemiological data relative to BRD in cattle from three Brazilian states, as well as its economic impacts on livestock production during a two-years-period (2019-2020).

Material and methods: This study analyzed data from 10

beef cattle feedlots in Brazil with animals of various breeds (nellore, nellore mixed breed, dairy cross, and industrial crossing), located in the states of Goiás, Minas Gerais and São Paulo. The cattle in these feedlots came from different Brazilian states and were non-castrated steers aged 24 to 36 months, with an initial average body weight of 369.7 ± 74.97 kg. A total of 699,526 animals in these feedlots were monitored daily by technicians and veterinarians under the supervision of a veterinarian responsible for the feedlot welfare and health. All animals exhibiting significant signs of any pathology were promptly isolated, examined and treated individually by the veterinary staff. The data obtained on clinical diagnosis, therapy, morbidity, and mortality were tabulated and used as data for the results of this study.

Results: The origin of the animals was 61.2% (428,103/699,526) from São Paulo, 20.5% (143,413/699,526) from Minas Gerais and 18.3% (128,010/699,526) from Goiás, with five, four and one feedlots, respectively. The animals were fed for an average of 106.4 ± 18.23 days on feed until they reached approximately 525.1 ± 73.89 kg of body weight, with an average daily weight gain of 1.46 ± 0.31 kg. The study's overall crude morbidity was 3.6% (25,132/699,526), while general mortality was estimated at 0.38% (2,661/699,526). The morbidity rate attributed to BRD was 2.08% (14,559/699,526) and the mortality rate from BRD was 0.08% (588/699,526). When the mean morbidity frequency distribution for BRD (n=14,559) was evaluated, it was shown that 29.1%, 62.4%, and 82.6% of cases occurred in cattle within the first 15, 30, and 60 days on feed, respectively. The distribution of mortality frequency caused by BRD (n=588) was 16.8%, 40.1%, and 64.2% of deaths occurred within the first 15, 30, and 60 days on feed, respectively. The average daily gain (ADG) in healthy bovines was 1.47 ± 0.31 kg/d, while in cattle with BRD was 1.33 ± 0.36 kg/d (*P<0.0001*). The projected loss associated with BRD mortality was $777.98/animal and morbidity was $51.4/animal.

Conclusion: In conclusion, the BRD complex significantly impacted morbidity and mortality in the feedlots of the three Brazilian states evaluated, during the period 2019-2020.

Keywords: BRD complex, beef cattle, economic impacts, mortality, morbidity.

FE-P08

Metabolic profile of cattle fed with high-grain diets

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Objectives: We aimed to evaluate to metabolic profile of cattle fed with two different high-grain diets, with 70% and 100% of concentrate.

Materials and methods: The study was conducted at Santarém, Amazon, Brazil. Twelve non-castrated health cross-breed cattle aged 14-16 months and with 281 ± 28 kg of body weight were divided into two groups (n = 6): the conventional diet group (C), which received 70% concentrate [90% ground corn + 10% protein and mineral supplement (Engordin 10®, Agrocria Animal Nutrition, Brazil)] and 30% corn silage on dry matter basis; and the whole-grain group (WG), which received a diet with 100% concentrate diet [85% whole-grain corn and 15% protein and mineral supplement (Engordin 38®, Agrocria Animal Nutrition, Brazil)]. The diets were isoproteic and the ingredients were mixed and offered as a complete meal twice a day (07:00 and 17:00). The animals were housed in individual stalls with feeder and water supply. Feed remains were weighed daily and each animal received an amount of feed enough to provide a 5% surplus. The experimental design was in randomized blocks and the animals were blocked by weight. The study lasts 80 days, with 16 days of diet adaptation period and 64 days of evaluations. The body weight was determined weekly for diet correction. Blood samples were obtained in the morning, before feeding, at the following days (D): D0 (baseline, after the adaptation period), D7, D21, D35, D49, D64. Serum and plasma samples were used for biochemical analyses, which were performed through an automated biochemical analyzer (Labmax 240, Labtest Diagnóstica S.A., Lagoa Santa, MG, Brazil). Serum was used to determine the concentration of (TP), albumin, urea, creatinine, cholesterol (COL), triglycerides (TRI), total bilirubin (TB), direct bilirubin (DB), calcium (Ca), phosphorus (P) and magnesium (Mg), as well as aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and creatine kinase (CK) enzyme activities. Plasma samples were used to evaluate glucose, lactate, β-hydroxybutyrate (BHB) and non-stratified fatty acid (NEFA). Data were analyzed using the PROC MIXED repeated measures of the SAS with 5% significance. Statistical analysis considered the effects of time, treatment and interaction.

Results: The serum urea was the only variable different among WG and C animals. Urea concentration was higher (p <0.001) in C (13.5 ± 0.8 SEM mmol/L) compared with WG (8.9 ± 0.9 mmol/L) animals, probably due to the greater amount of non-fibrous carbohydrates in the whole-grain diet that resulted in greater microbial utilization of nitrogen in the rumen. Both groups had sharp decrease in BHB (0.34 ± 0.02 at D0 to 0.18 ± 0.02 at D7 mmol/L), lactate (5.4 ± 0.9 at D0 to 1.8 ± 0.4 at D7 mmol/L) and NEFA (0.2 ± 0.01 at D0 to 0.09 ± 0.01 at D7 mmol/L) concentrations starting as earlier as D7 and remained with low values until the end of the study. Mg, P, AST, GGT, CK, BT, BD TP and ALB did not present any difference within the factors analyzed (diet, time and interactions). The COL and TRI concentrations were affected by time but no pattern could be observed. Regarding energy metabolism variables, glucose increased during the study (p = 0.004) but lactate (p= 0.0006), BHB (p < 0.001) and NEFA (0.0003) decreased, what corresponds to a pattern of high energy availability due to the high grain diets. Cattle fed with whole-grain diet probably have a higher efficiency of microbial protein synthesis observed by the lower serum urea values.

Conclusions: Cattle fed whole-grain diet had minimal metabolic alteration compared with the conventional feedlot diet used in Brazil (70% concentrate).

Keywords: Bovine, feedlot, whole-grain, energy metabolism.



GB-P01

Decoding the polymorphisms of heat shock protein (HSP) genes in native Portuguese bovine breeds

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Global warming is one of the most challenging and dangerous environmental factors affecting livestock animals' sustainability. High environmental temperature and humidity induces heat stress (HS), which leads to negative repercussions on animal fertility and productivity. Heat Shock Proteins (HSPs) allow maintaining cellular function, physiological parameters and fertility during HS. This study aimed to identify genetic polymorphisms of HSP70/90 family genes in two native bovine breeds well adapted to the particularly extreme environmental thermal conditions of southern Portugal. Genomic DNA was extracted from the venous blood of Alentejana (n=191) and Mertolenga (n=190) cattle and 74 SNPs of both HSP70 and HSP90 gene families were identified by using the Axiom™ Bovine Genotyping Array. Chi-square test of independence and Fisher's exact test were conducted between genotype information (heterozygosity/homozygosity) at each SNP position and breed (Mertolenga/Alentejana). Preliminary results showed that in 74 SNPs studied, 14 were homozygotic (HSP70, n=12 and HSP90, n=2). There was a significant association ($p < .05$) between genotype and breed in both HSP70 (60% of SNPs) and HSP90 (86% of SNPs) genes, namely in the SNPs associated with the following isoforms: i) HSP70 (HSPA12B, HSPA13, HSPA14, HSPA2, HSPA4 AND HSPA9), ii) HSP90 (HSP90AB1, HSP90AA1, HSP90B1). Among these, the association (Cramer's V effect size) was Large (3%), Medium (38%) and Small (59%) in HSP70, and Medium (67%) and Small (33%) in HSP90 genes. Overall, a significant association ($p<0.05$) between genotype and breed was also observed among all SNPs. These results indicate that high-density SNP arrays constitute alternative platforms for genome-wide SNP genotyping, representing a valuable tool for research into the genetics of cattle Heat Shock Protein gene families. Furthermore, the observed association between breed and genotype, unravel new horizons to a sustainable selection that can help tackle future climate changes.

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Keywords: Heat Stress, Portuguese bovine breeds, Polymorphisms, Heat shock protein.

GB-P02

Genome-wide association study for tail alterations in German Holstein dairy cows

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Objectives: Tail injuries and pathological alterations have been reported in many species. In cattle, they were investigated mainly in fattening bulls and feedlot cattle. In dairy cows high prevalences for different tail alterations were found. However, aetiology and pathogenesis of this health trait are still unclear and need further investigation. Out of 4443 phenotypes of different tail alterations we assorted seven groups common in dairy cows: 1. very tip of the tail , 2. ring-like, 3. scurf, 4. swelling, 5. thinning, 6. axis anomaly, and 7. verruca-like mass. The objective of this study was to identify genomic regions that may influence the occurrence of different tail alterations in dairy cows, which could be useful for a potential implementation of a genomic selection tool for more robust and healthy cows in the future.

Material and methods: Occurrence data of each tail alteration group were collected monthly from 167 German Holstein cows. The cows originated from a German 1300 cows dairy herd. Data collection was performed from May to December 2021, since calving of all included cows was from April to May. The cows were in their first to seventh lactation. The phenotype was encoded binary, where 0 means the absence and 1 the presence of a tail alteration group within the whole timespan.

For 118 cows, Illumina EuroG10k genotypes were available and imputed up to 45k (FImpute). The remaining cows were genotyped with the Illumina EuroG MD (V1, V1.1, V2) with 45613 SNPs. After quality check (only segregating SNPs, at least two groups with a minimum of 10 observations, no duplicated markers, a minor allele frequency of 1%, and within Hardy-Weinberg-Equilibrium $P>0.01$), 41062 SNPs remained.

A genome-wide association study was performed using the software GEMMA and the univariate linear mixed model. Each tail alteration group was treated as a separate phenotype. A standardized relatedness matrix was included in the model and calculated on SNP chip data to consider the population stratification, since many half-sib groups were present. The lactation (1st, 2nd, ≥3rd) was included as covariate. The genotype matrix was included in the model and the effect size per marker was estimated and tested for significance using a Wald test.

For positional candidate gene analysis, genomic regions around top markers ($P < 0.0001$) of 325kbp were considered, since the linkage disequilibrium decay analysis gave a mean r^2 of >0.61 within this distance. The marker positions are given

on the ARS-UCD 1.2 *Bos taurus* genome assembly.

Results: In total 51 top markers resulted for all seven tail alteration groups, whereof one marker reached Bonferroni-corrected genome-wide significance threshold for tail alteration group "thinning" (BTA1: rs42577957, $-\log_{10}(P)$ = 9.22). The markers were found on 18 different chromosomes. Close to these markers, 65 positional candidate genes reside. Among them *CCDC122* (rs42421906, $-\log_{10}(P)$ = 5.46), which was associated with the phenotype "scurf" in our analysis. *CCDC122* is one of the top differentially expressed genes in liver metabolism in pigs showing swine inflammation and necrosis syndrome (Ringseis et al., 2021). This syndrome results in severe tail alterations in pigs as well.

Conclusions: This first genetic investigation of tail alterations in dairy cows showed the potential of finding genetic markers for this novel health trait. Nonetheless, it is recommended to increase the sample size of cows and to further investigate the cause of tail alterations, to substantiate the reported phenotypes.

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Keywords: animal health, GWAS.

GB-P03

Genetic parameters and genome-wide association study of digital cushion thickness in Holstein cows

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Objectives: The digital cushion of the bovine claw is composed of three parallel pads of soft fat and loose connective tissue and thought to play an important role in protecting the corium from mechanical forces in the foot (Lischer and Ossent, 2002; Räber et al., 2004, 2006).The digital cushion is implicated in the development of sole lesions (sole hemorrhage and sole ulcers). The objectives of this study were to estimate genetic parameters for digital cushion thickness (DCT) and sole lesion severity at different stages during a production cycle, and to identify candidate genes associated with DCT.

Materials and Methods: A cohort of 2,352 Holstein cattle were prospectively enrolled on four farms and assessed pre-calving, immediately after calving, in early lactation, and in late lactation. At each time point, sole lesion severity was recorded by veterinary surgeons, and ultrasonographic images of the digital cushion were stored and retrospectively measured at two anatomical locations. Animals were genotyped and pedigree details extracted from the national database. Genetic parameters were estimated for sole lesion severity and both DCT measurements using univariate linear mixed models, following a single-step approach, implemented in BLUPF90 software; bivariate models were used to estimate genetic correlation between DCT and sole lesion severity at each stage and between stages. Single-marker and window-based genome-wide association analyses of DCT were conducted using repeatability models; candidate genes were mapped to the genomic markers or windows with the largest effects.

Results: Heritability estimates of DCT ranged from 0.18 – 0.31 and 0.12 – 0.22 depending on the location of DCT measurement, genetic correlations between these different DCT measurements were 0.96 – 1.00. The genetic correlation between DCT and sole lesion severity was generally negative, notably between DCT immediately after calving and sole severity in early or late lactation, and between DCT in early lactation and sole lesion severity in early or late lactation. There was a polygenic architecture to DCT, the top markers and windows were mapped to genes which have roles in inflammation, fat deposition, bone development, and carcass weight.

Conclusion: The heritability of DCT indicates an opportunity to change this trait in a population through selective breeding. The pattern of genetic correlation between DCT and sole lesions at different stage of production lends support to the prevailing hypotheses of sole lesion pathogenesis. Candidate genes highlighted add further information regarding the biological control of DCT, but further studies are needed to explore and corroborate these findings.

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Keywords: lameness, genetics, digital cushion thickness, genomics.

HH-P01

Swedish dairy cattle veterinarians spend little time on advisory visits despite highly satisfied clients: Can training in Motivational Interviewing help to stimulate efforts?

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Preventive medicine is an important means to improve animal health and welfare as well as animal production and farm profitability. It is also important as a way to reduce antimicrobial drug use and reduce risks of antimicrobial resistance. Although dairy veterinarians have long been encouraged to increase their engagement in veterinary herd health management (VHHM) advancements are slow and veterinarians struggle to move into the role of proactive health advisors.

Objectives: The present study aimed to 1) estimate the time Swedish cattle veterinarians involved in VHHM spend on VHHM visits, 2) estimate client satisfaction with veterinarians in VHHM, and 3) investigate whether training in the client-centered communication methodology Motivational Interviewing (MI) may encourage veterinarians to increase their efforts in VHHM.

Materials and methods: The 36 veterinarians involved in VHHM, who volunteered to participate in the study, were randomized into two groups. MI veterinarians participated in a 6-month training program in MI before they conducted VHHM visits in cattle herds, whereas control veterinarians performed visits without such training. Approximately two weeks after the visits, we telephoned the clients of both groups of veterinarians and asked them to grade their satisfaction with the veterinarians' behaviour, competence and time efficiency on a 6-point Likert scale. Veterinarians recorded (weekly during a 6-month period) the number, type and length of their VHHM visits to cattle farms and the percentage of a 40 hour-week that they worked with cattle. A gamma model was used to analyze the effect of MI training on time allocated to VHHM visits. Trained veterinarians were divided into subgroups based on MI skills: poor, near moderate and moderate. Extra explanatory variables were the type of veterinarian (animal health veterinarian/general practitioner) and veterinarians' experience in VHHM (<1, ≥1-≤5, >5-≤15, >15 years).

Results: Veterinarians performed 1115 advisory visits to which they allocated a median 56 (interquartile range: 17-107; range: 6-202) hours per 6-months of full-time veterinary work with cattle. The most common visit types were service visits (521 h), strategic VHHM visits (406 h) and biosecurity visits (202 h); length of visits was a median of 1 hour (interquartile range: 0.5-2; range: 5 minutes – 8 hours). Clients were highly satisfied with their veterinarians, with median Likert scores for behaviour, competency and time efficiency of 6, 5 and 5 respectively, where 1=highly unsatisfied and 6= highly satisfied (interquartile range: 5-6). The median (interquartile range) amounts of time veterinarians who were untrained in MI, trained in MI but with poor skills, trained in MI with near moderate skills and trained in MI with moderate skills allocated to VHHM were 22 (8-78), 70 (59-107), 38 (16-53) and 106 (73-136) hours, respectively. There was no evidence of a difference between the groups (P=0.31).

Conclusions: Veterinarians allocated little time to VHHM. The high satisfaction level indicates a large potential for veterinarians to be trusted allies in herd health management work on farms. Further studies – utilizing quantitative and/or qualitative methodologies – are needed to evaluate the potential of training in MI to stimulate efforts in VHHM.

Keywords: preventive medicine, veterinary herd health management, Motivational Interviewing, veterinarian-client communication, farmer satisfaction.

HH-P02

Reticulorumen ph, temperature and cow activity as indicators of diseases after calving

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Objectives: The objective of current study was to determinate can reticulorumen ph, temperature and cow activity, registered before calving, be as indicators of diseases after calving.

Materials and methods: Lithuanian Black and White dry dairy cows (n=30) were selected according to those fitting a profile of having had a 2nd or more lactations (on average 2.9±0.13 lactation). The clinical examination (identification of diseases after calving) performed from 60 days before calving, till 60 days after calving. Clinical mastitis (CM) cases were characterized by the demonstrated clinical signs, including abnormal appearance of milk (watery, flakes, fibrin clots, and so on; mild); Milk fever (MF) were characterized by sternal recumbency with typical S-shaped curve on the neck, weakness and inappetance. On clinical examination, the general health-condition was found to be poor. The body temperature was subnormal and pulse was undetectable. The cows that did not expel their placenta within 12 h were diagnosed with placental retention (PR). Cows without any clinical sign of disease after calving were characterized as clinical healthy (H). The pH temperature of the contents of cow reticulorumens and cow activity were measured using specific smaX-tec boluses manufactured for animal care.

Results: The healthy cows before calving showed the highest pH and temperature of reticulum. The highest level of activity was in PR-cows, the lowest in the CM group before calving. Reticulum pH and temperature were positively related in all groups of cows. Base of our funding pH of the reticulum statistically reliably correlated with the activity of the cows — positively in the PR group and negatively in the CM, MF and H groups. The temperature of the reticulum was negatively related to the activity of the cows. In all groups of sick cows,

we found a sharp decrease in the activity of animals 3-4 days before calving, while the activity of healthy cows increased. The temperature of reticulum sharply decreased in all groups of cows 6-7 days before calving in groups H, MF and PR, on day 4 - in group CM. In the PR group, the reticulum temperature increased 2 days before calving.

Conclusions: The highest pH and temperature before calving can be as biomarkers of healthy cows after calving. The lowest reticulum temperature before calving, can be as indicator of MF after calving. Positively correlation of reticulum pH and temperature before calving can be as biomarkers of PR.

Keywords: cow, reticulorumen, after calving, diseases.

HH-P03

Effect of age at first calving and parity number on colostrum total solids content

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Objectives: Colostrum total solids (TS) is a reliable proxy for its immunoglobulin content and, therefore, its quality. The objective of this study was twofold: a) to evaluate the effect of age at first calving on colostrum TS and b) to test whether colostrum quality of primiparous cows is lower than that of multiparous ones.

Materials and Methods: The study was conducted in 10 commercial dairy herds in Northern Greece, from February 2015 to September 2016. A total of 387 primiparous (27±3.8 months at calving) and 687 multiparous Holstein cows were included in the study. All cows were milked completely 232±195 minutes after calving; colostrum yield was recorded and a sample was collected from each one. A Brix refractometer was used cowside to measure %TS (a value ≥22% is indicative of high quality). Calendar season, parity, age at calving (months), time interval between calving and colostrum collection (TI) and body condition score (BCS) at calving were also recorded. Primiparous cows were grouped by age at first calving as follows: ≤24 months (110 cows), 25-27 months (148 cows), 28-30 months (78 cows) and >30 months (51 cows). All cows were grouped by parity as follows: 1st parity (387 cows), 2nd parity (311 cows), 3rd parity (181 cows) and 4th+ parity (195 cows). Effects of farm, season, age/parity group, colostrum yield (≤4kg, 4.1-8.5kg and >8.5kg), TI (<120min, 120-360min, >360min) and BCS (5-point scale with 0.25-unit increments) on colostrum TS were assessed using univariate linear models. Farm was fitted as a random effect and all other factors as fixed ones in the model. Moreover, percentage of samples with Brix value <22% were calculated for primiparous cows' age groups, for all cows' parity groups, for TI, colostrum yield groups and for BCS. Differences among groups were estimated using a chi-square test.

Results: Age at calving of primiparous cows had no effect on colostrum TS (P>0.05); for age groups of ≤24 months, 25-27 months, 28-30 months and >30 months, mean %(±SD) TS was 26.0±4.4, 26.0±5.1, 26.2±3.7 and 25.5±4.6, respectively. The percentage of samples with Brix value <22% in primiparous age groups were 4.4%, 7.2%, 2.1% and 3.4%, respectively. No statistical difference (P>0.05) was detected among these groups, either. Farm, calendar season, TI and BCS had significant effects (P<0.05) on TS. The latter was highest in autumn and when TI was <360 min. When TI was >360min, 31.0% of samples had values below 22%; respective percentages for <120min and 120-360min were lower, at 11.4% and 14.9% (P<0.05). Primiparous cows with a BCS ≥3.5 had the lowest mean TS (P<0.05) but percentage of samples with Brix value <22% was not affected by BCS. Parity had a significant effect on colostrum TS (P<0.05). Mean %(±SD) TS of parity 1, 2, 3 and 4+ cows was 25.8±4.6, 24.7±4.5, 25.9±4.6 and 27.1±4.8, respectively. The percentage of samples with Brix value <22% in parity 1, 2, 3 and 4+ cows was 17%, 26%, 18% and 13%, respectively (P<0.05). Interestingly, and contrary to common belief, colostrum from 2nd parity cows had an inferior quality than that from primiparous cows. Farm, calendar season, colostrum yield and TI had also a significant effect on TS. Lowest values were observed during spring and summer (P<0.05), when colostrum yield was ≥8.5kg (P<0.05) and TI ≥6h (P<0.05). When yield was >8.5kg, 27.4% of samples had values below 22%; for yield classes ≤4kg and 4.1-8.5kg failure percentages were lower, at 16.8% and 17.3%, respectively (P<0.05). When TI was >360min, 28.0% of samples had values below 22%; respective percentages for <120min and 120-360min were lower, at 15.2% and 18.0% (P<0.05).

Conclusions: Age at calving of primiparous cows had no effect on colostrum TS. Therefore, farmers can continue their efforts to decrease age at first calving up to the suggested 22 months, without worrying about colostrum quality. Moreover, the notion that primiparous cows produce low quality colostrum proved to be false in this study. Colostrum from primiparous cows should not be rejected. Its quality, as that of colostrum from older cows, should be assessed on farm with a Brix refractometer before administration to calves.

Keywords: dairy cow, colostrum, parity.

HH-P04

A pilot study on the association of daily rumination time with fat and muscle mobilization during the transition period in Holstein cows

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Objective: Daily rumination time (DRT) is mainly associated with NDF intake and under the same feeding management, it represents a proxy of dry matter intake. Optimizing dry matter intake during the pre-partum period is probably the most important strategy for a successful transition. The objective of this study was to investigate the association of DRT with body fat and muscle mobilization during the transition period in dairy cows.

Materials and methods: Thirty-two multiparous Holstein cows in different parities from a commercial dairy farm in Greece were enrolled in this study. Dry cows were housed in a straw bedded barn providing at least 10m^2 per cow. After calving, cows were moved to a typical 2-row free-stall barn. Cows were equipped with commercial tags (SCR, Israel) recording rumination time in two-hours recording sessions. DRT recordings (min/day) were available from 3 weeks pre-partum to 2 weeks post-partum for each cow. The following parameters were calculated: the areas under the curve for DRT during the pre-partum (AUC_Pre) and the post-partum (AUC_Post) period, the coefficient of DRT variability during the pre-partum period (CV_Pre), and the DRT at day of calving (DRT_Calv). Body condition score (BCS), backfat thickness (BFT) and *longissimus dorsi* muscle thickness (LDT) of each cow was assessed at 5 time-points relative to calving: -21d; -8d; 0d; +8d; +21d, by the first author. Cows were scored for BCS on a 5-point scale with 0.25-unit increments; BFT and LDT were measured by ultrasonography, using a 5.0-7.5 MHz linear transducer. BFT was measured in the pelvic region and LDT on the transverse process of the 4th lumbar vertebra at the site of the larger diameter of the muscle between the fasciae. Fat and muscle mobilization were defined as the decrease in BFT and LDT measurements from -8d to +21d, respectively (ΔBFT and ΔLDT). Medians in ΔBFT and ΔLDT were used as cut-offs to distinguish cows with high from those with low fat or muscle mobilization, respectively. Comparisons between the above groups for DRT parameters were performed with t-tests or Mann-Whitney tests, for normally distributed data or not, respectively. Analysis was performed with IBM SPSS v.25.

Results: Cows had a mean (±sd) BCS =3.34 (±0.52), mean (±sd) BFT =17.33 mm (±6.72) and mean (±sd) LDT =34.81 mm (±5.64) at -21d. Medians for ΔBFT and ΔLDT were 4.4 mm and 12.0 mm, respectively. Cows with low ΔBFT had higher DRT_Calv than cows with high ΔBFT (357 min/d vs. 268 min/d; P=0.019). Moreover, cows with low ΔLDT had higher AUC_Pre (9860 vs. 9059; P=0.022), lower CV_Pre (9.94% vs. 13.95%; P=0.011), higher DRT_Calv (368 min/d vs. 272 min/d; P=0.011) and higher AUC_Post (7378 vs. 6589; P=0.015) than those with high ΔLDT.

Conclusions: Daily rumination time during the transition period is associated with mobilization of body fat and muscle reserves. Thus, rumination time during the pre-partum period and at the day of calving could serve as a tool for predicting the onset and the degree of negative energy and/or protein balance. A larger scale study should aim on identifying cows at high risk for significant fat and/or muscle mobilization from their daily rumination time patterns as early before calving as possible.

Keywords: rumination, transition, backfat, longissimus dorsi, dairy cattle.

HH-P05

Postpartum identification of cows at risk of disease

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Introduction: The consumer requires ever greater transparency in the production of milk and meat and the social sensitivity towards the welfare of production animals and food safety is progressively and steadily increasing. The resistance to antibiotics cannot be underestimated, as well as the need/possibility to achieve productive and reproductive performances, without a systematic use of hormones. The major limitation of modern reproductive management are a low heat detection rate and a low conception rate. These problems are solved only with a different approach of the transition phase reducing the risk of having sick cows in the immediate postpartum and identifying animals at risk as soon as possible.

Objectives: Define an operational strategy capable of highlighting cows at risk of pathology in the first 10 postpartum days. Define a clinical criterion that would allow us to distinguish between a sick animal and a simply symptomatic cow.

Materials & Methods: The pilot test was conducted in a herd of 590 cows in milk in the Parmigiano Reggiano area (province of Bologna), between October 2014 and December 2019. Daily, during the first 10 days in milk, cows are been observed to decide whether to subject them to a clinical visit on the basis of a screening based on front and rear observation. Frontally, the ears are analyzed (position and symmetry of the ears, temperature at the base of the ears, ear infection: purulent, unilateral/bilateral), eyes (shine/corneal opacity, epiphora mono/bilateral, type of epiphora) and the muzzle (dry, wet, nasal discharge: type, odor, mono or bilateral). Behind, the observation was including: the rumen score, the locomotion score and the mobility score, the shape of the abdomen (right side), the frequency and type of breathing (right

side), the mammary system (symmetry, color, edema, odor, size, shape and color of the teats, possible loss of milk), stool (consistency, color, odor, presence of indigestible: type and quantity of indigestible), vaginal discharge (color, odor, consistency), the vagina (presence of lacerations). Each parameter taken into consideration `s was given a score (from 0 to 3 according to the problem) and according to the overall score, each cow as been identify with a color red (score> 23), orange (score 11-23) and yellow (score 0-10). The red cows', must be visited immediately, the orange cows', must be monitored and checked again after 12 hours, while the yellow cows', can be considered normal and/or with a very low risk of developing pathology and therefore will be rechecked after 24 hours. The observation of each individual cow must be completed with the last milking production: cows that do not increase production or that decrease production, must undergo an immediate clinical visit. The score is made on mobile or tablet, which automatically establishes the list of cows that must undergo a clinical visit The postpartum technicians were veterinarians or animal science graduated.

Results: With this system, the animals subjected to daily clinical visits were 5-8% of the total cows hosted in postpartum burn. The consumption of antibiotics in postpartum has been reduced from 30.79% of the total drug cost to 20.26%. This method has accelerated the working procedures, allowing to keep the animals blocked for no more than 60 minutes a day, guaranteeing one of the fundamental principles of cow welfare. Between October 2013 and December 2019, the heat detection rate went from 58.79% to 71.61%, while the pregnancy rate went from 16.98% to 27.65%, also thanks to the introduction of the Double Ovsynch.

Conclusions: This clinical method allows the identification of cows at risk of pathology with great timing and precision. It allows us to distinguish between a sick cow and a symptomatic cow, that is, it allows us to establish a fair principle of use of antibiotics and other drugs. The method was subsequently exported to three other dairy farms, respectively of 800, 1500 and 3500 cows in milk, comparing it with collar/ear tag (SCR) clinical detection systems, demonstrating extreme accuracy. This clinical method of evaluation of postpartum cows, is complementary to the new forms of artificial intelligence, or in their absence, can replace them.

Keywords: Sick cows, Postpartum, early diagnosis.

HH-P06

Farmers say they like vets to be 'proactive' but what do they mean, exactly? Exploring proactive behaviour from a social science perspective

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Objectives: Previous work has shown that farmers rate 'proactive vets' higher than many other veterinary attributes, such as clinical knowledge or experience (Jefferson-Loveday et al, 2019). However, this research did not define proactivity, and while proactivity has been studied by social scientists in other contexts, to our knowledge there is an absolute paucity of studies exploring this construct in the context of the vet-farmer relationship. Set in the context of seasonal calving UK dairy farmers, the aims of this study were to i) explore dairy farmers experiences and perceptions of interactions with their vet that they considered to be proactive in nature (ii) explore any barriers to veterinary involvement on farms.

Methods: Semi-structured telephone interviews were conducted with 12 seasonal calving UK dairy farmers. A snow ball sampling strategy was employed which sought to capture a diversity of opinions. Interviews were audio recorded and transcribed verbatim. An inductive thematic data analysis was performed using NVivo software. The six-phase process described by Braun and Clarke (2006) was followed. Coding and theme generation was led by GG, with refinement following discussion with HMH and EB.

Results: There were 11 male participants and 1 female. Herds were a mixture of spring and autumn blocks, with varying lengths of breeding season from 9-20 weeks. Herd size ranged from approximately 150 – 750 milking cows and were selected from the south west of England, Wales, Herefordshire and Kent. Respondents age ranged from mid-twenties to mid-sixties and the vet practices used ranged from traditional mixed practices to large privately owned farm practices and corporate farm animal practices.

There were 3 themes generated in total. The first theme entitled 'Why farmers *don't* always trust their vet or turn to them for advice' highlighted reasons underpinning some farmers' decisions to not seek out input from their local vet or to act upon the veterinary advice they are given. This theme also included examples of missed opportunities when vets were on the farm and could have engaged with farmers but did not. It also shows that a relationship of trust takes time to build up and that changing vets will disrupt the farmer vet practice relationship. The second theme entitled 'Striking a balance: helping or pushing?' explored the fine line that farmers acknowledged could exist for vets between coming across as supportive versus appearing too forceful in their approach. Listening, timing and understanding farmers goals all featured in this theme. The last theme, 'what proactivity looks like to farmers' provides examples of behaviours vets undertook, or could undertake, that farmers liked and considered to be proactive, as well as behaviours and advice that vets did that annoyed or frustrated them. In general, there appeared to be a dichotomy with regards to farmers perceptions of their vet. In this study, farmers either had little involvement with their vet, questioned their expertise and were frustrated that their vet was not more proactive. Or, they had regular contact with their veterinary practice, valued the input they received and being 'pushed' into action by their vet and considered it helpful to have continual veterinary input to keep them on track.

Conclusions: The results provide useful new insights that can be used by vets in clinical practice to improve the way they interact with their clients, and ultimately help farmers to make changes that prevent and control diseases on their farm. Understanding the types of veterinary behaviours and advice that farmers value the most and consider to be proactive is paramount. Just as important, is to avoid the so-called dark

side of proactivity, as recognised by social scientists. These are behaviours that while unquestionably proactive in nature, nonetheless, on some occasions and in some contexts, prove unhelpful or even counterproductive. Our work is suggestive that this can arise by a lack of intrinsic motivation by the farmer on a certain subject topic, which the veterinary surgeon must identify and then change his/her approach to the perceived problem for there to be a successful outcome.

Keywords: facilitating behaviour change, engagement, proactive.

HH-P07

Prevalence of piroplasmosis in cattle in some grazing reserves in Nigeria

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Objectives: The Nigerian livestock resources was conservatively estimated to the tone of USD 6 billion and contributes significantly to the Agricultural component of the Gross Domestic Product (GDP) of which cattle production contributes up to 40% . Disease is one of the numerous debilitating factors affective livestock productions in Nigeria which result in substantial economic loses of which piroplasmosis forms an important component. Parasitic diseases have devastating impact on human and animal health worldwide particularly in developing countries. The losses caused by the parasites comprises acute illness, death, premature slaughter, rejection of some body parts at meat inspection and reduction of productive potential such as decreased growth rate, weight loss in young growing animals and late maturity of slaughter stock. The study was carried out to determine the prevalence of piroplasmosis in cattle in some grazing reserves in Taraba State, Nigeria.

Materials and Methods: The study was conducted in six (6) randomly selected gazetted grazing reserves (Sunkani and Dinding in Northern zone, Mayo-Kam and Maihula in Central zone and Gankwe Assen and Jibu in Southern zone) of Taraba State, Nigeria. A total of 1005 blood samples were collected from apparently healthy cattle and examined for the presence *Anaplasma, Babesia and Trypanosoma* species using thick and thin blood smear. Identification was carried out to genus and where possible, species level. Information about sex, age, breed and location of cattle were collected using structured questionnaire.

Results: The result revealed overall prevalence of piroplasmosis of 153 (15.2%). The organisms examined were *Babesia, Trypanosoma* and *Anaplasma* species. The *Babesia* species isolated were *Babesia bigemina* (7.2%) and *Babesia bovis* (3.7%) which accounted for 110 (10.9%). *Trypanosoma vivax* (1.5%) and *Trypanosoma congolense* (0.7%) were the two species of *Trypanosoma* 22 (2.2%) identified. Only *Anaplasma marginale* was isolated in the study 21 (2.1%).

In relation to sex, out of the 118 males and 887 females examined, high infection was recorded in females 136 (15.3%) than males 17 (14.4%). On age groups, 948 adults and 57 young cattle were examined, the result showed that the infections are higher in adults than young with 145 (15.3%) and 8 (14.0%) respectively. Based on seasons, 499 and 506 cases were recorded during dry season and rain season respectively. The occurrence of piroplasmosis was found to be higher during the rainy season with 96 (19.0%) and least in the dry season with 57 (11.4%).

Regarding breeds, Rahaji, Bokoloji, Bunaji and Muturu breeds were examined. The highest infection was observed in Bokoloji 10 (16.4%) and no infection in Muturu. The occurrence of the infections based on locations revealed the highest prevalence in northern Taraba 96 (28.5%) with Dinding grazing reserve 58 (34.3%). The least prevalence was recorded in Taraba South 19 (5.7%) with Gankwe Assen 7 (4.2%).

Conclusions: The results confirmed the presence of carrier populations of piroplasmosis in cattle in the study area which may favour the spread of the infections to other susceptible animals and humans. Animals of all sexes and age groups are carrying the diseases. Females are more susceptible because they are kept longer for breeding and milk production purposes while calves have rapid immune responses to primary infection through a complex immune mechanism by colostral immunity. Some of the factors responsible for the spread of the disease include; distinction in geo-climatic condition, increasing the upsurge of tick population and animal exposure to vectors of the diseases. Better control measure against the vectors, regular strategic prophylactic treatment, breeding and improving resistance breeds and development of vaccines against the diseases should be adopted to prevent its spread.

Keywords: Piroplasmosis, Prevalence, Cattle, Grazing reserves.

HH-P08

Influence of the title of agrarian property in the zootechnical practices of beef cattle in Guanajuato, Mexico

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Objective: Analyze the zootechnical practices carried out by beef cattle producers with and without agricultural property title, in Guanajuato, Mexico, in order to identify how land ownership influences livestock production and zootechnical management.

Material and methods: The study was carried out in six localities located in the municipality of San Luis de La Paz, Guanajuato, Mexico. In the municipality, people who own cattle are governed by different types of agricultural property, mainly by the ejido and private property, and there are also producers who do not have legally official documentation proving the possession of land called “avecindados”. Through

non-probabilistic sampling, three localities with title of agrarian property (ejidatarios) and three without title of agrarian property (avecindados) were chosen; through the snowball method in the six localities 65 farmers were surveyed: 40 ejidatarios and 25 avecindados, which were available to participate in the study. The information was collected through semi-structured interviews and participant observation. The survey considered aspects related to the main zootechnical practices: genetics, reproduction, food practices, health and economics. With the information obtained, a frequency analysis was performed in Microsoft Excel.

Results: The main differences in zootechnical practices between ejidatarios and avecindados were: in genetics, the ejidatarios (44%) prefer the crossbreeding of charolais, zebu and native breeds, while 48% of avecindados are oriented more towards the racial purity of cattle Charolais; in reproduction, the ejidatarios (43%) are oriented to make a change of sire from 1 to 3 years, while the avecindados (34%) do so from 4 years onwards; in the feeding it was observed that in 25% of the ejidatarios tend to incorporate technological practices in the conservation of forages such as silage; in preventive medicine, 90% of ejidatarios vaccinate 1 to 2 times a year, while only 54% of avecindados have done so, for deworming 85% of ejidatarios and 54% avecindados do it frequently, and the application of vitamins, 88% of ejidatarios and 50% of avecindados do it; in the commercialization, it was obtained that 39% of the ejidatarios sells mainly calves and waste cows, while 36% of avecindados only sell cows; In other zootechnical practices, it was identified that 41% of ejidatarios keep records and none of avecindados do this practice. These differences may be due to 50% of ejidatarios having access to technical advice through the state extension program.

Conclusions: The study showed that although the avecindados do not have any legally official documentation, this is not a limitation to carry out the livestock activity, since it uses abandoned and idle land in the region. However, there are differences in zootechnical practices that can be reflected in the productive conditions of cattle, a situation granted by technology transfer program that they receive from the state, thanks to the agricultural rights they possess.

Keywords: livestock production practices, technology transfer, agricultural rights.

HH-P09

The selection of Johnes Disease Control Strategies for dairy herds engaged in the UK National Johnes Management Plan

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Objectives: The UK National Johnes Management Plan requires participating farms to select one of six control strategies to manage Johnes disease in their herds. This study looks at the strategy selection in over 2400 herds that are participating in the scheme, and investigates the selection according to Johnes Disease risk and status.

Materials and Methods: 2462 dairy herds are using a web based herd health management programme to engage in the UK National Johnes Management Plan, and comply with the requirements to asses risks, determine disease status and select an appropriate prevention and control strategy with the help of a trained Accredited Johnes Advisor.

Results: In this set of herds, 58% of herds have selected a strategy of managing Johnes disease using strategic testing, 17% use a test and cull strategy, 12% use improved farm management without testing and 5% selected biosecurity and monitoring to protect their herds. Only 0.5% use vaccination, and 5% breed to a terminal sire to control the disease.

Conclusions: Herds with high risks of Johnes disease are likely to select the resource intensive strategies designed to deal with high prevalence such as frequent strategic testing and risk management (63%), whilst herds with low disease risks are more likely to select less onerous strategies such as biosecurity, protect and monitor (27%), tests and cull (27%).

The strategic options for control are an important part of the UK National Johnes Management Plan, allowing trained and accredited veterinary advisors to select the most appropriate control program for herds dependent upon risk, status and resource. The results of this study show that appropriate strategies are being selected to ensure continued engagement and economic benefit.

Keywords: Johnes, management. control strategies.

HH-P10

Preliminary study: Factors influencing dystocia and stillbirth on four east-german dairy cattle farms

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Dystocia and stillbirth have negative effects on the following production period and cause direct losses, therefore they are of considerable economic importance for the farmer. The problem is well known, however still around 6 - 8% of dairy calves are born dead or die shortly after delivery.

Objective: Sex, parity of the dam, season of birth and monitoring intensity are a few factors influencing stillbirth and dystocia rates on a farm, and we aimed to analyze some of them on four large east german dairy farms.

Materials and Methods: In a retrospective observational study on four farms in eastern Germany we analyzed calving data submitted by the farmers to the herd management software “Herde” (dsp agrosoft). 7314 calving’s over 22 months were included, and data to parity of the dam, stillbirth rate and ease of calving were collected. For calving ease 3 grades were distinguished. Grade 1 without assistance, grade 2 easy pull and grade 3 heavy pull and veterinary assisted calving's. Dystocia rate was calculated as percentage of calvings with a grade 3 calving. Stillbirth rate was calculated as percentage of

calves born dead or dying within 24 h after birth.

Results: Dystocia occurred in 1 - 6% (Mean 2.4%) of the calvings and stillbirth rate was calculated to be 4 - 6% (Mean 5.2%). In male calves, dystocia rates were 2 – 8% (Mean 3.5%) and stillbirth rates for male offsprings was 5-10% (Mean 6.3%). Female calves were born in 1 - 4% (Mean 1.75%) under dystocic conditions and stillbirth rates were 4-5% (Mean 4.25%) (P>0.05). In heifers dystocia occurred with 1 - 8% (Mean 3.5%) of the calvings whereas in cows in with 1 - 4% (Mean 2.0) (P>0.05). Stillbirth rates for heifers were with 3-7 % (Mean 4.7%) similar to cows (Mean 4.9) (P>0.05). Further analyses showed that in dystotic calvings the stillbirth rate was higher (Mean 39.25%) than in eutotic calvings (Mean 3.25%) (P<0.05).

In *conclusion*, in our preliminary study, in a limited number of farms, classical factors favoring stillbirth as parity of the dam and sex of the calve might not be the only main risk factors, and herd specific factors need to be studied more in detail. Further studies with a larger number of farms are necessary.

Keywords: Calving, eutocia, heifer.

HH-P11

Effect of a monensin controlled-release capsule on milk production and methane emissions of dairy cows

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The objective of this study was to evaluate monensin controlled release capsule (Kexxtone™, Elanco Animal Health) impact on milk production and methane emissions (CH4) up to 90 days in milk (DIM) in dairy cows at the first two Dairy Herd Improvement (DHI – Africor Lugo) tests after calving. Individual information about farm identification, cow number, parity, days in milk, and milk yield at DHI tests (DHI 1 was performed between 5 to 35 DIM; and DHI 2 was performed ≤ 90 DIM) were recorded. In addition, milk samples (bronopol preserved) were collected and analyzed for somatic cell count (SCC), fat%, protein% and fatty acid profile at Laboratorio Interprofesional do Leite de Galicia (LIGAL; A Coruña – Spain). Chillard et al., (2009) indicated that milk fatty acid profile could be considered as a potential indicator of *in vivo* CH4 output reporting different predictive equations. CH4 emissions were estimated based on the following formula: CH4 (gr/cow/day) = 16.8 x Milk 16:0 (% Total Fatty Acids) – 77 (r^2=0.82, Chillard et al. (2009). Animals were enrolled during the dry period and data was collected from January to October of 2017. Season was defined as summer (April to September) and winter (October to March). All data were analyzed with PROC MIXED (SAS 9.4, Kenward Rogers ddfm) and included the fixed effects of treatment, season, and treatment x season and the random effects of farm, parity, and DIM. Number of treated cows differed among farms (range: 9 to 126), but each farm enrolled between 25 up to 75% of total cows. A total of 1429 cows from 18 commercial dairy herds from Lugo province (Galicia, Spain) were enrolled in this study. A total of 796 cows received monensin in controlled-release capsules 3 to 4 weeks prior to expected calving date, and 633 did not receive any treatment (control group). Treatment with monensin increased milk production by 1.8 Kg/ day at DHI 1 (*p-value* = 0.001) and by 3.3 Kg/day at DHI 2 (*p-value* <0.001) compared to control group. Cows from control group produced more estimated CH4 compared to cows treated with monensin (354 g/cow/day versus 344 g/cow/day, respectively; *p-value* = 0.012) at DHI 1. However, there was no difference for estimated CH4 between control and treated groups at DHI 2 (416 g/cow/day versus 412 g/cow/day, respectively, *p-value* = 0.367). At DHI 2, cows produced greater estimated CH4 during winter compared to summer (*p-value* = 0.003). Monensin increased total fatty acids at DHI 1 compared to control group (*p-value* = 0.004) but there was no difference at DHI 2. In conclusion, monensin decreased estimated CH4 emission at first milk test after calving and increased milk production during first two tests.

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Keywords: Methane, milk yield.

HH-P12

What are the areas of greatest opportunity in the dairy cows' management around calving in CEE farms?

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The 90 days period (V90D, beginning approximately 60 days prior to calving and continues through the first 30 days of lactation) represents key period of the success of future lactation (Rapnicki and Overton 2014). Housing and facilities, feeding and handling management during V90P have a direct impact on the health and well-being of holidays in transition and, consequently on their future productivity and fertility (Norlund 2005, 2009). Evaluation of those variables would provide to the veterinarian the opportunity to advise their farmers about design and management in order to cover the cow's needs during transition period (Cook and Nordlund 2004).

The risk analysis tool (*dRisk*™ Risk Assessment Tool, Elanco Animal Health) allows the management evaluation during the dry, close-up and post-partum period at farm level, by developing a 65-75 question survey about aspects related to facilities, food and handling management.

Objective and material and methods: With the aim of evaluate the far dry, close-up and maternity and fresh cows management in Easter Europe countries dairy farms, dRisk analysis was implemented in 124 dairy farms (Turkey=5, Po-

land=80, Hungary=6, Greece=3, Estonia=3, Czech Republic=22, Cyprus=1 and Bulgary=4).

Results: Those farms had, on average, 344 milking cows (range: 24-3500) and 40 dry cows (range: 2- 609), at inclusion date. 35% of farms involved (n=44) had separated prepartum facilities and 61.29% (n=76) had a separate calving pen. Bedded pack was the most common housing system for both Far dry (n=63) and Close up pens (52), followed by free stalls (n=42 and 23) and Dry Lots/pasture (n=18 and 5, respectively). Only 1 farm had dry cows on pasture. Main housing system used for maternity and fresh cow was free stall (n=87) and bedded pack (34). 2 farms used dry lots/pasture pens for maternity and fresh cows.

General evaluation of the conditions of those farms shown that the area with more improvement opportunites (fewer farms having optimal conditions) was dry period. So, 73.39% of the farms had poor housing conditions and facilities (n=21) or improvable (n=70) and only 26.61% (n = 33) of the farms visited the drying facilities met the optimal conditions. In addition, in 78.23% of farms, management of dry cow feeding was precarious (n=76) or improvable (n=21). On the opposite, postpartum period management was optimal in 74.19% (n=92) of the farms visited, and in only three of them it was precarious. Regarding with the postpartum facilities and nutrition, in 10.48 and 5.65% of the farms (n = 13 and 7, respectively) conditions were precarious, in 52.42 and 59.68% (n=65 and 74, respectively) could be improved and only 37.10 and 34,68% (n=46 and 43, respectively) of the farms had optimal conditions.

The main critical points more frequently repeated for each area were also analyzed. Thus, for far dry and close up period, the most frequent critical points were a) limited access to drinking water (lower that 4 ” linear water access per cow; not clean water or less than 2 locations in pen, n = 99), b) high body condition score (> 10% of cows had BCS in 5 points scale > 4.0, n = 74), c) cows and heifers were housed together (n = 73). The most frequent critical points in maternity and fresh pens were a) limited access to drinking water (lower that 4 ” linear water access per cow; not clean water or less than 2 locations in pen, n = 95), b) cows and heifers were housed together (n = 91) and c) limited bunk space (<30 ”of feed bunk access / cow, n = 82).

In conclusion, this work shows that there is ample room for improvement in the conditions of housing, management and feeding of cows during the transition period, especially in the dry cows group. Evaluating the conditions of the animals of this period gives the veterinarian the opportunity to advise their farmers about the improvements that may have a greater impact on the health and welfare of the cows and, consequently, on their future productivity and fertility. Doing so in a rigorous and standardized way allows you to compare the conditions of the different farms, monitor and establish the possible relationships between the driving conditions and the productive and reproductive parameters.

Keywords: Management, Dairy, Facilites, Transition.

HH-P13

Evaluation of IgG concentration and bacterial load of bovine colostrum in the Benelux region

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Objectives: Ingestion of sufficient amounts of good quality colostrum in the first hours of life is essential for the future health and performance of the calf. Colostrum quality reflected by its immunoglobulin G (IgG) concentration and its bacterial load can vary widely among cows. The qualitative standards for bovine colostrum are set at an IgG concentration of at least 50 g/l and a maximal total bacterial cell count of 100.000 CFU/ml. To our knowledge, there has been no recent peer-reviewed study evaluating the quality of colostrum from cattle in the Benelux region. The aim of the present study is to evaluate the colostrum quality, defined as IgG concentration and the total bacterial count, from cattle in the Benelux.

Materials & Methods: For this study bovine practitioners were invited to participate in the collection of colostrum from freshly calved cows. On 88 commercial farms located in Belgium and The Netherlands, a total of 162 colostrum samples were collected. Sampling occurred at the first feeding of colostrum to the calves. Colostrum samples were stored at -20°C until 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). Total bacterial cell count was determined using a bactoscan automatic bacterial count reader (Bactoscan™ FC+, FOSS, Denmark). Colostrum samples with IgG concentrations < 50 g/l or with a total bacterial cell count of > 100.000 CFU/ml were considered of low-quality. All analyses were performed using R software (R Core Team, 2017).

Results: The number of colostrum samples collected per herd ranged from 1 to 5. Colostral IgG concentrations were normally distributed with a mean of 66,4 ± 25,5 g IgG/l (range 14,4 – 150,0 g IgG/l). The bactoscan method resulted in a median total bacterial cell count of 7.000 CFU/ml (range 4.000 – 1.000.000 CFU/ml).

Based on an IgG concentration of at least 50 g/l, 22,3 % of the colostrum samples were of poor quality. Total bacterial cell count was above the norm of 100.000 CFU/ml in 10.2 % of the colostrum samples. Taking both quality parameters into account, 26,8 % of the colostrum samples were of low-quality.

Conclusion: The quality of bovine colostrum in the Benelux region can be improved, considering that nearly one third of the colostrum samples were classified as inadequate to be fed to newborn calves.

Keywords: Cattle, Colostrum, IgG concentration, Total bacterial count.

HH-P14

Bayesian latent class analysis of the characteristics of three diagnostic tests to assess the passive immunity transfer status in neonatal dairy calves

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Objective: The assessment of diagnostic tests for the evaluation of failure of passive transfer (FPT) in calves is usually performed applying the radial immunodiffusion (RID) test as the gold standard. The value of the RID test as gold standard is, however, debatable. The main objective of the study was to determine the diagnostic test parameters of three different tests for the evaluation of FPT in the absence of a gold standard applying a Bayesian latent class model. A second objective was to obtain prevalence data on FPT in dairy calves in Belgium.

Materials & Methods: Neonatal dairy calves (n = 95) from 6 commercial farms in Belgium were randomly selected to be enrolled in the study. Only calves aging between 2 and 7 days were eligible to be included in the trial. All enrolled calves had received at least 2L of colostrum in the first 24 h of life. Serum samples were tested by digital brix refractometry and by lateral flow sandwich immuno-chromatography for the evaluation of FPT according to the manufacturer's instructions. An independent, accredited lab (Zoolyx, Aalst, Belgium) performed serum electrophoresis. Failure of passive Transfer was considered as the positive outcome in all interpretations of the test results. In the serum electrophoresis, the concentration of the serum gamma-globulins was used as a proxy of the IgG concentration as described by Pardon et al. (2015). Pardon et al. proposed a cut-off value for FPT of < 10g/L of gamma-globulins in 2 to 7-day old calves. In the present study, this proposed cut-off value was applied, but additionally a cut-off value of 12g/l serum gamma-globulins was evaluated. For the digital brix refractometry data, the study by Elsohaby et al. (2015) was used as reference in which a cut-off value of < 8,3% brix indicated FPT. The cut-off value applied for the lateral flow sandwich immuno-chromatography method was the one proposed by the manufacturer, namely IgG < 10g/l indicating FPT. For the purpose of the Bayesian multivariable latent class analysis of three conditionally independent diagnostic tests, prior distributions of the prevalence and sensitivity and specificity of each diagnostic test were determined according to Dendukuri et al. (2009).

Results: The mean age of the calves at sampling was 3,5 days (SD ± 1,6). The median and 95% credibility interval (CI) for the prevalence of FPT was 48.2 (95% CI: 27.4-67.4%). The Bayesian multivariable latent class analysis of the characteristics of the three diagnostic tests indicated that the best combination of sensitivity and specificity was obtained with the digital brix refractometry method. Depending on the cut-off used for the electrophoresis, the sensitivity of the digital brix refractometry method varied between 83% (95%CI 69,2-93,0%) and 90,7% (95%CI 80,9-96,6%) and its specificity between 74,8% (95%CI 60,4-87,7%) and 84,9% (95%CI 73,2-92,4%). The commercial lateral flow sandwich immuno-chromatography test showed a very low sensitivity (34,5%, 95%CI 20,3-52,6) which resulted in a large percentage of false negative results. For the practitioner, the negative predictive value (NPV) of a test for FPT ("if the test indicates 'no FPT' how sure can I be that the calf is ok?") is the most important. Taking this into consideration, the commercial lateral flow immuno-chromatography method scored very poorly (NPV 61%, 95%CI 41-81%). Also, the NPV (71%, 95%CI 46-95%) of the serum electrophoresis is rather low when the cut-off value of 10g/L gamma-globulins was applied. Increasing this cut-off value to 12 g/L drastically improved the NPV (96%, 95%CI 81-99%) of the serum electrophoresis.

Conclusion: Our results indicate that, of the different test methods assessed, the best characteristics were obtained using the brix refractometry method for the evaluation of FPT in neonatal calves applying a cut-off value of 8,3% brix. The highest NPV, however, was recorded for the serum electrophoresis using a cut-off value of 12 g/L of gamma-globulins.

Keywords: failure of passive transfer, diagnostic tests, neonatal calves, Bayesian analysis.

HH-P15

Does the position of the food bucket (inside or outside the hutch) affect food consumption and growth in pre-weaning calves?

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The aim of the present study was to evaluate the effect of the position of the food bucket (inside or outside the hutch) on the feed intake and growth in pre-weaning calves kept in individual outdoor hutches. The study was conducted from October 2018 to June 2019 on a commercial dairy farm in Girona (Spain). Mean environmental temperature ranged from 2 ºC to 26 ºC, relative humidity from 22 % to 100 % and monthly rainfall from 30 mm. to 85 mm. A total of 151 female Friesian calves from the first day of life to weaning were studied. Calves were separated from their dams as soon as possible after calving. Afterwards, calves were kept in individual outdoor hutches bedded with straw bedding and randomly allocated into two different treatments that differed in the bucket position: bucket inside the hutch next to the resting area (IN group, n= 63) or bucket outside the hutch covered with a metal lid (OUT group, n=88). All calves received 4L of colostrum by oesophageal feeder tube and were fed pasteurized milk from the tank twice daily (4L each meal). Fresh water was offered ad libitum to calves after the first day of life. Calves were offered 300g of starter diet from 7 days of age onwards (week 2). The starter died consist of concentrate mixture with a 23.1% of protein and 18.5% of fibre. Every day, food was checked and changed ensuring that calves were fed ad libitum. Weaning was done at 8 weeks of life if calves reached a body weight

(BW) ≥ 80 Kg. A total of 121 calves were weaned as expected at 8 weeks of life, and 30 calves were weaned one week later than expected. Every day, the starter diet was weighted in order to obtain daily food intake from week 2 after birth to weaning. Calves were individually weighted at birth and every week from birth to weaning. Calves tended to eat more starter food when the bucket was allocated inside the hutch in week 2 (36.3 ± 3.5 g/day in the IN group vs. 14.1 ± 1.71 g/d in the OUT group; P=0.0512) and week 3 (92.3 ± 7.86 g/day in the IN group vs. 56.6 ± 2.77 g/d in the OUT group; P=0.0985) after birth. From week 4 to weaning, calves ate a similar quantity of starter food. Similarly, calves with delayed expected weaning tended to consume more starter food when the bucket was allocated inside the hutch but only in week 2 (P=0.0627). These tendencies were statistically significant when calves with non-delayed expected weaning were studied. Calves with non-delayed expected weaning significantly consumed more starter food when the bucket was allocated inside the hutch in week 2 (41.6 ± 4.15 g/d in the IN group vs. 16.3 ± 2.20 g/d in the OUT group; P=0.0004), week 3 (103.1 ± 9.22 g/d in the IN group vs. 60.2 ± 3.21 g/d in the OUT group; P=0.0019) and week 4 (153.1 ± 10.3 g/d in the IN group vs. 107.3 ± 4.96 g/d in the OUT group; P=0.0246) after birth. There was no interaction between the position of the food bucket and the time needed to wean calves. BW and Average Daily Gain (ADG) were not affected by treatment group from birth to weaning. Although ADG from week 2 to week 5 showed higher absolute values in the IN group (week 2: 548.0 ± 38.76 g/d and week 5: 903.9 ± 68.79 g/d) compared with the OUT group (week 2: 504.9 ± 34.8 g/d and week 5: 886.3 ± 73.60 g/d), these differences were not statistically significant. When calves are allocated in outdoor hutches, placing the food bucket inside the hutch increases food consumption until the fourth week of life. Factors such as the proximity of the bucket to the resting area or the fact that food is better protected from climatic conditions when the bucket is inside the hutch may enhance food consumption during the first weeks of life. Nevertheless, BW and ADG were not affected by the position of the bucket.

Keywords: Calves, Consumption, Growth.

HH-P16

Blood metabolism in Holstein calves fed milk replacer twice *vs.* thrice per day

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Objectives: Lactation is critical for the growth and health of cattle and nutritional strategies during this period can affect the subsequent development of the animal. Historically, calves have been fed twice a day. However, calves nurse their mothers more than 6 times per day. Therefore, our aim was to evaluate the effect of an isocaloric diet of 6l of milk replacer, fed two *versus* three times per day, on the metabolic status of diary claves at moments associated with stress peaks: weaning and grouping.

Material & methods: We included in the study 140 calves from a commercial rearing farm (Cowvet SL) in Valencia, Spain, 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 group "2Fd" (n=71) or "3Fd" group (n=69). The calves were fed 6l daily 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.

Blood sampled were taken at four moments. 1) Farm Entry (28.87±8.35; days old); 2) Pre-Weaning (49.54±5.94d); 3) one-week Post-Weaning (66.74±8.2d) and 4) "Grouping" 10 days after having been grouped in batches of 24 (140.78±19.33d).

Serum was assessed with clinical chemistry analyzer (Konelab 20; Thermo Fisher Scientific, Waltham, MA, USA) according to manufacturer's instructions. Serum total cholesterol (TC, mg/dL), triglycerides (TG, mg/dL), high density lipoprotein cholesterol (HDL-C, mg/dL), low-DL-C (LDL-C, mg/dL), glucose (GLU, mg/dL), fructosamine (FRU, mg/dL), lactate (LAC, mg/dL), β hydroxy butyrate (BHB, mmol/L), non-esterified-fatty acid (NEFA mmol/L) and urea (mg/mL) were measured.

We analyzed the effect between 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: Serum parameters were within physiological ranges described for cattle at all assessment moments. When age at entry resulted significantly (P<0.05) affecting the parameters, it was kept in the model of repeated measures. No metabolic parameter differed significantly between treatments at moments of Entry, Post-Weaning and Grouping. At Pre-Weaning, we observed significant differences (P<0.05) in NEFA (0.25±0.007 *vs*. 0.22±0.08), LAC (16.65±8.48 *vs*. 13.88±6.4), GLU (115.94±18.56 *vs*. 108.37±16.58) and TC (86.71±16.44 *vs*. 81.25±18.94 for 2Fd and 3Fd, respectively). These results indicate that metabolically, feeding three times per day induces a lower mobilization of metabolites related to energy metabolism, which may reflect a greater metabolic stability than calves fed twice a day. This effect is overcome one week after weaning, so it seems to be an immediate effect, while the calves are drinking milk, but not long-term. The repeated measures analysis detected significant differences in parameters related to energy-gluconic metabolism, specifically, in FRU (between Post-Weaning and Grouping), increasing for treatment 3Fd (from 328.2±68.34 to 344.5±83.25) but remaining steady in the group 2Fd (from 330.29±61.11 to 329.75±66.58); and GLU between Entry and Pre-Weaning, decreasing in group 3Fd (from 112.71±18.16 to 108.37±16.58) but being

stable in group 2Fd (from 116.25±17.27 to 115.94±18.56). Between Post-Weaning and Grouping, GLU increased in the two treatments (from 116.25±17.27 to 115.94±18.56 for 2Fd, and from 116.25±17.27 to 115.94±18.56 for 3Fd).

Conclusions: The nutritional regimen of three feedings/day induces a more stable lipid metabolism in Holstein calves, but only until weaning, with this difference disappearing medium-term.

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Keywords: Palabras clave: blood metabolism, Rearing, Dairy, performance.

HH-P17

Blood metabolic parameters in Holstein rearing calves up to 4.5m of age according to health status at entry

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Objectives: The health status at very early stages of life can long-term affect subsequent productivity and growth. Usually, at farm entry, the health status of the animals is determined, 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), indicating a higher score with worse health status (0-21). The aim of this study was to evaluate plasma metabolism over time, especially at stress peaks: weaning and grouping, in dairy calves with different health score values 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. All animals were evaluated by a modified version of the Wisconsin-Health-Score, and categorized 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).

Blood was sampled at four moments. 1) Farm-Entry (22.94±7.8; days old); 2) Pre-Weaning (49.63±9.4d); 3) one-week Post-Weaning (65.84±8.6d) and 4) "Grouping": 10 days after having been grouped in batches of 24 (137.54±17.8d).

Serum was assessed with clinical chemistry analyzer (Konelab 20; Thermo Fisher Scientific, Waltham, MA, USA) according to manufacturer's instructions. Serum total cholesterol (TC, mg/dL), triglycerides (TG, mg/dL), high density lipoprotein cholesterol (HDL-C, mg/dL), low-DL-C (LDL-C, mg/dL), glucose (GLU, mg/dL), fructosamine (FRU, mg/dL), lactate (LAC, mg/dL), β hydroxy butyrate (BHB, mmol/L), non-esterified-fatty acid (NEFA mmol/L) and urea (UR, mg/mL) were measured.

We analyzed the effect among groups at each time point and over time using ANOVA and Tukey test for the means comparison (P<0.05; SAS® 9.0). Data are expressed as mean ± standard deviation.

Results: Serum parameters were within physiological ranges at all assessment moments. At Entry urea values showed significant differences (P<0.05), increasing with HSc (12.3± 5^{a}; 14.5± 6.9^{ab}; 18.2± 10.3^{b} for HSc1, HSc2 and HSc3, respectively). Pre-Weaning was the moment where we detected more metabolic differences among health-groups, with UR (14±6.2^{a}; 17.3±5.9^{b}; 16.4±5.1^{ab}; for HSc1, HSc2 and HSc3, respectively), LAC (13.1±5.1^{a}; 16.9±9.1^{b}; 13.9±6^{ab}; for HSc1, HSc2 and HSc3, respectively), GLU (117±18.3^{a}; 107.5±17.8^{ab}; 104.3±11.2^{b}; for HSc1, HSc2 and HSc3, respectively) and CHO (87.2±21.1^{a}; 83.3±15.1^{ab}; 71.1±12^{b}; for HSc1, HSc2 and HSc3, respectively) being different. However, later, we did not observe differences at any metabolic parameter. This reflects the increased metabolic challenge of reduced health status, with values related to protein metabolism (urea) and stress (lactate) being higher in calves with higher HSc. Glucose and fat metabolism was lower in calves with less health, which may reflect a lower energy availability, probably due to the higher energy costs attributable to inflammatory and pro-inflammatory states.

Regarding the study over time, some variables showed a different evolution over time according to HSc at entry: Urea in HSc1 and 2-groups increased significantly up to Grouping, while HSc3 did not vary over time (HSc1: 12.3±5^{a}, 14±6.2^{a}, 15.7±5.6^{a} and 20.9±7.5^{b}; HSc2: 14.5±6.9^{a}, 17.3±5.9^{a}, 16.1±5.7^{a} and 22±8.9^{b}; HSc3: 18.2±10.3, 16.4±5.1, 18.6±6.3 and 20.43±3.9, for Entry, Pre-Weaning, Post-Weaning and Grouping respectively). The urea increase is expected with time, according to the muscular growth. However, in the less healthy animals, urea requirements are higher since the very early moment. Finally, no significant difference was found in metabolic parameters after weaning, among health-groups, reflecting the similar health scores achieved among groups after weaning.

Conclusions: An inadequate health status at rearing-farm entry is associated with a higher metabolic challenge in the calves up to weaning, even with low HSc scores. However, it seems that under an adequate environment and management, those calves can recover all parameters to physiological levels in weeks.

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References:

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: metabolism, energy challenge, Health Score, Dairy, weaning.

HH-P18

The first survey of bioexclusion practices on dairy farms engaged in contract heifer rearing

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Objectives: Abolition of EU milk quotas has triggered a period of expansion in Ireland's dairy industry; dairy cow numbers increased by 34% between 2010 and 2018. This has resulted in increased uptake of contract heifer rearing services. Contract rearing is a collaborative farming practice where heifers are moved from their source dairy farm to be reared at a contract rearing farm for an agreed duration and fee. By moving heifers off the milking platform, land and labour resources can be focused on the milking herd. However, contract rearing increases animal movements, and animal movement is the most significant route for disease transmission. So, contract rearing poses a potential threat to herd health and biosecurity. Hence, the objective of this study was to compare bio-exclusion practices (measures taken to exclude pathogens from the farm) used by source dairy farmers (SDFs) and farmers rearing their own heifers (control farmers; CFs).

Materials and methods: In total, 66 SDFs and 54 CFs were recruited to a wider nationwide longitudinal study investigating the animal disease risks associated with contract heifer rearing. These 120 farmers were surveyed by postal questionnaire between September and November 2018 to identify bio-exclusion practices as part of a larger survey to establish herd management and wider biosecurity practices. Questions relating to bio-exclusion practices (n=25) were selected for inclusion in the survey following an extensive review of published literature and consultation with a biosecurity expert group. From the original cohort of farmers recruited to the study, 94% and 91% of SDFs and CFs returned their postal survey pack, respectively. The results from the farm characteristics, management and bio-exclusion practices questions are reported here.

Results: The average herd size was 198 cows (60-380) for SDFs and 146 cows (60-501) for CFs. The majority of farmers from both cohorts (93% SDFs, 92% CFs) provided a foot dip for use by farm visitors. Source dairy farmers were 4.4-times more likely to use a hired contractor to spread slurry than control farmers. The most commonly reported method of transporting cattle to and from SDFs was using livestock hauliers (76%) while control farmers were most likely to use their own trailer (80%). All SDFs and 98% of CFs reported operating a policy for rodent control on-farm. In total, 68% of SDFs and 65% of CFs reported purchasing animals in 2018. With regard to purchasing strategy, SDFs were 2.3- and 3.25-times more likely to buy animals from a closed herd or a herd using bulk tank milk (BTM) disease screening than CFs, respectively. Both SDFs and CFs who bought in cattle were equally likely to operate a quarantine policy for newly purchased animals upon arrival on-farm (79%). Of the farmers operating a quarantine policy, the majority (54%) of SDFs and CFs most commonly quarantined animals in a separate house from other cattle groups. Most frequently, SDFs (85%) and CFs (89%) who quarantined animals did so for a period of ≤ 4 weeks. The CFs were 1.4-times more likely to test newly purchased animals for disease than SDFs. Nose-to-nose contact with cattle on neighbouring farmers was possible on 13% of SDFs and 14% of CFs.

Conclusions: The results of this survey, the first to be administered to dairy farmers engaged in heifer contract rearing, indicate aspects of good bio-exclusionary practices on both farm enterprise types (use of foot-dips, rodent control, quarantining incoming livestock and limited neighbouring livestock nose-to-nose contact). However, many biosecurity risks were identified equally on both farm enterprise types (increased herd size, open herds, not testing purchased cattle and not buying them from closed or BTM testing farmers, short and non-exclusionary quarantining and using external livestock hauliers and slurry contractors). More of the farmers who send their heifers out to contract rearers (SFD) appear to be taking greater risks with some of these practices (open herd status and not testing purchased cattle and use of slurry contractors, and livestock hauliers). This is despite the potential increased risk for disease transmission associated with contract rearing for SDFs.

Keywords: Dairy, Heifers, Contract rearing, Bioexclusion.

HH-P19

Do attitudes and personality of calf care workers affect calf mortality?

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Objectives: Calf care workers are the main persons responsible for the daily care and health surveillance of preweaned calves. Due to their high contact rate with calves, their attitudes and personality might affect calf health and welfare. The aim of the current study was to analyse the associations between calf care workers' attitudes, personality traits, job satisfaction and calf mortality.

Materials and methods: Random sample of 120 herds was taken from a list of all Estonian dairy herds with at least 100 cows. Calf care workers who had worked in the farm for at least one year were asked to complete an anonymous questionnaire. The questionnaire registered respondent´s characteristics (age, gender, level of education and working experience) as well as their attitudes towards calves and calf mortality, farm working conditions and satisfaction with different job-related factors. The statements also revealed respondents´ overall empathy, self-confidence and importance of quality of life. Ten-item personality inventory form was used to register calf care workers' personality dimensions. A 7-point Likert scale was used to record the responses.

Based on the farm records and Estonian Agricultural Registers and Information Board data, a yearly calf mortality risk was calculated for each participating herd for calves up to

21 days and 22-90 days. Spearman correlation analysis was used to analyse the associations between calf mortality and statements from the questionnaires and variables with a p-value of < 0.25 were further used in K-mean clustering analysis to identify different calf care workers´ clusters based on their attitudes and personality.

Results: A total of 176 filled questionnaires from 108 different farms were received. The mean number of calves born in the study herds per year was 535. The mean calf mortality risk was 5.4% (range 0-23.3%) during the first 21 days of age and 2.7% (range 0-12.7%) during 22-90 days of age.

Seven of the studied statements were significantly associated with calf mortality risk in the younger (0-21 days) age group. Based on these statements, cluster analysis revealed four clusters. The average yearly calf mortality risk was 7.5% in cluster (CL) 1, 5.6% in CL2, 3.7% in CL3 and 4.0% in CL4. Calf mortality risk of CL1 differed significantly from that in CL3 and CL4 ($p < 0.01$). Calf care workers of the highest mortality cluster (CL1) were less satisfied with calf mortality level. They also felt that calf health problems are not under their control and they cannot overcome these on their own. In such herds, calf care workers also thought that higher calf mortality increases their workload. They were also less satisfied with their working equipment. A weak positive correlation between respondent´s extraversion and calf mortality was identified in the correlation analysis, but it appeared insignificant in cluster analysis.

In the older calf group (22-90 days), 11 statements met the criteria for including in the cluster analysis. The average yearly calf mortality risk was 3.4% in CL1 and 2.1% in CL2 with statistically significant difference between the clusters ($p < 0.01$). Similarly, a higher level of dissatisfaction with calf mortality level and working equipment was identified in the cluster with higher calf mortality (CL1). Calf care workers from the lower mortality cluster (CL2) agreed more that the people working with calves affect calf mortality.

Conclusions: This study revealed an association between calf care workers attitudes and mortality of calves, and possibly a two-way direction of the effect exists. In farms where calf mortality is high, calf care workers are more likely to feel that their skills and knowledge are insufficient to deal with calf health problems and they need more support from the veterinarians and other farm personnel to avoid frustration and possible decline in work motivation.

In addition to promoting the good health of calves, the working environment and equipment should be improved where.

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Keywords: calf, mortality, calf care workers, personality.

HH-P20

In practice animal health management by a digital app and relevant biomarker monitoring methods for farmers and veterinarians

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Objectives: During the transition periode dairy cows are submitted to massive metabolic changes and challenges. There is therefore an increased risk for cows to fail, resulting e.g. in ketosis [1,2]. In time prognosis of cows at risk would allow sucessfull preventing measures [3,4]. In the predessing optiKuh2-Project a predective model for estmating the ketotic risk has been developed (Risk- and DataIndex=RuDI). The aim of this study was to integrate this model into a digital APP in order to allow farmers and veterinarians to reliably estimate the transition cow risk of cows and to precisely monitor the

Parameter	Calcium 1		Calcium 2		NEFA		BHB		IGF-I	
Method	Cobas Mira	Vet-Photometer	Cobas Mira	Horiba LAQUAtwin	Cobas Mira	Vet-Photometer	Cobas Mira	BHB Sticks Keto	RIA	ELISA
Number n	19		8		20		20		20	
Minimum	1,87	1,85	1,55	1,50	50	100	0,34	0,5	39	29
Mean	2,15	2,20	2,20	2,21	301	345	0,83	1,18	132	185
Maximum	2,50	2,42	2,48	2,50	1550	1570	3,33	5,30	254	381
Standard Deviatioin	0,16	0,16	0,29	0,31	336	330	0,64	1,03	69	99
Correlation-coefficient (r)	0,734		0,979		0,997		0,994		0,974	
95% Confidence-intervall	0,419 – 0,891		0,884 – 0,996		0,993 – 0,999		0,984 – 0,998		0,934 – 0,990	
P	0,0003		<0,0001		<0,0001		<0,0001		<0,0001	

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disease incidences for a better health and cow welfare. For the validation of the APP several on site practice methods were established.

Material & Methods: On 2 dairy farms (105 cows/76 cows) transition cows were blood sampled from the tail vein. Ca, BHB, NEFA and IGF-1 was evaluated in laboratory by photometric methods (Cobas Mira/RIA) and the results compared to on site methods (Ca: Vetphotometer/Horiba iCa Laquatwin, Quidee GmbH; BHB - BHB-check-ß Keton (Taidoc); Nefa: Vetphotometer, Quidee GmbH).

Results: (Table).

Conclusions: The onsite tests Ca, Nefa and BHB worked very well with a significant correlation compared to the laboratory methods. Only for IGF-1 there is actually no on site test available. These results can be entered into an animal health APP to better identify and quantify the risk of ketotic cows, being then displayed on this APP. This allows a much more preventive management of cows at risk for a transition disease.

Keywords: animal health; biomarker; calcium test; Nefa; ketosis.

HH-P21

Application Note for the Use of a Wireless Device Measuring Reticuloruminal pH under Practice Conditions in a Swiss Dairy Herd

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Objectives: Subacute ruminal acidosis (SARA) is caused by ingestion of diets high in rapidly fermentable carbohydrates and/or deficient in physically active fiber, often suspected in high yielding dairy cows. Although SARA has been defined as repeatedly occurring periods of low ruminal pH, both practical measuring methods and threshold values remain subject to research.

Smart farming represents application of new digital and high-tech technologies into farming. Reticuloruminal pH can be monitored continuously using indwelling boluses situated in the reticulum, linked to a wireless data transmission system.

The objectives of this study were i) to test one commercially available bolus type under field conditions and ii) to suggest a threshold value for SARA on herd level. Three aspects contributed to threshold proposition: testing strategies in published literature, continuity of measurement, and length of the possible insult due to low pH values.

Material and Methods: In a dairy herd of 58 Holstein Friesian cows in Switzerland, in 2021, one bolus (smaXtec animal care GmbH, Graz, Austria) per cow was administered orally to 15 cows in their first third of lactation. The average daily herd milk yield had dropped significantly, fever of unknown origin occurred in several cows, and liver abscesses were found in two cows at slaughter. After a diagnostic visit, SARA remained the most probable underlying cause. Boluses were used to measure reticuloruminal pH in 10-min intervals during 150 days as guaranteed by the manufacturer. Mean individual circadian pH curves were calculated and summarized into a herd level circadian pH curve. A critical number of three or more cows with a ruminal pH ≤ 5.5, adapted from Garrett and Oetzel 1999, and a minimum of 3 hours duration, adapted from Ghozo 2005, were defined to be suspicious for SARA.

Results: The application of the boluses was uneventful. Twelve out of 15 indwelling boluses (80%) provided reliable data. Data from three boluses had to be excluded due to their random unintentional malposition in the rumen instead of reticulum or failure of accurate pH measurement. Of all measurements, 0.032% were pH values ≤ 5.5 (range: 4.67 - 5.5). No event of three cows simultaneously showing a ruminal pH ≤ 5.5 during a ≥ 3 hours interval was recorded throughout the study period. Maximum time interval where pH was ≤ 5.5 in a single cow was 2 hours.

Conclusion: The bolus enables the demonstration of circadian pH changes in individual cows, and can be used for practical purposes, although both malposition in the rumen and failure of accurate pH measurement occur. On herd level, automatic calculation of mean herd values is not provided by default, and we propose establishment of such a data tool.

Our proposed threshold of three cows out of 12 with a ruminal pH ≤ 5.5 during ≥ 3 hours was applicable regarding data processing. The herd level dataset of mean pH values never met the criteria despite being collected in a population at risk. A critical view on validity of our threshold suggestion is therefore needed, involving a larger study population. Additional considerations need to include the possibility that our herd was truly positive for SARA, but we failed to proof. Although thresholds on herd level are the scope of herd health medicine and smart farming innovations nowadays, individual susceptibility on cow level might be an interesting field of research, considering that data on herd level may not represent the true sum of individual pathophysiological processes.

We therefore conclude that continuous monitoring of reticuloruminal pH is possible and applicable for herd level investigations, but both reliability of the elected threshold and data procession may need improvement.

Keywords: Dairy Herd Health Management, Subacute Ruminal Acidosis, Smart Farming, Reticuloruminal pH.

HH-P22

Immunoglobulin concentration and bacterial contamination of bovine colostrum samples

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Objectives: Feeding poor quality colostrum is one of the most important risk factors for calves facing failure of passive transfer of immunity (FPT). Good quality colostrum is defined

as an immunoglobulin concentration of >22.0% Brix and a low bacterial contamination (total plate count <100,000 colony forming units/ml; fecal coliforms <10,000 colony forming units/ml). The objectives of this study were to 1) assess immunoglobulin concentration and 2) bacterial contamination of colostrum samples originating from cows from the federal district of Salzburg, Austria.

Material and Methods: From November 2020 to January 2022, members of the Animal Health Service of the federal district of Salzburg were ask to participate in the study. The study participants answered a questionnaire regarding general farm characteristics and specific questions on colostrum management. Colostrum samples were taken from primiparous and multiparous cows and stored at the farm of origin at minus 20°C until they were transferred to the University Clinic for Ruminants for further analysis. Immunoglobulin concentration was assessed by using a digital Brix refractometer. Bacterial contamination was assessed by plating the samples on Columbia agar (containing 5% sheep blood) and McConkey agar for total plate counts and fecal coliform counts, respectively. The agar plates were incubated for 18 to 24 hours at 37°C. The colonies were counted using Fiji-Software. Colostrum samples were categorized in good and poor-quality colostrum using the aforementioned thresholds for Brix values and bacterial contamination.

Results: In total, 72 farms participated in the study. The farms were distributed within the federal district of Salzburg in the regions of Flachgau (n=28), Pongau (19), Pinzgau (14), Lungau (7) and Tennengau (4). The majority of farms were dairy farms (69 farms; 95.8%), one farms was a cow-calf operation (1.4%) and two farms were mixed farms (2.8%). Forty-seven farms (65.3%) produced according to the European standards for organic farming and 25 farms (34.7%) run a conventional farm. Forty-one farms (56.9%) were full-time and 31 (43.1%) were part-time farms, respectively. The number of calvings per farm were as follows: ≤20 calvings = 29 farms (40.3%) and >20 calvings = 43 farms (59.7%). The cows were between their 1st and 14th lactation. In total, 278 (26.5%) primiparous and 752 multiparous cows were included. Dry period length was <6 weeks in 34 multiparous cows (4.6%) and ≥ 6 weeks in 707 cows (95.4%).

In total, 1.051 colostrum samples were collected. The samples originated from 34 farms breeding Simmental cows (n = 605 colostrum samples), 32 farms kept multiple cattle-breeds (381), three farms bred Pinzgauer cows (35) and two farms bred Holstein cows (23). Immunoglobulin concentrations ranged from 7.3% to 36.1% Brix (median = 22.0%; mean = 21.8%). Brix values of ≤22% were found in 529 (50.3%) and >22% in 522 (49.7%) of the analyzed samples, respectively. Brix values of <18% were found in 198 (18.8%) of the analyzed colostrum samples. Total plate counts were examined in 528 colostrum samples, whereof 326 (61.7%) met the standards for good quality colostrum yielding <100,000 cfu/ml and 202 (38.3%) showed total plate counts of ≥100,000 cfu/ml. Fecal coliform counts were investigated in 611 colostrum samples. In total, 550 colostrum samples (90.0%) were within the threshold for good quality colostrum yielding <10,000 cfu/ml and 61 colostrum samples (10.0%) were ≥10,000 cfu/ml.

Conclusion: In terms of immunoglobulin concentration and bacterial contamination, the results show that colostrum quality needs to be improved on the investigated farms.

Keywords: total plate counts, fecal coliform counts, Brix refractometry, poor quality colostrum.

HH-P23

Q fever vaccination strategy

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Objectives: Q-fever is a common disease in dairy cow worldwide. It is due to *Coxiella burnetii* infection and is associated with abortion, higher risk for placental retention, metritis and deteriorated reproduction performances (delayed conception, longer calving calving intervals and extra artificial insemination). Average within-herd and inter-herd prevalence of Q-fever in dairy production is estimated to 40% and 20%, respectively.

Vaccination is a common tool for Q-fever control. Most of time, it is used when the presence of Q-fever within a herd is detected, so as to limit its spread within herd and to reduce clinical consequences. The present trial aims at defining the economic benefit of Q-fever vaccination in the situations with moderate and high level of within-herd infection.

Materials and methods: A bioeconomic static model has been developed to mimic 3-year herd dynamics after vaccination. The benefit of the vaccination is assessed through a partial budget analysis based on the difference of the situation with vaccination compared to without vaccination. The production losses before vaccination were equal to the total cost when no vaccination for one year, and was constant during a given analysis, years after years, assuming no change in the situation if no vaccination was done. The production losses after vaccination were the final impact of Q-fever, when vaccination is done (damage is only partly decreased) for the 3 years after vaccination started. It is considered that vaccination improves health and reproductive performances differently for cows and heifers. The vaccine costs were changing every year since two injections are needed the first year for all the animals and the following years for heifers only. The cows already vaccinated the previous year received only one injection. Two level of Q-fever infection intensity was modelled.

Results: Vaccination protection makes it possible to reduce almost completely the number of cows infected in the dairy herd after 3 years. Vaccination is cost effective starting year 2 after vaccination. For a 100 cows herd with a low prevalence of Q fever infection (20%) before vaccination, the 3-year benefit is estimated at 3,169 €. For a herd with a higher prevalence (40%) of infected cows before vaccination, the benefit reaches 11,937 €.

Conclusions: The model allows to mimic the changes in within-herd infection level when vaccination is done. The benefit of vaccination for the 3 years ranges from 3,000 € to al-

most 12,000 € depending on the initial infection prevalence in the 100 cows herd. Our model focuses on cost benefits directly linked to animals and thus don't take into account farmer labour cost, improvement of productivity due to a better welfare and a lower quantity of milk discarded for sanitary concerns. Public health costs are also not included in this study although Q fever is a zoonosis. In spite the method only provides simplified consideration of disease dynamics and complexity, it appears as sufficient considering data available in the literature for its calibration, and it permits to demonstrate the benefit of vaccination against Q-fever.

Keywords: Q fever, vaccination, bioeconomic model, economics.

HH-P24

Systematic approach to neonatal diarrhea in dairy farms

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Objectives: Neonatal calf diarrhea (NCD) is a major health and welfare challenge during the first month of life. It is associated with reduced weight gain, increased mortality, and morbidity rate. Moreover, it is a frequent cause of antimicrobial use in dairy farms. NCD is a multifactorial disease and clinical cases are triggered by the interrelation of several factors.

On large dairy farms, infectious pressure is a key aspect due to the high number of calving's and calves per unit of time. Therefore, the aim of this cross-sectional study was to evaluate the different environmental and management factors associated with an increased infectious pressure. A secondary objective was to develop a practical tool to monitor these risk factors in a structured way at farm level.

Material and Methods: Between 2019 and 2021, 6 dairy farms located in the Northeast of Spain with NCD problems (>20% morbidity) were analyzed at herd level to identify potential sources of exposure to NCD pathogens. Additionally, epidemiologic data such as sanitary program, passive transfer, age of onset of NCD, morbidity, and mortality were monitored. Moreover, to identify the etiology of NCD, fecal samples were collected from clinical NCD calves and sent to the laboratory.

To identify those potential sources of exposure to enteric pathogens and based on calves traffic patterns four points were sampled to determine total aerobic bacteria, coliform count (CFU/cm^3 or ml) and *Salmonella spp.* presence: calving area bedding, colostrum, calf housing bedding and calf feeding equipment.

Results: NCD morbidity and mortality ranged in the different farms 40-70% and 1.9-6.2, respectively. Although most farms (5/6) vaccinate the dams against NCD, in most of them (5/6) a high percentage of calves showed failure of passive transfer (FPT) (n=4308, range 1-29%). On the other hand, related with the NCD etiology, 68 fecal samples were collected: 32% of samples were positive to one pathogen (being *E. coli* the most prevalent isolated pathogen), while 59% were positive to 2 or more pathogens (being the combination *E. coli* with *Cryptosporidium spp.* the most frequent). Moreover, in 3 farms, *Salmonella spp.* was isolated.

The environmental bacteriological sampling showed:

- Calving area: all farms had group calving pens with straw (2) or compost (4) bedding. The TCC in the 18 samples analyzed in occupied pens was above the goal of TCC < 5x10^5 CFU/cm^3 suggested by McGuirk (2008) in a 45% (23% and 85% for compost and straw bedding, respectively). Interestingly, on the 3 farms with fecal samples positive to Salmonella in the calves, were already positive in the calving bedding.
- Colostrum: Colostrum on farms was used fresh (3) or heat treated (1) or both (2), finding as expected, a lower bacterial contamination in the pasteurized colostrum.
- Calf housing: In all farms, calves were housed in individual hutches for the first weeks of life, with different types of bedding by farm and/or season (straw, sawdust, or sand). The highest bacterial contamination was obtained in sand (n=5), with 60% of samples above recommended goal and 1.4x10^7 UFC/cm3 on average; while the same farm using straw bedding in winter (n=4), 60% were adequate and average TCC was on average below the limit 2.4x 10^4 CFU/cm^3.
- Calf feeding material: the samples collected from the feeding equipment at the end of milk feeding. There farms used different feeding systems being 100% of bottle with teats below the limit, while 100% were above the limit in the bucket with nipple and 43% above the limit in buckets). On the other hand, comparing the cleaning system (50% of samples were above the limit with manual cleaning while a 100% were below with the dishwasher).

Conclusions: Farms with the highest mortality rate in the first month of life also presented the highest FPT rate. This showed how colostrum management is still a must and a major risk factor for NCD on large dairy farms, that clearly reduce the positive impact of dam's vaccination. Related to the sources of exposure to enteric pathogens from the environment: bedding type, colostrum management and feeding material hygiene can be interesting points to control on farms with endemic NCD, in order to reduce its economic impact and improve the welfare and antibiotic use in the calves.

Keywords: Neonatal, diarrhea, colostrum, management.

HH-P25

Colostrum supply: how is it done and what to improve?

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Objective: It is well known that colostrum feeding is essential for the transfer of passive immunity and health of newborn calves. The general advised strategy is to provide colostrum with some alliterating key aspects in mind: Quantity, Quickly, Quality and cleanliness. The aim of the survey was to substantiate how Dutch farmers translate the use of this advice into practice.

Materials and Methods: We randomly selected 400 dairy farmers with a conventional milking parlour and 400 dairy farmers with AMS from a national database of milk equipment services organizations. The farmers were approached by email and asked to participate in an online questionnaire. After some questions about farm management, we asked the farmers about the supply of colostrum to the most recently born calf. Among others, we asked when the newborn calf was fed for the first time, how farmers stored the colostrum in between feedings, and how they heated the colostrum before providing it to the calf.

Results: A total of 62 farmers with AMS and 45 farmers with a conventional milking parlour completed the questionnaire, resulting in an overall survey response of 107 farmers and a response rate of 13.4%. Milk production levels of these herds were around the national Dutch average, indicating representativeness of the respondents. The majority of the farmers (82%) reported that they used the dams' own colostrum for the first feeding of the calves, while some of them allowed the calf to suckle (14%) their mother. From 84 responding farmers, the amount of colostrum at first feeding was known and was on average 7.2 liters (SD 2.2). Out of these 84 farmers, 66 (79%) fed at least 6 liters of colostrum. Calves born during the afternoon received significantly more colostrum at first feeding than calves born during the evening and calves born at night had a significant higher risk to receive first colostrum later compared to calves born in the morning. On most occasions, colostrum feedings consisted of fresh colostrum given to the calf directly after milking. When colostrum was stored before feeding, the refrigerator was most often used, followed by storage at room temperature. Nursing bottles were most frequently used to supply colostrum.

Farmers indicated that they did not differentiate colostrum feeding strategies based on sex of the calves, which was in agreement with other analysis results from this study. We also found no differences in colostrum management between AMS farms and farms with conventional milking systems for any of the dependent variables.

Conclusion: We concluded that there are no systematic differences in colostrum management between dairy farms using AMS or a conventional milking system and that heifer and bull calves are treated similar with respect to colostrum management. Moreover, we found that the majority of farmers follow the general guidelines on colostrum management. More attention could be given to the timely feeding of colostrum of calves born in the night and to the storage conditions of collected colostrum.

Keywords: colostrum supply, online survey, current status, perspectives.

HH-P26

Milk and conductivity deviation settings on an automatic data collection system for accurate detection of acute disease in Holstein dairy cows

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Objective: The objective of this study was to determine whether a specific algorithm of milk and conductivity deviations on automatically collected data could be used to accurately identify diseased animals on several dairy farms as determined by professional veterinary examination as opposed to lay on-farm personnel examination.

Materials and Methods: A longitudinal observational study was undertaken at 3 dairy farms in Chile. Milk production and conductivity were automatically measured every milking for every cow. Deviations from the previous 10-day average for each specific metric are reported by the automated system for each cow after each milking. Based on previous work, the algorithm for mastitis detection was set as either (1) a single milking with -30% milk and +30% conductivity deviation, or (2) two consecutive milkings with +25% in conductivity in each. The algorithm for all other acute disorders was set as two consecutive milkings with at least -30% milk deviation if ≤200 DIM or at least -40% milk deviation if >200 DIM.

A veterinarian visited the farms 1-3 times per week and evaluated the cows on the list. Cows identified by on-farm personnel as diseased that were not flagged by the system were to be presented to the veterinarian at next visit. Data for this study included only veterinary evaluations. Positive and negative predictive values for the detection algorithms were calculated.

Results: The systems evaluated approx. 66,800 cow-days during the days that a veterinarian visited the farms. A total of 492 cows were flagged by the system (incidence = 7.3 per 1000 cow-days). A single cow (*N*=1) was deemed to have pathological findings by the veterinarian but not identified by the system, resulting in a negative predictive value of 99.9% (1/66,800).

Of the 492 cows flagged, 163 underwent veterinary examination. The rest were either diagnosed by on-farm personnel or missed by the sorting system and evaluated the following milking. A diagnosis was established by the veterinarian in 78 (47.9%) of the cows. In-depth evaluation of the 85 (52.1%) cows with no veterinary diagnosis revealed 27% (23/85) milked in a malfunctioning stalls. Of the remaining 62 cows with no pathologic findings on veterinary examination: 51.6% (32/62) had abnormal TPR (temperature, pulse, respiration), and 12.9% (8/62) had incomplete TPR values. Of the

remaining 22 cows (no pathological findings and normal TPR), 2 were in estrus and retained milk, 2 has incomplete milkout (increased milk in next milking), and 3 had received multiple vaccines in the previous 48 hours. A total of 7 cows with no documented diagnosis or management intervention had delayed milk production recovery that extended on average 38.4 ±50.0 days (range 6-140), while the remining 8 cows never recovered and were either culled or dried off.

After detailed review, evidence of abnormal health status (including long-term milk reduction) was *N*=125, resulting in a positive predictive value of 76.7% (125/163).

Conclusion: Previous studies used diagnosis by on-farm personnel as the gold standard for comparison to automatic data-collection systems, and have typically shown low accuracy for disease detection by the technology. We expected higher accuracy when trained veterinarians did the physical examination compared to lay on-farm personnel. Our results show that this is not the case.

The most likely reason for the discrepancy is due to the automatic systems functioning as diagnostic tests in series, multiple times per day, every day, while a veterinarian visiting the farm on occasional days functions as a single isolated diagnostic test. Additionally, the use of very granular data collected 2-3 times per day allows technology to identify cows with subclinical disease or detrimental management events that resulted in temporary decreased milk production.

A major conclusion is that our diagnostic techniques as veterinary practitioners need to evolve with the capabilities of the new modern automatic data-collection systems that exist on many farms to detect small consistent changes in individual cows. Additionally, a standard setting for all farms may not be appropriate, and therefore systems that can be customized to each farm may have a practical diagnostic advantage in the field. Using the data provided by these systems will help clinicians and consultants to identify management practices leading to sustained milk production losses in dairy cows, and thus help improve animal wellbeing.

Keywords: Automatic data, disease detection, algorithm, dairy, diagnosis.

HH-P27

Restriction of free access to feed and resting area after last milking before dry-off associated with lower blood calcium levels in dairy cows

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Objectives: A recent publication has emphasized the fact that subclinical hypocalcaemia can be found in dairy cows immediately following dry-off (Krogh et al., 2018) and the condition of atypical milk fever (clinical hypocalcaemia in late gestation) has been described before (Radostitis, 2007). Despite considerable research into dry cow management and the increasing level of milk production at dry-off in the modern cow, there is limited information available on the prevalence of hypocalcaemia at dry-off and potential associated risk factors. This study aims to assess the prevalence of subclinical hypocalcaemia after the last milking before dry-off in dairy cows in United Kingdom and the effects of restricting access to feed and lying area on the mean serum total calcium value at this stage of the lactation cycle.

Materials and methods: A total of 15 farms selected across South West UK participated in the study. Within these farms, 197 cows were included in the study follow-up between the 22th of November, 2018 and the 28th of February, 2019. For all cows, information was recorded regarding the time without free access to feed and lying area after last milking. Blood samples were collected 8-12h after their last milking before dry-off and sent to a reference laboratory. Subclinical hypocalcaemia was considered when cow blood calcium was <2.200 mmol/L. Statistical analysis was performed using the computer software JMP (version 7) SAS Institute.

Results: Overall 67% (132/197) of cows were without access to feed or lying for ≥ 1 hour after their last milking before dry-off. Analysis of serum total calcium demonstrated that 2% of cows had hypocalcaemia (5/197) 8-12 hours after their last milking before dry-off. Statistical analysis demonstrated that cows with ≥ 1h without access to feed and lying area had a significantly lower mean serum calcium value (mmol/L) 8-12 hours after their last milking before dry-off compared to those that were without access to feed and lying area for < 1hr (2.400 vs. 2.441, P=0.018).

Conclusions: This study demonstrates that subclinical hypocalcaemia in dairy cows after dry-off occurs in United Kingdom, and that restriction of access to feed and lying area increases the likelihood of lower blood calcium level of cows in the hours after dry-off.

Keywords: Dry-off, Calcium, Welfare, Dairy, Cows.

HH-P28

Dry-off practices on UK commercial dairy farms

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Objectives: Dry-off is an extremely important, but also risky period for the dairy cow. Traditionally, dry period management has been focused on practices that are aimed at maximizing milk production in the subsequent lactation while optimizing the health and fertility of lactating cows. However, current dry-off methods present drawbacks. Abrupt dry-off has

a higher risk of new intramammary infections and excessive engorgement of the udder, and gradual dry-off stresses animals through feed restriction and social regrouping. In both cases there is a negative impact on dry cow health and welfare. A better knowledge and understanding of current dry-off practices would help practitioners to implement best practices and identify future research priorities. The study was aimed at investigating the prevalence of different dry-off practices, with a special focus on those procedures that can potentially adversely affect cow health and welfare.

Materials and methods: A total of 15 farms selected across South West UK participated in the study, with the size of herds ranging from 130 to 800 dairy cows. Information on production parameters, feeding and management practices at dry-off, both at herd and cow level, were collected through a farm audit and survey from participating farms between November 2018 and February 2019.

Results: The farms had a mean production (energy corrected milk) per lactation of 9,983.5 ± 1,310.6 kg milk. The majority of cows in participating dairy farms (70.8%) were dried-off at 10-30 kg/d of milk production (10-20 kg/d: 34.6 %; 20-30 Kg/d: 36.2 %). Cows dried-off with a daily milk production >30kg represented 9.4%. Just over half the herds (53.3%, 8/15), performed abrupt drying-off, while 46.7% (7/15) of the farms performed a gradual drying-off. Among the herds performing a gradual dry-off, 71.4% (5/7) reduced both milking frequency and feeding regime prior to drying-off, while 28.6% (2/7) only modified the feeding regime before drying-off. No herds reduced solely milking frequency. Changes in the feeding regime concerned changes of only feed quantity (n=4), only feed quality (n=2) or both (n=1). Following last milking, 26.7% (4/15) and 53.3% (8/15) of farms did not allow cows free access to water or to feed and resting area for ≥ 1 hour, respectively. In 100% (15/15) of farms, cows after their last milking after dry-off were moved to a dry cow shed and isolated from the lactating cows.

Conclusions: Potentially stressful procedures for cows such as abrupt cessation of milking in high yielding cows, changes to their diet, social regrouping and restricted access to water, feed or resting area at dry-off are part of dry-off management procedures on dairy farms. These results evidence the need to find a simpler method for abruptly reducing milk production without negatively impacting udder health and cow welfare.

Keywords: Dry-off, Dairy, Welfare, UK.

HH-P30

Evaluation of productive parameters associated with food manegement and well - being in beef cattle

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Objective: To evaluate the effect of addition of sodium bicarbonate and fodder in the diet, plus the increase of feeding space on the daily weight gain and food conversion at 30 and 50 days.

Material and methods: This study was carried out in a cattle fattening facility located in the state of Michoacán, México. Two groups were formed, Treated Group A, integrated by 225 animals Bos taurus x Bos indicus, with a weight range between 226-330 kg, conforming 5 pens, 45 animal in each pen. In the first 7 days a diet with 22% of pellets and 78% of fodder was offered; from day 7 to day 14 it was a diet with 40% of pellets and 60% of fodder; from day 14 to 21, they enter in a transition period, the first meal of the day is 40% pellets and the second 70% of pellets and the rest fodder until it´s completion. During the first 7 days corn stubble was added as fodder to stimulate animal consumption; additionally, sodium bicarbonate was administered in the drinking water in a dose of 180 gr/animal for 4 days, to contribute to the increase in consumption of dry material and a better digestion. The B Group (control) was formed by 326 animals Bos taurus x Bos indicus, with a weight range from 226-330 kg, separated in 5 pens of 65 animals each, they received the same diets as the treated group, except for forage stimulation and the addition of sodium bicarbonate in the drinking water. Both groups underwent a preventive medicine program consisting of deworming against gastrointestinal and pulmonary worms, vaccination against clostridiums and bovine respiratory complex, as well as the use of hormonal implants and the use of antibiotic – based metaphylaxis such as Tulatromycin and Enrofloxacin.

Results: The Treated Group A had an initial weight of 286.8 kg and the Control Group B of 288.7 kg. The daily weight gain (DWG) at 30 days of the Treated Group A was 1.31 kg compared to the Control Group B with a DWG of 1.09 kg ($P<0.05$). The DWG at 50 days (reimplantation weight) of the Treated Group A was 1.38 kg and the Control Group B of 1.1 kg, there being a significant difference ($P<0.05$). The feed conversion (FC) at 30 days of Treated Group A was 6.31 kg, while in Control Group B it was of 6.26 kg ($P>0.05$), while the FC at 50 days (reimplantation weight) for the Treated Group A was 5.83 kg and for the Control Group B, 6.03 kg, there was no significant difference ($P>0.05$). CONCLUSIONS: For the Treated Group A, with the addition of fodder and sodium bicarbonate, plus the increase in feeding space, the behavior of the DWG was better than in the Control B Group, there being a significant statistical difference, but not with the FC between the group.

Keywords: Fodder, feeding space, sodium bicarbonate.

ID-P01

An approach to investigating a herd level BVD breakdown via the Scottish Government eradication scheme.

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Objectives: The objective of this poster is to provide practitioners with a 'real life' approach to investigating BVD at a herd level in accordance to the compulsory Scottish Government scheme. The importance of investigating and eradicating this infectious disease from herds, and ultimately, whole countries is important due to the welfare, health and economic impacts on animals and farming enterprises. As the whole of the United Kingdom is now taking part in BVD eradication this abstract was accepted and presented at the British Cattle Veterinary Association Congress in 2019.

Materials and Methods: Annual compulsory BVD check test screening had been performed on a 280-cow dairy farm since 2010 in accordance with the Scottish Government requirements (*Scottish Government, 2011*). When the check test was performed in 2013, ten out of the ten heifers blood sampled had positive BVD antibody titres. A two-pronged investigation was then initiated, consisting of adult milking herd screening and individual animal ear tag testing.

Ear tag testing of calves, within two days of birth, was commenced. All calves born since the 2012 check test, that remained in the herd, were tested for antigen by ear tag. Only around 40% of these animals were available. The dams of all confirmed PI calves were antigen tested by blood sample.

The milking herd was screened for BVD antigen by bulk milk PCR.

Results: The bulk tank screening was negative for BVD antigen. Over the next twelve months seventeen antigen positive calves were found by ear tag and a blood sample was taken three weeks later to identify BVDV-PI animals. Of these seventeen, thirteen tested BVD antigen positive, three tested negative and one animal died before testing could occur. All confirmed PIs were culled as soon as possible. The dams of these calves were all found to be heifers. These animals had been vaccinated pre-insemination with an inactivated virus vaccine (Bovidec**,** Elanco Animal Health) whilst away from the main premises. After questioning the head stockperson, it was found that unbeknownst to the herd owner the rearing unit had started buying stores. The primary vaccine course was also not performed as detailed in the data sheet. Heifers are no longer reared away from home after a neighbouring farm ceased business and these sheds are now rented. The herd now uses a modified live virus vaccine (Bovela, Boehringer Ingleheim Limited).

Conclusions: Eradication of BVD from herds is worthwhile due to its economic significance although there have been few farm level estimates of the losses associated with BVD infection of the UK dairy herd (*Gunn et al., 2003*). Highlighted during this investigation is the need for good management compliance at farm level with regards to biosecurity and use of vaccines.

References:

1. Scottish Government 2011, *Scottish Government website*. January 2011. Available from: http://www.gov.scot/Publications/2011/01/17111217/4 [accessed on 26th July 2019].

Keywords: BVD, eradication.

ID-P02

Determination of the prevalence of *Salmonella* spp. in feces from two dairy herds located in the Sabana de Bogotá-Colombia

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Objectives: The objective of this study was to determine the prevalence of *Salmonella* spp. shedding in fecal samples in two dairy herds in the Sabana de Bogotá – Colombia.

Materials and Methods: Eighty cattle of Normande and Holstein breeds were sampled. They belonged to two herds, herd 1 had 16 cattle and herd 2 had 64 cattle. Each animal was individually sampled twice 15 days apart in order to increase the likelihood of detecting *Salmonella* spp. intermittent shedders. Each sample was obtained directly from the rectum and stored in ziploc bags. The samples were tagged with all the information of the animal to be tested. Each sample was initially pre-enriched in non-selective broth, followed by enrichment in selective broth and afterward were plated in selective and differentiating agar (Hektoen and XLT4). The colonies that were identified as *Salmonella* spp. were isolated using a chromogenic agar (Brilliance Agar) following the Oxoid *Salmonella* Precis™ methodology which is approved by ANFOR according to the norm ISO 16140. The biochemical identification was done using the RapidOne® galleries for enterobacteria. The colonies determined as *Salmonella* spp. were tested using a conventional Polimerase Chain Reaction (PCR), the PCR-Salmonella (CorpoGen® BM-00007) commercial kit to detect the 284 pb genic fragment that corresponds to gen invA using electrophoresis in 1% agarose gel. Finally, the DNA extracted samples were sequenced to identify the serovars present in the samples.

Results: *Salmonella* spp. was isolated from 25% (n=20) of the sampled cattle. All the samples from herd 1 were negative, while all the positive samples were from herd 2. Fifty-seven percent of the positive animals were 2-5 years, 24% were between 5-8 years old and 19% was older than eight years. Water source were different between the two herds, herd one has water from the municipal aqueduct, but in herd two, the water source came from built ditches. All the isolates were confirmed to be *Salmonella* spp., by detecting the 284bp fragment that is specific for gen invA.

Sequencing of the isolates indicated that the serovars present were *S.* Bredeney in 15% of the cases (n=3), *S.* Newport in 10% (n=2), *S.* Typhimurium in 10% (n=2), and the serovars *S.* Cerro, *S.* Anatum y *S.* enterica subsp. diarizonae in

5% each (n=1); however, in 50% of the isolates, the *Salmonella* serovar could not be identified.

Conclusions: A 25% prevalence of *Salmonella* spp. was observed in the studied two herds in the Sabana de Bogotá.

The most prevalent serovar was *S.* Bredeney

The younger cattle had a higher prevalence of *Salmonella* spp.

The presence of this pathogen in the feces could have been related to the quality of the animals' drinking water (water in ditches).

Keywords: Salmonella, feces, prevalence.

ID-P03

Comparative analysis of serological tests and fecal detection in the diagnosis of *Mycobacterium avium* subsp. *paratuberculosis* infection

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Mycobacterium avium subsp. *paratuberculosis* (MAP) is a causative agent of Johne's disease (JD), also known as paratuberculosis (PTB), which is a chronic granulomatous enteropathy in ruminants. MAP infection is characterized by chronic diarrhea, progressive wasting, edema, and eventual death because of hypoproteinemia caused by protein-losing enteropathy. JD occurs worldwide and produce significant losses to the livestock industry due to weight loss, reduced milk production, death. JD has long incubation period due to long generation time and intracellular survival of MAP. The intracellular survival of MAP induces complicated immune responses in the host. Also, fecal shedding of bacteria occur without any clinical symptoms and detectable antibody in the early stage of infection. Those things make accurate diagnosis difficult. In addition, the commercially available serological diagnostic kits have different characteristics, leading to confusion in accurate diagnosis of MAP infection.

Objective: Therefore, accurate diagnostic methods have been required to control the disease. Based on the current situation of diagnosis of MAP infection, we compared and analyzed serological diagnostic methods and fecal detection using PCR to find out some clues.

Materials & Methods: Two serological diagnostic kits currently used (A and B) were compared using 298 sera and, also, the results were compared with fecal detection with PCR.

Results: The A kit showed higher positive rate than B kit (37.5% vs 23.1%). The agreement between the two kits was found to be moderate (k=0.49, Cohen's kappa coefficient). However, the correlation of S/P ratio between the two kits was relatively high (R^2=0.747, Pearson's correlation coefficient). In addition, in comparison of serological tests at more than two time points with same samples, the two kits showed similar patterns in S/P ratio even though there was difference in the positive and negative results. There was a low agreement between serological and fecal detection tests (k=0.30, Cohen's kappa coefficient). In general, the detection rate of fecal and serological tests was highly correlated with age, so the fecal test showed a high detection rate in young age and serological test in old age.

Conclusion: These results indicate that a new diagnostic system needs to be established for the effective diagnostic protocol of MAP infection by combination of serological test and fecal detection test.

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Keywords: diagnosis, Mycobacterium avium subsp. paratuberculosis.

ID-P04

Prevalence and risk factors associated to *Coxiella burnetii* in Terceira Island dairy farms

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Objectives: The dynamics of *C. burnetii* infection in ruminant herds in Portugal is not well understood. Recently, a study reported that *C. burnetii* was endemic in dairy livestock in Central Portugal (Anastácio et al., 2016) but the epidemiologic situation of Azores islands, one of the main contributor to the dairy industry in Portugal is currently unknown. The main goal of this study was to determine the prevalence of antibodies against *Coxiella burnetii* in cattle from dairy farms in Terceira Island, Azores – Portugal, and investigate the potential risk factors, which could contribute to the infection and dissemination of Q fever in the herd.

Materials and methods: The survey was conducted from January 2018 to March 2018 in Terceira Island, Azores, Portugal. At the date of sample collection, no dairy farm had started vaccination for Q fever. Thus, it was possible to assume that the antibodies present were the result of contact with the agent. The survey relied on the detection of milk antibody in bulk tank milk (BTM) as BTM has been shown to be a suitable sample to screen for *C. burnetii* infection on lactating animals and the milk antibody analysis by ELISA testing is also a reliable tool to provide information about the exposure to *C. burnetii.*

Bulk tank milk (BTM) samples were obtained from 132 randomly selected dairy farms and analyzed using a commercial ELISA kit (ID Screen® Q fever indirect multi-species ELISA, IDVet Genetics). Sample size was representative of the existent 544 dairy farms in the island (CI 95%, margin of error of

7.5%). During February and March of 2018, a questionnaire was conducted to collect information concerning characterization of the farm, herd health and farm management from the selected farms. Statistical analysis was performed using R® program and Pearson's Chi-square tests were performed for the variables that were investigated as potential risk factors.

Results: The apparent prevalence of *C. burnetii* was 75.8% (CI 95%: 67.8-82.3). The proportion of negative and inconclusive herds was 18.2% (24/132) and 6% (8/132) respectively. The results demonstrated a uniform distribution of positive cases in both counties of Terceira Island. In the univariate analyses, three factors were identified as presenting a significant association with the presence of anti-*C. burnetii* antibodies in BTM, namely the size of the herd (>50 animals), the contact of the animals with cattle from other farms and the direct interaction of the animals with dogs or cats (P <0.05).

Conclusions: This study is the first to report the prevalence of *C. burnetii* and associated risk factors in Terceira island dairy farms. Our data provide evidence of a high prevalence of Q fever in Azores and contact with cattle from neighboring farms seems to be a relevant risk factor for the presence of antibodies against this agent. The problem of Q Fever should be seen from a global perspective of raising awareness among veterinarians and producers so that the most appropriate control strategies, such as vaccination and proper management conditions, can be established.

References:

Anastácio, S., Carolino, N., Sidi-Boumedine, K., Silva, G.J. da, 2016. Q Fever Dairy Herd Status Determination Based on Serological and Molecular Analysis of Bulk Tank Milk. Transbound. Emerg. Dis. 63, e293–e300.

Keywords: Q fever, Coxiella burnetii, Dairy cattle, Prevalence, Azores.

ID-P05

The risk of a naive dairy herd to obtain *Mycoplasma bovis* infection from *M. bovis* contaminated bull semen – preliminary results

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Objectives: *Mycoplasma bovis* affects animal welfare, causes financial losses and increases the use of antibiotics. The infection was detected in Finland for the first time in 2012. In 2016, we detected *M. bovis* in several lots of a bull (Bull A) that had been used in artificial insemination (AI) in Finland. Consequently, we could document how a naïve herd had obtained the infection from a contaminated semen lot of Bull A in AI. In this study, we analyzed the risk of a naive dairy herd to obtain *M. bovis* infection by AI from *M. bovis* contaminated bull semen.

Materials and methods: The usage of semen from Bull A in AI in Finland was collected from the database of the breeding company Faba Coop in 2019. A list of known *M. bovis* positive dairy farms was obtained from Animal Health ETT. We analyzed how the previous use of a contaminated semen associated with the *M. bovis* infection status of the farm. Univariable logistic regression model was performed with the positive semen lot used as a variable and the infection status of the dairy farm (positive/negative) as an outcome.

Results: Altogether 2006 cows were inseminated with semen from any lot of Bull A. Of these, 269 cows on 233 farms were inseminated with semen from contaminated *M. bovis* lots. Out of 8808 dairy farms in our data, 73 farms were known to have been *M. bovis* positive. Ten of these *M. bovis* positive farms had used semen from *M. bovis* contaminated lots. Based on the logistic univariable regression model, a dairy farm had 6.4 times higher odds of becoming *M. bovis* positive, if semen from a known *M. bovis* contaminated lot was used in insemination (P<0.001, CI= 3.2–12.55).

Conclusions: Insemination with *Mycoplasma bovis* contaminated semen significantly increases the risk of a dairy farm to get infected with *M. bovis.*

Keywords: Mycoplasma bovis, Cattle, Semen.

ID-P06

Molecular surveillance of pathogens associated with diarrhea in pre-weaned Korean native calves

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Objective: Calf diarrhea leads to severe economic losses due to poor growth and mortality of the affected young calves in livestock production worldwide. The aim of the present study was to investigate the prevalence of bovine coronavirus (BCoV), bovine norovirus (BNoV), bovine rotavirus (BoRVA), bovine torovirus (BToV), bovine viral diarrhea virus (BVDV) and *Escherichia coli* from pre-weaned Korean native calves according to fecal consistency and calf age, and to report any associations between these pathogens identified in this study and calf diarrhea.

Materials and methods: Between March 2017 and October 2018, a total of 689 fecal samples were collected from ≤60 days of pre-weaned calves from nine regions of the Republic of Korea (ROK). Total RNA was extracted from a 200 µL of fecal suspension. The BCoV, BNoV, BoRVA, BToV and BVDV were detected using one-step RT-PCR. Each fecal sample was inoculated onto MacConkey agar and blood agar. Pure colonies with appearance of *E. coli* were randomly selected and confirmed to be *E. coli* using standard biochemical tests (API 20E system, bioMerieux, France). PCR for ETEC and STEC was performed by multiplex PCR for two factors (*F17* and *saa*) and six factors (*F5* (=*K99*), *F41*, *stx1*, *stx2*, *eae*, and *sta*).

Results: Among the five viral pathogens, BVDV (15.8%) infection was the most frequently detected, followed by BNoV (12.2%), BCoV (5.4%), BToV (2.2%), and BoRVA (1.9%). *E. coli* were detected the most (56.3%) from all regions examined. The results showed that BCoV (*P*=0.032) and BoRVA (*P*=0.007) were significantly associated with diarrhea in these calves, whereas BNoV (*P*=0.796) and BToV (*P*=0.662) were not statistically associated with diarrhea. BVDV was more commonly detected in normal feces (*P*=0.021). There was no statistical significance between calf age and viral pathogens. Interestingly, the prevalence of *E. coli* is significantly related to age of calf (*P*=0.000).

Conclusions: The present study demonstrated that BVDV and *E. coli* were prevalent in pre-weaned Korean native calves. Moreover, BCoV and BoRVA are involved in calf diarrhea in the ROK, and *E. coli* infection is closely related to calf age. Therefore, the risk factors associated with the pathogens causing calf diarrhea may be identified for prevention and control.

Keywords: diarrhea, pathogens, pre-weaned calves

ID-P07

Anaplasma marginale infected calves detected by the electrophoretic profile of bovine serum proteins: preliminary results

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Introduction: *Anaplasma marginale* causes major economic losses for dairy and beef herds in Brazil. Infected calves are diagnosed by direct blood smear examination and clinical findings reducing the treatment efficiency. Unfortunately, direct examination lacks sensitivity and is a late method for diagnosing anaplasmosis.

Objective: The objective of this study was to verify the electrophoretic profile of serum proteins for differentiating between *Anaplasma* infected and non-infected calves.

Materials and methods: Calves from a dairy herd from Brazil were monthly examined for *A. marginale* infection and blood serum sampled for resolution in 10% sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE). Ten micrograms of whole bovine serum proteins were resolved by 10% polyacrylamide gel under reducing conditions (25 mA for three hours) and stained by Coomassie Blue R250 for 2 hours). Relative gel migration of serum proteins was obtained by comparing them with a molecular weight standard (200 kDa to 20 kDa). SDS-PAGE was stained with Coomassie Blue and analyzed by ImageJ and Image Lab software. These analyses included determination of the number of bands in gel, estimative of molecular weight and investigation of the electrophoretic profile from infected and non-infected calves. This study was approved by the ethical committee of animal experimentation (number 115/17) of Universidade Federal de Goiás.

Results: The average of protein bands were 17.3 ± 1.3 (maximum 19 and minimum 15 bands) and 19.4 ± 2.5 (maximum 23 and minimum 17 bands) for non-infected and infected calves respectively. Maximum and minimum molecular weight averages were 176.5 kDa ± 12.6/25 kDa and 221.4 kDa ± 29.7/24 kDa with a standard deviation of ± 2.01 for non-infected and infected calves respectively. Densitogram revealed differences in protein bands from non-infected and infected calves involving proteins with molecular weight from 140 kDa to 75 kDa and from 52 kDa to 37 kDa. Acute phase proteins (APP) detection such as ceruplasmin (132 kDa), haptoglobin (40 kDa) and alpha-acid glycoprotein (44 kDa) were included in these intervals. Additionally, *Anaplasma*-infected calves that present a low or a high parasitism rate apparently have a different profile of proteins including the APP.

Conclusion: SDS-PAGE of serum proteins is a promising method to differentiate between anaplasmosis infected and non-infected calves and identify low or high *Anaplasma* infected bovines. This method can identify protein targets for precocious diagnosis of *Anaplasma* infection by platforms for field use. At this moment, we are carrying out mass spectrometry experiments for precisely identification and measuring of those serum proteins.

Keywords: anaplasmosis; electrophoretic profile; acute phase proteins.

ID-P08

Correlation between anti-*Babesia bovis*, *Babesia bigemina* and *Anaplasma maginale* antibodies from calved cows and the serum immunoglobulins obtained by colostrum in calves

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Introduction: *Babesia bigemina*, *Babesia bovis* (babesiosis) and *Anaplasma marginale* (anaplasmosis) cause major losses in the cattle industry in Brazil each year. Passive immunity against babesiosis can be transmitted to calves from cows infected or previously infected with the parasite while immunity of calves against anaplasmosis develop independent from previous infection of cows.

Objective: The objective of this study was to correlate the levels of antibodies against *Babesia bigemina*, *Babesia bovis* and *Anaplasma marginale* of calved cows with the immuno-

globulins obtained by calves through colostrum, from the birth to weaning. Blood samples from 25 cows and 25 calves were collected on a dairy farm in the southeastern part of Brazil. The type of "compost barn" is the management animal system used for pregnant and lactating females. In addition, grazing of native field is allowed from weaning to puberty. Blood samples from the cows were collected 72 hours before calving and on the day of calving. Blood samples from the calves were collected on the day of birth until 84 days after birth at intervals of 14 days. Antibody titers were determined by ELISA using positive controls of bovine sera with high parasitemia and negative controls from sera from newborn animals that did not ingest colostrum and negative on PCR and ELISA / RIFI. The microplates were sensitized with crude *B. bigemina* and *B. bovis* extract antigens and purified from *Anaplasma.* Anti-bovine IgG labeled with alkaline phosphatase conjugate produced in rabbits was used. Statistical analysis was performed according to the type of comparison using the Fisher and Chi-square (χ2) tests. The ELISA cut-off was established with a 99.99% of confidence. The blood samples and the stages of parasites were evaluated and the parasitemia determined.

Results: Hematological parameters were evaluated and no changes were observed in the animals. In the analysis of blood smears, only four cows showed *Babesia bigemina* intra-erythrocytic corpuscles, with a 3.4% parasitemia. The presence of *B. bovis* or *Anaplasma* was not observed in cows and none of the offspring was positive. ELISA was positive for four cows infected with *Babesia bigemina,* two for *B. bovis* and none for *Anaplasma marginale*. No calf showed seropositivity for babesiosis. The cows that showed seropositivity for *B. bigemina*, were the same that showed *Babesia bigemina* intra-erythrocytic corpuscles on blood smears. There was no positive and significant correlation between the level of antibodies from the cows and the immunoglobulins of their calves.

Conclusion: Although, the presence of *Babesia* was detected on the property, the animals showed low levels of antibodies, which would be insufficient to produce passive immunity in the calves. If the calves enter in contact with the etiological agents, they can develop severe disease. The high number of non-reactive animals is probably due to the type of management, which reduces the number of ticks in the environment and in the animals, reducing their contact with the host.

Keywords: Babesiabovis, Babesiabigemina, Anaplasmamaginale, calvedcows, antibodies.

ID-P09

Molecular survey of severe fever with thrombocytopenia syndrome virus in cattle from the Republic of Korea

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Objectives: Severe fever with thrombocytopenia syndrome (SFTS) caused by the SFTS virus (SVTSV), an emerging tick-borne zoonotic infectious disease, has mainly been reported in many Asian counties. SFTSV is known to infect a wide host range. Between 2018 and 2019, the prevalence of SFTSV from cattle on ranches in 6 provinces in the Republic of Korea (ROK) was investigated using a total of 556 serum samples.

Material & Methods: Viral RNA was extracted from sera using viral RNA extraction kit. One-step RT-nested PCR were conducted to test SFTSV specific RNA of S segment. The sequence data were analyzed using Chromas and were aligned using CLUSTAL X. The phylogenetic analysis was constructed using the neighbor-joining method in MEGA7.

Results: The overall prevalence of SFTSV was 2.0% (11/556) in cattle. Among 6 different provinces (Chungcheongbuk-do, Jeollabuk-do, Jeollanam-do, Gyeongsangbuk-do, Gyeongsangnam-do, and Jeju-do) examined, SFTSV was detected only in two provinces, Jeollanam-do (3.1%, 6/193) and Jeju-do (8.3%, 5/60). The size of amplified product of S segment was 346 bp and the isolates obtained in this study belonged to all genotype B of SFTSV.

Conclusion: This study shows the presence of SFTSV infection in cattle in the ROK. The strategies for control and prevention of SFTSV infection should therefore be considered in cattle as well as humans.

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Keywords: Severe fever with thrombocytopenia syndrome (SFTS), tick-borne diseases, cattle.

ID-P10

Seroprevalence and factors related to infection of bovine viral diarrhea in dairy cattle in Western Thailand

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Objectives: Bovine viral diarrhea (BVD) is a disease that suppresses a cow's immune system, making the animal susceptible to a host of other infections and leading to silent economic losses. Although the epidemiological studies of BVD virus (BVDV) infection among dairy cattle herds in Thailand have been done since 1991, the lack of disease prevention and less awareness of herd biosecurity may have resulted in continuing viral spread in country. The aims of this study were to investigate the current situation of BVD disease in dairy cow in Western Thailand and to identify the factors related to infec-

tion of BVD disease.

Materials and methods: This study was carried out in 27 unvaccinated against BVDV dairy cattle herds located in four provinces of the Western part of Thailand. A commercial ELISA kit (BVDV Total Ab Test, IDEXX, USA) was used to detect BVDV- specific antibodies in 521 serum samples. Detected antibody levels were measured as corrected optical density (COD) and the sample to positive (S/P) ratio was calculated for each sample relative to the positive and negative control serum on each plate which a cut-off for positive samples was S/P >0.30. Animals and herds information such as lactation number, health problems (diarrhea, abortion and respiratory signs), herd size, herd management, purchased new cow during the last year were collected by interviewing the owner together with the observation by bovine practitioner during farm visit.

Results: In total, 521 samples collected from individual cattle were evaluated. Antibody-positive prevalence at individual animal level was 46.07 % (95%CI: 42.72- 49.42%) and at herd prevalence ranged from 6.67% (95%CI: 4.13-17.47%) and 96.55% (95%CI: 91.40-100%). In the univariable analysis, herd-level prevalence differed among provinces (*P* <0.0001), the prevalence varied from 25.74% (95%CI: 18.55-32.87%) and 86.32% (95%CI: 81.15-91.48%). Among positive individual, the first and the second lactation cattle revealed the highest proportion (20%) followed by the third lactation (18.75%). Most sero-positive herds were medium herd size with 20-50 animals (66.67%). Focused on the characteristics of farm, the most common raising type was free stall raising (62.96%) and no biosecurity in farm (66.67%) was reported. Majority of the farmer reported not purchase new cow during the past twelve months (70.73%), had abortion problem in herd (77.78%) and history of calf death with diarrhea and/ or respiratory problems (81.48%).

Conclusions: The sero-prevalence obtained from this study indicated that all herds had been exposed to the virus. Although we cannot identify either past or present exposure, however, positive cattle can be latent carriers of the BVDV and viral shedding within herd can be occurred continuously. The implementation of measures to control and eliminate BDVD in this area should be given consideration. Awareness of farmer according to BVDV and biosecurity in farm should be enhancing. This study provides a basis for the future monitoring and implementation of BVDV control program at the region or national level.

Keywords: Bovine viral diarrhea, seroprevalence, dairy cattle.

ID-P11

Serological detection of *Mycoplasma bovis* in beef calves in eastern Algeria

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Objectives: Bovine Respiratory Diseases (BRD) have always been predominant in beef calf farming. They are complex to diagnose and treat since they involve several etiological agents, bacteria as well as viruses. *Mycoplasma* (*M.*) *bovis* is a pathogenic bacteria, involved in young cattle BRD. It infects both the upper and lower respiratory tracts and induces a marked immunological response, easily detectable by ELISA kits. The aim of this study was to detect the circulation of *M. bovis* in Eastern Algeria, where BRD are frequent although the presence of *M. bovis* has never been reported so far.

Materials and methods: Circulation of *M. bovis* in calves was monitored through indirect diagnosis, in farms where BRD occurred. Blood samples were taken both from animals showing respiratory signs (n=3) and from apparently healthy ones (n=3) in each visited farm (n=60). Sera were analyzed with a commercial ELISA kit at the Anses Laboratory of Lyon, France.

Results: A total of 351 blood samples from 60 different herds of eastern Algeria were analyzed. The global proportion of seropositive animals was 69 %, 55 % of these positive being healthy animals, the serological status being logically not strictly correlated with the acute phase of the disease. However as 58 % of the samples are very high positive (S/P % ≥150), it might highlight a recent seroconversion. At the herd level, only 7 out the 60 herds were negative, i.e. had no seropositive animal in the six tested; they were located in the regions of Batna, Constantine and M'sila. This has to be interpreted with care as the number of sampled calves (n=6) is not always representative depending on the overall herd sizes ([17-450]).

Conclusion: Our study proved for the first time a frequent circulation of *M. bovis* in Algeria by detection of antibodies in calves with a high herd prevalence (53/60) and high seroconversion (84 % of positive samples having a S/P% > 150). As everywhere in the world, *M. bovis* may be an important pathogen implicated in BRD in this country. To confirm that, further studies are in progress to isolate and characterize the circulating strains (subtypes and antimicrobial resistance profiles).

Keywords: Mycoplasma bovis, ELISA, Algeria, prevalence.

ID-P12

Efficacy of pasteurization and freezing on the inactivation of bovine leukosis virus present in milk as a control strategy in lactating calves

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Objetive: The objective of this work is to evaluate the efficacy of pasteurization and freezing on the inactivation of bovine leukosis virus present in milk as a control strategy in

lactating calves.

Material and Methods: For the study, a sheep bioassay was performed, in which 16 sheep were used. The sheep received an inoculum intraperitoneally from milk with cells infected with bovine leukosis virus (VLB) previously treated with one of the virus inactivation methods. Four study groups were evaluated: a) control group: milk with cells infected by the VLB without any previous treatment, b) pasteurization group: milk with cells infected by the VLB treated by pasteurization, c) freezing group 12 hours: milk with cells infected by the VLB treated by freezing for 12 hours, d) freezing group 36 hours: milk with cells infected by the VLB treated by freezing for 36 hours.

Results: It was found that at the tenth week after inoculation, 100% of the animals in the control group and 75% of the animals in the freezing group for 12 hours were seropositive to the VLB, while no animal was seropositive (0%) to the VLB in the pasteurization and freezing group for 36 hours. The results indicate that the process of pasteurization and freezing for 36 hours is efficient to inactivate the bovine leukosis virus, while the freezing process for 12 hours fails to inactivate the virus.

Conclusion: The implementation of milk and colostrum processing received by calves could reduce the prevalence of VLB infection in stables that implement a parallel herd as a measure of control and elimination of VLB.

Keywords: pasteurization, freezing, milk, bovine enzootic leukosis, calves.

ID-P13

Outbreak of persistent infection (PI) of bovine viral diarrhea virus (BVDV) in a farm - Why so many PI calves were produced?

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In a dairy farm (about 400 milking cows), one lactating cow was diagnosed as persistently infected with bovine viral diarrhea virus (BVDV) (PI cow). After culling the PI cow from the farm, 35 newborn calves were diagnosed as BVDV infection. The detection rate was 9.7 % over a 7-month period (35 positive/ 359 newborns). The present study estimated the suspected causes of this prevalence. OUTLINEof the farm: Approximately 400 cows are usually lactated, fed in free stool and robot milking parlor. 300 to 400 calves and young stock are fed and growth up in the farm. The inactivated vaccine is used for respiratory problem including BVDV. All lactating cows were growth up in the farm from their birth time, pregnancy period and parturition are also within the farm. OCCURENCEof prevalence: On July 2019, bulk tank milk test indicated positive of BVDV. All cows in the farm were examined for BVDV PI. Eleven PIs including one pair of dam and calf were detected. The bulk tank milk without the PI dam converted negative for BVDV. The age of detected PI cows was 0.1- to 26-month old. All were infected with BVDV-2. In order to confirm the eradication of BVDV from the farm, all newborn calves were examined for BVDV.

Results & discussion: In 11 PIs at first time, BVDV-2 was identified, and the nucleotide homologies of their 5'-UTR were 100%. Their amino acid homologies of a part of E2 antigen of BVDV were 98.2-100%. During 6 months after the culling 11 PIs, 359 calves were borne, 35 of them were detected as BVDV positive at the birth time. There was no evidence of contact with primipara and multipara, detected viruses from new borne calves were almost homologus nucleotide sequence of 5'-UTR each other also with first lactating PI cow.

Conclusion: It was strongly suspected that the latent PI existed in the herd without immunized by it, or unknown route for infection in the uterine for establishment of PI.

Keywords: BVD, PI, outbreak.

ID-P14

Retrospective study on the surveillance on dairy cows infective abortions in Northeastern Italy from 2006 to 2019

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Objectives: Bovine abortion is an important cause of economic loss in dairy cattle, and has important implications in public veterinary health. Veneto region, in Northeastern Italy, has implemented since year 2006 an official surveillance plan on abortion in dairy cattle. The aim of this study is to describe the results of this surveillance, from 2006 to 2019, and to provide information about the occurrence of abortive pathogens and their prevalence.

Material and methods: Aborted fetuses, accompanied by the dam's blood sample, were delivered to the Regional State Veterinary Laboratory (IZSVe), and submitted to a panel of laboratory tests. The cows' sera were tested for antibodies against *Neospora caninum*, *Chlamydophila abortus, Coxiella burnetii*, IBR virus, and BVD non structural protein NS2-3 by mean of commercial ELISA tests, and to *Brucella abortus* and *melitensis* following the EU regulation mandatory method. Antibody against *Coxiella burnetii* were detected using also the complement fixation test according to OIE guideline. On all the fetuses were performed necroscopy, microbiological culture from abomasum, histopathology from lung, and detection, by PCR from spleen, of BVD virus, *Chlamydia spp.*, and *Coxiella. burnetii*. Detection of *Neospora caninum* by PCR from the brain was performed only on fetuses older than four month. *Brucella* spp isolation was carried out only if the abortion occurred after the fifth pregnancy month; while *Campylobacter fetus* isolation was performed on abortions occurred before

the fifth month. A PCR for the detection of Schmallenberg virus from the brain was introduced after year 2013.

Results: During these years (2006-2019) 4,562 bovine abortions were delivered to IZSVe, 668 of them were under the fifth month of gestation (14.6%). The most of fetuses delivered were autolytic (62.7%), without macroscopic lesions (30.8%) or mummified (4.5%). Histologically lung inflammatory lesions were present in 32.8% of cases. An infective agent was detected in 1451 fetuses (31.8%). *Neospora caninum* was the most frequent specific abortion agent isolated (22.2%), followed by BVD virus (5.7%), *Coxiella. Burnetii* (4.7%) and *Chlamydia spp* (0.7%). Schmallenberg virus was detected only in 3 fetuses delivered respectively in year 2012, 2013 and 2014, but only one showed congenital abnormalities (limbs arthrogryposis and jaw malformation). Microbiological culture was considered positive only when specific abortifacient pathogens were isolated: according to this criteria the 13% had a culture positive test. Among the bacteriological agents isolated the most relevant were *T. pyogenes* (47.4%), *Bacillus spp.* (29.4%), *Streptococcus spp.* (15.8%), *L. monocytogenes* and fungi (1.9%), *P. multocida* (1.7%), *Salmonella spp.* (1.1%), *M. haemolytica* (0.7%), *Corynebacterium spp.* (0.2%). *Campylobacter fetus* was isolated in one abortion and *Brucella spp.* was never isolated.

Serological tests showed a high percentage of cows had antibodies against BVD virus (44.8%), *Chlamydophila abortus* (41.3%), *Neospora caninum* (33.2%); IBR virus (25.4%), *C. burnetii* (15.8%). All tested sera and abortions were negative for Brucella spp.

The agreement between serological test and PCR for *Neospora caninum* was substantial (Cohen's kappa (k) = 0.667, while the agreement for BVD virus and *C. burnetii* was slight (k = 0.11; k = 0.16).

Conclusions: In our opinion, the abortion surveillance program provided many useful and interesting information about the health status of dairy farms and the diagnostic methods suitable for abortion diagnosis. Necroscopy findings showed the low prevalence of specific macroscopic lesion in fetuses, highlighting the importance to use a standardized protocol including tests for detection of the most relevant abortion agents. Infective abortions should be expected approximately in 30% of cases, several other causes should be considered as the source of pregnancy interruption. In order of importance *Neospora caninum* is the major abortive pathogen in Northeastern Italy, bacterial or fungal agents are the second, with prevalence ranging from 12-14%, while BVD virus and *Coxiella. burnetii* are less likely to be isolated.

Keywords: abortion, cow, Neospora, epidemiology, Italy.

ID-P15

Part 1: UK approach to JD control -Farmer Engagement using low cost surveillance, risk assessment and prevalence prediction

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Objectives: Farmer engagement must be achieved for success with Johne's Disease (JD) control. The farmer has to apply the necessary controls every day for several years to break transmission. In the UK this was achieved using education, risk assessments and targeted surveillance utilising 30 cow milk ELISA screens and prevalence prediction. The method of engagement and surveillance had to be simple with widespread uptake for success. JD incidence rose in the period 2005-2010 due in part to widespread restocking after Foot and Mouth disease in 2001 and subsequent multiplication within herds.

Materials and Methods: Milk processors were encouraged to host over a 80+ farmer events nationally to educate farmers on the importance of JD control. 1200 farmers engaged directly through milk processors and a further 1000 through a regional health scheme. Farmers were asked to submit milk samples from the 30 highest risk cows in the herd (disappointing milk yields, high cell counts, history mastitis/lameness of symptoms of JD). Sampling boxes were provided for non milk recorded herds. For herds that were recording the farmer and vet were tasked to generate a list of high risk cows for sampling. Contemporary risk assessments identified risk of JD introduction and spread. A proportion were entered into Myhealthyherd, a web-based health planning tool, for traffic light scoring. High risk herds with "red" or high risks of entry and spread were prioritised for further surveillance if test negative. The program combined the risk score and test prevalence to estimate of future true herd prevalence. The process was further extended over 10 period to engage the majority of UK farmers.

Results: The most important result was the creation of a social norm to test and manage for JD nationally. The targeted 30 cow screen and education was provided free by the milk processor. The sampling kit was dependent on the farmer attending the education program. Data from 2502 herds within the Myhealthyherd program revealed that 54% of dairy herds were high risk of disease entry with 77% high risk of within herd spread using the traffic light model. The modern dairy farming system with larger herds, communal maternity pens, the widespread use of slurry rather than manure and group housing of young calves often fed waste milk increased within herd prevalence. The management of the known risks highlighted from the Myhealtherd results collected in the early years of the program highlighted the challenges that had to overcome and explained why the traditional Biosecurity and Test and Cull policies had failed to appeal to modern dairy farmers.

General biosecurity risks relevant to Jones Disease n = 2993 dairy herds	**Frequently**	**Occasionally**	**Never**
The herd introduces cattle on to the farm	13.7%	62.1%	24.2%
Cattle share grazing or buildings with cattle of unknown disease status	2.9%	8.2%	88.9%
Slurry or farm yard manure is from another farm is spread on land	0.6%	4.9%	94.5%
Cattle have access to waterways that have passed through another livestock farm	14.5%	38.7%	46.8%
Cattle are fed with feeds that could have had contact with other animals	1.8%	16.1%	82.2%
Johnes Disease specific biosecurity risks n=2296 dairy herds			
The herd has introduced groups of animals of unknown Johnes status in last ten years	13.4%	39.6%	47.0%
The herd has introduced individual animals of unknown Johnes status over last ten years	11.0%	57.8%	31.2%
Slurry of farm yard manure from another farm is spread onto youngstock pastures	0.4%	4.2%	95.4%
Calves have access to streams or watercourses that have passed through another livestock farm	6.6%	28.2%	65.2%
Youngstock graze pastures that are heavily infected with rabbits	13.8%	48.9%	37.3%
Youngstock co-graze pastures with sheep of unknown disease status	8.0%	21.9%	72.1%
Calves are fed on colostrum from other herds that may be high risk of carrying MAP	2.0%	4.0%	94%

Fig 1. Data extracted from the Myhealthyherd Risk Assessments identifying the key risk of entry and spread of JD in the UK between 2010-14.

Discussion: Simple, cost effective methods of providing education, surveillance and risk management are crucial for JD engagement. The combined targeted 30 cow screen and risk assessment were easily implemented engaging the farmer and vet in next steps of JD control.

Keywords: Johne's Disease, Paratuberculosis, MAP, Dairy.

ID-P16

Part 2: UK approach to Johne's Disease control -Utilising web-based tools to enhance farmer engagement

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Introduction: The complexities of a long incubation period, tests of low sensitivity and discrepancies between test and true herd prevalence hamper Johne's Disease (JD) control resulting in farmer and vet despondency. Developing tools to explain risk and prevalence better has been central to the success of the UK JD program. The challenge was how to graphically present to a farmer how the disease would progress and what the test results actually mean.

Methodology: Two web-based tools have been the cornerstone of vet and farmer engagement with JD control in the UK.

Myhealthyherd- a web-based health planning tool based on a predict and prevent model of disease management. Risks are captured and traffic light scored to assess the likelihood of disease introduction and spread. Allogorithms exist within the program to translate the risks into scores which in turn are used to generate the traffic light score. Within the JD module a prevalence prediction graph was developed to illustrate future true herd prevalence. The risk score was combined with the surveillance results to generate a True herd prevalence taking into account the sample size and test sensitivity. Previous work had shown that a correctly selected sample of high risk cows increased the probability of finding a test positive cow by two fold.

The graphs were constructed to follow the epidemic curves published by other workers. The model was simple but provided an basic explanation of why a test prevalence of 5% was important to control as the True Herd prevalence was much higher and if the risks were in place for spread could rise even further.

Within Myhealthyherd a JD control planner with inbuilt robustness checker allows for effective JD plans to be created. The control plan was divided into segments and each control measure was scored. Failing to select the most robust control measures would trigger a partially compliant plan which could then be discussed with the farmer. With JD 80% control of the minor risks can end up with 100% failiure. Early analaysis of control plans found that vets and farmers selected the easiest tasks (controlling milk spread risk) rather than the more important faecal transmission routes (hygiene in maternity pens, segregation of high risk cows and culling the heavy shedding cows).

Herdwise- A web-based reporting system for interpreting quarterly milk ELISA results using a traffic light system based on the Danish control system. The program was developed by National Milk Records with over 2000 herds now using the program. Other Milk Recording Organisations now use the same test and methodology. Individual cows are categorised into high, medium and low risk and disease control progression is illustrated by graphs tracking prevalence and date of birth of infection.

Results: The use of graphical based web-based tools aids cost effective management of JD. The traffic light scoring of risks and results avoided the more typical binary positive/ negative reporting of results. The widespread adoption of these tools indicate that they are valued by farmers and they have both contributed to the success of the National Johnes Management Plan.

Discussion: JD is a complex disease requiring risk management and behaviour change to effectively control spread. Graphical web-based tools provide a simple, cost effective way of illustrating the complexity of JD whilst maintaining farmer engagement. The additional benefit has been the ability to analyse prior results and track progress with the program. This will form the basis of the UK program from 2020-2025.

Keywords: Johne's Disease, Paratuberculosis, MAP, Dairy.

ID-P17

Part 3: UK approach to Johne's Disease control -Top tips for effective control

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Introduction: The UK JD program has been driven by the use of milk ELISA testing and effective risk management. An estimated 79% of herds utilise improved farm management and strategic testing and management. A survey of 394 conducted in 2017 to evaluate the barriers to participation revealed that the major concerns were confusing test results (22%), Culling expense (18%) and Challenges of Segregation (37%) Effective segregation of high-risk cows and culling decisions are challenging areas to manage throughout the year and the advice provided by veterinarians had to be improved. New work indicated that calf to calf transmission may contribute to JD spread. A revised educational strategy had to be adopted.

Methodology: To improve farmer engagement with controls most recent veterinary education has focused on the targeted application of JD control to high risk places or time periods in the cow calendar. The quarterly milk testing program utilised by an estimated 2500 dairy herds in the UK generates a list of traffic light scored - red (highest risk for culling), amber (for segregation) and green (low risk). However challenges occurred as not all red cows progressed and some amber cows appeared to go into remission. The complications of latency, remission and progression risked jeopardising confidence of the JD milk testing program.

The revised education program focused on the creation of a low risk maternity and calf pen areas (“Green calving and calf line”) to minimise disease transmission rather than focus on test results per se. Challenging the farmers to keep the high risk areas "green" or low risk was a key message to reinforce as transmission will occur though indirect routes (bedding, fomites). No red tagged calves or cows were allowed in the green areas.

Utilising red ear tags to clearly identify high risk animals preventing them entering the ‘green calving line” is a clearly understood concept by farmers. Both Red and Amber cows should be tagged. The tag triggers a decision before breeding and aids segregation. Any replacement calf born from a red cow is tagged as a red calf and reared seperately from other "green" replacement calves.

A greater emphasis encouraging vets to help farmers categorise test results to aid decision into one of 4 categories dependent on the predicted lifespan of the cow- to cull immediately (losing control), end of lactation (deteriorating results), do not breed(unlikely to survive another lactation) or breed to terminal sire (infected but resilient cow likely to survive another pregnancy) has been encouraged.

Identifying times of the year where the “green calving line” can be most simply applied and the use of sexed semen has allowed for sourcing of low risk replacements from low risk environments greatly simplifying controls. In many herds simply sourcing replacements when the cows calve outdoors may be easier and lower risk than choosing replacements from an over-crowded maternity pen. This combined with sexed semen use can greatly ease generation of replacements from low risk cattle at times of low risk of transmission.

Discussion: A robust JD control plan can only be delivered by changes in farmer behaviour.

Utilising simple language and developing readily grasped concepts based on solutions rather than problems is central to JD control. Focusing the mind on creating ways of protecting 30% of the calf crop destined for herd replacements is easier to achieve than delivering robust control for 100% of the year. Protecting the maternity environment from contamination combined with cost effective breeding and culling decisions is vital for cost effective JD control.

Keywords: Johne's Disease, Paratuberculosis, MAP, Dairy.

ID-P18

Digital Dermatitis in beef cattle: a severe outbreak in Northern Italy

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Objectives: Digital dermatitis (DD) is multifactorial, infectious, presumably contagious disease originally described in Italy in 1974, almost exclusively in dairy cows, and subsequently reported from most parts of the world. It is currently considered as endemic in almost all countries where the cows are housed. DD is a polymicrobial condition associated with

infection by spirochete Treponema bacteria. Otherwise known as "hairy heel wart, strawberry footrot, footwarts, papillomatous digital dermatitis" and finally "Mortellaro's disease", this painful, superficial ulceration of the digital epidermis, is commonly found on the plantar surface of the hind foot, from the interdigital space and heel bulb to the dewclaws, being the skin-horn junction the preferred localization. The interdigital skin is not spared and represents sometimes the "entry door" of the disease. Digital Dermatitis is characterized by mild to severe lameness, thereby negatively affecting the welfare, causing furthermore significant economic losses through reduced productive/reproductive performance, weight loss and costs of treatment and prevention. The aim of this work is to report a severe outbreak of Digital Dermatitis occurred in a beef cattle farm in northern Italy in December 2019: clinical observations, pathological, histological and bacteriological findings were reviewed.

Material and methods: The present farm was located in Veneto region (northern Italy) and was surveyed on 11 November 2019. The breeder reported that the problem firstly occurred in August 2018 about 2 months after the arrival of the bulls from France. Since then many animals had been prematurely sent to slaughter due to severity of the lesions, not responding to systemic antibiotic and NSAID's, being the local individual therapy not practicable.

The clinical visit was made in a pen with 120 animals (4 boxes of 30) mainly Charolaise breed. About a third of the animals was showing forelimb and/or hind limb lameness of the 2rd-4th degree. Some animals have been closely inspected after deep sedation and safe restraint. Digital Dermatitis of the dorsal skin, vertical and/or horizontal horn fissures, ulcerative necrotic lesions consistent with interdigital phlegmon, septic pododermatitis of the wall, swelling of the pastern and coronal regions were detected.

Later on at the " Istituto Zooprofilattico delle Venezie" located in Legnaro (Padua) we carefully examined 18 isolated feet belonging to lame animals previously slaughtered, to detect and classify any lesions present. In case of gross pathologic lesions consistent with Digital Dermatitis, histological and biomolecular tests (PCR), for Fusobacterium necrophorum, Spirochetes and Treponema spp. (group 1, 2, 3), were performed.

Results: At necropsy in 78% (14/18) of samples Digital Dermatitis was detected, 71% of which had an M2 lesion. Most of the dermatitis were localized onto the skin above the anterior part of the interdigital space that in several cases was also involved by the pathological process showing single or multiple erosive lesions consistent with M1 stage. One foot was diagnosed with interdigital phlegmon with concurrent septic pododermatitis of the sole and third phalanx osteomyelitis. Three samples had no macroscopic lesions.

Histologically examined biopsies were characterized by severe epidermal hyperplasia and parakeratotic hyperkeratosis, with some developing frond-like projections. Epidermis was frequently eroded and necrotic with marked infiltration of neutrophils, forming intraepithelial aggregates. Mixed bacteria were associated with outer necrotic debris, whereas clusters of spirochetes were detected in deeper viable epidermis (Warthin-Starry stain). The dermis showed moderated to marked infiltration of neutrophils, eosinophils, lymphocytes and plasma cells and variable degree of fibrosis. Necrosis of dermal papillae, associated with edema and hemorrhage, was observed in some biopsies.

Spirochetes and Treponema spp. (group 2) were detected in 100% of samples; group 1 and 3 of Treponema spp. were detected in 83% of samples, instead 67% of samples were positive to Fusobacterium necrophorum.

Conclusions: Current therapy in dairy cattle involves topical antibiotic/non antibiotic application paired with a careful hoof trimming but in beef cattle seems now "utopian". Industrial research is needed to develop specific DD treatment in beef cattle farming.

Keywords: digital dermatitis, beef cattle.

ID-P19

Antimicrobial potential of Recombinant Host Defense Peptides produced as soluble and nanoclusters

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Antibiotic-resistant infections are rising sharply, currently being one of the most alarming public health threats. Remarkably, an important percentage of antibiotics are sold for use in livestock. In the last years, some progress has been made limiting the use of critically important antibiotics by banning their use for growth promotion in many countries. However, other strategies are also needed to minimize the antibiotic use in animal production. In this context, there is a need to develop alternative antimicrobial molecules and, among them Host Defense Peptides (HDPs) appear as a promising alternative. HDPs are cationic peptides of the innate immunity with a broad-spectrum antimicrobial activity. Although HDPs show desirable therapeutic activities, their production process through chemical synthesis and short half-life are critical drawbacks. In this scenario, the recombinant production of HDPs has emerged as a promising alternative, since it allows to produce proteins at high production yields through a cost-effective process. Recombinant technologies allow producing the HDP of interest not only in a soluble format but also as protein nanoclusters (Inclusion Bodies, IBs), which lead a low-cost product with a sustained release format, reducing treatment doses and avoiding undesirable side effects. Thus, we have focused our study in the development of a novel platform of recombinant HDPs against antibiotic resistant (AMR) bacteria, that can be produced in both soluble and protein nanocluster formats. For that, different HDPs including bovine lingual antimicrobial peptide (LAP), human defensin 5 (HD5) and human defensin 6 (HD6) were produced as fusion proteins while others as single polypeptides combining several antimicrobial domains (JAMF polypeptides). They were produced in *E. coli* as both soluble and nanostructured proteins. Next, all these antimicrobials proteins (soluble and nanoclusters) were purified and quantified by Western blot. The biological activity of all of them was measured *in vitro* with BacTiter-Glo™ Microbial Cell Viability Assay. Specifically, different concentrations ranging from 0.1

mM to 5 mM were tested. Data were analyzed using ANOVA. The analysis of the antimicrobial polypeptides and fusion proteins, produced either as IBs or soluble format, showed significant antibacterial activity not only against a model bacteria *Escherichia coli* DH5a (up to 84 % of inhibition), but also against multidrug-resistant bacteria such as several strains of *Enterococcus spp.* (up to 94 % of inhibition) and *Klebsiella pneumoniae* quinolone (qnrA) and carbapenem-resistant (91 and 96 % of inhibition, respectively) with an optimal concentration between 0,5 and 3 mM. Our results demonstrate that these constructs are a potential cost-effective alternative to conventional antimicrobials.

Keywords: recombinant protein, host defense peptide, antimicrobial, nanocluster.

ID-P20

Control, Monitoring and Eradication of Infectious Bovine Rhinotracheitis (IBR) in a Closed Dairy Herd Following Vaccination with a Live/Inactivated IBR Marker Vaccine Programme

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Objectives: An opportunity arose to study the epidemiology of IBR control in a closed (apart from occasional stock bull purchase) 230 cow Holstein dairy herd. Following an IBR breakdown in 2012, the breeding herd was vaccinated with a live/inactivated IBR marker vaccine programme with the aim of controlling clinical disease and reducing risk of subsequent virus shedding from latently infected animals. The herd was monitored by individual milk/serum IBR antibody testing over a 9 year period to follow IBR infection status with a view to achieving eradication of IBR from the herd.

Materials and Methods: The herd had excellent boundary biosecurity, and homebred heifers were reared on the same farm as cows but housed in separate accommodation until pregnant. Vaccination for IBR had been carried out 2002-2007 (marker vaccine used 2006/7) but ceased in October 2007. IBR status was then monitored by annual first lactation heifer cohort milk IBR antibody testing.

In May 2012, a 1st lactation animal showed clinical signs of IBR and died, with IBR confirmed by PCR testing and histopathology. Herd status was then investigated by testing all cows for milk or blood IBR gE antibody to define herd seroprevalance. Yearling and in-calf heifers plus the stock bull (IBR free at purchase) were blood tested for IBR gB antibody.

The breeding herd (all cows, pregnant and bulling heifers) were vaccinated intra-muscularly (i/m) with a modified live IBR marker vaccine (Rispoval® IBR Marker Live, Zoetis) followed 4 weeks later by an inactivated IBR marker vaccine (Rispoval® IBR Marker Inactivated, Zoetis) subcutaneously (s/c) and boosted annually with inactivated IBR vaccine. In subsequent years the yearling heifers received a primary vaccination course of live IBR vaccine i/m followed 4 weeks later by inactivated IBR vaccine s/c before breeding, and were then boosted annually with inactivated IBR vaccine.

Monitoring and vaccination continued 2013-2021 with annual individual IBR gB antibody blood testing of bulling heifers prior to vaccination (2012-2021) to monitor IBR seronegativity prior to primary vaccination. Individual milk IBR gE testing of cows (2012-2021) was performed to assess wild-type (WT) IBR seroprevalence.

Results: First lactation heifer cohort milk IBR gB antibody testing between 2008 and 2011 proved negative suggesting absence of active infection in the milking herd during this period.

The individual IBR antibody percentage seropositivity for herd groups following the IBR breakdown in 2012 were: bulling heifers: 1% (n=90), in-calf heifers: 68% (n=69), milking cows: 99% (n=230). The stock bull tested IBR antibody positive in 2012 having previously been confirmed IBR free at entry to herd. These sampling results showed that in 2012, IBR appeared to have spread widely in the milking herd. It is of note that in the six months prior to the index IBR clinical case, the herd had experienced no “classical” signs of IBR infection.

Following vaccination of the breeding herd in 2012, there have been no clinical signs of IBR in the herd. The adult herd IBR gE seroprevalence has declined steadily from 99% in 2012 to 0% in 2021, attributed to the standard herd culling policy resulting in the removal of seropositive animals. Bulling heifers tested prior to primary vaccination had an IBR gB seroprevalence of 0-3% between 2014 and 2021, indicating that they were not exposed to WT IBR virus prior to vaccination.

Conclusion: Within 5 years of ceasing an IBR vaccination programme, IBR appeared to recrudesce and spread widely in this herd. Vaccination with a live/inactivated IBR marker vaccine programme has to date controlled clinical disease and possibly reduced the risk of latent carrier cows shedding virus and causing infection of naïve heifers entering the milking herd.

Within 9 years, the adult cow herd IBR gE seroprevalance has fallen from 99% to 0% suggesting that eradication has been achieved within 8 years of initial diagnosis by utilizing IBR marker vaccine and removing existing latent carriers by routine herd culling policy.

Keywords: IBR, dairy cow, eradication.

ID-P21

Area vaccination program to prevent Bovine Viral Diarrhea at Betsukai town, Hokkaido, Japan

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Objective: Bovine Viral Diarrhea (BVD) virus is one of the most widespread cattle pathogens. Persistent Infection (PI) animals are the key to the maintenance of BVD in cattle since

PI animals ensure viral persistence in host populations. BVD is categorized as a notifiable disease by the Act on Domestic Animal Infectious Disease Control in Japan; therefore, if a PI animal is identified, it has to be reported to the authority to cull the concerned animal. In Japan, the most prevalent BVDV is BVDV 1b, followed by BVDV 1a, and the prevalence of BVDV type 2 is relatively low. Betsukai town located in the eastern part of Hokkaido holds 100,000 or more cattle. In 2006, an outbreak of BVD at one farm in Nakashibetsu town, adjacent to Betsukai town, prompted the strategic vaccination program of BVD for all herds in Betsukai town backed by subsidies in order to control the disease, in particular to prevent PI animals, as well as for minimizing the financial impacts on the cattle farmers and associated industries.

Materials and methods: A council composed of all key stakeholders such as bovine practitioners, agricultural cooperatives, municipal officers, milk and dairy products manufactures was established to decide the BVD vaccination strategy of all herds in Betsukai town since 2006. The scope of vaccination was all dairy and beef cattle in the town from the age of 3 up to 24 months old. Two types of BVD vaccines were chosen depending on the age of cattle at the time of each vaccination. When it comes to calves between 3 and 10 months old, a 5-way Modified live vaccine or MLV (BVDV 1a, IBRV, PI3V, BRSV and BAdV-7) was used as the maternal antibody level was taken into consideration. For the cattle older than 11 up to 24 months old, due to variable status of pregnancy, a 5-way Killed vaccine (BVDV 1a, BVDV 2a, IBRV, PI3V and BRSV) was administered twice with a 3-week interval for the initial year. Since 2015, the MLV was changed to the 6-way MLV (BVDV 1a, BVDV 2a, IBRV, PI3V, BRSV and BAdV-7), and the Killed vaccine was changed to another 5-way Killed that replaced the different genotype of BVD type 1 since 2017. The vaccines were administered simultaneously twice per year in spring and autumn. Half of the cost of vaccines, blood examination and PCR to detect PI animals were subsided by various grants available by the national and local governments. When a PI animal was detected, one third of the market price of the concerned cattle was subsidized to the farmer, and the concerned animals were culled accordingly.

Results: No single PI animal was detected in the cattle at Betsukai town from 2006 until 2013. Single cases of PI animals were detected in 2014, 2015, 2016, 2017 and 2019, respectively, whereas no PI animal was detected in 2018. The outcomes of adjacent towns in the same period, except for 2008 (n=0) and 2019 (n=19), the detections of PI animals were present with low single digits.

Conclusions: It was considered that the strategic vaccination program performed at Betsukai town has been shown to minimize the spread of BVDV in the concerned area. Unfortunately, certain years had a single animal that was positive with BVDV. None of the PI animals were bred in the town, but brought in from Honshu, the mainland of Japan, where the status of BVD vaccination was uncertain, and this was also seen at the farms in the adjacent towns. Unlike Betsukai town, the adjacent towns resisted using MLV due to their concern about vaccine reactions. N^{pro} and E^{rns} double mutated BVDV live vaccine (Bovela®, Boehringer Ingelheim Vetmedica GmbH, Germany) that can be used safely regardless of pregnancy status has been available in the EU since 2015, and it is expected that this vaccine has a potential to contribute to the BVD vaccination program in Japan once it is registered and commercialized in Japan. In addition, it is also considered critical that the vaccination program should be run on a national level to maintain the high herd health status against BVD.

Keywords: Bovine Viral Diarrhea, Persistent Infection.

ID-P22

Are Bovine Respiratory Syncytial Virus (BRSV) vaccine strains still aligned with circulating BRSV field strains ?

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Objectives: Bovine Respiratory Syncytial Virus (BRSV) is spread in cattle herds worldwide and is considered one of the most important etiological agents involved in Bovine Respiratory Disease Complex (BRDC), causing severe outbreaks especially in young cattle. BRSV, also known as *Bovine Orthopneumovirus,* is a single-stranded, negative-sense RNA virus belonging to the genus *Orthopneumovirus* within the *Orthopneumoviridae* family. Its genome encodes for 11 viral proteins, including 3 surface proteins: the small hydrophobic protein (SH), the fusion protein (F) and a glycoprotein (G). This last one mediates the viral attachment to the host cell, and it is suspected to have a role in interacting with the immune system. The ectodomain in the central hydrophobic region of the protein contains an epitope that appears to be immunodominant and this particular genomic region has been widely used for molecular epidemiological investigations throughout the years.

In this study, we reviewed the recent reports in literature and conducted an updated phylogenetic analysis to study BRSV evolution of circulating strains in the field during the last 30 years. Two vaccine strains were also included in the analysis in order to understand the diversity between the field strains and the ones used for prophylactic measures.

Materials & methods: Nucleotide sequences based on a partial sequence of the G protein of 268 strains collected from Genbank were aligned using ClustalW and manually optimized. Maximum Likelihood phylogenetic trees (Tamura Nei model, and 500 bootstrap values as statistical support) were constructed using Geneious Prime software.

Results: Our results confirm, as previously reported, the presence of eight genetic groups that, unlike in the past (Valarcher et al., 2000), are not geographically specific anymore. The most recent circulating strains in the field seem to belong to the genetic group II (Norway, Croatia, and Italy), group III (Brazil, Turkey, and China), VII (Croatia, and Italy) and VIII (subgroup of III, Italy, Croatia, and Belgium). Some other diverging groups were identified and could be classified as additional clusters, possibly representing new circulating BRSV. The genetic analysis revealed that the vaccine strain of an inactivated BRSV-Bovine Parainfluenza 3 – *Mannheimia haemolytica* vaccine (Bovilis® Bovipast® RSP, MSD Animal Health) belongs to the genetic group II, currently composed of mostly North European strains. On the other hand, the live at-

tenuated BRSV strain present in a bivalent BRSV-Parainfluenza 3 intranasal vaccine (Bovilis® INtranasal RSP® Live, MSD Animal Health) belongs to the genetic group III, composed of strains that are worldwide detected and from which derived actual strains circulating in Turkey, Japan, China, and several European countries.

Conclusion: Our phylogenetic analyses results show that BRSV is constantly evolving throughout the years. Despite this, the two vaccine strains (present in the inactivated Bovilis® Bovipast® RSP and in the live attenuated Bovilis® INtranasal RSP® Live) belong to the same genetic groups as the predominant strains currently circulating in the field. These results provide additional insight of BRSV evolution and could help the implementation of disease control measures.

Keywords: BRSV, sequencing, phylogenic tree, vaccination.

ID-P23

Clinical Disease and Histopathology Associated with Respiratory Infection by Bovine Coronavirus

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Objectives: Bovine Coronavirus (BCoV) is a member of a family of viruses associated with both enteric and respiratory diseases in a wide range of hosts including ruminants, pigs, chickens, mice, cats, dogs, humans, and many other species. BCoV itself has been well-established as a causative agent of diarrhea in cattle, however, its role as a respiratory pathogen has been controversial. The objective of this study was to demonstrate that BCoV can cause respiratory disease in cattle.

Materials & Methods: In this study, fifteen calves were infected intranasally with virulent BCoV in order to observe the clinical manifestation of the BCoV infection for up to eight days after initial administration, looking specifically for indication of symptoms, pathology, and presence of viral infection in the respiratory tract, as compared to six uninfected control calves. The virus was administered via a nebulizer to facilitate inhalation. Throughout the study, clinical signs of disease were recorded and nasal swabs were collected daily. Additionally, bronchoalveolar lavage (BAL) was performed at four days post-infection to test for BCoV isolation. Tissue samples were collected from calves at four, six, or eight days post-infection to be tested for the presence of pathology and BCoV by qPCR.

Results: Challenged calves displayed a high incidence of clinical symptoms, both enteric and respiratory in nature, including elevated rectal temperatures (5 of 15), diarrhea (7 of 15), nasal discharge (12 of 15), and coughing (6 of 15). Samples collected support that this BCoV infection resulted in respiratory infections as evidenced by the isolation of BCoV in BAL fluids (14 of 15) and positive qPCR in the nasal turbinates (14 of 15), tonsil (15 of 15), lung (10 of 15), trachea (11 of 15), and eyelids (6 of 15). Immunohistochemistry (IHC) showed BCoV in nasal turbinates in the infected animals (7 of 15) as well as in trachea (3 of 15), tonsil (7 of 15) and bronchus (7 of 15) with low occurrence of positive staining of eyelid (2 of 15), deeper bronchus (2 of 15), and lung (1 of 15). Histopathologic lesions in the upper and lower respiratory tissues were observed, with the most severe lesions in the tracheas (13 of 15) and some pathology in the nasal turbinates (10 of 15) of the majority of infected animals.

Conclusion: This study can be added to a growing body of data supporting that respiratory infection by Bovine Coronavirus results in histopathological lesions and clinical signs and is a contributor to respiratory disease in cattle.

Keywords: bovine coronavirus, BRD, challenge.

ID-P24

Detection of influenza d virus-specific antibodies in swedish bulk tank milk collected in 2019 and 2020

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Objective: Influenza D virus (IDV) is the most recently discovered agent among viruses involved in the bovine respiratory disease complex. Evidence based on detection of virus or specific antibodies supports that IDV circulates in cattle populations worldwide and is associated with the presence of clinical respiratory signs. Experimental studies demonstrated direct and short-distance aerosol transmission of IDV between calves, the induction of mild clinical signs during a single IDV-infection and an increase of the impact of other respiratory pathogens during co-infections. Based on the lack of information about the existence of the virus in Sweden and the potential importance that this pathogen might have, this study aimed to determine the seroprevalence and spacio-temporal distribution of IDV in the Swedish cattle population on two consecutive years.

Material and methods: In total, 810 bulk tank milk (BTM) samples from 810 dairy herds distributed throughout Sweden and collected in 2019 or 2020 were analyzed by an in-house indirect ELISA to detect the presence of IgG antibodies specific for IDV. Initially, the samples were submitted to the National Veterinary Institute (SVA) as part of the Swedish Surveillance Program for Bovine Viral Diarrhea Virus following a risk-based design. ArcGIS software was used to create maps and give an overview of the results in each county.

Results: The proportion of bulk milk samples with detectable IDV-specific antibodies was 32 % (n=461) and 40 % (n=338) in 2019 and 2020, respectively. Influenza D virus was

diagnosed in 9 counties in 2019 (6/10 in the south (Götland), 3/8 in the center (Svealand) and 0/5 in the north (Norrland)) and 12 counties in 2020 (6/10 in the south (Götland), 4/7 in the center (Svealand) and 2/5 in the north (Norrland)). For both years, the county of Halland had the largest proportion of positive samples: 84% in 2019 and 77% in 2020.

This is the first screening of IDV-specific antibodies in BTM and the first report of IDV in Sweden. The similarity of the geographic distribution in 2019 and 2020 indicate that it is possible to monitor the spread of IDV by BTM analysis. The largest percentage of positive samples were detected in the south, which suggests higher virus circulation, maybe related to the higher cow density in this part of the country. The increased number of herds and counties in which IVD antibodies between 2019 and 2020 suggests that this virus is increasingly circulating in Swedish dairy herds.

Conclusion: Influenza D virus is present in Sweden and most prevalent in the south of the country. Further studies are needed to monitor the circulation dynamic of the virus and measure the impact of the virus in the Swedish cattle population.

Keywords: Cattle, Influenza D virus, respiratory disease.

ID-P25

Frequency and molecular characterization of *Mannheimia haemolytica, Pasteurella multocida* and *Histophilus somni* among healthy and diseased calves in feedlots in Spain

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Objectives: The aim of this study was to investigate the frequency and molecular characteristics of the three main bacteriological agents of the *Pasteurellaceae* family associated with the bovine respiratory disease (BRD) complex in healthy calves at arrival and in diseased animals in feedlots.

Materials and methods: Mixed beef-breed calves (n=124) of eight different lots in one large feedlot in Valencia (n= 80) and a small feedlot in Madrid (n= 44), were studied at the arrival for the presence of *M. haemolytica*, *P. multocida* and *H. somni* between April and November 2021. Animals were vaccinated at the time of arrival against BRSV, PI3 and BVD virus and *M. haemolytica* A1 and daily exanimated by veterinarians for clinical signs of respiratory disease (fever and cough, depression, dyspnea and/or nasal discharge). Samples were taken from those with one or more clinical sign. Samples were taken by deep nasopharyngeal swab samples from the apparently healthy animals at arrival and by deep nasopharyngeal swab or bronchoalveolar lavage from diseased animals. The samples were cultured on Columbia agar + 5% sheep blood and Chocolate agar + PolyViteX (BioMérieux SA, France) and incubated aerobically or anaerobically at 37 °C by 24 and 48 hours, respectively. *P. multocida, M. haemolytica and H. somni* isolates were identified by mass spectrometry MALDI-TOF and PCR. *M. haemolytica* capsular types (A1, A2 and A6) and *P. multocida* capsular types (*capA, capB, capD, capE* and *capF)*, lipopolysaccharide (LPS) genotypes 1 to 8 and virulence factors (*hgbB, pfhA, tbpA, toxA*) were determined by PCR.

Results: *M. haemolytica*, *P. multocida* or *H. somni* were isolated from the 56.3% and 47.7% of healthy animals from Valencia and Madrid feedlots, respectively. Frequency of carriers was significantly different (P<0.05) by lots of animals ranging between 10-90%. *M. haemolytica* and *P. multocida* were isolated from virtually all the lots analyzed whereas *H. somni* was isolates only from one lot of Valencia feedlot. Nevertheless, *M. haemolytica* and *P. multocida* frequency of isolation was significantly different (P<0.05) by lots. Clinical cases of BRD were detected after arrival in all the lots in the first 15 days in Valencia with cumulative incidence ranging between 2.5 and 11.3%, whereas no clinical cases were detected in the same period in the feedlot in Madrid. *P. multocida, M. haemolytica* and *H. somni* were isolated in statistical significantly frequency from BRD cases by lot ranging from 0 to 50%. *H. somni* was isolated from 50% of clinical cases in the unique lot with *H. somni* carriers. Differences were detected among *M. haemolytica* molecular characteristic from healthy and diseased animals. The capsular type A2 was the most prevalent (79.3%) among healthy animals, followed by A6 (13.8%) and A1 (3.5%). Capsular type A1 was the unique detected among BRD cases in the first 15 days after arrival. All the *P. multocida* isolates from both healthy and diseased animal showed the same molecular profile characterized by capsular type A, LPS3, and *tbpA+/pfhA+/toxA-/hgbB-* virulotype.

Conclusions: The differences on carrier frequencies observed among lots are likely driven by the diverse origin of animals in lots. The relative low incidence of *P. multocida* and/or *M. haemolytica* associated BRD cases might be explained by the appropriate management and sanitary practices used in both feedlots. In addition, *P. multocida* and *M. haemolytica* from clinical cases exhibited genetic characteristics also identified in isolates from apparently healthy calves, result consistent with the importance of asymptomatic animals as origin of BRD cases in feedlots after predisposing conditions.

Keywords: Bovine respiratory disease, feedlot, Mannheimia haemolytica, Pasteurella multocida, Histophilus somni.

ID-P26

A scoping review of the testing of bulk milk to detect infectious diseases of dairy cattle caused by bacteria

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Objectives: Here we performed a scoping review to summarize the literature reporting on the testing of bulk milk (BM) samples to detect infectious diseases of dairy cattle caused by bacteria. Results from this review can be used to inform the development of surveillance initiatives that will integrate multifaceted programs aimed to mitigate impacts of endemic and (re)emerging infectious diseases of dairy cattle.

Material and methods: Original studies of any design reporting on the testing of farm-level BM samples for detection of infectious diseases of dairy cattle were eligible for inclusion. We screened five electronic databases for potentially relevant articles: 1) Agricola (via ProQuest), 2) CAB Abstracts (via CABI interface), 3) ProQuest dissertation and thesis, 4) SCOPUS, and 5) Web of Science (all databases). Records identified in the search process were uploaded into EndNote X9 (Clarivate Analytics, Philadelphia) and merged as a single database. A 2-step approach was used for screening for eligibility. From each eligible study, variables extracted included author, year, country(ies) of origin of BM samples, disease(s) or pathogen(s) screened, test(s) used, test cut-off value (e.g. S/P ratio, CT value), sensitivity, specificity and testing protocol.

Results: Our search yielded 8,829 records, from which 474 were retained. Overall, 575 eligible bacterial pathogens were screened using BM samples, ranging from 1 to 6 assessments per study. *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA), were the most commonly studied bacteria (179 studies), followed by *Streptococcus agalactiae* (86 studies), *Mycobacterium avium* subspecies *paratuberculosis* (MAP; 79 studies), *Coxiella burnetii* (79 studies), and *Mycoplasma* spp. (including *M. bovis*; 67 studies). Overall, culture-based protocols, ELISA, qPCR and PCR were the most commonly methodologies adopted to screen BM samples.

Sensitivity of BM testing to detect herds infected with bovine paratuberculosis was generally low and varied greatly according to the ELISA cut-offs adopted and herd-level definition of disease. In general, protocols had low to moderate sensitivities (<50%), which were notably higher if a greater within-herd proportion of positive samples was needed to define a positive herd. Specificity of bulk milk testing to detect herds free of paratuberculosis was high.

With respect to mastitis pathogens, BM testing demonstrated increased sensitivity and specificity to detect herds with and without *S. agalactiae*-infected cattle. Yet, we observed inconsistency among studies with respect to the sensitivity of BM culture to detect infected herds, partially due to the herd eligibility criteria; culture of a single BM sample to detect *S. agalactiae* positive herds had low to moderate sensitivity, which increased drastically if enrolled herds were heavily infected or have history of clinical disease. As for *Mycoplasma bovis*, sensitivity to detect herds with infected cattle was highest for ELISA, followed by qPCR and culture. Testing of BM samples to detect herds free of *M. bovis* had very high specificity (>95%), regardless of methodology.

Among *Salmonella* spp. pathogens, *S*. Dublin was the most frequent studied bacteria for which BM testing has been validated. Specificity to detect herds free of *S*. Dublin-infected cattle was high, ranging from 89.0 to 99.4. In contrast, sensitivity of BM testing to detect *S*. Dublin-positive herds varied greatly among studies, ranging from 50.6% to 100%. One of most important factors affecting sensitivity of BM ELISA to detect *S*. Dublin herds was whether non-lactating cattle such as heifers and calves were considered in the definition of true infection status. Other bacterial diseases or pathogens for which BM testing has been validated to detect animal-level infections include brucellosis (8 studies), *Coxiella burnetii* (8 studies), bovine tuberculosis (2 studies), digital dermatitis (2 studies) and *Listeria* spp. (1 study).

Conclusions: This scoping review focuses on the use of BM testing to detect infectious diseases of dairy cattle. In general, protocols analyzed had low sensitivities and high specificities, which varied according to the pathogen screened as well as protocols employed. For MAP, sensitivity increased according to within-herd prevalence of affected cattle used to define true infection status of herds. There was inconsistency with respect to the sensitivity of BM culture to detect herds infected with *S. agalactiae*, which varied according to the spectrum of enrolled herds. For *S*. Dublin, our findings support that one of most important factors affecting sensitivity of BM ELISA is whether non-lactating cattle are considered in the definition of herd infection status.

Keywords: bacteria, bulk milk, dairy, infectious diseases.

ID-P27

Nanopore sequencing as a rapid diagnostic tool for identification, strain typing, and antimicrobial susceptibility testing of Mycoplasma bovis

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Objectives: *Mycoplasma bovis* is an infectious bacterium causing mainly pneumonia, arthritis and otitis in calves, and mastitis and arthritis in cattle. This highly contagious pathogen leads to significant economic losses, impaired animal welfare, and high antimicrobial use. To be able to implement appropriate antimicrobial therapy during an outbreak of *M. bovis*, rapid identification and antimicrobial susceptibility testing (AST) are key, whereas information on strain types can support biosecurity measurements. However, current diagnostic methods (*e.g.* culture, PCR) are not sufficiently filling these needs. Nanopore sequencing, a third-generation sequencing technology, became rapidly affordable over the last few years, making it a potential all-in-one diagnostic tool for infectious diseases in veterinary medicine. Therefore, the objective of this study was to explore nanopore sequencing as a diagnostic tool for (1) identification, (2) strain typing, and (3) AST of *M. bovis*.

Material and methods: In the first experiment, 100 non-endoscopic bronchoalveolar lavage fluids (nBALf) were obtained

from diseased calves, and analyzed with (a) nanopore sequencing [MinION], (b) RIMM (Rapid identification of *M. bovis* with MALDI-TOF MS), and (c) triplex real-time PCR. In addition, 17 pooled samples, each containing 5 individual samples were analyzed by nanopore sequencing and RIMM. The diagnostic test accuracy for the individual samples was determined with a Bayesian Latent Class Model (BLCM), while the pooled samples were compared to the individual samples. In the second experiment, 100 Belgian *M. bovis* isolates obtained over 2014-2019 from different cattle sectors (dairy, beef, and veal) were fully sequenced, compared by Single Nucleotide Polymorphisms (SNPs) with *M. bovis* PG45 as a reference, using CSI phylogeny (Center for Genomic Epidemiology, Denmark), and a phylogenetic tree was created (MEGA-X). In the final experiment, a genome-wide association study (GWAS) on high-quality genomes of 100 *M. bovis* isolates was performed to identify genetic markers for seven antimicrobials of clinical importance and their association with antimicrobial resistance. Antimicrobial resistance was based on minimum inhibitory concentration (MIC) of the 100 *M. bovis* isolates, and epidemiological cut-off values previously obtained by microbroth dilution of 141 *M. bovis* isolates.

Results: In the first experiment, the BLCM showed good diagnostic test accuracy for the identification of *M. bovis* with nanopore sequencing from individual nBALf with a sensitivity of 77.3% (95% credible interval: 57.8-92.8%) and specificity of 97.4% (91.5-99.7%). For pooled samples a sensitivity of 85.7% (95% confidence interval: 59.8-111.6) and specificity of 90.0% (71.5-108.6%) were observed. The second experiment showed the presence of five *M. bovis* clusters, and one outlier strain in Belgium. No association between cluster and cattle sector, year, or geographic location could be identified. The GWAS showed (new) genetic markers for macrolides (tilmicosin, tylosin, gamithromycin), fluoroquinolones (enrofloxacin), and gentamicin. Also, point mutations previously described for *M. bovis* with elevated MIC for tetracyclines were observed.

Conclusion: Nanopore sequencing has a high potential as a diagnostic tool for the rapid identification, strain typing, and AST of *M. bovis* during acute, clinical infections. It can contribute to appropriate antimicrobial treatment for the control of *M. bovis* outbreaks, and provides useful epidemiologic information which can be of support for biosecurity measurements to prevent *M. bovis* from entering the herd.

Acknowledgments: We thank everyone involved in the collection of the isolates (Animal Health Care Flanders, ARSIA, Clinic of Large Animal Internal Medicine, veterinarians), and the analyses of the isolates (Department of Diagnostics and Crisis Organization, Wageningen Bioveterinary Research, and technical staff from the involved laboratories).

Keywords: Bayesian latent class model, bronchoalveolar lavage, genetic markers, genome-wide association study.

ID-P28

Evaluation of the effectiveness of cow colostrum on treatment and its effect on serum proteomes of calves with cryptosporidiosis

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Objectives: The aim of this study was to investigate the effect of using high quality cow colostrum in two different ways (with or without bicarbonate) in addition to paramomycin, on the treatment outcome and serum proteomes of calves naturally infected with cryptosporidiosis.

Materials and methods: The material of the study consisted of 30 Holstein calves which younger than 21 days old, infected with only *Cryptosporidium spp.*. Calves were divided into three equal groups and paramomycin was given to all groups orally at a dose of 100 mg/kg once daily for 5 days. While only paramomycin was administered to Group PC (control group), 250 ml colostrum was given 3 hours after the morning and evening feeding for 3 days to Group PBCOL and PCOL. The PBCOL group was also given 6 g of sodium bicarbonate 15 minutes before colostrum administration. While the clinical examinations and fecal scores of all calves were evaluated daily for 10 days from the initiation of the treatment, the fecal oocyst counts were determined on the 0, 1, 2, 3, 5, 7 and 10th days. Blood samples were collected to tubes without anticoagulant from all calves at 0 and 3rd day of the study and serum samples were stored -80 °C after separation. In these samples, serum proteomes were identified using LC-MS based proteome expression analysis and matched according to protein database for *Bos taurus.* Also, immunoglobulin G (IgG), gamma-glutamyltransferase (GGT), total protein, Brix% measurements were performed on the 0 and 3rd days sera.

Results: When comparing to pretreatment levels, fecal scores in PBCOL, PCOL and PC groups decreased significantly ($p<0.05$) on days 2, 2 and 8, respectively. Similarly, compared to pretreatment; fecal oocyst numbers in PBCOL, PCOL and PC groups decreased significantly on the 2nd, 3rd and 10th days, respectively ($p<0.05$). IgG, TP, Brix% and GGT levels were decreased in all groups with treatment. Eightythree, 93 and 99 proteomes were detected in PBCOL, PCOL and PC groups, respectively. From these proteomes; while with the colostral treatment significant changes were observed in A1AT, SAA, ACTG, HP20 in Group PBCOL and A1BG, S39AB, CTHL1, ACTB APOA4 proteins in Group PCOL; there was no significant change in any protein in Group PC. It was determined that these proteomes have roles mainly binding/transporter activity, catalytic activity, regulation of molecular functions, and regulation of structural molecular activity. Among these 9 proteomes which showed significant difference, SAA was the most dowregulated proteome with 2.16 fold change (in Group PBCOL), while APOA4 was most upregulated proteome with 1.47 fold change (in Group PCOL) during treatment. It was stated in the previous studies that A1AT has anticryptosporidial properties, but it was demonstrated for a

first time that A1AT has anticryptosporial activities in calves with cryptosporidiosis in this research.

Conclusions: While there was no significant changes in any serum proteomes with treatment in the control group, but there were significant changes in serum proteomes with colostral treatment and more pronounced healing and shorter clinical recovery time in the colostum groups (especially colostrum with sodium bicarbonate) revealed that these proteomes had positive effects in the treatment with their systemic and local effects in the intestines. So, it can be suggested that using paramomycin with sodium bicarbonate and colostrum give better outcome in treatment of cryptosporidiosis.

Keywords: calf, cryptosporidiosis, colostrum, proteomic analysis, paramomycin.

ID-P29

Fatal *Mannheimia haemolytica* infections caused by different serotypes in dairy cows and veal calves

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Objectives: *M. haemolytica* is part of the commensal flora of the upper respiratory tract in ruminants. However, this bacterium is known to cause respiratory disease and polyserositis in calves. Fatal *Mannheimia haemolytica* (*M. haemolytica*) infections in cattle in the Netherlands increased significantly between 2004 and 2018 (Biesheuvel et al 2021). Since it emerged in both dairy cows and veal calves, it was important to investigate whether these infections were caused by the same *M. haemolytica* genotype. Particularly as veal calves are born and raised for at least two weeks on dairy farms.

Materials and methods: To gain more clarity on this, Whole Genome Sequence(WGS) was performed on 96 *M. haemolytica* isolates. These isolates were cultured from necropsied veal calves with fibrinous polyserositis (n=47) and dairy cows with fibrinous pleuropneumonia (n=38) due to *M. haemolytica* as the cause of death.

The animals were submitted for necropsy by practitioners and farmers from 38 Dutch dairy farms and 35 Dutch veal farms between December 2018 and June 2020.

Results: M. haemolytica isolates from fibrinous pleuropneumonia (FPP) in dairy cows and fibrinous polyserositis (FPS) in veal calves appeared to be distinct serotypes. Most strikingly, *M. haemolytica* serotype A2, commonly assumed to be the commensal nonvirulent serotype in the nasopharynx of cattle, was cause of death in all fibrinous polyserositis cases in veal calves. These isolates contained antimicrobial resistance-genes for several groups of antibiotics, while these resistance-genes were virtually absent in isolates from FPP in cows, which were all serotype A1 of A6, except one.

Conclusions: This almost dichotomous pathology distinction between serotype A2 and serotype A1 of A6 seems to be more likely related to the bacterial serotype than to the animal category or its age. This is also confirmed by the only two calves with FPP (and not FPS) that were infected with serotype A1 and A6. Veal calves with FPS are born and raised for two to five weeks on dairy farms, where it seems plausible that all animals harbour the same M. haemolytica types. Since FPS rarely occurs among dairy calves or dairy cows, it seems likely that the trigger that causes this disease, FPS, in veal farms is not present on dairy farms. To determine to what extent this trigger is due to virulence characteristics of the bacterium, specific housing or management characteristics or vulnerability of the host further research is needed.

Keywords: bovine, genotyping, Mannheimia haemolytica, pleuropneumonia, polyserositis.

ID-P30

Prevalence of Bacterial Pathogens of Bovine Respiratory Disease in Portugal

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Objectives: Bovine Respiratory Disease (BRD) is the most common cause of death and disease in cattle[1]. BRD is an infectious respiratory disease of cattle, with multifactorial causes, including Mannheimia haemolytica, Histophilus somni, Pasteurella multocida and Mycoplasma bovis as important bacterial pathogens[2].

The aim of this study was to determine the prevalence of bacterial pathogens involved in BRD in cattle from different production systems in different regions of Portugal, as useful scientific evidence-based information for vet practitioners when choosing a suitable vaccination plan on farms.

Material and methods: To determine the presence of bacteria involved in a BRD problem, from October 2020 to December 2021, samples (n=1,636) from diseased animals were collected from 81 farms distributed throughout Portugal, of which 58 were dairy herds, 19 feedlots, and 4 cow-calf herds.

Samples from diseased animals were taken using BOVIRESPcheck, an in vivo diagnostic tool that allows the identification of the main pathogens associated with BRD. It uses a nasal swab as a sampling method and incorporates FTA card technology where the exudate is impregnated, allowing maximum stability and safety in the transport of the sample. Molecular diagnostic techniques (Real Time Multiplex PCR adapted from previous studies[5,6,7,8,9]) are used for the specific amplification of the genetic material of the different pathogens.

For the analysis of the information and the calculation of the prevalence of the different bacterial agents, a farm was considered positive if at least one of the samples analyzed

was positive.

The farm prevalence of each pathogen was calculated as the total number of positive farms/total number of farms and was presented as a percentage.

Statistical analysis was performed using R software v4.0.3.

Results and Discussion: The prevalence of M. haemolytica, H. somni, P. multocida and M. bovis found in this study over the total number of farms was 61.73%, 40.74%, 91.36% and 46.91%, respectively. When analyzed by production system, the prevalence of M. haemolytica, H. somni, P. multocida and M. bovis was, respectively, in dairy farms: 56.9%, 29.31%, 91.38% and 44.83%; feedlots: 78.95%, 68.42%, 89.47% and 57.89%; and cow-calf herds: 50%, 75%, 100% and 25%.

Feedlots are exposed to very significant stressing and risk factors and this explains why the occurrence of BRD is high, and the majority of farms show these findings at the highest rates. Dairy farm and cow-calf show lower rates of BRD in young calves. In the case of dairy, the long-term consequences of BRD in heifers are significant, as this at a young age is associated with higher rates of culling before first calving, as well as lower milk yield (233 Kg less) in the first lactation[10].

M. haemolytica and H. somni can start BRD outbreaks on their own or when there are other predisposing factors involved related to animal management, climactic conditions or previous viral infection. Their presence indicates that they are responsible for the outbreak as initiators or in combination with other pathogens[11].

The use of the PCR technique in BRD outbreaks has improved detection of H. somni when compared to conventional bacteriology. In this study, H. somni prevalence was higher in feedlots and cow-calf herds compared to dairy herds, where M. haemolytica was more prevalent.

Mycoplasma bovis particularly can act as a primary agent and it is related with chronic infections[10]. Unfortunately, there is no vaccine available against this pathogen, and the increasing antimicrobial resistance makes M.bovis infections difficult to control[12].

Despite being the most prevalent bacteria in all farms, P. multocida is considered a secondary agent that needs the involvement of a previous infection by other respiratory agents or management risk factors[10], so we cannot conclude that this bacteria by itself causes respiratory problems.

Conclusions: Given the need to use antimicrobials in a more rational way, PCR diagnostic tests to identify the pathogens involved in respiratory disease in cattle are increasingly used. Bacteria can act as primary or secondary pathogens within the BRD complex and cause lung damage in the affected animals, leading to poor production performance. The high prevalence found in this study in all production systems supports the need for bacterial pneumonia prevention measures on farms, such as vaccination, to reduce the use of antibiotics and improve farm profitability and animal welfare.

Keywords: Bacteria, Respiratory Disease, Prevalence.

ID-P31

Prevalence and risk factors of *Trichophyton verrucosum* in cattle from Republic of Korea

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Objective: Dermatophytosis is a zoonotic disease caused mainly by dermatophytes that infect both human and animals. The bovine dermatophyte (commonly known as ringworm) could cause erythematous alopecia and scabs on the skin lesions, which also negatively affects livestock growth and meat production worldwide. *Trichophyton verrucousm* (*T. verrucosum*) is most predominant aetiological agent of bovine dermatophytosis. The regional prevalence investigation is the first step for establishing of the effective control measures against bovine dermatophytosis. However, there was only one report which investigated the regional prevalence of *T. verrucosum* in Korea between 2000 and 2001. Therefore, this study aimed to update the prevalence of dermatophytes in cattle from Korea and analyze the risk factors in farms.

Materials and Methods: A total 172 samples, which included Korean native cattle (n = 123), dairy cattle (n = 10), and environment (pens, water bowl, and soil) (n = 39), were collected nationwide (31 farms in 10 cities in Korea). The 133 cattle samples were obtained from the lesion of the suspected dermatophytosis. Samples were scraped from the skin at the junction between healthy and skin lesion region. If there is no skin lesion, the swab of face skin from cattle were collected. Samples were test by rapid test kit and incubated on Sabouraud Dextrose Agar at 37℃ for 1–2 weeks. Suspicious colonies were subjected for further identification by sequencing the ITS region. Phylogenetic analysis of the obtained sequences from confirmed DNAs of *T. verrucosum* along with the sequences of closest relatives available in the GenBank data

Results: The study showed the prevalence of *T. verrucosum* in Korea was 15.7% (27/172 samples). The isolates of *T. verrucosum* from Korean native cattle (21/121 17.4%, $p < 0.05$) were significantly more than environment (2/39 5.9%). Cattle which have more than 10 points of skin lesion ($p < 0.05$) were more frequently isolated *T. verrucosum* than those have less than 10 points of skin lesion. The young calves (< 6 months) with dermatophytosis were more prevalent ($p < 0.05$) than the growing calves aged from 6 to 12 months. No *T. verrucosum* were isolated from the adult cattle (> 12 months). The isolation rate incidence of lesion in male and female were 20.63% and 18.46%, respectively. In the neighbor-joining phylogenetic tree, 27 present *T. verrucosum* isolates were clustered to together with *T. verrucosum* reference isolates from different geographical locations in Korea.

Conclusion: This study was the first report which determined the prevalence of dermatophytosis in cattle from Korea nationwide. The overall results suggested that bovine dermatophytes were more prevalent in cattle with severe skin lesions and in calves aged less than 6 months. These results could

help better understanding of the bovine dermatophyte in Korea, which contributed to minimize its impact.

Keywords: Dermatophytosis, *Trichophyton verrucosum*, identification, South Korea, Prevalence.

ID-P32

A droplet digital PCR assay for detection and quantification of *Mycobacterium avium* subsp. *paratuberculosis* DNA in whole-blood and fecal samples from MAP-infected Holstein cattle

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Bovine paratuberculosis (PTB) is an infectious disease that affects ruminants worldwide and is a burden for the dairy industry. PTB control measures include culling of *Mycobacterium avium* subsp. *paratuberculosis* (MAP)-infected animals from the herd and the enhancement of farm-biosecurity measures. Diagnostics tools for the direct detection of MAP are real-time PCR and bacteriological culture, the last one being considered the gold standard. However, both show limitations to detecting subclinical MAP-infected cattle with low bacterial load in feces and tissues. Droplet digital polymerase chain reaction (ddPCR) is a third-generation PCR method that shows high sensitivity for the detection and quantification of low DNA copy numbers. The objective of this study was to design a ddPCR assay to detect and quantify a fragment of the F57 MAP-specific sequence. Specifically, DNA was isolated from whole-blood and fecal samples from cows with no lesions detected by histopathology (N= 75) and from infected cows with PTB-associated focal (N=32), multifocal (N=21), and diffuse lesions (N=17) in gut tissues. After ddPCR, the fecal samples from the cows with diffuse lesions showed higher mean copies per microliter (13,791.2 copies/µl) than samples from cows with multifocal lesions (78.8 copies/µl), focal lesions (177.1 copies/µl) or control cows (4.8 copies/µl) (P≤0.05). Significant differences in mean copies/µl were also observed in the blood samples from cows with focal lesions (47.7) when compared with cows with multifocal and diffuse lesions; 18.1 and 12.4 copies/µl, respectively. When compared with other diagnostic tools, fecal ddPCR positively correlated with the results of a commercial ELISA for the specific detection of MAP antibodies, and with fecal and tissue PCR and bacteriological culture results. In contrast, blood ddPCR results negatively correlated with fecal PCR and fecal and tissue bacteriological culture results. In summary, our results suggest that the blood ddPCR could be used for MAP detection in cows with subclinical PTB with low amounts of MAP in feces and tissues.

Keywords: droplet digital PCR, paratuberculosis, diagnosis, blood, feces.

ID-P33

Evaluation in calves of a Bovine Viral Diarrhoea (BVD), tagged vaccine prototype (AQ06 - BoVLP-BVD) based on Virus-Like-Particles (VLPs)

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Objectives: Aquilón CyL intends to develop a new and disruptive recombinant vaccine against BVD using proprietary antigens in Virus-Like-Particles.

The main objective of this study was to evaluate preliminary safety and immunogenicity *in vivo* of an AQ06 - BoVLP-BVD vaccine prototype that includes BVD virus peptides shown to be naturally antigenic. Besides, the DIVA (Differentiating Infected from Vaccinated Animals) attribute of the vaccine was also tested.

Materials and methods: Twelve Frisian 300 kg male calves were enrolled. Eight of them were inoculated at day 0 and day 21 with a preliminary vaccine formula based on a first prototype of VLP-BVD active substance and an aqueous adjuvant; four calves were inoculated with "empty" VLPs (no active substance) with the same formulation. Safety parameters (general clinical signs, rectal temperature and inoculation site) were daily observed. Animals were scheduled for bleeding (caudal venipuncture) immediately before the first immunization, 21 days after de first immunization and before the second and, 21 days after the second immunization.

In order to test the ability of the VLPs to induce an immune response against a reporter antigen (DIVA attribute), sera of the experimental animals were tested with a specific commercial indirect ELISA for the VLP. Furthermore, sera samples were analyzed using a commercial ELISA test for BVD total antibody and an *in house* developed ELISA for the BVD selected peptide.

Results: Results indicated that all animals increased optical densities against the VLPs when comparing days 0 and 21, suggesting seroconversion to the proteins. Animals receiving the native -empty- VPL were the ones with the highest levels of antibodies at day 21. One of the animals showed a total antibody immune response at day 42. Results of the *in house* developed peptide ELISA indicated that most animals showed increased ODs after the second vaccination.

Conclusions: The results of the present experiment indicate that the VLPs used as the active substance carrier were immunogenic as shown by the induction of specific antibodies, confirming the DIVA attribute of the vaccine prototype ("positive" tagging). On the other side, seroconversion against the active substance (BVD peptide) by most animals suggested a potential immunizing effect of the vaccine, as well as the positive result of one animal for BVD total antibodies ELISA.

In summary, the product tested was safe, showed immunogenicity and induced a specific BVD response in some of the inoculated animals.

Keywords: BVD, VLPs, DIVA.

ID-P34

Vertical transmission of *Mycobacterium avium* subsp. *paratuberculosis,* Bovine viral diarrhea virus, Bovine herpes virus 1 and *Neospora caninum* in a dairy herd

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Objective: The aim was to determine the vertical and horizontal transmission of *Mycobacterium avium* subsp. *paratuberculosis (Map),* Bovine viral diarrhea virus (BVDV), Bovine herpes virus 1 (BHV1), and *Neospora caninum* (*N caninum*) in a dairy herd.

Materials and methods: This study was conducted in a commercial dairy farm located in Castelli, Buenos Aires, Argentina, between September 2017 and December 2018. Once a week, cows 60 and 30 days before expected calving were vaccinated against BVDV and BHV1 (inactivated vaccines) and a blood sample was taken from the coccygeal vein (n= 585), and were monitored for parturition. Also, blood samples were taken from jugular veins from calves immediately after calving (n= 85 Holstein dairy calves), at 1-7 day of age and from dams (n= 40) at calving. At the laboratory, indirect ELISA (in house) was used to detect antibodies against *Map* (cut off for a positive result was a percent positivity value of >70%), commercially available ELISA kits (PrioCHECK™ Bovine BVDV Ab Plate Kit, that recognize non-structural protein p80 and PrioCHECK® BHV-1 gB, that recognize surface glycoprotein B; Thermo Fisher Scientific Inc) were used to detect antibodies against BVDV and BHV1 (cut off for a positive result was a percent inhibition value of >50%). An indirect fluorescent antibody test was used to detect antibodies against *N. caninum* (serological titers of ≥1:100 and ≥1:25 were considered positive for dams and precolostral calves, respectively). The prevalence of seropositive animals was defined as the number of seropositive animals divided by the total number of animals tested. The vertical transmission was defined as the proportion of seropositive precolostrum calves born to seropositive dams, while the horizontal transmission was defined as the difference between seroprevalence in precolostrum calves and seroprevalence in dams. Descriptive analyses, proportion comparison, and logistic regression model were performed by SAS (9.4).

Results: Seroprevalences of *Map,* BVDV, BHV1, and *N caninum* in dams before calving was 18.82% (16/85), 70.59% (60/85), 80.77% (42/52), and 74.12% (63/85), respectively. These seroprevalences did not change at calving (BVDV, 75.68% [28/37]; BHV, 75.68% [28/37]; and *N caninum*, 64.10% [25/39]) except for the seroprevalence of *Map*, which decrease to 2.50% (1/40; *P* = 0.047). On the other hand, seroprevalences of *Map*, BVDV, BHV1, and *N caninum* in calves before colostrum intake were 1.18% (1/85), 27.06% (23/85), 10.59% (9/85), and 60.00% (51/85), respectively. In contrast, after colostrum intake were 24.39% (10/41), 92.68% (38/41), 78.05% (32/41) and 58.54% (24/41), respectively. Vertical transmission of *Map*, BVDV, BHV1, and *N caninum* was 6.25% (1/16), 36.67% (22/60), 18.18% (8/44), and 79.37% (50/63), respectively. In the case of *N caninum*, the odds of vertical transmission increased 11.07 times when cows had high antibody titers (≥400) compared with those that had low antibody titer (≤100) (95% CI = 1.34-91.70, *P* = 0.03). Calves born from positive dams with high antibody title had 6.67 times greater odds of having high antibody title (95% CI = 1.92-23.18, *P* =0.003). Considering the seroprevalence of *Map*, BVDV, BHV1, and *N caninum* in dams before calving and calves before colostrum intake, the horizontal transmission was 17.64%, 43.53%, 69.41%, and 14.12%, respectively.

Conclusions: Determining the most prevalent route of infection in a herd would contribute to developing preventive and control strategies towards these infections. As we expected, vertical transmission frequency varies between the diseases included. Although in some of them, such as *Map*, the frequency is low, it should also be considered because it can delay the success of control programs. On the other hand, in the case of *N caninum*, the antibody titers of the dams could be used as predictors of vertical transmission.

Keywords: infection diseases, seroprevalence, dairy farm.

ID-P35

Real time PCR (RT-PCR) in pooled blood samples as an economical monitoring technique in cattle populations with low Bovine Viral Diarrhoea Virus (BVDV) prevalence

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Objectives: The aim of this study was to determine whether the use of pooled blood samples and a commercially available real time PCR (RT-PCR) test are suitable for monitoring of BVD virus presence in cattle populations with low prevalence.

Materials & methods: A total of 2701 blood samples were collected from 62 different dairy farms located in seven geographical regions of Turkey. The samples were pooled in groups of eight, creating a total of 342 pools. Nucleic acid extraction from each pool and subsequent RT-PCR were performed using commercially available test kits with appropriate negative

and positive controls. Statistical differences between pools, farms and regions were assessed through Pearson's Chi-square test with p-value >0.05 regarded as statistically significant.

Results: The presence of BVDV nucleic acid was detected in 18 pooled samples out of 342 pooled samples tested. The BVDV-positive separate pools represented 11 individual dairy farms, and therefore 17.74% of all farms tested. Unfortunately, it was not possible to test individual blood samples within each pooled sample. Therefore, as the incidence of false positives in the tested pool of samples was unknown, the predictive positive value of the test could not be defined. Nonetheless, the standard approach would be to follow each positive result of a farm-specific pooled sample, with more in-depth antigen-testing down to the level of individual animals. No statistically significant differences between the regions where BVDV were detected in pools (P = 0.297).

Conclusion: The results presented here describe the detection of BVDV in pooled blood samples tested with a commercially available real time PCR test. It offers an economically viable approach to cattle owners who perceive the cost as a barrier to testing for the presence of BVDV in their herds This may be of interest in animal disease surveillance and herd certification programs. Moreover, periodical monitoring of cattle farms for the presence of BVDV should facilitate elimination of PI animals and adoption of appropriate control measures.

Keywords: BVD, monitoring.

ID-P36

Occurrence of enzootic bovine leukosis in dairy and beef cattle in São Paulo state, Brazil

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Brazil has the second largest cattle herd in the world and holds the third position in the world in number of animals slaughtered, making it even more important to prevent diseases that affect the quality of milk and meat, as well as the productivity and longevity of animals. Enzootic bovine leukosis is Bovine leukosis is a common neoplastic disease in cattle caused by bovine leukemia virus. The aim of the present study is determining the occurrence of enzootic bovine leukosis in properties of the state of São Paulo, Brazil. Three rural properties were included in this study. On property I, eighteen animals, females and males, aged between 2 and 8 years old, of the Senepol breed were evaluated. In property II, thirteen animals, females, aged between 2.5 and 6 years old, of the Holstein breed were evaluated. In property III, a hundred sixty animals, female, aged between 1.5 and 3 years old, of the Holstein breed were evaluated. Blood samples were collected by jugular vein puncture to perform an agar gel immunodiffusion test from all participating animals. In property I, fourteen animals (77.77%) had a positive result, being thirteen females and one male, in property II all animals had a positive result (100%) and in property III the agar gel immunodiffusion test was positive for sixty-one animals (38.13%). With the present study we concluded that the occurrence of enzootic bovine leukosis is high in the evaluated herds, reaching all the animals in one of the properties and more than 50% in another one. We also concluded that the disease is important in both dairy and beef cattle, although there are more studies about the prevalence of enzootic bovine leukosis virus in dairy herds. Thus, more studies must be carried out to determine the real presence of the virus in cattle herds in the state of São Paulo, Brazil. We also concluded that the disease is important both in dairy and beef cattle, thus further studies should be carried out to determine the presence of the virus in cattle herds in the state of São Paulo, Brazil.

Keywords: Leukemia virus.

ID-P37

A clinical case of enzootic bovine leukosis in a 5-year-old Senepol cow

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A 5-year-old Senepol cow was referred to the Veterinary Hospital with an increase in breast volume and dewlap. Owner reported weight loss and decreased performance for approximately 1 month and that the animal had anorexia and spent most of the time in recumbency in the last days. On physical examination chest and dewlap edema, increased volume of prescapular and subiliac lymph nodes, bed sores, prolonged skin tent test, enophthalmos, tachycardia (80 beats/min), tachypnea (40 beats/min), mixed dyspnea and bilateral mucopurulent nasal secretion. The hemogram showed remarkable leukocytosis (39,100 cells/mL) by lymphocytosis (76%) and the presence of atypical lymphocytes. Fine needle aspiration cytology (FNAC) of the prescapular lymph node was performed, which revealed high cellularity, with lymphocytes predominance and a high number of atypical lymphocytes (75%). Bone marrow puncture in the sternum and myelogram were also performed, which showed an increased granulocytic/erythrocytic ratio (2.06) due to erythroid hypocellularity, high presence of lymphocytes (52%), 29% of which were atypical. Finally, an agar-gel immunodiffusion test was performed which was positive for enzootic bovine leukosis. The animal was euthanized under anesthesia for necropsy. On the gross level superficial cervical, prescapular and subiliac lymph nodes were swollen. In the thoracic cavity, a whitish mass was observed at the base of the heart and also hydropericardium. In the abdominal cavity, there were whitish masses on the wall of the rumen, omasum, abomasum, in the liver, in the wall of the large intestine, in the mesenteric lymph nodes, right kidney and uterus. Histopathological examination of fragments of the aforementioned organs identified the presence of anaplastic lymphoid cells and atypical lymphocytes with sparse stroma

infiltrated in the affected tissues in a diffuse manner consistent with multicentric lymphoma. In the present study we described a clinical case of enzootic bovine leukosis with diffuse multicentric lymphoma in a beef cattle. We also demonstrated that enlargement of superficial lymph nodes is an important finding in this disease and exams like hemogram, fine needle aspiration cytology and myelogram can contribute to the clinical diagnosis.

Keywords: Leukemia virus, leukocytosis, myelogram, multicentric lymphoma.

ID-P38

Immunohistochemical identification of infectious disease agents associated with respiratory disease in fetal lungs of aborted bovine fetuses

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Introduction: Abortions in cattle may be sequelae to infections by pathogens associated with the development of bovine respiratory disease (BRD). The most frequent of these pathogens are alphaherpesvirus-1 (BoAHV1) and bovine viral diarrhea virus (BVDV). While information relative to the association of bovine parainfluenza virus-3 (BPIV-3), bovine respiratory syncytial virus (BRSV), and Mycoplasma bovis with fetal disease and/or abortions is sparse.

Objectives: Describe the histopathological patterns of BRD observed in the lungs of aborted bovine fetuses and associate these patterns with infectious disease pathogens of pulmonary disease.

Materials and methods: A retrospective study was done between January 2010-December 2019, to identify aborted bovine fetuses with pulmonary tissues submitted for post-mortem evaluations. All selected pulmonary tissues were reviewed for predetermined histopathologic patterns, and histologically categorized as: 0, normal lung; 1, circulatory, reversible, and irreversible cellular alterations; 2, interstitial pneumonia (IPN); and 3, suppurative bronchopneumonia (SBPN). Selected formalin-fixed paraffin embedded tissue sections were then submitted to immunohistochemical (IHC) assays designed to identify intralesional antigens of five pathogens associated with BRD: BoAHV1, BRSV, BVDV, BPIV-3, and Mycoplasma bovis. Positive and negative controls were included in al IHC assays.

Results: During the evaluated period 37 bovine fetuses containing pulmonary tissues were identified, most (91.2%; 34/37) of these were from Paraná State, Southern Brazil, with three being from the State of Minas Gerais, Midwest Brazil. Although the gestational age of the fetuses varied between 5 to 8 months, the age of most fetuses was not recorded. Normal pulmonary tissue was observed in 48.8% (18/37) of all cases, circulatory and/or cellular alterations were diagnosed in 18.9% (7/37), 29.8% (11/37) had IPN, with one case (1/37; 2.7%) of fetal BPN being identified. Positive immunoreactivity to antigens associated with BRD were observed in 11 fetal lungs: category-0 (n=0), 1 (n=5), 2 (n=6), and -3 (n=1). Twenty-four pulmonary fetal tissues (19 without pneumonia; 5 with pneumonia) did not demonstrate positive immunoreactivity to any of the agents evaluated. Interstitial pneumonia was associated with singular infections by BRSV (n=3), BVDV (n=1), and BoAHV1 (n=1). Furthermore, there was one concomitant manifestation of IPN due to BRSV and BoAHV1; one fetal lung with IPN contained antigens of BoAHV1, BVDV, and M bovis, while none of the agents investigated were observed in four fetuses with IPN. Alternatively, the fetal lung with SBPN contained antigens of BVDV and BRSV. Interestingly, M. bovis antigens were only identified in coinfections: in one fetus with circulatory and/or cellular manifestations infected by BRSV, and in the triple infections that was diagnosed in a fetus with IPN. All antibodies evaluated demonstrated intracytoplasmic immunoreactivity. Positive immunoreactivity to BRSV antigens occurred within degenerated and normal bronchial and bronchiolar epithelial cells, and epithelial cells of the mixed bronchial gland. Positive immunoreactivity to BVDV was observed within normal and degenerated bronchial epithelial cells. Intralesional BoHV-1 antigens were identified within normal bronchial and bronchiolar epithelial cells, chondrocytes of hyaline cartilage, and capillary endothelium. Infections due to M. bovis resulted in positive immunolabelling within normal, degenerated, and necrotic bronchial and bronchiolar epithelial cells, necrotic epithelial cells of the mixed bronchial glands, endothelial cells, and alveolar macrophages. BPIV-3 antigens revealed positive intracytoplasmic immunoreactivity within normal bronchiolar epithelia and alveolar macrophages.

Conclusions: These results have demonstrated interstitial pneumonia in the lungs of aborted bovine fetuses and this pattern of pneumonia was associated with the intralesional identification of BRSV, BVDV, and BoAHV1. These are infectious disease pathogens known to cause BRD. Furthermore, the identification of these infectious disease agents in fetal tissues suggest that these fetuses suffered intrauterine infections, probably via transplacental transmission. Although BVDV and BoAHV1 are well known abortive agents of cattle, the identification of BRSV, BPIV3, and M. bovis in fetal tissues suggest these pathogens may have contributed towards the development of fetal disease. Alternatively, these initial results may suggest that M. bovis is an innocent bystander, since this pathogen was only observed in mixed infections. Consequently, the role, if any, of BRSV, BPIV3, and M. bovis in the development of reproductive diseases in cattle must be thoroughly investigated.

Keywords: fetal pathology, interstitial pneumonia, BVDV, BoAHV1, BRSV.

ID-P39

Bovine gammaherpesvirus 6 associated with intestinal and pulmonary lesions in buffaloes (*Bubalus bubalis*) from Central-West Brazil

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Introduction: Bovine gammaherpesvirus 6 (BoGHV6), formerly known as bovine lymphotropic virus, belongs to the genus *Macavirus*, subfamily *Gammaherpesvirinae*, family *Herpesviridae*. This genus contains ovine gammaherpesvirus 2 (OvGHV2) and alcelaphine gammaherpesvirus 1 (AlGHV1) that cause malignant catarrhal fever in ruminants worldwide. While the diseases induced by OvGHV2 and AlGHV1 are well known and recognized, specific diseases or syndromes related to BoGHV6 are controversial. Nevertheless, there are reports associating BoGHV6 with reproductive and neoplastic diseases, while no associations between BoGHV6 and diseases were described in other studies. Consequently, the participation of BoGHV6 in the development of diseases is still not fully understood and must be investigated.

Objective: Describe the histopathological and molecular findings observed in the lungs and intestines of buffaloes infected by BoGHV6.

Materials and Methods: Fragments of the small intestines and lungs were collected from buffaloes slaughtered at an abattoir, under federal inspection service, located within the State of Goiás, Central-West, Brazil. All tissues were processed for routine histopathological evaluation; frozen fragments of these organs, collected in duplicate, were used in molecular assays designed to amplify the nucleic acids of bovine alphaherpesvirus 1 (BoAHV1), bovine viral diarrhea virus (BVDV), OvGHV2, and BoGHV6. The products amplified from all molecular assays were sequenced for confirmation.

Results: During this study, 19 intestinal and pulmonary fragments from buffaloes were collected. All pulmonary (100%; 19/19) fragments had mild, patchy, interstitial pneumonia, characterized by foci of proliferation of type 11 pneumocytes with accumulations of lymphocytes; in two (10.5%; 2/19) fragments, concomitant suppurative bronchopneumonia and interstitial pneumonia were observed. Histopathological evaluation of the intestinal fragments revealed mild to moderate atrophic enteritis characterized by varying degrees of fusion and blunting of the intestinal villi, mild accumulations of necrotic debris within intestinal crypts and mucosa, and lymphoplasmacytic inflammatory accumulations with the mucosa of all (100%; 19/19) fragments evaluated. BoGHV6 nucleic acids were amplified in 31.6% (6/19) of the pulmonary fragments containing only interstitial pneumonia, and in only one pulmonary tissue that contained simultaneous interstitial pneumonia and suppurative bronchopneumonia. Additionally, BoGHV6 DNA were amplified in 26.3% (5/19) of the intestinal fragments with atrophic enteritis. Sequencing confirmed that the products amplified in all PCR assays were BoGHV6. Additionally, nucleic acids of BVDV, OvGHV2, and BoAHV1 were not amplified in any of the tissues evaluated.

Conclusion: The histopathological evaluation of pulmonary tissues from these buffaloes revealed that all animals had interstitial pneumonia, with atrophic enteritis being diagnosed in some of the intestinal fragments evaluated. Additionally, PCR amplified BoGHV6 DNA from 31.6% (6/19) of the tissues with interstitial pneumonia and in 26.3% (5/19) of the fragments with intestinal lesions. The non-amplification of BVDV, BoAHV1 and OvGHV2 suggest that these organisms were not involved with the pulmonary and enteric lesions observed in these buffaloes. Consequently, these results may indicate that BoGHV6 was associated with the interstitial pneumonia and atrophic enteritis observed in these animals, suggesting a possible cause of pulmonary and enteric diseases in the buffaloes herein evaluated. The occurrence of BoGHV6 was not related to any disease in an investigation done in ruminants derived from several European countries, while this virus was associated with reproductive and neoplastic diseases in reports from other countries. Consequently, this is the first study to suggest the possible participation of BoGHV6 in the etiopathogenesis of pulmonary and enteric diseases. It must be highlighted that the intestinal and pulmonary lesions herein described were mild and since clinical manifestations of pneumonia and enteritis were not observed in these animals, all buffaloes were asymptomatic, demonstrating the resistant nature of this ruminant species in the development of diseases. Furthermore, additional analyzes are being carried out to include and/or exclude the participation of other agents associated with the development of pulmonary and enteric disease in ruminants, thus determining the real participation of BoGHV6 in the etiopathogenesis of the lesions herein described. Additionally, pulmonary and intestinal tissues from populations of buffalos maintained within several geographic regions of Brazil are being collected and analyzed to confirm the association of BoGHV6 with pulmonary and/or enteric disease. Nonetheless, the findings herein presented suggest the possible participation of BoGHV6 in the development of interstitial pneumonia and enteritis in buffaloes.

Keywords: Bovine lymphotropic virus, etiopathogenesis, enteritis, Macavirus, pulmonary disease.

ID-P40

Within-Herd Prevalence and Transmission Patterns of *Mycoplasma bovis* in Co-Mingled Holstein Dairy Heifers fro Two Different Parent Farms

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Objective: *Mycoplasma bovis* infection in dairy cattle is problematic due to asymptomatic carriers, multiple disease entities, limited treatments and poor resolution. This study assessed within-herd prevalence of asymptomatic *M. bovis* infection and evaluated associations between positive culture sites in heifers from two herds of origin (A and B) while co-mingled at a heifer-rearing facility raising only their calves.

Material and Methods: Thirty Holstein calves (15 from each farm) were enrolled every 4 months until 150 calves (75 from each farm) were entered into the study. Every enrollment added the first 15 heifers born at each farm. The first sample was collected from all calves at the parent farm, then monthly while co-mingled at the heifer facility, followed by quarterly sampling after return to the home farm. At each sampling, swabs were taken from four sites: intranasal, ocular, ear canal, vagina for a total of 10x per site. Swabs were cultured for *M. bovis* and confirmed by PCR. Both herds and the heifer facility were in southeastern Pennsylvania, US. Data were analyzed using STATA 17. Non-parametric Wilcoxon rank sum or Kruskall Wallis tests were used for count data. Categorical data were analyzed with a chi^2 or Fisher's exact test, where appropriate. Zero-inflated Poisson regression was used to compare rates of infection at all sites, or combinations of thereof when more than one site was positive. Inclusion of calf as a fixed effect and robust variance estimation were used to control for clustering in all regressions. Statistical significance was inferred when $P < 0.05$.

Results: Three heifers from each dairy were lost after enrollment leaving 144 heifers (72 from each farm) in the analysis. There was one positive on the first test day, a vaginal sample in a calf from farm A. While co-mingled, the number of positive calves increased over time. Only 19/144 heifers were never positive (13/72, 18.1% and 6/72, 8.3% from farms A and B, respectively; P = 0.09). Days of age to first positive were not different between farms (Farm A mean = 136, standard deviation [SD] 95, range 21-561 days; Farm B mean = 131, SD 72, range 33-404 days; P = 0.83). The only significant difference between farms was that Farm B had fewer calves (6/72, 8.3%) with ear canal positives (either alone or in combination) than Farm A (31/72, 18.1%; incidence rate ratio [IRR] = 0.43, 95% confidence interval [CI] 0.24-0.76; P = 0.004). There were no significant differences between farms in positives from any other site or combinations thereof.

Overall, 430 positive samples were obtained from 125 heifers. Most were from intranasal (168, 39.1%) swabs followed by ocular (52, 12.1%), vagina (43, 10.0%) and ear canal (19, 4.4%). Among combinations when more than one site was positive on a given test day, eye/nose (55, 12.8%) and nose/vagina (21, 4.9%) were most frequent. Other combinations were observed between 1-15 times, 0.2-3.5% of all positives.

At the second sampling (i.e., first co-mingled) 12 calves were positive (1 ear, 2 eye, 4 nose, 4 vagina and 1 ear/vagina). However, at samplings 3 and 4 there was an exponential increase in calves with positive intranasal swabs; 16 and 36 (peak frequency), respectively. Positive ocular swabs also increased and combinations of positives involving the nose began to appear but temporally lagged the intranasal positives and never reached the same frequency. The number of intranasal positives plateaued at 26 to 28 over samplings 5-7. During the study, intranasal positives alone or in combination with other sites occurred more frequently than sites not involving the nose (IRR 1.44, 95% CI 1.41-1.47: P < 0.001). After return to the home farm the frequency of intranasal positives declined precipitously, while other sites and positive combinations declined more gradually.

Conclusion: These data demonstrate that over time *M. bovis* can be cultured from all sites tested, but the nose is by far the most critical for establishing transmission between co-mingled calves. Moreover, when the level of intranasal carriage escalates at around 2-3 months of age, virtually all calves will subsequently be positive at least once at one or more sites. These results indicate how readily asymptomatic *M. bovis* can be perpetuated in a population of animals and potentially manifest as clinical disease.

Keywords: Asymptomatic carrier, dairy heifer, prevalence, co-mingled.

ID-P41

Investigation of disease hazards in cattle in South of Italy (Sicily)

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Objective: Infectious diseases represent a serious limitation of bovine production. The etiology of these diseases is diverse and comprises a variety of viral, bacterial, protozoan and chlamydial agents, some of which are zoonotic [1]. Infectious-parasitic agents associated with reproductive disorders in ruminants include *Neospora caninum, Coxiella burnetii*, *Chlamydia abortus* and *Toxoplasma gondii*; they cause the greatest economic losses for the livestock industry [2]. This is a cross-sectional study to assess the presence of antibodies in ruminants against selected pathogens including the zoonotic agents *C. burnetii, T gondii, N. caninum, Clamydia spp.* and *Theileria annulata* in cattle in Sicily region and to determine the molecular status for *T. gondii* in order to determine the serological and molecular status of bovine in the Sicily region.

Materials and methods: Livestock represents one of the most important resources for the Sicilian economy, involving more than 9000 farms with more than 380,000 heads of cattle,

with concentration in the province of Palermo (21%), Agrigento (4.5%), and Trapani (2.6%) [3]. The farms are registered in the National database (BDN) (Reg CE 1760/2000—BDN data) and their locations can be identified by geographic coordinates. Traditionally, the livestock system in Sicily consists of cattle reared in feral and semi-feral condition with extensive grazing methods that represent an ancient and traditional practice for using poor lands. Livestock farming differs between the production of farm animals for slaughter and the production of milk for the processing of dairy products.

A simple random sample was determined with proportional stratification and an expected prevalence of 5%. The simple random sample was stratified by province with weights proportional to the total number of cattle within the province. To ensure the representativeness of the sample and eliminate the bias, an extraction step of bovine serum from herds in each province was calculated. The extraction step is different for each province. Finally a total of 478 bovine blood samples were collected from all the farms in the province of Palermo, Agrigento e Trapani in the region of Sicily, taking two samples for each farm, between September 2020 and September 2021. An extensive range of serological and molecular diagnostic assays were used.

The collected sera were analyzed by Enzyme-Linked Immunosorbent Assay (ELISA) for *C. burnetii, T. gondii, Clamydia spp.* and *N. caninum* by using different commercial ELISA kits, following the manufacturer's instructions. The samples were analyzed for *T. annulata* by IFI test.

Results. Seroreactive animals were found for all diseases studied except for *Clamydia spp*, with general prevalence rates of 93.7% *T. annulata* (CI95% 0.91-0.95), 13.0% (CI 95% 0.09-0.16) for *N. caninum,* 4.2% (0.02-0.06) for *C. burnetii* and 6.1% for *T. gondii* (CI 95% 0.04-0.8).

T. annulata was mainly detected in province of Palermo (84.15%), followed by Agrigento (10.71%) and Trapani (5.14%). *N. caninum* was detected in 79.03%, 11,29% and 9.68% from serum collected in province of Palermo, Agrigento and Trapani respectively. The 90.00% of *C. burnetii* positive sare were collected form province of Palermo and the remaining 10.00% in Agrigento. Most sera (72.41%) seroactive for *T. gondii* were collected from province of Palermo, followed by Agrigento (20.68%) and Trapani (6.91%).

Conclusions: The prevalence rates of the diseases analyzed in Sicily among the bovine population fluctuate from medium to high and thus require official control measures, with the highest percentages in the province of Palermo, for all the infections detected 87%. followed by Agrigento (12,7%).

This is the first study that has identified *C. burnetii* in cattle associated with *N. caninum* and *T. gondii*. Thus, future studies should be conducted to investigate how widespread this pathogen is in sicilian cattle herds.

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Keywords: Neospora caninum, Coxiella burnetii, Chlamydia spp, T gondii, Theileria annulata

ID-P42

Serological investigation of *Leptospira* serovar Hardjo and Pomona in cattle in South of Italy (Sicily)

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Objective: Leptospirosis is a notifiable disease in Italy, and 45 outbreaks have been officially notified in cattle in the last 10 years (2011–2020) [1], but it is probably underreported. The cattle industry is a major driving force for the Italian agricultural sector totalling about 5.6 million heads for dairy and meat production together. Leptospirosis is an infectious zoonotic disease causing reproductive problems and economic losses in livestock. In ruminants it is mainly due to *Leptospira borgpertsenii* serovar Hardjo and *Leptospira interrogans* serovar Pomona. The aim of this study is to evalate the presence of antibodies against the two serovars mentioned above, in bovine sera collected in Sicily region**.**

Materials and methods: In Sicily, as in other Mediterranean areas, livestock represents one of the most important resources for the island economy. This sector involves more than 10,000 farms of cattle (15% of national production) which are actually increasing their number and more than 380,000 heads of cattle, with concentration in the province of Palermo 21%, Agrigento (4.5%), and Trapani (2.6%) [2].

A simple random sample was determined with proportional stratification and an expected prevalence of 5%, stratifying by province, with weights proportional to the total number of cattle within the province. To ensure the representativeness of the sample and eliminate the bias, an extraction step of bovine serum from herds in each province was calculated. Finally. a total of 478 bovine blood samples were collected from all the farms in the province of Palermo, Agrigento e Trapani in Sicily region, between September 2020 and September 2021 (two samples for each farm were collected). The sera were analyzed by Enzyme-Linked Immunosorbent Assay (ELISA) for *Leptospira* serovar Hardjo and *Leptospira* serovar Pomona by a commercial ELISA kit (*Leptospira pomona and hardjo* Antibody Test Kit, ELISA Bovicheck® Lepto HP, Biovet) following the manufacturer's instructions.

Results: Seroreactive animals were found for both diseases studied, with prevalence rates of 15.48% (CI95% 0.12-0.18) for *Leptospira* serovar Hardjo and 5.64% (CI95% 0.18-0.36) for *Leptospira* serovar Pomona.

Leptospira serovar Hardjo was mainly detected in province of Palermo (79.73%), followed by Agrigento (12.06%) and Trapani (8.21%). Most sera (96.29%) seroactive for *Leptospira*

serovar Pomona were collected from province of Palermo, the remaining (3.71) in Agrigento.

Conclusions: The epidemiological situation in cattle reported in this study does not appear particularly different from that described in previous serological findings [3]. Serovar hardjo was confirmed as the most prevalent agent of *Leptospira* infection in cattle confirming its role in determining infection in ruminants. Future studies should be conducted to investigate how widespread this pathogen is in sicilian cattle herds.

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Keywords: Leptospira serovar Hardjo, Leptospira serovar Pomona, ELISA, Sicily.

ID-P43

Measuring environmental faecal samples on dairy farms infected with paratuberculosis

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Objectives: Paratuberculosis is caused by the bacterium Mycobacterium avium subspecies paratuberculosis (MAP). The infection is usually spread through the faecal-oral route. Infected animals shed the bacteria mainly in faeces, but also with milk. The calves are the most susceptible to infection. Most Hungarian dairy farms use ELISA in individual serum or milk samples to determine the occurrence of the disease on a herd level, which is a time-consuming and expensive method in large herds. Measuring environmental samples can be a cost-effective and fast method to identify MAP infected herds. We aimed to find a relationship between the environmental faecal samples and the MAP infection rate at the farm level.

Material & Methods: We performed the measurements on five large-scale dairy farms, where the average lactating cow number was 590 ±116 animal/farm. The apparent prevalence of paratuberculosis infection detected by the ELISA method was 3.6 % (min.: 2%, max.: 5.4%) among the cows more than two years old, according to one-year herd-level blood sampling. We took environmental faecal samples from 5 different places (calving pen, workers boots, a fresh cow pen, dry cows barn, walking alleys) at the farm into sterile plastic tubes. Each sample consisted of a pool of an average of 50 g faeces samples from 4 different locations. We measured the samples by real-time qPCR using Macherey-Nagel NucleoSpin Tissue and Adiavet Rt-PCR kit. During the farm visit, we filled out a questionnaire with the local vet and a farm manager. The questions focused on the calving pen's hygiene, calving protocol and protocols for the cows diagnosed MAP positive.

Results: According to the Rt-PCR results, the presence of the bacterium was the lowest at the dry cow's barn (mean threshold cycle (Ct) value: 37.9 min.: no Ct, max.: 38.1), and the highest at the fresh cow pen (mean Ct value: 34.9, min.: 32.3, max.: 37.1). At farms where MAP positive animals calved individually and separated from the others, we found no bacteria at the calving pen. These two farms had the lowest ELISA positivity (2 and 3.6%) based on the herd-level blood testing. All samples taken from the alleys contained MAP (mean Ct: 35.4, min.: 32.2, max.: 37.8). MAP was detectable on workers' boots, albeit in minimal quantities (mean Ct value: 38.6, min.: 35.7, max.: 40.2).

Conclusions: Although the number of samples in our study is rather small, the result suggests that animals infected with paratuberculosis shed the least amount of bacteria during the dry period and the burden of MAP is the highest at the fresh cow pen. In case the calving pen's hygiene is quite good, and the farm has a protocol for handling the animals with paratuberculosis, environmental exposure is much lower. At the farms where the herd-level ELISA positivity was lower, a smaller number of bacteria were detected in the environmental samples, and the number of positive samples was also lower. Since we were able to find bacterial DNA at the alleys and on the worker's boots on each farm, we highlight the importance of boot washing, before entering the calf rearing facilities. According to our result, environmental faecal sampling can be a cost-effective method in the monitoring of control programs and also in finding find the hazard point for the spread of paratuberculosis at the farm.

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Keywords: Paratuberculosis, dairy cows, environmental samples.

ID-P44

Investigation for persistent infection of bovine viral diarrhea virus in Korean native cattle from 2015-2020

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Objectives: Bovine viral diarrhea virus (BVDV) is one of the most important viruses in cattle that can cause economical losses due to increased abortion, diarrhea, and respiratory symptoms in Korea and worldwide. Infection of a pregnant cattle lead to persistent infection (PI) of the fetus and birth of a PI calf that sheds the virus throughout its life. The PI releases continuously more virus than transient infection (TI) and acting as an important source of viral spread in cattle herd. The detection of PI cattle at early stage, after birth is of significant benefit to implement BVDV control.

This study aimed to detect the PI cattle in Korean native cattle farms and investigate post management of the PI cattle.

Material and Methods: From 2015 to 2020, ear notch tissue samples of 13,608 Korean native cattles were obtained and tested for BVDV antigen ELISA. The PI cattle was confirmed by repeated test of BVDV positive cattle after 4 weeks. The PI cattle were traced to identify the mortality rate and slaughter status.

Results: Among 13,608 cattles from 130 Korean native cattle farms, 165 BVDV PI cattles (1.21%) were confirmed in 58 farms (44.6%) over 6 years. The post management of PI cattle from 2017 to 2020 showed that 38 cattles (28.6%) were died, 22 cattles (16.5%) were killed voluntarily, and 28 cattles (21.1%) were slaughtered.

Conclusion:BVD is causing massive economic loss in Korean cattle farms. However, it is difficult for the farmer to determine whether to cull PI cattle, due to high price of Korean native cattle. Therefore, it is necessary to carry out screening for nationwide surveillance by an early stage detection of PI cattle and culling support by government should conduct to reduce the damage of BVD infection in Korea.

Keywords: Bovine viral diarrhea virus, Persistent infection, Korea.

ID-P45

Protective efficacy of recombinant proteins from heavy chain fragment of *Clostridium botulinum* B, C/D and D/C

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Objectives: Botulism is a paralytic disease of human beings and animals caused by neurotoxins produced by *Clostridium (C.) botulinum*. Botulinum neurotoxins (BoNT) B, C/D and D/C were the most common cause of cattle botulism in Korea. Vaccination has been reported to be the most effective way to control cattle botulism. The aim of this study was the expression of recombinant heavy chain (Hc) fragments with type B, C/D and D/C. And also, purified proteins were evaluated with mouse protection against BoNT challenge.

Materials and methods: DNA fragments encoding the BoNT-Hc genes were amplified from total DNA of *C. botulinum* using PCR. The PCR products were sub-cloned into TA- and pET-vector, and transformed in *E. coli* BL21 expression host. Recombinant proteins were over-expressed and purified by affinity chromatography. Mice were vaccinated subcutaneously with recombinant proteins plus aluminum hydroxide. After three weeks, mice were challenged intraperitoneally with 25 LD_{50} of neurotoxin.

Results: The results showing in this study demonstrated that recombinant proteins were successfully cloned and expressed in *E. coli* BL21. Mouse survival rate with recombinant proteins of BoNT-Hc was confirmed as 100% whereas survival rate in negative control group was 0%.

Conclusions: In this study, we established *E. coli* clones which produced recombinant proteins of BoNT-Hc with B, C/D and D/C, respectively. Recombinant proteins were successfully over-expressed and purified. Each purified protein was able to protect against 25 LD_{50} with a dose as low as 3 μg, 10 μg and 7 μg of B, C/D and D/C neurotoxin, respectively.

Keywords: Efficacy, recombinant, heavy chain, botulinum.

ID-P46

Pathological, immunohistochemical, and molecular findings associated with ovine gammaherpesvirus 2 infections in a goat

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Introduction: Ovine gammaherpesvirus 2 (OvGHV2), is a member of the genus *Macavirus*, subfamily *Gammaherpesvirinae*, family *Herpesviridae*, and produces malignant catarrhal fever (MCF) in a wide range of mammalian hosts. Sheep are considered as the asymptomatic hosts of OvGHV2 with clinical and pathological manifestations of MCF identified primarily in numerous ruminant species, that are considered as the terminal host, resulting in sheep associated-MCF (SA-MCF). Other important *Macavirus* members know to produce MCF and considered as malignant catarrhal fever virus (MCFV) include alcelaphine gammaherpesvirus 1 (AlGHV1) and caprine gammaherpesvirus-2 (CpGHV2). Furthermore, bovine gammaherpesvirus 6 (BoGHV6) is within the *Macavirus* genus but not associated with the development of MCF. Although there

are numerous descriptions of SA-MCF and/or infections associated with OvGHV2 in cattle and bison, cases of this infection due to OvGHV2 in goats are rare.

Objectives: Describe the pathological, immunohistochemical, and molecular findings observed with infections due to OvGHV2 in a goat.

Materials and methods: A 6.5-year-old female, Anglo-Nubian goat with a history of tetraparesis, apathy, hyporexia, amaurosis, and depression was admitted at the larger animal clinics, Universidade Estadual de Londrina, Southern Brazil. The animal remained hospitalized for four days, received adequate therapy, but was euthanized due to deterioration of clinical conditions and poor prognosis. The carcass was submitted for post-mortem evaluation and tissues were routinely evaluated for histopathologic alterations. Furthermore, selected formalin fixed paraffin embeded (FFPE) tissue fragments were used in immunohistochemical assays designed to identify the 15A epitope common to members MCFV and frequent systemic pathogens of ruminants, such as bovine viral diarrhea virus (BVDV) and bovine alphaherpesvirus virus 1 (BoAHV1). Moreover, duplicate sections of these tissues were used in molecular assays designed to amplify the nucleic acids OvGHV2, BVDV, and BoHV-1. Furthermore, the strain of OvGHV2 herein identified was phylogenetically compared with the reference strains for OvGHV2, AlGHV1 and -2, and BoGHV6 deposited in GenBank.

Results: Grossly, there was pulmonary edema, rib impression of the pleural surface of the lung, hydrothorax, ascites, and congestion of meningeal vessels. The most significant histopathological findings included interstitial pneumonia, purulent bronchopneumonia, pulmonary edema and congestion with thrombosis and proliferative vascular lesions (PVL), neuronal necrosis with laminar cortical edema at the cerebrum, and atrophic enteritis. The I5A IHC assay identified intralesional antigens of a MCFV within epithelial cells of the small intestine and lungs of the FFPE tissue fragments. The nested-PCR amplified the partial fragment of the tegument protein gene of OvGHV2 from intestinal fragments with atrophic enteritis. Sequence analysis demonstrated that the strain herein identified had 99.5% nucleotide (nt) sequence identity with the reference strain of OvGHV2, but 67.2%, 67.6%, and 68.9%% nt identity with reference strains of AlGHV1, AlGHV2, and BoGHV6, respectively. Additionally, nucleic acids and/or antigens of BVDV and BoHV-1 were not identified in the tissues evaluated.

Conclusions: The histopathological findings of interstitial pneumonia, neuronal necrosis, and atrophic enteritis with concomitant proliferative vascular lesions observed in multiple tissue of this goat were previously described in ruminants infected by OvGHV2. Additionally, the intralesional identification of tissue antigens of a MCFV within epithelial cells of the intestine and lungs demonstrated that these lesions were associated with a member of this group of infectious disease pathogens. Furthermore, PCR and sequence analysis confirmed that the MCFV identified by the 15A IHC assay was OvGHV2, being phylogenetically distant from other members of the *Macavirus*. Collectively, these findings demonstrate that OvGHV2 was associated with the development of the clinical manifestations and pathological findings herein described. However, since this goat did not demonstrate typical clinical manifestations of MCF, the disease herein presented represents asymptomatic infection by OvGHV2; similar syndromes have been described in cattle and bison naturally infected with OvGHV2. The proliferative vascular lesions (PVL) described in this goat refers to a wide spectrum of vascular alterations observed at the arterial walls of mammalians infected with OvGHV2 and include endothelial proliferation, vascular occlusion, and degeneration of the wall of arteries. The importance of this report lies in the documentation of one of the few confirmed infections of OvGHV2 in goats worldwide, contributing towards the understanding of the pathogenesis associated with infections by OvGHV2 in ruminants and the identification of terminal hosts.

Keywords: Atrophic enteritis, interstitial pneumonia, Macavirus, malignant catarrhal fever, proliferative vascular lesions.

ID-P47

Comparison of PCR and qPCR for the detection of *Mycobacterium avium* subsp. *paratuberculosis* in feces and milk of dairy cows in Uruguay: a longitudinal study

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Objectives: Paratuberculosis (PTBC) is a chronic infectious disease of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), with an important economic impact in the dairy sector. This study aimed at evaluating and comparing the performance of two molecular diagnostic tests (PCR and qPCR) for the detection of MAP shedding in feces and milk of seropositive dairy cows across time.

Materials and Methods: One dairy farm with PTBC seroprevalence of 7%, cattle with clinical history (chronic diarrhea, weight loss), and molecular confirmation of MAP was selected. Thirteen cows with serum anti-MAP IgG detected by a commercial ELISA were included. Fecal and milk samples were collected at 4 different time points (T1, T2, T3 and T4) with 15-day intervals and processed for the detection of MAP by PCR and quantitative real-time PCR (qPCR) targeting the IS900 sequence. The agreement between test by sample type was assessed by the kappa statistic, using R software. Values of kappa were interpreted as follows: 0–0.2 = no agreement; 0.21–0.39 = minimal; 0.40–0.59 = weak; 0.60–0.79 = moderate; 0.8–0.9 = strong, and >0.9 = almost perfect agreement. Fisher´s exact test was used assess differences in the proportion of positive cows by PCR and qPCR.

Results: The % of positive cows by PCR and qPCR in feces, respectively, were 54.5% (6/11) and 90.9% (10/11) at T1, 30.8% (4/13) and 76,9% (10/13) at T2, 46.2% (6/13) and 84.6% (11/13) at T3, and 46.2% (6/13) and 76.9% (10/13) at T4. Consistently across all sampling times, the % of positive cows detected by qPCR was higher than for PCR. The overall % of positivity was significantly higher ($p<0.05$) for qPCR (41/50, 82%) than for PCR (22/50, 44%). All PCR-positive samples

were also positive by qPCR. There was no to weak agreement between both test (kappa=0.29, 95% CI 0.12–0.47).

The % of positive cows by qPCR in milk at T1, T2, T3 and T4, respectively, was 27.3% (3/11), 30.8% (4/13), 38.5% (5/13), and 30.8% (4/13). All milk samples were negative by PCR, resulting in no agreement between tests (kappa=0). Fourteen of the 15 cows with a positive qPCR result in milk across all sampling times, also showed a concurrent positive qPCR result in feces, while in 1 cow MAP was detected by qPCR in milk but not in feces collected concurrently. MAP shedding in milk was intermittent.

Conclusions: The qPCR presented higher sensitivity than PCR to detect MAP in both types of samples, but especially in milk. Due to intermittent shedding in milk, care must be taken when using this type of sample for diagnosis. The highest % of positive cows was detected by qPCR in feces. Despite the no to weak agreement between tests, qPCR consistently detected as positive all PCR-positive fecal samples, suggesting that this agreement is likely due to a significantly higher sensitivity of the qPCR. In this context, the qPCR used in this study could be valuable in disease control programs in dairy herds, although further validation studies and determination of the specificity of the qPCR are necessary.

Keywords: "Paratuberculosis, cattle, molecular".

ID-P48

Simultaneous infections by ovine gammaherpesvirus 2 and bovine gammaherpesvirus 6 in a sheep

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Introduction: Ovine gammaherpesvirus 2 (OvGHV2), is a member of the genus *Macavirus*, subfamily *Gammaherpesvirinae*, family *Herpesviridae*, and produces malignant catarrhal fever (MCF) in mammalian hosts worldwide. Members of the *Macavirus* genus known to cause MCF in their respective hosts are referred to as MCFV, include alcelaphine gammaherpesvirus 1 (AlGHV1) and caprine gammaherpesvirus-2 (CpGHV2), and all share the 15A epitope. Sheep are the asymptomatic (adaptative) hosts for OvGHV2 with clinical and pathological manifestations of MCF occurring in numerous ruminant species (the terminal hosts), resulting in sheep associated-MCF (SA-MCF). Furthermore, while MCFV are known to produce diseases in numerous hosts, the participation of bovine gammaherpesvirus 6 (BoGHV6) and porcine lymphotropic herpesvirus, also members within the *Macavirus* genus, in the development of diseases is controversial. Although there are numerous descriptions of SA-MCF and/or infections associated with OvGHV2 in the terminal hosts, descriptions of diseases due to OvGHV2 in the adaptive hosts are rare, while there are few reports of simultaneous infections by *Macavirus*.

Objectives: Describe the pathological, immunohistochemical, and molecular findings associated with concomitant infections by OvGHV2 and BoGHV6 in a sheep.

Materials and Methods: A 4-month-old, male, Santa Ines sheep with a complaint of apathy and lateral decumbency was attended at the large animal clinics, Veterinary Teaching Hospital, Universidade Estadual de Londrina, Southern Brazil. Clinical evaluation revealed corneal opacity of the left eye, tachycardia, tachypnoea, ruminal hypomotility, congested mucous membranes, permanent lateral decumbency, diarrhea with opisthotonos, amaurosis, nystagmus and tetraparesis. The kid received adequate therapy and clinical care but died suddenly four days after admission. The carcass was submitted for post-mortem examination and routinely processed for histopathologic evaluation. Selected formalin fixed paraffin embeded (FFPE) tissue fragments (lungs, kidney, myocardium, and spleen) were used in immunohistochemical assays designed to identify the 15A epitope common to members of MCFV, and antigens of bovine viral diarrhea virus (BVDV) and bovine alphaherpesvirus virus 1 (BoAHV1). Duplicates of frozen FFPE fragments used in molecular assays designed to amplify commonly associated with pulmonary disease in ruminants from Brazil: including OvGHV2, BoGHV6, BoAHV1, BVDV, bovine coronavirus, bovine respiratory syncytial virus, *Mycoplasma bovis*, *Histophilus somni*, *Pasteurella multocida*, and *Mannheimia haemolytica*. Furthermore, frozen fragments of the myocardium, spleen, and kidney were used in molecular assays to amplify OvGHV2 and BoGHV6.

Results: The main gross findings were rib impressions at the pleural surface of the lungs, pulmonary abscesses with adherences, pulmonary edema, ulcerative parasitic abomasitis, purulent lymphadenitis, and hyperemia of cerebral vessels. Histopathology revealed interstitial pneumonia with multiple abscesses, non-suppurative encephalitis with cortical edema, neuronal necrosis, abscedative lymphadenitis, lymphoplasmacytic myocarditis and nephritis, and atrophic enteritis. Proliferative vascular lesions (PVL) were observed in the brain, liver, spleen, and lymph node. The IHC assays revealed positive intracytoplasmic immunoreactivity to the 15A monoclonal antibody within epithelial cells of the small intestine and lungs. Nucleic acids of OvGHV2 and BoGHV6 were amplified from fragments from the myocardium, spleen, and kidneys. Sequencing confirmed the molecular findings. However, all molecular assays done to identify agents associated with the development of pulmonary disease in ruminants were negative, including the OvGHV2 and BoGHV6 assays.

Conclusions: In this case a presumptive diagnosis of OvGHV2 infections were only suspected when typical histological alterations and PVL were observed in multiple tissue.

Positive intracytoplasmic immunoreactivity to the 15A monoclonal antibody suggested that these lesions were associated a MCFV, while the PCR assay confirmed that the MCFV identified was OvGHV2. Additionally, the molecular identification of BoGHV6 DNA in multiple tissues suggest that this sheep was also infected by this *Macavirus*, resulting in concomitant infections by two members of this genus, and suggesting that this *Macavirus* may not be specific for cattle. Nevertheless, the exact role of BoGHV6 in the development of diseases is controversial. Of extreme importance in this case was the identification of intralesional antigens of a MCFV from multiple organs with molecular confirmation of infection by OvGHV2, demonstrating infection by OvGHV2. These findings highlight the importance of the 15A IHC assay since the simple amplification of OvGHV2 DNA is not sufficient to associate this virus with infections in sheep. Additionally, this sheep suffered from simultaneous caseous lymphadenitis, which may have been the trigger for the development of OvGHV2-associated lesions, since sheep are not easily infected by this pathogen.

Keywords: Atrophic enteritis, interstitial pneumonia, Macavirus, malignant catarrhal fever, proliferative vascular lesions.

ID-P49

Detection of bovine gammaherpesvirus 6 in aborted bovine fetal tissues from Brazil

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Introduction: Bovine gammaherpesvirus 6 (BoGHV6), formerly known as bovine lymphotropic virus, is a member of the genus *Macavirus*, subfamily *Gammaherpesvirinae*, family *Herpesviridae*. Since the first identification of BoGHV6 in association with proliferative diseases in the USA, several investigations have identified this agent in association with reproductive diseases. However, the real participation of BoGHV6 in the development of diseases and/or disease syndromes continues to be controversial since studies done in several countries failed to determine any association between BoGHV6 and disease. This study investigated the participation of BoGHV6 in aborted fetal tissues.

Objectives: Determine the possible participation of BoGHV6 in the development of reproductive diseases and/or syndromes in fetal bovine tissues.

Materials and Methods: This study used the extracted nucleic acids from 13 fetuses and the dam of one of these, maintained at -80℃, and derived from a previous report that investigated the participation of *Histophilus somnus* in reproductive syndromes of cattle, during which some fetuses under appropriate conditions were evaluated for histopathologic alterations. These fetuses were obtained from eight dairy herds located within the North-eastern (n=2), Midwestern (n=1), and Southern (n=10) regions of Brazil. Extracted DNA from selected tissues (lung, kidneys, spleen, myocardium, and the brain) from 12 fetuses and cow of one of these fetuses, and the pool of organs from one fetus was used in nested-PCR (nPCR) assays designed to amplify the BoGHV6 polymerase gene. The nPCR products were then subjected to direct sequencing for confirmation.

Results: BoGHV6 DNA was amplified from nine fetuses and from the pool of organs from one fetus; direct sequencing confirmed the results of the nPCR assays. The ages of the infected fetuses varied between 45 to 270 days. In most (70%; 7/10) fetuses infected, BoGHV6 DNA was amplified in at least two different organs, with infections being identified in three organs from one fetus and in a single organ of two fetuses. BoGHV6 DNA was not identified in the cow nor her fetus, while infections were identified in five dairy herds, from two geographical regions (Southern and Midwestern) of Brazil. Additionally, BoGHV6 infections were more frequently identified in the kidneys (*n*=5), spleens (*n*=4), and myocardial tissues (*n*=3) of the aborted fetuses. It must be highlighted that from the previous study, all tissues and fetuses that contained BoGHV6 DNA were simultaneously infected by *H. somni*, while some fetuses were also infected by *Brucella abortus*, *Neospora caninum* (*n*=3), and bovine viral diarrhea virus, BVDV (*n*=2). Furthermore, these additional infections resulted in singular (*n*=3), dual (*n*=7), triple (*n*=1), and quadruple (*n*=2) infections in these fetuses, when all agents were collectively analysed. Additionally, the tissues from eight fetuses that were previously evaluated by histopathology, had histological evidence indicative of disease-related infections by *H. somni*.

Conclusions: This investigation amplified BoGHV6 DNA from most of the bovine fetuses evaluated that originated from several dairy herds located within two distinct geographical regions of Brazil and represents the first study to investigate the possible participation of this pathogen in diseases of cattle from the Southern Hemisphere. Since all fetuses infected by BoGHV6 were concomitantly infected by *H. somni*, while others were infected by *B. abortus*, *N. caninum*, and BVDV, the real contribution of BoGHV6 towards the etiopathogenesis of the pathological alterations observed in some of these fetuses cannot be confirmed nor totally discarded. Consequently, it must be investigated if BoGHV6 is just an innocent bystander or has some participation in the development of fetal disease, thereby contributing towards fetal mortality. Accordingly, experimental studies are being implemented to determine the participation, if any, of BoGHV6 in the development of fetal disease/mortality in cattle.

Keywords: Bovine lymphotropic virus, fetal pathology, reproductive diseases, Macavirus.

ID-P50

Diagnosis of infectious agents and molecular characterization of bovine coronavirus identified in calves with respiratory disease from dairy cattle herds

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Introduction: Respiratory disorders are considered a major cause of morbidity and mortality in suckling dairy calves worldwide. Bovine respiratory disease (BRD) is a multifactorial disease caused by a range of infectious agents that affect the respiratory system. BRD can be caused by a single infection or mixed infections as a result of the interaction of several viral and bacterial pathogens. Among the most common viral pathogens are bovine coronavirus (BCoV), bovine viral diarrhea virus (BVDV), bovine alphaherpesvirus 1 (BoAHV1), bovine respiratory syncytial virus (BRSV), and bovine parainfluenza virus 3 (BPIV-3). Viral pathogens are mainly present in primary respiratory tract infections. The bacterial agents frequently found are *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis* which can act as primary or more common as secondary pathogens. In South America, BRD reports are limited to postmortem examination and identification of specific pathogens only, without the complete description of the etiologic occurring. Additionally, the association of BCoV with enteric disorders is frequently reported in beef and dairy cattle herds from Brazil, causing important productive and economic problems. However, few studies conducted in Brazil report the association of BCoV infection with BRD in calves.

Objective: This study aimed to determine the frequency of viral and bacterial pathogens identified in calves with respiratory disease from high-production dairy cattle herds and perform the molecular characterization of S1 gene in BCoV strains identified.

Materials and Methods: Nasal swabs were collected from 166 heifer calves, namely, 85 symptomatic and 81 asymptomatic calves aged between 5 and 90 days, from 10 dairy cattle herds. Nasal swabs were evaluated using molecular techniques for the identification of viruses (BCoV, BoAHV1, BVDV, BRSV, and BPIV-3) and bacteria (*P. multocida*, *M. haemolytica*, *H. somni*, and *M. bovis*). To confirm the specificity of the amplicons different positive samples were randomly selected for nucleotide (nt) sequencing analyses. In addition, two BCoV field strains were submitted to S1 gene amplification and nt sequencing. Phylogenetic trees based on nt sequences of the S1 gene of BCoV were obtained using the maximum likelihood method with the general time-reversible model using MEGA software and the nt sequence identity matrix were constructed using BioEdit software.

Results: Only BCoV, *P. multocida*, and *M. haemolytica* were identified in nasal swabs of symptomatic and asymptomatic heifer calves from dairy cattle herds evaluated. The frequency of diagnosis of BCoV was higher (56%, 93/166) than the frequency of *P. multocida* (39.8%, 66/166) and *M. haemolytica* (33.1%, 55/166). The three microorganisms were identified in the calves of symptomatic (BCoV: 54.1%; 46/85; *P. multocida*: 41.2%; 35/85; and *M. haemolytica*: 28.2%; 24/85) and asymptomatic (BCoV: 58%; 47/81, *P. multocida*: 38.3%; 31/81, and *M. haemolytica*: 38.3%; 31/81) heifer calves groups. All other pathogens included in the analyses were negative. The nt sequence analyses confirmed the specificity of BCoV, *P. multocida*, and *M. haemolytica* amplicons obtained from the different nasal swab samples. The nt analysis of the partial S1 gene was performed based on the classification into genotypes. The nt sequences from representative BCoV strains of genotypes #1 to #14 were analyzed, including the reference strains Mebus and Quebec. The Brazilian strains formed a new branch, in addition to the observed identity data of nt (>98.8%), it can be suggested that the two Brazilian BCoV strains of respiratory origin analyzed, belong to a new genotype, called # 15.

Conclusion: This study describes high rates of diagnosis of BCoV, *P. multocida*, and *M. haemolytica* in heifer calves from high-production dairy cattle herds with clinical signs of respiratory disease. Additionally, the molecular characterization of the BCoV strains identified in this study shows that they are ancestrally different from the prototype strains and other enteric and respiratory strains previously reported in Brazil, and even from strains reports in other part of the studied region. Continuous studies focusing on multietiological diagnosis and molecular characterization are needed to provide an understanding of infections and the implementation of assertive prophylactic measures aimed at reducing respiratory disorders in dairy calves. Together, these actions can contribute to reducing the frequency and intensity of BRD in dairy cattle herds and increasing the productive indices of the Brazilian milk production chain.

Keywords: Dairy calves, bovine respiratory disease, spike protein, Pasteurella multocida; Mannheimia haemolytica.

IM-P01

Frequency of ocular pathologies in cattle (*Bos taurus*) of the department of Córdoba, Colombia

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Objetives: Veterinary ophthalmology is a relatively new specialty, being little applied in bovine medicine, so it is uncommon for bovine eyes to be examined if they do not have lesions large enough to be obvious, but it should be considered that eye problems in cattle affect the productivity and welfare of animals, since if they are not treated in time they can cause visual impairments. This study aims to report the frequency of presentation of ocular and peri-ocular diseases present in cattle from the department of Córdoba, Colombia.

Materials and methods: A study was conducted where 124 clinical cases of cattle of all races, and of both sexes, were evaluated. Descriptive statistics were performed and a distribution of macroscopically characterized ocular and peri-ocular lesions was performed.

Results: It was found that the most frequently affected ocular structure was the cornea (53.2%), followed by eyelids (17.7%). The most frequently diagnosed ocular alterations in descending order were: superficial keratitis (20.2%), corneal ulcer (16.9%), conjunctivitis (14.5%) and cell carcinoma (9.7%). The most affected sex corresponded to males (61%).

Conclusions: This is the first report of ophthalmic alterations in cattle made in the Department of Córdoba and Colombia and evidences a high presence of ophthalmic alterations, making this work an important contribution on the epidemiological state of the different ocular alterations in bovines.

Keywords: Squamous cell carcinoma, ophthalmology, keratitis.

IM-P02

Comparison between efficacies of intravenous hypertonic sodium chloride or bicarbonate solutions followed by intraruminal water for acute rumen lactic acidosis treatment

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The aim of this study was to compare the efficacy of intravenous (IV) administration of 7% hypertonic sodium chloride (HSC) and 6% hypertonic sodium bicarbonate (HSB) followed by intraruminal water administration for acute rumen lactic acidosis treatment (ARLA) in sheep. Six crossbred adult, healthy, non-pregnant and non-lactating sheep were used. The mean body weight (BW) was 50.62 ± 5.62 kg. The ewes were submitted to ARLA induction with 15 g/kg BW sucrose diluted in water and administered into the rumen via stomach tube after 18 h of solid fasting. Each sheep was submitted to induction twice, with the second induction done at least 30 days after full recovery from the first. Eighteen hours after induction was performed rumen lavage with 20 L of water. The HSC (4 mL/kg BW) or HSB (6.7 mL/kg BW) solution was IV administered over five minutes, followed by intraruminal water, administered via stomach tube in a volume corresponding to 8% of BW. The cross-over design was used, and all sheep received both solutions, one during each induction. Physical examinations and venous blood samples were collected before (0 h), immediately after infusion (5 min) and after the treatment (48 h). The variables pH, HCO_3^- and BE were measured and strong ion difference (SID), anion gap (AG) and percent change in plasma volume (PV) were calculated. Two way repeated measures analysis of variance was used to test the effects of time and treatment, and to evaluate the interactions between them. The Tukey test was used for multiple comparisions, when the F statistic was significant. P-values <0.05 were considered statistically significant.The induction protocol was effective in causing the disease and all sheep had rumen atonia (with rumen pH < 5.0), watery diarrhea and mild-to-moderate dehydration. The ewes had metabolic acidosis at 0 h with reductions of pH (mmol/L) (HSC: 7.201 ± 0.087; HSB: 7.245 ± 0.084), HCO_3^- (mmol/L) (HSC: 14.63 ± 2.37; HSB: 17.18 ± 2.64), BE (mmol/L) (HSC: -13.37 ± 3.76; HSB: -10.15 ± 3.90), SID (mmol/L) (HSC: 37.12 ± 1.27; HSB: 37.22 ± 1.83) and AG (mmol/L) elevation (HSC: 22.47 ± 1.89; HSB: 20.07 ± 2.28). The dehydration was observed with PV (%) decreases (HSC: -3.84 ± 6.83; HSB: -8.23 ± 4.70). The solutions infusion caused exacerbations of acid base imbalances with alterations in pH (HSC: 7.098 ± 0.060; HSB: 7.596 ± 0.036), HCO_3^- (HSC: 11.38 ± 1.53; HSB: 41.92 ± 3.39), BE (HSC: -18.28 ± 2.47; HSB: 20.30 ± 3.70), SID (HSC: 30.54 ± 2.37; HSB: 53.72 ± 3.03), AG (HSC: 19.15 ± 1.93; HSB: 11.78 ± 2.02) and increase in PV (HSC: 26.58 ± 9.76; HSB: 27.43 ± 9.17). The animals presented 48 h after treatment the following values: pH (HSC: 7.259 ± 0.097; HSB: 7.375 ± 0.049), HCO_3^- (HSC: 16.90 ± 2.31; HSB: 22.77 ± 3.15), BE (HSC: -10.17 ± 3.68; HSB: -2.40 ± 3.86), SID (HSC: 32.56 ± 2.42; HSB: 35.92 ± 0.92), AG (HSC: 15.65 ± 2.28; HSB: 13.15 ± 2.56) and PV (HSC: 1.20 ± 4.77; HSB: -2.89 ± 2.74). The results of PV confirmed the water imbalance correction in both protocols treatment. There was metabolic acidosis correction after 48 h of treatment using HSB, because pH, HCO_3^-, BE and AG returned to their originals values. The animals still presented metabolic acidosis at the same period with HSC protocol, as a result values of pH, HCO_3^-, BE and SID were not returned to their baseline values. The HSC causes iatrogenic hyperchloremic acidosis, nevertheless it was effective to correct the water imbalance. The ewes were accompanied throughout the time and the acidemia was corrected after 120 h of the treatment with HSC. Despite the HSB protocol corrected the metabolic acidosis earlier than HSC protocol, the animals showed clinical recovery, that is return of appetite and forestomach motor function, at the same time with both solutions. The both protocols corrects dehydration and mild to moderate metabolic acidosis in sheep with ARLA.

Keywords: Fluid therapy, hyperosmotic solution, 7% hypertonic saline solution, 6% hypertonic bicarbonate solution.

IM-P03

Clinical characterization of hemoparasitism by *Babesia* spp. and *Anaplasma* spp. in Wagyu cattle

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Objective: To describe the clinical signs, hematological, metabolic, electrolytic and acid-base alterations in Wagyu cattle with hemoparasitism due to *Babesia* spp. y *Anaplasma* spp. admitted to the Large Animal at the Veterinary Teaching Hospital, School of Veterinary Medicine, Universidad Nacional de Colombia (CGA-UN).

Materials and methods: All the records of bovine cases admitted to the CGA-UN between august 2017 and April 2018 were evaluated. The inclusion criteria in the study were: 1. That the patients were of Wagyu breed 2. They had clinical signs of hemoparasitism and were confirmed by demonstration of either or both *Babesia* spp. and/or *Anaplasma* spp. in peripheral blood smear. Each patient that met the inclusion criteria had signalment, history, clinical signs, clinical laboratory tests results, rule outs, treatment and outcome collected from its medical records.

Clinical characterization was based on the clinical signs at presentation and during hospitalization. Their frequency of presentation in the patients that met the inclusion criteria was determined. Descriptive statistics that included maximum, minimum range values and standard deviation were determined.

Results: During the study period 22 wagyu cattle were admitted to the CGA-UN and 10 patients met the inclusion criteria; the prevalence of hemoparasitism in this population was 45.4%. All the affected patients were older than 12 months of age. *Babesia spp* was found in 60% of the patients and the other 40% were positive for *Anaplasma* spp. The observed parasitemia irrespectively of the agents ranged between 0,1 y 12%. The history of these patients indicated that 90% (n=9) were imported from USA within the last 6 months before admission.

The observed clinical signs in the affected animals of which males and females were in equal numbers (n=5) included: Diarrhea in all cases, ruminal and intestinal hypomotility (90%), tick presence, depression, pale mucous membranes, dehydration and increased capillary refill time in 70% of the affected animals, followed by tachycardia, pulmonary crackles in 60% of the cases and in a lower frequency low body condition, anorexia, weakness, jaundice, pigmenturia, tachypnea and oral petechiae.

Hematology tests indicated severe anemia in four patients with PCV between 10-11%, while the rest had PCV between 25 and 38%. The animals with the lower PCVs were positive for *Anaplasma* spp. Total plasma proteins were variable, they ranged from hypoproteinemia (n=2) to hyperproteinemia (n=2), only one patient had hyperfibrinogenemia (1.0g/dL). Albumin levels were between normal ranges. Immature red cells were observed in 50% (n=5) of the cases. The white cell counts showed from leukopenia (3.400-4.200 cel/µL) in 20% of the cases to leukocytosis (12.800-25.000 cel/µL) in 50%, neutropenia was seen in 20% of the patients (884-1.112cel/µL), 30% had neutrophilia (9.396-11.750 cel/µL), 20% had lymphopenia (2.312 cel/µL) and 50% lymphocytosis (11.500cel/µL).

The clinical chemistry tests showed increased serum values of urea, creatinine, AST, GGT, glucose, lactate, and bilirrubins were increased to different values. Some of the subjects had acute renal failure. Ionic hypocalcemia and hypokalemia were present in all the animals. Arterial or venous blood gases and acid base status were evaluated indicating that 4 had a metabolic acidosis, hypocarbonetemia and increased base deficit. Three only had a mild respiratory alkalosis.

The patients were treated according to the agent and observed signs. *Anaplasma* spp. infection was treated with oxytetracycline and *Babesia* spp. with diminazene, hydration was done with saline 0.9%, metabolic acidosis was corrected with $NaHCO_3^-$ at 5% according to base deficit, fluid therapy according to dehydration, hematinics, and blood transfusion was done in cattle with PCV lower than 15%, animals with respiratory crackles were treated for pneumonia. The duration of the treatment was according to severity and duration of the clinical signs. Only one patient died possibly due to the severity of the anemia and systemic compromise at admission.

Conclusion: This is the first report of hemoparasitism in Wagyu cattle in Colombia. The anemia was more severe in Wagyu cattle infected with *Anaplasma* spp., the clinical treatment of the cases was highly successful and prevented an important mortality.

Keywords: Wagyu cattle, Anaplasma spp., Babesia spp., hemoparasitism.

IM-P04

Alkalizing effect of oral electrolyte solutions containing sodium acetate or bicarbonate in diarrheal newborn calves

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Objectives: The aim of this study was to compare the effects of two oral electrolyte solutions (OES), containing sodium bicarbonate or sodium acetate, as alkalizing agents, on the hydroelectrolyte and acid-base balance of newborn calves with induced osmotic diarrhea.

Material and methods: Ten healthy Holstein calves, male, with two to three days old, with effective passive immunity transfer, remained in adaptation until reaching 10 days old, ingesting milk in volume referring to 12% of body weight (BW), divided into two daily meals, with water and coast cross hay *ad libitum*. At ten days, they were submitted to osmotic diarrhea induction protocol, using milk (16.5 ml/kg) and sucrose (4 g/kg)

in 20% solution, spironolactone (2 mg/kg) and hydrochlorothiazide (2 mg/kg), every 8 hours, for two days. The calves were divided into two groups with five animals each, wich received the solution cointaining sodium acetate (SA: Glutellac®, Bayer Animal Health, Germany) or sodium bicarbonate (SB: Nutronlyt®, Nutron Alimentos, Brazil). On the day of treatment, each group received six liters of OES, divided into two feedings, at 11 am and 7 pm, using a bottle. Each OES was prepared according to the manufacturer's recommendations. Physical examinations were performed every 8 hours throughout the experimental period. Venous blood samples were collected at 48h (start of induction), 0h (start of treatment) and 72h, to measure pH, HCO_3^-, BE, Na^+, K^+, Cl^-, plasma total protein (PTP) and L lactate. Strong ion difference (SID_3), anion gap (AG), weak non-volatile acids (A_{tot}) and percentage change in plasma volume (PV) were calculated. Two way repeated measures analysis of variance was used to test the effects of time and treatment, and to evaluate the interactions between them. The probability of error of 5% was admitted.

Results: The induction protocol was effective in causing diarrhea with hydroelectrolyte and acid-base imbalances, evidenced by the reduction in skin turgor, decreased suction reflex, presence of enophthalmia, apathy and increased capillary filling time, varying in different degrees according to each calf. Before the diarrhea induction (-48h), the calves presented the measured variables within the normal range for the specie, for both SA and SB groups. At the beginning of treatment (0h), some variables underwent changes, such as pH (SA: 7.12 ± 0.11; SB: 7.21 ± 0.13), HCO_3^- (mmol/L) (SA: 13.98 ± 6.42; SB: 17.3 ± 5.48), BE (mmol/L) (SA: -15.28 ± 8.06; SB: -10.46 ± 7.64), Na^+ (mEq/L) (SA: 121.4 ± 7.31; SB: 124.62 ± 3.31), PTP (mg/dL) (SA: 6.8 ± 0.89; SB: 6.68 ± 0.86), L lactate (mmol/L) (SA: 1.11 ± 1.04; SB: 0.79 ± 0.29), SID_3 (mmol/L) (SA: 29.76 ± 6.91; SB: 33.17 ± 3.80), AG (mmol/L) (SA: 15.79 ± 1.51; SB: 15.88 ± 3.56) and A_{tot} (mmol/L) (SA: 27.88 ± 3.66; SB: 27.38 ± 3.55). PV (%) was -8.98 ± 10.37 and -15.22 ± 4.81 for the SA and SB groups, respectively. This results indicate dehydration, hyponatremia and metabolic acidosis. K^+ and Cl^- have not changed. At the end of the experiment, all values returned to baseline, with no statistical difference between the initial (-48h) and final (72h) values of any variable or OES. The animals presented 72h after treatment the following values: pH: (SA: 7,34±0,13; SB:7,32±0,14), HCO_3^- (mmol/L) (SA: 24,74±8,54; SB: 22,9±8,90), BE (mmol/L) (SA: -0,98±10,75; SB: -3,12±11,33), Na^+ (mEq/L) (SA: 130,34±6,20; SB:129,26±4,29), PTP (mg/dL) (SA: 6,12±0,30; SB: 5,76±0,47), L lactate (mmol/L) (SA: 0,87±0,53; SB: 0,47±0,11), SID_3 (mmol/L) (SA: 37,47±5,38; SB: 35,04±6,08), AG (mmol/L) (SA: 12,73±3,41; SB: 12,14±2,88), A_{tot} (mmol/L) (SA: 25,09±1,24; SB: 23,61±1,95) and PV (%) (SA: 0±0; SB: -2,07±5,12).

Conclusions: Both OES, with different alkalizing agentes (acetate and bicarbonate), used in the treatment of induced osmotic diarrhea in newborn calves, were equally effective in correcting water, electrolyte and acid base imbalances, since all parameters returned to normal at the end of the experiment.

Keywords: Osmotic diarrhea, fluid therapy, hydroelectrolyte imbalance, alkalizing agents.

IM-P05

Hydroelectrolytic and acid-base imbalance in calves with induced osmotic diarrhea fed with milk

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Objective: The aim of the present study was to evaluate the changes in the hydroelectrolytic and acid-base balance in newborn calves with induced osmotic diarrhea fed with milk.

Materials and Methods: Twenty-six Holstein calves, 10 days old, with 43.48 ± 3.90kg body weight (BW), fed with milk in a daily volume corresponding to 12% BW, were submitted to induction of osmotic diarrhea using protocol based on the intake of milk (16.5 mL/kg) and sucrose (4 g/kg, diluted in water, 20% solution) and the administration of diuretics, spironolactone (2 mg/kg) and hydrochlorothiazide (2 mg/kg), every 8 hours for a period of 48 hours. Venous blood samples were collected in three moments: -48h (start of the induction protocol), -24h (middle of induction protocol) and 0h (end of the induction protocol). The blood gas analysis consisted of blood pH, pCO_2, HCO_3^-, BE, Na^+, K^+ and Cl^- measurements. Hematocrit (HTC) was determined by the microhematocrit method and plasma total protein (PTP) concentrations by the refractometry method. Total concentration of weak non-volatile acids (A_{tot}), percentage change in plasma volume (PV), strong ions difference (SID_3) and anion gap (AG) were calculated. Analysis of variance of repeated measures was used to test the effect of time and a 5% probability of error was admitted.

Results: The protocol used was efficient to cause diarrhea in the studied calves, evidenced by the reduction of the skin turgor, decrease in the suction reflex, presence of enophthalmia and apathy and increased capillary filling time, varying in different degrees according to each calf. The values of HTC, PTP, A_{tot} and AG, at the time -48 h, were within the normal range (HTC: 34.15 ± 3.17%, PTP: 5.82 ± 0.51 g/dL, A_{tot}: 23,87 ± 2.09 mmol/L, AG: 11.90 ± 1.83 mmol/L) and increased in diarrheal calves from the time -24h (HTC: 39.11 ± 4.53%, PTP: 6.81 ± 0.67 g/dL, A_{tot}: 27.22 ± 2.67 mmol/L, AG: 15.05 ± 2.71 mmol/L), remaining altered at the end of the induction protocol (HTC: 40.50 ± 4.77%, PTP: 6.64 ± 0.65 g/dL, A_{tot}: 27.91 ± 2.76 mmol/L, AG: 15.91 ± 3.00 mmol/L). The reduction in %PV from the moment -24h (-12.05 ± 2.67) indicates the high loss of circulating volume. These changes together are indicative of the dehydration that the calves experienced. Severe acidemia was observed in the end of the induction protocol (pH = 7.175 ± 0.120). As a compensatory response, hyperventilation and consequent decrease in pCO_2 (39.48 ± 3.64 mmHg) were established. The HCO_3^- and BE values were, respectively, 14.88 ± 4.72 mmol/L and -13.56 ± 6.60 mmol/L at 0h, which characterize a moderate degree of metabolic acidosis. Na^+ values decreased as the protocol was instituted, falling below the reference value from the moment -24h (129.7 ± 4.42 mmol/L). Cl^- underwent a significant reduction at 0h (97.11 ± 4.02 mmol/L). The K^+ concentrations did not change significantly during the induction protocol. There was a reduction in SID_3 (mmol/L) at -24h (35.35 ± 3.40) and at 0h (30.80 ± 3.90). Hyponatremia and relative hyperchloremia led to a

decrease in SID_3, characterizing a condition of strong ions acidosis or hyperchloremic acidosis. Metabolic acidosis in calves with induced osmotic diarrhea and fed with milk was more pronounced than that observed in other studies in calves fed with milk substitute.

Conclusions: The osmotic and milk-fed diarrhea induction protocol was effective, resulting in dehydration, hyponatremia and hyperchloremic acidosis in newborn calves 48 hours after its onset.

Keywords: Neonatal diarrhea, hyperchloremic acidosis, hyponatremia, dehydration.

IM-P06

Effects of postpartum treatment with oral calcium formate on serum calcium, serum metabolites, and the occurrence of diseases in early lactation of dairy cows

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Objectives: This study aims to evaluate the effects of oral administration of calcium (Ca) formate in the postpartum of high-producing dairy cows on calcemia, on other blood biochemical markers and on the occurrence of diseases at the beginning of lactation.

Materials and methods: 120 healthy Holstein cows, distributed according to parity (first, second, third and fourth to sixth lactation), were treated or not with oral Ca formate (two doses: after calving and 24 hours later; equivalent to 50 g of Ca per dose; in a commercial liquid presentation), comprising 8 groups (n = 15). The cows belonged to the same farm, were handled in intensive system (compost barn), and received a low DCAD diet (3.33 mEq/100g DM) in the last three weeks before calving. BCS between 3.0 and 3.5 at calving and urinary pH between 6.2 and 6.8 in the week before calving were the inclusion criteria established for the study. Cows that had twin pregnancies or needed birth assistance were not included in the study. The following variables were measured in the blood serum of samples collected after calving (0h) and 24, 48, 72, and 96h after: total Ca, phosphorus, magnesium, non-esterified fatty acids, beta-hydroxybutyrate (BHB), glucose, total protein, albumin, urea, aspartate aminotransferase and gamma-glutamyltransferase. Blood BHB was also measured at 7, 14, and 21 DIM. For the assessment of disease occurrence, cows were distributed in treated (n = 60) and untreated (n = 60) and in hypocalcemic (n = 71) and normocalcemic (n = 49). Two-way repeated measures ANOVA and chi-square test were used for comparisons. This research proposal was previously approved by the institutional Ethics Committee on the Use of Animals (CEUA-UEL), under protocol number 13822.2017.94.

Results: parity did not influence the studied blood constituents, except for the highest calcemia in primiparous cows. Serum Ca was lower up to 24h and increased after 48h and subclinical hypocalcemia (SCH) ([Ca] <8.5 mg/dL) was more frequent in cows with a higher number of lactations. Treatment with Ca formate had no effect on the variation of serum Ca and the other studied variables in the first days of lactation. Treated and untreated cows did not differ in terms of milk production at 21 DIM (28.8±8.3 vs. 30.9±7.3 L; P=0.140), the presentation of diseases at the beginning of lactation (55.5% vs. 56.7%; P=1.000), the rate of discard (22.7% vs. 16.7%; P=0.263) or death (6.7% vs. 6.7%; P=1.000) up to 60 DIM, and the frequencies of SCH in the first days of lactation (60% vs. 58%; P=1.000), and of subclinical ketosis up to 21 DIM (43.3% vs. 53.3%; P=0.361). Hypocalcemic cows became more ill (64.8% vs. 42.9%; P=0.028) and had a higher frequency of retained fetal menbranes (43% vs. 20.7%; P<0.001).

Conclusions: Oral administration of Ca formate after calving and 24 hours later is not justified as a preventive measure to be adopted indiscriminately in dairy herds. Studies involving larger numbers of observation may clarify whether the selective treatment of cows with a higher risk of hypocalcemia is advantageous.

Keywords: Metabolic disorders, subclinical hypocalcemia, calcium homeostasis, transition period.

IM-P07

Prepartum anionic diet induces hyperchloremic acidosis in high-producing dairy cows without preventing subclinical hypocalcemia

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Objectives: The objectives of the study were to evaluate the effects of the pre-partum anionic diet on the electrolyte balance and calcemia of high yielding dairy cows in the first days of lactation, and to verify the impact on the frequency of subclinical hypocalcemia (SCH).

Materials and methods: 60 healthy Holstein cows, producing 30 kg of milk/day, were distributed in groups (n=15) according to parity: first, second, third, and fourth to sixth lactations. The cows belonged to the same farm, and were handled in intensive system (compost barn) receiving total mixed ration. BCS between 3.0 and 3.5 at calving was the inclusion criteria established for the study. Cows that had twin pregnancies or needed birth assistance were not included in the study. In the last three weeks before calving they received a diet with negative DCAD (-6 mEq/100g DM) and high chloride content. After calving, they received a diet with positive DCAD

(18 mEq/100g DM). Urine pH was measured in the last week before calving. Serum concentrations of sodium (Na^+), chloride (Cl^-), potassium (K^+), and total calcium (Ca), and strong ion difference (SID_3) were determined in samples taken soon after calving (0h), 24, 48, 72 and 96h after. The frequencies of SCH were determined considering the critical value of 8.5 mg/dL. Two-way repeated measures ANOVA and chi-square test were used for comparisons. This research proposal was previously approved by the institutional Ethics Committee on the Use of Animals (CEUA-UEL), under protocol number 13822.2017.94.

Results: the cows eliminated acidic urine before calving (6.65±0.30) without differences between groups (P=0.062). Na^+, K^+, Cl^-, and SID_3 values did not differ between groups. Na^+ and K^+ did not vary between days; Cl^- was elevated at calving (111.21±0.51 mmol/L) and decreased (P<0.001) until 72h (105.96±0.51 mmol/L); and SID_3 was reduced at calving (36.51±0.69 mmol/L) and increased (P=0.005) up to 48h (39.32±0.69 mmol/L). The Ca levels were reduced until 24h (8.26±0.11 mg/dL) and increased (P<0.001) up to 72h (8.62±0.11 mg/dL). Cows of third and fourth to sixth lactations presented lower values up to 24h (P=0.007). SCH was observed in almost half of the cows until 48h (48.3% at 0h, 55% at 24h, and 43.3% at 48h). The maintenance of SCH for two or more consecutive days was observed in 58.3% of cows with no distinction between groups (P=0.257): 40% in primiparous, and 53.3%, 73.3%, and 66.7% in second, third, and fourth to sixth lactations cows, respectively. The maintenance of SCH for three or more consecutive days occurred in 53.3% of third and fourth to sixth lactations cows, and was less frequent (P=0.004) in primiparous (6.7%) and in second lactation cows (13.3%).

Conclusions: Ingestion of a high chloride prepartum anionic diet led to hyperchloremic acidosis and this imbalance was reversed on the second postpartum day. The induced effects on electrolyte and acid-base balances were not able to prevent the occurrence of SCH in the first days of lactation.

Keywords: Transition period, serum electrolytes, ammonium chloride, calcium metabolism, serum calcium.

IM-P08

Effect of blood contamination on total nucleated cell count and protein concentration in cerebrospinal fluid from recumbent cows

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Many etiologies can be the cause of the downer cow syndrome. Among them, spinal cord lesions are usually associated with a poor prognosis. Analysis of cerebrospinal fluid (CSF) is a useful procedure to establish a diagnosis and prognosis. Red blood cells (RBC) are not a normal component of CSF. Total nucleated cells count (TNCC) and protein concentration (TPC) could be falsely elevated in the presence of blood contamination.

The aim of this retrospective study was to describe the variation of TPC, TNCC and RBCC using CSF analysis results from recumbent dairy cows admitted to the Centre Hospitalier Universitaire Vétérinaire.

Among the 235 samples included, RBCC varied between 0-869220 cell/µL (mean=4741.9, median=6.6), TPC varied between 0.04-6.51 g/L (mean=0.43, median=0.27), and TNCC varied between 0-7500 cell/µL (mean=35.5, median=1.1).

One hundred and seven samples had <30 RBC/µL. Among them, TPC and TNCC varied between 0.13-1.06 g/L (mean=0.29, median=0.27) and between 0-31.4 cell/µL (mean=1.8, median=0.6), respectively.

Eighty-four samples were less likely to be associated with a spinal cord lesion (TPC <0.25 g/L, TNCC ≤4.5 cell/µL). Among them, RBCC varied between 0-1290 RBC/µL (mean=61.5, median=4.7).

Twenty samples had absence of nucleated cells (TNCC=0 cell/µL). Among them, RBCC varied between 0-840 RBC/µL (mean=65.8, median=3.9).

A RBCC of <840 RBC/µL seems to less likely interfere with CSF results interpretation. The threshold of 30 RBC/µL (used in humans) appears to low in bovine. To study the TPC and TNCC levels of CSF samples before and after known dilutions of whole blood are added is suitable.

Keywords: Cattle, downer cow, cerebrospinal fluid analysis, blood contamination.

IM-P09

First quantification of propionic acid in serum of dairy cows from different farms

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Objectives: Propionic acid is produced by microbial fermentation of carbohydrates in the rumen of cattle (up to 54 mol/day). The liver metabolizes up to 75 % of absorbed propionic acid from the rumen and up to 25 % is directly metabolized by mammary gland tissue. Propionic acid is converted to glucose by two key enzymes (Propionyl-CoA Synthetase, EC 6.2.1.17; Propionyl-CoA Carboxylase, EC 6.4.1.3). The inhibition of gluconeogenic enzymes by phenolic substances in vitro was found in ovine hepatocytes. Therefore, it is possible that propionic acid accumulates in bovine blood by continuous high production in the rumen. Both very low and high concentrations of propionic acid in peripheral venous blood can be expected. No specification concerning levels of propionic acid in blood except experimental data are available from previous studies. The aim of this study was to analyze the variability of propionic acid in serum of dairy cows from different farms by gaschromatography.

Materials and methods: A total of 360 serum samples

from dairy cows were examined for the presence of propionic acid. Of these samples, 180 were from chronically sick and 180 from healthy animals of various control- and case-farms. Case-farms are dairy farms with a chronic, unspecific disease process.

The method is based on the derivatization of propionic acid by reaction with 2-chloroethyl chloroformate (2). The internal standard n-valeric acid was added to the serum and was deproteinized with highly concentrated hydrochloric acid and subsequently centrifuged. For the following treatment, samples were alkalized with sodium hydrate and vacuum-dried. Afterwards the reaction medium consisting of pyridine, acetonitrile and 2-chloroethanol was added to the dried sample. The 2-chloroethyl ester of propionic acid is formed by addition of 2-chloroethyl chloroformate. In the ultimate step, the derivative was extracted by chloroform and analyzed by gaschromatography.

N-valeric acid was developed as internal standard (determined limit of detection for propionic acid: 45.26 µmol/l; CV in series for n-valeric acid: 5 %; CV in series for propionic acid: 4.4 %). In addition, all samples with propionic acid contents under 45.26 µmol/l were analyzed with a modified method that aimed at lowering the detection limit. In these samples, the concentration of propionic acid ranged from 0.0001 to 0.004 µmol/l. The statistical analysis was performed with SAS Enterprise Guide (Wilcoxon's two-sample Test). To determine the reference limits for propionic acid the internationally acknowledged procedure of the IFCC (3) was used. The reference interval should contain the central 0.95 fraction (or 95 %) of the reference distribution.

Results: There were no significant differences in propionic acid concentrations between diseased and healthy cattle within one case and control farm (P>0.05). There were significant differences in propionic acid concentrations between diseased cattle within different case and control farms as well as between healthy cattle within different case and control farms (P<0.05).

The 2.5th and 97.5th percentile of the whole sample collective incorporates propionic acid concentrations from 0.01 to 1063 µmol/l.

Conclusions: The established method is cost-efficient (no ultra-filtration) and allows easy preparation and rapid processing. Under the given conditions, a first potential normal range for propionic acid in serum of cattle was elaborated: 0.01-1063 µmol/l. The high variability of propionic acid contents in the investigated collective can be explained by the factors that affect propionic acid levels in blood: the composition of the diet, the dry matter intake and the time of sampling after feed intake. Further studies are necessary to investigate the importance of those factors in order to define proper sampling schemes and to further examine the role of propionic acid as potential biomarker for animal health.

Keywords: Propionic acid, serum, dairy cows.

IM-P10

Bioactive compounds in organic and aqueous extract in plant species of Mexico

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The aim of this study was to identify the total phenolic compounds of organic (methanolic and butanolic) and aqueous extracts and evaluate their biological activity. The presence of phenolic compounds was determined by the technique of gelatin-sodium chloride, as well as the recognition of flavonoids with the Shimoda technique. Total phenols were quantified by ultraviolet light spectrophotometry and Folin-Cicalteau reagent, using the tannic acid standard, which results were expressed in mg of tannic acid/g. Thin layer chromatography was used to identify the flavonoid presents in each extract. The antioxidant activity was evaluated in the extracts with higher polyphenol content, using the commercial Trolox kit expressed as µMol / 100g. The results indicated that tejocote methanolic extract and peanut dichloromethane extract had a higher phenolic content of the condensed tannin group (759.12 mg / g; 103.61mg / g) and red corn with the lowest condensed tannin content (68.0 mg Tannic Ac / g) in the butanolic extract. It is concluded that the solvent used in the extraction of polyphenolic compounds is very important as well as the type of sample and its genotype, since methanol was better for the extraction of total phenols in tejocote; water for red corn and blue corn and dichloromethane for peanuts. The highest content of flavonoids was obtained in the tejocote leaves, comparing to blue and red corn and peanut. Chromatography allowed the identification of quercetin (Rf = 0.91) and hesperetin (Rf = 0.96) as the flavonoids present in extracts of tejocote leaves, therefor these extracts might be used for the treatment of bacterial diseases such as Salmonellosis, Colibacillosis, Listeriosis and Mastitis produced by *Staphylococcus aureus* or *Pseudomonas aeruginosa*. The antioxidant capacity of the tejocote leaves extract was 0.46 mmol Trolox / g, higher than vitamin E (0.20). The peanut had a high inhibitory activity of 0.65 mmol Trolox / g higher than vitamin C and vitamin E.

Keywords: Bioactive compounds, plant species, antioxidant activity.

IM-P11

Determination of Potassium Levels in Cows with metabolic diseases and in the Periparturient Period

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Objective: Detection and prevention of hypokalemia which causes economic losses and metabolic diseases in dairy cows have great importance. For this reason, the first objective of this study is to determine the level of blood potassium (K) in dairy cows in the periparturient period, and the second objective is to a determination of the relationship between metabolic diseases and blood potassium level.

Material and Methods: Healthy 40 cows (group I; 20 and group II;20) which are pregnant and multiparous and 20 cows with metabolic diseases (group III) were used 60 cattle totally. First blood sample was taken 2 weeks before calving, and the second blood sample was taken within two weeks after calving. Blood samples of 20 cows with metabolic diseases were taken from V.Jugularis once, as 10 ml at admission. All blood samples were centrifuged at 5000 rpm for 10 minutes. Serum samples were analyzed for Na, K, Cl, Creatinine, SGOT, LDH, Mg, P and CPK parameters.

Results: Clinical symptoms related to hypokalemia and metabolic diseases in healthy groups (I and II) were not observed in during the study. There was also no decrease in milk production and appetite. Serum potassium levels of healthy groups were mild-moderate decreased in after birth compared to before birth values. The mean potassium level of group I and II healthy cows after calving were 2,35 ± 0,17 mmol/l and 2,71 ± 0,12 mmol/l, respectively.

Hypokalemia had observed in cows with displacement of abomasum and ketosis (group III). The cows had also anorexia, weakness and decreased milk production. Mean serum potassium levels had 2,53 ± 0,06 mmol/l in cows with metabolic diseases. The potassium levels in cows with metabolic diseases (group III) had lowest compared to healthy groups.

In conclusion: development of negative K balance was detected in lactating cattle after calving. Additionally, cows with displaced abomasum and ketosis were also hypokalemic.

Keywords: Cow, hypokalemia, potassium, ketosis, displacement of abomasum.

IM-P12

An outbreak of *Vicia villosa* intoxication in a French Holstein dairy herd

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Objectives: The main objective of this report is to raise awareness among veterinarians about hairy vetch (*Vicia villosa*) poisoning in cattle through the description of an outbreak in a French dairy herd.

Material and methods: In mid-November 2019 a Holstein dairy farm located in the north-eastern part of France in the *Ardennes* reported several cases of severe hyerthermia and pruriginous dermatitis associated with high mortality rate. The cows had been kept indoors in their usual tie-stall barn since late-October 2019. A wide range of treatments, including antibiotics, anti-histamines, avermectins, steroidal- and non-steroidal anti-inflammatory drugs were administered with no avail. In late-November 2019, a 3 year-old Holstein cow – which had been sick for 12 days – was addressed to *Maisons-Alfort* Large Animal Hospital for examination and necropsy.

Physical examination revealed a wasted animal, with a mild hyperthermia (39.5°C) and superficial lymph nodes enlargement. Close examination of the skin showed multifocal alopecic scaly plaques becoming sometimes confluent, often associated with thickening of the skin on the head, neck, trunk, and escutcheon. Numerous papules and crusts were noted on the face, the limbs, the interdigital spaces and the udder. Pruritus was noted during the clinical examination. Lesions were restricted to the skin and independent of the pigmentation.

The association of pruriginous dermatitis with hyperthermia and lymph nodes enlargement in a herd of dairy cattle located indoor led to the following differential: malignant catarrhal fever (MCF), stachybotryotoxicosis, and sterile eosinophilic folliculitis and furunculosis. The implication of sarcoptic mange, dermatophytosis, dermatophilosis or staphylococcal folliculitis and furunculosis could not be excluded but could not explain the severity of this condition. In order to explore the eventuality of these hypotheses, skin scrapings, trichoscopic and skin cytologic examinations were performed and were unremarkable. MCF hypothesis was excluded based on a negative polymerase chain reaction (PCR) assay on whole blood. Skin biopsies at different locations were performed and processed for routine histopathological examination.

Results: At necropsy, besides the aforementioned skin lesions, decisive gross pathological findings concerned the liver, the heart and the lymph nodes. The liver was bulging, purplish-blue, mottled with clearly demarcated beige spots or large patches; on cut surfaces these beige patches deeply infiltrated the parenchyma . In the heart, the epicardial surface of the left atrium and of three quarters of the left ventricle was uniformly light brown. On its cut surface, the left ventricular wall was diffusively beige on a depth of 1 cm. The rest of the heart was studded with firm, grayish multifocal nodules visible on epicardial and endocardial surfaces. They were slightly protruding in the heart lumen.The lymph nodes were all markedly enlarged; on their cut surfaces there were raised multifocal nodules of 5 to 10 mm in diameter. Organ samples were processed for routine histopathologic examination.

Histopathological findings were similar for the skin, the heart and the liver and consisted in multifocal granulomatous eosinophilic inflammation with numerous multinucleated giant cells that varied in intensity from organ to organ. Comments by the reviewing pathologists were that these changes were highly suggestive of hairy vetch (*Vicia villosa*) poisoning.

Conclusion: Investigation in the farm pointed out that all the lactating cows had been grazing on an artificial pasture for two months prior to their return indoors. Unlike previous years, the farmer had mistakenly used hairy vetch seeds instead of common vetch (*Vicia sativa*). In the end, morbidity was evaluated at 17% (9/54) and lethality rate at 78% (7/9). These figures are consistent with previous reports of vetch-associated outbreaks and stress the lethality of this disease. To our knowledge, this is the first report of hairy vetch intoxication in cattle in Europe.

Vetch-associated diseases have been linked to the consumption of plants of only some species of *Vicia*: *villosa*, *dasycarpa*, and *benghalensis*, but not *sativa* which had been used on this farm for eight years before the seed supplier mistakenly changed the usual order. The farmer should have been alerted of this toxic risk but since hairy-vetch poisoning seems occasional and limited to some breeds of cattle and has never been described in Europe, it is not surprising that this information was omitted by the supplier.

Keywords: 'hairy vetch' 'systemic granulomatous disease' 'intoxication'

IM-P13

Is antimicrobial treatment for calf diarrhea a risk factor for pneumonia?

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Calf pneumonia is a major cause of morbidity and mortality in preweaning dairy calves. Risk factors include sudden changes in temperature and humidity, transportation and stress. However, the role of antimicrobial for preceding illnesses such as diarrhea as a risk factor for pneumonia remains unknown. The objective of this study was to evaluate the association between antimicrobial treatment for diarrhea with the risk of being treated for pneumonia during the first 30 days of calf life.

This study used a retrospective methodology. Calf health records from 10 dairy farms were reviewed and the following information was recorded: antimicrobial treatment for diarrhea, and antimicrobial treatment for pneumonia.

Treatment records of 4300 calves were available. The incidence of diarrhea was 76% (3271/4300). The incidence of pneumonia was 46% (1997/4300). During the study period, 59% (1923/3271) of the diarrheic calves were treated with antimicrobials. Univariable logistic regression model revealed that calves treated for diarrhea had a higher odds (Odds Ratio (OR): 1.99; P < 0.001; 95% Confidence Interval (CI): 1.73 to 2.31) of being treated for pneumonia. Additionally, of the calves that were treated for diarrhea, if they received an antimicrobial treatment as part of their diarrhea therapy, they had a 2.22 higher odds (P < 0.001; 95% CI: 1.92 to 2.57) of being treated for pneumonia.

Calves that receive antimicrobial treatment for diarrhea had higher odds of being subsequently treated for pneumonia than diarrheic calves without antimicrobial treatment. Antimicrobial therapy for diarrhea may have a detrimental effect on the respiratory microbiota potentially predisposing to infection, however this hypothesis warrants further investigation.

Keywords: pneumonia, diarrhea, antibiotics, antimicrobial stewarship.

IM-P14

Bacteria involved in sepsis in calves and their antimicrobial resistance

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Objective: Sepsis is a life-threatening condition in calves, often necessitating the use of broad-spectrum and bactericidal antimicrobials. As in human medicine, critically important antimicrobials such as cephalosporins and fluoroquinolones are often preferred for this indication. However, legislation in food-producing species warrants their prudent use. In cattle, insufficient information is available concerning the bacteria involved and their resistance profiles. Therefore, the objective of this study was to determine the occurrence of sepsis in critically ill calves, the bacteria involved and their antimicrobial susceptibility.

Material & methods: A retrospective cohort study was conducted on 230 calves in a critically ill state. Routine hemocultures on enriched aerobic medium (BACTEC™) were done in all 230 calves. The hemoculture positive critically ill animals were considered in sepsis and the isolates were analyzed, focusing on identification of etiologic agents, antimicrobial susceptibility testing, presence of multidrug resistance (MDR, ≥ 3 different classes), and determination of the appropriateness of the used antimicrobial treatment. Inappropriate antimicrobial use was defined as an antimicrobial therapy to which the pathogen was resistant, taking the intrinsic resistance against pathogens into account. (In)appropriateness was evaluated per case and for each antimicrobial.

Results: Sepsis (positive hemoculture) was evidenced in 34% (79/230) of the critically ill animals. In 67 of those animals one or more bacteria were isolated and identified to species level. In total 62.5% (45/72) Gram-negative and 37.5% (27/72) Gram-positive bacteria were found. The most frequently isolated species was *Escherichia coli* in 30.6% of the cases (22/72), followed by *Staphylococcus* spp. (15.2% (11/72)) and *Salmonella* spp. (9.7% (7/72)). Of the isolated bacteria, 61.7% of the bacteria displayed MDR, and was especially high in *E. coli* (90.9%). Treatment was appropriate in 78.7% (37/47) of the suitable cases. Inappropriate treatment of the animals with sepsis did not significantly increase their odds at mortality *(P* =0.76). When appropriate treatment was initialized, 64.9% (24/37) of the calves died, versus 70.0% (7/10) in case of inappropriate treatment. Inappropriate treatment against isolated bacteria was frequent in potentiated sulfonamides (50.8%) and aminopenicillins (65.0%), whereas it was more limited for ceftiofur (28.5%) and enrofloxacin (19.6%).

Conclusion: Hemoculture was positive in 34% of the critical ill calves, suggesting sepsis. Enterobacteriaceae were the most frequently isolated causative agents, but also Gram-positive bacteria were found. Antimicrobial resistance against aminopenicillins and potentiated sulfonamides was more prevalent in contrast to the critically important quinolones and cephalosporins. High MDR in various bacteria is worrisome and close monitoring of appropriateness of antimicrobials in the future is warranted.

Keywords: Cattle, Hemoculture, Multidrug resistance, Appropriate antimicrobial treatment.

IM-P15

Abomasitis associated with halofuginone intoxication in pre-weaned calves

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Objectives: Halofuginone is widely used to prevent calf diarrhea due to infections with Cryptosporidium parvum. Reported clinical signs of intoxication are diarrhea, blood in feces, anorexia, dehydration, apathia and exhaustion. Here, we report a case of long-time overdosing of halofuginone in preweaned calves, mainly presenting as abomasitis.

Material and methods: Seven Belgian blue beef calves (4 days – 28 days of age) from the same farm were presented to a referral clinic with complaints of anorexia and weight loss. Recently, three calves from the same farm had died after showing comparable clinical signs. Cases were examined clinically and by ultrasound. In lethal cases, gross pathology and histology were performed.

Results: All animals showed signs of dehydration, but only two animals had diarrhoea. Based on the ultrasound, four animals were diagnosed with pneumonia. Six animals had an enlarged abomasum with an oedematous wall. After administration of intravenous fluid therapy, antibiotics and proton-inhibitors four animals reached clinical cure after 7 – 19 days. On the necropsy of the other three animals an abomasitis was detected with different severities going from oedematous to haemorrhagic and ulcerative. Further histological examination of the abomasum identified lesions ranging from oedema to necrosis in the mucosa. No lesions were observed in the mouth, pharynx and oesophagus in any of the animals. Further questioning of the farmer revealed that his original dose was to high (194 µg/kg, 100 µg/kg according to leaflet recommendations) and that in response of diarrhea in the calves he further increased the dose to 389 µg/kg. After correction of halofuginone dosing, no new cases occurred.

Conclusions: Halofuginone is known to be a drug with a narrow therapeutic-margin, but abomasitis has not previously been mentioned in case of intoxication. Halofuginone intoxication should be included in the differential diagnosis of milk refusal (anorexia) in 1-3 week old calves.

Keywords: Intoxication, calves, halofuginone, abomasitis.

IM-P16

Comparison of the content of selected heavy metals in liver tissue of wild boar, red fox and red deer living in north-eastern Poland

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Objectives: The aim of this study was to determine the content of selenium (Se), zinc (Zn), copper (Cu) and cadmium (Cd) in the liver of predominantly plant-eating omnivore wild boar (Sus scrofa), predominantly meat-eating omnivore red fox (Vulpes vulpes) and herbivore red deer (Cervus elaphus), from North-Eastern Poland, in order to verify distribution of this elements in the trophic pyramid. Furthermore, the study was used to assess the risk of eating venison.

Material and methods: The research was carried out on three species of wild mammals- wild boar (Sus scrofa), red fox (Vulpes vulpes) and red deer (Cervus elaphus) from the region of Warmia and Mazury. The research was conducted during the hunting season from October to December 2015. Tissue samples were obtained from 10 red deer, 10 wild boars, and 10 red foxes. The animals were shot by hunters under the hunting law and did not show any external signs of disease. Liver samples (200g) were collected from the animals immediately after the shot and the concentration of Cu, Zn, Se, Cd was measured in the homogenates by flame atomic absorption spectrometry using AAS Solaar M6. The obtained results were subjected to statistical tests using the Mann-Whitney-U test with Python 3.7 software.

Results: The average concentration of Se was 3.9 (0.32±0.09 mg/kg, $P<0.001$), and 1.8-fold higher in wild boar (0.14±0.05 mg/kg, $P<0.001$), and red fox in comparison to red deer (0.08±0.01 mg/kg) and 2.1-fold higher in wild boar comparing to red fox ($P<0.001$). There was no significant difference in the average concentration of Zn between red fox (28.86±10.35 mg/kg), wild boar (30.97±5.57 mg/kg), and red deer (28.60±1.85 mg/kg). The average concentration of Cu was 9.3 and 5.4-fold higher in red deer (27.64±9.51 mg/kg) in comparison to wild boar (2.96±0.61 mg/kg, $P<0.001$) and red fox (5.06±3.30 mg/kg, $P<0.001$). The average concentration of Cd was 1.9-fold higher in wild boar (0.15±0.05 mg/kg) in comparison to red fox (0.08±0.06 mg/kg, $P<0.029$). The average Cd level in red deer was 0.15±0.01 mg/kg.

Conclusions: In conclusion we can say that the liver concentrations of selenium (Se), zinc (Zn), copper (Cu), cadmium (Cd) in selected species of wild animals from the hunting areas of Warmia and Mazury do not exceed from standard values. These results do not diverge from the literature data. A low concentration of selenium may be related to the low level of this element in the studied area. The cadmium and zinc level correspond with the low level of this heavy metal in the other parts of Poland.

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Keywords: Wild animals, liver, heavy metals.

IM-P19

Comparison of the efficacy or enrofloxacine hcl-2h2o and oxytetracycline in cases of metritis in pospartum cows

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Objectives: To evaluate and compare the efficacy of the treatment of clinical metritis, through the use of enrofloxacin hydrochloride dihydrate applied intrauterine route plus prostaglandin F2 α using parenteral route in the Treated Group (A); Oxytetracycline applied intrauterine route plus prostaglandin F2α in a parenteral route in the Control Group (B).

Material and methods: The study was carried out in a commercial milk production facility, located in the State of Hidalgo, México.

The target population was 100 animals with clinical metritis. Two groups were formed, the Treated Group (A) received 3 grams of enrofloxacin HCl-2H2O, diluted in 50ml of physiological saline solution, via IU and a support therapy with 2ml of prostaglandin F_2α using a parenteral route (only in the first treatment).

The Control Group (B) received 2.5 grams of injectable oxytetracycline via IU, and 2ml of prostaglandin F_2α parenterally as support therapy (only in the first treatment).

Results: Regarding the number of treatments required to achieve the healing of the animals, an average of 2.1 treatments with enrofloxacin were required, with a total of 29 cured animals at the first treatment, 13 at the second and 7 at the third for the Treated Group (A).

For the Control Group (B) with oxytetracycline, an average of 2.26 treatments were required, 13 animals responding to the first treatment, 13 to the second and 11 to the third; but there were no significant differences between groups (P> 0.1).

In relation to the reproductive parameters, it can be observed that as regards to the period of delivery at first heat in the Treated Group (A) it was 44 days on average, while in Control Group (B) it was 71.36 days on average. Therefore, from the statistical point of view, it was significant between the groups (P <0.05).

As for the period between delivery to first insemination, in the Treated Group (A) it was up to 82.33 a day on average, leaving 15 animals pregnant at the first service; while in the Control Group (B), it was 105.9 per day on average, with 10 animals being pregnant at first service. From the statistical point of view, a significant difference is shown (P <0.05).

Conclusions: The efficacy of enrofloxacin in metritis problems in Holstein Friesian cows at the first treatment was 58%, while for oxytetracycline it was 26%.

Keywords: Metritis, enrofloxacine, oxytetracycline, dairy cows.

IV-P01

Immune response and status of the upper respiratory tract of dairy calves challenged with BVDV2 and BHV1 after intranasal vaccination and trace minerals injection

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Bovine viral diarrhea virus (BVDV), and *Bovine herpes virus 1* (BHV1) are major pathogens involved in bovine respiratory disease (BRD). These viruses cause immunosuppression and damage of the upper respiratory tract (URT), increasing host susceptibility to secondary bacterial infection. Vaccination is an important tool to prevent BRD. The use of injectable trace minerals (ITM) has demonstrated beneficial effects on the immune response elicited by vaccination against respiratory pathogens. Endoscopy is a diagnostic tool that allows evaluation of the URT of affected animals before lung lesions appear. The objective of this study was to determine if administration of ITM improves the immune response and protection elicited by intranasal (IN) modified-live virus (MLV) vaccine in dairy calves challenged with BVDV2 and BHV1. In addition, we compared the status of the URT mucosa using endoscopy after challenge with BVDV2 and BHV1 in dairy calves vaccinated and treated or not with ITM. Twenty-four dairy calves (1 mo) were prime vaccinated with a MLV IN vaccine containing BHV1, BRSV, PI3V (Inforce 3®), and randomly assigned to subcutaneous (SC) administration of ITM (ITM, n=12; Multimin®90 containing Se, Cu, Zn & Mn) or saline (Sal, n=12). Ten weeks later, both groups received a booster of the same IN vaccine, and a second dose of ITM, or saline, according to the treatments. Calves in both groups were administered a MLV SC vaccine containing BVDV1 & 2 (Bovi-shield gold BVDV®). Additionally, 12 calves did not receive vaccine or treatment, serving as a control group (Unvac, n=12). Seven weeks after booster vaccination, all calves were intranasally inoculated with BVDV2 (5 x10^5 $CCID_{50}$); and seven days later with BHV1 (8 x10^6 $CCID_{50}$). Health status was evaluated every day using the University of Wisconsin's system. Blood samples were collected on days -7, 0 (BVDV inoculation), 3, 6, 7 (BHV1 inoculation), 10, 12 and 14, relative to BVDV challenge. Serum neutralizing antibodies (SNA) to BVDV2 and BHV1 were measured on days 0, 7 and 14. Total leukocyte count and T cell populations ($CD4^+$, $CD8^+$, $WC1^+$) were measured using a hematology analyzer (HT5, Heska®) and flow cytometry, respectively. Endoscopic evaluation of the URT (nasal cavity, pharynx, larynx, trachea, and bronchi) was performed before inoculation and five days after BHV1 challenge, in a random subset of calves (ITM=5; Sal=5 & Unvacc=3) using Tele Pack Vet X-led endoscopy (Karl Storz®). The URT was visually assessed for vascularization, integrity of the mucosa, and secretions by three evaluators blinded to treatment assignment. An endoscopic score (ES) from 0 to 3 (0: normal; 1: mild; 2: moderate; 3: severe) was assigned for each characteristic, then an overall ES was calculated for each calf and the means were compared among groups. Health scores were higher ($P < 0.05$) in Unvac calves on d6, d10 and d12 compared to the vaccinated groups. Significant differences were not found between vaccinated groups. Vaccinated calves had higher SNA titers to BVDV2 on d7 and d14 compared to Unvac calves ($P<0.001$). Sal calves had higher SNA response to BHV1 on d14 compared to ITM ($P=0.06$) and Unvac ($P<0.001$). Leukopenia occurred between d3 and d10 in the Unvac calves, but not in the vaccinated groups. A significant reduction in $CD4^+$ and $CD8^+$ T cells was observed in the Unvac group between d3 and d10 ($P<0.05$). A greater number of $CD4^+$ T cells was observed in ITM (d3 and d6) and Sal calves (d6) compared to Unvac calves ($P<0.05$), with no significant differences between vaccinated groups. ITM calves had higher ($P<0.05$) number of $CD8^+$ T cells than Unvac (d3, d6 and d7) and Sal groups (d6). $WC1^+$ T cells dropped in all groups between d3 and d6 post BVDV challenge. Unvac calves had the highest URT ES (27.5±4.5) after BVDV2 and BHV1 challenge, showing marked mucosal congestion, severe lymphoid hyperplasia and ulcers at pharynx and larynx with abundant purulent secretions along the URT. Calves treated with ITM showed significantly lesser ES (18.2±1.3) compared with Sal (24.9±1.0) and Unvac groups ($P<0.01$). In conclusion, vaccination was effective preventing BVDV2+BHV1 clinical disease. Administration of ITM concurrent with IN vaccination mitigated the reduction in circulating $CD4^+$ and $CD8^+$ T cells, and was associated with a lower level of inflammation and mucosal damage after inoculation with BVDV2 and BHV1.

Keywords: Trace minerals, dairy calves, vaccination, BVDV, BHV1.

IV-P02

Stability of an inactivated Rotavirus-Coronavirus-E.coli combination vaccine until at least 28 days after first opening and storage at +2 - +8°C

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Introduction and objectives: Inactivated vaccines for cattle are typically filled in multi-dose ready to use vials and according to the label they should be used within a few hours after broaching. This is the case for a registered vaccine containing inactivated antigens of Bovine Rotavirus (BRV), Bovine Coronavirus (BCV) and *E. coli* K99 (K99) (Rotavec® Corona) which should be used within 8 hours after first opening. However, such use is not always possible in practice, especially

for small herds, which is inconvenient for the user and lead to medical waste.

The objective of the study was to assess the stability of the vaccine when stored at 2-8°C for at least 28 days after first opening.

Material and methods: From 3 different batches, at T0 the required volume of vaccine was withdrawn using a syringe and used for the different T0 tests. Additional volume was removed from the broached vials in order to keep 50% of the original volume in each vial. The broached vials were kept in horizontal position, refrigerated (+2 °C – +8 °C), protected from light for at least 35 or 28 days until tested again. All procedures above were performed using a sterile needle.

The first two batches were tested at T0 and after subsequent storage for 28 days (T28) for the physical-chemical parameters relevant for stability. In addition, the preservative efficacy was determined on a T28 sample from each of the two batches. The third batch was tested for potency at first opening (T0) and after subsequent storage for 35 days (T35).

The testing of the different stability parameters was performed according to standard procedures for batch testing and in line with the requirements of the European Pharmacopoeia.

Results: For all three antigens, the potency test results were above the respective specifications both at T0 and D35.

The actual values were very close to each other without obvious decline in potency.

Likewise, the results for the physical-chemical parameters for both batches passed the requirements at the T0 and the T28 time point. Finally, the preservative efficacy was confirmed at 28 days after broaching.

Conclusions: Under the defined conditions of use, acceptable potency and microbial safety was demonstrated over the in-use shelf life of 28 days. These data indicate that the inactivated Rotavirus - Coronavirus - *E. coli* K99 (K99) combination vaccine tested here (Rotavec Corona) is stable for at least 28 days after first opening if stored at +2 – +8 °C and protected from light.

Keywords: Scour vaccine, extended shelf life, Rotavec Corona.

IV-P03

Nine-week duration of immunity against a virulent *Mannheimia haemolytica* challenge in 60-day-old calves vaccinated with a multivalent vaccine containing a *M. haemolytica* toxoid

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Bovine respiratory disease complex (BRDC) is one of the most important health problems in the cattle industry. One of the major bacterial causative agents of BRDC is *Mannheimia haemolytica* which causes disease in cattle of all ages and types of production systems. The objective of this study was to establish a 9-week duration of immunity against virulent *M. haemolytica* challenge in 60-day-old calves administered a modified live bovine rhinotracheitis-virus diarrhea-parainfluenza3-respiratory syncytial virus-Mannheimia haemolytica toxoid combination vaccine [Bovi-Shield GOLD One Shot™]. Fifty-seven, 60-day-old Holstein cross calves were vaccinated subcutaneously with a 2 mL dose of either a placebo containing only the modified-live viral fractions (T01, n = 29) or the full combination vaccine containing the *M. haemolytica* toxoid fraction (T02, n = 28). At 9-weeks post-vaccination, all animals were challenged trans-tracheally with a virulent strain of *M. haemolytica*. Animals were observed for six days post-challenge; mortality during this period was evaluated to determine efficacy. In response to *M. haemolytica* challenge, mortality was significantly ($p < 0.05$) reduced in the vaccinated (T02) animals compared to the placebo (T01) calves. The placebo (T01) group had mortality of 92.9%, while the vaccinate (T02) group had 58.6% mortality. In conclusion, the study successfully demonstrated a 9-week duration of immunity for the *M. haemolytica* toxoid fraction of a modified live bovine rhinotracheitis-virus diarrhea-parainfluenza3-respiratory syncytial virus-Mannheimia haemolytica toxoid combination vaccine in 60-day-old calves.

Keywords: BRD, M. haemolytica, Vaccine, DOI.

IV-P04

Efficacy of the intranasal respiratory vaccine against IBRV, PI3V, and BRSV in early weaned calves

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Objectives: Bovine respiratory disease (BRD) is a one of the most money costing disease in bovine production. Farmers usually use commercial vaccines which are modified live or killed vaccines around weaning period to prevent BRD outbreak. For many years, veterinarians and scientists have experienced that young animals with circulating maternal derived antibodies cannot initiate active immune response in Korea, where BRD vaccinations are performed at around 2 or 3 month of age. To overcome immune interference due to maternal antibody, vaccines which can be administered intra-nasally were developed. This study was conducted to evaluate the efficacy of intranasal respiratory vaccine in early weaned calves.

Materials and methods: From three beef farms, 18 calves ranging from 2 to 8 weeks of age and 42 calves from 3 to 6 months of age, were randomly selected. Ten (10) calves were randomly selected as vaccine group and 10 as control group for each farm. Two ㎖ of the modified live vaccine for bovine respiratory syncytial virus (BRSV), infectious bovine rhinotracheitis virus (IBRV), and parainfluenza-3 virus (PI3V) (INFORCE-3, Zoetis, USA) was inoculated into each nostril as recommended by the manufacturer using a 1.0 ㎖ syringe in

a total of 30 calves. Two ㎖ of saline was inoculated into 30 control animals. Incidence of respiratory disease, severity of symptom, mortality rate, treatment days and treatment cost of vaccine group and control group were observed. Observations of respiratory clinical symptoms were based on the Calf Health Scoring Criteria developed by Wisconsin University. Serum neutralizing (SN) antibody titers and ELISA titers of sIgA of nasal secretin against BRSV, IBRV, and PI3V were determined based on Korean National Veterinary Research & Quarantine Service Notice 2009-5.

Results: The incidence of respiratory disease between vaccine and control group was significantly different at two weeks, four weeks, and during 5 months after vaccination (p<0.05). Severity of the respiratory disease, the number of treatment of respiratory disease, and the number of deaths due to respiratory disease were also lower in the vaccine group than the control group. There was a significant difference in SN antibody titers against BRSV, IBRV, and PI3V at 2 weeks, 4 weeks, and 8 weeks after vaccination between vaccine group and the control group (α = 0.05). Calves having high level of maternal SN antibody at the time of vaccination did not increase SN antibodies against BRSV, IBRV and PI3V, However, a sufficient level of immune stimulation was induced in the calves carrying low level of maternal antibody at vaccination. Nasal vaccination increased the sIgA antibody titers in nasal secretion, a localized specific antibody against BRSV, IBRV, and PI3V, within two weeks after vaccination, whether or not the calves had maternal antibody.

Conclusions: Nasal vaccination with respiratory vaccine containing BRSV, IBRV, and PI3V in calves from 2 weeks to 6 months of age increased local nasal antibodies and SN antibody against BRSV, IBRV, and PI3V. And nasal vaccination reduced the incidence of respiratory disease, alleviated clinical symptom of respiratory disease, and contributed to lower mortality.

Keywords: Nasal vaccine, efficacy, early weaned, calves.

IV-P05

Case study report: Less individual antibiotic treatments after intranasal vaccination against respiratory viruses in rose veal calves

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Objectives: The objective of this case study was to evaluate whether vaccination against Bovine Respiratory Syncytial Virus (BRSV) and Parainfluenza 3 (PI3) on a rose veal farm resulted in less individual antibiotic treatments for respiratory disease. A second objective was to evaluate the effect of vaccination on mortality rates, slaughter weight and uniformity within the herd.

Material and methods: The farm has two identical units for both starters and finishers. Calves between 2 to 3 weeks of age arrive on the farm within a one-week period and are randomly housed in the two starter units. After acclimatization, during the first week of arrival, both groups were vaccinated intranasally with a live BRSV and PI3 vaccine (Bovalto Respi Intranasal®, Boehringer Ingelheim Animal Health Netherlands B.V.). The oral antibiotic regime was the same for both groups and comparable to previous rounds of calves. After 12 weeks, group 2 was re-vaccinated intranasally with the same vaccine; three weeks later both groups moved into the finishers units. BVD and IBR serology were monitored, 5 animals from each group were sampled at 4 different points in time; 2 weeks, 6 weeks, 11 weeks and 6.5 months after arrival. Both groups together were compared with the previous round and the two groups were also compared with each other. Data from farmer, veterinarian and integrated producer were collected. Microsoft Excel 2016 was used to build a database and www.socscistatistics.com was used for statistical analysis.

Results: In total 410 calves were housed in the starter units with 216 calves in group 1 and 194 calves in group 2. Average weight at arrival did not differ between groups. The previous round received 0.32 (131/410) individual antibiotic treatments per animal and the vaccinated round (group 1 and 2) 0.22 (91/ 410) treatments per animal (p< 0,05). Overall, group 1 received more treatments than group 2; 60 and 31 respectively (p<0,05). In the starter unit, group 1 seroconverted for BVD, while group 2 did not. 50 of the 60 antibiotic treatments in group 1 were in the starter unit, 10 treatments were in the finisher unit. All (31) antibiotic treatments in group 2 were in the starter unit, group 2 did not have any treatments in the finisher unit. Mortality rates did not differ between rounds and groups. Average slaughter weight did not differ between groups. The average weight at arrival of the previous round was not recorded and therefore slaughter weight between rounds could not be compared. Looking at uniformity; in group 1 more calves (33/216 = 15%) had a slaughter weight < 160kg compared to group 2 (17/ 194 = 9%), this difference was significant (p< 0,05).

Conclusions: The BRSV/PI3 (Bovalto Respi Intranasal®, Boehringer Ingelheim Animal Health Netherlands B.V.) vaccinated round needed less individual treatments overall compared to the previous round, despite seroconversion to BVD virus in group 1. Group 2 had no individual treatments in the finisher unit and slaughter weight was more uniform. In this study, BRSV/PI3 vaccination reduced the individual antibiotic treatments required for respiratory disease, presumably leading to less labour requirements and increased peace of mind for the farmer. An additional BRSV/PI3 vaccination further resulted in no treatments in the finishers and a more uniform slaughter weight.

Keywords: BRSV, PI3, vaccination, antibiotic, treatments.

IV-P06

Comparison of milk production of dairy cows vaccinated with a live double deleted BVDV vaccine and non-vaccinated dairy cows cohabitating in endemic BVDV herds

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Objective: Daily milk production and reproductive performance of cows vaccinated with a live double-deleted Bovine viral diarrhoea virus (BVDV) vaccine were compared to those of non-vaccinated animals, cohabitating in endemic BVD herds.

Materials & methods: All animals in the treatment group were vaccinated at a single point in time (day 0) irrespective of lactation or gestation status, while control animals did not receive any treatment. The endemic presence of BVDV in the herds was demonstrated by the detection of BVDV in the bulk tank milk using PCR, and seroconversion was evaluated at the beginning of the study. For individual animals, the day of calving was taken to be the start of the lactation for the calculation of days in milk (DIM). The standard lactation period of 305 days was divided into three separate periods: early lactation (EL, from DIM 8 to DIM 102), mid lactation (ML, from DIM 103 to DIM 204 and late lactation (LL, from DIM 205 to DIM 305). For each farm and each lactation period, a mixed model statistical analysis was performed with daily milk production as response, and group, day as well as the interaction between those two factors as fixed factors. Chi-square test was used to compare abortion rate and the prolonged inter-oestrous interval rate between treated and control groups.

Results: A total of 1463 cows were enrolled in the study from four different farms (farm 1, 2, 3 and 4) from three different countries (UK, Italy, France). On study day 0, totals of 471, 316, 499 and 177 animals were included in the study at farm 1, 2, 3 and 4, respectively. A significant increase in milk production in the vaccinated group was seen in farm 1 (1,023 L/day) and 3 (0,611 L/day) during EL ($p<0.001$) and in farm 2 (1,799 L/day) during ML ($P<0.001$). In addition, at farm 2, vaccinated animals produced more milk than the non-vaccinated animals starting from 80 DIM.

Conclusion: Data demonstrate that cows in herds endemically infected with BVDV and vaccinated with live double-deleted BVDV vaccine produce more milk than the control cows in same BVDV endemic herds. The difference in milk production occurs during early lactation.

Keywords: Bovine viral diarrhoea,immune suppression,milk production,endemic,lactation.

IV-P07

Spot testing in multiple vaccinated herds with a live BVDV vaccine in The Netherlands

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Objectives: In many countries spot testing of young stock is an applied method to monitor the BVD status of herds. In the Netherlands a mandatory BVD control program for dairy farms is in place since April 2018. Biyearly testing of 5 animals between 8 and 12 months of age is one of four available routes to maintain a BVD-free status. When 2 or more out of 5 animals have antibodies against BVDV the spot test is considered positive. BVD vaccination, especially with live vaccines, leads to development of p80 antibodies in vaccinated animals and the detection of maternal antibodies in their offspring after colostrum uptake. This could interfere with BVD control programs using spot testing. A retrospective analysis of Dutch herds which have vaccinated with a live BVDV vaccine (Bovela®, Boehringer Ingelheim Vetmedica GmbH) and applied spot testing for multiple years was performed to check whether this combination is feasible in practice. The secondary objective was to observe whether the month of gestation at time of vaccination and number of vaccinations of the mother would influence the results of spot testing.

Methods: In total 12 BVD-free certified farms were selected. All herds applied spot testing and vaccinated with a live BVDV vaccine (Bovela) for multiple years (in a voluntary program initially and, since April 2018, in the mandatory program). Once a year whole herd vaccination of all animals above 8 months of age was performed. All farms additionally vaccinated young stock at another moment to ensure that all animals were vaccinated before their first breeding. Vaccination dates and results of spot testing per herd were obtained from the practice management program. Birth dates of young stock and their mothers (dams) were obtained from the national Identification and Registration App. Microsoft Excel 2016 was used to build a database. The age of the young stock at the time of spot test sampling, the month of gestation at time of the latest vaccination (before birth of the tested young stock) and the number of vaccinations of the dam (from 8 months of age until birth of the tested young stock) were calculated using Excel 2016. Descriptive statistics were performed.

Results: Thirty spot tests, including 150 young stock animals in total, were obtained from the 12 farms and used for the analysis. One of the 150 animals tested seropositive; the corresponding spot test was considered negative, since only 1 of 5 animals was seropositive. The age range of the animals at time of spot testing was 6 to 15 months. The seropositive animal was 12 months of age at spot test sampling, the mother had been vaccinated twice and was 7 months pregnant at time of last vaccination. 82% of the dams were vaccinated multiple times (ranging from 2 - 4). 73% (110 of 150) of the mothers received the latest BVD vaccination before birth of the tested young stock (during that pregnancy). With 35, 38 and 37 animals vaccinated in 1st, 2nd and 3rd trimester respectively.

Conclusions: Theoretically, in herds using live BVDV vaccines, spot testing for monitoring can be applied, with alignment of vaccination and sampling of non-vaccinated animals for spot testing at 8 - 12 months of age. The goal in The Netherlands is to perform spot testing in animals from 8 - 12 months of age. If not enough animals in this age group are present animals up to 15 months can be sampled. The results of this analysis show that in multiple vaccinated herds with a live BVDV vaccine, all spot tests were seronegative, the age of the animals used for spot testing ranged from 6 to 15 months of age. The month of gestation at time of vaccination and the number of vaccinations of the mother did not seem to influence the outcome of the spot test results, since they were all negative. Spot testing of young stock can be used for BVD monitoring in vaccinated herds with a live BVDV vaccine (Bovela®, Boehringer Ingelheim Vetmedica GmbH), regardless the number of vaccinations of the mother and the stage of gestation at time of vaccination.

Keywords: BVDV, vaccine, spot testing, antibodies, program.

IV-P08

BVD antibody testing in young stock from 6 months of age in herds vaccinated with a live BVDV vaccine

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Objectives: BVD vaccination, especially with live vaccines, leads to the development of BVD p80 antibodies and the presence of maternally derived antibodies after colostrum uptake in their offspring. This could interfere with BVD control programs that use spot testing. A diagnostic pilot study was performed in two Dutch BVD-free certified farms vaccinating with a live BVDV vaccine (Bovela®, Boehringer Ingelheim Vetmedica GmbH) for multiple years, to test for BVD antibodies in young stock from 6 months of age.

Methods: The two selected farms were certified BVD-free and started vaccination with a live BVDV vaccine in 2015. Biyearly spot testing (5 animals between 8 and 12 months of age) was performed to monitor and maintain the farms' BVD-free status. One farm (farm 1) applied once a year whole herd BVD vaccination of all animals above 8 months of age. On the other farm (farm 2), all cows were vaccinated after each calving. Both farms additionally vaccinated young stock to ensure that all animals were protected against BVDV before their first breeding. For this pilot study animals present on both farms, between 6 to 8 months of age, were sampled and tested for BVD antibodies. Vaccination dates were obtained from the practice management program. Birth dates of young stock and their mothers (dams) were obtained from the national Identification and Registration App. Microsoft Excel 2016 was used to build a database. Descriptive statistics were performed.

Results: In the period from September 2019 until November 2019, 10 animals from farm 1 and 13 animals from farm 2 were sampled. All animals tested BVD antibody negative. The age at sampling ranged from 6 months to 8 months with 10, 11 and 2 animals sampled at 6, 7 and 8 months of age respectively. The number of Bovela vaccinations of their dams ranged from 1 to 5. The majority of the dams (17 of 23) were vaccinated multiple times (ranging from 2 - 5).

Conclusions: As observed in this pilot study, farms and veterinarians in practice choose to apply different vaccination schemes. Theoretically, and shown in practice, herds using live BVDV vaccines can apply spot testing for BVD monitoring, with alignment of vaccination and sampling of non-vaccinated animals for spot testing at 8 - 12 months of age. In this pilot study all young stock ranging from 6 to 8 months of age tested BVD antibody negative despite their dams having been vaccinated at least once with a live BVDV vaccine. These results support the fact that spot testing of young stock can be used for BVD monitoring in vaccinated herds that use a live BVDV vaccine (Bovela®, Boehringer Ingelheim Vetmedica GmbH).

Keywords: BVDV, vaccine, antibodies, spot testing, program.

IV-P09

Q fever: Development and persistence of phase-specific antibodies in individual milk samples after vaccination

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Objective: *Coxiella (C.) burnetii* is classified as a zoonotic pathogen. Small ruminants are regarded as a major source of human infection; however, the pathogen is frequently detected in dairy cattle farms, too. During periods of active infection *C. burnetii* is shed at calving; additionally, individual cows shed *C. burnetii* persistently in their milk. Phase (Ph) II-antibodies in non-vaccinated cows in first lactation indicate an active infection at herd level. Persistently infected cows are characterized by elevated Ph I-titres (>100 in milk). Consequently, milk samples are screened for Ph I-titres >100, subsequently, a quantitative PCR (IS1111) is performed on those pre-selected samples to confirm infection. Coxevac™ is an inactivated vaccine based on a Ph I-strain. To assess whether vaccination interferes with serological screening for persistently infected cows two dairy cattle farms were primary vaccinated. Milk samples were then tested for PhI- and PhII-antibodies before and 1-7 months after vaccination.

Materials and Methods: Two dairy cow farms with 175 (Farm A) and 53 cows (Farm B) were included in this study. All animals (>12 months) were primary vaccinated with Coxevac™ according to the manufacturer's instructions. Individual milk samples were collected automatically at milking. Cows were tested 4 (t0) months before and 1 (t1), 3 (t2) and 5 months (t3) after vaccination (Farm A), and 2 months before (t0) and 2 (t1) and 7 months (t2) after vaccination (Farm B).

Ph-specific antibodies were assessed as described by

Böttcher et al. (2013). Based on the pre-vaccination testing, the cows were grouped as 'PhI^{-}/PhII^{-}' (farm A n=20/farm B n=10), 'PhI^{-}/PhII^{+}' (13/6) and 'PhI^{+}/PhII^{+}' (8/4). The data were analysed by MedCalc Statistical Software version 19.1.3 (MedCalc Software bv, Ostend, Belgium; https://www.medcalc.org; 2019). Serial measurements per group were compared by Kruskal-Wallis-test.

Results: Farm A: Geometric mean PhI-/PhII-titres in groups 'PhI^{-}/PhII^{-}', 'PhI^{-}/PhII^{+}' and 'PhI^{+}/PhII^{+}' over time were (t0) <5/<5, (t1) 9/150, (t2) 11/126 and (t3) 7/69, (t0) <5/42, (t1) 27/230, (t2) 61/418 and (t3) 43/308 and (t0)144/224, (t1) 280/303, (t2) 419/347 and (t3) 555/395, respectively. Only groups 'PhI^{-}/PhII^{-}' and 'PhI^{-}/PhII^{+}' showed a significant rise of both titres upon vaccination. Additionally, a significant decrease and at least a tendency to decrease was observed for PhII- and PhI-titres at 5 months after primary vaccination for both groups, respectively.

Farm B: Geometric mean PhI-/PhII-titres of groups 'PhI^{-}/PhII^{-}' and 'PhI^{-}/PhII^{+}' over time were (t0) <5/<5, (t1) 25/457, and (t2) 10/135 and(t0) <5/150, (t1) 54/431 and (t2) 13/129, respectively. Group 'PhI^{-}/PhII^{-}' showed a significant increase of PhI- and PhII-titres after vaccination. Subsequently, both titres decreased at 7 months after primary vaccination. PhI-titres of group 'PhI^{-}/PhII^{+}' significantly increased and decreased after vaccination, whereas changes of PhII-titres were not significant.

Five months after primary vaccination 1 out of 20 cows in group 'PhI^{-}/PhII^{-}' and 6 of 13 cows in group 'PhI^{-}/PhII^{+}' scored positive (PhI>100) in farm A, while no cow of the respective groups in farm B scored positive at 7 months after vaccination.

In both farms no significant changes upon and after vaccination were observed for group 'PhI^{+}/PhII^{+}'.

Discussion: The antibody status at primary vaccination influenced the antibody response after vaccination: Both PhI- and PhII-antibodies were not affected by vaccination if PhI-antibodies were present at vaccination and no decline was observed thereafter. Therefore, vaccination does not affect PhI-screening for probably persistently infected cows.

A transient rise of PhI-titres was observed in cows with a 'PhI^{-}/PhII^{-}' and 'PhI^{-}/PhII^{+}' pattern at vaccination. At least in one farm these titres persisted longer than 5 months in cows with a 'PhI^{-}/PhII^{+}'-pattern at vaccination.

Currently, a screening for persistent shedders is recommended before vaccination. However, a PhI-testing might even be used after vaccination to detect chronic-shedders or for monitoring purposes. Five to 7 months after vaccination the rate of positive screening is only slightly increased as compared to pre-vaccination status. Primary vaccination of seronegative heifers preferentially induces PhII-titres, however, a single booster vaccination after first calving induced PhI-titres (Böttcher et al., 2018). Consequently, the persistence of these antibodies needs to be determined.

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Keywords: Q fever, Coxiella, vaccination, antibody, chronic shedding.

IV-P10

Combined approach of vaccination with test & cull of persistently infected animals delivered successful control of Bovine Viral Diarrhea (BVD) – A field experience

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Objectives: Bovine viral diarrhea virus (BVDV) is a diverse group of viruses responsible for causing disease in ruminants worldwide. Infection by BVDV leads to substantial costs for cattle producers through decreased milk yield, respiratory disorders, congenital defects, growth retardation, extended calving intervals, reduced first service conception, and increased mortality of affected animals. It is believed that successful control of BVDV requires a multidimensional approach, involving vaccination, biosecurity, and identification and removal of BVDV reservoirs, i.e. persistently infected animals (PI) (Walz et al., 2010). The aim of this study was to verify that such combined approach is efficacious to control BVDV infection under field conditions.

Materials and methods: This trial was carried out in a dairy herd of more than 250 lactating cows in the province of Modena (Italy) that experienced an acute episode of BVDV infection in February 2017. Prior to this episode, the herd was tested negative for IBR and positive for BVDV in the bulk tank milk (PCR). Moreover, a sentinel group of 25 animals, aging from six to 12 months of age, tested in February 2016, showed a 36% of seroprevalence for BVDV (NS antibodies). At that time, it was decided to monitor the herd without implementing any control program as there were no obvious clinical signs that could be associated with BVDV infection in the herd.

In February 2017, the herd presented an increased incidence of bovine respiratory disease (BRD) cases in young calves and heifers. Bovine Respiratory Syncytial Virus (BRSV) and *M. haemolytica* were isolated from lungs at necropsy and from broncho-alveolar lavage. Further on, in October 2017, four heifers aged 14-17 months experienced an acute and severe diarrhea. One heifer died while the three others were slaughtered. BVD Type 1 was detected at necropsy by PCR. Concomitantly, BVD was isolated from the faeces of young calves with diarrhea (PCR), confirming that BVDV was actively present in the herd. Decision was then rapidly taken to start implementation of a combined approach using extensive screening of PI animals together with vaccination using a modified live vaccine able to prevent fetal infection (Mucosiffa®, Ceva Santé Animale). The vaccination protocol implied the vaccination of all the animals aging more than 6 months. Heifers received a booster every six month and cows every 12 months.

Results: On November 2017, 261 animals aging from four to 30 months of age were tested, in pool of 20 animals, for the detection of BVDV by PCR. Positive animals to BVD virus were tested again after 1 month to confirm their viremic status. On January 2018, 38 young heifers were also tested in pool of 20 animals by PCR. Animals of the positive pools were tested individually for the detection of the BVD virus (by BVDV antigen ELISA test). Subsequently, PI research on ear notch samples, by ELISA antigen test or by PCR, was performed on all newborn calves. Moreover, bulk milk tank was tested by PCR on a six-monthly basis.

Following this detection scheme, 17 animals of the 299 animals tested in pool were found to be persistently infected (PI) and were removed from the herd. All these animals were born from unvaccinated dairy cows. Since the implementation of vaccination with the modified-live vaccine until the end of November 2019, no PI animals were detected in newborn calves. All the tests on bulk milk also came back negative for BVDV antigen.

Conclusion: This field report illustrates the successful approach of using vaccination with a modified-live vaccine (Mucosiffa®, Ceva Santé Animale) in combination with a test and cull strategy to control an acute BVDV infection. This combined approach resulted in the absence of new PI calves, the absence of BVDV circulation as assessed by RT-PCR in the bulk tank milk and the absence of any new clinical manifestations of BVDV.

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Keywords: BVD, control, vaccination, PI cattle.

IV-P11

Interest of clostridial vaccination in pregnant cows to reduce omphalitis in beef calves. Results from a field study in France

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Objectives: Several French practitioners reported that the proportion of calves with omphalitis was lower in beef herds that vaccinate pregnant cows against clostridial disease compared to herds that does not vaccinate their pregnant cows. This study was performed to confirm this field observation.

Materials and methods: The study was carried out during two calving campaigns (2016-2017 and 2017-2018) and involved four cattle practitioners located in central France. Eight herds with an history of high incidence of omphalitis in calves participated in the study and a total of 299 cows were enrolled. In each herd, half of the pregnant cows were randomly selected to be vaccinated with a clostridial vaccine (Coglavax®, Ceva Santé Animale). The vaccination protocol included two injections 4 to 6 weeks apart, with the second injection to be given no later than 2 weeks before calving. The other cows were left unvaccinated.

After calving (D0), each calf was evaluated by the farmer at D1, D3 and D5. The attending veterinarian was asked to assess the calves at least once before D14 using a scoring sheet designed to reflect the severity of omphilitis (no omphilitis, mild omphalitis or severe omphalitis requiring parenteral antimicrobial treatment). Routine neonatal and umbilical care were kept as it was in each herd prior to this study. Comparisons were made between calves born from vaccinated pregnant cows and calves born from unvaccinated cows using chi-squared test with a .05 significance level.

Results: A total of 222 calves were included in the study; 114 born of vaccinated cows (group V) and 108 of unvaccinated cows (group U). A significant difference was found regarding the prevalence of omphilitis. In group V, the prevalence of omphalitis was 26.3% (n=30) compared to 40.7% (n=44) in group U (P = 0.02). The difference between calves from group V and U was even more important for severe omphilitis, with a prevalence of 8.8% (n=10) in group V vs. 25.0% (n=27) in group U (P = 0.001).

Conclusions: Results from this field study confirm cattle practitioner's observation and support the fact that clostridial vaccination of pregnant cows can be beneficial to reduce the prevalence of calf omphilitis. The interest of this clostridial vaccination seems particularly relevant for cases of severe omphilitis that generally require large spectrum antibiotic treatment. As such, this preventive strategy can contribute to reduce the consumption of antibiotic in beef herds with a high incidence of omphalitis. Future studies should be carried out to further investigate the relative importance of *Clostridia* and of others pathogens implicated and the potential benefits of clostridial vaccination in the various type of omphilitis.

Keywords: Omphalitis, clostridial vaccine, Clostridium perfringens, cow-calf.

IV-P12

Evaluation of immunogenicity of an inactivated Korean bovine viral diarrhea virus 1a and 2a vaccine in calves against virulent viral challenge

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Objectives: Bovine viral diarrhea virus (BVDV) 1a and 2a strains are the predominant sub-genotypes in South Korea. Because of the genetic and antigenic variability among different BVDV strains, multivalent vaccines are recommended to help prevent infection by varied field strains of BVDV. In this study, the immunogenicity of an inactivated vaccine containing

Korean BVDV 1a and 2a with different antigen dosages was evaluated by virulent BVDV challenge.

Material and methods: Four-month-old calves were intramuscularly vaccinated with three different dosage ($10^{4.0}$ $TCID_{50}$/ml, $10^{5.0}$ $TCID_{50}$/ml, and $10^{6.0}$ $TCID_{50}$/ml/dose), respectively. And then animals were boosted at three weeks after the first vaccination, with mock immunized calves serving as a control group. At 2 weeks after second vaccination, all animals were challenged with virulent BVDV type 1 and 2. Clinical signs including leukocyte count were observed. Virus shedding in nasal and rectal swabs was also detected by virus-specific RT-PCR and serum was tested for virus-specific antibody by serum neutralization test.

Results: The average neutralization antibody titer to BVDV 1a and 2a of calves immunized with high dosage group ($10^{6.0}$ $TCID_{50}$/ml/dose) vaccine reached high levels at 14 days post the second vaccination. After challenge, calves immunized with high dosage group showed less clinical signs (fever and leukopenia, etc.) and viral shedding was also prominently lower than a mock control group challenged.

Conclusions: Our results suggest that the induction of virus-specific antibodies following immunization was strongly dependent on the vaccine antigen dosage. The Korean inactivated BVDV vaccine at high dosage efficiently protected the calves against virulent BVDV challenge and may be considered as next vaccine candidate for the control of BVDV infection in Cattle.

Keywords: Bovine viral diarrhea, Vaccine, Immunogenicity, Protection.

IV-P13

Efficacy of inactivated gE-deleted *marker* vaccines against *Bovine alphaherpesvirus 1* (BoHV-1) in pregnant cattle to evaluate passive immunity

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Objectives: *Bovine alphaherpesvirus 1* (BoHV-1) is a member of the subfamily *Alphaherpesvirinae* and is responsible for significant economic losses in the cattle industry worldwide. The virus causes a variety of clinical signs including a respiratory disease called infectious bovine rhinotracheitis (IBR).

In Europe, modified live or inactivated *marker* vaccines are marketed. Through specific ELISA tests these products can differentiate animals immunised with *marker* vaccines and those infected or injected with traditional non-*marker* vaccines.

They are known to induce a high humoral and cell-mediated immune response, while to date only little information is available regarding their induction of passive immunity.

In this study we evaluated the mother-to-calf antibody response after vaccination with two different inactivated gE-deleted *marker* vaccines against BoHV-1.

Materials and methods: Eighteen Friesian cattle of approximatively 4-5 years of age devoid of neutralizing antibodies of BoHV-1 were selected. The animals were at their 5th month of pregnancy, and were divided into 3 groups of 6 animals each. The first two groups (A, B) were immunized with 2 different commercial inactivated gE-deleted *marker* vaccines, via intranasal and intramuscular route, respectively. The third group (C) was used as negative control. The possible adverse reactions after vaccination were evaluated.

After calving, the 18 newborn animals became part of their respective group, 6 for each group.

Clinical and serological investigations were conducted in all animals. In the pregnant cattle, the humoral immune response was evaluated before calving and subsequently at different times until post-calving day (PCD) 180. Moreover, passive immunity was detected in colostrum, milk and in serum samples of the newborn calves at different times until PCD 180.

Two commercial ELISA tests (IDEXX IBR gE Ab Test, Maine, USA; IDEXX IBR gB X3 Ab, Maine, USA) were used in parallel to examine the collected sera, colostrum, or milk samples, while indirect ELISA (IDEXX BHV1 Bulk Milk Ab, Maine, USA) was used only for colostrum and milk samples.

Moreover, a virus neutralisation test was performed using the protocol described by the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.

Results: According to our observation, no adverse reactions were evidenced for the vaccines used. In addition, no animal showed clinical signs and remained seronegative to gE-ELISA and seropositive to gB-ELISA throughout the entire experimental period.

On the calving day, the vaccinated cattle showed high titres (Vaccine A = 2.82 log_2; Vaccine B = 3.06 log_2) of neutralising antibodies against BoHV-1, that gradually declined until PCD 180 (Vaccine A, B = 1.86 log_2).

The day of calving, same antibody response was observed for passively immunized calves with lower titres (Vaccine A =2.51 log_2; Vaccine B=2.76 log_2).These values declined up to PCD 180 (Vaccine A = 0.85 log_2; Vaccine B = 0.75 log_2). No seroconversion was detected in the control group. During the experimental study, both colostrum and milk samples were seropositive to gB-ELISA and to indirect ELISA, while they were seronegative to gE-ELISA. The results showed that passive immunity transferred to calves can be detected up to PCD 180.

Conclusions: The vaccines used in this experiment proved innocuous for the pregnant cattle. Moreover, the transfer of passive immunity from dams to calves was demonstrated up to PCD 180. Therefore, these vaccines can be suitable for immunisation in IBR eradication programs. However, further studies will be necessary to determine if the passive immunity titres evidenced in our experimental calves are protective against virulent strains of BoHV-1.

Keywords: IBR, BoHV-1, passive immunity, marker vaccines.

IV-P14

A new live attenuated BRSV vaccine triggers both local and systemic cellular immune responses in newborn calves after intranasal administration

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Objectives: A new vaccine (NASYM®) that reduces respiratory clinical signs and Bovine Respiratory Syncytial Virus (BRSV) shedding has recently been registered. One of the most important features of this new modified live vaccine (MLV) is its flexibility in administration schedules.

Newborn calves were studied after intranasal application and after challenge with a heterologous BRSV strain, with the aim of characterising the immune response.

Materials and methods: With this aim, three different trials were conducted[1,2,3]. Calves were distributed randomly into 'Vaccinated' (NASYM®) and 'Control' (phosphate-buffered saline, PBS) groups, including at least seven seronegative animals per group. The age of the calves was <14 days at vaccination. Immunological testing characterised different immune responses: (i) specific IgA antibodies against BRSV in nasal secretions (in-house ELISA); (ii) serum antibodies against BRSV (INgezim BRSV); and (iii) cellular response (IFNγ production after stimulation of peripheral blood with BRSV, BOVIGAM ELISA kit). After vaccination, the animals were sampled and immunological parameters were monitored weekly for 3 weeks and 2 months (Trial 1 and Trial 2, respectively). Animals were challenged 3 weeks after vaccination with a European strain (Trial 1) or after 2 months with an American strain (Trial 3). Immunological testing was repeated after the challenge.

Results: Intranasal vaccination induced an increase in local IgA response in the upper airways from 2 weeks and detectable 8 weeks after vaccination, in addition to a systemic cellular (IFNγ) response 1-4 weeks after vaccination. Once the animals had been infected with either American or European challenge strains, vaccinated animals produced a statistically significant increase in IgA content in nasal secretions 1-2 weeks after challenge ($p < 0.05$). This kind of response may neutralise BRSV locally, preventing it from entering the target respiratory tissues and protecting animals against viral infection. Additionally, although no antibodies were detected in sera with intranasal vaccination, a significant serological anamnestic response appeared 2 weeks after challenge ($p < 0.05$). This systemic response was equivalent to that seen in young animals that received intramuscular vaccinations followed by a BRSV challenge[3].

Conclusions: In conclusion, intranasal vaccination (NASYM®) of newborn animals induced local IgA production and a systemic cellular response, and according to the booster response observed after challenge, it also primed lymphocytes. All of these mechanisms may contribute to protecting animals against BRSV infection.

The immune response varies with different administration routes or ages of cattle. When an intramuscular vaccination schedule is used, a systemic response is observed that confers long-term protection (not less than 6 months) to young calves from 10 weeks of age, including both humoral and cellular responses. However, IgA production is absent in their nasal secretions.

Despite this difference, both vaccination schemes demonstrated the same high efficacy in terms of reduction of respiratory clinical signs and virus shedding. These results help us to better understand immunological behaviour when efficient vaccination plans are used. Overall, intranasal and intramuscular administration routes induce local and/or systemic responses that protect against BRSV infection in the short and long term, covering the most critical period of the calves' production life cycle.

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1. Efficacy of NASYM against heterologous challenge with a virulent bovine respiratory syncytial strain in young calves 5 and 21 days after vaccination. Montbrau et al. (2019) Cattle Practice, 27 (2):113
2. Efficacy of NASYM against a heterologous challenge with a virulent BRSV strain in the presence and/or absence of MDA. Montbrau et al. Poster presented at the *BRD Symposium* 2019*,* Denver.
3. A new live attenuated BRSV vaccine triggers both humoral and cellular immune response in young calves of 10 weeks of age after intramuscular administration. Marzo et al. Poster presented at the WBC 2020, Madrid.

Keywords: BRSV, vaccination, intranasal, immune response.

IV-P15

A new live attenuated BRSV vaccine triggers both humoral and cellular immune responses in young calves of 10 weeks of age after intramuscular administration

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Objectives: The main goal of this work was to characterise the immune response triggered by a live attenuated BRSV vaccine (NASYM®) after intramuscular administration in young calves.

Materials and methods: Two studies were conducted. In the first (onset of immunity; OOI)[1], animals were challenged 21 days after vaccination with NASYM®. In the second study (duration of immunity; DOI), animals were challenged 4 months after vaccination (note that DOI was also demonstrated for 6 months in another study[2]). A heterologous challenge with a virulent BRSV strain was used in both studies. Animals of 10 to 15 weeks of age that were seronegative to BRSV were selected. In each study, animals were randomly distributed into two groups: vaccinated (NASYM®, OOI n=5; DOI n=6) and control (phosphate-buffered saline (PBS), OOI n=5; DOI n=6). The vaccine or PBS were applied intramuscularly (2 mL) at about 10 weeks of age and again 4 weeks later.

The immune response was studied after vaccination and challenge. ELISA techniques (INGEZIM BRSV) were used to determine BRSV-specific antibodies in serum. BRSV-specific IgA was studied in nasal secretions (in-house ELISA). Cellular response was assessed by measuring IFNγ production (BOVIGAM ELISA kit) after stimulating peripheral blood with the vaccine strain.

Results: A serologic response was already observable one week after completion of the vaccine schedule (2 doses), reaching its peak 3 and 4 weeks after vaccination in the OOI and DOI studies, respectively. However, the serological titres of BRSV antibodies decreased two months after vaccination (DOI study). Nevertheless, an anamnestic response was observed in the vaccinated groups after challenge, reaching significantly higher titres of serum antibodies compared to the control groups (both studies, $p < 0.05$).

Cellular response was already observable after the first dose in vaccinated animals during the DOI study. Likewise, after completion of the vaccination schedule, a cellular response was also seen in the OOI study ($p < 0.05$, Mann–Whitney U test). After the challenge, a cellular response was observed in both vaccinated and control groups, but it was higher in the vaccinated groups. In the DOI study, the response was assessed at further post-challenge timepoints, finding a significant increase that peaked 7 to 10 days after challenge ($p < 0.05$, mixed-effects analysis).

The vaccine (NASYM®) was shown to be effective after the challenge for both OOI[1] and DOI[2].

Conclusions: After intramuscular vaccination (NASYM®), a systemic immune response was triggered, including both humoral and cellular responses. Although the humoral response was not maintained throughout the four-month period, an anamnestic response was observed after challenge. Similarly, a cellular response was observed after vaccination and was also boosted to significant levels after challenge.

When this vaccine (NASYM®) is administered to newborn calves by the intranasal route[3], it also triggers a local IgA response in the upper airways, together with a systemic cellular response. After the challenge, an IgA increase in the nasal mucosa and a serologic anamnestic response are observed.

Hence, when the vaccine is administered intranasally, it protects newborn calves by triggering a local response (IgA) as well as a memory response (IFNγ and anamnestic serologic response). Moreover, when it is administered intramuscularly, it triggers a systemic response that confers long-term protection (not less than 6 months) to young calves from 10 weeks of age. Therefore, by combining both routes of administration, this vaccine triggers both local and systemic responses, conferring short and long-term protection against BRSV during the most critical period of the calves' production life cycle in terms of respiratory disease.

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2. "*NASYM confers 6 months of duration of immunity by an intramuscular booster at 3 months of age after neonatal primovaccination by intranasal route*". Marzo et al. (2019) Cattle Practice, 27 (2):111.
3. "*A new live attenuated BRSV vaccine triggers both local and systemic cellular immune responses in newborn calves after intranasal administration.*" Moreno et al. Poster presented at the WBC 2020, Madrid.

Keywords: BRSV, vaccination, intramuscular, immune response.

IV-P16

A clear decline in antibiotic use and mortality rate on a veal calf farm in belgium after implementing a brd vaccination protocol

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Objectives: A BRD vaccination protocol was implemented on a veal calf farm in Belgium due to periodic, recurrent respiratory problems. This enabled different parameters of the fattening period, in which vaccination was administered, to be compared with three previous fattening periods, during which vaccination was not performed. In particular, the antibiotic use and mortality rates were compared.

Materials and methods: On this farm, 700 veal calves are present and there is no 'ALL IN – ALL OUT' system. Each week approximately 50 calves arrive, originating from different farms, without knowledge of their immune status. At arrival, the calves are aged between 12 and 32 days. In the barn, there are different separate areas, either side of a central corridor, which can contain 40 – 50 calves each. During the first 4 weeks, calves are housed individually and later reside in groups of 4 to 6. Vaccination in the veal calf industry is not common and treatment of BRD usually consists of oral administration of antibiotics. On this farm, farmer and veterinarian were often confronted with respiratory disease expressed by signs ranging from slight coughing to death.

Regarding this particular fattening period, the calves arrived between the end of March and end of July 2019. A fattening period takes 8 to 9 months; the first calves were slaughtered in November 2019 and the last calves went to the slaughterhouse in February 2020. There is an overlap between the arrival of new calves for the next fattening period and the departure of the last calves of the current period.

A BRD vaccination protocol was implemented within 5 days of arrival at the farm; calves were aged between 12 and 32 days. The veterinarian vaccinates the calves intranasally with an MLV vaccine containing BRSV (Bovine respiratory syncytial virus) and PI_3 virus (Bovine parainfluenza 3 virus) (Bovalto Respi Intranasal*). Within the following two milk feeds Sodium Salicyl 80% WSP (sodium salicylate 800 mg/g water soluble powder) was administered orally to all vaccinated calves at a dose of 50mg per animal in order to prevent a possible inflammatory reaction triggered by manipulation or vaccination. Two to three weeks later the veterinarian vaccinated all

calves subcutaneously, with an inactivated vaccine containing the same parental viral strains plus Mannheimia haemolytica (Bovalto Respi 3*). Three weeks later the veterinarian repeated this vaccination.

Data were recorded in the files of the veterinarian e.g. every administration of medication, mortality, diseased calves. Any diseased calves were sampled; one calf was sent to the laboratory of reference for autopsy.

* For more detailed information see SPC Bovalto Respi Intranasal and Bovalto Respi 3.

Results: It is the veterinarians experience from previous rounds that extended oral antibiotic administration is necessary to achieve a satisfying start to the fattening period; this was not necessary during this round. The mortality rate in previous periods was between 7 and 10 %, in the fattening period of these vaccinated calves this reduced to approximately 4,5 %. Furthermore, the use of antibiotics decreased by 41,2%. None of the analyses performed following disease or mortality identified any of the pathogens present in the administered vaccines. Other causes of disease or mortality were identified e.g. Mycoplasma bovis, Salmonella typhimurium, Fusobacterium necrophorum and trauma.

Calves presenting at the slaughterhouse were subjectively assessed as good quality (exact data regarding carcass weights are still being processed as the last calves arrived at the slaughterhouse recently).

Conclusions: These data show that, on this particular veal calf farm, the implementation of a vaccination protocol, which consists of early IN vaccination with an MLV BRD vaccine, containing BRSV and PI_3, followed by two administrations of an inactivated vaccine containing BRSV, PI_3 and Mannheimia haemolytica, can result in lower mortality and a clear decrease in antibiotic use compared with previous fattening periods, with the same management.

Keywords: Bovine respiratory disease, Antibiotics, vaccination.

IV-P17

Preliminary evaluation in calves of a Bovine Viral Diarrhoea (BVD), tagged vaccine prototype (AQ06 - BoVLP-BVD) based on Virus-Like-Particles (VLPs)

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Objectives: Aquilón CyL intends to develop a new and disruptive recombinant vaccine against BVD using proprietary antigens in Virus-Like-Particles.

The main objective of this study was to evaluate preliminary safety and immunogenicity *in vivo* of an AQ06 - BoVLP-BVD vaccine prototype that includes BVD virus peptides shown to be naturally antigenic. Besides, the DIVA (Differentiating Infected from Vaccinated Animals) attribute of the vaccine was also tested.

Materials and methods: Twelve Frisian 300 kg male calves were enrolled. Eight of them were inoculated at day 0 and day 21 with a preliminary vaccine formula based on a first prototype of VLP-BVD active substance and an aqueous adjuvant; four calves were inoculated with "empty" VLPs (no active substance) with the same formulation. Safety parameters (general clinical signs, rectal temperature and inoculation site) were daily observed. Animals were scheduled for bleeding (caudal venipuncture) immediately before the first immunization, 21 days after de first immunization and before the second and, 21 days after the second immunization.

In order to test the ability of the VLPs to induce an immune response against a reporter antigen (DIVA attribute), sera of the experimental animals were tested with a specific commercial indirect ELISA for the VLP. Furthermore, sera samples were analyzed using a commercial ELISA test for BVD total antibody and an *in house* developed ELISA for the BVD selected peptide.

Results: Results indicated that all animals increased optical densities against the VLPs when comparing days 0 and 21, suggesting seroconversion to the proteins. Animals receiving the native -empty- VPL were the ones with the highest levels of antibodies at day 21. One of the animals showed a total antibody immune response at day 42. Results of the *in house* developed peptide ELISA indicated that most animals showed increased ODs after the second vaccination.

Conclusions: The results of the present experiment indicate that the VLPs used as the active substance carrier were immunogenic as shown by the induction of specific antibodies, confirming the DIVA attribute of the vaccine prototype ("positive" tagging). On the other side, seroconversion against the active substance (BVD peptide) by most animals suggested a potential immunizing effect of the vaccine, as well as the positive result of one animal for BVD total antibodies ELISA. In summary, the product tested was safe, showed immunogenicity and induced a specific BVD response in some of the inoculated animals. Optimization of peptide epitopes content and exposure in the VLP, vaccine dosing and adjuvancy are ongoing.

Keywords: Vaccine, BVD, tagged, VLPs.

IV-P18

Bivalent and polyvalent clostridial vaccines induce a similar serological response against C and D botulinic toxoids in cattle

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Objectives: Botulism is one of the most important clostridial diseases causing mortality in bovine herds in tropical and subtropical regions of South America. Botulism caused by

Clostridium *(C.) botulinum* type C or D toxins in extensive cattle breeding is associated with osteophagy triggered by a lack of or insufficient phosphorus supplementation, environmental contamination by *Clostridium botulinum* spores, presence of dead animals on pastures and absence or lack of immunization. Vaccination with *C. botulinum* type C and D toxoids is one of the main preventive measures. Currently, more than 110 million doses are annually marketed in Brazil. Until the early 2000s, clostridial vaccines sold in the country were bivalent (C and D botulinum toxoids) or polyvalent (blackleg, gas gangrene, malignant edema, necrotic hepatitis, enterotoxemia) without botulinum toxoids. The first classical anti-clostridial commercial polyvalent vaccine (blackleg, gas gangrene, malignant edema, necrotic hepatitis, enterotoxemia) including C and D botulinic toxoids were marketed in Brazil in 2001. This study aims to compare the humoral response observed with two Brazilian commercial vaccines.

Material and methods: Two clostridial vaccines produced by the same manufacturer, one bivalent containing only C and D botulinic toxoids (Botulina, MSD Animal Health - Vallée, Brazil), and another polyvalent containing bacterins and classical clostridiosis toxoids (symptomatic carbuncle, gaseous gangrene, malign edema, necrotic hepatitis, and enterotoxaemia), and C and D botulinic toxoids (Poli-Star, MSD Animal Health – Vallée, Brazil), were evaluated in a commercial herd. In a longitudinal study 60 multiparous cows were vaccinated (n = 20 for each vaccine and control group), boosted 42 days later and monitored at six different moments over approximately one year (day 0, 42, 75, 160, 250 and 342). Humoral kinetics were measured by identification of specific antibodies (Immunoglobulin G) against C and D botulinic toxins through Elisa.

Results: The antibody response against C and D botulinic toxoids demonstrated a good immune response against the vaccines. There was no significant difference between the two vaccinated groups ($p>0.05$). The antibody response to C and D botulinic toxoids was significantly higher at day 75 in the vaccinated group compared to the control groups ($p<0.05$). From day 160 onwards, serological levels in vaccinated animals remained higher than control animals (except for C toxoids at day 342 for the bivalent vaccine) but were no longer significantly different.

Conclusion: The use of a polyvalent clostridial vaccine containing C and D botulinic toxoids offers a broader protection than a bivalent vaccine while offering the same serological response against C and D botulinic toxins. This information is useful for production systems with high exposure and potential risk for the occurrence of botulism.

Keywords: Clostridial, vaccination, bivalent, polyvalent, serological response.

IV-P19

Isoprostanes can modify vascular functions in a bovine model of oxidative stress

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Oxidative stress is associated with several inflammatory-based diseases in dairy cattle and results in damage to tissue macromolecules. Isoprostanes (IsoP) are molecules generated from interactions between free radicals and membrane phospholipids, thus serving as excellent indicators of free radical-mediated lipid damage during times of oxidative stress. During periods of increased inflammation, IsoP have been detected in both the blood and milk of dairy cows. Although IsoP are recognized as excellent biomarkers of oxidative stress, however, their impact on inflammatory cells remains largely unknown. The vascular endothelium is a primary target of lipid peroxidation during excessive inflammation and oxidative stress. Thus, the goal of this study was to determine how certain IsoPs influence bovine endothelial cell functions during oxidative stress conditions. Bovine aortic endothelial cells (BAEC) were incubated in the presence of an arachidonic acid-derived IsoP alone and in combination with known oxidizers 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) and lipopolysaccharide (LPS). Exposure to IsoP significantly decreased ROS production in BAEC when incubated with AAPH for 12 hr compared to cells incubated with AAPH alone. Additionally, IsoP significantly decreased apoptosis in BAEC incubated with LPS for 12 hr when compared to cells incubated with LPS alone. The results of this study indicate that certain IsoPs may have a cytoprotective role during times of oxidant challenge. Future studies should be directed towards investigating if IsoPs alter other factors associated with vascular damage during oxidative stress, such as endothelial cell barrier integrity. This research benefits the industry by providing insight into how a well-known biomarker of oxidative stress in dairy cattle may contribute to the pathophysiology of economically important inflammatory-based diseases.

Keywords: Dairy cattle, inflammation, oxidative stress, isoprostane.

IV-P20

Defining protective immune signatures in dendritic cells draining vaccination sites

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Objectives: An urgent need exists to understand why current vaccine formulations incorporating defined antigens fail to induce protective cell-mediated immune responses, and how to design more effective, deployable vaccines. To address this, it is necessary to identify both the features of the early immune response associated with generating protective responses, and targets for its manipulation.

Dendritic cells (DCs) play a key role in initiating T cell responses and determining their functional differentiation. This study utilises well established models to generate protective cell-mediated immune responses, and aims to determine the functional, phenotypic and transcriptomic profiles of DCs draining from sites of vaccination. Defining protective signa-

tures will facilitate targeted vaccine design and the rational selection of adjuvants.

Materials and Methods: Our approach exploits two technologies in a combined novel approach to identify protective immune signatures induced by vaccination: 1) a bovine lymphatic cannulation system which provides direct access to DCs draining from inoculation sites and 2) next generation transcriptome sequencing and network analysis.

We surgically cannulated the afferent lymphatic vessels draining the skin enabling collection of cells trafficking in the lymph. The, vaccine preparations were inoculated above these sites enabling collection of cells dynamically as they respond to vaccination. Using a combination of cellular analytical tools (flow cytometry, ELISA, ELISPOT) and single cell RNASeq we will define signatures associated with protective immunity.

Results: Previous studies demonstrated that the DCs draining the skin were functionally and phenotypically heterogeneous [Howard & Hope, 2000]. These DCs were shown to carry vaccine antigens including *Mycobacterium bovis* Bacille Calmette Guerin (BCG) [Hope et al, 2012], which is known to protect young calves against bovine tuberculosis (TB) [Thom et al, 2012]. Here, we extend our findings to examine the single cell transcriptome of DCs in naïve, and BCG vaccinated calves. Alongside this, alterations in the surface phenotype, and cytokine producing capacity of DCs draining vaccination sites were examined.

Conclusions: Defining immune signatures will facilitate rapid screening of vaccine candidates and delivery systems to enable selection for those capable of inducing protective signatures, which can then be taken forward for further study. This will be important for a range of diseases that affect cattle, including bovine TB and East Coast Fever.

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Keywords: Dendritic cells, vaccines, gene expression.

IV-P21

The association between colostral density and immunoglobulin concentrations of Dairy cattle

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Objectives: The colostrum contains diverse and numerous immunoglobulins. The neonatal calf obtains majority of passive immunity from colostrum, which especially has huge effects on calf health. Historically, the amount of immunoglobulins in colostrum has been predicted through the density of the colostrum, but it has not yet been clearly determined whether the density of the colostrum and antibody amount against each pathogen are correlated. In this study, we determined the correlation between the density and the immunoglobulin concentration, the difference between Holstein and Jersey cattle, and the impact by parity number in colostrum.

Material & Methods: Colostrum samples were collected from Holstein and Jersey cows at National Institute of Animal Science in Cheonan, Korea. Colostral density was measured by Kruuse colostrum densimeter. Antibody titers from colostrum were measured by using the enzyme-linked immunosorbent assay (ELISA). We used indirect ELISA to measure total immunoglobulin G (IgG) and antibody for against corona-rota virus and Competitive ELISA for Foot-and-Mouth Disease Virus (FMDV) antibody. IBM SPSS 21.0 software was used for analysis.

Results: Colostral density significantly showed positive correlation with total IgG (r=.697, p <.001), FMD virus antibody (r=.711, p <.001), and corona rota virus antibody (r=.441, p <.001). Total IgG had significant positive correlation with FMDV antibody (r=.502, p <.001), and corona-rota virus antibody (r=.414, p <.001). Holstein cows produced colostrum with density of 1.057 ± 0.002, total IgG of 351.25 ± 34.98 ng/ml, FMDV antibody of 55.09 ± 3.96 PI value, and corona-rota virus antibody of 58.68 ± 8.99 relative value whereas Jersey cows produced colostrum with density of 1.044 ± 0.003, total IgG of 82.34 ± 15.27 ng/ml, FMDV antibody of 22.35 ± 12.87 PI value, and corona-rota virus antibody of 14.045 ± 5.58 relative value. Colostrum produced by multiparous cows had 1.062 ± 0.002 density, 407.00 ± 39.69 ng/ml total IgG, 65.23 ± 4.59 FMDV antibody PI value, and 65.62 ± 12.18 corona-rota virus antibody relative value while colostrum of primiparous cows had 1.048 ± 0.002 density, 218.99 ± 48.07 ng/ml total IgG, 34.66 ± 5.24 FMDV antibody PI value, and 39.42 ± 10.25 corona-rota virus antibody relative value.

Conclusion: The concentration of total IgG and antibody against FMDV tend to increase following the density of the colostrum. However, the level of antibody against corona-rota virus was not strongly correlated with the density. Regarding species and parity effects, interestingly, Holstein cows had significantly higher values in total IgG, FMDV antibody, and corona-rota antibody than Jersey cows. Multiparous dairy cows produced colostrum with higher concentrations of total IgG, FMDV antibody than primiparous dairy cows.

Keywords: Colostrum, density, immunoglobulin.

IV-P22

BVDV Monitoring By Pooling and Real Time RT-PCR as Economical Monitoring Technique With Low BVDV Prevalence

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The aim of this study was to determine whether the use of pooled blood samples and real-time RT-PCR are feasible for detecting BVDV in viremic or PI animals. For this purpose, blood samples obtained from 2701 cattle, brought from 62 different farms, were used to test for the presence of bovine viral diarrhoea virus (BVDV) at the Veterinary Virology Department of Aksaray University Faculty of Veterinary Medicine. The sampling was done from all geographical regions in Turkey. Blood samples were pooled in groups of eight, creating a total of 342 pools. Each pool was checked for BVDV with a real-time RT-PCR test. BVDV nucleic acid was detected in 18 (5.26%) of a total of 342 pools. BVDV was detected in 18 of 62 farms. The most important result obtained from this study is that BVDV monitoring by pooling and real time RT-PCR can be done very economically when the disease prevalence is low (<10%). A risk/benefit estimation can be done for breeders who want to start vaccination programs.

Keywords: BVDV, Pooling, Real Time RT-PCR.

IV-P23

Benefits of vaccination against IBR & BVD on the reproductive performance in extensive cattle

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Introduction: The Lidia breed (usually called "Brava") is very popular in Portugal and Spain, being the production system mainly in extensive. Although most of the breed is raised in the Portugal mainland, using around 800 hectares, there are also a small production of 30ha in Terceira Island (Azores). The production profitability on these herds is closely related to the efficiency reproduction indexes, being November to April the mating months. It is described that non pregnant animals within the herd have an important economic cost; some authors estimate the cost of each barren cow between €500 to €900 per year(1). Therefore, one of the main goals on these farms is to improve the fertility rate, management, nutrition and animal health. Focussing on animal health, IBR/ BVD viral circulation has demonstrated a negative reproductive impact on unvaccinated farms. Vaccination is most of the times part of the strategy to control the impact of both viruses.(2)

Objectives: Our objective was to show that vaccination against BVD/ IBR produces an improvement in the reproduction ratios and, therefore a benefit in the economy of the farm.

Material and Methods: From 2015 to March 2018, in Terceira Island (Azores), a study was developed using 94 Lidia females aged more than 24 months and raised in an extensive system in a geographical area with high concentration cattle. The animals were randomly divided into two groups, 43 of them were vaccinated (being the experimental group) and 51 not vaccinated were used as a control group. During the first year of the study, cows were vaccinated with a tetravalent vaccine (HIPRABOVIS® 4, BRSV, IBR, BVD and PI3); the heifers joining the study were first vaccinated one month before mating, following the SPC. From the second year of the study onwards (2016 to 2018), the vaccine was switched to 2 products, HIPRABOVIS® IBR MARKER LIVE (IBR) and HIPRABOVIS® BALANCE (BRSV, BVD and PI3). The difference is that from 2016 animals were vaccinated with a live, marker vaccine against IBR allowing the identification of infected animals. Data, for the characterization of the reproductive indexes, were obtained using the reproductive registers made by the animal's owners as well as data obtained through the sanitary and reproductive visits. Moreover, for each animal, all data inserted in the National Bovine Register System (SNIRB), namely parturitions and dead animals, were also considered, and the reproductive parameters were calculated according to Anderson (3). Statistical analysis using the software SPSS and the results are presented as mean ± standard deviation (table 1).

Results: The objective of this study was to compare the reproductive efficiency when animals are vaccinated. According to the data obtained, in all the parameters studied (age at first calving, calving interval and real fertility), the vaccinees presented significantly superior indexes compared to the unvaccinated group (Table 1).

Table 1. Reproductive results obtained for both groups, vaccinated and non-vaccinated with the HIPRABOVIS® vaccines.

Reproductive Parameters	Vaccinated Group (43 animals)	Non-vaccinated group (51 animals)
Age 1st calving	40.8 months	41.8 months
Calving Interval	471 ± 35 days	889 ± 25 days
Real Fertility	71.1%	28.6%

Discussion: In extensive cattle, having a calf per cow per year is the main goal, meaning that the calving interval should be around 365 days; however, a calving interval of 412 days is considered acceptable. IBR and BVD viruses produce persistently infected animals, which keep viral circulation within the herds, being those farms endemic for the diseases. Strategies based on vaccination have demonstrated efficacy to control the negative impact of these viruses. Unvaccinated

animals are unprotected against these diseases, being very vulnerable to manifest infertility and therefore not being economically profitable.

Conclusion: The results herein clearly demonstrated that vaccination of female cattle against IBR/BVD with HIPRABOVIS® substantially improved their reproductive performance. The results indicated that the most pronounced benefit was the calving interval, where values decreased from 889 ± 25 days in not vaccinated cattle to 471 ± 35 days in vaccinees. This dramatic improvement led to a raise of the real fertility from 28.6% in the unvaccinated group, to 71.1% for the vaccinees.

Keywords: Vaccination, IBR, BVD, reproductive, extensive.

IV-P24

ELISA antibodies against bovine rotavirus, coronavirus and ETEC in serum from cows and calves as indicator for the protective status against neonatal calf diarrhea

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Objectives: Neonatal calf diarrhoea (NCD) is a common problem affecting calves in the first few weeks of life. The most common aetiological agents are *Cryptosporidium parvum*, bovine coronavirus (BoCV), bovine rotavirus (BoRV) and enterotoxigenic *E. coli* (ETEC). Protection against clinical disease is provided by the ingestion of specific antibody present in colostrum and early milk. Most vaccines that aim to control neonatal calf diarrhoea are therefore designed to increase the magnitude and duration of specific antibody levels in mammary gland secretions. In some recent studies in herds not vaccinating against NCD, the antibody levels in the cow's serum, colostrum and calf serum were relatively low against BoCV, BoRV and ETEC based on ELISA tests from BIO X (Belgium). In this preliminary study, we checked if this is also a constant finding in some commercial herds in the Netherlands, knowing that the 3 pathogens are endemic and test kits are available for detection of protective antibodies. If results show low levels, the Elisa test may be a tool to address the importance of vaccination against NCD.

Material & methods: On 6 Dutch dairy farms with no history of NCD, 3 to 6 healthy fresh cows (first week post calving) that were never vaccinated against NCD and their respective healthy calf were blood sampled. Antibody titres in the sera against BoCV, BoRV and ETEC were assessed by measuring the inhibition of optical density (percentage of inhibition, PI) by competitive enzyme-linked immunosorbent assay (ELISA). The BIO K 392 Monoscreen Ab ELISA Bovine Coronavirus/ Competition, BIO K 126 Monoscreen Ab ELISA Bovine Rotavirus/ Competition; and BIO K 295 Monoscreen Ab ELISA E. coli F5 (k99)/ Blocking (all from BIO-X Diagnostics, Belgium) were used for the indirect quantification of antibodies against BoCV, BoRV and ETEC F5 (K99), respectively.

Descriptive statistics were generated, including the mean, median and standard deviation for the PI of each antigen. Anova, a t-test or the corresponding non-parametric tests were performed to compare between farms and between cows and calves for each type of antibody (p=0.05).

Results: In total 25 calves and their respective mothers were sampled. The mean PI of samples from cows and calves together (median, 95%-confidence interval) were 56.82 (55.45, 50.78-62.87), 36.32 (29.65, 28.38-44.27) and 5.31 (4.45, 3.35-7.28) for respectively BoCV, BoRV and ETEC.

The mean PI (median, confidence interval) in adult cows corresponded to 54.3 (53.8, 47.08-61.52), 26.56 (18.7, 17.82-35.29) and 5.44 (5.6, 3.23-7.65) for respectively BoCV, BoRV and ETEC.

The mean PI (median, standard confidence interval) in calves correlated with 59.35 (62.8, 49.20-69.49), 46.09 (43.1, 33.37-58.81) and 5.19 (4.00, 1.76-8.62) for respectively BoCV, BoRV and ETEC.

The PI were significant different between the farms for BoCV (p=0.03) and ETEC (p<0.01) but not for BoRV (p=0.13). On the contrary, the PI value was significantly higher in calves versus cows for BoRV (46.1 vs 26.6, p<0.01) but not significantly different for BoCV (59.4 vs 54.3, p=0.20) and ETEC (5.4 vs 5.2, p=0.54).

The PI values observed in this study from cows and calves together are in line with the results obtained in Spain in a non-vaccinated group on a farm with no history of vaccination against NCD (Gonzalez et al, 2021). The median values in the Spanish study were slightly higher for BoCV and BoRV but lower for ETEC.

In general, it can be stated that PI values in non-vaccinated herds are intermediate (40-70) for BoCV, intermediate to low for BoRV (20-60) and low (<10) for ETEC. In contrast, PI values observed in vaccinated cows and calves that received colostrum from vaccinated animals are high (>70) for BoCV, and intermediate to high (40-99) for BoRV and ETEC (Gonzalez et al, 2021). Finally, high PI are also expected in herds that recently had an infection with BoCv, BoRV or ETEC.

Conclusion: Measuring ELISA antibodies against bovine coronavirus, rotavirus and enterotoxigenic *E. coli* in serum from cows and calves are a good indicator to differentiate herds vaccinating against NCD from non-vaccinating herds. Moreover, it provides information for farmers and vets on the possible benefits of vaccinating the cows to increase the specific antibodies against BoCV, BoRV and ETEC in the serum of cows and calves to improve the protection against NCD in the herd.

Keywords: Neonatal calf diarrhea, antibodies, serum, vaccination.

IV-P25

Intranasal vaccination of calves with a live vaccine against respiratory coronavirus combined with a bivalent vaccine against respiratory syncytial virus and parainfluenza type 3 virus

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Objectives: Bovine Respiratory Disease (BRD), a major cause of calfhood mortality, results from the interactions between microorganisms in the respiratory tract and unfavorable environmental factors which negatively affect the animal's immune status. Bovine Corona Virus (BCV), Bovine Respiratory Syncytial Virus (BRSV) and Bovine Parainfluenza 3 Virus (BPIV3) are generally considered the major viral pathogens in BRD outbreaks in Europe.

Several vaccines against BRSV and BPIV3 are licensed in Europe but to our knowledge, no vaccines for active immunization against respiratory BCV. The objective of the studies reported here was to investigate the effect of combined application of an intranasal live attenuated vaccine against respiratory BCV (in development) and a commercial intranasal live bi-valent BRSV-BPIV3 vaccine.

Materials & Methods: The investigations comprised two studies in colostrum deprived calves at approximately one week of age. In both studies, 18 animals were allocated to three groups of six animals: one unvaccinated control group, one group vaccinated both with the live attenuated respiratory BCV vaccine and the live attenuated BRSV/BPIV3 combination vaccine (Bovilis® INtranasal RSP Live; MSD Animal Health) and the third group was vaccinated with only the BCV vaccine (study 1) or only the bivalent BRSV-BPIV3 vaccine (study 2).

One week after the vaccination, all animals in study 2 were challenged with a BPIV3 field isolate and two weeks later with a BRSV field isolate. Three weeks after the vaccination, all animals in study 1 were challenged with a BCV field isolate. The challenge viruses were administered by aerosol (using a mask).

The animals were monitored for general and respiratory clinical signs following the challenge infection. Samples were collected and tested for viral RNA specific for the respective challenge viruses. The studies ended 14 days after the (second) challenge infection and the animals in study 2 were necropsied to investigate typical BRSV lung pathology.

Results: Study 1

The nasal BCV challenge virus shedding as well as the challenge virus load in lung lavage samples and rectal swab samples was statistically significantly lower in the vaccinated groups compared to the control group.

After the BCV challenge infection, mild signs of upper respiratory tract disease (URTD) were observed in all control animals and most vaccinated animals. The course of disease was milder in the two vaccinated groups, yet the differences in clinical scores and rectal temperatures between the control group and vaccinated groups were not significant.

Study 2 – BPIV3

The amount and duration of nasal BPIV3 challenge virus shedding was significantly reduced in both vaccinated groups compared to the control group.

Moreover, after the BPIV3 challenge infection, mild signs of URTD were observed in all control animals and most vaccinated animals. The course of disease was milder in the two vaccinated groups, and the differences in URTD scores between the control group and vaccinated groups were significant.

Study 2 - BRSV

The duration of BRSV nasal challenge virus shedding and maximum viral load in nasal discharge was reduced in both vaccinated groups compared to the control group. Moreover, the average BRSV viral load in lung lavage samples was significantly lower in the samples from vaccinated animals than in the control group.

After BRSV challenge infection, all animals developed mild signs of URTD. In addition, all control animals and five out of six animals in each vaccinated group developed signs of lower respiratory tract disease (LRTD). The clinical scores in the vaccinated groups were lower than in the control group, but the differences were not significant.

At necropsy, most animals had macroscopical lung lesions. The total percentage of consolidated lung surface area was larger in the control animals compared to the vaccinated animals but the difference was not statistically significant.

Conclusion:

- The challenge virus infections led to clinical signs of respiratory tract disease. For all three viruses, the disease scores in the vaccinated groups were lower than in the control groups, but the differences were only significant after challenge with BPIV3.
- Moreover, vaccinated animals shed less challenge virus as judged by the lower amount of viral RNA in swab samples.
- Viral load (only tested for BRSV and BCV) in lung lavage samples was significantly lower in the vaccinated groups.
- Within both studies, the results after application of only one or both vaccines were comparable.

Keywords: Intranasal vaccination, combination, BRSV, PI3, coronavirus.

IV-P26

Characterization of F5(K99) and F41 fimbria present on *E. coli* used in a multivalent cattle vaccine and its associated immunological responses in pregnant cows

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Objectives: Neonatal calf diarrhea is an important cause of morbidity and mortality worldwide. Enterotoxigenic *Escherichia coli* (ETEC), rotavirus, coronavirus, and *Cryptosporidium* are the four major pathogens associated with neonatal calf diarrhea. Almost all ETEC bacteria are known to adhere to receptors on the small intestinal epithelium by their fimbriae without inducing significant morphological changes. The most observed fimbriae on ETEC from calves with diarrhea are F5, also named K99, and F41. These two fimbriae often occur together but may be present independently. The objective of the study was to further characterize the *E. coli* antigen used in a commercial neonatal calf diarrhea vaccine (Bovilis® Rotavec Corona, MSD Animal Health). More specifically, the study looked at the presence of the F41 fimbriae on top of the already characterized F5 (K99) fimbria and the associated immunological responses in serum and colostrum of vaccinated cows.

Material & methods: *In vitro* characterization: The purified cell free *E. coli* antigen was analyzed using Liquid Chromatography - Tandem Mass Spectrometry (LC-MS/MS) after digestion of the denatured, reduced, and alkylated proteins using trypsin. The reported peptides were identified using a protein database (Uniprot) with published *E. coli* protein sequences. Relative protein quantification was performed by calculating the sum of the intensities of the three most intense peptides of 13 independently produced batches of the *E. coli* antigen. The most dominant fimbriae found were reported as well as the peptide coverage after digestion with trypsin. Descriptive statistics were generated, including the average protein ratio of the most dominant fimbriae found with the standard deviation over the 13 batches analyzed.

In vivo characterization (immunological responses in cows): One group of 21 pregnant cows was vaccinated with (Bovilis® Rotavec Corona, MSD Animal Health) and one group of 19 pregnant cows served as unvaccinated control. All animals were blood sampled before vaccination and six weeks later. Colostrum samples were collected within 4 hours after calving. Anti-F5 antibody titers in the colostrum and serum samples were assessed by measuring the inhibition of optical density by competitive enzyme-linked immunosorbent assay (ELISA, BIO K 295 Monoscreen Ab BIO-X Diagnostics, Belgium). The F5 titers were reported in PI (percentage of inhibition). For F41, antibody titers in the colostrum and serum samples were assessed by measuring the relative signal of the samples compared to an assigned reference sample with known anti-F41 titer in an in-house developed ELISA. The F41 titers were reported in Log_2. Descriptive statistics were generated of the average titers reported including the standard deviation and distribution. The mean differences (and 95% confidence interval) between groups in the sera and colostrum were estimated separately. The sera and colostrum samples were analyzed using ANCOVA model.

Results: LC-MS/MS analysis of the purified cell free *E. coli* antigen batches identified two fimbriae, F5(K99) and F41. The F41 identity was confirmed with peptide fragments covering 89.4% of the sequence published for *E coli* F41 (P11900) without the leader sequence. The F5 identity was confirmed with peptide fragments covering 74.8% of the sequence published for *E coli* F5 (P18103) without the leader sequence. Analysis of 13 independent antigen preparations revealed a relative constant F41/F5 ratio of on average 0.67±0.15.

Before vaccination, similar F5 (3.7±6.4 PI) and F41 (4.39±1.23 Log_2) antibody titers were found for both vaccinated and non-vaccinated animals. Six weeks after vaccination, serum antibody titers against F5 remained low (5.8±5 PI) in the control animals but significantly increased (74±23 PI, $p<0.0001$) in the vaccinated animals. Serum antibody titers against F41 significantly decreased (2.55±0.69 Log_2, $p<0.0001$) in the control animals and strongly increased (5.21±1.07 Log_2) in the vaccinated animals. Colostrum antibody titers against F5 were low (4.7±5.4 PI) in the control animals and were significant higher (63.4±25.4 PI, $p<0.0001$) in the vaccinated animals. Colostrum antibody titers against F41 were also significantly higher in the vaccinated animals (9.45±1.04, $p<0.0001$) compared to the control animals (6.32±0.83).

Conclusions. Purified cell free *E. coli* antigen in the multivalent enteric vaccine (Bovilis® Rotavec Corona, MSD Animal Health) expresses both *E. coli* F5(K99) and F41 fimbriae. The ratio of both fimbriae was found constant in 13 independently produced antigen batches. After immunization of pregnant cows, a strong antibody response against both F5 and F41 can be measured in the serum and colostrum of vaccinated animals.

Keywords: Neonatal calf diarrhea, antibodies, vaccination, ETEC, E. coli K99(F5/F41).

IV-P27

Intranasal vaccination of calves with a live vaccine against bovine respiratory coronavirus in the presence of maternally derived antibodies

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Objectives: Bovine Respiratory Disease (BRD), a major cause of calfhood mortality, is a multifactorial disease induced by unfavorable environment factors and a number of microorganisms in the respiratory tract. Bovine Respiratory Syncytial Virus (BRSV), Bovine Parainfluenza 3 Virus (BPIV3) and Bovine Corona Virus (BCV) are generally considered the major viral pathogens in BRD outbreaks in Europe. BCV is also associated with enteric disease.

This study was performed with a live attenuated vaccine against respiratory BCV. As the majority of calves have antibodies against BCV, the immunogenicity of the live attenuated vaccine was studied in calves with maternally derived antibodies.

Materials & Methods: The study was performed in 12 conventional calves at approximately one week of age. The animals were allocated to two groups of six animals each: one unvaccinated control group and one group vaccinated intranasally with the live attenuated respiratory BCV vaccine.

Three weeks post vaccination all animals were challenged with a BCV field isolate by aerosol (using an inhalation mask). The animals were observed for general, respiratory and enteric clinical signs following challenge infection for two weeks.

Multiple samples were collected and tested for the presence of BCV challenge virus.

Results: All animals were positive for BCV antibodies prior to vaccination with comparable average antibody levels in both groups and no obvious change over time throughout the study.

After challenge infection, all animals developed signs of mild to moderate upper respiratory tract disease (URTD), such as nasal or ocular discharge, and coughing. A non-significant, but notable reduction of URTD was observed in the vaccinated group compared to the control group. All control animals developed enteric disease after challenge infection for one or more days, whereas only one vaccinated animal had enteric disease on a single day. The scores for enteric disease were statistically significantly lower in the vaccinated group compared to the control group ($p<0.01$).

All animals shed BCV challenge virus with nasal discharge for at least six days. The duration of nasal virus shedding and maximum viral load in nasal discharge was significantly reduced in the vaccinated animals compared to the controls ($p<0.02$). In addition, the virus load in a post-challenge lung lavage sample was statistically significantly lower in the vaccinated group compared to the control group ($p<0.04$).

In all animals, BCV challenge virus was detected in rectal swabs for at least two days. The duration of virus excretion with feces and maximum viral load in the feces was significantly reduced in the vaccinated animals compared to the controls ($p<0.04$).

Conclusions: From this study in which conventional calves were vaccinated with an intranasal live attenuated BCV vaccine, the following can be concluded:

- The BCV challenge induced clinical signs of respiratory tract disease and enteric disease. The disease scores in the vaccinated group were notably lower compared to the control group, and the differences for enteric disease reached significance.
- Vaccinated animals shed significantly less BCV challenge virus as judged by the lower amounts of virus in nasal and rectal swab samples and lung lavage samples.
- The reduction in clinical signs and virus shedding was comparable as measured in studies with colostrum deprived calves.

Keywords: Intranasal vaccination, bovine coronavirus, maternally derived antibodies.

IV-P28

Co-administration of a plasmid encoding CD40 or CD63 enhances the immune responses to a DNA vaccine against bovine viral diarrhea virus in mice

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Objectives: Bovine viral diarrhea virus (BVDV) causes bovine viral diarrhea (BVD). BVDV infection has caused huge economic losses to the global animal husbandry industry through reduced milk production, abortions, and the decreased lifespan of infected animals. Vaccination is used to control BVDV infection. However, several problems exist in the current BVDV vaccines. Compared with conventional modified live virus and killed virus vaccines, DNA vaccines are more stable, easier to manufacture, and safer during handling. DNA vaccines induce not only humoral immune responses but also cell-mediated immune responses by directly expressing antigens on antigen-presenting cells. However, DNA vaccines for BVDV have not been sufficiently immunogenic on its own. Here, we investigated the molecular adjuvant effect of CD40 and CD63 on the immune responses to a BVDV DNA vaccine in mice.

Materials and methods: We constructed pUMV-C4a-based plasmids encoding the BVDV E2 protein (pE2), mouse CD40 (pCD40), or mouse CD63 (pCD63). Protein expression by each plasmid was assessed through Western blot analysis and immunofluorescence staining of cultured cell lines. BALB/c mice were immunized intradermally twice with pE2 in combination with, or without, pCD40 or pCD63, with 3 weeks between the two doses. After immunization, serum and spleen cells were collected to evaluate humoral and cellular immune responses.

Results: pE2, pCD40, and pCD63 expressed recombinant BVDV E2 protein, CD40, and CD63, respectively, in eukaryote cells in vitro. Virus neutralizing antibody titers were significantly higher in the groups that received pE2 with pCD40 than in those that received pE2 only. In addition, BVDV-specific lymphocyte proliferation and IFN-γ production were significantly higher in the group that received pE2 with pCD40 or pCD63 than in those that received pE2 only.

Conclusions: The results indicate that co-administration of a plasmid encoding CD40 or CD63 enhances humoral and cellular immune responses to a DNA vaccine encoding BVDV E2 protein in mice. Further studies are needed to investigate whether the plasmid encoding CD40 or CD63 enhances immune responses to and protection by the BVDV E2 DNA vaccine in cattle.

Keywords: Bovine viral diarrhea virus, CD40, CD63, DNA vaccine, E2.

IV-P29

Improvement of the natural service conception rate in beef cows after vaccination with a multivalent reproductive vaccine

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Objectives: In Uruguay, reproductive performances in beef cow herds have historically been considered low. Among the causes that may be underlying low reproductive performances, the importance of enzootic infectious diseases is comprehended. Such diseases are communicable and frequently sexually transmitted. They cause genital infections, then infertility, abortions and perinatal losses. Over the years, many reports have pointed out the prevalence of Campylobacteriosis, Leptospirosis, Brucellosis, Trichomoniasis, Neosporosis, Rhinotracheitis, and Bovine Viral Diarrhea in the country. The current study aimed to evaluate the impact of preventing reproductive infectious diseases with multivalent vaccines on beef cows before being naturally mated.

Material and Methods: The authors carried out this study in an 11.000 hectares commercial cow-calf operation of Northern Uruguay with documented low reproductive performances from September 2018 to July 2019. Cattle and sheep have been being herded together for a long and graze on semi-extensive pastures with an average stocking rate of 0,6 head/hectare. Reproductive management of the selected farm has always been natural mating. Two to 4-year-old animals, pure or crossbreed Hereford and Aberdeen Angus, without a history of vaccination against any reproductive infectious diseases, both heifers and cows were enrolled. All animals were healthy, with a body condition score of 2.5-3.5 out of 6. Cattle were eartagged and randomly allocated into 2 groups, G1V, 3/4 cows, and 1/4 heifers received 2 doses of 5 mL SQ of an inactivated multivalent reproductive vaccine (BOVISAN®TOTAL SE [BHV-1, BHV-5, BVDV-1, BVDV-2, Leptospira interrogans ser. Canicola, Grippotyphosa, Hardjo (Hardjo-prajitno), Icterohaemorrhagiae, Pomona, Tarassovi, Wolffi, L.borgpetersenii ser. Hardjo (Hardjo-bovis), Campylobacter fetus ssp. fetus, C.fetus ssp. venerealis, C.fetus ssp. venerealis bio. Intermedius, Selenium 10 mg/dose, Aluminium hydroxide 10 %], Virbac Santa Elena, Uruguay), 21 days apart before the planned start of the natural mating season, and G2C where animals were dosed with 5 mL SQ of saline solution with AlOH 10 %, 21 days apart. All bulls were clinically assessed and tested for venereal diseases (Trichomonas and Campylobacter) one month before breeding. Bulls were introduced in the trialled herd two weeks after the booster vaccination and stayed with the cows for three months and a half. Pregnancy status was assessed by trans-rectal examination with an ImaGo® Veterinary ultrasound scanner equipped with a 5 MHz probe 40 days after the end of the mating period. The Chi-square test was used to compare pregnancy rates, and the significance level was 0.05.

Results: Six hundred twelve (612) unvaccinated females and 24 bulls were enlisted and put together on the same pastures from November 2018 until mid-February 2019. G1V, as well as GC2, consisted of 223 cows and 83 heifers. Pregnancy rates in the two latest mating seasons (2017 and 2018) were 55 % and 68 %, respectively. In 2019, overall pregnancy rates at the end of the mating season were 62,1% ($CI_{95\%}$[56.7;67.5]) and 75.8% ($CI_{95\%}$[71.0;80.6])(P<0.001), in the G2C and G1V groups, respectively. When analyzing the results of cows and heifers separately, it is observed that the pregnancy rate was higher in the vaccinated group for both categories (77.6 vs 63.2% for cows in G1V and G2C, respectively, P<0.001, and 71.1 vs 59.0% for heifers in G1V and G2C, respectively, P=0.104).

Conclusions: The low pregnancy rate in beef cow operations might be associated with infectious diseases. Several studies have been done combining fixed-time artificial insemination with reproductive vaccines, however, to the knowledge of the authors, this is the first study in animals under natural mating. In this study, proper vaccination of naive cows under natural mating as breeding management resulted in a significant impact on pregnancy rates. In countries like Uruguay where natural mating is predominant in cow/calf operations, the use of a multivalent vaccine can contribute to increasing the number of pregnancies, the calf crop rate and the profit of the farm.

Keywords: Beef cattle, abortion, vaccination, Uruguay.

IV-P30

Multimineral (Se, Cu, Zn, Mn) injectable supplementation improves the passive transfer of immunity to calves born from cows subjected to oxidative stress

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Objectives: Oxidative stress (OS) is a term used to describe various deleterious processes resulting from an imbalance between the excessive formation of reactive oxygen species (ROS) and/or reduced antioxidant defences. In cows, the progressive development of OS during the transition period is thought to be a significant underlying factor leading to dysfunctional immune cell responses. The ability to control intracellular ROS accumulation and oxidative stress is a common beneficial effect of supplementing animals with selenium (Se), copper (Cu), zinc (Zn), chromium (Cr), and manganese (Mn), as well as vitamins A and E. Parenteral supplementation with Se, Cu, Zn and Mn was found increasing antibody response to vaccination against bovine herpesvirus type 1 (BoHV-1) and bovine viral diarrhoea virus (BVDV) in calves. The objective of this field study was to investigate the impact of multi-mineral injectable supplementation on the immune response to prepartum vaccination against neonatal calf diarrhoea in dairy cattle.

Material and Methods: This trial took place in the Northwest of France. One hundred thirty-eight pregnant heifers (⅔) and cows (⅓) from 7 specialized dairy herds were enrolled in this study. At the time of vaccination with a multivalent (BoCV, RVA, *E. coli* F5) calf scour vaccine (BOVIGEN®SCOUR, Virbac, France), cows were randomly allocated to a control group (CONT, n=68) or a treatment group (MM, n=70) and dosed with a parenteral multimineral solution (MULTIMIN®, Virbac, France, according to the product label). The immune status against the 3 antigens (various commercial ELISA test kits by Bio-X, Belgium, optical density (OD) expressed as % of inhibition) in vaccinated animals was measured at the time of vaccination (Day 0), and on Day 25±3 after vaccination. The immune status of newborn calves was evaluated between 2-7 days after birth. The oxidative balance status (ROS and serum

antioxidant capacity [SAC]) was assessed in selected dams (n=36), shortly before vaccination. ROS and SAC were evaluated using commercial test kits (Diacron International, Italy) and expressed as CarrU (Carratelli unit) and µmol HClO/mL, respectively. The oxidative stress index (OSi) was as ROS/SAC, as published elsewhere; thus, an increase in the ratio indicates a higher risk for OS due to an increase in ROS production and/or defensive antioxidant consumption. Data were processed using XLSTAT and the level of significance was 0.05.

Results: Vaccination induced a highly significant ($p<0.001$) immune response against the three antigens as measured by the change in OD. Ultimately, the calves' immune status was also considered satisfying, being equivalent (BoCV) or better (*E. coli* F5, RVA, n.s.) than the dams' status on D25, thanks to proper colostrum management. Median OD was higher (n.s.) on D25 in group MM than in group CONT for the two viruses. In calves born from supplemented dams, median OD was higher (n.s.) for the three antigens. Median OSi on D0 was 0.40 (95%CI[0.38;0.43]), higher than reported in previously published works (same methodology). In CONT cows, the initial OSi did not correlate (Pearson's $|r|<0.200$) with the response to vaccination to RVA but had a detrimental impact for *E. coli* F5 (r=-0.356) and the BoCV (r=-0.255). In supplemented cows, the initial OSi had a neglectable ($|r|<0.200$) impact for the RVA and BoCV, whereas the response to *E. coli* F5 changed dramatically compared to CONT cows (r=-0.356 vs r=+0.283, P=0.047). Initial OSi status of the dams had a serious negative impact on the immune status of their offspring. In calves born from mineral-supplemented dams, the correlation between the initial OSi and OD tended to change for BoCV (r=-0.547 vs r=+0.055, P=0.046, CONT and MM calves, respectively), RVA (r=-0.591 vs -0.134, P=0.085) and *E. coli* F5 (r=-0.237 vs r=+0.278, P=0.092).

Conclusion: In pregnant cows vaccinated against neonatal calf diarrhoea prior to calving, the oxidative stress has a detrimental effect on the immune status of their offspring. In this study, injectable trace mineral supplementation slightly improved the immune response to BoCV, RVA and *E. coli* F5, but also mitigated the correlation between the redox status at the time of vaccination and the immune status of newborn calves. Therefore, this study also enlightens the need for further research on the relationship between the redox status and efficacy of therapeutic actions in the transition period of dairy cows.

Keywords: Trace minerals, cattle, oxidative stress, vaccination.

IV-P31

Immunization Japanese Black heifers against gonadotropin-releasing factor (GnRF) improves carcass characteristics and meat quality.

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Objectives: Female cattle with suppressed estrus provide more economic value because their behavior is less aggressive during estrus. Anti-gonadotrophin-releasing factor (GnRF) vaccine suppresses the hormonal action of GnRF and animals can avoid estrus immunologically.

The suppression of GnRF can reduce accidents during the fattening period and deterioration of carcass quality due to less aggressive actions of the cattle and will bring economic benefits for beef cattle producers.

To the authors' knowledge, there are no studies reporting the effects of anti-GnRF vaccination on Japanese Black female cattle carcasses. The efficacy of anti-GnRF vaccination on carcass score of Japanese Black heifers is evaluated and reported here...

Material and Methods: An experiment was conducted in a farm in Kagoshima prefecture, Japan. Forty Japanese black heifers were randomly separated into two groups of 20, one was treated with anti-GnRF vaccine (Bopriva®, Zoetis Japan) and the other were untreated controls. These cattle were fattened by common procedure at the farm, 585 days on average in pens of four. After the fattening period, they were shipped to a slaughterhouse and carcasses were evaluated.

Animals were injected twice at an interval of three to four weeks with 1ml of Bopriva® subcutaneously in the neck to suppress the hormonal action of GnRF through immunological mechanisms mediated by immunogen of 2-10-GnRF/diphtheria toxoid. The 1st dose of Bropriva was administered at 565 days old, followed by 2nd dose at 590 days of age on average.

Meat and carcass quality outcomes measured included: beef quality grade graded by Japan Meat Grading Association; the number of hard spots and internal hemorrhages in carcasses; shipping weight; daily gain in fattening period; carcass weight and carcass yield. The results were statistically analyzed using Fisher's exact test.

In Japan, beef traceability is mandated by law and all cattle are identified for food safety reasons, using this system, cattle information was individually managed.

Results

1. Grading

   In the treated group, 8 of 20 (40%) animals were graded the most premier value beef grade (A5), compared with 3 of 20 (15%) in the control group (P=0.07).

2. The number of hard spots and internal hemorrhages in carcasses.

   In the treated group, 2 of 20 (10%) animals had hard spots and internal hemorrhages present in carcasses compared with 8 of 20 (40%) in the control group (P=0.06).

3. Weights

   As average, Shipping weight was 736.4kg in experimental group and 705.9kg control group, daily gain in fattening period is 0.77kg and 0.75kg, carcass weights are 472.9kg and 455.0kg and carcass yield is 64.4% and 64.4% respectively.

   Regarding shipping weight and daily gain in the fattening period, the treated group performed numerically better than the control group, no statistically significant differences were observed.

Conclusion: Japanese Black heifers treated with anti-GnRF vaccine showed higher incidence premium grade (A5) carcasses. Some possible explanations include the avoidance of losing appetite related with estrus; increasing feed conversion by relaxed behavior; and reducing of wasting energy by aggressive actions. There are previous reports that mentioning that castration through ovariectomy which was practiced in some traditional rearing system contributes to improvement of meat quality, thin subcutaneous fat, high marble grade and brighter color but the reason is still unclear and needs further research.

The total incidence of the hard spots and internal hemorrhages in carcasses were lower in the treated group than the control. This may be due to the suppression of estrus and reduced aggressive actions. Cattles in the control group is less aggressive and easier to handle. This is one of the important advantages for workers in the farm from workplace safety viewpoint.

Due to the fact that ovariectomy is a traumatic procedure, this research demonstrates the potential economic merit of using an anti-GnRF vaccine as an animal welfare friendly alternative on Japanese Black heifers. Further research on larger groups is required to further demonstrate the effect and quantify the return on investment.

Keywords: Estrus, Japanese black, carcass characteristics, meat quality, Bopriva.

IV-P32

The effect of pegbovigrastim injection on oxygen metabolism of peripheral blood granulocytes in *Mycoplasma bovis* affected calves

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Objectives: Bovine granulocyte colony stimulating factor (bG-CSF) is a naturally occurring cytokine which affects some functions of leukocytes. Previous data showed some prophylactic properties of modified form of bG-CSF – pegbovigrastim (PEG bG-CSF) in preventing clinical mastitis in cows, however studies in calves have not been conducted so far. The aim of this study was to determine the effect of PEG bG-CSF injection on oxygen metabolism of peripheral blood granulocytes in calves infected with *Mycoplasma bovis*.

Materials & Methods: The study was carried out in accordance with the unified requirements of the Local Ethics Committee on Animal Experimentation, which also meet the EU standards. The study was performed on calves divided into three groups: experimental (E) and two controls: positive (PC) and negative (NC). The E group was injected with PEG bG-CSF once and then repeated after 7 days. On day 7 post the PEG bG-CSF injection the E and also the PC group were infected with the *M. bovis* field strain. The NC group received PBS. Blood samples were collected just before the first injection of PEG bG-CSF and on days 2 and 4 after this injection, on day 7 (just before the second PEG bG-CSF injection), and then on days 2, 4 and 7 post this injection and every 7 days up to week 3 after the second injection. In the blood samples oxygen metabolism of granulocytes was assayed according to the manufacturer's instruction and analysed using flow cytometer. The oxygen metabolism of peripheral blood granulocytes was determined as the percentage of cells activated by *E. coli* as well as mean fluorescence intensity (MFI) for the measurement of phagocytic activity of granulocytes. The differences between the mean values recorded in the experimental and control groups at the same time point were analysed using t test with a statistically significant level of $P < 0.05$.

Results: The analysis of oxygen metabolism of peripheral blood granulocytes showed a significantly higher percentage of activated cells on day 4 and 7 post the first injection of PEG bG-CSF as compared to the NC group. Additionally, on day 4, the significantly higher values than in the NC group were recorded for the PC group. Post the second injection of PEG bG-CSF a significant increase in the percentage of activated cells was observed on days 4 when compared to the both control groups despite subsequent *M. bovis* infection. At the same time the significantly higher values than in the NC group were also recorded for the PC group. The oxygen metabolism of peripheral blood granulocytes expressed as MFI rapidly increased in response to the first injection of PEG bG-CSF and reached a statistically significantly higher values on day 2 after the cytokine administration compared to the both control groups. The second injection of PEG bG-CSF caused a further increase in the MFI which reached a significantly higher value on day 4 when compared to the both control groups despite subsequent *M. bovis* infection.

Conclusion: In response to the injection of PEG bG-CSF in calves a general increase in the oxygen metabolism of peripheral blood granulocytes was observed. This effect was also shown despite subsequent infection with *M. bovis* which may indicate a beneficial effect of PEG bG-CSF on the innate immunity of the animals.

Keywords: Calves, pegbovigrastim, Mycoplasma bovis, oxygen metabolism, granulocytes.

IV-P33

Saccharomyces boulardii CNCM I-1079 improves vaccine response to *Histophilus somni*, *Pasteurella multocida*, and *Mannheimia haemolytica* in young Japanese Black calves: a field trial

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Objectives: This study was aimed at evaluating the supplementation of the probiotic strain *Saccharomyces boulardii* CNCM I-1079 ("ProTernative," Lallemand-Biotech Co., Ltd.,

Tokyo) as a potential method to enhance vaccine efficacy in young Japanese black (JB) calves in the field.

Materials and methods: Healthy young JB calves in a commercial farm served as subjects for this study. All calves were born in the farm and were grown with their dams from birth to 2 weeks of age. After the dam–calf separation, the *S. boulardii*-group (SB group) calves (n = 15) were fed 2.0 × 10^{10} CFU/day of *S. boulardii* CNCM I-1079 in a milk replacer, whereas the control-group calves (n = 10) were only fed milk replacer. All calves received primary intramuscular immunization at the age of 3 weeks and booster immunization 3 weeks later. The vaccine contained inactivated antigens of *Histophilus somni*, *Pasteurella multocida*, and *Mannheimia haemolytica* ("Cattlebact3," Kyoto Biken Laboratories, Inc., Uji, Japan). The blood samples were collected at each immunization and 3 weeks after the booster immunization. Antibody titers and cytokine mRNA expressions of interleukin (IL)-4, IL-10, IL-12, IL-17A, and interferon-gamma in peripheral blood mononuclear cells (PBMCs) were investigated. Antibody titers above the cutoff values (*H. somni*: 0.604, *P. multocida*: 100, and *M. haemolytica*: 200) were determined to be high enough to offer protection. The calves with antibody titers above the cutoff at the final blood sampling were categorized as positive for each of the three bacteria.

Results: Three weeks following the booster vaccination, the SB-group calves showed a higher antibody titer against *H. somni* than the control calves. The number of *M. haemolytica*-positive calves in the SB group was significantly higher than that in the control group. Additionally, the mRNA expressions of IL-4 and IL-10 in the PBMCs at the booster immunization of the SB group were significantly higher than those of the control group.

Conclusions: In young JB calves, supplementation with *S. boulardii* CNCM I-1079 is likely to improve the antibody response to the inactivated bacterial vaccine with increased mRNA expression of cytokines involved in humoral immunity. The findings from the present study suggest that *S. boulardii* can provide a promising means of improving the vaccine efficacy in young calves.

Keywords: Bovine respiratory disease, S. boulardii, early vaccination, cytokine expression.

IV-P34

Study the safety and efficacy of different IBR marker vaccines in Buffalo species

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Objectives: Both buffaloes and cattle are known to be susceptible to heterologous infection by Bovine alphaherpesvirus 1 (BoHV-1) and Bubaline alphaherpesvirus 1 (BuHV-1), which means that these viruses have undergone a process of "adaptation" to ensure a higher survival rate in the environment, already using latent infection for this purpose. The presence of Infectious Bovine Rhinotracheitis (IBR) in our territory may have negative implications in the marketing and movement of seropositive animals between different areas; seronegativity to IBR is required for bulls destined to genetic centers, and therefore routine measures for infection control must be adopted, starting from direct to indirect prophylaxis measures (VACCINE PLAN). The presence of this virus in buffalo is not associated with severe clinical signs usually observed in bovine infections, but the importance of this species in the role of BoHV-1 reservoir has been demonstrated, especially in cases where cattle and buffaloes are raised together on the same farm. BuHV-1 has been predominantly associated with subclinical disease in buffalo . Therefore, the present work aimed to test the use of systematic vaccination to evaluate the safety and efficacy of several commercially available single (gE-) and double (gE-,tk-) deletions in the buffalo species to thoroughly study the buffalo immune system during vaccination. In addition, drafting a vaccination protocol allows buffalo species to avoid unjustified restrictions in the context of IBR eradication plans.

Materials and Methods: Two different commercial IBR marker vaccines were used. An inactive marker vaccine with gE deletion was administered intramuscularly; an inactive marker vaccine with gE-thymidine kinase (tk) deletion was injected intramuscularly. Vaccines were administered according to the manufacturers' package inserts.Thirteen 3-month-old buffalo calves lacking neutralizing antibodies to BoHV-1 and BuHV-1 were used. Neither of the two selected herds had ever used a BoHV-1 vaccine, and neither had ever been diagnosed with clinical symptoms attributable to herpes infection. Buffalo calves were transferred to the experimental enclosure at the Istituto Zooprofilattico Sperimentale del Mezzogiorno. Animals were divided into three homogeneous groups (A, B, C). The first group (A) received a live attenuated marker vaccine with double deletion (gE-, tk-); the second (B) received an inactivated marker product with single deletion (gE-); the third (C) was the negative control. Thirty-five days after the first vaccine intervention, all animals underwent challenge infection with a wild-type BuHV-1 strain. Each water buffalo received 5 mL X 10 5.50 TCID50/mL administered intranasally. Nasal swabs and serum samples were collected from all animals at 0, 2, 4, 7, 10, 15, 30, and 63 days post-challenge (PCD). Nasal swab samples were used for two RTPCRs against BoHV-1 and BuHV-1, respectively. Serum samples were tested for specific antibodies by competitive gB-ELISA, gE-ELISA, and virus neutralization assays against BoHV-1 and BuHV-1.

Results: The results showed the neutralizing antibodies (NA) against BuHV-1 with a titer of 1:2 (Groups A, B), and NA against BoHV-1 with a titer of 1:4 (Group A) 14 post-vaccination days. All animals' NA against BuHV-1 titer increased after challenge infection, reaching values from 1:2816 (Group A) to 1: 1:1321 (Group B) on 63 post-challenge days (PCD). On the same days, high NA titres were detected against BoHV-1 with values from 1:1024 (Group A) to 1:608 (Group B). NA against BoHV-1 and BuHV-1 were detected in the controls on 10 PCD

with a titer of 1:2. These titers reached values from 1:256 against BuHV-1 to 1:128 against BoHV-1. Group A evidenced specific gB antibodies 14 PCD, while in Group B, the same antibodies were detected 34 PCD. The gB positivity was detected until the end of the experiments. RTPCR BuHV-1 positivity was detected from 2 to 7 PCD. The vaccinated groups produced specific gE antibodies 63 PCD, while group C developed antibodies to gB and gE at 10 and 15 PCD, respectively.

Conclusion: Regulation (EU) 2016/429 provides for IBR testing using gB and gE ELISA tests and authorizes DIVA vaccination. Currently, there are no specific vaccines for the buffalo species, so vaccines developed for cow were used. The two vaccines tested in the trials can be considered innocuity and efficacy for the buffalo species and can therefore be used in areas where, due to a high prevalence of BuHV-1 infection, it is considered useful to control viral circulation through vaccine immunisation.

Keywords: Water Buffalo, BoHV-1, BuHV-1, marker vaccines.

IV-P35

Immune status of beef cows vaccinated against enterotoxinogenic *E. coli*. Do we measure the right things?

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Objectives: In cattle, neonatal calf diarrhoea (NCD) remains a scourge. Among the recognized control methods, the vaccination of mother cows against the primary pathogens (bovine RVA, BoCV, enterotoxigenic *E. coli*) is a well-documented and effective measure. It takes advantage of the massive transfer of type G1 immunoglobulins (IgG1) between cow plasma and colostrum during colostrogenesis. Besides the clinical benefits (reduction of morbidity, mortality, and epidemiological improvement), the evaluation of the IgG1 concentration using tests carried out on serum or colostrum is a means of evaluating the immune response. Routinely, available laboratory tests are commercial ELISA tests, and one is entitled to wonder whether they are predictive of the level of protection offered by vaccines.

Material and Methods: In three beef cow operations without vaccination history in the Burgundy region (France), 160 Charolais cows pregnant of 245±7 days were enrolled. Prior to the experiment, mineral dietary supplementation of the animals was checked, in particular, that the Selenium supplementation was sufficient. In addition, a coprological examination was performed on pooled samples. Herds suspected of harbouring too many strongyle-infested animals have been treated (IVOMEC®Pour-On, Boehringer Ingelheim AH, France). Animals were randomly blocked by three and vaccinated against major NCD pathogens. The first animal (BS group) received 3 mL of a vaccine against RVA, BoCv, and *E. coli* F5 (BOVIGEN®SCOUR, Virbac, France), the second (IMC group) received 5 mL of a vaccine against *E. coli*, different serotypes, carrying the virulence factors K99, Y and CS31a (IMOCOLIBOV®, Boehringer Ingelheim AH, France) and the last one (BSIMC group) received one dose of each vaccine in order to broaden the vaccine spectrum. The blood of the animals was collected just before vaccination and the colostrum immediately after delivery. The titers of antibodies to *E. coli* F5 and CS31a were measured in serum and colostrum using commercial ELISA tests (Monoscreen AbELISA, Bio-X, Belgium). The results expressed semi-quantitatively from 0 to ++++. Animals with ⩾++ results were considered seropositive. The anti-RVA and anti-BoCV antibody titers were measured in the same way to serve as a control of the immune reaction. Colostrum density was measured using a Brix's refractometer (°B). Clinical data were collected for calves, in particular the appearance of diarrhoea. The stools of diarrheal animals were subjected to an immunochromatographic test (SPEED V DIAR® 5, Virbac, France).

Results: Considering obstetric contingencies and reasons for exclusion, data from 145 cows and their calves were available, with no significant imbalance between the groups (n=48, 48, and 49, for the BS, IMC, and BSIMC groups, respectively). All colostrums had a density ⩾20°B and 137/145 ⩾22°B. The response to vaccination was satisfactory, with colostrums predominantly seropositive (⩾++) to RVA and BoCV in the BS and BSIMC groups. In contrast, the proportion of colostrums seropositive to *E. coli* F5 was 46% (67/145), all groups combined, with no apparent differences between the groups (Chi-2, P=0.605). For *E. coli* CS31a, this proportion was 22% (21/97, IMC and BSIMC groups together, no difference between the groups, P=0.493). In addition, two (2) calves presented with diarrhoea unrelated to the pathogens targeted by the vaccination; both calves survived.

Conclusion: This study confirms the interest in the vaccination of dams prior to calving against RVA, BoCV, and two major ETEC virulence factors (F5 and CS31a) responsible for neonatal calf diarrhoea. However, although a high colostral antibody status after vaccination is satisfactory, this measure probably does not reflect the actual immune status of the vaccinated dams nor their calves after the passive transfer of immunity. In particular, commercial ELISA tests are poor predictors of clinical risk for diarrhoea caused by ETEC. Indeed, the cellular component of immunity against *E. coli* is neglected with serological tests, although it better explains the excellent clinical results observed in calves from vaccinated mothers.

Keywords: Beef cattle, neonatal calf diarrhea, ETEC, K99, CS31a.

IV-P36

Phylogenetic analysis of Korean bovine coronavirus and cross reactivity analysis between vaccine strain BC94 and field strain BR-1 in mice

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Objectives: Bovine coronavirus (BCoV) is a member of the family *Coronaviridae* of the order *Nidovirales*. BCoV causes severe diarrhea, is associated with winter dysentery and respiratory infections in calves and cattle, resulting in significant economic losses. In this study, we performed the phylogenetic analysis for complete spike (S) and hemagglutinin/esterase (HE) genes of BCoV circulated in Korea. We also investigated the antigenic characteristics of strain BR-1 isolated from calf by cross reactivity test and evaluated the potential of the strain BR-1 as vaccine candidate.

Material and methods: Total 140 calf diarrheic feces were collected from eight provinces in South Korea. Total RNA was extracted from the feces using Qiagen RNeasy kit and the cDNA were synthesized by Helix Cript Easy cDNA synthesis kit. S and HE genes were amplified by each specific-PCR. The multiple sequence alignments and phylogenetic analysis were carried out using Bio Edit Sequence Alignment Editor version 7.2 and Mega 6 program. To evaluate cross reactivity between the Field strain BR-1 and vaccine strain BC94, mice was intramuscularly inoculated with inactivated BR-1 ($10^{5.0}$ $TCID_{50}$/mL) and inactivated BC94 ($10^{5.0}TCID_{50}$/mL), respectively. To select optimal adjuvant, inactivated BR-1 strain vaccines containing 3 different types of adjuvants (Carbopol, Montanide01 or IMS1313) were prepared and each vaccine intramuscularly inoculated to mice.

Results: In the phylogenetic analysis for spike genes, the Korean BCoV strains belong to group Ia and – Ib, but the vaccine strain BC94 used in South Korea belong to group III. In the HE phylogenetic analysis, the vaccine strain BC94 and the Korean BCoV strains were also included in different clusters. The serums immunized by the vaccine strain BC94 showed high antibody titers against the homologous virus, but it showed a relatively low antibody titers against the strain BR-1. The inactivated BR-1 vaccine with Montanide01 induced highest antibody titers, so Montanide01 was confirmed as most optimal adjuvant.

Conclusion: In this study, the vaccine strain BC94 and Korean wild strain BR-1 were included in the different genotypes and poor cross reactivity was identified between these two strains. Thus, it may be required to develop a new BCoV vaccine against recent circulating BCoV strains in Korea and the strain BR-1 is expected as a potential vaccine candidate.

Keywords: BCV, COW, DIARRHEA, ANTIBODY.

IV-P37

Immunogenicity of an inactivated bovine viral diarrhea virus 1 and 2 vaccine in calves

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Objectives: Bovine viral diarrhea virus (BVDV) is a single-strand, positive-sense RNA virus belonging to the genus *Pestivirus* within the family *Flaviviridae*. BVDV is widespread among cattle and cause significant economic losses to the livestock industry. In South Korea, although BVDV type 1 and -2 strains are the predominant genotypes, only the BVDV type 1 vaccine is being used in South Korea. Due to the genetic and antigenic diversity of BVDV, multivalent vaccines are recommended to prevent infection of various field strains. In this study, the immunogenicity of an inactivated vaccine containing Korean strain BVDV-1a and -2a with different antigen dosages was evaluated by virulent BVDV challenge.

Material and methods: Four-month-old calves were intramuscularly vaccinated two times with three different doses ($10^{4.0}TCID_{50}$/ml, $10^{5.0}TCID_{50}$/ml, and $10^{6.0}TCID_{50}$/ml) and control group calves were not inoculated. The animals were boosted at 3 weeks after the 1st vaccination. At 2 weeks after 2nd vaccination, all animals were challenged with virulent BVDV-1 and -2. Clinical sign and leukocyte count were checked. For virus shedding, viral antigens were detected from nasal and rectal swabs by specific RT-PCR and collected serums were tested for virus-specific antibody by serum neutralization test.

Results: The average neutralization antibody titer to BVDV-1a and -2a of calves immunized with $10^{6.0}TCID_{50}$/ml/dose was the highest among the vaccinated groups and the antibody titer peaked at 14 days post the 2nd vaccination. After the challenge, the calves immunized with $10^{6.0}TCID_{50}$/ml/dose showed less clinical signs (fever, leukopenia, etc.) and significantly low viral shedding compared to the control calves.

Conclusion: The virus-specific antibody induction following immunization was strongly correlated to the antigen dose in this study. The challenge test results suggested that the inactivated BVDV (-1 and -2) vaccine at high dose may be helpful to prevent BVDV-2 as well as BVDV-1, together.

Keywords: BVDV, COW, VACCINE, ANTIBODY.

IV-P38

Efficacy of prepartum vaccination against neonatal calf diarrhea in Nelore cattle

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Objectives: The aim of this study was to evaluate the efficacy of prepartum single dose vaccination against NCD, especially Bovine Rotavirus type A (BoRVA) and Bovine Coronavirus (BCoV), in Nelore dams and offspring. To the best of our knowledge, studies regarding the effectiveness of prepartum vaccination in beef cattle for prevention of NCD are scarce; furthermore, this research is the first in Nelore's breed.

Material & Methods: A total of 117 pregnant cows (n= 81) and heifers (n=36) were randomly distributed in two groups, vaccinated (VAC: cows=40; heifers= 19) and non-vaccinated (NVAC: cows= 41; heifers= 17). Vaccination occurred between 60 to 50 days before the expected calving date with a single dose (2ml, i.m) of a vaccine containing inactivated BoRVA, BCoV and *E.coli* antigens (Bovilis® Rotavec® Corona, MSD Brasil), and NVAC group received a single dose of saline solution 0.9%. Blood samples were collected immediately before vaccination and 30 days after to evaluate the antibody (Ab) response. Calves' births were monitored, and the passive transfer of maternal Abs was evaluated between the second and third day of life. Diarrhea was monitored in the first 30 days of age, and fecal samples were collected for identification of the etiological agent in diarrheic calves. Specific IgG1 Abs against BoRVA and BCoV were measured by using a in-house antibody Enzyme Linked ImmunoSorbent Assay (ELISA).

Results: The statistical analysis of serological response against BoRVA and BCoV from dams revealed an effect of group, time and interaction group*time. The mean serological response at 30 days after vaccination showed significantly ($P<0.0001$) higher IgG1 Ab titers against BoRVA and BCoV in the VAC group compared to the NVAC group. Regarding the calves, there were no significant differences ($P>0.05$) between groups for the total protein (TSP) concentration and the success of the passive immune transfer. However, the titer of specific IgG1 Abs in the calves' serum reflected the dam's response, observing higher IgG1 Ab titers against BoRVA ($P<0.0016$) and BCoV ($P<0.0095$) in the offspring born to VAC cows and heifers. A statistically significant ($P<0.05$) difference in incidence of diarrhea was observed in the calves born to the VAC and NVAC group, of respectively 18.6% (11/59) and 29.3% (17/58). Among diarrheic calves, the most prevalent agent detected with PCR was BoRVA.

Conclusion: Prepartum vaccination with a single dose of the vaccine tested increased the titers of IgG1 Ab against BCoV and BoRVA resulting in a significant decrease of NCD occurrence in Nelore calves.

Keywords: Bovine Rotavirus, Bovine Coronavirus, calves, Serological response, Nelore.

IV-P39

Colostrum quality and management effect on dairy cattle health

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Objective: To study the effect of colostrum quality and management on dairy cattle health and increase awareness among farmers.

Materials and methods: Colostrum quality and passive immunity transfer (PIT) were measured in four dairy cattle farms in Northern Spain. For this purpose, fresh colostrum samples were collected from 7-10 calvings cows per farm along with blood samples from newborn calves at 24-48 hours of age. When animals reached the age of *ca.* 3 months, blood was extracted from the same animals to assess immune cell populations. Farmers were asked to estimate colostrum IgG concentration with a portable digital refractometer, feed sufficient colostrum volume immediately after birth and record all infectious episodes and antibiotic treatments. At the laboratory, colostrum and serum IgGs were estimated by digital refractometry and measured by radial immunodiffusion (RID). Mononuclear cells from colostrum (CMCs) and mononuclear cells from peripheral blood (PBMCs) of heifers were isolated by density gradient and analysed by flow cytometry to assess T lymphocyte populations (CD4, CD8 and gd).

Results: Correlations were observed between in-farm and laboratory IgG refractometry measurements ($p<0.0001$) and between digital refractometry and RID ($p<0.0001$) measured in the lab for colostrum. Also, a positive correlation was observed between IgG refractometry and RID measurements in serum ($p<0.0001$) when data from all farms was included in the analysis. Differences were observed between farms in colostrum quality and PIT. Colostrum IgG content ranged between 8.8-35.6 % Brix and 3.8-90.3 mg/ml, whereas serum IgG content ranged between 6.6-11.3 % Brix and 2.3-49.8 mg/ml. Although colostrum quality was not always good (40% with > 22% Brix or > 50mg/ml IgG), successful PIT (> 9 mg/ml) was achieved in most cases (60%) indicating that factors other than immunoglobulin concentration can influence a positive transfer outcome. Lymphocyte T sub-populations showed differences among animals in both colostrum and blood.

Conclusions: Farmers can easily and efficiently measure IgG content in colostrum with digital refractometers to aid feeding strategies and enhance passive immunity transfer. Refractometry may slightly overestimate effective PIT in serum of calves. Associations with colostrum quality, PIT, lymphocyte T cell populations and health status of heifers between farms will be presented and discussed.

Keywords: Colostrum, passive immunity transfer, lymphocyte subpopulations.

IV-P40

A prevalence survey to understand the excellence of transfer of passive immunity (ETPI) of calves across Europe, using on-farm Brix refractometry

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Denmark; [11]Boehringer Ingelheim Animal Health Austria, Vienna, Austria; [12]Boehringer Ingelheim Animal Health META, Dubai, United Arab Emirates; [13]Boehringer Ingelheim Schweiz, Basel, Switzerland.

Objectives: The objective of this prevalence survey is to evaluate the effectiveness of transfer of passive immunity of calves under 1 week of age across Europe, using a Brix refractometer as an indirect measurement of IgG, as described by Deelen *et al.* (2014). This will provide a broad view on variation within and between farms and countries, when it comes to excellent transfer of passive immunity (ETPI) through colostrum feeding.

Materials and methods: The prevalence survey is being undertaken during the spring calving season in April 2022. The survey will include between 3 and 5 farms, in each of 10 countries across Europe. Each farm will include up to 10 calves, between 2 and 7 days of age.

Whole blood will be obtained either through jugular venepuncture or caudal coccygeal venepuncture from calves by the attending veterinary surgeon, using a 20-gauge, 1-inch hypodermic needle and sterile vacutainer tube without coagulant. The samples (6ml) will either be left to settle in a refrigerator or be spun down to provide serum, 2 drops of which will be placed on the clean platform of an optical Brix refractometer.

A reading will then be obtained through the eyepiece which will be recorded in an MS Excel spreadsheet, along with production type, breed, weight, age and sex of calf. The time of birth, time of initial feeds, volume and quality of colostrum fed, and whether fresh or frozen and method of feeding will also be recorded.

The Brix % reading will then be converted and categorised to an IgG band according to those proposed by Godden *et al.* (2019).

Results: It is anticipated that less than 40% of calves will achieve levels of IgG defined as 'Excellent' by Godden *et al.* (2019), with serum IgG being <25g/l, and more than 10% of calves classed as 'Poor', with serum IgG <10g/l. These levels were recommended through consensus of calf health experts following review of IgG levels associated with morbidity data (Godden *et al.*, 2019).

Conclusions: Results are expected to show that transfer of passive immunity within herds shows large variability, with variation within herds being as great as variation between herds. Levels of IgG are expected to be below levels required for excellence in calf rearing.

This paper will provide unique insight into the current status of calf immunity across a large number of countries. It will highlight this readily available calf-side tool to assess a key indicator of the future success of the calf and will support the role of the veterinarian in advising farmers on transfer of passive immunity through more widespread use of on farm testing, to achieve excellence in calf rearing.

Keywords: Colostrum, Passive Transfer, FTPI, ETPI, Immunity.

IV-P41

Towards a Vaccine for Malignant Catarrhal Fever

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Introduction & Objectives: Ovine herpesvirus-2 (OvHV-2) is the causative agent of sheep associated malignant catarrhal fever (MCF), a generally fatal disease with a wide range of hosts including but not limited to cattle, buffalo and bison. Sheep largely present asymptomatically so act as a reservoir for OvHV-2. Viral transmission to MCF susceptible hosts in mixed farming systems and thereby sporadic MCF is a constant threat to food production, particularly during periods of stress when virus is shed more frequently e.g. around lambing. No specific treatments exist and empirical treatments are largely ineffective. Currently there is no vaccine licensed for the prevention of OvHV-2, although the urgent need is recognised.

Large animals are physically and financially impractical for modelling disease in vivo, making controlled vaccination trials implausible. The susceptibility of rodents to OvHV-2 has been previously described (Buxton et al. *J. Comp. Path*. (1988) **98**, 155.). The objectives of the study were:

- Develop a model for infecting golden Syrian hamsters with OvHV2.
- Describe the pathological changes associated with OvHV2 infection in hamsters.
- Investigate the efficacy of a novel modified vaccinia Ankara (MVA) vector vaccine previously designed by our lab, in hamsters against OvHV2 infection.
- Assess the antibody response of bison to the MVA vaccine.

Methods: Syrian golden hamsters (*Mesocricetus auratus*) were infected intra-peritoneally with spleen and lymph node cells from a hamster previously infected with OvHV-2. Body temperature and behaviour of the hamsters was monitored daily. Hamsters were culled following a sustained increase in temperature, and/or clinical signs including respiratory distress.

Lungs, spleen and lymph nodes were collected and analysed by histopathology and OvHV-2 quantification by qPCR.

A second cohort of hamsters was given either phosphate buffered saline (PBS) (as a control), or OvHV-2 vaccine, then challenged with OvHV-2. Again, they were monitored daily and culled following a sustained increase in temperature and/or clinical signs. Tissues and blood were collected for histopathological examination, OvHV-2 quantification by qPCR and antibody titre by ELISA.

A mixed species farm with bison, cattle, sheep, poultry and deer was involved in the study. The novel vector vaccine was administered twice, one month apart, to bison already on farm. Blood samples were taken for antibody titre by ELISA before vaccination as well as one month and one year post vaccination.

Results: Hamsters readily became infected with OvHV-2 when inoculated with lymphatic tissue cells from another hamster infected with OvHV-2. Clinical signs developed 30-40 days post infection, including a sustained temperature increase, swollen feet, respiratory distress and/or scruffy coat. The post mortem examination revealed splenomegaly and enlargement of Peyer's patches. Histologically, vasculitis of pulmonary vessels was the most consistent finding. OvHV-2 viral load varied across different tissue samples.

No adverse reactions were observed in either the hamsters or the bison following vaccination with the MVA construct. Bison had significantly higher antibody titre to MVA one month post vaccination with our OvHV-2 MVA vaccine than pre-infection. Antibody titres specific to OvHV-2 were not significantly different.

Conclusions: Hamsters provide an affordable, practical option for modelling OvHV-2 disease and can be used to assess vaccine efficacy in a controlled challenge trial. The novel MVA OvHV-2 vector vaccine could potentially aid the control of OvHV-2 in ungulate hosts, however further work is required to elicit a virus specific neutralising antibody titre great enough to protect against OvHV-2 infection.

Keywords: Ovine Herpes Virus-2, Malignant Catarrhal Fever, Bison, Hamster.

IV-P43

Mastitis-associated Mastitis-associated *Staphylococcus aureus* induced PD-L1 expression on macrophage cell line *aureus* induced PD-L1 expression on macrophage cell line

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Staphylococcus aureus is one of the pathogens most frequently isolated from cases of mastitis worldwide. Thus, to decrease the impact of S. aureus mastitis in dairy farming, alternative strategies to control mastitis should be evoked that mainly depend on a better knowledge of protective immunity. Besides that, *S. aureus* mastitis caused by this pathogen is still one of the biggest concerns in dairy herds, because it has a great diversity of evasion mechanisms of the immune system. For this reason, it is well-now that T cells are very important in the response against *S. aureus* infections, as they are responsible for generating memory cells that are able to readily recognize the agent when there is a second exposure, making the immune response faster and more efficient. However, there are a number of regulatory proteins associated with the T cell response, among which the program death receptor (PD-1) and its respective ligand (PD-L1) have stood out. These surface proteins are found in antigen presenting cells such as dendritic cells and macrophages, and act by mediating signaling to the T cell receptor (TCR). Some studies have shown that some bacteria are able to regulate the function of PD-1 and PD-L1 leading to decreased immune response. Interaction of PD-1 with PD-L1 can induce T cell exhaustion, which is characterized by loss of effector functions, decreased proliferation, and apoptotic cell death. Thus, here we evaluate the induction of PD-L1 expression in murine macrophage cell line by two dissimilar bovine-associated *Staphylococcus aureus* strains. For the present study a macrophage cell line, so-called raw cells, was used. These cells were cultivated in RPMI medium supplemented with 20% of fetal bovine serum and 2% of antibiotic-antimycotic solution. The cells were adjusted to a concentration of 2.5×10^5 per well in 24-well plates, and afterwards the cellular suspension was incubated for 1 h in CO_2 chamber at 37ºC to adhere to the plate. After the incubation period the cells were challenged with two dissimilar representative *S. aureus* bovine-associated strains, one isolated from persistent intramammary infection (IMI) and the other from muzzles/nozes using two different concentrations [multiplicity of infection (MOI) 1:1 and MOI 1:5]. An unstimulated control was also used. Furthermore, for the present study two incubation times (3 and 18 h) in CO_2 chamber at 37ºC were used. The expression of PD-L1 was determined using PE Rat Anti-Mouse CD274 antibody and the sample readings were performed using a FACSCalibur ™ flow cytometer. For this assay, 30,000 cells were examined in each sample. Flow Jo Tree Star software (Becton, Dickinson and Company, Oregon, United States) was used to analyze the data. Here, we observed no statistical difference after 3 h of challenge. However, after 18 h of incubation a higher percentage of macrophages expressed PD-L1 when *S. aureus* isolated from persistent IMI (MOI 1:1 = 43.68 $\pm$ 0.98; MOI 1:5 = 49.98 $\pm$ 1.34) was used when compared with *S. aureus* isolated from an extramammary niche (MOI 1:1 = 32.27 $\pm$ 1.30, P = 0.005; MOI 1:5 = 32.92 $\pm$ 0.71, P = 0.0005) and unstimulated control (basal; 33.22 $\pm$ 0.67, P = 0.002 for MOI 1:1, and $P \leq 0.0001$ for MOI 1:5) independently of the MOI used. Thus, our study showed that *S. aureus* modulation of PD-L1 is strain dependent and time-dependent, and *S. aureus* isolated from bovine mastitis can negatively modulate T cell response by inducing PD-L1 expression in macrophages.

Keywords: Mastitis, Staphylococcus aureus, immunocheckpoint inhibitor, flow cytometry, dairy cattle.

IV-P44

Is vaccination against endemic dairy herd diseases economic or not?

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Objective: The most commonly adopted biosecurity practice on most dairy farms internationally is vaccination; however, even this practice is not universal. One of the barriers to farmer adoption of such biosecurity practices is the lack of economic data underpinning their cost-effectiveness. Hence, this research explored Irish dairy farmers' adoption of biosecurity practices and compared economic outcomes of vaccine adopters and non-adopters.

Materials and methods: In order to establish biosecurity practice adoption rate, supplementary questions were added to the 2019 on-farm, recorder-collected, Teagasc National Farm Survey (NFS). The main survey included questions on farm characteristics such as farm size, input costs and output. We used these NFS responses to compare economic outcomes for farmers who used more than three vaccines (the median) to farmers who used less. We focused on vaccination because this was the most commonly adopted biosecurity practice. In total a nationally representative cross-sectional sample of 267 farmers were surveyed.

Results: The herds surveyed had an average of 90 cows on 68 hectares. The adoption rates for biosecurity practices varied widely with highest adoption rates for vaccination (86%) and lowest for (non-mandatory) testing of purchased cattle (13%), (Table 1).

Table 1. *Distribution of biosecurity practices adopted by Irish dairy farmers.*

Biosecurity practices	%
Vaccinate cattle	86.1
Test bulk tank milk (BTM) for diseases (other than SCC)	64.6
Carry out other biosecurity measures	55.5
Do not pool colostrum (from more than one animal)	45.0
Maintain a closed herd	35.6
Quarantine bought-in cattle[1]	30.2
Test bought-in cattle for diseases[1]	13.4

n=267; [1]percentage refers to farmers who buy in stock.

Biocontainment practices were adopted more commonly than bioexclusion practices. Cattle vaccination is a legacy practice on most dairy farms while testing of BTM is a relatively recent practice promoted by the agri-industry. Despite the national concern about Johne's disease (paratuberculosis), the majority of dairy farmers (55%) still pool colostrum. The relatively low proportion of farmers reporting a closed herd (36%) reflects the current national dairy herd expansionary era. Neither quarantining (30%) nor post-purchase testing (13%) were widely adopted despite being low cost practices. Very few farmers (4.5%) sent their heifers out to be contract-reared.

Dairy farmers were more likely to vaccinate against cow diseases than calf diseases. The high vaccination rates against leptospirosis and IBR reflect legacy practice and the recent Animal Health Ireland-IBR programme, respectively.

The empirical analysis of the economic impact of vaccination revealed that, on average, farmers who vaccinated their cattle against more than three diseases achieved €78 higher gross margin/cow (GM/cow) when compared to dairy farmers who vaccinated against one or no diseases. Additionally, farmers who vaccinated their cattle against two or three diseases achieved, on average, €67 higher GM/cow compared to farmers who vaccinated against one or no diseases. This suggest that, the higher the number of diseases farmers vaccinated against, the higher the economic gains from such vaccination measures. These significant effects were found after controlling for regional characteristics, farm management ability, farmer's attitude to risk and farmer characteristics.

Conclusions: Biocontainment practices (preventing disease spread within farm) were more commonly adopted than bioexclusion practices (preventing disease spread into a farm) on Irish dairy farms. Vaccination was the most common, and testing bought-in cattle the least common, biosecurity practice adopted. Vaccination was significantly associated with better economic outcomes for dairy farms.

Keywords: Dairy herd, Diseases, Vaccination, Economics.

LA-P01

Frequency of hoof disorders in dual purpose cattle in Cordoba department, Colombia

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Objetives: A podal upheaval is all present alteration in the bovine hoof, of structural, functional or infectious type, that can or not ends with a halt. The animal production systems in many parts of the Córdoba department, show a high incidence of pedal alterations, most of the lameness of the bovine cattle have their origin in the hooves, mainly caused by pedals upheavals; for this reason, every day is more important for the Veterinary Doctor to have the necessary knowledge about the main affections that afflict the extremities of these animals and how to face them in a convenient way. The present work was made with the purpose of determining the frequency of pedal upheavals in double purpose bovines in the Córdoba department.

Materials and methods: A study was done of cross section using 170 greater cows of three year selected at random in different property which were evaluated by means of the accomplishment of the clinical examination of the hooves, determining normality or then abnormality that these displayed and in agreement with the found injury, it was identified and characterized.

Results: The frequency of pedals upheavals in Córdoba department was of 64.70% of the total of the studied sample. 30.59% of the studied animals displayed alteration of the relation 2:1 wall-heel and 47.05 % displayed some type of podal pathology. The found pedals pathologies were: Hoof in corkscrew (36.47%), digital granuloma (17.06%), septic pododermatitis (4.71%) and interdigital callus (2.35%) the incidence of lameness was of 78.75% with respect to the animals that displayed pathologies and 37.05% with relation to the total of the sample.

Conclusions: In addition was dependency between the type of land with relation to the podal upheaval and independence between podales adjustments and presence of footbaths with relation to the podal adjustment

Keywords: Podology, hoof, lameness

LA-P02

Locomotion score and days open in Jersey cattle in the humid tropics

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Lameness is reported as the third cause of discard in cattle around the world and it is directly related to reproductive and productive performance. Systematization of lameness examination has resulted in locomotion score, a qualitative analysis that lets to evaluate the presence of lameness and its effects on the animal locomotion from a more accurate perspective. Different studies have shown the influence of locomotion score in reproductive health during the oestrus cycle, pregnancy and puerperium, however, the conception post-partum seem to be the most affected reproductive parameter. In order to analyse the relation of locomotion score and days open in Jersey cattle in the humid tropics, 53 milking cows from 2 to 8 years old were scored once (1 non-lame, 2 slight-lame, 3 evident-lame, 4 moderate-lame and 5 severe-lame) for an expertise unique evaluator and scores were confirmed by using a locomotion score software. A number of inseminations and days open were followed. Further, interval of days from hoof treatment to conception in 3, 4 and 5 scored cows was also registered. Each reproductive variable was related with locomotion score through an ANOVA test and a Tukey test was applied to analyse significant differences between each locomotion score and those variables. A significant relation between locomotion score on number of inseminations, days open and interval of days from hoof treatment to conception was found, $P< 0.000$; $P<0.000$; $P<0.009$, respectively. Number of inseminations was similar in 1 to 3 and 5 scored cows, 3,1 ± 0,9; 3,2 ± 1,6; 4,3 ± 2 and 3,2 ± 1,6, respectively, but significant influence of 4 scored cows was observed, resulting in 7,5 ± 2,9 inseminations. No significant differences of days open were found in 1 to 3 scored cows (142,9 ± 43,9; 158,3 ± 61,9; 3 and 230,7 ± 95,7), but days open were significantly larger in 4 and 5 scored cows, 287,9 ± 94,3 and 383 ± 91,3, respectively. With respect to interval of days from hoof treatment to conception, a shorter time was observed in 3 scored cows (85, 6 ± 58,3), however, a significantly larger time from hoof treatment to conception was found in 4 and 5 scored cows, 136,3 ± 51,4 and 185,7 ± 55,5, respectively. In this study, about three or four inseminations were required to successfully conceive in healthy, slight, evident or severe-lame cows but it was doubled in moderated-lame cows. Otherwise, moderated and severe lameness increased three times the average of days open compared with healthy and slight-lame scored cows. Besides, it is to highlight that evident-lame treated cows were successfully pregnant sooner after treatment compared with moderated and severe-lame cows, in which, a successful conception kept larger even when a hoof treatment had been performed. Days open are considered hidden costs in dairy herd and locomotion score should be considered as a routine analysis to reduce the influence of lameness on the reproductive performance of cattle. Moreover, it should be more taken into account when cattle are raised under tropical conditions where drastic changes of humidity and temperature can rapidly worse a slight-lame cow to advanced stages of lameness, increasing not only the costs related with days open as number of inseminations, drop in milk production and delayed cyclicity but also the costs of hoof treatment.

Keywords: Lameness, reproductive performance, fertility, tropical enviroment.

LA-P03

Prevalence of digital dermatitis using mirror scoring in Finnish dairy herds

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Objectives: Digital dermatitis (DD) is a severe bacterial claw disease found worldwide. The disease can be classified into 5 different M-stages by clinical examination. The aim of this study was to find the herd level prevalence of acute (M2, M4.1) and chronic (M3, M4) DD lesions in Finnish dairy cattle population and test scoring in milking parlors and pens. Prevalence studies concerning DD have not been conducted on national level earlier.

Materials and methods: Altogether 81 randomly selected herds were visited by the project veterinarians. During the farm visit, hind legs (14 020 feet) of all lactating cows were scored for digital skin lesions with the help of a mirror and a flashlight. In addition, 260 feet were inspected in a trimming chute (gold standard) and the results were compared with the results of mirror scoring. Sensitivity and specificity for the tests were calculated based on the comparison.

Results: We found that 32 % of the herds had acute lesions (M2, M4.1) but cow level prevalence was less than 2%. In most farms only 1-2 cows were affected. Sensitivity for scoring acute lesions was low (37.5%), but specificity for the test was very high (98.1%). Sensitivity for scoring any DD lesions was 79.6% and specificity 92.1%. Despite the low sensitivity, none of the herd level classification (DD present, DD absent) changed.

Conclusion: In our study, the prevalence of acute DD lesions was higher than previously thought. Scoring in the pen or milking parlor had low sensitivity but could be used to monitor within-herd DD dynamics regularly. The scoring method could also be used to investigate national prevalence of DD in herd level (DD present, DD absent), but a careful approach should be used when interpreting the results. Education concerning DD is needed for all involved in the cattle field. Measures for controlling and preventing DD in Finland must be introduced without delay.

Keywords: Digital dermatitis, prevalence, mirror scoring, infectious hoof diseases.

LA-P04

Herd and cow level risk factors for different stages of Digital Dermatitis

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In a recent study, risk factors for different DD stages have been investigated separately and the results will be presented at WBC 2022.

Material and methods: Between June 2017 and August 2018, 7 trained WUR and Veterinary Faculty students were trained to collect information about presence and stage of DD on the hind claws of 6766 cows on 88 farms were scored for DD by seven students and a claw health expert, using the M-scoring system (M0 – M4.1). Farms used in this study were selected by convenience sampling, based on their willingness to participate, geographical location and last measured DD prevalence. Data were collected as part of a project to validate a Treponema Elisa by Royal GD in Deventer, The Netherlands. Information like ID and date of trimming were recorded and a questionnaire was completed by the student to acquire information regarding management and housing. Information about breed, parity and previous calving date was obtained from the Dutch herd book organization NRS, after written permission of the farmer.

Statistical analysis: The analysis comprised first an univariable logistic regression analysis using PROC GENMOD (SAS 9.4). This analysis was performed 4 times; each with one of the M-stages (present/absent) as the response variable, except for the M3 stage as only 48 M3 lesions were scored in 13,532 observations, indicating a low prevalence for this stage of DD in the study population. The low prevalence makes it numerically challenging to analyse M3 lesions. Also, this stage is not part of the natural disease progression and only occurs when a footbath was applied.

Results: *Prevalence*

In total 6766 cows on 88 farms were included in the risk factor analysis. The number of records collected per farm varied between 47 and 131 (mean = 87.3; SD = 21.4). From these records, 49.8% of animals were diagnosed with one of the DD stages other than M0 in one or both hind legs. The foot-prevalence for each of the DD stages is shown Table 1.

Table 1. Foot-prevalence of all M-stages of Digital Dermatitis in hind legs of Dutch dairy cows (n=13532).

M-stage	Prevalence	Frequency
M0	61.45 %	8315
M1	1.37 %	186
M2	7.12 %	964
M3	0.35%	48
M4	20.93%	2832
M4.1	8.77%	1187

The prevalence of DD (M1, M2, M3, M4 or M4.1) in the study population was 38.84% and 38.26% for respectively right and left feet (p=0.49).

Risk factors

The results of the risk factor analysis of the different M-stages will be presented at the WBC as given here for the M2 stages.

Table 2. Multivariable analysis of Digital dermatitis stage M2 (n=936) versus stage M0 (n=8030) at foot level.

Variable	Class	Frequency (n)	Prevalence M2 (%)	OR	95% CI		P-value
Manure scraping frequency							
	Rarely	158	2.53	0.30	0.11	0.83	**<0.01***
	1 - 3 x a day	2848	11.31	1.04	0.61	1.77	0.90
	3 - 10 x a day	2723	7.60	0.54	0.29	1.02	**0.06**
	≥10 x a day	3237	12.45	1.0**			
Active compound footbath							
	Footbath not used	3645	9.44	1.08	0.56	2.08	0.82
	Formalin	2240	8.79	1.0**			
	Copper sulphate	170	8.24	0.43	0.10	1.85	0.26
	Other composition	322	20.50	3.00	1.35	6.65	**< 0.01***
	Formalin + copper sulphate	2589	12.17	1.46	0.49	2.83	0.26
Rubber in walking path							
	No	8259	11.04	3.08	1.68	5.66	**<0.01***
	Yes	707	3.39	1.0**			
Age							
	≤2	2521	8.57	1.0**			
	3	2156	12.01	1.41	1.11	1.79	**<0.01***
	4	1579	12.03	1.45	1.16	1.80	**<0.01***
	5	1050	12.10	1.56	1.12	2.16	**<0.01***
	6	696	8.48	1.14	0.85	1.54	0.37
	≥7	964	8.92	1.07	0.74	1.55	0.72

Significant P-value; **reference category; exchangeable working correlation: 0.08

Keywords: dairy cattle, digital dermatitis, risk factors.

LA-P05

First study of dairy cattle hoof pathologies in Ecuador

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The dairy cattle hoof diseases in Ecuador are underestimated by the livestock sector minimizing the real problem affecting the dairy herds, considering the negative effects they have on the production, reproduction and animal welfare. For this reason, we have decided to determine the prevalence of hoof pathologies in discarding dairy cows destined for slaughter, through external observation and serial cuts of front and back limbs in the city of Cuenca-Ecuador. The study is a descriptive, prospective longitudinal cohort, in which 200 distal ends of front and back limbs. Of dairy cows were sampled, obtained at the municipal slaughterhouse of Cuenca city. The samples were cut at the level of the tarsometatarsal joint; the diagnosis of lesions was made by macroscopic observation of claws fixed on a mechanic clamp. Then proceeded to cut serially, transversely and longitudinally, with an interval of 5 mm between each cut, in order to measure with a caliper, the depth, extent, and degree of the lesion, the cut was in an anteroposterior direction. The results are as follows, the hoof overgrowth is the first pathology with 61.5 % (123/200); the double sole with 48.5 % (97/200); erosion of the heel was 41.0 % (82/200); white line disease shows 25.5 % (51/200), corkscrew claw with 10.5 % (21/200), digital dermatitis was 4.5 % (9/200); scissor claws only 4 % (8/200), finally ulcers represent 3.5 % (7/200). The study identified the presence of 398 problems in the sample studied, showing 86.68 % (345/398) in comorbidity or interaction with each other, interacting up to 4 pathologies simultaneously in the hoof. According to the injury commitment degree on average of the specimens, the hoof overgrowth presents a high level of affectation, leading this pathology to generate others simultaneously, the white line disease, double sole, heel erosion, hoof ulcers, digital dermatitis, are pathologies that presented moderate level injuries. The back limbs are those with the highest prevalence of pathologies, with 32.1 % for the right limb and 24.8 % for the left limb, the front limbs have 18.7 % and 24.5 % for the right and left respectively. Finally, the lateral claw of the right back limb is the one with the highest frequency of hoof pathologies with 17.8 %, followed by the right back medial finger with 14.4 %.

This study leads us to conclude that hoof pathologies are very common in dairy herds in Ecuador. The high prevalence rates it may be due to the lack of preventive trimming, demonstrated in cases of hoof overgrowth, which leads to other pathologies; masking the economic losses they generate, in addition to the lack of specialists in this area, and the limited knowledge of farmers about the importance of hoof problems in dairy cattle production and the factors that predispose them. This conclusion suggests massify specialists in hoof trimming, at the same time to consider livestock research to establish the prevalence of lameness and other hoof pathologies.

Keywords: Dairy cattle, Hoof lesion, Lameness, Claw trimming.

LA-P06

Change starts in mind – Farmers´ awareness towards lameness and associated factors

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Objectives: Lameness is probably the most challenging problem concerning dairy cow health and welfare, nowadays. Several studies have shown that most farmers underestimate the prevalence of lameness in their herds. We assume that this underestimation results in farmers being less motivated to take measures to control lameness. The objective of this study was to gain further insight into the farmers' awareness towards lameness and possible associated factors. Characteristics of the farm, farmers' attitude and education as well as farmers' personality were presumed to potentially influence the farmers' awareness.

Material and Methods: In a cross sectional study approach, trained veterinarians visited 253 dairy farms in the northwest of Germany. Inter alia, locomotion scoring was performed on the dairy cows. Cows with a locomotion score ≥ 3 according to Sprecher et al. (1997) or at least two fulfilled criteria of the Stall Lameness Score according to Leach et al. (2009), respectively, were considered as lame. During a standardised face-to-face interview, the farmers were asked how many cows they estimated not to walk sound or be lame at the day of the farm visit. Information on farmers' attitude and education as well as the characteristics of the farm was collected during the interview, too. The farmers' self-reported personality was assessed using a modified version of the 24-item Brief HEXACO Inventory (BHI; de Vries, 2013). Descriptive analyses and univariable linear regression were performed on farm level.

Results: The mean herd prevalence of lame cows assessed by the researchers (RP) was 25.6 ± 15.3% (median=23.1%, n=251). Mean prevalence of lame cows estimated by the farmer (FP) was 12.5 ± 11.9% (median=9.3%, n=253). The concordance correlation coefficient (CCC) between RP and FP was 0.34, indicating that the self-assessment of the farmers was improvable. The mean Farmers' Detection Index (FDI=FP/RP*100; Leach et al., 2013) was 55.2 ± 50.4% (median=42.0%, min=0%, max=458.3%, n=242). Hence, on average, farmers were only conscious of every second lame cow in their herds.

Significant differences were apparent concerning the characteristics of the farms: Farmers of organic farms (n=10) had a better estimation of the lameness situation in their herds (FDI: mean=102.4% ± 89.4, median=83.8%, p=0.0023) than farmers of conventional farms. Farmers who kept their cows mainly in straw yards or on pasture had a higher FDI (mean=99.0 ± 87.7%, n=11) than farmers who kept their cows mainly in freestall barns (mean=53.5 ± 47.8%, n=210). Farmers who kept their cows mainly tied (n=5) had a lower FDI (mean=22.0 ± 26.8%) than farmers with a freestall barn. The more cows a farmer kept, the lower was the FDI (log of herd size: regression coefficient -14.5, p-value=0.0055). Concerning the HEXACO personality domains (emotionality, extraversion, agreeableness, conscientiousness, openness to experience) and the farmers' attitude (health status of the herd, workload, and other items), no significant differences were apparent concerning the FDI. Farmers with a higher degree of education tended to have a higher FDI. Surprisingly, farmers who assessed lameness as a separate work step (n=7) and farmers who stated to participate in a veterinary herd health program including lameness control (n=42) had no better self-estimation than farmers who assessed lameness during stable work and farmers who stated not to participate in such herd health programs.

Conclusions: This study provides further evidence on the theory that most farmers considerably underestimate the lameness situation of their herds or define a cow lame differently than veterinarians. Therefore, a first step to reduce the number of lame cows in dairy herds would be the improvement of the farmers' awareness.

Farmers who provided an alternative housing system or ran their business according to organic criteria had a better awareness of lameness. However, due to small sample sizes and potential interaction of influencing variables, further research is required. Awareness seems to decline with larger herds, indicating that farmers might lose track of their cows' health due to higher workloads. However, for the future, education might be an important key to take the first step and help farmers to a better self-assessment of the lameness situation in their herds.

Keywords: Lameness, awareness, farmer, personality, attitude.

LA-P07

Assessment of potassium monopersulfate as a disinfectant solution for footbath to control digital dermatitis in dairy cows

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Objectives: The objective was to assess potassium monopersulfate as a disinfectant used in footbath to control digital dermatitis (DD) in dairy cows. We hypothesized that a potassium monopersulfate solution would control DD.

Materials and methods: A 180-day nonrandomized clinical trial was conducted in a 265-Holstein free-stall facility. Throughout the trial, foot bathing was performed twice weekly using a split (left *vs.* right feet) footbath: one tub filled with 1% potassium monopersulfate (treatment), the other with tap water (control). DD lesions were scored during trimming chute examinations of the unwashed hind heels (sampling units) every 90 days using the modified M-scoring system.

DD lesions were re-categorized into four variables: 1) inactive; 2) active; 3) any; 4) inactive or no DD lesions. Three longitudinal outcomes were characterized: risks of 1) developing a DD lesion; 2) reactivating an inactive DD lesion; 3) curing a DD lesion. A generalized linear model was used to compare the variables and longitudinal outcomes between treated and control groups.

Results: Prevalence of active DD lesions increased from 12.5% to 39.9% between days 0 and 90. This significant increase in prevalence justified the discontinuation of the study on day 90 for ethical reasons. There was no statistical difference between treated and control groups in the risks of developing a new DD lesion (RR: 1.0; 95% CI: 0.62, 1.7), reactivating an inactive DD lesion (RR: 1.0; 95% CI: 0.62-1.7); or curing a DD lesion (RR: 0.88; 95% CI: 0.37, 2.1).

Conclusion: A 1% potassium monopersulfate footbath solution appears ineffective to control DD in this study design.

Keywords: Digital dermatitis, footbath.

LA-P08

Susceptibility of aerobic bacteria isolated from hoof lesions in cattle to preparations containing silver- and copper nanoparticles

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Objectives: Hoof diseases in dairy cattle, in addition to metabolic disorders and diseases of the udder, are one of the most common causes of problems in obtaining high yield and quality of milk. The occurrence of hoof diseases in cattle has many negative effects, such as increased animal culling and economic losses, resulting from costs of therapy and withdrawal of milk. Commonly used methods of treatment are associated with a threat to the environment, exposure of animals to discomfort or the need to incur high costs of antibiotic therapy. The aim of this study was to identify aerobic bacteria isolated from hoof lesions in cattle and to assess their susceptibility to silver- and copper nanoparticles.

Materials and methods: The research was carried out on 42 bacterial isolates originating from 26 animals living on 7 farms. Isolated bacteria were identified using both phenotypic (ID 32 STAPH, API 20 STREP tests [bioMérieux, France]) and genotypic (sequence analysis of the 16S rRNA gene) methods. The minimum inhibitory concentration (MIC) of silver- and copper nanoparticles was assessed by the microdilution method using 48-well culture plates.

Results: Members of the genus *Acinetobacter* were the most common isolated bacteria (27 strains). In addition, microorganisms belonging to the genera *Corynebacterium*, *Staphylococcus*, *Bacillus*, *Streptococcus* and *Enterococcus* were also identified. Our study has shown that silver nanoparticles have a higher antibacterial activity (minimum inhibitory concentration = 0.1 – 30.0 ppm) than copper nanoparticles (MIC = 0.25 – 50.0 ppm), moreover, it has been shown that Gram-positive bacteria are more resistant to silver and copper preparations than Gram-negative ones. The most resistant bacteria turned out to be Gram-positive *Bacillus licheniformis* and *Bacillus glycinifermentans*.

Conclusions: The conducted research indicates that preparations containing silver or copper nanoparticles can be an effective alternative to commonly used methods of treatment because they show high bactericidal activity against a number of isolates obtained from hoof lesions in dairy cattle.

Keywords: Cattle, hoof lesions, aerobic bacteria, copper nanoparticles, silver nanoparticles.

LA-P09

An observational study on managing digital dermatitis through risk assessment and veterinary advice on 19 Dutch dairy herds

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Objectives: (1) identify associations between digital dermatitis (**DD**) prevalence and a DD risk score, (2) determine the effect of veterinary advice based on the risk score on DD prevalence and management by farmers and their veterinarians

Material & Methods: 19 farms with routine veterinary herd

health visits from one practice were visited between March and April in 2016 and 2018. Selection criteria were (1) presence of DD, (2) miking parlour suitable for in-parlour M-scoring and (3) willingness to participate in the study. During the farm visit, DD prevalence was determined and a DD risk assessment (**RA**) survey done. At the end of the visit, farmers were given a list of cows with M2-lesions, eligible for treatment. The DD RA survey was an interim version of the lameness RA questionnaire (University of Calgary). It consisted of 22 MCQ with sections on foot health, housing and general management. Each answer was given a risk score based on published literature, with higher scores indicating higher risk for DD. Risk scores of all questions summed up to a total risk score (**TRS**). Two veterinary students, one in 2016 and one in 2018, performed the farm visits and were trained in M- and leg hygiene scoring using published literature, classroom training with digital color photographs and in-parlour scoring of washed hind feet of around 50 dairy cattle using a mirror glued on a spatula and a powerful headlight. Dairy herd improvement data were extracted from farm management software with consent.

Before the start of the study, farmers and their veterinarians were invited for a DD meeting. Study design together with general information on DD and its control on dairy farms were presented. In 2016, one-page summaries with advice on DD control were compiled and emailed to the farmers and their veterinarians after completion of all farm visits, in 2018 these were compiled and emailed within 14 days following each farm visit. At the end of the study, farmers and their veterinarians were again invited for a group meeting where anonymised results were presented.

Descriptive statistics were calculated for TRS (% of maximum risk score) and herd DD prevalence (cow level). The association between TRS and DD prevalence was tested using linear mixed model (**LMM**) analysis with TRS as predictor and DD prevalence as outcome variable, year as factor and herd as random effect. The association between ΔTRS (2018-2016) as predictor and ΔDD prevalence as outcome variable was visualised with a scatter plot.

In 2019 farmers and veterinarians were asked via email what they had done with the given advice in both study years.

Results:

Table 1. Descriptive statistics for TRS and DD prevalence of 19 Dutch dairy herds

variable	year	mean	standard deviation	minimum	maximum
TRS (%)	2016	42	13	13	65
	2018	41	13	20	68
DD prevalence (%)	2016	39	13	15	59
	2018	49	10	27	69
ΔTRS (%)	-	-1	9	-17	16
ΔDD prevalence (%)	-	10	11	-14	31

LMM analysis indentified that higher TRS was associated with higher DD prevalence (0.45, 95%CI 0.19-0.73) and DD prevalence was higher in 2018 compared with 2016 (10.48, 95%CI 3.47-17.50). Scatter plot indicated that there was no important relation between ΔTRS and ΔDD prevalence.

For advice given in 2016 and 208, the response rate from farmers 53% (10/19), with 4 farmers indicating some form of change in their DD management in 2016 and 7 farmers in 2018. The response rate from veterinarians was 89% (8/9) for 2016 and 90% (9/10) for 2018. They had discussed the advice and DD control with the farmer but the majority admitted lack of follow-up.

Conclusion: The DD RA survey can be used to raise awareness of strong and weak points of DD control on dairy farms. However, veterinary advice based on the DD RA survey is insufficient to intiate behavioral change, in both farmers and veterinarians, that results in a decrease in DD prevalence under field conditions.

Keywords: Behavioral change, dairy cow, digital dermatitis, veterinary advice, survey.

LA-P10

Transdermic flunixin-meglumine for white line disease treatment in dairy cows, a clinical trial

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Objective: To evaluate the effect of transdermic flunixin-meglumine on healing and reproductive performance in lactating dairy cows with white line disease (WLD).

Material & Methods: The study was performed in a commercial dairy farm with 2.500 lactating cows in the Nord-East of Spain in 2019. Cows were included in the study if they had WLD in one leg and hoof, 0- 120 days in milk and no concomitant health problems (mastitis, metritis, etc.) or medical treatment in the 30 days prior enrollment.

The day of enrollment (day 0) a functional trimming for the WLD affected hoof and a block treatment for the healthy hoof was performed in all cows. Additionally, dams were blindely randomized into one of both treatment groups: anti-inflammatory treatment with a single dose of flunixin pour-on administered on day 0 (group FTD, Finadyne® Transdermal 50 mg/ml, MSD AH) or without anti-inflammatory treatment (control group).

Cows were again evaluated between Day 7 to 21 after enrollment. The progress of the lesion was considered as healing if the hoof was reconstructed and no blood was present. Moreover, conception rate was evaluated if the animal was inseminated between -30 to +60 d of treatment (n=31). The following data was recorded for each animal: days in milk, parity (primiparous vs multiparous), affected limb (fore vs hind limb) and claw (medial vs lateral), severity of the lesion depending of the size (mild vs large), healing rate and conception rate.

Data were statistically analyzed by Chi square´s test using SPSS 18.0 (SPSS, Chicago, USA).

Results: Fifty cows were finally included in the study (23 were primiparous and 27) multiparous cows. Twenty-eight

cows were in the control group and 22 in the FTD group). The lesions registered were 33 mild and 17 large lesions, affecting 29 cases the fore limb and 21 the hind limb, and being 31 and 19 in the lateral and medial claw, respectively.

Our results showed that 50% of the cases did not progress properly; being necessary to replace a new block in 20% of the dams. Cows with a medial claw affection presented an increased healing rate in the FTD group compared to control animals [10/17(58,8%) vs 3/14(21,4%); p<0.05). Moreover, cows with the medial claw of the hind limb affected included in the FTD group had a better progression of the claw lesion than control cows [4/4(100%) vs 1/3(33,3%), p=0.05). When primiparous cows were analysed, FTD animals tended to present an increased healing rate compared to control cows [8/14(57,1%) vs 2/9(22,2%); p=0.09), with no limb or claw interaction.

Thirty one lame cows were inseminated in the study period. Finally, dams with severe hoof lesions had a decreased conception rate compared to cows with mild lesions [12/22 (54,5%) vs 0/9(0%); p<0,05].

Conclusions: Animals with severe hoof WLD lesions had an impaired conception rate compared to cows with mild disease. Combination of flunixin transdermal pour-on solution with a functional hoof trimming in cows with WLD in the hind limb and medial claw increased the healing rate compared to functional hoof trimming alone. Then, the use of flunixin transdermal could be an appropriate clinical tool to improve the healing rate for WLD lesions.

Keywords: Lameness, dairy cow, claw, white line disease, hoof.

LA-P11

Approaching digital dermatitis as a wound instead of an infectious dermatitis: efficacy of a non-antibiotic enzymatic alginogel

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Objectives: The aim was to investigate the efficacy of an enzymatic alginogel (EAG) on M1 and M2 lesions of digital dermatitis (DD), compared with a gel containing copper and zinc chelates (CZG), usinig (1) the M-score and (2) wound healing parameters as outcome.

Material & Methods: The hind feet of 536 lactating cows from 7 different Dutch dairy farms were foot trimmed and examined for the presence of DD using the M-score. Feet with M1 or M2 lesions were included in the study. Feet were alternately allocted to the EAG or CZG treatment group and photographed, treated and bandaged on day 0 and 3. On day 7, lesions were photographed and received a third treatment and bandage if the lesion was still an open wound. All treated lesions were photographed for examination on day 10. Clinical improvement of DD lesions was defined as lesions that transferred from being classified as M1 lesions to M0, M3, M4 or M4.1, or M2 lesions that transferred to M0, M1, M3, M4 or M4.1. The effect of EAG and CZG on clinical improvement was analysed using logistic regression models, including several important covariates.

All treated and photographed lesions were assessed in seriel (e.g. foot 1 on day 0, 3, 7 and 10, foot 2 on day 0, 3, 7 and 10, foot 3...) by a specialist in veterinary wound healing on possibility to assess wound healing on the photograph, presence of skin necrosis, presence, level and vitality of granulation tissue presence of wound contraction, presence of epithelisation and overal improvement compared with the previous photograph of the same foot.

M-score and wound healing assessment occured blinded from treatment group.

Results: A total of 202 hind feet from 160 cows were treated and included in the analysis. The clinical improvement rate of feet treated with EAG was 29% (29/99, 95%CI 20-38%) and with CZG 93% (96/103, 95%CI 87-98%). M1-lesions treated with EAG mainly remain an M1-lesion (32%) or transfer to an M2, M3 or M4.1. M2-lesions treated with EAG mostly remain an M2-lesion (76%). The lesions treated with CZG, are likely to transfer to an M3-lesion (72%), or in case of an M1-lesion on day 0, transferring to M0 (17%) or M4 (22%). Feet treated with EAG were less likely (OR 0.03, *P*<0.001) to improve than feet treated with CZG. The covariate 'farm' was identified as a significant risk factor for clinical improvement of the treated DD lesions in the logistic regression.

Wound healing evaluation is in progress and will be presented during the congress.

Conclusion: Treatment of M1 and M2 lesions with EAG results in a marked lower M-score improvement rate than treatment with CZG. Only 5 lesions transitioned into M0, all of them were treated with CZG.

Note: The M-score results were presented at the European Bovine Congress 2019 in 's-Hertogenbosch, the Netherlands.

Keywords: Wound healing, M-score, dairy cow, digital dermatitis, alginate gel.

LA-P12

The 7-point plan to improve claw health

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Despite extensive investigation, improved insight into risk factors and knowledge of the pathogenesis of infectious and non-infectious claw disorders, claw health at herd level has not been structurally improved in recent decades. Various studies have shown that claw disorders have a negative effect on, milk production, fertility, longevity and job satisfaction of the farmer. Enough reasons to reduce claw disorders on dairy farms and starting this, is primarily a matter of prevention and rapid intervention.

Most diagnoses are nowadays made by the farmer and the

claw trimmer during regular claw trimming. Registration of the noticed claw disorders is not always carried out consistently, so the prevalence (= number of cows with a claw disorder) is frequently an underestimation of the actual situation (Tunstall et al., 2019). In order to be able to provide well-fitting advice, insight into the real prevalence of the various claw disorders in a herd should be the first step.

However, good advice alone is no guarantee for improving the claw health situation in a herd. Research has shown that in addition to a high-quality, well-founded advice, the correct communication between adviser and farmer is essential. The form of communication is important to motivate the farmer to have the given advice been implemented. With the more complicated herd problems, it appears that farmers appreciate a conversation at the kitchen table. A clear plan can be formulated quietly to serve as a guideline for the improvement process in which it is clear what is expected from all parties involved.

In a presentation a scientifically based claw health approach (also called 7-point plan) will be introduced as a tool for advisors who want to focus on guiding and improving claw health at herd level. It provides both advisers and farmers tools to identify risk factors and improve claw health structurally.

For the production of a good quality horn the provision of certain vitamins and trace minerals essential. For example, biotin and zinc play a key role in the formation of good quality claw horn and manganese is essential for growth and function of cartilage and bone. For monitoring and optimization the provision of these elements, GD-AHS has launched in 2018 a voluntary program for dairy farmers, in which bulk milk samples are analyzed for biotin, zinc and manganese four times a year. The objective of this research was to evaluate the results from this bulk-milk monitoring program in the first three seasons. These results are not representative for the Dutch dairy population, because only a small proportion of farms participate in this voluntary program. Bulk milk samples were analyzed for biotin using a commercial ELISA and for zinc and manganese the inductively coupled plasma mass spectrometry method was used.

Results: Biotin concentrations in bulk milk of dairy farms in 2019 (mean ± SD) were 23.4% too low for optimal provision, 70.2% good provision and 6.4% too high provision. These values were for manganese 22.5%, 77.1% and 0.4% and for zinc 23% too low for optimal provision and 77% good provision respectively.

To the knowledge of the authors, this is the first bulk milk program monitoring the supply of the key elements like biotine and zinc in dairy herds to guide feeding strategies for monitoring and possible improvement of the provision for the best claw horn quality.

Keywords: Claw health, structural approach, bulmilk, biotin, minerals.

LA-P13

Occurrence of sole ulcer in dairy cows with laminitis

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Objectives: Laminitis is a multifactorial syndrome with complex pathophysiology and significant economic impact on dairy industry. One of the effects it has on the welfare of cattle is that it predisposes to the development of other lesions on the foot, predominantly sole ulcers and white line disease. Sole ulcer is one of the most important causes of lameness in dairy cattle worldwide which are housed on concrete and hard flooring. The incidence is also variable depending on predisposing causes such as housing, hoof trimming, nutrition and claw care. The most frequent forms of the laminitis in dairy herds are subclinical and chronic inflammations. The aim of the study was to investigate if the subclinical and chronic laminitis predispose dairy cows to the development of sole ulcer.

Material and methods: The data used in this study were obtained on 221 Holstein Friesian dairy cows during two sessions of routine orthopaedic and claw trimming visits (spring and autumn) as well as within a period between them when lame cows were treated. All of the cows were kept on manure solid bedding and fed TMR. The average milk year yield was 9000 kg. At the first visit the cows with subclinical and chronic laminitis were identified (LS and LC group). The diagnosis of the subclinical laminitis was based on an observation of *symptoms of sole haemorrhages and yellowish-coloured* soles. A "slipper foot" was used to diagnose a chronic laminitis. They had to be free from other claw diseases. The occurrence of the sole ulcer was checked at the following visits in all the cows. Statistical analysis was performed by running a chi-squared test to test a difference between sole ulcer incidence in LS, LC and control (healthy) group.

Results: Out of the 221 dairy cows examined during spring 11 and 12 cows were affected by the subclinical (LS group; 4.97 %) and chronic laminitis (LC group; 5.43 %), respectively. 72 dairy cows were free of claw diseases (control group; 32.6 %). In the following orthopaedic controls the sole ulcer was detected in two cows in the LS group (20 %), five cows in the LC group (41.7 %), and only in three cows in the control group (4.17 %). The chi-squared test revealed a significant difference ($p<0.05$) in the sole ulcer occurrence between laminitis and healthy cow groups.

Conclusions: The results of this study indicate that there is an association between laminitis and prevalence of sole ulcer in dairy cows. Therefore, a dairy farm management should pay more attention to avoid all known risk for laminitis development on the farm to prevent higher incidence of lameness.

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Keywords: Laminitis, sole ulcer, dairy cows.

LA-P14

Local treatment of interdigital phlegmon (Footrot) by the regional intravenous administration of tylosin in cows

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Introduction: Interdigital phlegmon (IP) or footrot is a common infectious disease in bovine. It produces lameness in dairy cattle and feedlots. In some severe cases, the animal does not respond favourably to the parenteral administration of antibiotics such as tylosin or ceftiofur. Possibly, the farmer does not pay enough attention and treats without veterinary supervision. There are some studies conducted with the intravenous regional administration of quinolones in IP treatment. (Varsano et al., 2015.; Celani et al., 2017). Due to the current EU regulation on the use of antibiotics, quinolones and cephalosporines (category B) can only be used in the case of laboratory tests that justify their use. The hypothesis of the study is that IP could be treated by regional intravenous tylosin rather than the use of quinolones.

Objective: The objective of the study was to evaluate the efficacy of IP treatment by the regional intravenous administration of tylosin and a tourniquet for 3 consecutive days.

Materials and Methods: The study was performed in 19 cows diagnosed with IP during May 2019 to February 2020. The affected cow breeds were 18 Holstein and one beef cattle (Asturiana de los Valles). The animals were housed in freestalls with cubicles (n=10), tie-stall (n=4) or tie-stall with a daily pasture exit (n=5). A tourniquet was maintained for 30 min before the intravenous administration. This tourniquet consisted of three flat rubber compressors.

After application of the tourniquet, 1/3 of the systemic dose of tylosin (PHARMASIN 200 MG / ML) was administered through the saphenous vein, cephalic vein or common dorsal digital vein (10cc of PHARMASIN tylosin 200 MG / ML). The treatment was repeated for 2 more days. The antibiotic administration was carried out with a butterfly Venofix 23G 0.65x 20mm and 30 cm length of plastic conduit to avoid movement issues.

In order to evaluate the effectiveness of the treatment, the locomotion score (LS) was evaluated on days 1,3, 5 and 15 based on the 5-point scale of Sprecher et al., 1997. The measurement of the limb circumference was also done at 1 centimetre below the accessory hooves and 1 cm above the accessory hooves on days 1, 2, 3, 5 and 15.

Results: IP was diagnosed in all of the limbs: Right forelimb (5,3%; n= 1)left forelimb (10,5%; n=2), right rear limb (36,8%; n=7) and left rear limb (47,4%; n=9). The LS punctuation was as follow: day 1 – LS5 (31,6%; n=6), LS4 (52,6%; n=10) and LS3 (15,8%; n=3); day 3 - LS4 (36,8%; n=7), LS3 (31,6%; n=6) and LS2 (31,6%; n=6); day 5 - LS4 (10,5%; n=2), LS3 (31,6%; n=6) and LS2 (57,9%; n=11); day 15 - LS4 (5,3%; n=1), LS3 (5,3%; n=1), LS2 (31,6%; n=6) and LS1 (57,9%; n=11).

The reduction of the measured circumference taken 1 centimetre above the accessory hooves was more than 0,5 cm from day 1 to 2 (73,7%; n = 14), day 2 to 3 (57.9%; n = 11), day 3 to 5 (68,4%; n = 13), day 5 to 15 (84.2%; n = 16) and, from day 1 to 15 the reduction was more than 1 cm (89,5%; n = 17). The reduction of the measured circumference taken 1 centimetre below the accessory hooves was more than 0,5 cm from day 1 to 2 (63,2%; n = 12), day 2 to 3 (57,9%; n = 11), day 3 to 5 (78,9%; n = 15), day 5 to 15 (84,2%; n = 16), and from day 1 to 15, the reduction was more than 1 cm (94,7%; n = 18).

Conclussion: The tylosin treatment was administered to 19 animals. Results over 15 days showed that 17 of the 19 animals resulted in no lameness (LS 1, 2) vs 19 animals with lameness (LS 3, 4, 5) on day 1. In this study, the use of tylosin with a local venous application ended up in an effective alternative to the IP treatment vs the parenteral use of quinolones and cephalosporines.

Keywords: Lameness, interdigital phlegmon, footrot.

LA-P15

A novel biomechanically designed orthosis for the bovine claw

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Objectives: To develop a novel orthopedic orthosis based on the anatomy and biomechanics of the bovine claw that can be used for claw-friendly unloading in the therapy of claw diseases.

Materials and Methods: The orthosis is made of different synthetic materials and has a two-layer structure consisting of a cushioning layer and a support layer. It is intended for application to the healthy claw of a limb with a damaged second claw to relieve the latter. The hardness of the cushion layer matches the hardness of the claw horn. In addition, the cushioning layer is extended in the region of the heel to reduce the additional load of the entire weight on the healthy claw. The supporting layer has a reinforcement in the area of the bearing edge, so that at this point the pressure forces are absorbed and transferred by the suspending structures of the claw. The orthosis has rounded ends so that it is easier to put on and take off. Attachment to the healthy claw is accomplished by an activatable adhesive that is already applied to the surface. The adhesive connection transmits forces while maintaining a durable connection.

The pressure distribution between the claw and the orthosis was measured in a compression test device at a load of 200 kg using pressure sensors from TecScan on 20 cattle distal limbs from the abattoir. The pressure sensors were put between the claw and orthosis or block respectively. The pressure distribution was first measured on trimmed claws and then on orthoses and wooden blocks attached with epoxy resin. The measurement data from 3 measurements per setup were then comparatively evaluated and statistically analyzed. The following parameters were considered: Area loaded, magnitude of compressive forces, distribution of compressive forces, peak pressures occurring, correlation between the localization and dimension of the pressure and the anatomical

structures located in the claw above.

Results: In direct comparison to trimmed claws or to wooden blocks, the orthosis achieves a significantly larger surface area (36qcm) for pressure absorption under 200kg load. The absorption surface to trimmed claws (24qcm) is increased by 1.5 times, compared to a glued block (11qcm) by 3 times. The data show that the pressure on the sole and the load of anatomically sensitive regions are lower compared to conventional wooden blocks. A considerable portion of the pressure is transmitted to the bearing edge of the claw. This ensures a physiological load on the claw-bone connection, which suspends the limb skeleton in the horn shoe. On the other hand, the sole and heels are uniformly loaded with low to medium pressure. Local pressure peaks, which represent a risk for the development of claw ulcers, were not observed in the pressure measurements with the orthoses.

Conclusions: The orthosis considers the anatomy and biomechanics of the claw and thus ensures a more claw-friendly pressure load and pressure distribution. In addition, the activation of the adhesive makes the application of the orthosis much easier and also allows a very precise application, which is crucial for the stability of the blocks and the slip resistance. Our pressure measurement data provide good evidence that this is an innovation with great potential to improve animal welfare by reducing secondary claw damage. Evidence will be strengthened in the next step by pressure measurements on larger numbers of live animals in farm settings. The use of the orthosis to relieve a damaged claw will most likely significantly reduce the number of claw lesions developing on the claw that is blocked for unloading the primary damaged claw.

Keywords: Orthesis, lameness, bovine claw.

LA-P16

Monitoring Bulk-Milk for Less Lameness

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Lameness in dairy cattle is mainly a consequence claw disorders (Toussaint Raven, 1977; Murray et al., 1996). Lameness is a multifactorial problem resulting from interactions between farm management, housing conditions, nutrition and cow characteristics (Ranjbar et al., 2016). As an advisor for the dairy farmer you have limited influence on the housing conditions and the cow characteristics. About the cow's claw characteristic you should advice the farmer about the breeding bull's to use regarding claw conformation and risk for disorders, if this information is provided by the breeding organization. Good claw-horn quality is dependent also of good nutrition. Good nutrition means intake/production of carbohydrates, protein etc. but also essential vitamins like biotin en trace elements like copper, zinc and manganese. Objective: Investigation of vitamin and mineral supply related to claw health.

Material and methods: Since 2018 GD Animal Health in The Netherlands offers a monitoring estimation for estimation bulk milk concentrations of zinc, manganese and biotin and Treponema antibodies in dairy herds' bulk milk . Based on a system of subscription the test is performed 4x per year (n=2000).

Results: Based on this information the biotin supply was year-round at more than 40% of the herds investigated too low and supply of trace elements was in more than 50% of the herds too low in summer months. We had no information about the rations of the dairies or yes/no pasturing in this herds.

Conclusion: Structural improvement of the vitamin and mineral supply in dairy cattle's ration will result in less claw-health problems.

References:

Murray RD, Downham D.Y, Clarkson MJ, Faull WB, et al. (1996). Epidemiology of lameness in dairy cattle: description and analysis of foot lesions. Vet Record, 138, 586-591.

Cattle Footcare and Claw Trimming by E. Toussaint Raven.

Keywords: Dairy cattle, lameness, prevention, nutrition.

LA-P17

Herd level risk factors for active digital dermatitis lesions in Finnish free stall dairy herds

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Objectives: Digital dermatitis (DD) is a severe bacterial hoof disease found worldwide. The disease can be classified into five different stages, of which DD M1, DD M2 and DD M4.1 are considered active lesions. Based on earlier studies, prevalence of active lesions in Finland was low. The aim of this study was to find herd-level risk factors for active DD lesions.

Materials & Methods: Free stall dairy herds were randomly selected from Finnish dairy herds that had > 45 lactating cows. Study population included 81 herds and 6877 cows. We scored all hind feet of adult cows for DD with a mirror when cows were standing in a pen or in the milking parlor. If at least one active lesion was detected in a herd, the herd was considered as an active DD lesion herd.

Farmers also filled out a questionnaire, which included 67 closed questions. Questions were divided into five categories: overall management, herd and barn characteristics, animal purchasing history, hoof trimming and hoof bath practices, and hoof disease history on the farm.

A multivariable logistic regression model was used for modelling risk factors. Selection of potential risk factors for a full model was based on a causal diagram and univariate screening. The risk factors for the model were chosen with univariate analysis. Herd and area were used as random effects. A predictor variable with $p < 0.25$ in an univariable analysis was selected for the multivariable model.

Multicollinearity was evaluated between categorial explanatory variables using phi-test and Cramer's V. Most of the explanatory variables were strongly correlated which led to the selection of the predictor variable to the final model that best described the situation. All biological meaningful two-way interactions were tested in the final model.

Results: The mean herd size was 87 cows (range 49–241). Of all the study cows, 67% were milked in an automatic milking system (AMS) and 33% in a milking parlor. Out of study herds, 33% had active DD lesions. No significant biological interactions were detected.

Larger herd size was a risk factor (OR 1.8 / cow nr $\log_e$; p=0.05) for active DD lesions. Compared to AMS, barns with parlor milking were associated with the presence of active DD lesions (OR 3.7; p=0.02). Occurrence of interdigital phlegmon during the previous year was associated with higher odds of active DD lesion (OR 3.6; p=0.04).

Conclusions: In this study, larger herd size, barn with a parlor milking and occurrence of interdigital phlegmon were risk factors for having active DD lesions in the herd. As AMS farms are more common in Finland, further research on management practices and their effect on prevalence of active DD lesions in the herd is warranted.

Keywords: Digital dermatitis, risk factor, dairy cattle.

NU-P01

Early onset of acidosis correction in neonatal calf scours, a comparison between two commercially available products containing different alkalizing agents

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Objectives: Diarrhoea remains the most common cause of death in neonatal calves, with significant economic impact. A number of metabolic disturbances are associated with neonatal calf scours, and acidosis is an important issue because it contributes to death from heart failure. Correction of acidosis results in a quicker return to normal demeanour.[1] Therefore, rapidly correcting acidosis is an important therapeutic target, mainly achieved by administering alkalizing agents. However, there remains confusion on the onset of alkalizing compounds. In fact, it was determined that during intravenous administration, sodium bicarbonate had the most rapid alkalizing effect.[2]

There are currently a number of oral formulations available and most having either sodium bicarbonate or sodium acetate as their primary alkalizing agent. This trial aimed to evaluate the onset of moderate acidosis correction between two different commercially available formulations.

Materials & methods: Data was collected from farms in Germany, late 2017 and early 2018. Calves (age ≤ 30 days) displaying clear diarrhoeic signs were tested and randomly assigned to two groups: 1) n=31 Calf-Lyte PLUS® (Vetoquinol) - alkalizing agent sodium acetate, 1 pouch (90g) per 2 liters of water (bicarbonate equivalent of 100 mmol/l), 2) n=32 Effydral® (Zoetis) - alkalizing agent sodium bicarbonate and citrate, 2 tablets per 2 liters of water (bicarbonate equivalent of 80 mmol/l). Two liter formulations were administered *ad libitum* by pail or feeder bottle with nipple, within the first hour for both groups. Calves not drinking the entire formulation were excluded from the study. Two blood pH measurements were performed using an electrolyte and blood gas analyser (IDEXX VetStat®). The first measurement was taken immediately before the first administration of either treatment, the second measurement was done 4 fours later (+/-1hr).

All transmitted data were used in the analysis and none of the 63 calves were discarded. For statistical analysis a mixed model ANOVA with repeated measurements was used, and applied by MIXED procedure Statistical Analysis Software (SAS/STAT 9.4), (SAS Institute, USA). The treatment, the time and their interaction (treatment*time) was used as fixed effects, while the animal was used as random effect in the model. Satterthwaite approximation for the denominator degrees of freedom was used to prevent errors linked to heteroscedasticity and normality of the residuals were verified with the Shapiro-Wilk test from the UNIVARIATE procedure. All data are presented as means ± standard deviation (SD).

Results: It's reported that healthy and diarrhoea free calves blood pH is on average 7.42.[3] Moreover, it's also reported that calves dying of diarrhoea have a blood pH ranging 6.50 to 7.05. Thus moderate acidosis can be ascertained as a blood pH between 7.05 and 7.40.[1] There were no significant differences in acidosis levels between either group at the first blood test, with most having on average "moderate" acidosis. Mean pH readings were 7.22 (†0.05) and 7.23 (†0.04) for the Calf-Lyte PLUS® and Effydral® groups, respectively. At the second measurement pH readings were 7.40±0.06 and 7.40±0.05 for the Calf-Lyte PLUS® and Effydral® groups, respectively. The previous translating into mean blood pH improvements by 0.173 and 0.175 for the Calf-Lyte PLUS® and Effydral® groups, respectively.

The mixed model revealed no statistically significant treatment effect (p=0.577) nor interaction treatment*time effect (p=0.887).

Conclusion: Within an interval of approximately 4 hours, both Calf-Lyte PLUS® and Effydral® groups saw a net improvement in their blood pH, indicating that moderate acidosis correction was underway. Considering that both groups were performed in comparable way, it can be argued that in an oral rehydration formulation, sodium acetate provides a quick treatment onset of moderate acidosis correction, similar to sodium bicarbonate. These results arrive to similar conclusions using different models and formulations.[4] Furthermore, in contrary to sodium bicarbonate, some other alkalizing agents such as sodium acetate are reported to facilitate sodium and water absorption in the calf small intestine, and when metabolized to produce energy. Finally, sodium acetate does not alkalinize the abomasum, while sodium bicarbonate does. As a reminder, low abomasal pH is a natural defense mechanism against bacterial proliferation, favouring the use of sodium acetate.[5] This study was focusing on acidiosis correction, not taking into account some possible differences, such as clinical scores and duration of the treatment, which can be related to specific product compositions.

Keywords: Acidosis, Calf, Scours.

NU-P02

Determination of dry matter and crude protein ruminal degradability in situ of moringa (*Moringa oleifera*)

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Objective: The aim of this work was to estimate the percentage of *in situ* ruminal degradability of dry matter (DM) and crude protein (CP) of the foliage of *Moringa Oleifera* harvested in summer time in the Irrigation of Majes, Arequipa, Peru.

Material & Methods: Sampling was done in three ages of cut (30, 60 and 90 days) and ruminal incubation was done in seven times (0, 2, 4, 8, 16, 32 and 48 hours) in a completely randomized design with factorial arrangement (3x7) with four repetitions for each ruminal incubation time. All incubation parameters were analyzed using the general linear model GLM of SPSS.

Results: The soluble fraction (A) of the ruminal degradability of DM varied from 27.77 to 30.50%, with no significant differences between days of regrowth (P> 0.05). The potentially digestible insoluble fraction (B) varied from 52.70% at 90 days to 58.69% at 30 days of regrowth with highly significant differences between 30 days and 60 and 90 days (P <0.01) but without significant differences between 60 and 90 days (P>0.05). The digestion rate (Kd) fluctuated from 0.08 to 0.16 h^{-1} with significant differences between 90 days and the rest of days of regrowth (P<0.01) except those between 30 and 60 days of regrowth (P> 0.05). The effective degradability (ED) for a constant passage rate (Kp) of 6% / h varied from 59.38% at 90 days to a maximum of 71.81% at 30 days and was highly significant between all days (P<0.001). An average of 65.69% of ED was determined among all the cutting times. Finally, the potentially digestible fraction (PD) varied from 80.93% at 60 days to 89.19% at 30 days of regrowth similarly to the ED was highly significant between all cutting times (P <0.001). Regarding to the CP, differences were found (P <0.001) within both: the days of regrowth and the incubation times. There were highly significant differences between the 30 days of regrowth with a percentage of 63.36% respect to the rest of days. But without differences between 60 (56.11%) and 90 days of regrowth (54.09%). The soluble fraction (A) varied in general from 18.47 to 19.10%, with no significant differences between all the days of regrowth (P> 0.05). The insoluble fraction (B) varied from 72.04% at 60 days to 78.46% at 90 days of regrowth without significant statistical differences between all cuts states (P> 0.05). The Kd fluctuated from 0.06 to 0.15 h^{-1} with highly significant differences between 30 days and other days of regrowth (P <0.01) but without differences between 60 and 90 days of regrowth (P> 0.05). The ED of the CP at a (Kp) of 6% / h varied from 58.32% at 90 days of regrowth to a maximum of 73.38% at 30 days with highly significant differences between all the days of cut (P <0.001). An average of 63.39% ED of the CP was determined among all the cutting times. The % Ruminal Non-Degradable Protein for a (Kp) of 6% / h varied from 29.62% at 30 days of regrowth to 41.68% at 90 days, with highly significant differences (P <0.001) comparing 30 days with respect to the other days except between 60 and 90 days of regrowth where no statistical differences were observed. The results demonstrated a significant increase in the percentage of non-degradable protein or "by pass" with the increasing of the days of regrowth. The potentially digestible non-degradable protein fraction (PDNDP) ranged from 71.79% at 30 days to 94.07% at 90 days of regrowth**.**

Conclusions: In conclusion *in situ* ruminal degradability of DM and CP of the foliage of *Moringa Oleifera* revealed that that the ruminal degradability of the DM decreased significantly as the time of cutting increased and the CP degradability decreased significantly between 30 and 60 days as the cut time increased but then tended to be stable, demonstrating a significant decrease in the ED of the CP as the days of forage cut in the summer season increase.

Keywords: Moringa oleifera, degradability in situ, ruminal kinetics.

NU-P03

The effect of calcium boluses at parturition on milk yield and plasma calcium in grazing Holstein cattle

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Hypocalcemia is a common metabolic disorder affecting dairy cows around parturition. A successful strategy to prevent hypocalcemia is the use of anionic diets; however, older cows may still experience certain degree of clinical hypocalcemia. The aim was to assess the effect of oral calcium boluses at calving on daily milk yield up to 30 d postpartum (pp) and plasma Ca during the early postpartum (pp). The study was conducted on a grazing dairy in Chile. At 30 d before expected parturition, cows were moved to a prepartum lot receiving 80% of their dry matter (DM) as a mixed ration and 20% from pasture (DCAD -109 mEq/kg DM). In May 2019, 30 cows with 2 or more lactations were randomly assigned at parturition to either a treatment group (T), receiving 2 boluses of a commercial product based on calcium chloride, providing 44 g of Ca, 24 h apart, or a control group (C). A blood sample for plasma collection was obtained at d 1 (within 6 h pp), and at d 2, 3, 4 and 7 pp. After that, cows returned to their group and were fed the pp diet as a mixed ration (70% of their expected DM intake) in an open barn. Then, cows were directed to their pasture (ryegrass) to provide the rest 30% of their DM. Cows from both groups were kept in the same group and handled homogeneously until 30 d pp. Plasma Ca, was assessed by standard laboratory methodologies. Daily milk yield up to 30 d pp was recorded in a computerized milking system. Metabolites and milk yield were analyzed by ANOVA for repeated measures using the PROC MIXED of SAS 9.4. In second parity, plasma Ca was not statistically different between experimental groups, but milk yield was lower in the T group than the C group. In parity 3 and ≥ 4, Ca boluses at calving tended to increase the concentration of Ca at day 2 pp, and this difference persisted until day 3 pp in parity 3. In addition, milk yield was higher in parity 3 and ≥ 4 in the T group than the C group. It is concluded that in grazing cows that were fed an anionic diet during the prepartum period, the supplementation of 2 boluses of Ca at parturition, given 24 h apart, increased the concentration of Ca during the early pp and milk yield up to 30 d pp in cows within parity 3 and ≥ 4.

Keywords: Calcium, milk yield, supplementation, anionic diets, grazing.

NU-P04

Changes in oxidative stress parameters in healthy and unhealthy Holstein cows during the transition period

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Objectives: Oxidative stress, which reflects an imbalance between oxidant and antioxidant levels, increases the risk of metabolic and infectious diseases by eliciting dysfunctional inflammatory responses. Furthermore, oxidative stress causes oxidative damage to macromolecules such as lipids, proteins, and DNA, and increases during early lactation in cows. In addition, oxidative stress increases susceptibility to metabolic and infectious diseases such as ketosis, displaced abomasum, mastitis, and uteritis. However, there is little information on the changes in oxidative stress that occur during the transition period, or on the role of oxidative stress in postpartum disease. To clarify the relationships among oxidative stress, postpartum transition, and disease, we investigated malondialdehyde (MDA; an end product of lipid peroxidation caused by reactive oxygen species) and glutathione peroxidase (GPx; an antioxidant enzyme involved in lipid peroxidation that converts hydrogen peroxide to water in the presence of glOutathione) levels, and the potential antioxidant capacity (PAO; total antioxidant capacity in serum based on the reduction of copper ions), of healthy and unhealthy Holstein cows during the transition period.

Materials and Methods: Healthy transition (HT) Holstein cows (n = 9) were used to explore longitudinal changes during the transition period. Unhealthy postpartum (UHP) cows with ketosis (n = 10), displaced abomasum (n = 9), and acute mastitis (n = 10) were compared to control cows (n = 10). Blood samples were collected at 2 weeks prepartum, and at 1, 2, 4, 6, and 8 weeks postpartum, in the HT group, and on the first day of examination within 60 days postpartum in the UHP group. The plasma MDA and GPx levels, and the serum PAO, were measured using commercial kits. Other blood components were assayed using an automatic analyzer (AU-680; Beckman Coulter, Tokyo, Japan) and a high-performance liquid chromatograph (Nanospace; Osaka Soda, Osaka, Japan). Milk yield and composition were measured at 2 and 4 weeks postpartum. Repeated-measures analysis of variance and Tukey's test were used for intragroup comparisons of blood parameters. Pearson's product-moment correlation coefficients or Spearman's rank correlation coefficients were also calculated. Results In the HT group, the plasma MDA level was significantly ($P < 0.05$) higher at 2 and 6 weeks postpartum compared to that at 2 weeks prepartum. There was a significant ($P < 0.05$) negative correlation between the plasma MDA level and the BCS and Glu levels, and a significant ($P < 0.05$) positive correlations between the plasma MDA level and the milk yield, free-fatty acid (FFA) β-hydroxybutyrate (BHB), and aspartate aminotransferase levels. The plasma GPx level and serum PAO did not significantly change between 2 and 6 weeks postpartum. In the UHP group, the plasma MDA level was numerically higher in cows with ketosis and displaced abomasum, and significantly ($P < 0.05$) higher in cows with ketosis, than in control cows. There was a significant ($P < 0.05$) positive correlation between the plasma MDA and FFA levels, and a significant ($P < 0.05$) negative correlation between the plasma MDA and Glu levels. The serum PAO was low in the diseased cows, and was significantly ($P < 0.05$) lower in cows with ketosis than control cows. The FFA and BHB levels were significantly ($P < 0.05$) higher in cows with ketosis and displaced abomasum than in control cows.

Conclusions: Postpartum oxidative stress was associated with hypermetabolism (which increases milk production) and peripheral blood components (Glu, FFA, and BHB) related to NEB status. Furthermore, NEB status influenced oxidative stress in cows with postpartum diseases; ketosis exerted the largest influence on oxidative stress. A higher MDA level increases milk production, but in the presence of postpartum diseases, especially ketosis, MDA shows stronger associations with peripheral biochemical components, such as Glu, FFA, and BHB. Therefore, controlling oxidative stress in transition or postpartum cows may improve their productivity. Further examination of the relationships among oxidative stress, productivity, and disease in Holstein cows during the transition period is needed.

Keywords: Blood parameters, Healthy and unhealthy cows, Oxidative stress, Periparturient period.

NU-P05

Evaluation of the clinical effect of an oral rehydration treatment in either water or milk, in young calves with E. coli-induced diarrhoea

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Objectives: Neonatal calf diarrhoea instigates several physiological and metabolic changes including acidosis, dehydration and hypoglycemia.[1] Correctly used, oral electrolyte treatments (ORT) effectively address related metabolic disturbances. Controversy remains on whether feeding, or not, milk to diarrhoeic calves, especially that "on-farm" practices often mix directly ORTs in milk. Inversely, it's reported that feeding milk to diarrheic calves is beneficial, if sodium bicarbonate based ORTs are not used.[2,3] Derived from a trial with broader observational targets, this abstract emphasises on metabolic and physiological parameters of diarrhoeic calves fed an ORT diluted in water, or in a commercial milk replacer.

Material & methods: Conducted at the CEBIPHAR experimental facilities, this trial was performed under Good Laboratory Practice principals and standing ethics committee approval. This specific assessment considers 18 randomly selected Prim'Holstein male calves, age 7-10 days of similar body weight (ANOVA p=0.67). After acclimatisation and assessed as healthy, calves were administered 2 separate doses (D0, D1) of a 10ml oral inoculation (pathogenic *E.coli* strain CS31A, 2.109 CFU/mL). When determined ill per clinical score criteria, animals were divided into 3 groups: n=6 "control" (milk replacer only), n=6 ORT+water (1.5L ORT preparation and interval administration of 0.5L milk replacer), n=6 ORT+milk replacer. ORT therapy consisted of: 90g/2L BID, Calf-Lyte® PLUS (Vetoquinol), diluted in 2L of water or 2L of milk replacer (Starmilk, Evialis), both served at ~37°C and administered in a

pail. Treatments lasted ≥ 3 days (6 doses minimum), and until clinical signs disappeared, or up to 7 days (14 doses maximum), whatever came first. With clinical score assessments, 5ml jugular vein blood samples with heparin-lithium were collected and analysed (Idexx® VetStat™) on D0 (before inoculation), on T0 (before the first treatment administration), on T2 (24h after first administration), and 24h after the last treatment (Tx). Observations/clinical assessments done from D0 (inoculation) to D9. Statistics were performed using validated Stata® statistical software (StataCorp. College Station, TX, USA; version 15). All data represented as mean ± SD.

Results: Under the conditions of this this trial no statistical differences were found, although certain trends were noted. Numerical data show that ORT treated animals body weight loss was less from D0 to D9 ("control" -3.1kg ± 1.1kg, ORT+water -2.8kg ± 2.2kg, ORT+milk -1.0 ± 1.8kg), and they had a faster recovery per total clinical score before T7, when the minimal ORT treatment schedule (6 doses) was given to all animals ("control" 5.0 ± 1.7, ORT+water 1.7 ± 2.0, ORT+milk 2.7 ± 1.6).

Blood assays data show no statistical differences for all clinical assessments/endpoints at D0 versus TX. To name a few, blood pH: "control" 7.40 ± 0.05 versus 7.35 ± 0.04, ORT+water 7.43 ± 0.01 versus 7.38 ± 0.06, ORT+milk 7.38 ± 0.07 versus 7.39 ± 0.10. HC03 mmol/L values at D0 versus TX: "control" 29.8 ± 1.6 versus 24.0 ± 3.8, ORT+water 29.0 ± 1.8 versus 32.1 ± 5.8, ORT+milk 28.0 ± 4.6 versus 30.1 ± 4.0. Na+ mmol/L at D0 versus TX: "control" 136 ± 3 versus 127 ± 8, ORT+water 139 ± 1 versus 136 ± 5, ORT+milk 136 ± 7 versus 136 ± 3.

Conclusion: Hypertonic ORT solutions may potentially instigate transient dehydration and hypomotility, perhaps accentuated by "on-farm" practices by incorporating directly to milk. Inversely, it's reported that using hypertonic ORT solutions (~725 mOsm/L) induced a transient dehydration of <30 minutes in diarrhoeic/dehydrated calves, considered as of no clinical importance.[4] Moreover, hypertonic oral rehydrating therapy in the range of ~700 mOsm/L can be preferred as osmolarity at the top of intestinal villi is around 600mOsm/L, therefore absorption is enhanced, but the use of concomitant milk is questioned.[5,6] On the other hand, ORT reconstituted in water can be more quickly absorbed as compared with dilution in milk due to faster abomasal emptying.[7]

This trial did not include a sufficient number of calves to arrive at a statistical power which would indicate if the use of concomitant milk with the ORT had either beneficial or detrimental effect. Nevertheless, excerpt of blood assays show that data trends at 24h after the last treatment (Tx), were not different from the normal baselines established before inoculation and also reported as normal physiological values.[8,9]

Keywords: Oral, rehydration, diarrhoea, water, milk.

NU-P06

Influence of sample volume and time on rumen juice analysis in cattle

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Objectives: Literature describing rumen juice (RJ) analysis as a diagnostic tool is based on analysis of a 10mL sample directly after sampling. However, it may be challenging to obtain 10mL of RJ from some patients, and clinical circumstances may delay the RJ analysis. This study quantified the effect of sample volumes, 2, 5, 10, 50 and 100mL; and time-to-analysis on RJ analysis after 30 and 60 minutes (min).

Material & Methods: Prospective experimental study. Two liters of RJ were obtained from a cannulated donor cow 21 times. The samples were subdivided into 1 set per time point. A set was composed of 2 duplicates of each sample volume. Samples were analyzed at 0, 30, and 60 min after sampling. Analysis of RJ consisted of pH, methylene blue reduction time (MBRT), and protozoal motility scoring.

Results: At 30 and 60min, pH of the 2mL and 5mL samples were significantly ($p < 0.05$) higher than pH of the 10mL, 50mL, 100mL samples. The MBRT was significantly prolonged for 2mL samples compared to 10, 50 and 100mL samples at all time points. The pH and MBRT at 60min were significantly higher than at 0min for all sample volumes. There was no significant difference in protozoal motility between sample volumes or times.

Conclusion: This study indicates that the interpretation of RJ analysis may be altered by sample volumes less than 10mL or 30 to 60min delays in analysis.

Keywords: Rumen juice, Rumen juice analysis, Cattle, Indigestion, Rumen sampling.

NU-P07

What are the long-term effects on health, fertility and production of copper toxicity in dairy calves?

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Objectives: To assess the impact of an episode of copper toxicity as pre weaned calves on the subsequent parameters of Holstein heifers on one farm in North Somerset.

Materials and methods: Copper toxicity in a cohort of heifer calves on a commercial Dairy in North Somerset UK was investigated after the confirmation of copper toxicity in one animal within the cohort that died. Toxicity was confirmed due to a kidney copper sample (6970 umol/kg DM, ref: 0-787).

Blood test results from a cohort of 30 heifers that suffered

copper toxicity in-feed in January 2018 were made available from veterinary practice records of the on-farm investigation at the time and collated using Excel spreadsheets.

The surviving heifers from this cohort have subsequently calved and are being milked as part of the commercial dairy herd. Data from this cohort of heifer calves from 2017 to present have been collected from veterinary practice records, farm records, fertility records and milk recording records and collated using excel spreadsheets.

Comparative data will be collected from cohorts of heifer calves reared on the same farm, born since February 2018 when the in-feed toxicity was detected and corrected.

Ethical approval for the collection of data was obtained from the University of Bristol vet school ethics committee.

Results: The ages of the calves in the investigation ranged from 1 week to 15 months. Calves aged 3 to 5 months were most severely affected at the time; as defined by measurement of blood glutamate dehydrogenase (GLDH) levels. Maximum GLDH level recorded on one calf was 1160 iu/L (ref 0 – 25 iu/L), range 15 – 1160 iu/L. Notably, blood copper levels analysed at the same time were rarely elevated in these animals.

A group of 12 animals that were most affected (as defined by blood GLDH levels) were identified and were followed up with further blood tests. All 12 had raised GLDH levels 2 months after the initial toxic insult. Of these 10 animals calved into the herd and survived through their 1st lactation.

Data from both 'affected' and 'non-affected' cohorts of heifers will be further analysed using excel and SPSS software. Paired T tests will be used to assess differences both between and within cohorts of affected and non-affected animals.

Analysis of the data will assess the impact of copper toxicity on parameters such as; survival to first service, age at 1st service, number of serves, age at calving, milk yield and other health events in these animals.

Conclusions: While the clinical symptoms, diagnostics and treatment of copper toxicity in cattle are well documented in the literature, there is very little evidence to suggest how a defined subclinical toxic insult may affect the future health and production of the affected animals.

This cohort study will investigate whether an incident of copper toxicity in pre-weaned calves had any detectable impact on their survival, health and production in comparison with other, non-affected calves that were reared on the same farm in the same management conditions.

It is hoped that the findings of this study will help clinicians to better advise their clients on the likely future survival and productivity of animals that suffer sub clinical or clinical copper toxicity but that survive this event.

Keywords: Copper, toxicity, dairy, calves, production.

NU-P08

The relationship between hypocalcemia and urine pH during periparturient dairy cows

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Objectives: Hypocalcemia has been reported as a problem during peripaturition in dairy cows. Urine pH is necessary to reflect the cow's acid-base status and can use as an indicator for preventing subclinical hypocalcemia. The objective of this study was to determine the relationship between hypocalcemia and urine pH in periparturient dairy cows.

Material & Methods: A prospective, observational cohort study was undertaken in primiparous and multiparous Holstein cows (n=54) during 7 d before calving to 1 d after calving. Cows were divided into 2 groups by parity; group 1 (1st and 2nd parity cows, n = 37) and group 2 (>2nd parity cows, n = 17). Urine samples were collected and determined immediately after urination during 7 d (4 ±1.7 d) before calving. Urine pH level was classified into two groups; alkaline urine group (pH >6.8) and acid urine group (pH ≤6.8). Blood samples were collected within 24 hours after calving and were determined for serum calcium concentration. Statistical analyses were performed using the statistical program R.

Results: The average calcium concentration of group 1, group 2 and total was 8.56, 8.32 and 8.48 mg/dL, respectively ($P < 0.140$). The prevalence of subclinical hypocalcemia in this study was 22.2%. The prevalence of subclinical hypocalcemia in cows with acid and alkaline urine was 7.14 and 27.5%, respectively. The odds of subclinical hypocalcemia with alkaline urine was 4.9 (odds ratio) times more likely than the odds of subclinical hypocalcemia with acid urine (95%CI= 0.5749-42.2959, $P < 0.145$).

Conclusion: The prevalence of subclinical hypocalcemia was low in this study. Urine pH did not significant association with hypocalcemia. However, cows with alkaline urine before calving tended to have hypocalcemia, while acid urine was not associated with hypocalcemia. Hypocalcemia and urine pH level may result in nutrition management of cations and anions during dry period.

Keywords: Dairy cow, Urine pH, Hypocalcemia.

NU-P09

Plasma methionine response for the evaluation of two rumen-protected methionine prototypes

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Objectives: Plasma methionine responds linearly to intestinal absorption and, thus, can be used to assess relative differences in bioavailability among products. Two previous experiments evaluated plasma methionine response to feeding two rumen protected methionine (RPM) methionine prototypes that differed in their encapsulation technology. The primary objective of this work was to develop a method to retrospectively compare plasma methionine responses across studies. The second objective was to determine whether plasma methionine response to feeding the more bioavailable product differed according to supplementation level.

Materials and methods: This was a retrospective comparison of results from two previous studies conducted as a replicated 5×5 Latin squares with 7 d periods. For this work, only three of the five treatments were compared: control without RPM (CON), dietary supplementation with a low level of RPM (LO), and dietary supplementation with a high level of RPM (HI). The first experiment (EXP1) evaluated response to feeding 14 g/d (LO) or 29 g/d (HI) methionine in a prototype protected by a previous protection technology (P1; Kemin Animal Nutrition and Health, Herentals, Belgium). The second experiment (EXP2) supplemented cows with 22 g/d (LO) or 44 g/d (HI) methionine in a prototype protected with a new technology based on a pH sensitive polymer (P2; Kemin Animal Nutrition and Health, Herentals, Belgium). Plasma samples were collected from jugular catheters on day 7 (EXP1) or days 5-7 (EXP2) of each period. Plasma amino acid concentrations were measured by cation-exchange chromatography at the Missouri Agriculture Experiment Station Chemical Laboratories (USA). Free plasma methionine (μM) was converted to percent of total amino acids minus the sulfur containing amino acids (Met%). To standardize the results across studies, regression analyses were conducted in SAS (version 9.4, SAS Institute Inc., Cary, NC) to determine the effect of g/d supplemental methionine on plasma Met%. Separate regression analyses with results from P2 only were conducted to compare fit statistics of the full model (0, 22, and 44 g/d methionine) to incomplete models (0 and 22, 22 and 44, and 0 and 44 g/d).

Results: In EXP1, the regression yielded an intercept of 1.102 and a slope of 0.006 (R^2=0.17). For EXP2, the intercept estimate was 1.047 and the slope estimate was 0.033 (R^2=0.78). These results indicate that supplementation of 1 g/d methionine in P1 increased plasma Met% by 0.006 percentage units while supplementation of 1 g/d methionine in P2 increased plasma Met% by 0.033 percentage units. Thus, bioavailability of P2 was over 5 times greater (0.033/0.006) than that of P1. In addition, comparison of the R^2 between experiments indicate that only 17% of the variation in Met% was explained by level of supplementation of P1, but 78% was explained by level of supplementation with P2. In EXP2, slope estimates did not vary substantially when regressing only results from the CON and LO (0.031, R^2=0.71), CON and HI (0.033, R^2=0.82), or LO and HI (0.035, R^2=0.52) treatments, indicating a consistent result independent of feeding level.

Conclusions: Determining the plasma methionine response to feeding different RPM prototypes was an effective way to compare results across separate experiments. Results of this work indicate that P2 was over 5 times more bioavailable than P1. In addition, the Met% response was highly consistent independent of supplementation level.

Keywords: Bioavailability, methionine.

NU-P10

Analysis of the b-hidroxybutyrate blood concentration in Lidia bulls and relationship with the animal mobility

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Objectives: In its vast majority, the lidia cattle maintain a regime of extensive exploitation, but everything changes in the case of the males that are put under a final bait during 5-9 months. According to several authors, this bait could be harmful to the animal and produce different metabolic problems. Therefore, the concentration of β-hydroxybutyrate in the blood was analyzed as an indicator of a metabolic and physiological disorder resulting from fasting, stress and intensive bait. In turn, the influence of different variables related to management (duration of transport, time in pens, type of food supply, frequency of administration), as well as the bull's behavior in the fight, the number and type of falls and variables such as weight and age.

Material and methods: Blood samples were collected and the behavior of 72 fighting animals (54 bulls, 12 steers, 6 erales) was recorded. The blood ketosis test was performed with the βHB ControlFreeStyle® system, a method that indicates the level of β-hydroxybutyrate in blood (mmol/l) and the animal's behavior was assessed using the software and methodology published at the University of León.

Results: Only four animals showed pathological levels of β-hydroxybutyrate being the mean = 0.46 μml / l with a maximum concentration of 2,6 μml / l and a minimum of 0.1 μml / l. No correlations are observed with the number and degree of falls experienced by animals. On the other hand, if we observe a negative correlation between the concentration of ketone bodies and the duration of the training, which leads us to consider its positive effect. It is also observed positive correlations with age, perhaps due to their longer bait time, and also with the distance of transport of the animals, which could result in a longer fasting time and the consequent mobilization of reserves and metabolization of themselves reflected in the accumulation of these ketone bodies in blood.

Conclusions: The concentration values of ketone bodies in blood do not exceed the indicative threshold of a pathological state, however, they are significantly correlated with different parameters of productive management, showing no relation to the degree of mobility of the animal during the fight.

Keywords: β-Hydroxybutyrate, lidia cattle, fall symdrom.

NU-P11

Serum calcium levels and its relationship with the presentation of endometritis in postpartum dairy cows from intensive dairy farms

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Objetive: The aim of this study was to evaluate the relationship between serum calcium levels and the presentation of endometritis in post-partum dairy cows.

Material and Methods: The study included 213 Holstein dairy cows from three intensive dairy farms in the province of Lima for one year. We excluded cows that died before the diagnosis of endometritis, cows with dystocia, double births or stillbirths, placental retention, puerperal metritis, clinical hypocalcemia and those that were treated with calcium or had another pathologies. Cows blood samples were collected during the first postpartum week. The analysis of serum calcium from was done by the GenesysTM 20 spectrophotometer [ThermoSpectronic] and the AA Calcium Color diagnostic kit [Wiener]. The diagnosis of endometritis was made by evaluating the vaginal discharge of cows with 35 ± 3 days postpartum. The samples was collected using the Metricheck device and were classified (0 to 5) according to McDougall Score. Samples were cows with a McDougall Score greater than 1 were considered positive for endometritis. The cut point was determined by ROC analysis (Receiver Operating Characteristic). The odds ratio was calculated to evaluate the relationship between NCa and the presentation of endometritis.

Results: The cut point determined was 7.44 mg/dl. The 68% of cows with NCa calcium levels <7.44 mg/dl during the first week post-partum had endometritis compared to 55% of endometritis presentation in cows with NCa> 7.44 mg/dl during the first week post-partum. Cows with NCa calcium levels <7.44 mg/dl during the first week post-partum had 1.78 times more odds of having endometritis compared to cows with NCa calcium levels >7.44 mg/dl during the first week post-partum (95% IC: 0.98-3.23).

Conclusions: The study concludes that subclinical hypocalcemia (<7.44 mg / dl) during the first week post-partum is associated to presentation of endometritis in post-partum dairy cows.

Keywords: Calcium level, endometritis, dairy cows, postpartum, Lima-Peru.

NU-P12

Fresh cow management supplemented with a gluconeogenic substrate and its effect in milk production, lactation persistence and fertility in Holstein cows in Torreon, Mexico

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Objectives: The objective of this study was to evaluate the inclusion of a gluconeogenic substrate in fresh cows on average milk production at the peak (day 50 of lactation), lactation persistence at 210 days, and fertility in a dairy farm in Torreon, Mexico, a region with a semidry climate.

Materials and methods: 90 fresh Holstein cows, with same birth number and body condition, were housed under similar conditions and fed with the same formula. Animals were randomly divided in two groups of 45 cows each: T1 group were fed with a gluconeogenic substrate [Lipofeed ™] and T2 witness group without this substrate; T1 was given 60g/cow/ per day of the product mixed in the ration for 30 days previous to calving.

Lipofeed ™ contains propanediol as a gluconeogenic substrate and calcium propionate. The statistical analysis was performed with least significant difference (LSD).

Results: Milk production at the peak was different between T1 (53.9L/ per cow) and T2 (51.3L/ per cow), with a significantly higher production in the gluconeogenic substrate group (2.6L plus per cow) ($p<0.05$).

Similarly, average milk production during 210 days of lactation was higher in T1 (46.59 L) vs T2 (44.37L) group ($p<0.05$).

Previous studies suggested that the propanediol stores in the liver in form of glycogen, and unfolds slowly according to the cow requirements for milk production; instead of the bypass fat products which store excessively in the liver. This probably caused the increase in milk production observed in our study.

Another result shows the gestation percentages obtained in days 50, 70 and 90, showed that better percentages were obtained in T1; suggesting that there is a close relationship between the energy consumed and the cattle fertility. It was previously suggested that energy consumption increases LH hormone pulsation to cause ovulation and also causes a better ovulatory rate due to a raise in IGF1.

Conclusion: Considering the economic income, the supplementation of a gluconeogenic substrate to fresh cows is profitable.

During the dry period, cows are fed more fiber and less energy so as to avoid a high body condition (3.5 -4). This means that approximately 10-12 days before calving, the cow needs to increase her feed consumption but she has not the intake capability so when the calf is born and the lactation starts, she enters a negative energetic balance which causes body fat mobilization and fatty acid esters formation, which degrade into ketone bodies, causing severe ketosis in dairy cows. A gluconeogenic substrate at this moment avoids the degradation of adipocytes, shortening the period of negative energetic

balance, reducing the risk of ketosis and thus promoting a better liver health.

Overall, a sound dry cow program is a critical key to improved fresh cow performance.

Keywords: Propanediol, fresh cows, lactation persistence, fertility.

NU-P13

Effects of Glucose Precursors on Ruminal Fermentation and Blood Parameters in Cows

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In this study; propylene glycol and glycerol, one of the commonly used glucose precursors to meet the increasing energy demand in cows, was aimed to determine which of these two glucose precursors was more effective by observing the changes that dairy cattle had undergone. In this context, the introduction of propylene glycol and glycerol into cows; rumen volatile fatty acids (VFA), ammonia nitrogen, rumen pH values, blood glucose and insulin levels, locomotion score of cows, body condition score and dry matter intake (DMI) were investigated.

This research was carried out in the cattle unit in Uludag University, Faculty of Veterinary Medicine, Animal Health and Animal Production Research and Application Center. In this study, 3 dairy cows with black-pied breed who had more than one delivery and a rumen cannula were used. These animals were randomly selected as one animal in each group and divided into three groups as propylene glycol, glycerol and control in 13 day intervals according to 3 x 3 latin square method. The cows were orally administered propylene glycol (300 ml / day), glycerol (450 ml / day) and water (400 ml / day) for a total of 13 days, for 10 days of acclimation and 3 days of sampling. Blood samples at 0 (before drinking) and after 10, 20, 30, 60, 90, 120, 180, 240 and 360 minutes, and rumen fluids at 0 (before drinking) and after taken at 10, 20, 30, 60, 90, 120, 150, 180, 210, 240 and 360 minutes. The amount of dry matter intake by the cows during the study was recorded daily, locomotion score and body condition scores were recorded weekly. SPSS 20 software was used for statistical analysis of the data. In the study, the BMI value of the cows was found to be the highest (22,9 kg / day) in the glycerol-treated group and the lowest (17,3 kg / day) in the propylene glycol-treated group ($P < 0,02$). Blood insulin levels (22,2 ng / dL), glycerol (18,5 ng / dL) and control (16,8 ng / dL) were higher in the propylene glycol-treated group ($P < 0,05$). No difference was found between the propylene glycol and glycerol groups in rumen pH values. However, rumen pH values of propylene glycol and glycerol-treated groups were higher than control group ($P < 0,05$). Similarly, rumen propionic acid values of propylene glycol and glycerol-treated groups were higher than the control group ($P < 0,05$). On the other hand, rumen ammonia nitrogen values were higher in the glycerol-treated group than the control group ($P < 0,05$).

From this research; it is concluded that propylene glycol fed to cow's increases blood insulin levels, while decreasing DMI significantly, so these effects should be taken into consideration in use.

Keywords: Propylene glycol, glycerol, rumen fermentation, blood parameters.

NU-P14

Effects of prepartal body condition on postpartal energy metabolism and paraoxonase-1 activity in dairy cows

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Objective: In early lactation, energy availability from dry matter intake is often insufficient to meet energy requirements of dairy cows. Overconditioned dairy cows usually mobilize more lipids from adipose tissue than cows with optimal body condition resulting in increased ketone compound formation and possibly development of subclinical and clinical ketosis. Additionally, lipid mobilisation is accompanied with increased formation of reactive oxygen species (ROS) and oxidative stress development. Thus, the aim of the study was to evaluate glucose, nonesterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) concentrations and antioxidant paraoxonase-1 (PON1) activity in overconditioned cows and those with optimal body condition.

Material and Methods: The study included twenty-four Holstein dairy cows assigned into two groups according to body condition 30 days before parturition: (1) the optimal cows (n=12) that comprised cows with optimal body condition score (BCS) between 3.25-3.75, and the adipose group (n=12) that comprised overconditioned cows with BCS ≥ 4. Blood samples were taken on days -30, -10, -2, 0, 5, 12, 19, 26 relative to parturition. Glucose concentration was measured by the commercial kit reagent (Beckman Coulter Biomedical Ltd., Ireland) on the biochemical analyser Beckman Coulter AU 680 (Beckman Coulter Biomedical Ltd., Ireland). Serum BHB and NEFA were analysed by the commercial kits (Randox, Ireland) on the biochemical analyser Beckman Coulter AU 640 (Beckman Coulter Biomedical Ltd., Ireland). The PON1 activity was measured by the spectrophotometric assay using paraoxon as a substrate on Beckman Coulter AU 680. Statistical analysis was performed by SAS 9.3 Software.

Results: Adipose cows had significantly higher ($P<0.05$) glucose concentration at the day of parturition (4.66 mmol/L) than optimal cows (3.11 mmol/L). However, in early lactation,

glucose level was significantly lower (P<0.05) in adipose cows on days 12 and 19 (2.33 and 2.46 mmol/L, respectively) compared to cows with optimal condition at the same sampling points (3.21 and 3.18 mmol/L, respectively). Serum NEFA was significantly increased in both optimal and overconditioned cows on day of parturition (1.01 and 0.80 mmol/L, respectively) and 5 days of lactation (0.93 and 0.88 mmol/L, respectively) compared to values before parturition. Serum BHB was significantly higher (P<0.05) in overconditioned cows (2.3 mmol/L) compared to cows with optimal condition (1.8 mmol/L) considering all samples in each group. There were no significant differences (P>0.05) in NEFA and BHB concentrations between the two groups at each sampling points. Nevertheless, even not statistically significant, BHB level increased in adipose cows after parturition on day 12 and 19 (3.00 and 3.26 mmol/L, respectively) indicating subclinical and clinical ketosis. In optimal cows, BHB level was the highest 5 days after calving (2.25 mmol/L) suggesting subclinical ketosis. Serum PON1 activity was higher in adipose cows 2 days before parturition and lower 5 days of lactation compared to the optimal cows, even without statistical significance.

Conclusion: Transition period in dairy cows is accompanied with negative energy balance resulting in lipid mobilisation indicated by increased NEFA concentration in blood and decreased antioxidant status. Overconditioned dairy cows had lower glucose availability in early lactation and thus produced more ketone compounds to satisfy energy requirements of the body being susceptible to subclinical and clinical ketosis.

Keywords: Body condition, lipid mobilisation, nonesterified fatty acids, β-hydroxybutyrate, paraoxonase-1.

NU-P15

The effect of beta-carotene administration to pregnant cows on biochemical and haematological parameters of their calves

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Objectives: The aim of this study was to determine the effect of beta-carotene subcutaneous administration to pregnant cows on the biochemical and hematological parameters of their calves after birth and after colostrum intake at certain time intervals.

Material and Methods: Twenty pregnant cows were randomly divided into two equal groups - control group and experimental group. In the period of 10-14 days prior to parturition, the first blood collection was performed in cows from *v. coccygea media* and at the same time the beta-carotene in experimental group was administered subcutaneously (20 ml per cow). The second blood collection in cows was performed immediately after parturition and simultaneously the colostrum sample was obtained and third sampling of blood was done 5-7 days after calving. Colostrum from first milking was used for calf's first feeding within 2 hours and second feeding up to 6 hours after born. Colostrum from second milking was used for at least two consecutive feedings. In case of calves, four blood samples were obtained from *v.jugularis* - right after birth, 2 hours and 24 hours after colostrum intake and at the age of 5-7 days. Beta-carotene was analyzed in the samples by HPLC method. Biochemical profile was determined by spectrophotometric method and hematological parameters were determined on an automatic hematology analyzer. The data were processed statistically by one-way analysis of variance (ANOVA) followed by the Fisher LSD post-hoc test.

Results: A statistically significant (P<0.01) increase of beta-carotene concentration in cow's serum was observed in experimental group (6.05μmol/l) compared to the control group (3.61μmol/l) at the day of calving. A statistically significant difference (P<0.001) in beta-carotene concentration in cows was also observed between the experimental group (5.35μmol/l) and the control group (3.41μmol/l) at 5-7 days after delivery. There was no significant increase in beta-carotene serum levels in calves and colostrum, although tendencies to higher levels in serum and colostrum were observed in the experimental group. There were no statistically significant differences between groups of calves within individual WBC counts and biochemical parameters. On the other hand, there was a statistically significant increase in RBC count ($8,14*10^{12}$/l vs $8,96*10^{12}$/l, P<0.05; 2 hours after birth), hemoglobin concentration (92g/l vs 104g/l, P<0.05 in 2 hours after birth; 82g/l vs 96g/l, P <0.01 in 24 hours after birth) and hematocrit concentration (34,9% vs 40,1%, P<0.05 in 2 hours after birth; 30,3% vs 35%, P <0.01 in 24 hours after birth) in the experimental group.

Conclusions: We did not observe any significant changes in biochemical parameters and WBC count in calves. Elevated levels of RBC, hemoglobin and hematocrit in calves from experimental group could be explained by the fact that beta-carotene is part of the antioxidant system and protects the erythrocyte phospholipid membrane from lipoperoxidation.

Keywords: Beta-carotene, calves, colostral nutrition.

NU-P16

Effect of potassium carbonate on milk yield and quality in a commercial dairy with a concentrate-based diet

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Dairy cows are fed a high amount of concentrates to achieve their requirements for high production and to minimize the effect of the negative energy balance. Under these conditions, rumen fermentation can be altered, which may result in a decrease in ruminal pH, which can promote the production of biohydrogenation (BH) intermediates, derived from polyunsaturated fatty acids (PUFA) in feed, such as trans-10, cis-12 CLA, and trans-9, cis-11 CLA recognized for their ability to inhibit the production of fat by the mammary gland. The potassium carbonate (K_2CO_3) has been used in dairy cow diets to improve the amount of fat in milk.

The effect of K_2CO_3 on milk production and quality was evaluated in a commercial dairy farm, with 800 lactating cows averaging 174 days in milk. During the first 30 days of the experiment, we incorporated in the diet a Control buffer mix consisting of 240 g of calcium carbonate, 120 g of sodium bicarbonate and 80 g of magnesium oxide (control). Then, in the following 30 days, we tested a buffer mix consisting of 140 g of K_2CO_3, 120 g of sodium bicarbonate, 100 g of calcium carbonate and 80 g of magnesium oxide (K_2CO_3 treatment). Results were collected in the last 15 days of feeding each solution. The total complete ration (TMR) was prepared daily, consisting of 64% concentrate and 36% roughage. The roughage fraction was comprised by 78% of corn silage and 22% of wheat straw. The diets supplied 37% non-fibrous carbohydrates, 24% starch and a dietary cation-anion difference (DCAD) of 255 meq/kg DM for the control diet and 342 meq/kg DM for test diet. Diets were fed ad libitum with distribution of 105% of the feed consumed on the previous day. Milk yield was measured daily and analysed for milk fat, urea nitrogen (MUN), somatic cell count (SCC) and for fatty acid (FA) composition. The milk fat tended (P = 0.08) to be higher with the test treatment (3.14% compared to 3.07% control). The fat corrected milk yield, fat yield, SCC and MUN did not differ between treatments.

The K_2CO_3 treatment changed the FA profile of milk fat by increasing (P < 0.01) the saturated FA (664 vs 641 g/kg FA) and decreasing (P < 0.001) the trans-FA (29.8 vs. 45.5 g/kg FA), and PUFA (35.6 vs. 42.1 g/kg FA) compared to control. The major trans FA in milk was the trans-10 18:1 followed by the trans-11 18:1 and both isomers decrease with the K_2CO_3 treatment. Despite that, the decrease of the trans-10 18:1 was much larger than the decrease of trans-11 18:1, and thus the t10/t11 ratio also decreased. The FA data suggest that the K_2CO_3 treatment resulted in a more stable rumen ecosystem as indicated by the more extensive BH (i.e. less PUFA in milk), more complete BH (i.e. fewer trans intermediates and more saturated FA in milk), and more healthier trans FA isomeric distribution (I,e, lower t10/t11 ratio) than control.

Keywords: Potassium carbonate; milk fat; t10/t11 ratio.

NU-P17

The effect of organic selenium supplementation to pregnant aberdeen angus heifers on colostrum quality, birth weight of calves and selenium concentration in blood

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Objectives: Beef cows are often diagnosed as selenium deficient which has a negative impact on foetal development, colostrum quality, viability of neonatal calves and calf growth and health. The objective of this study was to investigate the effect of organic selenium supplementation to pregnant heifers on colostrum quality, birth weight of calves and blood selenium levels in mothers and calves.

Materials and methods: The study was performed in an Aberdeen Angus (AA) herd with suboptimal selenium status. 16 pregnant AA heifers whose daily ration consisted of meadow hay of average quality, straw and 0.5 kg of wheat bran, were randomized into 2 groups. 8 Control heifers were received the above basal diet until the calving. The dietary Se concentration was 0.18 mg/kg on dry matter basis. 8 organic selenium supplemented heifers (Organic Se group) received the same basal diet, but from 60-80 days prepartum to the calving day were supplemented with 5 mg of organic Se (Sel-Plex, Alltech Inc., USA). Blood samples were collected from the heifers on a day before the start of supplementation and on the calving day, and from the calves on days 3, 7 and 30 of age. Selenium was measured in whole blood by the hydride method using atomic absorption spectrophotometry (AAS). IgG was measured by radial immunodiffusion both in the first colostrum and in calf serum. Body weight of the calves was measured. The data were processed with one-way Anova.

Results: The initial whole blood selenium level was 53.13 µg/l in the Control heifers and 52.24 µg/l in the Organic Se heifers. On the calving day, the Control and Organic Se heifers had blood Se concentrations of 59.60 µg/l and 95.34 µg/l, respectively. The difference was statistically significant (P<0.001). The newborn calves before colostrum ingestion showed mean blood selenium concentrations of 59.65 µg/l and 92.50 µg/l in the Control and Organic Se groups, respectively. Mean birth weight was 33.55 kg and 34.98 kg in the Control and Organic Se groups, respectively. Colostrum IgG levels were 56.51 g/l and 73.89 g/l in the Control and Organic Se groups, respectively. Serum IgG levels in the calves during the first week of live were 8.83 g/l in the Control group and 11.55 g/l in the Organic Se group.

Conclusions: The organic selenium supplementation (Sel-Plex) to pregnant heifers at 5 mg/day for 60-80 days pre-calving increased blood selenium levels in both the mothers and calves, as well as birth weight of calves and IgG levels in the first colostrum. The organic Se supplemented calves had significantly higher serum IgG levels, showed better viability and their body weight at 30 days of age was higher.

Keywords: Heifers, calves, selenium, colostrum, IgG.

NU-P18

Utilization of prepartum blood parameters related to lipid metabolism on the prediction of subclinical ketosis in multiparous dairy cows

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Objectives: Postpartum in dairy cows is a particularly critical time to meet high energy requirements following the onset of milk production. Under the conditions in which the energy requirements exceed the capacity of glucose, body fat stores can be used as an alternative energy source. Ketosis is a pathophysiologic state described as an excessive increase in ketone bodies in body fluid, but there are not enough prepartum parameters to predict the postpartum ketosis. The objectives of this retrospective study were to evaluate the various prepartum plasma biochemical parameters related to lipid metabolism in ketotic or non-ketotic cows and to examine proper predictors of the occurrence of postpartum ketosis.

Materials and methods: In this retrospective study, a total of 157 parturition data (90 primiparous and 67 multiparous cows) from a dairy farm were included. The blood samples were taken once a two-week after dry-off for the regular transition period management, and plasma was collected at least within one hour after blood sampling. All plasma samples were analyzed immediately, and the concentrations including triglyceride (TG), total cholesterol (T-Chol), low-density lipoprotein (LDL), high-density lipoprotein (HDL), β-hydroxybutyrate (BHB), and non-esterified fatty acids (NEFA) were recorded. Subclinical ketosis was defined as [BHB] ≥1.4 mmol/L or [NEFA] ≥ 0.7 mmol/L after calving. To confirm the parameters showing a significant difference during the entire dry period, biochemical parameters related to the lipid metabolism were compared between ketotic and non-ketotic cows in primiparous and multiparous cows, respectively. Then, data from multiparous cows were divided into the early (49 to 29 days before calving) and the late dry period (28 to 8 days before calving), and biochemical parameters (TG, T-Chol, LDL, HDL) with a modified parameter, non-HDL (T-Chol minus HDL), were compared according to the period. Student's *t*-test was used as statistical analyses.

Results: Subclinical ketosis was diagnosed in 60 cows (30% of primiparous, 49% of multiparous cows). In primiparous cows, there were no significant differences in all plasma biochemical parameters between ketotic (n = 27) and non-ketotic (n = 63) cows. In multiparous cows, the plasma concentrations of TG, LDL, HDL, and non-HDL in all dry period samples represented significant differences between ketotic (n = 33) and non-ketotic (n = 34) cows ($P < 0.05$). In addition, in the early dry period, significant differences were detected in T-Chol, LDL, HDL, and non-HDL ($P < 0.05$), whereas only TG was significantly different in the late dry period ($P < 0.05$).

Conclusions: In primiparous cows, subclinical ketosis occurred with lower incidence rate and was unpredictable with prepartum biochemical parameters related to the lipid metabolism. On the contrary, the prepartum blood parameters in multiparous cows represented a significant difference in the plasma concentration of T-Chol, LDL, HDL, non-HDL between ketotic and non-ketotic cows, especially at the early dry period. In conclusion, various prepartum-parameters related to lipid metabolism reflect the postpartum incidence of subclinical ketosis in multiparous cows.

Keywords: Subclinical ketosis, blood biochemistry, lipid metabolism, dairy cows.

NU-P19

The Effect of Amylase Supplementation to Different Starch Level Calf Starters on Growth and Blood Parameters in Calves

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The purpose of this study is to evaluate effects of amylase supplementation to calf starter feeds with different starch levels on feed intake, body weight gain, feed conversion rate, body sizes, clinic and respiratory score and fecal score, blood beta hydroxy butyric acid (BHBA), insulin, growth hormone, NEFA and glucose levels and, rumen parameters in calves. The research was carried out in calf unit in İtimat Agriculture and Animal Husbandry in Yenişehir-Bursa. Therefore, 6 groups were formed and 90 Holstein-Friesian female calves were used totally as 15 calves in each group. Starter feed containing 23%, 28% and 33% starch in 88% dry matter and same feeds with amylase enzyme (RumiStar, DSM Animal Nutrition & Health Turkey) added at a dose of 1 kg / ton were given to groups, respectively. The groups were named as 23E-, 23E +, 28E-, 28E +, 33E- and 33E + according to the starch content of the calf starter feeds and whether or not the enzyme is contained. Body weights of the calves measured at 0^{nd}, 28^{th}, and 56^{th} days; calf starter consumption and feed conversion were calculated weekly. On 56^{th} day, approximately 10 ml of blood sample was taken; insulin, growth hormone, NEFA, BHBA and glucose values were measured. pH measurement was made and volatile fatty acids profile and NH_3-N were measured in rumen fluid taken on 56^{th} day. Starting from 0^{nd} day, clinic, respiratory and fecal score and wither height, heart width and body length were measured. Statistical analysis of data was performed with SPSS package program. The mean starter feed consumption until 56^{th} day was 12,2 kg and there was no difference (*P>0,05*) between groups. No difference (*P>0,05*) was found among groups for daily body weight gain, feed conversion rate and blood BHBA, NEFA, insulin and growth hormon values and clinic, respiratory and fecal score and wither

height, heart width and body length. However, the fecal score taken at 8th week in 23 E (+) group was statistically different from groups 28E (-) and 28 E (+) (*P<0,05*). The pH value of 23E + feds was higher than 33E + feds (*P<0,05*). Then, 23E- feds had the lowest blood glucose levels (72,9 mg / dL) and were found different (*P<0,05*) from 23E +, 28E- and 33E + feds. That is concluded; the 23E + feds have higher rumen pH, which may contribute to health and performance.

Keywords: Calf Starter, Starch, Amylase, Growth and Blood Parameters.

NU-P20

Effect of palmitic acid supplementation on lactating dairy cow performance

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The demand for butterfat in the US together with higher milk fat prices have increased the use of fat supplements enriched with palmitic acid (C16:0). Recent studies have shown that feeding palmitic acid to lactating cows increases milk fat production. The objective of this study was to conduct a meta-analysis to evaluate the effects of dietary C16:0 intake on lactating dairy cow performance. The study included eleven trials conducted between 2007 and 2019, with 33 treatment means in which C16:0 enriched fatty acid supplements were fed to dairy cows. Palmitic acid supplements were added to the diets at 0 – 6.0% DM so cows consumed up to 1,248 g of C16:0 daily. A mixed model meta-analysis was conducted using the random effect of study weighing by the inverse of the standard error of the means squared. Linear and quadratic effects of dietary C16:0 on dry matter intake (DMI), energy-corrected milk (ECM) yield, and milk component concentration and yield were evaluated. The best fit model was chosen based on the lowest Akaike information criterion (AIC) and Root mean squared error (RMSE). Residual vs. fitted values and Q-Q plots were used to identify the heteroscedasticity and normality of the final models, respectively. Results (Table 1) indicated that increasing dietary C16:0 intake linearly increased ECM (*P* = 0.005), and milk fat concentration (*P* = 0.005) and yield (*P* <0.001). However, increasing dietary C16:0 intake did not affect DMI and milk protein yield. Interestingly, milk protein concentration decreased as dietary C16:0 intake increased (*P* = 0.01). In conclusion, these results demonstrate the potential of C16:0 enriched fat supplements to improve milk fat concentration and yield in lactating dairy cows.

Keywords: Palmitic.

NU-P21

Electrolyte imbalance in paretic cows out of puerperal period

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Objectives: Recently, a number of recumbent, paretic cows during a lactation period has increased in Finland. The purpose of this study was to estimate the electrolyte balance of these cows. For this reason, we determine levels of the electrolytes in blood and plasma (calcium, potassium, phosphorus and magnesium) from acute paretic cows out of the puerperal period.

Materials and methods: The study was carried out on 25 commercial dairy farms during the routine practice of the Production Animal Hospital of the University of Helsinki from October 2015 to January 2017. A paretic cow was included in the study outside the periparturient period (±7 days). All cows had become acute recumbent (<6 h) and not treated before for this reason in 2 month. A total of 33 cows were clinically examined and blood sampled (lithium heparin tube) from jugular vein before any treatments by veterinarians. The data from the history of the cow was collected.

Ionised calcium and potassium were determined with a 9180-Electrolyte Analyzer (Roche) in the laboratory of Production animal hospital from lithium heparin blood within six hours. Blood samples were centrifuged to collect plasma and plasma phosphorus and magnesium levels were determined with a Dri-Chem NX500i (Fujifilm). The analysis of calcium and potassium levels of two cows failed. The statistical analysis was performed with Stata IC ver 14.

Table 1. Coefficients and respective standard error (SE) for predictors of the final models in dairy cows fed C16:0-enriched fat supplements.

Item	n	Coefficient	SE	*P-value*	RMSE	AIC
DMI, kg/d	33	0.00012	0.0008	0.88	0.13	151.6
ECM, kg/d	33	0.00439	0.0014	0.005	0.06	204.9
Milk fat, %	33	0.00033	0.0001	0.005	0.12	31.2
Milk fat yield, kg/d	33	0.00024	0.00004	<0.001	0.04	-11.2
Milk protein, %	33	-0.00007	0.00003	0.01	0.07	-41.2
Milk protein yield, kg/d	33	0.00005	0.00005	0.31	0.08	-3.6

Results: In total, 33 blood samples were included in the experiment. The lactation stages of recumbent cows were 70 % during the milking period, 20% during drying off and 10% during the dry period. Five of the cows had their first lactation and the range of rest 28 cows´ lactation was two to five. Some feeding protocol changes had been implemented on the 43% of the cows within a few days before recumbent. The range of temperature of paretic cows was 35.7-39.5 C. The most of the sampled cows were hypocalcemic, n=22/31 (reference value 1.0 -1.3 mmol/l) and one of the sampled cow was hypercalcemic and the rest (n=8/31) were normocalcemic. The range of the ionised calcium levels was 0.46-1.46. Approximately half of the cows were hypokalemic, n=16/31 (reference value 3.6-6 mmol/l) and one of the sampled cow was hyperkalemic and the rest (n=14/31) were normokalemic. The range of the potassium levels was 2.3-7.5. Hypophosphatemia was observed in the 26 cows (reference value 1.8-2.1 mmol/l) and the two cows were hyperphosphatemic and the rest (n=5/33) were normophosphatemic. The range of the phosphorus level was 0.42-2.31. The four of the sampled cows (n=33) were hypomagnesemic and 15 of the cows were hypermagnesemic and the rest were normomagnesemic (n=14/33). The range of magnesium levels was 0.30-2.42.

Almost all hypermagnesemic cows were hypocalcemic too (n=13/15). The hypomagnesemic cows were mostly normocalcemic (n=3/4). The most of the hypophosphatemic cows were hypocalcemic too (n=20/26).

Conclusions: Our results show that in the acute stage, paretic and recumbent cows were mainly hypocalcemic as the clinical signs predict also out of the periparturient period. The amount of hypermagnesemia in hypocalcemic cows was high in this study contrary to literature. Frequently, feeding of the cow was changed before electrolyte imbalances.

Keywords: Paretic, cow, calcium.

NU-P22

Concentration of essential and toxic metals in milk from the state of Pernambuco, Brazil

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Objectives: Thus, the objective of this work is to determine the concentration of heavy metals in whole raw bovine milk produced in Agreste Meridional, in the state of Pernambuco.

Material & Methods: 142 milk samples were collected from dairy cows raised on 14 farms located in the mesoregion of Agreste de Pernambuco, microregion of Garanhuns. The milk was collected (15 ml) during milking in a sterile plastic container with a 15 ml Falcon lid and kept at 4 ° C for transportation, it was stored in a freezer (- 20 ° C) for further analysis. Each sample was digested with nitric acid at 69% PA by microwave at the Research Support Center (CENAPESQ) of the Federal Rural University of Pernambuco (UFRPE). Subsequently, the metals lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn) and iron (Fe) were determined by inductively coupled plasma atomic emission spectrometry (ICP-OES) at the Soil Chemistry Laboratory (DEPA) of UFRPE. The samples were analyzed in duplicate, the average calculated and the results expressed in mg / L. In the statistical analysis, the data were verified by the Kolmogorov Smirnov test, the data sets were not normally distributed and, therefore, were recorded before the analysis and the data were presented as geometric means. A General Linear Model (GLM) was used to evaluate the effect of the farm's proximity to the main roads (O: no; 1: yes); effluents (O: no; 1: yes); and type of milking (0: automatic, 1: manual) and their interactions in metal concentrations. The chemometric analysis was performed, the data of this work were organized in an X142x5 data matrix by two unsupervised multivariate chemometric techniques, the principal component analysis (PCA) and the hierarchical cluster analysis (HCA).

Results: Pb (0.0431 mg / L), Cd (0.0069 mg / L), Zn (0.6212 mg / L), Cu (0.0195 mg / L) and Fe (0.0545 mg) levels were measured / L) in raw cow's milk. The general linear model to evaluate the effect of the type of milking (M), proximity to the main routes (R) and effluents (E) and their interactions in the concentration of toxic and essential trace elements in milk in this study, the only ones that presented the results in relation to the points were Cd (R = **) R, Pb (M = *) and (E = **) in addition to Cu (E = *). These concentrations are above the maximum limits by Brazilian law. The proximity of the farm to the main roads was a significant effect of Pb, Cd and Cu on milk. The effluents were significant for Cu. In the PCA analysis, the samples were separated with proximity to highways and roads, except for the presence or absence of effluents in the vicinity and by the milking system. In the analysis of the HCA, it revealed the presence of samples belonging to farms far from the main routes of communication, in addition to samples from farms close to the highways and main roads. In addition, milk from farms far from the highways was more similar as a group than those closer to the highways.

Conclusion: It was possible to determine the presence of Pb, Cd, Zn, Cu and Fe metals in raw milk from cows in the semiarid region of the State of Pernambuco, with Pb being the heavy metal present at levels above the tolerance limit established by ANVISA (National Surveillance Agency) Sanitary). The knowledge of these mineral concentrations provides relevant information regarding the exposure of humans and animals to higher concentrations of Pb, showing a risk to public health. This fact was attributed to the greater proximity to the highways, since the highest concentrations of these elements were evidenced in properties close to the highways. It is necessary to adopt practices aimed at controlling possible deficiencies and / or intoxications in dairy cows, since this can increase the productivity and profitability of herds, in addition to improving the sanitary control of milk, thus reducing the dangers of its ingestion. for public health.

Keywords: Bovine milk, contamination, heave metals, public health, toxicology.

NU-P23

Responses in milk performance of dairy cows supplemented with protected dl-methionine in pelleted feed

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Objectives: This research work had two main objectives, evaluate the effect of the pelleting process on the product (BYMET®) effectiveness, and assess the responses of the supplementation with protected DL-methionine in pelleted feed on milk performance.

Materials and methods: The protected DL-methionine was mixed with other raw materials at a rate of 4.5% and was subjected to a pelleting process at 75º C. Then the feed was bagged and stored under normal conditions until it was used in the total mixed ration (TMR) of the "in vivo" trial. One hundred and two Holstein cows were enrolled in the trial and were housed in the farm, where environmental conditions were controlled with fans. Cows were randomly assigned into two groups (n =51) and exposed for 8 weeks to 2 treatments. Treatments consisted in the supplementation with 40 g/cow/day of BYMET® (39% of DL-Methionine) in the TMR (Crude protein (CP) 15.6%, Neutral Detergent Fiber (NDF) 28%, Crude fat (CF) 3.1%) in the methionine supplemented (MET**)** group. The control (CTL) group did not receive any amino acid supplementation in the TMR (CP 16.7%, NDF 28%, CF 3.1%). MET cows received a lower amount of CP than CTL in order to reduce TMR cost. Daily milk production was recorded individually. A milk sample per animal was collected weekly, and milk protein, milk fat, lactose, urea, and somatic cell count (SCC) were analyzed. Dry matter intake (DMI), feed efficiency (FE), and protein efficiency were measured at group level. A completely randomized block design was performed, using the animal as the experimental unit. Data were analyzed using a mixed-effect model for repeated measures, where treatment (T), week (W), days in milk (DIM), and the interaction TxW were fixed effects. Cow within treatment was the random effect.

Results: Significance difference was observed in the TxW interaction (P = 0.05) for milk yield. Milk yield of cows in the MET group was higher during the first 7 weeks of the trial. On average milk yield of MET cows was 28.8 kg/cow/d, in the case of CTL ones, the milk yield was 27.8 kg/cow/d. Milk composition (fat, protein, and lactose) was not affected by treatment (P > 0.3). Milk urea content was different (P < 0.05) among treatments, the mean was 184 mg/l in MET cows and 227 mg/l in CTL cows. These values could be indicative that MET cows consumed less total nitrogen (CP) than CTL ones. There were no differences (P > 0.4) in SCC between treatments. Dry matter intake was different among treatments, it was 24.5 kg of DMI/cow/d in the MET group, and 25.2 kg DMI/cow/d in the CTL one. It was observed a significant (P < 0.02) interaction of TxW for FE, it was higher in MET group during the first 7 weeks, and was similar in both groups in the 8th week. The protein efficiency (PE), is the ratio between the N in the milk and the N ingested, and it was significantly (P <0.001) higher in the MET group, than in CTL one.

Conclusion: It was concluded that the pelleting process did not affect the effectiveness of the BYMET®. The supplementation with DL-methionine in pelleted feed improved milk yield but did not modify milk composition. The feed efficiency and protein efficiency were improved with MET supplementation.

Keywords: Methionine, pellet process, milk performance.

NU-P24

Characteristics of Holstein cows predisposed to ketosis during the postpartum transition period

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Objectives: Ketosis, which is caused by negative energy balance, is a common metabolic disorder during the postpartum transition period of the modern high-producing dairy cattle. Despite reports on the characteristics of dairy cows associated with ketosis, researchers have not investigated the weight of calves and not classified ketosis into subclinical and clinical types. In addition, some factors are still disputable.

Material & Methods: We divided 186 Holstein cows into three groups based on the β-hydroxybutyrate (BHBA) concentration during the postpartum transition period, namely non-ketosis (NK, <1.2 mmol/L, n = 94), subclinical ketosis (SCK, ≥1.2 mmol/L and <3.0 mmol/L, n = 58), clinical ketosis (CK, ≥3.0 mmol/L, n = 34). We monitored the BHBA once in 3 days for 21 days from parturition, and recorded the average of daily milk yield during the last 3 days.

Results: No evident association of the severity of ketosis was found with method of reproduction, parturition time, pregnancy wastage, premature delivery, retained placenta, and type of calf. The severity of ketosis was significantly associated with the calving season, body condition score (BCS) at parturition, age, parity, daily milk yield during early transition, and the weight of calves (p<0.05). Cows calving in spring and especially summer were at high risk of severe ketosis. Cows with increased BCS at parturition, age, parity, and calving interval likely developed severe ketosis. Clinical ketosis cows produced the most milk from day 4 to 6, whereas non-ketosis cows produced the least. Cows calving a heavy calf showed high risk of severer ketosis, due to more milk yield during the early lactation.

Conclusion: This study is the first to investigate the associations between ketosis and the characteristics of Holstein cows by dividing ketosis into subclinical and clinical, and the association between ketosis and the weight of calf. Our findings would help predict cows at risk of ketosis and take precautions.

Keywords: Ketosis, Characteristics, Holstein cows, Transition period.

NU-P25

Effectiveness of Industrial Spot Coolers for Heat Control in Pregnant Black Japanese Cattle

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Objectives: Oxidative stress is caused by an imbalance between the production of reactive oxygen species and their antioxidant activity, and induces a variety of cellular injuries and diseases. Hot environments increase the body temperature of livestock and increase oxidative stress in the body, which has a negative effect on physiological functions and reduces productivity and fertility. It has been reported that the installation of blowers and fine misters reduced the body temperature and improved the respiration rate of cattle. On the other hand, details of heat stress in pregnant black cows before and after calving are still unclear. Therefore, the present study aimed to clarify the effect of industrial spot cooler on heat stress reduction and oxidative stress related factors in peripheral blood of pregnant Japanese Black Cattle during summer.

Materials and methods: The cows were 1farm-bred Japanese Black pregnant 9 cattle and their 8 calves and were divided into two groups: cooled (5 mothers and 4 calves) and uncooled (4 mothers and 4 calves). The cows in the cooled group were cooled in an industrial spot cooler from the 4week before the expected calving week to the 4days after calving. Blood samples were taken from the jugular vein of the cows on the day of moving the calving cell before the expected 4 week of calving, during the preparation period 14 to 18 days before calving, and during the week and 2 week after 1 calving to determine malondialdehyde (MDA) as an indicator of oxidants, potential antioxidant capacity (PAO), glutathione peroxidase (GPx), VA, VE, VC, and general Biochemical tests (Glu, NEFA, T-Cho, TP, Alb, BUN, AST, GGT, IP, Ca) and cortisol (Corti) were measured. Colostrum was collected within a 1day after calving and the concentration of IgG in colostrum was determined. Calves were bled from the jugular vein on the 1, 4, and 7 day after the birth, and serum IgG concentrations and general biochemical tests (Glu, T-Cho, TP, Alb, BUN) were measured. The environmental temperature and humidity of each group were measured and recorded every 15minute, and the temperature-humidity index (THI) was calculated and compared at the end of the experiment.

Results: Corti in sows was significantly higher in the non-cooled group compared to the cooled group during the calving preparation period. In relation to oxidative stress, VC decreased after calving in both groups and was significantly lower in the uncooled group compared to the cooled group in the postpartum1 week. There were no significant differences in colostrum IgG or serum IgG between groups, and more data with THI 72higher in the uncooled group (3539) than in the cooled group (3116).

Conclusion: Based on the report that heat stress increased plasma Cori, the uncooled group was more exposed to heat stress than the cooled group during the calving preparation period. The report that parturition under heat stress decreased blood VC concentration and increased oxidative stress suggested that the cooling group may have been less affected by parturition than the non-cooling group. Heat stress was quantified by THI, and the report that dairy cows began to feel heat stress when THI was higher 72than that of the cooled group suggested that the uncooled group may have been exposed to more heat stress than the cooled group. The calves were separated from their mothers and reared in different environments for each sex. The use of industrial spot coolers is also in line with the recent SDGs, as it will help to combat global warming in the future and will make a sustainable contribution to milk and meat production.

Keywords: Black Japanese Cattle, Cortisol, Heat control, Oxidative stress, Temperature-humidity index.

NU-P26

Effect of parenteral use of minerals in antioxidant response capacity and health and production parameters in high-producing dairy cows

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Objectives: The peripartum period of high yielding dairy cows is a highly stressful period with possible metabolic, health and production disorders. Oxidative stress (OS) is associated with the calving itself, but also with the intensity of the negative energy balance (NEB), which in turn is conditioned by the genetic potential of the cow, milk production and nutritional status prior to calving. The objective of this work was to evaluate the effect of the strategic use of minerals during the peripartum of high-producing dairy cows on the antioxidant response and its relationship with health and productive parameters.

Material and methods: A total of two 250 Holstein cows fed according to NRC recommendations (NRC Dairy Cattle, 2001) and housed in the same herd were randomly assigned to 1) Group F (N=125), supplemented by injection with 10 mL of a multi-mineral solution(575.12 mg P; 31.36 mg K; 29.88 mg Mg; 14.91 mg Cu; 10.89 mg Se, FOSFOSAL®, Virbac Santa Elena, Uruguay) applied 20 days before calving, on the day of delivery and 14 days postpartum, and 2) Control group (C) (N=125), with identical treatment schedule, but using sterile saline solution. Information collected was a) Health parameters: calving difficulty, clinical hypocalcemia, mastitis, retained placenta, metritis, torsion of the abomasum, clinical ketosis, and mortality up to 30 days postpartum; b) Productive parameters: milk production at 15, 30, 60 and 90 days postpartum and total milk solids, protein, butterfat and somatic cell count (SCC) in milk. Moreover, blood samples of 50 randomized selected animals from each group were obtained by puncture of

the jugular vein on days 0, 4 7 and 14 postpartum. Samples were used for determinations of superoxide dismutase (SOD) activity, glutathione peroxidase (GPX) activity, antioxidant capacity (CA), TBARS, cortisol, NEFA, BHBT, and glucose. The Oxidative stress index (OSi) was calculated as ROS/SAC, expressed as µM of malondialdehyde (MDA)/CA in nM. Parametric data were analyzed using a completely random design, with a mixed model with repeated measures over time, where treatment, time and interaction were the dependent variables and animals the random variable. Qualitative data were compared using c^2 test. Data analysis were performed using the SAS® Statistical Software (SAS Institute Inc., Cary, NC, USA). A statistical significance of 5% was considered.

Results: Cows from group F had higher SOD activity on day 7 postpartum compared to group C (0.524 ±0.097 vs. 0.357 ±0.165 U/mL), higher GPX activity at calving (75.41 ±1.96 vs. 67.22 ±2.42 U/mLPack-Cell-Volume) and 14 days postpartum (88.87 ± 3.06 vs 78.33 ±3.43 U/mLPCV), and higher AC at delivery (1.008 ±0.038 vs 0.887 ±0.047 nM) and 14 days postpartum (1.056 ±0.051 vs 0, 94 ±0.047 nM). The TBARs were higher in animals from group C on day 7 postpartum (0.771 ±0.023 vs. 0.721 ±0.014 µM). The overall OSi was higher in animals from group C (1.003 ±0.028 vs 0.923 ±0.022; p=0.014). Additionally, OSi were higher in group C at calving and 7 days postpartum (p< 0.05). BHB and glycemia were higher in control animals at 4 days postpartum (1.114 ±0.032 vs 1.118 ±0.034 mmol/L 62.5 ±1.19 vs 68.54 ±0.89 mg/dL respectively). In the second lactation, supplemented cows had a lower incidence rate of mastitis (p< 0.033) than not supplemented animals. The overall mortality rate was lower in Group F than in group C (7 vs 18%; p= 0.02) and a trend towards higher milk production in the first 15 days postpartum exists (36.71 ±1.31 vs 33.38 ±1.47 L/day; p= 0.067). No differences were found for NEFA and cortisol, and the other health and productive parameters.

Conclusions: The use of this parenteral mineral supplementation scheme during the peripartum of high yielding dairy cows, however fed according to the criteria established by the NRC, increased the antioxidant capacity and reduced peroxidative damage of membrane lipids (Lower OSi) during the first two weeks after calving. The lowest values of blood glucose and BHB at 4 days after calving suggest a better adaptive response to stress and metabolic stress. Related to these results, and perhaps as a consequence of them, supplemented animals showed a significant decrease in the mortality rate and a marked tendency to produce more milk than control animals in the first 2 weeks of lactation.

Keywords: Trace minerals, dairy cattle, oxidative stress.

NU-P27

Effect of injetable mineral supplement on metabolic health of holstein cows during the transition period

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Objective: The aim of this research was to evaluate the effect of a mineral supplementation program by injection on metabolic health in Holstein cows during the transition period.

Material and Methods: The study was conducted in a commercial dairy operation composed by approximately 400 lactating Holstein cows, with an average daily milk production of 35 L per animal, located in São Pedro, São Paulo, Brazil. Close-up and fresh cow diet was formulated by the farm's nutritionist, and complied with NRC recommendations for dairy cows. Healthy cows were enroled in the study by 30 days before calving, or so, and allocated to either the control group (group C, n= 123) or the treatment group (group F, n= 189). Treated cows received three injections (10 mL) of a multi-mineral supplement by intramuscular route 30 to 20 days before calving, at calving, and 11-17 days after parturition. A 10mL dose of the mineral complex (FOSFOSAL®, Virbac Brasil, SP, Brasil) provided 575.12 mg P; 31.36 mg K; 29.88 mg Mg; 14.91 mg Cu; 10.89 mg Se. Ketosis was monitored on days 5 (d5) and 10 (d10) after calving by using portable device for measuring blood β-ketone that is suitable for bovine samples. The β-ketone cut-off used to interpret the data was 1.2 mmol/L. Animals were classified in transitory (high levels [β-Ketone] ≥1.2 on d5), persistent ([β-Ketone] ≥1.2 on d5 and d10) and late ketosis ([β-Ketone] ≥1.2 on d10). Blood samples was harvested at calving (d0) and the 4th day after parturition (d4) to measure total calcium by using biochemical laboratorial techniques. The cut-off used to interpretate data was [Ca]=8.0 mg/dL. Animals were classified in transitory ([Ca]<8.0 on d0), persistent ([Ca]<8.0 on d0 and d4) and late hypocalcemia ([Ca]<8.0 on d4).

Results: Injectable minerals supplementation had an effect on the incidence rate of ketosis on d5 in ≥3 lactation cows (C= 51.22 vs F = 31.34 %). The C group had 2.3 more chances (Odd Ratio, OR) to develop ketosis (CI 95% [1.032;5.123]). The incidence of persistent ketosis on ≥3 lactation cows was 46.34 and 25.00% for group C and F, respectively (OR =2.59, CI95% [1.124;5.969]). This data reflects on the multiparous category results, observing 1.98 (CI95% [1.021;3.832]) more chances for the group C animals to develop persistent ketosis. On d0, the incidence rate of hypocalcemia was higher in group C than in group F (40.00 vs 17.07%; OR=3.238, CI95% [1.085;9.663]) for cows in their 2nd lactation. Similar results are observed also on d10 with incidence rates of hypocalcemia of 28.57 and 10.91% for group C and F, respectively (OR=3.267, CI95% [1.109,9.621]). Persistent hypocalcemia ([Ca]< 8.0 mg/dL, on d0 and d4) was much more frequent in all group C cows than in group F (OR=3.128, CI95% [1.129;7.595]), more specifically in multiparous cows (OR=2.981, CI95% [1.175;7.567]) and 2nd parity animals (OR=4.605, CI95% [1.105;19.186]). Not

any difference was noted in the incidence rates of mastitis, endometritis or retention of the placenta. In contrast, cows dosed with the mineral complex presented less puerperal metritis (OR=2.101, CI95% [1.218;3.625]). This observation remained significant for multiparous cows (OR=2.073, CI95% [1.072;4.008]) and 2nd lactation cows (OR=3.294, CI95% [1.192;9.106]).

Conclusions: The results obtained in this study suggest that a series of three injections of the mineral complex tested, 3 to 2 weeks before calving, at calving and 2 weeks later, significantly improves the critical biomarkers of energy and mineral metabolism in Holstein cows with high yield, and incidence rate of puerperal metritis as well. Because ketosis and hypocalcemia underlie most other conditions of the perinatal period, the effects of such a prophylactic treatment regimen on production diseases deserve further investigation by more focused and more powerful studies.

Keywords: Ketosis, hypocalcemia, metritis.

NU-P28

Live yeast additive improves the performance and abundance of beneficial rumen bacterial populations in dairy calves

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Objective: Weaning is a challenge for calves and their producers. During the first months of its life, the digestive tract of a young ruminant undergoes significant and extensive changes as it evolves from its initial monogastric function. Calves usually are separated from their dam before this process is complete. As a consequence, rumen development becomes a concern with possible subsequent performance downfalls over the first months of life. The live yeast *Saccharomyces cerevisiae CNCM I-1077* is known to boost the pre-ruminant rumen maturation (Chaucheyras-Durand and Fonty, 2002) and favor calves growth and rumen fibrolytic bacteria (Terre et al., 2015).

The objective of this study is to evaluate the impact of a specific live yeast strain of *S. cerevisiae* on baby calves' zootechnical performance and to investigate the supplementation effect on ruminal microbiota.

Material and Methods: Fourteen Holstein male calves (47.6±6.7 kg; 10.3±3.3 days' old) entered the study for 9 weeks in sand bedded individual hutches. They were randomly allocated to a negative control (C) or a live yeast supplemented treatment group (*S. cerevisiae CNCM I-1077*, Levucell SC, Lallemand SAS; LSC) at 1.5x10e9 cfu/kg started feed (i.e. 1x10e9 cfu/kg complete feedingstuff). Animals were fed 2 x 2L/d milk replacer (MR) + starter feed (SF) ad libitum up to 28d of age, then 1 x 2L/d MR + SF ad libitum from d28 to weaning (d54), and after weaning SF ad libitum until departure. MR was composed of crude protein (CP, 21.7%), fat (16.4%) and lactose (47.2%). Starter Feed consisted in flour meal (CP: 16%, NDF: 13.5%, Starch+sugars: 49%) and was offered once a day manually for ad libitum consumption. They had no access to straw or other forage. Calves were weighed weekly and feed intake monitored accordingly. Rumen content was sampled at arrival and d 60 by esophageal tubing.Genomic DNA was extracted from rumen samples with Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (Zymo Research). Rumen microbes were quantified by qPCR targeting total bacteria, protozoa, *Ruminococcus albus, Ruminococcus flavefaciens, Fibrobacter succinogenes*, and fungi. The diversity and taxonomic composition of bacterial population were also evaluated through 16S rDNA MiSeq Illumina sequencing.

Results: At zootechnical level, ADG (482 vs 611 g/d; P<0.05) and feed efficiency (2.40 vs 2.18; P<0.10) were significantlyincreased in LSC supplemented calves during the first two months of life and starter intake was increased across the whole period (P<0.10). Overall, calves were very poorly colonized by ruminal fibrolytic populations (protozoa and fungi were not detected) and only a subset of calves harbored fibrolytic bacteria. This was probably linked to the high level of starch in the diet. However, a beneficial effect of supplementation could be observed on microbial data as bacterial colonization of fibrolytic species (ie *Fibrobacter succinogenes* and *Ruminococcus flavefaciens*) was enhanced in rumen contents of LSC supplemented calves. The initial groups could not be balanced according to their ruminal bacterial populations, and statistical differences were observed at the genus level for few OTUs. However, those differences were not impacting the global bacterial community structure as observed through the β-diversity measures. Sequencing data showed the effect of supplementation on lactate utilizers belonging to *Megasphaera* and *Selenomonas* genera which, although found in low relative abundance (<0.2%), were observed in higher proportion in LSC supplemented calves (P<0.05). None of these genera, both belonging to *Negativicutes*, was identified in T0 samples.

Conclusion: LSC improved the growth performance of calves around weaning, notably driven by a greater daily starter intake, as a sign of good rumen health at this stage, suggesting an easier transition to solid feed and a better rumen development. Further insight,, LSC supplementation increased the relative abundance of some beneficial functional bacterial populations in the rumen of supplemented calves, helping then the animal to maintain a good rumen function and health. More precisely, lactate utilizers play a role in lactate removal from the rumen and thus have a protective action against acidosis, and fibrolytic bacteria are key in plant cell wall degradation.

This study is bringing complementary information at zootechnical and microbial levels, all indicating positive effects of LSC supplementation on performance and ruminal health of dairy calves.

Keywords: Dairy calves, live yeast, rumen bacteria abundance.

NU-P29

Relationship between 7th day metabolic parameters and day of abomasum displacement diagnosis in postpartum dairy cows

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Objectives: Displacement of abomasum (DA) is among the main health problems encountered in high yielding dairy herds in the postpartum (PP) period. Metabolic parameters can be used to predict the animals at high risk and thus to decrease the incidence of the disease in herds. In the presented study, the animals that were diagnosed to suffer DA within the first 30 days PP were assigned to three goups based on postpartum timing of DA diagnosis, and betahydroxybutyric acid (BHBA), blood urea nitrogen (BUN), gamma glutamyl transferase (GGT), aspartate aminotransferase (AST), cholesterol (Chol), albumin (Alb), calcium (Ca), magnesium (Mg) and creatinine (Crea) levels were measured in blood samples collected on the 7th day PP and relationship with PP day that DA had been diagnosed was investigated.

Materials and Methods: 177 Holstein-Fresian cows in 1.-4. lactation (mean 1.8) from the same herd that were diagnosed to suffer DA (172 left and 5 right) in PP 1-29 days (mean= 9.75 days PP), were used in the study. As the standard operating procedure of the herd, blood samples were collected on day 1 PP for Ca and day 7 PP for BHBA, BUN, GGT, AST, Chol, Alb, Ca, Mg and Crea analysis. Cows were assigned to three goups based on the time of DA diagnosis; E(early) group consisted of cows that were diagnosed in the first 7 days PP (n=90), M(mid) group from that diagnosed on day 8-14 (n=48) and L(late) group on day 15-29 (n=39). Shapiro-Wilk test was used for evaluation of normality within groups, data that was normally distiributed was analysed by One Way Analysis of Variance and data that was not normally distributed by Kruskal-Wallis One Way Analysis of Variance.

Results: PP day 1 Ca of 177 cows was measured as 8.19±0.1 mg/dl and 41 of 177 cows (%34.7) suffered from hypocalcemia (Ca ≤ 8mg/dl). PP day 7 levels of BUN, AST, Chol, Alb, Ca, Mg and Crea levels did not differ between groups. BHBA levels were 0.97±0.11, 1.18±0.18 and 0.63±0.15 mmol/l in E, M and L groups respectively and BHBA was significantly (P=0.04) higher in M when compared to L group. GGT levels were measured as 26.8±2.8, 37.7±5.8 and 23.1±2.0 IU/l in E, M and L groups respectively and GGT was again significantly (P=0.03) higher in M when compared to L group.

Conclusions: The fact that the calcium level on the first day of PP was below 8 mg/dl in only 34.7% of the animals is a finding indicating that the role of hypocalcemia in the etiology of displacement in the herd is probably only limited. Previous studies have demonstrated that incidence of abomasum displacements increase significantly in herds that subclinical ketosis is detected. On the other hand, the fact that the BHBA and GGT levels were significantly higher in the M group when compared to the L group indicated that the relationship between elevated BHBA and GGT levels with abomasum displacements may be more apparent in a certain period of time.

Keywords: Displacement of abomasum, BHBA, GGT, prediction.

NU-P30

Changes in blood glucose levels, insulin, cortisol and ammonia concentrations in the hepatic portal, hepatic and jugular veins in Holstein cattle during the Feeding Period

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Objectives: Generally, blood glucose, insulin, cortisol and ammonia concentrations are measured with blood samples taken from the jugular vein. In this study, catheters were installed in the hepatic portal and hepatic veins by transdermal method under ultrasound guidance, and blood collection was carried out. We also investigated the transitions of the respective nutrient and hormone concentrations in the blood from 15 minutes before feeding to six hours after feeding.

Materials and methods: Five Holstein calves (34 ± 8.3 days, 51.9 ± 5.1kg) was used in this study. The calf received milk replacer (430g/ 3.5L/ time×2 times/ day), calf starter (800g/ day) and hay (500g/ day). An ultrasound imaging system with a convex probe (25Hz) (**ProSound α6, Hitachi-Aloka Medical,** Tokyo) was used in the catheterization of the portal and hepatics veins. The catheterization was performed under adequate sedation with subcutaneous injection of xylazine (0.05mg/ kg) and procaine hydrochloride (1mg/ kg). Blood samples were collected from the hepatic portal, hepatic and jugular veins 15 minutes before feeding, and 15, 30, 45, 60, 90, 120, 180, 240 and 360 minutes after feeding. Blood glucose, insulin, cortisol and ammonia concentrations were measured, and the transitions were compared. Measurements of aspartate aminotransferase (AST) and γ-glutamyl transferase (GGT) were carried out at the last blood withdrawal. Blood collection was started 2 ~ 24 hours after catheter installation, by using standard EDTA vacuum blood collection tubes. Protein-free tubes were used for blood samples collection for ammonia measurement. The implanted catheters were filled with heparin (1000 units/ml) to prevent blood clotting.

Results: Blood glucose levels were significantly higher in the jugular vein at 30 minutes after feeding and 15 minutes in the hepatic portal vein and hepatic vein compared to before feeding. Jugular veins were lower than that of hepatic portal veins and hepatic veins.

Insulin showed a significantly higher value 15 minutes after feeding in the hepatic portal vein compared with before feeding. In the jugular vein, the value was significantly higher in 120 minutes. Ammonia was significantly higher from 30 minutes after feeding compared with before feeding. Cortisol concentrations showed similar changes in the portal, hepatic and jugular veins, however, there was no significant difference. No abnormalities were found in the AST and GGT of the jugular

venous blood taken at the end of the study. All results were within the normal range during the study period.

Conclusions: Catheterization of the portal and hepatic veins was effective for repeated blood collection over a period of time. The blood ammonia concentration in the portal vein remained elevated during the feeding period, but it was metabolized in the liver and fell back to normal level in the hepatic and jugular veins. A lower blood glucose level was observed in the jugular vein in comparison with those in the portal and hepatic veins, suggesting a connection to the insulin concentration.

Keywords: Ammonia, glucose, hepatic portal, hepatic vein, insulin.

NU-P31

Reticular pH of transition cows fed grass-silage based partial-mixed-ration in a commercial dairy farm

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Objectives: Rumen has a complex entity of microflora, responsible for digestion of the ingested feed. If the stability of the microflora becomes disturbed because of a decline in rumen pH, energy and protein metabolism will be suboptimal and lead to various problems concerning cow health, productivity, and well-being. During transition period a cow undergoes significant changes: calf is born, milk production starts and feed changes from low- to high-energy roughage and concentrates with high protein and starch content. This could potentially lead to lowered pH of rumen (sub acute ruminal acidosis (SARA)). The aim of this study was to find out whether cows have SARA in transition period in a modern commercial Finnish farm. We also wanted to describe dynamics between reticular pH, energy balance of the cow, markers of inflammation and activity.

Materials and methods: Reticular pH of ten cows was measured during Autumn 2020 in a free-stall dairy barn with one milking robot. Oral boluses (SmaXtech animal care®, Graz, Austria) measured pH and temperature of the reticulum and activity of the cow using wireless, online technique at ten-minute intervals. The boluses were inserted 14 days before the expected calving, and the cows were observed until 35 days in milk (DIM). Cows were inspected d -14, -5, 5 and 35 relative to calving. Rumen fill, body condition, manure and vaginal discharge were scored from 1 to 5 and rectal temperature measured. Udder was palpated, milk inspected and California Mastitis Test carried out. If the test was 3-5/5, a bacterial sample was sent to laboratory for PCR analysis.

Serum for BHB (beta-hydroxy-butyrate), NEFA (non-esterified fatty-acids), SAA (serum amyloid-A) and haptoglobin analysis was separated from blood and frozen *in situ*. Blood calcium and serum fibrinogen concentrations were kept cold and analysed 1 to 4 days after sampling. The milking system recorded the timing of visits to the robot as well as milk yield and concentrate consumption.

The cows were fed grass-silage based mixed-ration complemented with concentrates up to 6 kg from the milking robot. The proportion of concentrates was approximately 55% in the whole ration and proportion of fiber from roughage was > 297 g/kg dry matter. Feeding space for milking cows was 55 cm/cow. The barn had facilities for close-up cows with bedded pack of straw. Dry cows were fed total mixed ration consisting of grass silage and straw complemented with less than 1 kg /d of concentrates. Associations between pH, blood concentrations and production parameters will be analyzed using Stata/MP 16.0.

Results: All ten cows experienced a decline in reticular pH 1 to 3 days after calving. The pH was normalized by 9 DIM. Four cows had pH < 5.8 for more than 3 hours per day, which fulfills the definition of SARA. However, additional 3 cows having low pH for < 3h/day were close to being classified as having SARA because reticular pH is approximately 0.2 higher than in rumen. Before calving, reticular pH fluctuated for 0.5 units following feeding every other day. Two of the cows with SARA had retained fetal membranes which reflected to low BCS and rumen fill, and high body temperature and fibrinogen throughout the study. Before calving they had high NEFA concentration. After calving they had high SAA and haptoglobin concentrations and low milk yield (20.4 vs. >27 kg) and concentrate consumption (2.4 vs. 3.4 kg) and high BHB concentration 35 DIM. Cows suffering from SARA without retained placenta had high serum BHB concentration after calving. Blood calcium was in reference level at 5 DIM. Seven cows had subclinical NAS infection in one quarter. Cows without SARA visited the robot 4.8 and cows with SARA 3.6 times per day.

Conclusions: In spite of modern dairy farming with good facilities, good care, grass-based silage feeding with high level of fiber and cows with optimal BCS, at least 4/10 cows developed SARA soon after calving. Besides dietary manipulation, there should be calm and spacious facilities for fresh cows to eat roughage to control SARA. To keep rumen pH steady before calving, feed should be delivered at least once per day.

Keywords: Reticular pH, dairy cow, grass-silage, transition cow.

NU-P32

Association of dietary intake and plasma concentrations of vitamin E in transition dairy cows

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Objectives: Vitamin E is usually supplemented to dry cow diets to meet requirements. Differences in dry matter intake between cows in a herd may, however, lead to variation in the vitamin status of animals around parturition. Objectives of the present study were to assess the association between dietary

intake and plasma concentrations of vitamin E in transition dairy cows and to study how variation in dry matter intake around parturition can effect the vitamin status of animals in this period.

Materials & Methods: The study was performed at the dairy farm of Schothorst Feed Research B.V (Lelystad, the Netherlands) between September 2018 and February 2019. Sixty cows of all parities were enrolled in the study at approximately four weeks before the expected calving date. Animals were housed in a loose housing system equipped with individual feeding places and individual daily feed intake was registered from four weeks before the expected calving date until four weeks after calving. Animals had ad libitum access to a basal diet (~5% residuals) and water. The dry cow diet consisted of grass silage, corn silage, straw, a protein supplement, and 1 kg of concentrates per cow per day. The basal diet for lactating cows consisted of grass silage, corn silage, pressed beet pulp, and a protein supplement. Concentrates were fed to lactating cows according to a fixed scheme based on age and days in milk.

Samples of roughages (grass silages and corn silage) were taken once a week, stored frozen until the end of the experiment, and pooled per batch for analysis. Concentrates were sampled at delivery. Vitamin E concentrations in feedstuffs were analyzed using high-performance liquid chromatography. The total vitamin E concentration in the dry cow diet was 108 IU/kg DM and in the lactation diet this concentration averaged 87 IU/kg DM. Plasma samples were collected at approximately one week before the expected calving date and at four weeks after parturition. Vitamin E concentrations in plasma were analyzed using high-performance liquid chromatography. Feed intake data were available for 57 out of 60 cows. Prepartum and postpartum plasma vitamin E concentrations were available for 56 and 54 cows, respectively. Descriptive statistics were performed and the association between the average dietary intake of vitamin E in the three weeks preceding the week of sampling and the vitamin E concentration in plasma of the cows was assessed by linear regression using separate models for prepartum and postpartum data.

Results: Descriptive statistics are shown in Table 1. Considerable variation existed in the dry matter intake of transition dairy cows, even under the conditions in this study where animals had unlimited access to the diet via individual feeding places. As a result, differences in the dietary vitamin E intake also occurred between animals. Plasma vitamin E concentrations were larger at four weeks after parturition than at one week before calving ($P<0.001$). Dietary intake of vitamin E was significantly associated with plasma vitamin E concentrations ($P<0.01$) in both prepartum and postpartum cows.

Table 1: Descriptive statistics of dry matter intake (DM), vitamin E intake, and plasma vitamin E concentrations in transition dairy cows on an experimental dairy farm.

Parameter	N	Average	SD	Median	Min	Max
DM intake (kg/day)						
Prepartum (week -4 to -2)	57	14.6	2.2	14.4	10.6	19.9
Postpartum (week 1 to 3)	57	19.8	3.4	19.6	11.1	28.3
Vit. E intake (IU/day)						
Prepartum (week -4 to -2)	57	1545	318	1529	947	2376
Postpartum (week 1 to 3)	57	1785	452	1759	713	2906
Plasma Vit. E (µmol/L)						
Prepartum (week -1)	56	12.5	4.1	12.1	4.1	23.1
Postpartum (week 4)	54	14.4	3.9	14.4	6.4	21.3

Conclusion: Dietary intake of vitamin E was associated with plasma vitamin E concentrations in transition dairy cows. Differences in dry matter intake of cows in the transition period may lead to variation in the vitamin E status within a group of cows fed on the same diet.

Keywords: Vitamin E, transition period, dairy cows.

NU-P33

Usefulness of bulk milk selenium analysis as screening method for selenium deficiency in dairy herds

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Objectives: Investigations into herd health or performance problems often involve analyses of the selenium status of the herd. Usually this is done via blood sampling a representative number of animals. Identification of selenium deficient herds via analysis of a bulk milk sample would cause less effort, costs and no stress for the animals. Information on the value of using bulk milk in this context is sparse in the current literature. Therefore, the aim of this study was to evaluate the usefulness of selenium analysis in bulk milk as a screening method for selenium deficiency in dairy herds.

Materials and methods: Selenium status was investigated in 318 dairy farms by veterinarians of the Bavarian Animal Health Service in 2019. In addition to 9 or 10 serum samples from cows on each farm a bulk milk sample was submitted for analysis. Analysis was performed through inductively coupled plasma optical emission spectrometry. A mean selenium concentration of below 70 µg/l in the serum samples was defined

as a selenium deficient herd. This definition was based on the fact that in herds with mean serum selenium concentrations of 70 µg/l and above, no individual animal showed a concentration below the reference value of 60 µg/l (Raven J., 2013, Untersuchungen zur Diagnostik der Selenversorgung von Milchkühen. Diss. med. vet., Freie Universität Berlin). Data were analysed using IBM SPSS Statistics 24.0.0.1. A receiver operating characteristic (ROC) analysis was used to test the reliability of the bulk milk result for the prediction of a selenium deficiency on a herd level.

Results: Correlation between bulk milk selenium and mean serum selenium concentration was significant but only moderate (r_s= .606). The area under the curve (AUC) is .78 (95% CI: .729 - .830). Analysis of sensitivity and specificity revealed that below a cut-off of 12.9 µg/l selenium deficiency can be diagnosed with a certainty of 99% and above 23 µg/l a deficiency can be ruled out with a certainty of 95%.

Conclusions: The results of the reported study showed that selenium analysis in bulk milk can at the best be used as a very rough predictor of selenium deficiency, since a wide range of values does not allow for a clear conclusion on the status of the herd. This also means that selenium excretion in milk is not closely correlated to the selenium status of the cow. This information is of interest for the investigation of calf health related problems, since it means that it cannot be concluded from an adequate selenium status of the cows that calves receive enough selenium via the milk fed.

Keywords: Selenium deficiency, bulk milk analysis.

NU-P34

Iron supplementation in dairy calves – necessary or superfood for bugs?

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Objectives: Recent investigations into risk factors for calf diarrhea as a herd health problem identified iron supplementation soon after birth as associated with a higher risk (Lorenz et al., 2021). On the other hand, recommendation of iron supplementation for calves by agricultural advisors is not uncommon. Therefore, in this study we evaluated the risk of anemia in dairy calves in relation to iron supplementation and feeding.

Materials and methods: The hematological status of healthy calves (n=261) was investigated on 55 dairy farms by veterinarians of the Bavarian Animal Health Service in 2020. On each farm 2 to 8 EDTA blood samples were taken from calves up to an age of 3 months. Details on feeding practice, iron supplementation and the frequency of calf diarrhea were inquired by means of a face-to-face questionnaire. Calves with (Group IS1, n=55) and without (Group IS0; n=206) iron supplementation were compared, as well as calves with iron supplementation and/or milk replacer feeding (Group Iron1, n=93) vs. no iron supplementation and whole milk feeding (Group Iron0, n=168). Data were analyzed using IBM SPSS Statistics 24.0.0.1. by means of student's t-test or Chi-Square-test.

Results: Over the whole range of calves aged up to 3 months there was a significant difference in hematocrit between IS1 and IS0 (36.24% vs. 33.13%, p= .016) but not in hemoglobin concentration. There were significant differences between Iron1 and Iron0 in hematocrit (35.29% vs. 32.95%, p= .034) and hemoglobin concentration (11.07 vs. 10.51 g/dl, p= .049). However, if only calves up to an age of 3 weeks were compared, no differences could be found. Calves from groups IS1 and Iron1 had a significantly higher risk to originate from a farm with a high incidence of calf diarrhea (above 25% of calves affected).

Conclusions: Iron concentration is low in colostrum and milk of all mammals. A reason for this might be that iron is necessary for the multiplication of many infectious agents. In natural environments young animals would start consuming iron rich food early in life. Only if this is prevented by husbandry conditions, the risk of iron deficiency anemia arises. However, an early supplementation of iron does not improve hematological values in calves, but is associated with a higher risk of calf diarrhea. Therefore, it is not recommended to supplement calves with iron orally or by injection within the first 3 weeks of life.

References:

Lorenz I, Huber R, Trefz F (2021). A High Plane of Nutrition Is Associated with a Lower Risk for Neonatal Calf Diarrhea on Bavarian Dairy Farms. Animals, 11(11), 3251; https://doi.org/10.3390/ani11113251.

Drakesmith H, Prentice AM (2012). Hepcidin and the iron-infection axis. Science, 338, 768 – 772.

Keywords: Iron supplementation, calves, calf diarrhea.

NU-P35

Impact of phosphorus depletion in dry period on calcium homeostasis, productivity and metabolism in following lactation period

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Objectives: Restricting the phosphorus supply is beneficial for the calcium homeostasis of periparturient cows but is otherwise thought to present a risk for health and productivity. The objective of this study was to investigate the impact of restricted phosphorus supply during the dry period on calcium

homeostasis, productivity and metabolism in the following lactation in dairy cows.

Material and Methods: Thirty late pregnant cows were assigned to either a dry cow ration with low (0.16%, LP) or adequate (0.30%, AP) phosphorus content for the last four weeks before parturition. After calving all cows received a ration with adequate phosphorus content (0.46%). Blood and liver tissue samples were obtained regularly. Body weight, feed intake and milk yield were also recorded.

Results: Plasma phosphate was decreased before calving but was higher shortly after parturition in LP cows compared to AP cows. Total plasma calcium was higher at 6 and 12 hours, and 2 and 4 days post-partum in LP cows compared with AP cows. No treatment effect was observed on feed intake, parameters related to energy-, protein- or lipid-metabolism in blood or liver, body weight and milk yield.

Conclusion: Dietary phosphorus restriction during the dry period of dairy cows had a beneficial effect on the calcium homeostasis around calving. Negative effects on feed intake, metabolic activity or productivity were not observed.

Keywords: Phosphorus, dry cow.

NU-P36

Performance responses to malate supplementation in beef cattle

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Objectives: The aim of this study was to evaluate the effect of supplementation with malate (RUMALATO®), on productive performance of beef cattle.

Materials and methods: Eighty-two beef calves were enrolled in the study, they were individually identified and had free access to drinking water. Animals were housed under standard production conditions. The housing system was a free stall on straw bedding. The animals had an area of 10 m²/animal. Calves were splited into two homogeneous groups (n=41) with an average of 128 kg of body weight (BW) each one. Animals received complete feed, supplemented with 2 kg/tone of RUMALATO® (RUM), or not supplemented in the CONTROL group (CTL). Animals received straw ad libitum. Animals were weighed at the beginning and at the end of the trial. Total increase of BW (BWI) and average daily gain (ADG) were measured individually. Dry matter intake (DMI) of feed and feed conversion ratio (FCR) were measured at group level. The trial followed a complete randomized design, with animal as the experimental unit. Data were analyzed using a general linear model (GLM). In the model, treatment was the main factor, and total days was a cofactor. Mean values were compared with Tukey test ($P < 0.05$).

Results: Total increase of BW (BWI) was significantly different ($P = 0.02$) among groups. Calves in the CTL group showed a mean of 189.5 kg BW, and RUM animals showed a mean of 195.8 kg BW. Average daily gain of the RUM calves was higher ($P = 0.01$) than CTL ones, it was 1.45 kg BW/d for RUM group and 1.40 kg BW/d for CTL group.

Regarding DMI of feed, it was significantly lower ($P = 0.05$) in RUM animals (5.86 kg DM/animal/d), than in CTL ones (6.00 kg DM/animal/d). FCR showed a tendency ($P = 0.1$) to be different among treatments, calves in RUM group need 4.20 kg DM to gain 1 kg BW, comparing with CTL calves that needed 4.30 kg DM.

Conclusion: It was concluded that the supplementation with malate (RUMALATO), improved the productive performance of beef calves. Calves that received malate increased the average daily gain and tended to decrease the feed conversion ratio.

Keywords: Malate, daily gain, beef calves.

NU-P37

Rumen fermentation, methane production, and microbial composition following in vitro evaluation of *Cannabis sativa* byproduct

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Objectives: Hemp is a material commonly used in the pharmaceutical, food, textile and many other industries. All fractions present in them show very high biological activity. The positive influence of natural compounds, including essential oils (thyme, rosemary, garlic) as well as terpenoid fractions etc. on rumen fermentation is well known. The aim of this study was to determine the influence of essential oils (EO) from Cannabis sativa L., extracts containing elevated amounts of cannabinoids as well as pomace on rumen fermentation process on DAISY in vitro model, including methane production.

Materials and methods: Essential oils from Cannabis sativa L. were obtained by steam distilling the inflorescences on a Deryng apparatus. Extracts containing cannabinoid fractions were obtained using a mixture of chloroform-methanol and supercritical CO2. On the other hand, the pomace was was the waste after obtaining hemp oil from the seeds. The collected rumen fluid was poured into thermal flasks, filtered through 4 layers. In vitro ruminal fermentations was performed using the Ankom Daisy^{II} in vitro incubator. The EO and hemp pomace were separately placed in Ankom F57 filter bags. Concentrations of 0.1, 0.05 and 0.01% active fractions were used. Markers of effect were microflora change, volatile fatty acid and low molecular lipid profiles. The chemical composition of the fractions, as well as the profile of short-chain volatile fatty acids and methane, was determined by gas chromatography coupled to a mass spectrometer (comparison of mass decays and Kovat indices) and a flame ionization detector. In addition, the structures of the dominant compounds were confirmed by nuclear magnetic resonance (NMR) technique comparing the 13C signals of the main compounds with literature ones. The methods of microbiological analyzes were applied on the basis

of Mott et al. (2022) The relative change in abundance of the target bacterial species was calculated using qPCR amplification of16S rDNA genes. Methane concentrations were analyzed using a gas chromatograph Shimadzu GCMS-QP2020.

Results: For the first time, the effects of essential oils from sativa and indica varieties, extracts containing elevated amounts of cannabinoids as well as pomace on rumen fermentation were investigated using the DAISY model. More than 60 volatile compounds in both fractions were characterized by gas chromatography coupled to mass spectrometry (GC-MS). For both cannabis cultivars, the sesquiterpenoid caryophyllene and its oxide, as well as the monoterpenes myrcenes, limonene, and pinenes, dominated the loric fraction. In organic extracts (analyzing products after BSTFA derivatization), acidic as well as neutral forms of CBD, THC, CBG were determined by GC-MS technique. Positive change of short chain acids (acetic, propionic butyric) after 24 hours of fermentation in presence of essential oils and cannabinoid fraction was proved. The effect on microorganisms was inconclusive, especially since only some bacteria were identified.

Conclusions: The positive effect of EO from Cannabis sativa on rumen fermentation was shown for concentrations of 0.1 and 0.05%. For all concentrations used, hemp pomace had the least effect on the course of fermentation.

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Keywords: Fermentation, hempseed, essential oils, volatile fatty acids.

NU-P38

Effects of Iron Supplementation on Metabolism, Oxidative and Immune Status in Calves receiving whole milk

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Young animals in the early stages of life are most susceptible to iron deficiency, however neonates do have some iron reserves in their body. Moreover, in the case of feeding calves with cow's milk, which has low iron concentration, rapid growth rates can lead to the development of temporary iron deficiency. This condition may be exacerbated by the immaturity of molecular mechanisms of iron absorption. Iron supplementation is based primarily on inorganic compounds. However, these compounds can undergo oxidation and transform into insoluble forms. For the purposes of animal nutrition and to increase the bioavailability of this element, research focuses on the use of chelates or proteinaceous iron preparations. Casein proteins have very good iron binding properties, thereby decreasing their susceptibility to oxidation and therefore have high bioavailability. The addition of a protein-iron complex (PIC) to the calves' diet, developed with the use of an enzyme isolated from the *Yarrowia lipolytica* strain, may have a beneficial effect on physiological changes and production parameters of dairy calves. Additionally, the protein-iron complex supplemented with whole milk may improve the immune status of calves and as well iron metabolism.

Material Methods: The study was carried on 20 Polish Holstein Friesian calves of the black-and-white variety. The animals were put into randomized groups, taking into account the age (7 days old), body weight (40 ± 1.65 kg) and sex. The calves were divided into: control group (FM), fed with full milk r (n = 10); experimental group (FMFe), receiving a PIC additive in milk at 16 g/day (n = 10). Blood was drwan from the external jugular vein (*vena jugularis externa*) from all calves in 7th, 14th, 28th and 35th day of life. Hematological parameters analyses were performed using ABC Vet analyzer (Horiba ABX, France). The biochemical and iron metabolism parameters were determined using a blood biochemistry analyzer Pentra 400, company HORIBA ABX (France). Among the antioxidant parameters there were parameters marked: glutathione peroxidase activity (GPx), total antioxidant capacity (TAS), glutathione peroxidase (GPx), superoxide dismutase (SOD) in erythrocytes. Serum IgG and IgM immunoglobulin was assayed using a IgG ELISA Kit. Tumor Necrosis Factor (TNF-α), IL-6 and insulin-like growth factor 1 (IGF1) was assayed in serum by the Bovine Tumor necrosis factor ELISA Kit from MyBio Source.

Results: There were no statistically significant differences in the concentration of haematological parameters, however, the concentration of HGB in the experimental group was higher than in the control group. The applied supplementation resulted in a successive increase in transferrin, TIBC and UIBC, with an increase in Fe concentration up to the 28th day of calves' life. The mean activities of the antioxidant status parameters were higher in the experimental group, indicating the enzymatic involvement in the reactive oxygen species (ROS) removal. At the end of the study, SOD activity was highest in the group FMFe and there was also a significant increase in TNFα concentration in this gruop.

Conclusion: The addition of a protein-iron complex (PIC) had a significant impact on the iron metabolism indicators and contributed to an increase in the concentration of iron in callves' whole blood, as well as TIBC, UIBC and the concentration of transferrin in the experimental group FMFe. The conducted evaluation of the effectiveness of protein-mineral chelate indicates its functional features that should be used in practice as an anemia prevention in calves.

Keywords: Calves, iron, metabolism, immune status.

NU-P39

Serum mineral, metabolite and hormonal profile as an indicator of possible reproductive conditions to occur in bovine reared on natural pastures in a semi-arid area

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This study aimed to examine the serum minerals, metabolites and hormonal profile in cows as a predictor of possi-

ble reproductive conditions to occur in bovine using animals that presented with these reproductive conditions. Mineral, serum metabolites and hormones plays a significant role in cattle production and reproduction. Minerals are known for their contribution to several physiological processes such as cell formation and participate in activities of vitamins, enzymes and hormones. Measures of serum metabolites are good indicators in disease diagnosis and nutritional status of animals. The role of hormones in cows requires careful consideration prior to and during pregnancy, as any imbalance can negatively impact cow's overall reproductive health. A total of 179 blood samples were collected from cases of cows experiencing dystocia (n=50), downer cow syndrome (n=34), vaginal prolapse (n=16), retained placenta (n=13), and abortions (n=69) following reports submitted to the North West University (NWU) Animal Hospital in Mafikeng Campus. Analysis of minerals, serum metabolite and hormones were done using ICP-MS, IDEXX Catalyst Chemistry Analyser and ELISA Kits, respectively. The data were coded and analysed using the Statistical Software for Social Scientists (SPSS) version 25. *Descriptive statistics such as mean, standard deviation and skewness were used to describe the distribution of the minerals, serum metabolites and hormone levels across the reproductive conditions.* The results were significantly different at 5% ($P < 0.05$). Low zinc levels were seen in cows with abortions and dystocia. In downer cow syndrome, the levels of iron, magnesium and phosphorus were high. Low Iodine levels were seen in cows with vaginal prolapse, retained placenta, abortion and dystocia. Low phosphorus levels were noted in cows with vaginal prolapse. Significant differences were seen in the concentrations of Urea/BUN, Total bilirubin, aspartate amino-transferase (AST), ammonia and lipase. Estradiol levels were significantly higher in cases of abortion, downer cow syndrome and dystocia. Oxytocin levels differed significantly in cows with the higher dystocia and aborting cows. Significantly low progesterone levels were seen in abortions, dystocia, vaginal prolapse, downer cow syndrome and retained placenta. Mean concentrations of magnesium, phosphorus, copper, zinc, iodine and selenium may influence the incidences of reproductive conditions. Serum metabolites may be useful predictors of peri/postpartum disorders and productivity. Hormonal imbalance during the transition period may influence reproductive performance in cows. Further study of the relationship between incidence of reproductive conditions and the level of minerals, serum metabolites and hormones in a controlled environment is necessary for definitive results confirmation.

Keywords: Minerals, serum metabolites, hormones, reproductive conditions, bovine.

NU-P41

Fumonisin's Occurrence in Maize Silage and its Enzymatic Degradation during Ensiling

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Maize silage constitutes 50-70% (DM) of a dairy cow's diet in modern dairy farming and its contamination by mycotoxins is of high relevance due to the diversity and concentration that can be found in a silage sample. These mycotoxins pose a risk to dairy cows by compromising animal health and general performance in many different ways. Fumonisins (FUM) are of particular interest among the mycotoxins because of the rumen's poor capacity to degrade them. Major clinical signs are decreased appetite together with serum biochemical and histological signs of hepatic damage.

Objectives: To describe the occurrence and concentrations commonly found in maize silage of certain regions, thus understanding the risk of intoxication through FUM via maize silage.

To evaluate the efficacy of FUM*zyme*® (an enzyme capable of degrading FUM) as a maize silage additive in order to reduce FUM concentration.

Materials and Methods: The data was obtained by the World Mycotoxin Survey 2019 by Biomin® which received feed samples (maize silage, in this case) submitted during the whole year and analysed by LC-MS/MS, HPLC and ELISA methods. The regions considered for the survey were North America (NA, 221 samples), Europe (E, 348 samples), Asia (A, 259 samples) and South Africa (ZA, 40 samples).

Under lab conditions, chopped maize plants with a FUM contamination of nearly 2800 ppb were sprayed with a dose of 20 U FUMzyme® /kg fresh matter. Concentrations of FUM were analysed at 0, 2 and 7 days of ensiling to monitor any changes.

Results: From all the samples (889), zearalenone (ZEN), deoxynivalenol (DON) and FUM were the most commonly found with 65, 63 and 50 percent occurrence; the aflatoxins (AF) on the other hand presented only a 1% occurrence. The percentage of FUM contamination, average concentration of positives and maximum values found per region were 77%, 344 and 100,000 ppb for E, 78%, 597 and 5,632 ppb for A, 70%, 492 and 5, 314 ppb for ZA and surprisingly the lowest FUM prevalence was in NA with 23%, 367 and 72,950 ppb. These results highlight the relevance of considering not only AF (which are commonly addressed by the industry), but to also manage the risk of other harmful mycotoxins, namely FUM.

A biotransformation was observed by obtaining a reduction of 32.4 and 87.5% of FUM after 2 and 7 days of ensiling respectively, indicating a rapid and efficient FUM degradation.

Conclusion: FUM being present in the majority of silage samples received, underlines the necessity of managing the risk it poses to animal health and performance. FUM*zyme*®

applied as an additive during the ensiling process proved to be a successful solution to decrease a great part of FUM present in maize plants.

Keywords: Mycotoxins, Fumonisins, silage, enzymatic degradation.

PA-P01

Prevalence of *Rhabditis bovis* parasitic otitis in cattle farms Gyr in Córdoba, Colombia

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Objetives: Within the genus *Bos indicus*, the Indubrasil and Gyr breeds are more susceptible to *Rhabditis bovi*s parasitic otitis. The Gyr breed due to its characteristics, in the Colombian Caribe has been selected for milk production. The objective was to determine the prevalence of *R. bovis* parasitic otitis in Gyr cattle farms in different livestock enterprises in Cordoba, Colombia.

Materials and Methods: A prospective descriptive study was implemented by convenience. Swabs of cerumen from ear canals of 136 cattle were collected in six cattle farms. The diagnostic criterion was the direct observation of the mass movement of cerumen and visualization of nematodes.

Results: The prevalence of parasitic otitis was 83,82%. Significant difference was found for the variables clinical otitis, otorrhea and odor, the sex variable was not analyzed.

Conclusions: Parasite diagnosis shows the existence of *R. bovis* as the cause of parasitic otitis in cattle in livestock farms in Cordoba (Colombia).

Keywords: Earwax, diagnosis, dual purpose, nematode.

PA-P02

Efficacy of a topical application of Cypermethrin, Clorpyriphos, Piperonil butoxide or Fluazuron on cattle naturally infested by *Rhipicephalus microplus* under grazing conditions of Brazil

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Objectives: This experiment aimed to compare the efficacy against *Rhipicephalus (Boophilus) microplus* in naturally infested cattle of two similar presentations of a topical formulation containing cypermethrin (5.0%), chlorpyrifos (7.0%), piperonil butoxide (5.0%) and fluazuron (2.5%), and a solution of Fluazuron (2.5%) alone, for pour-on application.

Materials & Methods: The study was designed as a comparative study with a negative control group. The research was carried out in a cattle farm at São José de Rio Pardo, São Paulo, Brazil (21º35 S, 46º53 W). Forty Simmental cattle were selected on the following criteria, 1) animals with at least 20 engorged females ticks in left side, parasite size ranged 4.5< Ø < 8.0mm, counted from each animal on three consecutive days before treatment (days -3, -2 and -1), 2) live weight ranged between 350 to 450 Kgs, and 3)no history of application of acaricide within 60 days before enrolment. Animals were ear-tagged and then randomly allotted in one of the four experimental groups of ten animals, 1) G01 (cypermethrin, chlorpyrifos+piperonil butoxide and fluazuron. FORTIK 4, Virbac do Brasil, Brazil), 2) G02 (a product similar to G01. FLURON GOLD POUR ON, Ceva Saúde Animal Ltda, Brazil), 3) G03 (fluazuron. ACATAK, Elanco Brasil), and 4) G04, no treatment. A tick count was done daily on the restrained animals in all the body regions, including the head and ears, from 3 to 49 days post-treatment (DPT). Tick count is expressed as a geometric mean. Efficacy of treatment strategies is calculated from the relative risk for an animal to harbour more ticks than the control group at the same time point.

Results: Over the study period (DPT 3 to 49), the average *R. microplus* count significantly differed ($p<0.001$) between G01, G02, G03 on the one hand and G04 on the other hand (ANOVA, repeated measures). There was no statistically significant difference ($P=0.56$) in the mean tick counts between G01 and G02 during the experiment. Also, animals in G01 and G02 had mean tick counts significantly lower ($P\leq0.05$) than the animals from G03 on DPT3 and 7. Ultimately, there was no significant difference ($p=0.17$) between the three treated groups. Overall efficacy was ≥95% from DPT 14 to 21 for the three products, and ≥80, from DPT 7 to 35, DPT 7 to 42 and DPT 14 to 42, for G01, G02 and G03, respectively. Then, the efficacy gradually declined and reached 56.9, 68.1 and 61.1 by DPT 49 for G01, G02 and G03, respectively (n.s.).

Conclusion: Based on the results from this experiment, the new combination of cypermethrin, chlorpyrifos, piperonil butoxide and fluazuron administered as a single pour-on treatment, showed residual protection against *Rhipicephalus (Boophilus) microplus* on naturally infested cattle managed under extensive conditions. This efficacy is similar to what is observed with a product formulated the same way. However, tick protection starts as soon as 3 DPT, which is not the case with a fluazuron 2.5% only-based product.

Keywords: Boophilus;flazuron.

PA-P03

Prevalence and molecular characterization of *Cryptosporidium* and *Giardia* in pre-weaned native calves in the Republic of Korea

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Objective: *Cryptosporidium* spp. and *Giardia duodenalis* are protozoan parasites that cause diarrhea in humans and animals. Molecular data on *Cryptosporidium* spp. and *G. duodenalis* in calves in the Republic of Korea (ROK) is limited. This study aimed to investigate the prevalence of *Cryptosporidium* and *Giardia* in pre-weaned calves, analyze the association between these parasites and diarrhea, and identify the

zoonotic potential of *C. parvum* and *G. duodenalis* subtypes/assemblages.

Materials and methods: Between January and October 2018, 315 fecal samples were collected directly from the rectum of pre-weaned Korean native calves aged 1-60 days from 10 different farms in the Republic of Korea (ROK). Genomic DNA was extracted from 200 mg of each fecal sample using the QIAamp Fast DNA Stool Mini Kit. *C. parvum* was subtyped by targeting the 60-kDa glycoprotein (*gp60*) gene using nested PCR. *G. duodenalis* assemblage types were analyzed using the triose phosphate isomerase (*tpi*) gene, β-giardin (*bg*) gene, and glutamate dehydrogenase (*gdh*) gene. In this study, only samples showing a good sequencing result were considered to be positive for *C. parvum* and *G. duodenalis*.

Results: Overall prevalence of *Cryptosporidium* spp. and *G. duodenalis* was 4.4% (*n*=14) and 12.7% (*n*=40), respectively. Co-infection was not detected. All *Cryptosporidium*-positive samples were identified as *C. parvum* after sequence analysis of a small subunit rRNA fragment and further subtyped into zoonotic ⅡaA15G2R1 (*n*=13) and ⅡaA18G3R1 (*n*=1) by DNA sequencing of the 60-kDa glycoprotein gene. Based on b-giardin (bg) gene, *G. duodenalis*-positive samples belonged to assemblages E (*n* = 36) and A (*n* = 4), with the latter belonging to subtype A1, the zoonotic genotype. Six subtypes of assemblage E were identified at the *bg* locus: E1 (*n*=6), E2 (*n*=3), E3 (*n*=13), E5 (*n*=1), E8 (*n*=1), and E11 (*n*=1). The occurrence of *C. parvum* and *G. duodenalis* was not associated with diarrhea in pre-weaned Korean native calves. The prevalence of *C. parvum* is not related to calf age; in contrast, the prevalence of *G. duodenalis* was significantly higher in 41-50-day-old calves (odds ratio=9.90, 95% confidence interval: 2.37-41.34; *P*=0.001) than in 1-10-day-old calves.

Conclusions: These findings suggest that calves may be an important source of zoonotic *C. parvum* and *G. duodenalis* infections. Because cryptosporidiosis and giardiosis prevention is important for maintaining good health of calves and humans, the risk of diseases caused by these parasites should be reduced by minimizing the infection pressure resulting from contamination of environment with *C. parvum* and *G. duodenalis* oocysts/cysts and by improving the immunity of calves.

Keywords: Cryptosporidium, Giardia, diarrhea, age, preweaned calves.

PA-P04

Rhipicephalus microplus resistance to acaricides in Brazil

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Introduction: *Rhipicephalus microplus* ticks have developed resistance to the majority of acaricides available on the market in Brazil over time.

Objective: The objective of this study was to determine the resistance levels of *R. microplus* against commercially available acaracides at randomly selected commercial farms in Brazil. This study was a randomized, open label, resistance study.

Materials and Methods: For the study, 44 commercial farms were selected from seven states in Brazil for tick collection. The adult Immersion Test (AIT) was used to evaluate serial concentrations of fluazuron. Impregnated papers with alpha-cypermethrin were used as an indicator of resistance against synthetic pyrethroids, chlorfenvinphos as a general indicator of organophosphorus resistance, and amitraz as an indicator for formamidine resistance in the Larval Packet Test (LPT). Serial concentrations of moxidectin and fipronil were evaluated by the Larval Immersion Test (LIT). In order to perform paired tests, the Porto Alegre strain (POA), characterized as susceptible to all acaricides, was used as a reference strain. The criteria for classifying a sample as resistant with the LPT was effectiveness below 95% at the discriminating dose. For the LIT with moxidectin and fipronil, the criteria for the diagnosis of resistance was: a) susceptible - when the half maximal effective concentration (EC50) of the field isolate was not statistically different from the reference strain), b) incipient - when the EC50 of the field isolate was statistically different from the reference strain and resistance ratio (RR) < 2, and c) resistant - when the EC50 of the field isolate was statistically different from the POA strain and RR > 2. For fluazuron, resistance was considered when there was significant difference in index of fecundity between the field isolate and the susceptible reference strain. For each acaricide, the percentage of samples with resistance and the 95% confidence interval (CI95) were calculated.

Results: All evaluated samples were resistant to alpha-cypermethrin (CI95: 88.97 to 100), demonstrating the severe resistance status of *R. microplus* to synthetic pyrethroids in Brazil. The second active ingredient with the largest number of resistant samples was amitraz, with 87.5% of the farms indicating resistance of *R. microplus* (CI95: 71.93 to 95.03). Critical fipronil resistance status was also observed in 75.75% of the samples (CI95: 58.98 to 87.17). Sixty percent (CI95: 23.07 to 88.24) of the samples were resistant to fluazuron. However, only samples from five farms were evaluated for fluazuron resistance, because females had already started laying eggs before arriving at the laboratory or due to reduced number of viable ticks. Resistance to moxidectin and chlorfenvinphos was observed in 33.33% (CI95: 18.64 to 52.18) and 24.32% (CI95: 13.36 to 40.12) of the samples, respectively, indicating that moxidectin and organophosphorus products can still be used to control ticks in cattle on some farms in Brazil.

Conclusion: These results demonstrate that there is resistance to all active ingredients available on the Brazilian market for the control of *R. microplus*.

Keywords: Rhipicephalus microplus, resistance, acaricide.

PA-P05

The prevalence of the cattle parasite, *Babesia divergens*, in ticks collected from Irish farms and woodland

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Objectives: In the past bovine babesiosis (redwater fever) caused by the protozoan parasite, *Babesia divergens* was common along the River Shannon catchment and the western seaboard of Ireland representing a serious economic and animal welfare problem to Irish livestock farmers. While clinical cases have declined significantly over the last 30 years, the reasons for this decline are poorly understood. This study aimed to determine the current risk of bovine babesiosis by screening questing ticks in formerly endemic areas for the presence of *B. divergens*.

Materials and methods: Between 2018 and 2019, 736 *Ixodes ricinus* nymphs from 11 woodland sites and 448 *I.ricinus* nymphs collected from 14 farms (5 dairy, 9 beef) located in the midlands, the River Shannon catchment area and the west and southwest of the country, were screened for the presence of *B. divergens* using a TaqMan PCR protocol aimed at an 83bp fragment of the HSP70 gene. All ticks were collected by blanket dragging in accordance with ECDC tick survey guidelines. This involved 35 x 5 meter sweeps of a 1x1m piece of cotton fabric over vegetation and recording the number of ticks collected on the cotton sheet after each sweep.

Results: In total, 2.0% (15 out of 736) (95% confidence interval: 1.0% to 3.1%) of nymphs collected in woodland and 3.1% (14 out of 448) (95% CI 1.5% to 4.7%) of nymphs collected from livestock farms were found to be infected with *B. divergens*. All infected ticks were collected on dairy farms.

Conclusions: *B. divergens* infection levels in questing ticks were comparable to those reported from other European countries such as Poland (1.6%), Norway (0.9%) Italy (0.85%) and Switzerland (1.9%). The fact that there was no difference in prevalence rates between ticks collected from woodland compared to those collected from farms is probably a reflection of the fragmented nature of the Irish landscape.

Keywords: Babesia divergens, Ixodes ricinus.

PA-P06

A pilot study on the ectoparaciticides used by Irish farmers against lice and their efficacy

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Objectives: The objectives of this pilot study were to assess whether cattle lice are an issue on Irish beef cattle farms, to investigate what measures were used to control them and whether they were effective.

Materials and methods: During the winter of 2018/2019 and 2019/2020, 17 beef cattle farms were visited to interview the farmers about the louse control measures implemented on their farms. On each farm the level of louse infestation was assessed by examining an average of 35 animals. Lice were returned to the lab for identification to species level. Lice from 3 herds were tested for susceptibility to deltamethrin using bioassays according to FAO guidelines.

Results: The farmers surveyed used a range of ectoparasiticides to control lice including ivermectin, doramectin, deltamethrin, cypermethrin, diazinon and a product containing both ivermectin and closantel. 14 farm visits took place within 1 to 8 weeks post louse treatment while no treatment had yet been applied on the remaining 3 farms. At the time of the visit, 16 farms (94%) were positive for lice, with lice being detected on 10-95% of the animals sampled. Heavy infestations were recorded on 11.8% of farms. Lice were identified as *Bovicola bovis* (87.5% of farms) and *Linognathus vituli* (56.25% of farms*)*. Bioassays on *B. bovis* lice collected from 3 farms indicated that the parasites were deltamethrin-resistant.

Conclusions: Cattle farmers in Ireland rely on a broad spectrum of ectoparaciticides to control lice control measures. Our survey provides preliminary data that indicate that on a small proportion of farms louse control measures failed to maintain infestation levels at low or medium levels. This study provides the first evidence of deltamethrin-resistance in chewing lice in Ireland.

Keywords: Lice, ectoparasiticides, resistance.

PA-P07

First report of anthelmintic efficacy in cattle received in Feedlot in Mexico

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Currently, one of the worldwide challenges in parasite control is to generate efficient protocols that help to reduce the impact of neglected parasitic diseases in cattle. Due to the growing problem of anthelmintic resistance, and its expansion against most of the active chemical principles, they represent a threat to the viability of bovine production systems that directly affect the economy of local farms. The objectives of this study were to evaluate the anthelmintic efficacy in bovines received in fattening pens, as well as measure the daily weight gain for 99 days. The work was carried out in June 2019 at a feedlot in Tamuin, San Luis Potosi, Mexico (Praderas Huastecas SPR de RL). 180 male bovines (Swiss x Zebu) with 10-15 months of age and an average weight of 320 kilograms were used. Therefore, 24 hours after the reception of the cattle, stool samples were collected directly from the rectum of each animal. Through the Mc Master technique, 80 positive animals were diagnosed with gastrointestinal nematodes (300 eggs per gram of feces on average), which were randomly distributed to six experimental groups: Control group (n = 13) that remained without anthelmintic treatment; Group 2 treated with 1% ivermectin (IVM) (n = 13), with a dose of 0.2 mg / kg body weight subcutaneously; Group 3 treated with 1% Moxidectin (MOX) (n = 13), with a dose of 0.2 mg / kg body weight subcutaneously; Group 4 treated with IVM 2% and triclabendazole (TCB) 12% (n = 13), with an orally a dose of 0.2 mg / kg and 12 mg / kg body weight respectively; Group 5 treated with IVM 2%, TCB 12% and MOX 1% (n = 13), with an orally a dose of 0.2 mg / kg of IVM, 12 mg / kg of TCB and 0.2 mg / kg of MOX, subcutaneous route; Group 6 treated with 10% albendazole (ABZ) (n = 15), with an orally a dose of 5 mg / kg of live weight. At the start of treatment, there was no statistically significant difference in the elimination of eggs per gram of feces (P> 0.05). All animals were weighed before treatment and at the end of the experiment in order to calculate the daily weight gain (GDP). Anthelmintic efficacy (AH) was determined according to Abbot's formula (P> 0.05), with samples collected individually before and after treatment (day 0 and 14). The results indicate that the highest AH efficacy was obtained in the group treated with IVM / TCB / MOX with 100%, followed by the group treated with MOX with an efficiency of 98.5%, the group treated with ABZ 97.4%, the group treated with IVM 39.39% and finally, the group with the lowest efficacy (27.4%) was treated with IVM / TRC. Larval culture results indicated the presence of *Cooperia* spp. (61.4%), *Haemonchus* spp. (29.8%) and *Oesophagostomum* spp. (8.7%). The greatest weight gain was obtained in animals where Moxidectin with 265 and 211 grams per day was included in the control group, followed by the group treated with Albendazole 155gr and finally the animals treated with Ivermectin 133 and 94 grs. It is concluded that according to the efficacy of the molecules used in this study, the groups that presented the highest efficacy were those that included MOX with 100% and 98.5% efficiency.

Keywords: Fattening livestock, Feedlot parasites, Efficiency.

PA-P08

Preventive efficacy of toltrazuril (15mg / kg), against eimeriosis in experimentally infected calves

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Objective: The present study evaluated the preventive efficacy of toltrazuril 5% (Baycox® - Bayer Animal Health), administered orally at a dose of 15mg / kg, against *Eimeria* spp. in experimentally infected calves.

Material and Methods: The study was conducted on a commercial farm located in the municipality of Jataí, Goiás, Brazil, from December 2019 to February 2020. Forty two calves (average age 30 days) which have not been treated with any anticoccidial drug since birth were recruited in the study and were kept in coccidia free clean environment before allocation to different groups. Feces from animals were examined for oocyst by McMaster technique on days -46, -45 and -42 pre challenge. Animals not shedding *Eimeria* oocysts were randomly allocated to 7 treatment groups (T01-T07) of 6 animals each.The animals of groups T01, T02,T03,T04, T05, T06 were treated with totrazuril 5% (Baycox® - Bayer Animal Health) at 15 mg/ kg body weight on day -42,-35,-28,-21,-14, and -7, respectively. Animals of group T07, received saline solution at day -42. Each group was placed in a separate collective pen, with wood shavings litter, during the experimental period. On day 0 of the study, each animal in all groups was challenged with 100,000 sporulated oocysts of *Eimeria* spp from a mixed field isolate containing 68.2% *E. bovis*, 24.7% *E. zuernii,* 3.5% *E. alabamensis* and 3.5% of *E. ellipsoidalis*. The preventive efficacy of toltrazuril was calculated by comparing mean oocysts per gram (OoPG) of feces counts, from faecal samples collected at different days in different groups with control group.

Results: Two animals of Group T07 (control) exhibited diarrhea with the presence of blood on day 14 post challenge, and diarrhea with or without blood was not observed on subsequent days. The calves of other groups (T01-T06), did not show any clinical signs (diarrhea and / or dehydration) suggestive of infection by *Eimeria* spp. during the study period. The preventive efficacy was calculated as 67.8%, 47.9%, 85.4%, 86.2%, 97.7% and 100 %, for calves treated days -42, -35, -28, -21, -14 and -7, respectively at day 28 post challenge. On day 35 post challenge, the preventive efficacy reached 59.9%, 53.1%, 83.3%, 78.1%, 94.8% and 100% for day -42,-35,-28,-21,-14 and -7 treated animals respectively. The mean OoPG counts in the control animals were significantly higher (P≤0.05) than those in the animals of the groups treated with toltrazuril on days -28, -21, -14 and -7.

Conclusion: The toltrazuril, administered orally at a dose of 15mg / kg, demonstrated preventive efficacy against *Eimeria* spp. The preventive efficacy was calculated as 100 % on day 21, 28 and 35 post challenge in animals which were treated 7 days before challenge . However, preventive efficacy on day 21, 28 and 35 post challenge in animals treated on 14 before challenge was 90.32 ,97.70 and 94.79 % respectively whereas, for animals treated 21 days before challenge was 80.65,86.21 and 78.13 % respectively.

Keywords: Bovine, diarrhea, oocysts.

PA-P09

Efficacy of paromomycin against cryptosporidiosis, a field trial in two large Slovenian dairy herds

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Objectives: To test the efficacy of paromomycin on dairy farms with a known cryptosporidiosis problem.

Material and Methods: Thirty calves naturally infected with Cryptosporidium sp. from each of two large Slovenian farm were enrolled in the study (15 test calves treated with paromomycin and 15 control calves treated conventionally : fluid therapy, NSAID, antibiotic if indicated).Calves were separated from their mothers immediately after birth and raised in individual boxes until they moved to group boxes at about 3 weeks of age. In farm A, paromomycin treatment was started on day 4 after birth (in clinically healthy calves with positive HuveCheck® Crypto test) for 7 consecutive days; in farm B, treatment with paromomycin was started after cryptosporidiosis was confirmed in calves with diarrhoea. Dosing was in accordance with the manufacturer's instructions: 50 mg/kg bw per day (Huvepharma, Belgium). All calves were weighed at birth and at one month of age. In addition, oocysts were counted in faeces smears on days 11 and 16 using modified Ziehl Neelsen staining.

Results: In farm A, we found statistically significantly better weight gain and fewer Cryptosporidium oocysts in faeces smears in the treated calves compared to the control calves. Although weight gain and oocyst count were on average better in the treated calves on farm B, the difference was not statistically significant. A better effect of paromomycin was observed when it was given before the onset of diarrhoea.

Conclusion: Since paromomycin is an antibiotic, prophylactic treatment cannot be recommended. However, metaphylactic use is beneficial during the calving peaks once cryptosporidiosis has been diagnosed.

Keywords: Cryptosporidiosis, paromomycin, calves.

PA-P10

Latitudinal region affects the prevalence and hematological parameters of *Theileria orientalis*-infected dairy cattle in the Republic of Korea

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Objectives: The increasing distribution of tick-borne parasites and the re-emergence of tick-borne diseases as a result of climate change pose a global threat to dairy cattle. In this study, we evaluated the effects of a climatic factor, the latitudinal region, on the prevalence and hematological profile of dairy cows infected with *Theileria orientalis* in the Republic of Korea.

Materials and methods: Blood sampling was done on 365 non-grazing, clinically healthy, lactating Holstein-Friesian cows, from 27 dairy farms located in seven provinces in Korea, assigned based on latitude as northern, central, and southern regions. Samples were subjected to hematology analysis using an automated hematology analyzer and molecular detection of *T. orientalis* major piroplasm surface protein gene using polymerase chain reaction.

Results: The overall prevalence was 20.00%, from 70.37% of farms. The prevalence was significantly higher in the southern region (35.94%) and tends to become lower in the central (21.95%) and northern (12.92%) regions. The RBC and its extended parameters, HCT, HGB, MCV, and MCH were significantly downgraded among infected cows in the southern region.

Conclusion: This is the first nationwide study of *T. orientalis* prevalence in Korea, that assessed the difference between latitudinal regions among non-grazed, asymptomatic, lactating dairy cows, revealing *T. orientalis* is more prevalent in areas with warmer weather. With the influence of this climatic factor, more animals could have impaired health and productivity due to the downgraded blood profile incurred by subclinical *T. orientalis* infection.

Keywords: Theileria orientalis, Dairy cattle, Latitudinal region, Hematology.

PA-P11

Concentration of antibodies against *Tritrichomonas foetus* surface antigen TF1.17 from sythetic mRNA-transfected cells

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Objectives: Antibodies against the TF1.17 surface antigen of *Tritrichomonas foetus* (TF) inhibit attachment to host cells and decrease parasite viability. Synthetic mRNA encoding these antibodies could be applied to the urogenital epithelium of bulls to prevent or treat trichomoniasis. Antibodies against TF1.17 have been produced by mRNA transfected preputial keratinocytes, but in concentrations too low to demonstrate biologically relevant effects. The objective was to develop an assay to concentrate functional expressed antibody against TF1.17.

Materials & Methods: Synthetic mRNAs for membrane anchored, bovine IgG antibodies against 2 epitopes of the TF1.17 antigen (TF1.15 and 1.17) were used to transfect (Messenger Max) A549 cells and bovine preputial keratinocytes (BPKs) for 24 hours. Phospholipase C (PLC; 1 U/mL) was used to cleave the antibodies from the cells and the supernatant was concentrated via centrifugal filtration. The concentration of bovine IgG was determined via ELISA (Abcam) and TF were treated with 1.0 µg/mL of TF1.15 or 1.17, and assayed for cytotoxicity via a commercially available kit (Promega CellTox Green).

Results: Bovine IgG yield from PLC treated A549 cells were determined to be 10.0 µg/mL and 5.5 µg/mL for TF1.15 and 1.17, respectively and were 10.0 µg/mL and 1.9 µg/mL for PLC treated BPKs. This confirmed that cells were expressing antibody and that transfection efficiency was higher for the TF1.15 construct in both cell types. Cytotoxicity evaluated via a fluorescent marker for membrane permeability revealed a 4-fold and 12-fold increase in relative fluorescent units from TF treated with TF1.15 and 1.17, respectively, as compared to untreated controls. While TF treated with both antibodies exhibited decreased viability, treatment with TF1.17 caused a larger decrease.

Conclusion: This method is feasible to concentrate expressed functional antibody for ongoing research to determine the efficacy of mRNA therapy to treat or prevent TF infection in bulls.

Keywords: mRNA, transfection, trichomoniasis, urogenital, cytotoxicity.

PA-P12

Revised control methods for psoroptic mange in cattle in the face of emerging drug resistance

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Objectives: Current recommendations for the control of psoroptic mange in cattle were revised in the face of emerging acaricide resistance.

Material and methods: The published results of interviews with farmers, field efficacy studies and data simulation were used to revise control strategies for psoroptic mange in cattle.

Results: Treatment of psoroptic mange, caused by *Psoroptes ovis,* relies on the use of acaricides. Available chemical products in Europe are the macrocyclic lactones (MLs), flumethrin and, in certain countries, phoxim. Current recommendations for the control of psoroptic mange comprise of two treatments with a 7 – 10-day interval, with an acaricide at the recommended dose, of all affected and in-contact animals after removal of the crusts and shearing of the hair. Multiple studies have reported treatment failure after the use of MLs. This can be caused by suboptimal application of the treatment, e.g. underdosing, incorrect treatment interval or formulation and partial treatment of the affected group. In 2012, 54% of the farmers with problems with psoroptic mange in Flanders (Belgium) made at least one of these mistakes. Another possible cause of treatment failure is acaricide resistance. Results from an efficacy study from 2016-2019 in Flanders, Belgium detected treatment failure on 12 out of 16 farms with ML-resistance as most likely cause.

The presence of acaricide resistance imposed the need to re-evaluate current control recommendations. It will be essential to identify per farm the acaricides with an adequate efficacy, in order to achieve on-farm eradication of the disease. The mite count reduction test (MCRT) is the only available method to measure treatment efficacy in cattle and calculates the reduction in mite counts, based on pre- and post-treatment skin scrapings. A mean reduction in mite counts after correct treatment of ≥95% with a lower limit of the 95%-confidence interval of ≥90% is regarded as adequate drug efficacy by the authors. When the mean reduction and the lower limit are below these thresholds after correct treatment execution, drug efficacy is regarded as reduced. In other cases with a mean reduction or a lower limit of the 95%-confidence interval below their threshold, the efficacy is considered doubtful. Data simulation has identified the total number of skin scrapings per farm as the most important parameter for the diagnostic performance of the MCRT. For the detection of severely reduced drug efficacy (post-treatment mite count reduction <70%), a total of 36 skin scrapings per farm will provide reliable diagnostic performance. In order to detect early signs of reduced efficacy (70% - 86% efficacy), higher numbers of skin scrapings are needed.

Due to the limited number of available acaricides, it will be better for some farms to limit the presence and consequences of *P. ovis* infestations on their farm instead of aiming for disease eradication, as it is a realistic option that treatment

with any given acaricide will not result in a normal efficacy on certain farms. Even though, flumethrin resistance has not yet been identified in bovine *P. ovis*. However, *in vitro* resistance against pyrethroids has been reported in *Psoroptes cuniculi* in rabbits.

The recommendation to treat all in-contact animals can thus lead to further increased selection pressure towards more severe acaricide resistant mites. Therefore, it is recommended to evaluate per farm if it is necessary to treat all animals or only the clinically affected, based on the fraction of clinically affected animals and present on-farm possibilities to separate a group of animals. A group of clinically affected animals could be separated from the rest of the herd for treatment in order to sustain a susceptible refugee population within the animals without lesions.

Conclusion: The emergence of resistance has complicated the control of psoroptic mange. It is important for the farmers to know the efficacy of the used acaricides and use farm specific control strategies to prolong the longevity of these products on their farm.

Keywords: Psoroptic mange, control, resistance, diagnostics.

PA-P13

Milk production and faecal egg counts in lactating dairy cattle

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Objective: Although research has indicated that anthelmintic use in lactating dairy cattle can result in increased milk production, much of this research has involved herds utilising housed dairy systems where cattle spend a portion of winter in stalls. Australian dairy systems are predominantly pasture-based. This may involve sustained exposure of cattle to gastrointestinal nematodes throughout life, allowing continued infestation but also ongoing immune system stimulation. Our aim was to assess the relationship between milk production and faecal egg counts (FEC) for individual cows in early lactation to determine whether there is a production deficit in animals with a higher FEC.

Materials and Methods: We measured the FEC of recently calved cows (less than 30 days in milk) in 11 commercial, pasture based dairy farms in south-west Victoria, Australia. 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. FEC was measured for individual animals at a sensitivity of 2.5 eggs per gram of faeces (epg). Farm data such as recent anthelmintic use, management, and cow body condition score (BCS) at sampling was recorded. Six farms used daily milk meters to analyse milk production whilst another six obtained regular herd tests (individual cow milk production assessments) through a contractor. These data were extrapolated to produce 100d production figures. Data analysis was then undertaken using the Jamovi statistical package.

Results: Preliminary data from the herds with daily milk monitors (six farms) indicates a milk production deficit of 2.85L per day for the first 100 days in cows with FEC greater than 2.5 epg. This relationship is not consistent once farm or origin is accounted for. Analysis of the production data from the remaining six farms is required before an explanation of the data can be attempted.

Conclusion: FEC at a sensitivity of 2.5 epg may be an appropriate measurement of worm burden in pastoral dairy systems, and in some instances may be correlated with changes in milk production. However, further investigation of this data set is required before application of FEC can be endorsed as an appropriate tool for decision making.

Keywords: Milk production, FEC, Dairy, pasture-based, Ostertagi.

PA-P14

Evaluation of condemned livers from major cattle due to lesions compatible with parasites in Spain

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Objectives: Parasitic lesions are one of the main causes of bovine liver condemnation notified at slaughterhouses by the Official Veterinary Service (OVS) in Spain. The objective of this study was to characterize condemned livers from major cattle due to the presence of lesions compatible with parasitic infection in a slaughterhouse in southern Spain, to confirm their aetiology.

Material and Methods: To carry out this study, a total of 29 livers of major cattle from 16 different origins were selected (2 from dairy and the others from beef) at the slaughterhouse. Selection criteria was the presence of gross lesions representative of the main lesions observed during each sampling day that originated the condemnation of the organ due to lesions compatible with parasites by OVS. Whole livers were transferred to the laboratory where the following analyses were performed:

(1) Macroscopic evaluation of the surface and serial sections of the parenchyma with a distance of approximately 3 cm in search of internal lesions.

(2) Microbiological culture in blood agar supplemented with 5% defibrinated and sterile sheep blood incubated at 37ºC in aerobiosis and anaerobiosis for 24 to 72 hours. When granulomatous content was observed, a duplex PCR against *Mycobacterium tuberculosis* complex and *Mycobacterium avium* complex was also performed (n=4).

(3) Evaluation of the content of the lesion under a stereoscopic magnifying glass and microscope to assess the presence of specific structures as protoscoleces or parasitic membranes.

(4) When necessary three different real time PCR (qPCR) assays were used in order to identify Cestode spp. on these samples (n=13), but also for *Echinococcus granulosus* (hydatidosis) and *Taenia hydatigena/Cysticercus tenuicollis* (n=12).

(5) Microscopic evaluation of liver samples containing parasitic-like lesion or any other type of lesion previously fixed in 10% neutral buffered formaldehyde and routinely processed to carry out the histopathological analysis. A record was performed identifying cystic lesions, hepatocyte degeneration, inflammation, fibrosis and any other lesion of interest.

Results: The most frequent causes of condemnation were hydatidosis (hydatid cysts formed by the metacestodes of *Echinococcus granulosus*), with or without hepatic fibrosis or abscesses (51.7%) and chronic nonspecific parasitic processes in which it was not possible to confirm the causal agent (17.2%). Only 2 out of 15 (13.3%) examined hydatid cysts produced protoscoleces and could be considered fertile cysts. Other diagnosed processes were fibrosis with or without liver degeneration (6.9%), abscesses, lithiasis, non-specific chronic hepatitis and hepatodystrophy (3.4% respectively). Regarding the nonspecific fibrosis diagnosed, the high frequency of parasitic lesions could indicate that it could be associated with the migration of parasitic forms. Although visceral cysticercosis due to *C. tenuicollis* is the most frequent parasitic disease in other livestock species evaluated in the same geographical area (sheep and pigs, data not shown) all samples analysed in this study were qPCR negative to this pathogen. Abscesses diagnosed in our study (10.3% analysed samples) were originated by anaerobic filaments, mainly *Fusobacterium necrophorum* (necrobacillosis).

Conclusions: Hydatidosis was the most frequent cause of major cattle liver condemnation in our study. As in previous reports, most cattle livers carried infertile cysts that do not produce protoscoleces and are unable to continue the life cycle of the parasite. This result highlights the importance of applying molecular techniques (qPCR) to confirm the aetiology of these lesions, especially when there are no preserved structures in chronic lesions or infertile cyst. In addition to dogs, other canids such as foxes, frequent in the geographical area of study, are also definitive hosts of *E. granulosus*. Therefore, in order to reduce parasitic lesions in the slaughterhouse, the presence of definitive hosts must be equally controlled through biosecurity measures that prevent the coexistence of these animals and domestic livestock as well as the onset of periodic antiparasitic treatment in domestic dogs.

Keywords: Hydatidosis, cattle, liver, parasites.

PA-P15

In vitro anthelmintic activity of Praziquantel and Nitazoxanide for controlling *Eurytrema coelomaticum*

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Objectives: The objective of the present study was to verify, separately, the *in vitro* anthelmintic activity of praziquantel (PZQ) and nitazoxanide (NTZ) in the *E. coelomaticum* adult parasite and the histopathological lesions induced by these drugs. Until now, anthelmintic drugs capable of controlling this parasitosis are unknown, since despite the proven effect of praziquantel, there are no commercial formulations with this pharmacological basis for ruminants. The affected animal usually has subclinical pancreatic disease, but the damage caused to health and animal production are subdued. Therefore, the evaluation of new molecules in order to control this globally disseminated parasite with high prevalence in the southern region of Brazil is justified.

Materials and Methods: *E. coelomaticum* specimens were obtained from the pancreases of naturally infected cattle, collected from animals slaughtered in the city of Concordia-SC, Brazil. A total of 180 parasites of uniform size and weight were used, distributed in tissue culture plates with six wells each and ten helminths per well, forming three groups: Group I negative control group (n = 60); Group II positive control group (n = 60) treatment with 80µg/mL of PZQ, and Group III treated group (n=60) treatment with 200µM of NTZ, The drugs were diluted in dimethylsulfoxide (DMSO) and separately added to the culture medium at a maximum concentration of 0.02% (v/v). In the negative control group, sheep serum and DMSO were used at the maximum concentration of the treatment groups, but without the drugs. Parasites in culture medium were kept in incubators at 37ºC with 5% of CO_2, being evaluated for motility after 3, 12, and 15 hours of incubation in a Stereomicroscope with a 20x magnification, using the following criteria: 3 (normal movement), 2 (slow movement), 1 (very slow movement), and 0 (no movement, dead). After the incubation period of 15 hours, the parasites were placed in a 10% buffered formalin solution for a minimum of 24 hours and sent for histopathological analysis. The parasites were dehydrated in increasing alcohol solutions, added in paraffin blocks, microtomized (3µm), and stained by the hematoxylin and eosin (H&E) staining method. Under an optical microscope, histopathological alterations in the organs of *E. coelomaticum* were analyzed comparatively between the negative control group, NTZ, and PZQ in 100x and 400x increase. The motility degrees between the groups were analyzed using the SAS program 9.3, submitted to the chi-square analysis (PROC

FREQ). Significant statistical differences were considered when P<0.05.

Results: After 12 hours of incubation all parasites of the NTZ and PZQ groups were motionless or dead, while in the negative control group, 82% (5/60) presented normal motility after 15 hours of incubation (p<0.001). Histopathological examination showed severe damage in the vitellogenic gland, intestine, parenchyma, integument, and testicle in both treated and positive control groups. The vitellogenic gland showed disorganization of the acinus, necrosis, and a presence of glandular secretory content dispersed throughout the parenchyma. In the intestine, flattening of villi with a decrease in basal cells that line the organ was observed. The integument showed decreased eosinophilia, basal lamina discontinuity, and subtegumentary vacuolization.

Conclusions: It was concluded that PZQ and NTZ showed *in vitro* anthelmintic action against the parasite, as they caused significant lesions in the evaluated organs and reduced the parasite's motility. The NTZ may be an alternative drug to the use of PZQ in the controlling of *Eurytrema coelomaticum.*

Keywords: Euritrematosis, histopathology, nitazoxanide, pancreas, praziquantel.

PA-P16

Eurytrema coelomaticum infection: correlation between parasite burden and impairment of pancreatic exocrine enzyme secretion

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Objectives: The objective of the present study was to determine if there is an impairment of exocrine pancreatic function and correlate it with parasite burden in *Eurytrema coelomaticum* infection. Despite some authors pointing out the disease as a cause of death, it is almost a consensus that the disease does not produce clinical signs, but is a silent disease that causes losses in milk and meat production. In addition, the infection of humans with this pancreatic trematode is also possible. Therefore, an accurate clinical and laboratory diagnosis is important for the best elucidation of the disease, since the definitive diagnosis is currently only reached with the necropsy of the animal or post-mortem examinations in slaughterhouses.

Materials & Methods: Pancreases, blood, and fecal samples were collected from 119 bovines at an abattoir. Stool samples were subjected to the gelatin and x-ray film digestion tests (to detect the presence of trypsin in feces). Using blood samples, the following biochemical tests were performed: amylase, lipase, glucose, fructosamine, cholesterol, triglycerides, total protein, albumin, and globulins. The renal function of the animals (urea and creatinine) was also evaluated, as renal diseases can increase the enzymes amylase and lipase due to deficiency in excretion, thus, interfering with the results of the exocrine pancreatic function by causing a false positive for pancreatic lesions. The pancreases were placed in individual trays, isolated, and washed. All the water used in the washing was sieved through nylon sieves to separate the trematodes. Subsequently, the pancreatic ducts were opened with the aid of a scalpel, forceps, and surgical scissors, and the trematodes were removed using surgical tweezers and counted. The data were analyzed using the SAS Software. In order to analyze the relationship between the number of *Eurytrema* sp. with blood variables, the data were evaluated using Spearman's correlation analysis (PROC CORR). Logistic regression analysis (PROC LOGISTIC) and the odds ratio calculation were carried out to identify the odds ratio of the low and high parasitemia groups to present changes in blood variables concerning the non-parasitized group.

Results: Initially, the animals were classified as parasitized or non-parasitized by *Eurytrema coelomaticum*, with 71 positive and 48 negative cases, resulting in a frequency of 59.66%. The mean number of specimens per infected pancreas was 628 parasites, ranging from 6 to 3,829. Cattle with a high parasitic load presented a higher incidence of negative tests in both gelatin digestion and x-ray film digestion tests ($P < 0.001$) when compared to non-parasitized animals and those with a low parasitic load. Changes in those tests only occurred if the parasitemia was moderate or severe. The activity of the amylase and lipase enzymes was significantly higher in animals with low parasitemia ($P < 0.05$), compared to non-parasitized animals and with a high parasitic burden.

Conclusions: There was an absence of pancreatic digestive enzymes in the feces of highly or moderately parasitized animals. In addition, elevated serum levels of lipase and amylase were seen in animals with low burden, which also indicated the disease is compromising pancreatic exocrine functions.

Keywords: Amylase, Euritrematosis, lipase, pancreatic insufficiency, pancreatitis.

PA-P18

Cryptosporidiosis in calves of Croatian dairy farms: A neglected condition without reason

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Introduction: Apicomplexan parasite *Cryptosporidium parvum* is one of the most important pathogens of young ruminants, particular newborn calves. Infected animals may suffer from acute watery diarrhoea, inappetence, lethargy leading to dehydration and in severe cases with lethal outcome. Even though cryptosporidiosis is widespread and has been reported

as a significant cause of enteritis worldwide, little is known on the occurrence in South-Eastern Europe, including Croatia.

Materials & Method: In the current study, 98 faecal samples were collected from individual calves under 21 days of age with diarrhoea from 10 farms. Faecal samples were examined using direct immunofluorescence test (MERIFLUOR Cryptosporidium/Giardia, Meridian Bioscience Inc., USA) and commercial point-of-care immunochromatographic test kit (SPEED V DIAR 4, Virbac, France). Samples were considered positive when the outcome was positive at one of the two tests at least.

Results: The infection with *Cryptosporidium* sp. was detected in 8 out of 10 (80%) farms surveyed with an overall prevalence of 38% (27/98) infected calves. In the positive farms, prevalence varied from 11% up to 50%. Interestingly, the immunochromatographic test showed higher sensitivity (27/27; 100%) compared to direct immunofluorescence assay (25/27; 92.6%).

Conclusions: Result of the current study has clearly shown that *Cryptosporidium* sp. is an essential and highly prevalent causative agent of neonatal diarrhoea in dairy calves and should consider more attention from both veterinarians and farmers. Furthermore, results from the present study showed that the immunochromatographic test performed slightly better than the direct immunofluorescence test, and could be used for fast detection of cryptosporidiosis on farms.

Keywords: Cryptosporidium, calf, Croatia.

PA-P19

Seroprevalence and risk factors associated to *Neospora caninum* and *Toxoplasma gondii* in breeding sheep in the west of Mexico

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The objective of this study was to determinate the seroprevalence and risk factors associated to *Neospora caninum* and *Toxoplasma gondii* in breeding sheep in the west of Mexico. They were collected 184 blood samples of six municipalities in the state of Jalisco, Mexico. The taking of samples was made through venipuncture of the jugular vein by using tubes without anticoagulant, after this they were taken to the laboratory where they were centrifuged for the collection of serum, placing it in tubes of 1.5 milliliters and stored -20°C until their use. At the moment of realizing the visit of the collection of samples it was made a survey to the producers with the finality of identifying the risk factors. For the diagnose of *Neospora Caninum* and *Toxoplasma gondii* it was used the commercial kit of indirect ELISA following the recommendations of the manufacturer. The results showed a general seroprevalence of 16.32% (28/184) for *Neospora caninum* and 66.16% (114/184) for *Toxoplasma gondii*. Among the risk factors that were identified they stand out the presence of the definitive hosts (dogs and cats) in the production unit, being proper or foreign, the direct contact with the definitive hosts, the manage of aborts as part of the personnel, and the closeness to the urban core. As a conclusion the, animals present a high seroprevalence with the two evaluated parasites if we compare it to other studies made where it was applied the same diagnose technique, this indicating that sheep are exposed to the infection of *Neospora caninum* and *Toxoplasma gondii*, meaning that it can cause lose in the production related to reproductive problems and abortions.

Keywords: Seroprevalence, Neospora, Toxoplasma, Sheep.

PA-P20

Efficacy of two novel oral formulations of Flubendazole and Flubendazole/Triclabendazole combination in lambs in Uruguay

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Objectives: The objective of this study was to evaluate the efficacy of two novel oral formulations of flubendazole against Haemonchus contortus and Fasciola hepatica in lambs: flubendazole alone and a flubendazole/triclabendazole combination.

Materials and Methods: Study 1: Thirty lambs (24,3 ± 4.8 kg of BW) were artificially-infested orally with 2,500 3 status of Haemonchus contortus larvae, 37 days prior to initiating the Controlled Faecal Egg Count Reduction (FECR) test. The animals were allocated into three groups: FBZ group: treated with Flubendazole at 10 mg/kg (Flubenzin Suspension®, Calier, Uruguay); FBZ/TCZ group: treated with a combination of Flubendazole [10mg/kg] and Triclabendazole [10 mg/kg] (Flubenzin Forte®, Calier, Uruguay) and CTR group (untreated group) . Individual faecal egg counts were performed prior (-10 days) and post (14 days) treatment.

Study 2: Eighteen lambs (30,1 ± 4.9 kg of BW) were artificially-infested with 200 metacercaria of Fasciola hepatica. The animals were allocated into two groups: FBZ/TCZ group: treated with a combination of Flubendazole [10mg/kg] and Triclabendazole [10 mg/kg] (Flubenzin Forte®, Calier, Uruguay) and CTR group (untreated group). All animals were necropsied at day 84 post treatment. Efficacy was assessed by the reduction of the number of parasites in the treated group in relation to the control group.

Results: Results in the study 1 showed a FECR of 95% [CI 94%-96%] and 92% [CI 91%-94%] in FBZ and FBZ/TCZ groups, respectively. The FECR in CRT group was 0% [CI 0 – 4%].

Results in study 2 showed a mean [range] of 39 [20-48] flukes in CTR group, whereas the fluke burden in the FBZ/TCZ

group was 0, resulting in 100% efficacy.

Conclusion: These results confirm the efficacy and sensitivity of two novel formulations of Flubendazole when used in lambs to control Haemonchus contortus and Fasciola hepatica. This adds two more alternatives to the parasite control in sheep farms.

Keywords: Fasciola, Haemonchus, Flubendazole; Triclabendazole; sheep.

PA-P22

Trypanosoma sp. infection in cattle in Argentina. Distribution and characterization of diagnosed cases

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Trypanosoma spp. are protozoan hemoparasites that cause different clinical manifestations in various animal species, including man. The animals can be carriers or develop acute to chronic clinical signs. These may be of different severity, even with an important economic-social impact. In America, the main agent is *T. vivax*, of mechanical transmission by hematophagous vectors such as *Stomoxys* sp. and *Tabanus* sp. It is also spread by iatrogenia. In Argentina, *T. vivax* was diagnosed for the first time by Monzón in Formosa in 2006. Since 2016, we have observed several clinical presentations in different dairy and beef herds from central Argentina, located in the province of Santa Fe, Córdoba and Santiago del Estero. The most frequent signs were anemia, weight loss, lower production, abortions, edema, diarrhea, sudden deaths. In some cases, there were misdiagnosis, confusing the disease with anemic diseases (anaplasmosis, babesiosis), abortigenic diseases (fetopathies such as leptospirosis), digestive processes (mycotoxicosis). The values of packed-cell volume and proteinemia were variable, the liver profile (GGT and GOT) was altered, and the Woo technique positive in some cases. The main lesions correspond to a syndrome of anemia and a generalized lymphoadenomegaly. Some cases with hemoperitoneum, without rupture of the spleen or large blood vessels. Microscopically, lymphadenitis and anemia lesions were observed, such as centrilobular necrosis, erythrophagocytosis. The severity of the cases was diverse, influenced by the productive system (beaf or dairy), level of production (advanced gestation, peripartum, high production), stressful situations (sudden death in caloric stress). The severity of the cases was diverse, influenced by the productive system (beef versus milk), level of production (advanced gestation, peripartum, high production), stressful situations (sudden death in caloric stress). Some cases were closely related to massive vaccinations, and in very few there was a co-infection with *Anaplasma marginale*. In some of them it was possible to have a PCR molecular analysis corresponding to *T. vivax*. The treatment established had a positive effect with few recurrences. There were untreated parasitized animals with no signology (asymptomatic). In some of these asymptomatic herds, the reproductive indexes were lower than in previous years. It is essential to identify the hematophagous vectors present, to know their ecology, distribution and behavior, in order to prevent and control the diseases transmitted by them, and to determine how weather conditions may influence the dynamics of infection. The productive conditions of domestic animals must be considered in order to improve their immunological status. In addition, the trade and movement of animals between regions must be known, understood and analyzed. Applying the concepts of spatial epidemiology and medical geography is essential to prevent the consequences of this and other diseases.

Keywords: Trypanosoma, cattle, Argentina.

PA-P26

Prevalence associated with natural infection of *Sarcocystis spp.* in fattening sheep in the state of Jalisco, Mexico

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The objective of the present investigation was to determine the prevalence of *Sarcocystis spp.* in fattening sheep in the state of Jalisco, Mexico. 132 tissue samples were collected from slaughtered sheep in the municipality of Yahualica de Gonzalez Gallo Jalisco. The animals came from farms located in the municipalities of Cañadas de Obregón, Cuquío, Mexticacán and Yahualica de González Gallo. The collection of samples was carried out after the sacrifice, taking approximately 15 centimeters of the esophagus and 100 grams of the heart, which were placed in a plastic bag. The samples were transferred to the zoology laboratory of the Autonomous University of Guadalajara, where they were stored at -20ºC until analysis. Prior to analysis, the excess fat in the tissues was removed to facilitate handling and to have a better view. The diagnosis was made by direct observation of the tissues, with the help of a stereoscopic microscope, dissection forceps and a scalpel. They were considered positive when observing white, round, or oval cysts on the tissue, with measurements ranging between 1.5 x 1.0 x 1.0 cm, with a white capsule and translucent gelatinous material inside. An overall prevalence of 2.27% (10/132; 95% CI 2.41 – 2.13) was identified. When performing the analysis according to the type of tissue, it was observed that the prevalence for heart was 10.61% (7/66; 95% CI 10.74 - 10.46) while in the esophagus the prevalence was 4.55% (3/66; 95% CI 4.68 – 4.40). A chi square test was performed to determine the association between the presence of cysts and tissue type; it was found that there is no significant difference P>0.05. In conclusion, *Sarcocystis spp.* It is found naturally in sheep farms in the state of Jalisco. Although the

prevalence is low, its presence should be considered as a potential risk to public and veterinary health as it is a zoonosis; therefore, when performing the macroscopic identification of cysts, it is considered a first step, to be able to implement other diagnostic techniques that allow determining the species present in the area; with the aim of establishing control programs that are useful to producers.

Keywords: Parasite, Protozoan, macroscopic diagnosis, cysts, Mexico.

PA-P27

Acaricidal activity of commercial topic formulations against Cattle tick (*Rhipicephalus microplus*) from Lower Amazon

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Objectives: The objective of this work was to evaluate the efficiency of three topical acaricides (Amitraz, Cypermethrin, and Deltamethrin) in the control of the cattle tick (*Rhipicephalus microplus*) from the Lower Amazon region.

Material & Methods: To carry out the study, adult ticks (engorged females) were obtained from a commercial farm located in the municipality of Santarém, Pará, western Amazon (02º10'17" S; 56º44'42" W). The engorged ticks were manually removed from cattle without receiving acaricidal treatment for the past 60 days. The ticks were stored in plastic tubes with the lid pierced for air circulation and sent to the laboratory for the adult immersion test, which was performed on the same day of tick sampling.

Adult immersion test

For the adult immersion test, a total of 150 engorged female ticks were selected, washed in running water, and dried with paper towels. The test was performed according to classic protocol. The 150 ticks were divided by weight into 15 Petri dishes with 10 ticks each (1.9 ± 0.4 mg). Of the 15 Petri dishes, 4 were used for each of the 3 acaricidal drug treatment groups and 3 were used as a negative control. Three commercial products with Amitraz (125 g/L), Cypermethrin (150 g/L), and Deltamethrin (25 g/L) were used.

For the adult immersion test, the acaricidal concentration was used according to manufactures indications. Drugs were diluted and homogenized using distilled water. Final concentration used was: Amitraz: 0.125 g/mL, Cypermethrin: 0.15 g/mL, Deltamethrin: 0.025 g/mL.

Females ticks from each group were submerged in the designated solution for 5 minutes, and then removed and dried with paper towels and returned to the same plate. The control group was submerged in distilled water.

These plates were incubated in a B.O.D. (Biological Oxygen Demand) with a temperature of 28°C (±1°C) and relative humidity ≥ 80%. For adult mortality evaluation, ticks were examined after 24 hours with counts of live and dead ticks.

The engorged females were kept in the Petri dishes under the controlled environment until the oviposition. After oviposition, the eggs were weighed and separated into syringes. The tick egg hatchability was visually estimated by trained personnel.

To determine if *Rhipicephalus microplus* is resistant to treatment, we first calculated the estimated reproduction index (ER), where ER = egg mass/female weight before oviposition X hatching percentage X 20,000. Then, acaricidal efficacy was calculated using the mean values of the plates from control and treated groups resulting in the *in vitro* acaricidal efficacy using the formula:

Acaricidal efficacy (AE) = (mean ER control group – mean ER treated group) / mean ER control group X 100.

Results: The topical acaricidal drugs tested presented limited adult (engorged female) mortality with 0, 2.5 and 7.5% adult mortality for Deltamethrin, Amitraz, and Cypermethrin, respectively. The negative control group had zero adult mortality.

Table 1 presents the complete evaluation of tick oviposition, egg hatchability, the estimated reproduction index, and the in vitro acaricidal efficacy.

Table 1. In vitro acaricidal activity results of *R. microplus* from Lower Amazon

Groups	Mean tick weight (mg)	Mean egg mass (mg)	ER	Mean egg hatchability (%)	AE (%)
Amitraz	176.5	13.3	7.1	53.7	90.5
Cypermethrin	195.2	84.4	42.7	88.7	10.4
Deltamethrin	202.9	79.7	39.6	80.0	26.6
Control	184.2	80.2	42.3	98.3	0.0

ER: Estimated reproduction index. AE: Acaricidal efficacy.

The egg hatchability was partially impaired by acaricidal, with Amitraz presenting lower results among the drugs tested. The Amitraz group was the most effective compared to the other two with 90.5% AE, however, considering the FAO regulations for chemical acaricidal drugs, Amitraz was not effective, since it had effectiveness lower than 95%.

The pyrethroids (Cypermethrin and Deltamethrin) presented limited acaricidal effectiveness indicating the tick has already developed drug resistance to this class of acaricidal drugs.

Conclusion: Among the tested acaricides, Cypermethrin, Deltamethrin, and Amitraz showed efficacy below 95% and cannot be considered effective to control cattle ticks. *R. microplus* from the Lower Amazon presented high resistance to pyrethroids. As far as we know, this is the first report to evaluate tick resistance to commercial topical acaricides in the Lower Amazon region.

Keywords: Ectoparasites, Amitraz, Cypermethrin, Deltamethrin, acaricidal efficacy.

PH-P01

Susceptibility of Escherichia coli and Salmonella field isolates to paromomycin

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Objectives: Paromomycin is an aminoglycide registered in both pigs and cattle for the treatment of gastro- enteric pathogens. As part of the prudent use of antibiotics and to monitor evolution of antimicrobial resistance, susceptibility testing is of great importance and performed on E. coli and Salmonella isolates from diagnostic cases by DGZ.

Material & Methods: Antimicrobial resistance was determined by the disk diffusion method on 2045 isolates of E. coli, hemolytic E. coli and Salmonella strains isolated from pigs and cattle clinical samples over the period of 01/2017 till 08/2019. Cultures were inoculated on Muller Hinton agar plates and susceptibility discs for paromomycin (Mast Diagnostics) 1000 µg were used. Plates and the inhibition zone were read after 24 h of incubation at 37°C. To categorize the strains in susceptible (S), intermediate (I) or resistant (R) the following breakpoints are defined S≥14mm –I : between 13 -11 mm and R<11mm.

Results: A total of 2045 isolates of which 1654 E coli strains (19.7 % was hemolytic) and a total of 391 Salmonella were tested. In total 79.80% of the Enterobacteriaceae strains were susceptible, 6.26% of the strains were resistant and 13.94% intermediate to paromomycin. If we look to the different species of origin the highest level of resistance was found in cattle stains 9.95% whereas in strains of pig origin only 0.95% of resistance was observed. Especially the E. coli and hemolytic E. coli isolates from cattle gives the highest level of resistance, 11.40%, while in Salmonella only 0.62% of resistance was noticed.

Conclusion: In conclusion we can state that, based on these results, a high proportion of Escherichia coli and Salmonella strains in Belgian farms shows susceptibility to paromomycin, but it is important to analyse the data by animal origin and by bacterial species to evaluate better the real development of resistance.

Keywords: Antimicrobial resistance, E. coli, Salmonella, Paromomycin.

PH-P02

In vitro induction of resistance in digestive *Escherichia coli* strains of bovine origin collected in France under the selective pressure of paromomycin

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Objectives: Paromomycin is an aminoglycoside antibiotic labelled in France to treat calves with diarrhoea due to *Escherichia coli* strains. This antibiotic is also used off-label in France, to treat calves with diarrhoea due to *Cryptosporidium parvum* but also as a preventive treatment for cryptosporidiosis, by a daily distribution during at least one week. However, few studies exist on the impact of this oral antibiotic therapy on digestive coliform bacteria, but in turkey farming. The aim of this study was to assess the evolution of *in vitro* sensitivity to paromomycin of digestive *E. coli* strains isolated in France in 2019 from diarrhoeic stool samples of calves, after repeated treatment, through a model with repeated passages.

Material & Methods: The study population consisted in fifty *E. coli* strains isolated from stool samples of calves with diarrhoea and sent to the laboratory (LABOCEA, Fougères, France) in 2019 for the purpose of diagnostic. Tested strains were picked up at random from the specimen bank of the laboratory. At the time of identification, MIC of paromomycin that inhibits the growth of all strains was determined using a broth microdilution method according to the CLSI VET01 standards and recorded. Since there are no validated breakpoints (CLSI, EUCAST or CA-SFM) for paromomycin, breakpoints to kanamycin for *E. coli* were used instead, as indicated in CA-SFM 2012 for a human being (susceptible, ≤ 8 µg/ml; resistant, >16 µg/ml). Of these 50 strains, 5 strains susceptible to paromomycin were selected (3 with MIC≤4µg/mL, and 2 with MIC≤2µg/mL), and the evolution of their sensitivity was assessed by a broth microdilution method in 96-well plate, derived from a method published previously.

Results: Among the 50 selected strains and according to breakpoints derived from kanamycin, only 38% were considered as sensitive to paromomycin (62% resistant), with MIC≥256µg/mL. After repeated passages, all the 5 sensitive strains became resistant to paromomycin. The ratio between MIC after and before repeated passages varied from 8 to more than 256.

Conclusion: This *in vitro* study is a first step, based on repeated passages with a fixed paromomycin concentration, corresponding to a continuous selective pressure, which is unlikely in the field. This first result confirms data gained by the RESAPATH survey for the sensitivity to paromomycin in *E. coli* (estimated from the sensitivity to kanamycin) reported for 2018 in France, with the same percentage of strains susceptible to kanamycin (38 %). In addition, this study is a phenotype-based approach and does not presume whether this resistance is linked to the genetic background of the bacterial species. Further in vivo research should be performed to assess the evolution of *E. coli* (commensal and/or pathogenic strains) sensitivity to paromomycin after a repeated treatment in field conditions in the calf. Nevertheless, this *in vitro* study illustrates that *E. coli*, exposed to a repeated selective pressure against paromomycin, that can occur on a long-course cryptosporidiosis preventive treatment, can quickly adapt to resist to paromomycin. This study finally shows that *E. coli* sensitivity to paromomycin should be specifically followed by an official observatory in France and in Europe.

Keywords: Paromomycin, cryptosporidium, E. coli, antimicrobial resistance.

PH-P03

Serological evidence of exposure to Q fever in humans after an abortion outbreak in dairy cattle due to *Coxiella burnetii*

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Objectives: Q fever is a zoonosis caused by *Coxiella burnetii*, a small bacterium strictly intracellular that is able to infect a wide range of hosts and to survive for long periods in the environment. In cattle, *Coxiella burnetii* is associated with abortions, stillbirths, retained placenta, weak newborns, metritis and infertility. Many studies confirmed the major role domestic ruminants have as the main reservoir for human infection. Veterinarians and farmers that are in contact with infected animals are considered at risk (Groten et al., 2020) through the inhalation of contaminated aerosols containing bacteria like the products of parturition. Among humans, clinical symptoms are often asymptomatic but in some cases acute flu-like symptoms, abortion and chronic form like endocarditis, vascular infections and "fatigue" syndrome can be present. Aim of this study was to investigate an abortion outbreak in cattle related to *Coxiella burnetii* and the possible correlation between the infection in ruminants and the seroprevalence observed in humans in contact with infected cattle.

Materials and methods: The case: between April and August 2019 ten 6 to 8 months' pregnant cows aborted in a dairy cattle farm with 220 milking cows located in Ragusa (Sicily – Italy). The cattle or the people from this farm never were in close contact with small ruminants. Two fetuses were submitted to the Diagnostic Lab of the Istituto Zooprofilattico Sperimentale Della Sicilia A. Mirri in Ragusa, for the detection of the major abortigenic pathogens namely IBR, BVD, *Neospora caninum*, Leptospira, Brucella, Chlamydia and *Coxiella burnetii*. Moreover, 246 animals (heifers and cows) were serologically tested for the same abortigenic agents. In order to investigate the potential transmission of the infection to humans into the farm, sample sera were collected from 16 people three months after the first abortion outbreak in cattle. This serological investigation involved the farmers, their families and farm workers and was based on testing for *Coxiella burnetii* antibodies (IFA test) to evaluate phase I and phase II IgM and IgG.

Results: The two aborted fetuses were found positive for *Coxiella burnetii* in PCR and were negative for all the other pathogens tested. Seroprevalence in dairy cattle for *Coxiella burnetii* was found to be around 50% . A high number of animals were found positive in serology for IBR (≥92.1%) and BVD (91.4%); this could be explained by previous vaccination implemented with a multivalent vaccine containing IBR (non-marker), BVD, BRSV and PI3.

Overall prevalence for *Coxiella burnetii* IgG in human cases was 50% (Table 2). If we consider the people strictly in contact with cattle, the seroprevalence was found to be about 67% for phase I IgG and 78% for phase II IgG. None of the people showed clear symptoms of Q fever infection with the exception of headache and, in one case, diarrhea.

Conclusion: Data obtained during the study highlight the possibility of transmission of *Coxiella burnetii* from cattle to humans and underline the need to increase epidemiological surveillance and the opportunity to implement infection control plans, including vaccination, in cattle.

Table 2 – Serology in humans (IFA Test).

	Strict contact with cattle	*Role in the farm*	*Age*	*Phase I IgM*	*Phase II IgM*	*Phase I IgG*	*Phase II IgG*
1	**yes**	worker	44	**neg**	**neg**	**positive**	**positive**
2	**yes**	worker	42	**neg**	**neg**	**positive**	**positive**
3	**yes**	Farmer	25	**neg**	**neg**	**positive**	**positive**
4	**yes**	Farmer	57	**neg**	**neg**	**neg**	**positive**
5	**yes**	Farmer	48	**neg**	**neg**	**positive**	**positive**
6	**yes**	worker	34	**neg**	**neg**	**neg**	**neg**
7	**yes**	worker	35	**neg**	**neg**	**neg**	**neg**
8	**yes**	worker	32	**positive**	**neg**	**positive**	**positive**
9	**yes**	son	13	**positive**	**neg**	**positive**	**positive**
10	no	daughter	14	**neg**	**neg**	**neg**	**neg**
11	no	daughter	11	**positive**	**neg**	**neg**	**neg**
12	no	wife	40	**neg**	**neg**	**neg**	**neg**
13	no	daughter	20	**neg**	**neg**	**positive**	**positive**
14	no	wife	51	**positive**	**neg**	**neg**	**neg**
15	no	son	24	**positive**	**neg**	**positive**	**neg**
16	no	worker	42	**neg**	**neg**	**neg**	**neg**
% pos				**31.2%**	**0%**	**50.0%**	**50.0%**

Keywords: Q fever, Coxiella burnetii, dairy cattle, zoonosis, abortion.

PH-P04

Susceptibility of *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni* to florfenicol in Europe, US and Canada

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Objective: Bacterial bronchopneumonia (BP) caused by *Mannheimia haemolytica*, *Pasteurella multocida* and/or *Histophilus somni* remains a major cause of morbidity and mortality in beef and dairy cattle worldwide. Among antimicrobials licensed for BP treatment, florfenicol is often selected as first line antimicrobial because of its high level of efficacy (Thiry et al., 2011).

In the current context of increased prevalence of antimicrobial resistances (AMR), it is crucial to regularly evaluate sensitivity of *Pasteurellaceae* against antimicrobials licensed to treat BP to ensure best selection of first line antimicrobials for BP treatment. Therefore, a systematic review of the literature was conducted to evaluate sensitivity to florfenicol (FFE) of *M. haemolytica, P. multocida*, and *H. somni* recently isolated from cattle in Europe, USA and Canada.

Material and methods: PRISMA guidelines were followed. Original articles in English, French and Italian published between 2014 and 2019 were searched in CAB (Commonwealth Agricultural Bureau) Abstracts and PubMed/MEDLINE databases on Feb 2nd, 2019. The following keywords were used for the search: (antibiotic resistance OR antimicrobial resistance) AND (*Mannheimia haemolytica* OR *Pasteurella multocida* OR *Histophilus somni*). Titles and abstracts were read and original articles that were relevant to the subject of AMR in *Pasteurellaceae* in cattle were selected for further review.

Articles were included in the review based on the following criteria: clinical isolates originating from Europe, USA or Canada; sensitivity/resistance defined based on CLSI guidelines; reporting phenotypic resistance against FFE; clinical isolates obtained after 2010. The following data were extracted from each study (where available): basic study characteristics (authors; year of publication); clinical isolate characteristics (year and location of isolation); number of herds and type of production (cow-calf herd; beef feedlot; dairy herd); type of sample (nasal swab; trans-tracheal aspiration/broncho-alveolar lavage; lung tissue; etc.); health status (healthy, bovine respiratory disease [BRD], dead with pneumonia) and treatment history of the animals at time of sampling; days on feed (for stocker/feedlot cattle only); method of sensitivity testing (disk diffusion of Kirby-Bauer or broth microdilution) and outcomes (sensitive; intermediate or resistant to FFE).

Results: Twelve original articles were included. Based on the data extracted from these articles, sensitivity to FFE was high in *P. multocida* and *H. somni* strains isolated in Europe, US or Canada during the last 5 to 10 years (>86%; Table 1). Sensitivity to FFE were also high in *M. haemolytica* isolated from Canada and Europe. However, sensitivity to FFE was sometimes reduced in *M. haemolytica* recovered from cattle previously exposed to antimicrobials as curative or preventive treatment (e.g. metaphylaxis) in the US. Presence of integrative and conjugative elements (ICEs) containing resistance genes against macrolides and FFE (e.g. *flo*R) can explain the relatively high prevalence of *M. haemolytica* resistant to florfenicol observed after metaphylaxis in US studies (Snyder et al., 2017).

Table 1. Proportions of *M. haemolytica, P. multocida* and *H. somni* isolates sensitive to florfenicol in Europe, USA and Canada (based on 12 recent original scientific articles).

	Number of strains	Europe	USA	Canada
M. haemolytica	2067	78 – 99% (n=285)	11 – 100% (n=1136)	96 – 100% (n=646)
P. multocida	1207	99 – 100% (n=185)	99 – 100% (n=410)	86 – 99% (n=612)
H. somni	732	100% (n=66)	100% (n=307)	98 – 100% (n=359)

Conclusion: To the authors' best knowledge, this systematic review provides the most up-to-date information available in the scientific literature regarding susceptibility to FFE of *M. haemolytica, P. multocida,* and *H. somni* recently isolated from cattle in Europe, USA and Canada.

The relatively low prevalence of *Pasteurellaceae* resistant to FFE indicates that genes conferring resistance to FFE such as *flo*R or *fexA* are not widely distributed among *Pasteurellaceae* originating from cattle and that new mechanism of resistances to FFE are probably slow to develop.

References:

Thiry, J., Rubion, S., Sarasola, P., Bonnier, M., Hartmann, M., de Haas, V., 2011. Efficacy and safety of a new 450 mg/ml florfenicol formulation administered intramuscularly in the treatment of bacterial bovine respiratory disease. Vet Rec. 169, 526.

Snyder, E., Credille, B., Berghaus, R., Giguere, S., 2017. Prevalence of multi drug antimicrobial resistance in isolated from high-risk stocker cattle at arrival and two weeks after processing. J Anim Sci 95, 1124-1131.

Keywords: AMR, rational use, florfenicol, BRD, susceptibility.

PH-P05

Which antimicrobials are used and dispensed most frequently as dry cow therapy tubes? An analysis of Defined Course Doses (DCD_{vet}) based on veterinary treatment data

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Objectives: The aim of the current study was to analyse which antimicrobial active ingredients were used most frequently by a convenience sample of Austrian farm veterinarians as dry cow therapy.

Materials and Methods: A total of seventeen veterinary practices treating over 6700 dairy cows provided antimicrobial use data for 250 dairy farms over a period of one calendar year. All antimicrobials administered by the veterinarians themselves and those dispensed to farmers were recorded. Individual animal IDs, diagnosis data, as well as the number and amount of drug used were provided to the authors electronically. Animal movement and production data were provided from the Cattle Database (RDV) of the Austrian Federation of Cattle Breeders. The analysis presented here includes only intramammary dry cow tubes. Antimicrobial metrics were calculated using the European Medicines Agency unit of veterinary Defined Course Doses (DCDvet), where 4 dry cow tubes are equal to 1 DCDvet. Using the mean calving interval for each dairy farm as well as the annual replacement rate, DCDvet values per cow and year were adjusted to account for these factors as follows:

$$Correction\ factor = \frac{Calving\ interval\ (d)}{365} \times \frac{100}{(100 - replacement\ rate\ (\%))}$$

Results: The median adjusted DCDvet/cow/year for the study farms was 0.611 (mean 0.551). Each veterinary practice enrolled different numbers of farms in the study. For this reason and to ensure participant anonymity, the data here refer to the proportions (in percent) of adjusted DCDvet/cow/year for antimicrobial dry cow therapy (DCT) used by each practice on the study farms. The only "Highest priority critically important antimicrobials" (HPCIA, as defined by the World Health Organization) licensed for use in Austria for DCT and identified in this analysis was the fourth-generation cephalosporin, cefquinome. Of 17 veterinary practices, 12 (70.6%) did not use HPCIAs for dry cow therapy. Of those practices which did use HPCIAs, the highest level of use for one practice was 46.4% cefquinome of all dry cow therapy administration and prescribing, as calculated as a proportion of total DCDvet/cow/year. This practice used cloxacillin (44.3%) and the first-generation cephalosporin, cephalonium (9.3%) for the remainder of their dry cow therapy. Of all 17 practices, the most commonly used antimicrobial active ingredients for dry cow therapy were cloxacillin and ampicillin & cloxacillin combinations.

Conclusions: Overall, the veterinarians included in this study were responsible in their use of antimicrobials for dry cow therapy with the vast majority using non-HPCIA active ingredients. However, veterinarians in practice need to be made aware of their options with respect to prudent use and antimicrobial stewardship. The use of antimicrobial classes of critical importance to human medicine should be restricted to specific cases where alternative less critical classes of antimicrobials have been proven not to be effective through bacteriological culture and sensitivity testing.

Keywords: Antimicrobial, antibiotics, dry cow therapy, mastitis, antimicrobial resistance.

PH-P06

Evidence for Coxiella burnetii infection in Shepherds and sheep milk Cheesemakers of Portugal – an occupational exposure study

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Objectives: Q fever is a zoonosis caused by *Coxiella burnetii* and transmission to humans is often associated with abortion outbreaks in ovine livestock. Infection usually occurs through inhalation of contaminated aerosols but also by the alimentary route and through the bite of ticks, causing a vast clinical range, ranging from asymptomatic seroconversion presentation to acute (mild, flu-like to severe pneumonia) or chronic infection (endocarditis manifestation) and death. Those exposed to ovine are particularly at high risk of infection. Recent studies show that Q fever is rising in ovine in Portugal raising alerts on spill-over to humans, which we sought to evaluate.

Materials and Methods: In the present study, a cross-sectional serological evaluation of *C. burnetii* antibodies in Shepherds and sheep milk cheesemakers of central Portugal (N=96) and general population controls (N=260, matched by age, sex and region) was performed to assess the impact of occupationally acquired Q fever, by using an anti-*C. burnetii* IgG commercial enzyme immunoassay.

Results: Anti-*C. burnetii* IgG were detected in 27 (28.1%;

95% confidence interval [CI]:19.4-38.2%) of 96 Shepherds and sheep milk Cheesemakers (at-risk), as well as in 21 (8.1%; 95%CI:5.1-12.1%) of the 260 controls (P=0.0001), pointing to an increased risk of *C. burnetii* infection in individuals with occupational exposure to sheep in Portugal.

Conclusions: Little is known regarding *C. burnetii* circulation in Portugal, both in animals and in humans. We alert for increased surveillance of Q fever in Shepherds and sheep milk cheesemakers, in a One Health vision.

Keywords: Coxiella burnetii, infection, Shepherds, Cheesemakers, Portugal.

PH-P07

Exploring Dairy Cattle Ruminal Microbiota as a reservoir of Antimicrobial Resistance Genes for the One-Health Initiative

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Objectives: Antimicrobial resistance (AMR) is the ability of a microorganism to stop an antimicrobial from working against it. In consequence, the main tool to prevent and cure infections and zoonoses is under threat. Although the phenomenon of acquiring resistance can occur naturally through mechanisms of microbial adaptation to environment or ecosystem, the inappropriate and excessive use of antimicrobials in food and companion animals, and in the medical practice have exacerbated this threat. From birth, bovine neonate acquires bacteria from the dam, partners, feed, housing and environment. As the rumen develops, it becomes colonized by a complex and genetically diverse group of microorganisms with complex interactions and symbiosis. Rumen microbial community composition is affected by feeding management and bovines could consequently be exposed to resistant microorganisms and antibiotic resistance genes (ARGs) through the consumption of feed produced on land with different risks of dissemination of ARGs. As a result, rumen could play an important role as a ARGs environmental reservoir and could also acts a reactor to facilitate horizontal transmission of ARGs between bacteria in the rumen microbiota and those from gastrointestinal tract.

Therefore, the objective of this study was to determinate the prevalence of ARGs in the ruminal ecosystem. This will allow evaluating the potential risk of transmission in commensal and pathogenic bacteria or through fecal discharge and saliva to other bovines, humans or in the environmental.

Material & methods: DNA was extracted from the ruminal content of 472 Friesian cows from 14 commercial farms in Spain. The project was approved by the Basque Institute for Agricultural Research and Development Ethics Committee.

Ruminal samples were collected using the oral stomach tube technique. To extract the microbial DNA we used a commercial kit "DNeasy PowerSoil".Then, it was sequenced using the nanopore technology on a MinION device, following the standard protocol from Oxford Nanopore (Oxford, UK) with sample multiplexing and library preparation.

After quality control, the sequences were analysed using the SQMreads tool from SqueezeMeta (Tamames and Puente-Sánchez, 2019), a pipeline for metagenomics. We implemented a custom integration of the Comprehensive Antibiotic Resistance Database (CARD). Genes were assigned to the CARD ontology for taxonomy and function annotation. Data were treated as compositional.

Results: The results of this study are a preliminary insight into the ruminal resistome. A total of 69 genes with relative abundance (RA) higher than 0.005% associated with AMR were detected, representing 7.0% of the total AGRs abundance detected in the rumen samples. From those, 24 AGRs were widely represented in the core of the ruminal ecosystem and were detected in all the herds (0.13%<RA<0.83%).

These 69 AGRs most prevalent could be classified into 22 AMR gene families, although 54.5% of them were grouped into 4. Regarding the resistance mechanism, 36 of the 69 AGRs confer antibiotic resistance through alteration, replacement or protection of the antibiotic target, another 28 via the transport of antibiotics out of the cell, promoting a decrease of intracellular concentration of antibiotic, and the rest through the inactivation of the antibiotic.

According to CARD information, several AGRs detected are carried by pathogenic bacteria to humans and public health concern (*Clostridium difficile*, *Campylobacter jejuni*, Salmonella Enterica,) and/or veterinary health (*Staphylococcus aureus*, *Streptococcus agalactiae*) or by commensal and opportunistic bacteria such as *E. coli*, *Enterococcus faecalis* or *Proteus vulgaris*.

The prevalence of AGRs within herds ranged between 39.1% and 97.1%. The distribution of AGRs between herds seems to be very similar. Antibiotic resistance gen macB presented the highest RA in all herds (from 0.037% to 0.089%) and is associated with the macrolide antibiotic class (approved for use in food-producing and companion animals). However, the set of AGRs related to tetracycline resistance, a Veterinary Critically Important Antimicrobials according to the OIE, presented the highest RA (1.34%) in the data set. No AGRs of particular relevance to human health (ESBL, AmpC and carbapenem resistance) were detected.

Conclusions: The detection of ruminal microbiota harbors resistance genes against a wide range of antibiotics is confirmed and could represent a risk for both animal and human health and food safety. Further research is needed to elucidate the role of the ruminal ecosystem as a vector for AGRs and which ruminal microorganisms are involved, and a more detailed analyses of the sort of microorganisms that carry the ARG.

Keywords: Cattle, antimicrobials, resistance, rumen, contamination.

PH-P08

Monitoring of antimicrobial drug use in northwestern Italy beef calves fattening operations

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Objective: The present study aimed to monitor the antimicrobial usage in fattening operation units in northwestern Italy.

Materials and methods: The present study was carried out in fattening operation units located in Piedmont region that imports beef heifers and bulls at the age of 8-12 months from France.

Antimicrobial drug usage was obtained from the official farm records of two consecutive years (2014 and 2015) and it was quantified based on the Animal Daily Dose (ADD) calculation. The ADD is defined as the average maintenance antimicrobial dose of a drug for the main indication in a specified species in 24 hours. The number of ADD (nADD) was obtained by dividing the total amount of drug administered (mg) by the ADD value (mg/kg) and the animal weight (kg) (mean between the animal's weight recorded at the beginning and at the end of the production cycle). For long-acting preparations, the ADD was calculated from the recommended dosage into a 24 h dose, by dividing by the long-acting factor (LA factor), defined as the number of days considered under treatment after one application of the drug.

The nADD administered for each animal in the two years of the study was obtained by dividing the nADD by the total number of animals at risk of being treated. Antimicrobial treatments were then categorized based on two criteria: oral *vs* parenteral and individual *vs* group treatments.

Furthermore, the dose actually administered to the animals, represented by the Used Daily Dose (UDD), was calculated for each drug. The UDD was represented by the ratio between the total amount of drug administered (mg) and the average animal weight (kg) multiplied by the number of applications. The ratio between UDD/recommended daily dose (RDD) was then calculated to assess the compliance with dosing. A ratio between 0.8 and 1.2 was considered appropriate, while lower than 0.8 and higher than 1.2 were considered underdosed and overdosed, respectively.

The nADD was reported as mean ± standard deviation (SD) (median, max) and the percentage of under, normal or over dosed treatment performed in each farm was reported as mean ± SD.

Results: A total of 26 fattening operations were included. The total number of animals at risk during the two years of the study was 64,719, with a median number of animals per farm per year of 886 (min=151, max= 4200). The median weight of the animals used for the calculation of the ADD was 494.5 kg (min = 335, max = 613).

Overall, 821.7 kg of antimicrobials were used during the two years period (2014 = 474.6 kg, 2015 = 347.1 kg). The average nADD used during the study period on each farm was 3 (± 2.1, 2.6, 8.3). Group antimicrobial treatments, representing 57.5%, were administered orally in 70.4% of the cases and parenterally in 29.6%. Oral formulations were composed primarily by doxycycline (97%). Parental formulations were composed mainly of tulathromycin (41.5%), tildipirosin (26.8%) and florfenicol (6.8%). Individual treatments were principally administered parenterally (98.1%) and the most used molecules included florfenicol (19.9%), marbofloxacin (19.5%) and tylosin (12.4%).

Based on UDD calculation, on average 23.5% (± 35.9%) of the oral group treatments were under-dosed. Moreover, 75.5% (± 34.3%) and 48% (± 21.9%) of parenterally administered antimicrobials in group and individual treatments, respectively, were under-dosed.

Conclusion: The number of ADD found in the present study was lower than those reported for poultry, veal calves and pigs and comparable with those calculated for dairy cattle in other nations. Based on ADD calculation, north-west Italian beef calves fattening operations seems to use less antibiotics than other meat production animals. The main active compounds used, both for group and individual treatment, had BRD as their main indication for treatment, confirming the importance of this disease in the beef cattle fattening operations. Critical antimicrobials for human medicine were largely used and a tendency to under-dose these antimicrobials, especially those orally administered, was identified. This result suggests the need for interventions in order to achieve a more responsible use of antimicrobials in this category of meat production animals.

Keywords: Antimicrobial, beef, cattle.

PH-P09

Gastrointestinal pharmacokinetics and microbiome analysis of different dosing regimens of danofloxacin administration in steers

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Objective: To gain insight on the gastrointestinal pharmacokinetics of danofloxacin and how it correlates with bacterial load, composition and resistance. We hypothesized that the gastrointestinal pharmacokinetics of danofloxacin for the lower, repeated dose would be below a therapeutic level for a longer period of time. We hypothesized the prevalence of resistant isolates would be higher in the steers administered the repeated, lower dose of danofloxacin.

Materials and Methods: Twelve steers (6-7 months) underwent gastrointestinal surgery to facilitate placement of an ultrafiltration probe in the ileum and colon. Intestinal ultrafiltrate, interstitial fluid and blood were collected for the pharmacokinetic analysis. Twenty-four after surgery, the steers were either administered 6 mg/kg danofloxacin subcutaneously (SC) (n=6) 48 hr apart or 8 mg/kg danofloxacin SC (n=6) once. Feces was collected manually until day 38. To determine the pharmacokinetic parameters of danofloxacin, high perfor-

mance liquid chromatography was completed. A two compartmental analysis was performed with individual student t tests to determine significance between dosing groups. Minimum inhibitory concentrations of *E. coli* and *Enterococcus* isolates was determined using broth microdilution. To assess microbial composition, 16s rRNA gene sequencing was performed with microbiome analysis using Dada2, Phyloseq and DESeq2.

Results: Area under the curve (AUC) was significantly different between the dosing group for plasma (p value <0.05). The mean AUC for the low group was 14.08 hr*ug/mL and for the high dose group was 19.39 hr*ug/mL. The half-life for interstitial fluid was also significantly different between dosing groups (p value < 0.05); it was higher in the low dose group. All other parameters were not significantly different between dosing groups for plasma, interstitial fluid, ileum or colon ultrafiltrate. However, there is high intestinal penetration following administration of both the high (743.6% +/- 241.7) and low (769% +/- 109.9) doses of danofloxacin. The mean log growth of both *E. coli* and *Enterococcus* was greater for the high dose group compared to the low dose group over time. The median MIC for *E.coli* was not different at any time point between the low and high dose group, but spiked after 96 hours. The median MIC for *Enterococcus* was varied between groups and did not return to baseline throughout the study period. Between both the high and low dose group, the *Euryarchaeota* phyla appears to be decrease over time, specifically due to a decrease in *Methanobrevibacter*.

Conclusions: The high level of intestinal penetration of danofloxacin alters the gastrointestinal microbiome regardless of dosing group. While difficult to interpret danofloxacin resistance based on MIC due to lack of a break point for enteric organisms, a spike in MIC can be seen for *E. coli* after dosing, indicating the bacteria are able to develop an increased tolerance after antibiotic pressure.

Keywords: Antibiotic Resistance, Microbiome, Gastrointestinal pharmacokinetics.

PH-P10

Lead poisoning in a dairy herd

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Objectives: Following a diagnosis of lead posioning in a dairy herd, a longitudinal study was conducted to monitor blood lead levels. The aim was to demostrate that whilst the bulk milk produced from the cows quickly returned to regulatory levels, the blood lead levels would fall at at a slower rate. This impacted on the issues faced by cull animals in such cases in regards to their entering the food chain and demonstrated the challenges faced by owners of such animals when exposed to lead.

Material and Methods: A diagnosis of lead posioning in a large dairy herd was brought to the attention of the veterinary officers responsible for the Chemical and Food Safety Project operated by the United Kingdom's Animal and Plant Health Agency. This small group of specialist veterinary surgeons act on behalf of the Food Standards Agency (FSA) and both report such issues to them and provide advice on the management of whilst uncommon but often highly significant and one health orientated type of cases.

As a dairy herd the immediate risk to the food chain was the liquid milk being produced on site. Analysis of bulk milk samples was conducted by Eurofins to establish when the milk was considered safe for human consumption.

As the lead source in this case was a battery, included by accident into the mixer wagon; prolonged raised blood lead levels were considered likely. It was agreed with the FSA that a longitudinal study of the blood lead levels of the exposed cows would provide a useful insight into this scenario. Heparin bloods were collected from all the cows on three occassions and lead levels assessed by the Scottish Rural College biochemistry unit.

Results: Bulk milk lead levels dropped rapdily so within a few weeks the herd was able to sell its milk again.

A large proportion of cows had raised blood lead levels at the first sampling exercise. A number of these had developed clinical lead poisoning with some responding to supportive treament and others culled on welfare grounds. The majority of the blood lead levels dropped dramtically between the first and second sampling events. The reduction was less marked between the second and third sampling events.

Conclusion: Despite the severity of the situation, with a number of adult animals exhibting acute lead toxicty; the bulk milk lead levels quickly returned to within regulatory levels. The issue of raised blood levels in ruminants following ingestion of battery fragements is well described in the literature. This study demonstrated this phenomenon succintly whilst confirming that after acute exposure removal of the point source results in a rapid fall in blood lead levels. The levels recorded at the second and third sampling event were considered not to be of immeadiate clinical concern to the animal. The guidance by the European Food Standards Agency is to continue to reduce both dietary and non dietary routes of lead exposure. Ensuring farmers and their advisors are aware of such public health concerns is important for confidence in the agricultural industry of the future. It is also important that regulatory bodies have robust, evidence based data upon which to make risk assessments on a case by case basis.

Keywords: Lead, Food Safety, Risk Analysis.

PH-P11

Monitoring antimicrobial resistance in dairy farms using high somatic cell counts dairy cows

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Due to the emergence of multidrug resistant bacteria, the requirement of a proper microbiological diagnostic based on

antimicrobial susceptibility testing is urgently needed to select the best therapy for treating mastitis in dairy cows. In six dairy farms, 10 cows with high somatic cell counts were selected in two different periods (March and October 2019) and the evolution of antimicrobial resistance was followed. The aim of the study was to evaluate the use of susceptibility testing to implement antimicrobial stewardship plans in these dairy farms. Milk samples were taken from each udder quarter and plated onto both, Blood and Polivitex agar plates. Following an overnight incubation at 37C and 5% CO_2, different colony morphologies were selected from each plate and identified by VITEK2 system. For Staphylococci, Streptococci and Enterococci isolates, minimal inhibitory concentration (MIC) against 10 different antimicrobials (ampicillin, penicillin, oxacillin, cephalotin, ceftiofur, erytromicin, novobiocin, pirlimycin, tetracycline, sulphadimexothine) was performed using a microdilution method (Sensititre). A total of 40 *Staphylococcusspp*. and 13 *Streptococcus spp*. were isolated during the first sampling point from 20 and 11 dairy cows, respectively. Furthermore, 56 *Staphylococcusspp*. and 20 *Streptococcus spp.*were isolated from 33 and 18 dairy cows, respectively, during the second sampling. Although 30 *Enterococcus spp*. were isolated from 26 dairy cows their MIC results were not used for the study since there are no clinical breakpoint described for most of the antimicrobials tested (oxacillin, cephalotin, ceftiofur, novobiocin, pirlimycin, sulphadimexothine). in*Streptococcus spp*the highest percentage of resistance were found for erythromycin, pirlimycin, and tetracycline, andin the case of *Staphylococcus spp.*for penicillin, pirlymicin and sulphadimexothine.In*Staphylococcus spp*the occurrence of pirlimycin resistances tended to increase (*P* = 0.08) from the first to the second sampling period (4.5 vs 22.8 ±5.8 %, respectively). Furthermore, the percentage of isolates presenting multidrug resistant profiles (≥3 antimicrobial families) was greater during the second sampling when compared with the first sampling period (17.5 vs 5.2 %, respectively). The most common antimicrobial resistance profile found in multidrug resistant isolates was pirlimycin, erytromicin and tetracycline, but as the number of resistance increased within the same isolates, resistance to penicillin, oxacillin and cephalotin also increased. Although the number of resistances increased during the last year in these dairy farms, the main resistances observed were for antimicrobials not used in these farms during the last year.

Keywords: Antimicrobial resistances, dairy cows, high somatic cell counts.

PH-P12

Molecular research of *Listeria monocytogenes* in bovine milk samples from bulk tank of small dairy farms

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Objectives: The aim of this study was to investigate the occurrence of *L. monocytogenes* in milk samples from cattle of small rural properties in the state 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 0018/2021, of the Faculty of Veterinary Medicine and Animal Science, UNESP, Botucatu. Milk samples were extracted and subjected to the molecular biology technique Conventional Polymerase Chain Reaction (cPCR) to detect *L. monocytogenes*, using *primers* PRS (which detects the *Listeria* genus), LM1/LM2 (which detects virulence gene hylA responsible for encoding listeriolysin O), InlA and InLJ (encoding internalins), which are important virulence factors. Then, the products generated from the PCR were subjected to electrophoresis in a 2% agarose gel.

Results: PRS gene was amplified in 21 (20.58%) samples and InLJ in 22 (21.57%). There was no amplification product in the LM1/LM2 and InLA *primers*. Positive samples were submitted to genetic sequencing. Samples that were positive with the InLJ *primers* showed 98.91% to 100% similarity for *L. monocytogenes* (Blastn accession: CP054846-1). **Conclusion:** In view of the lethality of *L. monocytogenes* and the involvement of raw milk and its derivatives, such as cheese and butter, as potential causes of listeriosis outbreaks, the presence of the *L. monocytogenes* DNA in the samples alerts to the importance of sanitary surveillance and emphasizes the need to develop public health policies for better epidemiological understanding due to its potential risk of foodborne diseases, linked to no reporting as well the lack of diagnosis.

Keywords: Listeriosis, Bulk tank, Bovine milk, Molecular diagnosis.

PH-P13

The association of treatment with 3rdand 4th generation cephalosporins and occurrence of ESBL-producing *E. coli* in dairy cows

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Objectives: Antibiotic use data are critical for drawing conclusions about the epidemiological connections between antibiotic use in farm animals, antibiotic resistance, animal and human health. The purpose of the study was to analyze the use of 3rd-4th generation cephalosporins in Estonian dairy farms. Secondly, we aimed to identify an association between the use of cephalosporins and the occurrence of extended-spectrum beta-lactamase (ESBL) producing *Escherichia* (E.) *coli* at the herd level.

Materials and methods: The invitation for participating in the study was sent to 70 large (with ≥100 dairy cows) Estonian dairy farms in 2018-2020. In total, 51(71.8%) dairy farms where 34 000 dairy cows were reared agreed to share their last 12 months of cow treatment data. The herd size of the participating herds ranged between 100 and 2,398 dairy cows, the average herd size being 660 dairy cows. Cow antimicrobial treatment data were registered in the farms' own electronic registers. All cow-level treatment records including ceftiofur and/ or cefquinome were extracted for the 12 months period and the total consumption of ceftiofur and cefquinome in milligrams of active ingredient per population corrected unit (mg/ PCU) were calculated for each farm.

From each farm, a pooled fecal sample was collected from the farm environment and was sent to the Estonian Veterinary and Food Laboratory for bacteriological analysis. Phenotypic ESBL-positive *E.coli* was determined by using selective media (MacConkey agar (Oxoid) with cefotaxime (1 mg/L), with prior enrichment in buffered peptone water in accordance to EU Reference Laboratory Antimicrobial Resistance protocol.

A univariable logistic regression model was composed with farm ESBL status (negative/positive) as an outcome variable and total consumption of cephalosporins as categorical predictor variable dichotomized at the median value (1.5 mg/PCU).

Results: From all antibiotic treatment cases (n = 65,650), the 3rd - 4th generation cephalosporins were used in 14.8% (n = 7,736) of times. In total, 3rd - 4th generation cephalosporins were used in 48 (94.1%) dairy farms, where the most common indications were foot diseases and metritis. The median amount of used cephalosporins across the farms was 1.53 mg/ PCU.

From all antibiotic-treated foot diseases and metritis, 3rd - 4th generation cephalosporins were used in 48.9% (n = 3,788) and 25.8% (n = 2,000) of treatment cases, respectively. Clinical mastitis and fever of unknown reason were treated with cephalosporins in 6.1% and 8.8% of cases, respectively.

ESBL producing *E. coli* were found in 14 out of 46 (30.4%) dairy farms. The use of 3rd-4th generation cephalosporins was on average 2.1 times higher in ESBL *E. coli* positive dairy farms (median 1.76 mg / PCU) compared to ESBL negative dairy farms (0.68 mg / PCU). The probability to isolate ESBL producing *E. coli* was on average 17% (p = 0.091) higher in herds using cephalosporins more frequently (over the median value of 1.5 mg/ PCU).

Conclusions: The use of 3rd-4th generation cephalosporins is high, especially in the treatment of foot and uterine diseases in Estonia. The frequent use of cephalosporins increased the production of ESBL producing *E. coli* and therefore has a negative impact on the general antimicrobial resistance level in the farm.

Keywords: Antibiotic use, cephalosporins, resistance

PH-P14

Development and implementation of an on-farm animal health and diagnostic educational training program for farm workers in antimicrobial stewardship in adult dairy cattle

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Objective: Antimicrobial stewardship (AMS) is a holistic approach, which promotes judicious use of antimicrobial drugs to preserve their effectiveness and availability. Dairy farm workers are commonly responsible for disease diagnosis and routine treatment decisions. This highlights the importance of farm workers knowledge and skills to successfully implementing judicious use of antimicrobials in livestock production systems. Knowledge gaps in animal health and behavior have been recognized before as an important reason to provide training to farm workers. A better understanding of cattle sickness behavior will improve early identification of clinical signs of disease and improve treatment success. The main objective of this project was to develop and implement an on-farm educational training program for farm workers in antimicrobial stewardship in adult dairy cattle.

Methods: A longitudinal quasi-experimental study design was used by enrolling a total of 18 conventional dairy farms in the United States (9 in California and 9 in Ohio), with six farms allocated to the training intervention group (TG) and three in the control group (CG) in each state. For the TG, farm worker(s) responsible for treatment decision on the farm participated in a didactic and hands-on 12-weeks training program led by the investigators. All the training materials were pilot tested to evaluate overall understanding of the training program. All materials were available in Spanish and English. Interactive short videos with audio were developed to cover objectives for each of the 6 teaching module (antibiotic resistance, treatment protocols, and visual identification of sick animals, clinical mastitis, puerperal metritis, and lameness). Printed materials were used to reinforce the learning objectives of the modules, and a hands-on session focused on clinical examination of sick animals was also included as part of the training program. Pre- and post- training assessments were administered to evaluate changes in knowledge about AMS. Change in knowledge was evaluated through 27 multiple choice/single answer and multiple choice/multiple answer questionnaire, with a maximum score of 27 points overall. Paired t-tests were used to confirm whether scores were significantly higher post-training compared with pre-training.

Results: A total of 25 workers completed the training program. Of the 25 participants, 48% (n=12) were enrolled in CA and 52% (n=13) in OH. A median of two workers were enrolled in each farm (range: 1 - 5). Self-identified females represented 12% of the participants (n=3) while 88% (n = 22) self-identified as male. Training assessments were answered in Spanish by

68% of the participants (n=17) and in English by 32% (n=8). Results from the pre-training assessment showed than participants had important knowledge gaps for the identification of sick animals and disease diagnosis (e.g., correct identification of disease, classification of disease severity). Based on the results from the post-training assessment, improved knowledge on antimicrobial stewardship practices and diagnosis of sick animals were detected (e.g., use of clinical signs for disease identification). A significant difference was detected between the mean pre- and post-training assessments scores at 10.85 (range: 1.69 – 23.42) and 19.15 (range: 4.18 - 24.27), respectively; paired *t*-test: t (24) = -4.62, *P* <0.0001].

Conclusion: Knowledge of participants on AMS and identification of sick animals improved after completing the educational training program. This improvement emphasized the potential value for educational training program tailoring farm workers for improving antimicrobial stewardship knowledge.

Keywords: Antimicrobial resistance, education, cattle.

PH-P15

Antimicrobial Resistance of Causative Agents of Bovine Mastitis in Andalusia

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Objectives: Bovine mastitis is one of the most prevalent infectious pathologies on the dairy sector and causes a big impact on production, animal well-being, and also on economy. Antimicrobial therapy is the most recurrent treatment for these infections. However, the emergence of multi-drug resistant bacterial isolates represents one of the main causes of antimicrobial treatment failure. The growing emergence of cases of antimicrobial resistance in bacterial species to many classes of antimicrobial agents represents a global issue in recent years. In response to this, we stablish the goal of this work: to determine resistance against different antibiotics in pathogens causing bovine mastitis in Córdoba, Andalusia.

Material and methods: A total of 180 strains (80 *E. coli*, 52 *Staphylococcus* spp., and 48 *Enterococcus spp)* solated from milk of animals suffering from clinical and subclinical bovine mastitis were analysed. Antimicrobial susceptibility against different antibiotics of those strains were studied by the automated VITEK® drug sensitivity analysis system and commercial VITEK® 2 AST-GN96 and AST-GP79 cards were used for *E. coli* and *Staphylococcus* spp. and *Enterococcus* spp.*,* respectively.

Results: The most important antimicrobial resistances for *E. coli* were found against beta-lactams (from about 4% for amoxicillin/clavulanic acid to 37% for ampicillin) and tetracycline (35%). With regards to *Staphylococcus* spp., there were resistances up to 35% against β-lactams (benzylpenicillin) and 31% against macrolides (tilmicosin). *Enterococcus* spp. strains showed resistances up to 21% against aminoglycosides (streptomycin high load) and lincosamides (clindamycin), and around 31% and 46% against tetracyclines and fluoroquinolones (enrofloxacin), respectively. Multiresistant strains were found among *E. coli* isolates (14%), *Enterococcus* spp. isolates (23%) and *Staphylococcus* spp. ones (8.3%).

Conclusions: This study has shown the existence of multiresistant strains from dairy cattle origin and resistance against different groups of antibiotics in the three groups of microorganisms studied: *E. coli*, *Enterococcus* spp. and *Staphylococcus* spp. These relevant findings represent an issue to the dairy sector, and encourage to take action and develop new strategies to control bovine mastitis.

Keywords: Mastitis, multiresistant, E. coli, Enterococcus, Staphylococcus.

PH-P16

Analysis of antimicrobial use in sheep and goats referred to the University Clinic for Ruminants at the University of Veterinary Medicine Vienna between 2005-2019

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Objectives: The aim of the current study was to analyse the use of antimicrobials in sheep and goat patients referred to the University Clinic for Ruminants of the Vetmeduni Vienna between 2005 and 2019. Furthermore, the study aimed to investigate whether national prudent use recommendations, introduced in 2013, made a difference to the use of certain antimicrobial drugs over time.

Materials and Methods: Treatment data were downloaded from the medical records of the clinic. Patient records covered the period from 1st January 2005 to 31st December 2019. All animals were either local to the Vienna city area (i.e. hobby/smallholder farms) or had been referred by their local veterinarian to the university clinic. Liveweight and age were not always available for all animals and were therefore estimated using established growth curves. Primary and secondary diagnoses entered into the hospital system were coded using a standardised diagnosis code system established for use on Austrian cattle farms. This enabled a division into diagnoses by organ system. Antimicrobial use was compared using milligram per kilogram metrics, and further divided by prudent use category as defined by the European Medicines Agency. Category A (avoid) antimicrobials were not included here as they are not licensed for use in veterinary medicine. Category B (restrict) refers to antimicrobials which are critically important for human medicine (such as 3rd/4th generation cephalosporins). Category C (caution) includes antimicrobials which should not be used as first choice treatments (such as aminopenicillins (e.g. amoxicillin & clavulanic acid), macrolides, and lincosamides); and Category D (prudent) includes

antimicrobials that may be used as a first line treatment in veterinary medicines (such as penicillins, tetracyclines, sulphonamide combinations etc.).

Results: A total of 2502 sheep and 2419 goat cases were treated with antimicrobials at the university referral clinic over the 15-year period. The most frequent diagnosis requiring antimicrobial treatment was for disorders of the urinary tract system (28% of all cases over all years), followed by treatment in combination with surgical procedures (17%) and gastrointestinal disorders (12%). Similarly, when divided by species, sheep were most likely to be treated for urinary tract disorders (29% of cases), in combination with surgical procedures (24%) and for respiratory or gastrointestinal disease (9% each); while goats were also most likely to require antimicrobial treatment for urinary tract conditions (28%), followed by gastrointestinal disorders (14%) and surgical procedures (9%). When antimicrobial use in mg/kg was analysed, it was determined that overall use had fallen from a maximum of 8.68 mg/kg in 2009 to a minimum of 3.38 mg/kg in 2018. By European Medicines Agency category, the most critical Category B antimicrobial use ranged from 4.08 mg/kg in 2019 to 0.75 mg/kg in 2014 (mean 2.08; median 2.04). While Category C (use with caution) antimicrobials ranged from 17.73 mg/kg in 2007 to 4.80 mg/kg in 2019 (mean 11.87; median 12.44) and Category D (prudent use) ranged from 13.03 mg/kg in 2015 to 3.92 mg/kg in 2018 (mean 7.92; median 7.83).

Conclusions: At the University Clinic for Ruminants in Vienna, the majority of small ruminants receiving antimicrobial treatments between 2005 and 2019 were suffering from urinary tract disorders. As a city-based referral clinic, sheep and goat patients were primarily pet animals from smallholdings, with a smaller number of valuable breeding animals from commercial herds. Overall antimicrobial use reduced over time, with a particularly noticeable decrease determined among antimicrobials in the European Medicines Agency's Category C (use with caution), such as aminopenicillins and macrolides.

Keywords: Antibiotics, treatment, prudent use.

PH-P17

Comparative assessment of antibiotic residues using HPLC-LC MS/MS and rapid screening test kits in colostrum and milk in postpartum Holstein dairy cows

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Objectives: The aim of this study was to compare residues levels and their differences according to the detection method, in order to determine the residual presence of antibiotics when using the Rilexine 500N™ drying tube (cephalexin benzathine 250mg/neomycin sulfate 250mg, Virbac Laboratories).

Material and Methods: The study was conducted in Tizayuca Hidalgo, Mexico; 23 animals at 230 days of gestation were randomized with an average of 45 + 5 days of the dry off period; milk and colostrum samples were taken postpartum at an interval of 12 hours (0 to 108h). Colostrum and milk concentrations were determined by a validated High-Performance Liquid Chromatography tandem mass spectrometry method (HPLC-LC MS/MS) and three commercial kits (Comet 4™, Trisensor™, Snap Beta ST Plus™). Method validation was performed according to the European Medicines Agency 2011 (Guideline on bioanalytical method validation) and the following parameters were evaluated: analytical curve, linearity, sensitivity, precision (intra- and inter-day repeatability), accuracy, and the limit of detection (LOD) and limit of quantification (LOQ). The method presented adequate linearity with correlation coefficients above 0.99 for both analytes in the dynamic range of 5–200 ng/mL of cephalexin and 75-3000 ng/mL of neomycin average accuracies between 84–110%. Method selectivity was verified by the absence of interfering peaks in the retention regions of the analytes and the internal standard when a blank sample was tested.

Results: Results of the analysis showed inferior cephalexin concentrations to the established lower limit of quantification (LLOQ). In the case of neomycin at 0h 65.21% positive with 263.108 ng/mL, 12h 65.21% positive with 205.76 ng/mL, 24h 21.74% positive with 142.114 ng/mL, 36h 4.35% positive with 86.15 ng/mL, 48h 4.35% positive with 90.11 ng/mL and after 60h 100% of the neomycin concentrations inferior to the established LLOQ was observed.

Comet 4™, 100% of the animals were negative from hour 0 in cephalexin and neomycin. Trisensor™ based on visual assessment, 8.7% of the animals were positive for β-lactams from 0 to 36h and 4.35% from 36 to 108h. Snap Beta ST Plus™ based on visual assessment, 100% of animals were negative for cephalexin from hour 0, however, 100% of the animals were positive for β-lactams, dividing into time intervals that remained positive (0-24h 13.04%, 0-48h 34.80%, 0-72h 26.08%, 0-96h 13.04% and 0-108h 13.04%). Comet 4™ detects concentrations from 50 ng/mL of Cephalexin and 200 ng/mL of Neomycin, which yielded negatives for both antibiotics in all samples; without detecting eight animals that in the first 24h had concentrations above 200 ng/mL of Neomycin. Trisensor™ detects concentrations from 700-800 ng/mL and showed three animals positive for β-lactams between 24h and 108h, which differs from what was obtained in the HPLC-LC MS/MS which was of 0 animals.

Conclusion: Considering the Maximum Residue Limits (MRLs) and Risk Management Recommendations (RMRS) for Residues of Veterinary Drugs in Food of the Codex Alimentarius (2018), which indicates 100 µg/kg for cephalexin benzathine and 1500 µg/kg for neomycin sulfate, it was determined that the animals that were positive for neomycin had a lower concentration, with 655.20 ng/mL being the highest concentration obtained, therefore there is no risk to human health. Nevertheless, when using the EMA WT software v.1.4 using the Safe Concentration per Milking Method (SCPM) and considering a tolerance limit of 95/95, a 12-hour milk withdrawal

is recommended. Snap Beta ST Plus™ gave positive results to β-lactams in 100% of the animals, observations made by Fernández-Molina D. *et al* 2012, suggest that somatic cells are capable of generating false positives; an important consideration, 100% of the animals were negative for cephalexin. We strongly recommend conducting more in-depth studies considering that colostrum and transition milk are high in total solids, thus being able to generate false positives in the Snap Beta ST Plus™ tests and determine the effect of the type of test reading (Visual vs SNAPshot Reader™), since, in Mexico, most of the establishments test results are based on visual assessment. It is important to consider that the golden test for evaluating antibiotic residues is HPLC-LC MS/MS, nevertheless, its high cost and difficult access complicates its use; giving the opportunity to the use of simple, rapid and qualitative methods of antibiotics detection, which can present important differences compared to HPLC-LC MS/MS.

Keywords: Cephalexin, neomycin, residues, milk, screening test.

PH-P18

Experimental Assessment of Copper Ion Treatment Efficacy against Penicillin G Contained in PBS and Milk

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Objectives: Antibiotics are widely used in animal production to treat bacterial infections and to improve animal performance, but their overuse has raised concerns related to public health due to the potential development of antibiotic-resistant microorganisms. Intramammary infections are one of the most frequent bacterial diseases in dairy cows and the main cause of antibiotic use in adult cattle[1]. Milk from cows with intramammary infections treated with antibiotics is called waste milk (WM) and includes milk contaminated with other types of drugs, milk from cows with clinical expression of intramammary infection, milk with high somatic cell counts and post-colostrum transition milk[2]. Waste milk causes significant economic losses for the dairy industry, because producers cannot market that milk for human consumption. Despite the above, this milk is widely used in raising calves[2], which represents a serious risk of infections transmission[3] to calves as well as the generation of bacterial strains resistant to antibiotics.

Several methods have been evaluated to degrade antibiotic residues in WM, however none of these methods have been used at a field level[2]. There is evidence about the efficacy of the electrochemical oxidation generated by metals to degrade antibiotics in waste milk[4]. As a contribution in this field, our research group has evaluated an antibacterial principle based on copper ions to decontaminate bovine milk mainly to control *Mycobacterium avium* sp. *paratuberculosis*, *S. aureus* and *E. coli* as bacterial models[5]. A subsequent study showed evidence that this antibacterial principle did not generate any negative effect on calve's health when this treated milk was intended for calf feeding[6]. The aim of this study was to evaluate the effect of copper ions on Penicillin G integrity in milk.

Material & Methods: To fulfill the proposed aim, an *in-vitro* experimental study was designed. Phosphate saline buffer (PBS) and UHT commercial milk were used as liquid matrices. Volumes of 500 mL were obtained from each matrix in which 20, 200 or 1,000 ppb of Potassium penicillin G (Merck KGaA, Darmstadt, Germany) were added. Each sample was treated for 30 minutes with a copper treatment device consisting of a glass receptacle in which two high purity copper plates were immersed. The copper plates were stimulated with a low voltage (24 V) electric current (3 Amperes) to release copper ions. PBS and milk samples without antibiotic were used as negative controls. In addition, pH controls were added using PBS and milk contaminated with 1,000 ppb of penicillin using a 30-minute incubation at pH 11.5 as treatment. All samples were tested for antibiotic detection before and after treatments with a commercial enzyme-linked receptor-binding assay, IDEXX SNAPduo* ST Plus Test (Idexx Laboratories Inc., Westbrook, ME). Additionally, pH, copper concentration and temperature were evaluated. Descriptive data analysis was performed using Excel 2016 (Microsoft Corp., Redmond, WA).

Results: In all PBS and milk samples where the three concentrations of penicillin (20, 200 and 1,000 ppb) were added, antibiotic residues could be detected before treatment with copper ions. However, after 30 minutes of treatment, no antibiotic residues were detected in any sample. Interestingly, when samples were incubated at an alkaline pH as treatment, antibiotics residues could be detected before and after treatment.

In the PBS the average pH was 8.06 (SD 0.06) in pretreated samples and 11.74 (SD 0.37) in post-treated samples. The average of copper concentration was 0.03 mg/L (SD 0.03) in pretreated samples, meanwhile 525.91 mg/L (SD 07.39) in post-treated samples. The average temperature was 20.80 °C (SD 1.28) in pre-treatment samples and 27.53 °C (SD 1.36) in post-treatment samples. In milk samples, the average pH was 6.58 (SD 0.03) in pre- treated samples and 9.10 (SD 0.159) in post-treated samples. The average copper concentration was 0.40 mg/L (SD 0.25) in pre-treated samples and 1,698.82 mg/L (SD 162.06) post-treated samples. The average temperature was 19.98 °C (SD 1.04) in pre-treated samples and 33.98 °C (SD 1.24) post- treated.

Conclusion: Treatment with copper ion on penicillin-contaminated milk and PBS samples potentially affects antibiotic integrity. Further trials with other antibiotic detection techniques and evaluation of their post-treatment biological activity are needed.

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Keywords: Dairy cows, waste milk, antibiotics, copper.

PH-P19

Prevent breakage of vials containing antibiotics with an innovative shock-absorbing silicone shell

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Objectives: The container of injectable pharmaceutical products must meet multiple and often antagonistic constraints. First, the primary packaging must preserve the integrity of the bottled product; in particular, it must not interact with the constituents of the drug. The packaging must strictly prevent the interaction between the external environment and the product and preserve the sterility of the drug if necessary. Then the packaging must prevent the user from coming into contact with the product and the drug from spreading into the environment. Glass, a millennial material, meets most of these requirements. However, it is fragile and manufacturers have therefore developed plastic-based packaging, capable of better absorbing shock and more suitable for veterinary use. These bottles made of organic materials are likely to interact with their contents and reduce the product's life. In addition, everyone should pay attention to the breakage of vials and accidental spillage of pharmaceuticals into the environment. Since 2015, the EU Commission has added certain macrolides to the watch list of contaminants of emerging risk to the environment. Recent work has shown that macrolides released into the environment can reach deep sediments and groundwater and persist there for up to ten years. Therefore, the objective of this work was to evaluate the relative resistance of a glass vial protected by a silicone shell and intended to receive a 10% solution of tulathromycin compared to a plastic reference vial.

Material and methods: An equal number (n=160) of two different types of vials for veterinary injectable pharmaceutical specialities were entrusted to a laboratory specializing in evaluating packaging (Metropack, Reims, France). The first one is a glass bottle (FL1) protected by a blue silicone soft shell at the shoulder and the bottom of the bottle marketed with a new drug (TULISSIN® 100 mg/mL, Virbac, France) and the other a multilayer bottle of plastic materials (FL2) already on the market (Clas® vial). An equal number of 250 and 500 mL bottles were tested. Three drop tests were carried out, on the bottom of the bottle (0°)(n=10, by type of bottle (glass/plastic) and size (250/500 mL)), with the bottle tilted (45°)(n=20/vial/size) and with the vial lying down on its side (90°)(n=10/vial/size). Each test was duplicated with two drop heights, h=800mm or h=1200mm. The results were photographed, and the relative risk (*RR*) and the confidence interval (95%CI) of breaking FL1 compared to FL2 were calculated.

Results: All drop conditions and packaging (250 and 500 mL) combined, the risk of breakage of FL1 vs FL2 was *RR*=0.480 (95%CI[0.312; 0.741], P<0.001); no FL1 of 250 mL (0/80) was broken against 20/80 for FL2 (*RR*=0.028, 95%CI[0.002; 0.397], P=0.009). Similarly, no FL1 of 500 mL (0/40) was broken up to h=800 mm, against 12/40 FL2 (*RR*=0.040, 95%CI[0.002; 0.654], P=0.024). At the height of h=1200 mm, an equivalent number of FL1 and FL2 were broken (24/40 vs 18/40, respectively, *RR*=1.333, 95%CI[0.871; 2.041], P=0.186). One can assume that a falling bottle generally hits the ground at a random angle, the expected value of which must be close to 45° (opening of the bottle upwards). Under these conditions, no FL1 was broken (0/80) while 23/40 FL2 were broken (*RR*=0.021, 95%CI[0.001;0.344], P=0.007).

Conclusion: In the event of an accidental fall, the antibiotic drug contained in the glass bottle protected by its silicone shell is significantly better protected than in the multi-layer plastic bottle. The glass also improves the life of the product. The glass bottle and its silicone soft shell are easily separable and recyclable.

Keywords: Antibiotic, packaging, environment, pollution, recycling.

PH-P20

Residues of Betalactam antibiotics in colostrum from dairy cows

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Objectives: Reports on the risk of antimicrobial residues in colostrum of dairy cows after dry cow treatment (DCT) are rare in the scientific literature. One reason could be that the specificity of antibiotic residue screening tests is poor (Andrew, 2001), so that advanced and expensive laboratory methods are required. The aim of the present study was therefore to identify the risk of residues of antimicrobial substances in first milking colostrum of dairy cows after dry cow treatment.

Materials and methods: Colostrum samples of the first milking after parturition from 286 cows on 30 farms were analysed by liquid chromatography–mass spectrometry (LC–MS) confirmation for the presence of _-lactam antimicrobials. According to the history obtained from the owner 200 cows underwent dry cow treatment, 79 did not; in 7 cases it was not known.

Results: Residues of Betalactam antimicrobials were found in 77 samples of cows treated with DCT, in 7 samples of cows not treated with DCT and in one sample were treatment was not known. Cloxacillin was found in 49 samples, in 34 (69 %) samples the concentration was above the MRL (30 µg/kg). DCT containing cloxacillin was used in 88 of the sampled cows, whereby four different commercial products were utilized (product: pos/neg): Cloxacillin – TS - 1000: 1/8; Cloxin – TS – Retard: 1/5; Orbenin Extra: 42/9; Wedeclox TS: 1/21. Other findings were: benzyl penicillin (n=8); amoxicillin (n=3);

cephalosporins (n=9). In all but two cases where benzyl penicillin was found, the substance found did not correspond to the DCT used but were rather due to systemic treatment prior to parturition.

Conclusions: The presented study shows that residues of _-lactam antimicrobials are rarely found in first milking colostrum of dairy cows. An exception constitutes the use of Orbenin Extra (Zoetis Deutschland GmbH), a preparation containing cloxacillin which is specifically composed for long lasting presence and action in the udder after treatment. Therefore, colostrum harvested from cows treated accordingly bears a high risk of containing cloxacillin in a concentration above the MRL for milk. A weakness of this study is that the duration of the dry period is not known for individual cows and that only lactam antimicrobials were tested.

Reference:

Andrew S. M., 2001. Effect of Composition of Colostrum and Transition Milk from Holstein Heifers on Specificity Rates of Antibiotic Residue Tests. J. Dairy Sci. 84:100–106.

Keywords: Antibiotic residues, colostrum.

PH-P21

Study of antimicrobial resistance during the calf rearing period in dairy farms

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Objectives: To determine antimicrobial (AM) resistance patterns of *E. coli* from dairy calves and their environment during the rearing period.

Materials and Methods: Six commercial dairy farms located in Buenos Aires, Argentina, were each visited twice, once during winter (June-August 2021) and once during spring (September-December 2021). Rectal swabs were obtained from healthy and no AM-treated calves at different ages (<3 days [d] old, n=2±1 [calves0], 25±10 d old, n=4±1 [calves1] and 55±10 d old, n=4±1 [calves2]). Environmental samples were collected from the calving and calf-rearing areas. In addition, milk, water, calf starter and pooled samples from feeding and milking buckets were also obtained. A total of 13 samples from calves and 7 samples from the environment were taken per farm per visit. Samples were delivered in a cooling container within 24 h of sampling to the Pharmacology and Toxicology Laboratory (LEFyT-FCV, UNLP). Each sample was cultured on 6 plates with chromogenic medium (CHROMagar™ Orientation) which had one of 6 AMs added (ampicillin, cefotaxime, ciprofloxacin, chloramphenicol, gentamicin and oxytetracycline). Plates were incubated at 37ºC for 24 h. All colonies that presented pink to reddish coloration were considered to be *E. coli*. A generalized linear model (GLM) with a binomial distribution was fitted to assess differences AM-resistant *E. coli* across the fixed effects of season (i.e., winter, spring), environmental samples (i.e., milk, walter, calf-starter, feeding, milking-buckets), areas of farm areas (i.e., calving, calf-rearing) and calves ages (i.e., calves0, calves1, calves2) using GLM and ANOVA.GLM in RStudio.

Results: A total of 234 samples were taken during the study. Ampicillin-resistant *E. coli* were isolated from 69.2% (162/234) of samples. In contrast, *E. coli* resistant to oxytetracycline, ciprofloxacin, chloramphenicol, cefotaxime, and gentamicin were isolated from 59.4% (139/234), 44.4% (104/234), 32.9% (77/234), 24.4% (57/234) and 16.2% (38/234) of samples, respectively. No AM-resistant *E. coli* was isolated from the calf starter. A low percentage of AM-resistant *E. coli* (to at least one antimicrobial) was found in feeding buckets (16.7% [2/12]), followed by water (33.3% [4/12]). The highest percentages of AM-resistant *E. coli* were found in calving area and calf-rearing area samples (100% [12/12], 100% [12/12]), followed by fecal samples (calves0 64.7% [22/34], calves1 96.6% [56/58] and calves2 86.8% [46/53]). Of resistant *E. coli* samples isolated, 69.2% (117/169) were resistant to ≥3 AMs; these were found most frequently in milk (100% [5/5]), calf-rearing areas (100% [12/12]), and fecal samples of the youngest calves (calves0 86.4% [19/22] and calves1 80% [44/55]). Out of total samples, 25.4% (43/169) were resistant to ≥5 AMs, the most frequent being found in samples from the calf-rearing areas (75% [9/12]) and milk (60% [3/5]). The most common AM resistance patterns were to ampicillin and oxytetracycline. There was a higher odd of isolating ampicillin and oxytetracycline-resistant *E. coli* from calves1 than from calves0 and calves2 (OR=6.4, 95%CI=1.9-21.1, *P*=0.002; OR=4.2, 95%CI=1.5-11.9, *P*=0.03, respectively) and there was a tendency to increase the odds to isolate chloramphenicol-resistant *E. coli* from calves1 than the others (OR=2.0, 95%CI=2.0-3.05, *P*=0.07). On the other hand, there was a lower odd to isolate ampicillin and oxytetracycline-resistant *E. coli* from calves during spring than during winter (OR=0.28, 95%CI=0.11-0.71, *P*=0.007; and OR=0.31, 95%CI=0.14-0.70, *P*=0.004; respectively). Furthermore, there was a higher odd of cefotaxime-resistant *E. coli* isolation from the calf-rearing area (OR=15.4, 95%CI=1.5-160.9, *P*=0.02) and milk (OR=13.75, 95%CI=1.2-156.6, *P*=0.03), and a higher odds of ciprofloxacin-resistant *E. coli* isolation from the calf-rearing area (OR=121, 95%CI=6.7-218.8, *P*=0.001) than from the rest of the samples.

Conclusions: The prevalence of AM-resistant *E. coli* was high in dairy calf-rearing areas as well as in fecal samples from calves that had never received antimicrobial therapy. Transmission of AM resistance to newborn calves through milk seems likely. In addition, the highest prevalence of resistance in *E. coli* was to ampicillin, an antimicrobial commonly used on dairy farms in Argentina for mastitis treatment. Finally, there were associations among the prevalence of *E. coli* resistance and the age of calves as well as differences in resistance between winter and spring. Recognizing these critical points will help develop strategies to reduce AM resistance during calf-rearing on dairy farms.

Keywords: Calves, environment, E. coli, resistance.

PH-P22

Prevalence of multidrug resistance of *Escherichia coli* in the immediate calves' environment on Swiss dairy farms

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Objective: The calves' housing environment is proposed as being one of the sources of colonization of calves' gut by antimicrobial-resistant bacteria, but there is limited data on the role of this reservoir, especially on small-scale dairy farms. Therefore, the aims of this study were to describe the prevalence of antimicrobial resistance (AMR) and multidrug resistance (MDR) of *Escherichia coli* isolated from preweaned calves' housing environment on Swiss dairy farms, and to explore associated factors.

Materials and Methods: In 60 farms, 2 environmental swabs were taken from the walls and/or railings of the pens or hutches housing 30 to 60-day-old calves. The swabs were incubated in Mueller-Hinton broth at 37°C overnight, then a loopful of the broth was used to inoculate *E. coli* selective media (BROLAC). Two colonies were selected from each culture (4 per farm, 240 isolates in total), identified by Maldi Tof, and tested for antibiotic susceptibility against a panel of 15 antibiotics by broth microdilution. Minimum inhibitory concentrations were interpreted based on EUCAST threshold values. Multidrug resistance was defined as resistance to at least three antimicrobial drugs. Farm characteristics and calf management practices were recorded by use of a questionnaire.

Results: At least one isolate exhibited AMR and MDR in 39 and 30 of the 60 farms, respectively. Among all isolates, 62% were pan-susceptible, whereas the remaining isolates showed resistance to 1 (2.9%), 2 (7.1%), 3 (8.8%), 4 (12.9%), 5 (3.8%), 6 (2.1%), or 7 (1.3%) antimicrobials. Therefore, 29% of all isolates were exhibiting MDR. The most common resistances were to sulfamethoxazole (44.6%), tetracycline (35.4%), ampicillin (29.2%), trimethoprim (21.7%), and chloramphenicol (19.2%). Among resistant isolates, AMR against 4 different antimicrobials was the most common observation, with the most prevalent pattern (15/69, 21.7%) being AMR to ampicillin, tetracycline, trimethoprim, and sulfamethoxazole. Calf management practices such as housing type or the feeding of calves with milk containing antimicrobial residues were not associated with MDR in calves' environment.

Conclusions: Our results show that AMR is a common observation in calves' environment on Swiss dairy farms, with MDR isolates having been found on half of the participating farms.

The associations between farm management practices, AMR in calves' environment, as well as inoculation by and carriage of resistant *E. coli* in young dairy calves need to be investigated more in-depth in future studies.

Keywords: Antimicrobial resistance, calf, environment.

PH-P28

Revealing the resistome of dairy cattle in a heavy metal contaminated area using shotgun metagenomics

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The aim of this research was to evaluate the antimicrobial resistance profile of dairy cattle in a heavy metal contaminated area, using shotgun-sequencing technology. Thirty-two dairy cows from two different farms located in the same state were enrolled in this study. Farm A was located in a heavy metal contaminated area (N=16 cows) and farm B in a non-contaminated area (N=16 cow), both located within 526km of each other, in Minas Gerais, Brazil. Fecal and deep nasopharyngeal swabs and rumen fluid were collected from each cow, and kept in liquid nitrogen until processing. DNA extraction was performed using the *MagMAX™ CORE Nucleic Acid Purification Kit.* Samples were sequenced using shotgun whole metagenome sequencing (Illumina NovaSeq 6000 sequencing at 2x150 bp). The demultiplexed Fastq files were uploaded in the Metagenome Rapid Annotation Subsystem Technology (MG-RAST), to determine the abundance of the antimicrobial resistance genes. The normality of the data was accessed using Shapiro-Wilk test. Variances were analyzed using Bartlett's test. The relative abundances of each resistance profile were compared using t-test or Wilcoxon test. All calculations were performed using R software, and variables with *P*-value < 0.05 were considered significant. Overall, the analysis of antibiotic resistance genes revealed five predominant profiles: resistance to fluoroquinolones, beta-lactamases, erythromycin resistance, resistance to vancomycin, and methicillin-resistance in Staphylococci (MRS). The heavy metal resistance profile analysis revealed cobalt-zinc-cadmium (czc) resistance, arsenic resistance, resistance to chromium compounds, mercury resistance operon, zinc resistance, cadmium resistance and multidrug resistance efflux pumps (MREP). Regarding sample type and farm location, antimicrobial resistance analysis revealed that most profiles were increased in farm A (affected by heavy metal environmental contamination) in all three sample types. Czc resistance (P = 0.002), MREP (P < 0.001) and the mdtABCD multidrug resistance cluster (P < 0.001) in fecal samples. Beta-lactamase (P = 0.003) and MRS (P = 0.02) in the rumen fluid, and MREP (P = 0.005) in deep nasopharyngeal swab samples. On the other hand, resistance to fluoroquinolones (P = 0.006) and czc resistance (P = 0.03) were increased in farm B when found in fecal swab and rumen fluid, respectively. Differences in the antimicrobial resistance profile were perceived especially in the contaminated area (farm A). We demonstrate here that the presence of both antibiotic and heavy metal resistance profiles may be evidence of a co-resistance enrichment for antimicrobial and heavy metal resistance genes, as their higher abundance in farm A may be related

to the heavy metal environmental contamination. New studies in similar environments are mandatory to confirm that heavy metal environmental accidents are linked to rapid spread of antimicrobial resistant bacteria.

Keywords: Heavy metal, Antimicrobial resistance genes, Sequencing.

PH-P29

Phenotypic antimicrobial resistance in *Escherichia coli* strains on clinical and non-clinical isolates from cattle in Germany and France

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Objetives: The increase of antimicrobial resistance (AMR) is a global public health concern. In animal husbandry, prevalence of resistant isolates is typically highest in young animals. In this study, *E. coli* resistance data on clinical and non-clinical isolates from calves and dairy cows are compared.

Material & Methods: Antimicrobial susceptibility testing (AST) data from 2014 to 2017 based on the microdilution method were collected from clinical *E.coli* isolates from Germany and from non-clinical *E.coli* isolates from Germany and France. French AST data from clinical isolates were gathered as disc-diffusion diameters (DD). Non-clinical isolates were collected from the German Zoonosis-Monitoring program and the ANSES database. Data on clinical isolates were collected from the German National Antibiotic Resistance Monitoring system (GERM-Vet) and from RESAPATH (France) covering clinical isolates from mastitis samples and isolates from young cattle with diarrhea. Antimicrobial panels included were based on antimicrobial agent overlaps between both system types, i.e, clinical and non-clinical, within countries.

Results: Antimicrobial panel of Germany showed an overlap on 7 seven antimicrobials (ampicillin, ciprofloxacin, cefotaxime, nalidixic acid, gentamicin, tetracycline and colistin). French prevalence levels on clinical and non-clinical isolates were described but not compared due to lack of laboratory protocol harmonization.

Conclusion: Data from clinical and non-clinical isolates indicate differences in AMR between populations and between clinical and non-clinical isolates. Preliminary results show that the level of resistance is higher in clinical isolates compared to non-clinical isolates. Factors contributing to these differences need to be investigated.

Keywords: AMR, calf, dairy cattl.

PH-P30

Milk, The only Superfood I know

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After several years or even centuries in which nobody doubted that milk was the best and most accessible food for humans. Lately a large number of detractors have emerged and are leading a change of attitude. We didn't have to worry but also, we had to defend many qualities and benefits of milk. We needn't have to advertise those advantages, but the constant and successive attacks force us not only to defend ourselves, but also to be proactive.

I say milk is the unique available superfood and has a lot of nutritional and economics advantages in comparison to the new vegetable drinks.

We define superfood. It is a compound noun. food.1. Set of products that people and animals eat or drink to continue living.2. Each of the substances that a living being takes for performing the nutrition function. super-.1. It means "over" "on top of ".2. It also means preeminence and excellence. Superfood: substance which is taken by the living beings for their nutrition and stands out for their preeminence and excellence. Could it be milk?

Milk, RAE . 1. white liquid produced by the mammary gland of mammals for feeding their babies. RD 1679/1994: product secreted from the mammary gland of domestic animals. JMF: the only superfood which is capable by itself of keeping people and animals alive and guaranteeing healthy growth, in the most vulnerable stage of their lives. Apart from its nutritional qualities, offers Food Safety levels.

The milk products reaches the market undergoes several tests: microbiological, counting of somatic cells, aflatoxins, inhibitors, pollutants and adulterations, etc.

Analysis of each collecting, from the tanker before the unloading. In the reception tank, pasteurized tanks, the intermediate and final product.

In what concerns to its composition, in Spain we produce milk that has a low content in fat and proteins. If we want to compete in the international market, we have to make an effort to get closer to the European standards and to the main exporter countries. The current percentage of fat (3,73 %) and proteins (3,30 %) are far from the European average (4,09 % and 3,43%) and too far from the percentages in New Zealand (4,75 % y 3,87%).

We have a superfood with huge food safety guarantees whit an avalanche of detractors, continuous attacks and an increasing campaign of different vegetable drinks.

Is it fashionable? Are there unacknowledged interests?

Let's compare the nutritional value and the price of milk with the new fashionable drinks that I call "mixture" "concoction" 1. Drink, especially the one composed of unpleasant ingredients to the palate.; From potion *apocima*. 1. Medicinal cooking of vegetal materials.2. Medicinal drink.3. Unpleasant liquid to drink.

If we compare these "mixtures" to semi-skimmed milk, which has no additives, vegetable drinks have no less than

four additives. Soy provides similar calories, fats and proteins. It is 33% more expensive. Coconut is water at a price of 298% higher than milk price. Oatmeal has the same number of calories, half of the fat, 60% more carbohydrates, 60% less protein and a price which is 298% higher. Rice has zero % proteins, similar calories, double carbohydrates, 40% less fats and its price is 298% higher. Almonds, have a few more calories, 30% more fats, 40% more carbohydrates, a quarter of proteins and a price 233% higher. The tiger nut milk "Spanish Horchata" has 50% more fats, 30% less carbohydrates, 80% less proteins and a price which is 225%.

On top of all these, we can add that, if calcium is not added, the contribution of this mineral salt is insignificant.

Not less important. There is a significant difference in calcium bioavailability and protein digestibility.

Finally, does anyone like these products?

Milk is the most complete food that exists, and the product which has undergone the most exhaustive quality controls, from the origin to the final consumer.

All the vegetable drinks that are sold to us as healthier, are nutritionally far from milk, they have been produced industrially through ultra-processing. They have included a great number of additives and are much more expensive for consumers. Is it a good deal for manufacturers? Is it a bargain for marketers or a scam for consumers?

Keywords: Milk Superfood Quality Controls Health.

PT-P01

Assessment of a new injectable tolfenamic acid formulation

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Objectives: Tolfenamic acid (TA) is a non-steroidal anti-inflammatory drug (NSAID) with proven antipyretic and analgesic properties, registered in Europe as a 4% injectable solution (Tolfine®/Tolfedine®CS) in cattle and pigs. A higher concentrated formulation (Tolfine®8%) was developed because of the interest in reducing the administration volume, while maintaining the same active dosing. Apart from slight excipient variations, both formulations retain the same active ingredient. Per GLP and validated procedures, trials were conducted to produce a registration docket for market authorisation review by relevant regulatory bodies. This paper conveys the results of three different trials. First, bioequivalence of TA between both formulations. Second, the activity over time profile of plasma creatine kinase (CK), a reliable biomarker of tissue damage at the injection site. Third, the assessment of local tolerance of the 8% formulation.

Material & methods: First trial, the bioequivalence between both TA formulations (reference 4%, test 8%), conducted in ruminant cattle (n=32 males) between 205 and 295kg. Both formulations were administered in 2 periods, 2 sequences in a cross-over design, with a 2 week wash-out from a single intramuscular injection (2mg/kg). After injection, blood samples were taken at different time points and TA plasma analysis was performed by a validated HPLC-UV method. The individual concentration-time profiles were submitted to a non compartmental pharmacokinetic (PK) analysis. AUC_{last} and C_{max} were then submitted for analysis. The second trial, also a cross-over design, was conducted in ruminant cattle (n=8), 2 males, 6 females, between 358 and 454kg. Blood samples were taken at different time points and plasma creatine kinase (CK) activity was evaluated by spectrophotometric method, using an automatic analyzer according to IFCC standards. Individual CK activity-time profiles were submitted to a non compartmental PK analysis to calculate AUC_{last} values, then translated into equivalent damaged muscle tissue weight using a published method. A third trial assessed local tolerance with the 8% formulation, with 2 IM injections administered 48 hours apart, each side of the neck (left first, right second), at 2mg/kg, in heifers (n=20), between 503 and 688kg. Observations were done within 5 minutes of injections using a scoring scale; (0) no reaction, (1) skin quiver and/or muscle contraction, (2) animal tries to scratch or lick its neck, (3) animal has "defensive reaction" during injection and displays major discomfort.

Results: First trial, bioequivalence was reached with both PK parameters. The calculated confidence intervals of the ratio AUC_{last} and C_{max} obtained with the 8% solution, divided by the values obtained with the 4% solution, were included within the priori interval of [0.80 - 1.25]. The mean AUC_{last} values were 28.66µg.h/ml and 27.23µg.h/ml, after injection of the 8% and 4% solutions respectively. The mean C_{max} values were 1.77µg/ml and 1.95µg/ml after injection of the 8% and 4% solutions respectively. Second trial, the mean equivalent damaged muscle weight after injection of the 8% solution was 12.64g (sd=6.57g) and it was 23.99g (sd=7.95g) after injection of the 4% solution. Third trial, the assessment of local tolerance with the 8% solution, score observations were for the left side of the neck, n=18 at (0), n=2 at (1), n=0 at (2), n=0 at (3). For the second injection 48 hours later, right side of the neck, n=13 at (0), n=4 at (1), n=3 at (2), n=0 at (3).

Conclusion: Bioequivalence between the 8% (Tolfine®8%) and 4% (Tolfine®/Tolfedine®CS) formulations was clearly demonstrated, and therefore well-established therapeutic efficacy of TA as a 4% formulation is also confirmed with the 8% formulation. The analysis of the CK plasma activity-time profiles showed that the muscular tolerance of the 8% formulation was also enhanced. The lower amount of damaged muscle tissue with the 8% formulation is directly linked to the lower injection volume. Considering the results from the injection site tolerance observations, the 8% formulation (Tolfine®8%) demonstrated good local tolerance in most animals, with only a few displaying mild swelling at palpation, on the day of administration.

Keywords: Tolfenamic acid, bioequivalence, creatine kinase.

PT-P02

Exploring the use of complementary and alternative approaches to health care on UK dairy farms

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Objectives: Evidence suggests that some farmers use complementary and alternative medicine (CAM) to manage a range of conditions in dairy cows. Exploration of how CAM is viewed, used, and understood by dairy farmers may provide valuable insight since there is little research to explore the current use of CAM on farms or its impact in reducing antibiotic use. This is something which warrants further consideration for two main reasons; (1) to determine if CAM use has potential to reduce unnecessary antibiotic use and support the global efforts against antimicrobial resistance, (2) to ensure that antimicrobials and other conventional treatment approaches are used where appropriate. This is not only important to avoid unnecessary suffering, but also to ensure that responsible use of antibiotics still occurs whereby farmers use *'as little as possible, but as much as necessary'* (RUMA, 2009).

Materials and methods: A heterogeneous purposive sample of 20 farms with a range of management systems, herd sizes and production goals were recruited to this study. Interviews were conducted with 24 farmers through a mixture of face-to-face, telephone and videoconferencing modalities necessitated by movement restrictions during the Covid-19 pandemic. In addition, 16 farms were visited to collect observational data using ethnographic fieldnotes and photographs. Interviews were conducted using a topic guide and explored participants' experience of CAM including drivers/barriers to

CAM use, experiences of CAM use and how CAM might influence the use of conventional medicines such as antibiotics. Observation visits involved collecting data on conventional and CAM medicine storage, CAM usage and farm management systems. Interviews were audio recorded, transcribed and analysed using NViVo computer software. Thematic analysis was employed to identify key themes in the data. Ethnographic fieldnotes and photographs were incorporated into this data analysis reflexively and iteratively.

Results: Data indicate several drivers for UK dairy farmers to use CAM approaches, including their own personal [or friends' and relations'] experiences, the views of influential people and advisors, networks within the farming community and the fact that CAM use allows a greater sense of autonomy in health-based decision making. Farmers often refer to milk buyers and organic guidelines as factors which influence their use of CAM. They further refer to a desire to 'do something' for the animal and to minimise animal welfare related concerns. A range of CAM information sources were also consulted by farmers including, holistic health management organisations/ courses, online materials, and pharmacies. Participating farmers associate the use of CAM approaches with other holistic health management practices, human-animal interactions, the actual character and physical characteristics of an animal and animal welfare. This indicates that CAM use is seen by farmers as part of a wider ethos and belief about holistic farming practices and land use. Additionally, data implies that some farmers value their positive personal experiences of CAM use over scientific evidence. In contrast, barriers to CAM use were also identified including: the perception that CAM approaches are reserved specifically for organic systems, little access to CAM and related resources and some existing tensions between farmers and other stakeholders' views. Data suggest that farmers' use of CAM is influenced by a range of individuals within the sector including veterinary surgeons (some of whom practice or prescribe CAM), other farmers, mainstream farming press and pro-CAM organisations and advisors.

Conclusions: Farmers continue to use complementary and alternative approaches to health care, and the conceptualisation of CAM is complex and subjective. A range of sources and stakeholders are consulted by farmers to understand herd health approaches including CAM or mainstream healthcare practices. It is proposed that the use of such approaches would benefit from being acknowledged and discussed between all stakeholders, including vets. It may be that by encouraging more open discussion surrounding the use of CAM on dairy farms, we are able to identify ways in which dairy farmers can reduce their antimicrobial use in a responsible way, with the support of their vets and other stakeholders. These findings provide evidence to support further exploration of CAM use on dairy farms, and how these practices might influence antibiotic use.

Reference:

RUMA (2009) RUMA promotes 'as little as possible, but as much as necessary' Antibiotic use, for the good of Animal Health and Welfare https://www.ruma.org.uk/ruma-promotes-little-possible-much-necessary-antibiotic-use-good-animal-health-welfare/ accessed 03/06/202

Keywords: Complementary Alternative Medicine Antimicrobials.

PT-P03

Acceptability of a new dose regimen for an oral solution of paromomycin after its dilution in milk or milk replacer in newborn calves

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Objective: Cryptosporidiosis is a frequent parasitological infection of neonatal ruminants. In calves, cryptosporidiosis is characterised by diarrhoea, dehydration, delayed growth, and weight loss, resulting in considerable economic losses. Current treatment options for clinically affected newborn calves are limited. Paromomycin is considered as a valuable option and recently a new dose regimen has shown great results to cure calves sick from cryptosporidiosis and to control their oocyst burden (Achard et al., 2022, under review). This new dose regimen consists of 150 mg/kg of paromomycin sulfate (Gabbrovet Multi®, Ceva Santé Animale) to be administered daily to newborn calves for 5 days via oral route. Generally, oral administration is performed with a syringe or after dilution in milk or milk replacer. To ensure that young calves would accept this new dosage when mixed with milk or milk replacer, a palatability study was performed with Gabbrovet Solution® (Ceva Santé Animale) as a positive comparator. Gabbrovet Solution® is indicated for colibacillosis and indeed frequently used diluted in milk or milk replacer with no impact on feed intake at the maximum dosage of 50 mg/kg/day for 5 days (data on registration dossier).

Material and methods: A GCP compliant study was performed in 30 newborn calves according to a blinded, randomized, and positive controlled design. At arrival at the investigational site, the animals were randomized into two feed regimens with age and body weight as blocking factors. One feed regimen consisted of fresh whole milk (WM) and the other of milk replacer (MR) throughout the study. At day 0, newborn calves aged between 4-14 days were enrolled if they present with no apparent sign of disease, and a normal feeding behavior/appetite. Any calf with diarrhoea for > 24 hours or elevated rectal temperature (above 40°C) was excluded. On day 0, calves in each feeding group were randomly allocated to three study groups. Calves in group A (N=12) received 150 mg paromomycin sulfate/ kg b.w. (Gabbrovet Multi®), once daily, for 5 days diluted in 2L of WM or MR (morning feeding), calves in group B (N=12) received 50 mg paromomycin sulfate/ kg b.w. (Gabbrovet Solution®), once daily, for 5 days diluted in 2L of WM or MR (morning feeding), while sentinel calves in group C (N=6) received the same amount of WM or MR (2L) without any supplementation. The following acceptability and safety parameters were monitored during the 5 days of the study: feed intake, duration of the meal, health status. Comparison of the morning feed intake of calves for five minutes after meal distribution into bucket between calves in group A and B was the primary criteria to evaluate palatability. More precisely, the quantity in kg of WM or MR drunk by the calf was calculated by the difference of the amount distributed and the leftover amount and compared from day 1 to day 5 between the two groups. Effect of food type (WM or MR) on acceptability, duration of the meal, feed intake by study day were considered as sec-

ondary criteria. Other criteria such as physical examinations and adverse events were also assessed.

Results: Overall, intake values were close to 2L for all calves. Mean feed intake over the treatment period was 1948 ± 205 g in group A, 1902 ± 309 g in group B and 1997 ± 18 g in group C. Complete/uncomplete feed intake was found to be similar in group A and B according to a generalized mixed model (p = 0.0752). By study day, similar values were observed regarding meal duration scores and feed intakes, which highlights a regular acceptability over the treatment period. Calves managed to finish their supplemented meal within 5 minutes for 91.7 % of calves group A and 81.8% in group B in the whole D1-D5 period. Effect of the diluent (WM or MR) was not significant. This mode of administration was well tolerated as no adverse event were recorded to be related to the use of the product.

Conclusion: In this experimental study, daily oral treatment with 150 mg/kg of paromomycin (Gabbrovet Multi®, Ceva Santé Animale) for 5 days was found to be safe and very well accepted by newborn calves when diluted in whole milk or milk replacer for their morning meal.

Keywords: Newborn calves, cryptosporidiosis, treatment, paromomycin.

PT-P04

Germicidal activity of a combination of *Melaleuca armillaris* and *Laurus nobilis* essential oils for teat dip against *Staphylococcus aureus* strains isolated from bovine mastitis

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Objective: To evaluate and develop a phytotherapeutic topical formulation alternative to commonly used disinfectants by combining essential oils (EOs) from *M. armillaris* and *L. nobilis* as a pre and/or post-dipping solution.

Materials and methods: The EOs of *M. armillaris* and *L. nobilis* were obtained from plants located in Coronel Brandsen, Buenos Aires, Argentina by steam distillation. Minimum inhibitory concentration (MIC) by microdilution method and minimum bactericidal concentration (MBC) of each EO against 3 wild isolates and *S. aureus* ATCC 29213 (control strain) were determined. Then, the checkerboard test was carried out to evaluate the synergism between both EOs. They were combined using the range of 50-0.09 µL/mL for each one. The inhibitory fractional concentration index (CFI) was calculated [synergism (S) if CFI ≤ 0.5, partial synergism (SP) if 0.5 < CFI < 1, indifference (I) if 1 ≤ CFI < 2 and antagonism (A) when CFI ≥ 2]. After analyzing the results, it was decided to use a combination consisting of 0.6% of both EOs for the development of the experimental formulation. Two formulations containing different amounts of distilled water and ethyl alcohol were prepared in order to establish their antimicrobial capacity against *S. aureus*. Distilled water or ethyl alcohol without the addition of EOs were used as control solutions. 5 mL of each formulation were confronted with an inoculum of 10^7 CFU/mL of *S. aureus* ATCC 29213. 10 µL were taken at two different times (30 and 120 sec.), which were plated on nutrient agar to observe later the presence or absence of bacterial growth. A drop (10 µL) of the inoculum was seeded simultaneously as positive growth control. The formulation with the mixture of EOs that demonstrated greater antimicrobial activity in less time was selected. Its efficacy was evaluated by the counting technique using excised teats from cows, obtained from a commercial abattoir. The inoculum of *S. aureus* ATCC 29213 was adjusted in physiological solution (PS) to match a 0.5 McFarland standard. For the germicidal power evaluation of the sanitizing solution, 20 teats divided into 4 groups were used according to the subsequent treatment: (i) control without treatment, (ii) sterile PS, (iii) formulation with 0.6% EOs each (1x), and (iv) formulation with EOs at 1.2% each (2x). The teats were hung on a horizontal rod and then all were immersed in nutrient broth with the *S. aureus* inoculum for 10 sec. It was allowed to drain for 5 min and later each group of teats was treated as previously mentioned. After 30 sec., a wash was performed for 20 sec. with a sterile PS to carry out a subsequent bacterial count. Wash solutions were diluted from 1:10 to 1:100.000 (10^{-5}). 10 µL of the serial dilutions were seeded in triplicate (count by drops on a nutrient agar plate). Statistical analysis was performed using a Bayesian mean comparison analysis with a 95% confidence interval using the Epidat 3.1 statistical program.

Results: The MIC against wild isolates was the same for both EOs (25 µL/mL), which showed synergism with each other when combined, observing CFI values ≤0.5. The CBM of both EOs was 50 µL/mL, that is, the CBM/CIM ratio for both EOs was equal to 2 (bactericidal effect). The mixture of 6 µL/mL (0.6%) of both EOs was selected for the elaboration of an experimental formulation. The formulation that contained the lowest proportion of ethanol and the highest proportion of distilled water presented bactericidal activity against *S. aureus* because of the EOs activity, so it was selected to continue the study. A reduction of 2.88 Log_{10} CFU/mL was obtained using the EOs at a concentration of 0.6% each, and by doubling the concentration of the EOs (1.2% of each). The reduction compared to the control was 3.03 logarithms. The use of EOs produced a significant decrease in the bacterial load compared to washing with PS ($p<0.05$).

Conclusions: The combination of *M. armillaris* and *L. nobilis* EOs was synergistic against *S. aureus*, constituting an interesting phytotherapeutic alternative for the disinfection of bovine teats in dairy farms. This was observed for both inhibitory and bactericidal antimicrobial activity.

Keywords: Phytotherapy, Teat dip, Melaleuca armillaris, Laurus nobilis, Staphylococcus aureus.

RB-P01

Biological efficiency the Nelore cows in a grazing system of the Bolivian tropics

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Understanding the factors that affect the biological efficiency of beef cows in grazing systems would be an important step in the identification of the most adapted genotypes. The objective of the study to evaluate the biological efficiency in Nelore cows in a grazing system of the Bolivian tropics. For the research study, retrospective data were used corresponding to the period between 1998 and 2018, belonging to the Centro Tecnológico Agropecuario en Bolivia (CETABOL) in Colonia Okinawa. (17° 13′ 12″ of south latitude, 62° 53′ 39″ of west longitude). The Japanese community is located at 257 m.a.s.l. and it has a tropical climate, with significant rains in most months of the year and a short dry season with little effect on the general climate. The average annual temperature is 24.3 ° C with average rainfall of 986 mm. The data corresponding to 900 Nelore cows, primiparous and multiparous, were used, with a total of 3734 calving cows with records covering all their productive life. The feeding of the rodeo was grazing managed under intensive conditions, on 229 hectares with cultivated pastures *Brachiaria brizantha* and *Brachiaria decumbens* occupying 80% of the fodder offered. The first artificial insemination (AI) is performed to the cows in October, then the second AI in early December. At the end of December, a natural service is made to cows that are still empty. The cows were characterized according to the values of the following variables: Longevity (L) in days: Discard date or death - date of birth, Total weight of weaned calf (WWC) in kg: Σ of weights at weaning of each cow), Calf Index (CI) in kg/days: Total production of weaned calf / longevity (adapted by Marini y Oyarzabal, 2002 a&b), Number of calving (NC): The number of births (Σ of births of each cow). Average and standard deviations for L, WWC and CI, and medians and ranges for NC were obtained. In addition, WWC scatter plot was performed in CI. Then, several regression models were adjusted and a quadratic polinomial regression model was chosen, since it was the one that provided the best fit to describe the functional relationship of the variables. The average and standard deviation of WWC (772 ± 639 kg), L (2403 ± 1244 days), CI (0.277 ± 0.121 kg / day), the median of the NC was 3 and a range of (1-14) which It means a minimum annual replacement of 33%. The chosen models had an adjusted R2 0.84 (p≤ 0.001). As WWC increases, the IC is also increased to 1500 kg, growth continues with less slope up to 2250 kg, and then as the WWC increases, the ratio changes and the CI values begin to decrease. This is suggesting a quadratic relationship between both variables. These results demonstrate that a higher production of weaned calf at the end of the cow's productive life, would not always guarantee a better productive efficiency. In these productive systems based on direct grazing there would be a limit on the expected production of a cow above which it would resent and generate the opposite effect. It is concluded that it is possible to use Nelore cow biological efficiency indicators such as the CI, showing that higher production does not always guarantee greater efficiency.

Keywords: Biological efficiency, Nelore cows, Grazing system.

RB-P02

Analysis of different reproductive parameters in Pyrenean cattle

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Introduction: Navarre (Spain) contains the 37,5% of the pirenaica breed national census distributed mostly in farms of 25 cows. This breed has a meat aptitude and therefore, its economic performance is obtained through the sale of calves, so the whole cycle revolves around the production of these. One of the factors that varies the most and produces the most losses is the duration of postpartum due to ovarian aciclity. These losses are both economic and reproductive. These losses are due in large part to nutrition, since the interval between births of the Pyrenean cow is influenced by 19% by nutrition in pre-school, 16.4% by postpartum nutrition and 13% by number of lactations.

Objectives: The aim of this work is to compare various Pyrenean cows 'exploitations' reproductive averages between them and with the national and navarre average. It will also be compared with the spanish and french average of the Limousine and the Blonde of Aquitaine.

Material & Methods: For this work, we have collected all the information since 2017, from 12 selected farms (4 farms of 3 different regions).

Results: The area influences in the interval between parts (IBP), interval part-insemination (IPI), body condition (BC), age and fertility of the heifers. Also the fertility is not related with the region where the cow lives. In the farms used for this study, the average has improved the results in IBP and IPI. The results that we have obtained show that using artificial insemination and giving the requirement of food that the cow needs in every step of production will improve their productivity.

Conclusions:

1. The establishment of a fixed-time artificial insemination decreases postpartum anestrus, in turn decreasing the IBP.
2. The variables: IBP, IPI, BC, age of insemination and the fertility of the heifers, are conditioned by the characteristics of the area in which they are raised.
3. When comparing our results with those published from the Pyrenean race and other races, we observed both differences in fertility and the interval between births, possibly due to feeding conditions, linked to grass in

France and to the genetic characteristics of the breed itself in each case.

Keywords: Pirenaica, Beef, Bovine, Artificial Insemination.

RB-P03

Comparison of a portable CASA device with a laboratory CASA device for determination of sperm concentration, motility and kinetics of bull semen

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Objectives: Bull breeding soundness evaluation is performed in the field and, in such conditions, portable, cheaper CASA devices enable better accuracy and precision in the evaluation of bull sperm quality. The present study evaluated the determination of sperm concentration, motility and kinetics using iSperm, a portable CASA system in comparison with SCOPUS, a laboratory CASA system.

Material & Methods: A total of 86 bull semen samples were evaluated. Concentration and kinetics were determined in diluted semen samples with an iSperm® (Aidmics Biotechnology) which consist of an iPad mini, the iSperm® software, a clip-on microscope and a warming unit. A disposable chip was connected to the clip-on microscope. The semen sample was applied to the bottom/tip of the chip, and a cover was then positioned over the chip. The analysis consisted of a 7 seconds video recording, after which the data were calculated and displayed on the iPad. When required, raw semen was diluted to around 100 × 10^6/ml in saline solution, according to the recommendations of the manufacturer.

To measure sperm concentration and kinetics in the CASA SCOPUS (Bioproyect SL), standard instrument settings of SCOPUS 1.0 for the bull semen were used. The same diluted sample evaluated in the iSperm was used with the SCOPUS system. Approximately 3 µL of the sample was loaded into a preheated 20 mm deep Leja-4 chamber (Leja Products B.V.) slide. The heated plate of the microscopic stage was 37–38ºC.

Statistical comparisons were made with the SPSS statistical software. Correlation and regression analysis were performed to evaluate the relationship between both methods. A p-value < 0.05 was considered statistically significant.

Results: The results confirmed a good correlation between the 2 CASA systems when measuring sperm concentration and kinetics (Table 1).

Table 1: Pearson correlation coefficient (r) and regression coefficient (r^2) between both devices, for the parameters sperm concentration, percentage of motile sperm and percentage of progressively motile sperm.

Coefficient	Concentration (Million Spermatozoa/ml)	Total Motility (%)	Progressive Motility (%)
Correlation (r)	r=+0.995, p<0.001	r=+0.760, p<0.001	r=+0.522, p=0.011
Regression (r^2)	r^2=0.88	r^2=0.58	r^2=0.27

Conclusion: The results of this experiment demonstrate that the iSperm is a suitable device to evaluate the sperm concentration and motility of bull semen in the field.

Keywords: Bull, semen, CASA, sperm motility, sperm concentratin.

RB-P04

Injectable progesterone and follicular development in acyclic Zebu beef females

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Objectives: Brazil is the largest beef exporter in the world. More than 70% of the females of the Brazilian beef cattle are from Zebu cattle. One of the main factors that harm the reproductive efficiency of females with this genetic is the delay in ovarian activity after partum. The study aimed to evaluate the effects of two different doses of Progecio™ (Agener União–Brazil), an injectable progesterone product, with concentration of 70mg/mL, in the P4 concentrations and follicular development in acyclic bovine beef females.

Materials and methods: Forty acyclic zebu beef cattle (Nellore breed), from 20 to 40 days postpartum, with body weight ranging from 300 to 700 kg, between 40 and 72 months of age, primiparous or multiparous were homogeneously distributed among the experimental groups according to the days postpartum, body condition score (BCS) and size of the largest follicle. The animals were randomized into G1: 2.5mL of Progecio® (N = 15); G2: 5mL of Progecio® (N = 15) or saline (N=10), all treatments applied via intramuscular. Blood samples were collected and progesterone (P4) dosed via electrochemiluminescence (ECL-Elecsys™ Roche-Germany) in D0, immediately before product application, aiming at indicating the absence of endogenous production of this steroid. Other samples were carried out 12, 48, 96, 120 and 168 hours after treatments. Ultrasound evaluations were performed on the same blood collection days in order to identify and measure the diameter (mm) of the largest follicle in each ovary and the absence of a corpus luteum. For the evaluation, a portable ultrasound equipment (Mindray™ M5 - China), was used, coupled to a linear transducer of 5MHz. Additionally, other ultra-

sound evaluations were performed in D+14, D+21 and D+28, aiming at determining the follicular diameter and existence of luteal tissue, as an indication of the beginning of cyclic luteal ovarian activity. On each day, the means of P4 concentration and follicle diameter were compared between the treated and control groups, using analysis of variance (ANOVA). Analysis were considered at the 5% significance level (SAS Software, v9.4).

Results: In D1, P4concentrations of in cows treated with 5.0mL of Progecio™ were higher than those found in those treated with 2.5mL and control group (P<0.05). On this day, females treated with 2.5mL of Progecio™ also showed higher concentrations (P <0.05) than control group. From D3, there were no differences (P>0.05) between P4 concentrations in females two treated groups (2.5 and 5.0mL of Progecio™). Until D5, concentrations were similar between treatments and higher (P<0.05) than those observed in females of control group. Between D1 to D5, females treated with Progecio, at different doses, had a lower mean follicular diameter (P<0.05) than those in the control group. This was probably because the higher P4 concentrations provided by these treatments, mainly in D1, which can be considered supraluteal, blocked the hypothalamic-pituitary axis, reducing the gonadotropins release and consequently reflected in follicular development. Between D5 and D9 there was no difference (P>0.05) in follicular development in the different treatments. From D14 until the end of the study (D28), the follicular diameter in cows treated with 2.5 or 5.0mL of Progecio™ were higher (P <0.05) than those found in females of control group. There was no difference (P>0.05) in follicular diameter between females treated with 2.5 or 5.0 ml of Progecio™.

Conclusions: According to the results, although there is a transient initial reduction in the diameter of the follicles, after 14 days post treatment of the different doses of Progecio™, there was an increase in the diameter of the follicles in the treated females.

Acknowledgment: Biotran, Agener União, Fapemig, CNPq, and CAPES.

Keywords: Bovine, reproduction, reproductive efficiency.

RB-P05

Comparison between 2 Progesterone-based synchronization programs (7-Day CoSynch+PRID vs PRID+eCG) for fixed time artificial insemination in suckled beef cows in European field conditions

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Objectives: The objective of this study was to compare the effectiveness of two different oestrous synchronization programs to facilitate fixed time artificial insemination (FTAI) in suckled beef cows under French field conditions.

Materials and Methods: A total of 540 suckled cows located on 35 farms in the South East of France were enrolled across one breeding season. After a minimum voluntary waiting period of 40 days postpartum, cows were body condition scored (BCS) on a scale of 1 to 5 and then enrolled in one of the two synchronization treatments. Cows were randomly assigned to receive: Treatment 1 (n=269) on day 0 cows received a progesterone-releasing intravaginal device (PRID Delta®, Ceva Sante Animale, Libourne, France), on day 6 a luteolytic dose of prostaglandin F2α (Enzaprost T®, Ceva) was administered and on day 7 cows received 500 IU of equine chorionic gonadotropin (eCG, Syncrostim®, Ceva) and the PRID was removed, all the cows were inseminated at fixed time 56h after PRID removal.

The cows in Treatment 2 (n=271) received a GnRH analogue (Cystoreline®/Ovarelin®, Ceva) at PRID insertion and a luteolytic dose of prostaglandin F2α (Enzaprost T®, Ceva) at PRID removal on day 7, all the animals were inseminated at fixed time 72h after in combination with an intramuscular injection of GnRH (Cystoreline®/Ovarelin®, Ceva). Pregnancy diagnosis was carried out 35-40 days after AI by transrectal ultrasonography. Data were analyzed using the GLIMMIX procedure in SAS.

Results: The two treatments achieved similar pregnancy per AI (P/AI) (Treatment 1 64.3%, Treatment 2 64.2%, P>0.05), neither BCS nor the interval postpartum-AI had an effect on pregnancy outcome. There was a treatment by parity interaction (P=0.048).

This effect was expressed by having the primiparous cows presenting a P/AI of 68.2% in Treatment 1 vs 54.6% in Treatment 2, instead for multiparous cows (parity ≥ 3) the P/AI was 58.12% in Treatment 1 compared to 69.4% in Treatment 2.

Conclusions: The use of PRID based FTAI programs for suckled beef cows, are a very relevant tool to establish pregnancy in a large proportion herds at appointed timing. The two treatments showed overall equivalence in terms of fertility/AI, which suggests that due to a lower number of handlings the use of 7-Day CoSynch+PRID is the most valuable solution for breeding suckled beef cows under European conditions.

The use of GnRH at the time of PRID insertion, to control follicular wave emergence, and the administration of GnRH at the time of AI to time the ovulation, resulted in a numerically higher P/AI in multiparous cows, this outcome can be justified by the fact that a more precise control of cyclicity guarantees higher chances of conception.

The supplementation of 500 IU of eCG at PRID removal, showed a tendency to increase P/AI only in primiparous cows (P=0.09), this finding is supported by the fact that is more frequent for these animals to suffer from extended anovulation condition post-partum when compared to multiparous cows (STEVENSON; HILL; BRIDGES; LARSON et al., 2015) and an exogenous source of gonadotropins can allow higher fertility. Despite the increase in P/AI, it must be taken in account the potential induction of twin pregnancies, which has been reported with the use of eCG (LANE; AUSTIN; CROWE, 2008), *this is an undesirable outcome for primiparous beef cows, being responsible of higher incidence of dystocia, retained foetal membranes and extended anovular period (ECHTERNKAMP; GREGORY, 1999).*ECHTERNKAMP, S. E.; GREGORY, K. E.

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Keywords: Progesterone, synchronization, fixed time AI, beef cows.

RB-P06

A case report of a Dexter bull with a persistent penile and preputial prolapse caused by a penile hypertrophy and a severe balanoposthitis

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Objective: A case report of a ten-year-old Dexter bull with a persistent penile and preputial prolapse caused by necrotic masses and overgrown granulation tissue involving the penis and the prepuce accompanied by a severe general balanoposthitis.

Material & Methods: The bull was hospitalized at the Clinic for Cattle, University of Veterinary Medicine Hannover (Lower Saxony, Germany) with a penile and preputial prolapse, which had probably persisted approximately three weeks prior to admission. The owner had recognized the prolapse roughly one week after its appearance. No causative trauma related to a mating act or any other reason had been observed. The local veterinarian carried out a manual reposition of the prolapsed tissue for several days. Due to reoccurrence, he fixed the non-prolapsed penis with two stitches passing through the prepuce cranial to an abnormal tissue growth, which in fact seemed to be the cause of the inability to retract the penis into the prepuce. Hence, after one week, the surrounding tissue became severely swollen and the sutures loosened up, causing the reoccurrence of the prolapse. The bull was subsequently admitted to the clinic in order to preserve the animal's life for further use as seeking bull. The animal showed a good general condition at initial examination. A detailed examination revealed a severe balanoposthitis and partial penile and preputial prolapse due to a prominent hypertrophy at the base of the prepuce, where fixation stitches had previously been placed. For a non-surgical reposition, the prolapsed and altered tissue was gently washed using a diluted iodine solution (0.5 % of a PVP-iodine solution). Due to the severe swelling, repositioning was only possible through constantly applied high pressure and a local application of a glucocorticoid solution to reduce the volume. A temporary partial closure of the prepuce opening with a modified method according to Bühner (a technique for partial closure of the vulva to treat prolapsed vaginal tissue), knotted over a rubber tube, was performed and antibiotic (amoxicillin) as well as antiphlogistic drugs (carprofen) were administered parenterally. The prepuce was flushed with 0.5 % PVP-iodine solution and an antibiotic salve (oxacillin) was applied locally twice a week for two weeks. Thereafter, the tissue had recovered sufficiently to allow a partial surgical removal of the hypertrophic granulation tissue at the base of the prepuce. The procedure was repeated two weeks later. A histological examination of the removed tissue was performed after both surgeries. For repositioning and surgical interventions, a high epidural anaesthesia was administered using 0.16 mg xylazine-hydrochloride / kg BW diluted with 25 ml isotonic saline, which additionally ensured a proper relaxation of the retracting muscles of the penis, thereby facilitating manipulations of the penile tissue whilst treatment stages. Supportive care had been continued twice weekly until the bull was released from the clinic by eight weeks.

Results: Effective reposition of the prolapsed tissue, regular care and surgical removal of the proliferated tissue led to a satisfactory healing of penile and preputial structures. Histological examinations of the altered tissue revealed formation of hypertrophic granulation tissue with absence of druses or the Splendore-Hoeppli phenomenon. This result excluded an infection with Actinobacillus lignieresii. Additional to the intent to preserve the animal's life as a seeking bull, potentia coeundi could be re-established.

Conclusions: Periodic breeding soundness examinations, including thorough inspections of the penis and the prepuce in bulls, are of great importance to detect genital disorders in early stages. Proper cleaning, repositioning and gentle care of any penile structure may result in good healing tendencies. However, the high self-healing and granulation tissue formation tendency of the mucosae may cause necessity of surgical interventions to reduce tissue overgrowth and re-establish full function of the organ. Surgical interventions often need several stages and a prudent modus operandi with a detailed anatomic knowledge of the penile structures to avoid iatrogenic damage.

Keywords: Penile prolapse, balanoposthitis, penile hypertrophy, surgical treatment.

RB-P07

Incidence of subclinical endometritis in extensive beef cattle of Comunidad Autónoma de Madrid(C.A.M.): Impact on reproductive rates

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Objectives:

- Analyze the incidence of subclinical endometritis (S.E.) in beef cattle and extensive production in Comunidad Autónoma se Madrid (C.A.M.).
- Determine its impact on reproductive rates of cows that present three or more natural mount or artificial inseminations(A.I.) without becoming pregnant.

Material and methods: A total of 145 animals from 16 extensive beef cattle farms in C.A.M. were analyzed. These farms have their own reproductive indexes register.

The checked cows are between 50 and 250 days post-calving, present 38.0°C-39.5°C in body temperature and no purulent vaginal discharge is appreciated in them.

Sampling by *Cytobrush* and May-Grünwald Giemsa staining. Then, the observation was made by immersion microscope (x100).

Results: Considering as positive animals at S.E. those which present a polymorphonuclear neutrophils (PMNs) count superior to 5%, there was a 11% of positivity in cows between 50 and 90 days postpartum. The percentage of positive animals increases significantly in cows that had three or more repetitions of heat after covering or A.I. Thus, in cows between 90 and 250 days postpartum the percentage of positive animals reached 63%.

Conclusion: The high percentage of positive cows to S.E. found in repeater animals indicates that the presence of this disease can be one of the causes which affects decisively the reproductive and productive indexes of a farm.

The detection and treatment of S.E. can be essential for the profitability of extensive beef cattle farms. However, other factors that could also affect these rates should be considered. All this means that it would be necessary to continue making studies and gathering information.

Keywords: Subclinical endometritis, beef cattle, reproduction.

RB-P08

Bull and seasonal effect on fresh and frozen-thaw semen characteristics in crossbreed beef bulls

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Objectives: The aim was to study the effect of bull and season on fresh and frozen-thawed semen in crossbreed bulls (*Bos taurus* x *Bos indicus*)

Materials and methods: A dataset of a commercial bovine artificial insemination facility (S 33º 41.795´ / WO 61º 57.049´, Venado Tuerto, Santa Fé, Argentina) from September 2016 to November 2019, was used for this study. This dataset contained 2471 fresh and 1561 frozen-thaw semen samples data from a total of 24 crossbreed bulls (*Bos taurus X Bos indicus*). Fresh and frozen-thawed semen parameters were analyzed by CASA software: sperm concentration, fresh progressive motility (FPM) and frozen-thaw motility (TPM; Androvision®, Minitube®). Besides, total sperm production (TSP) and freezability index (FI) per bull were calculated. The association between TSP, FPM, TPM and, FI was assessed by a repeated measures model with the season as a unique fix predictor and bull as random effect (PROC GLIMMIX, SAS 9.4). Statistical significance was set at $P < 0.05$. This study was approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Sciences, National University of La Plata, Argentina (code no. 102-6-19T).

Results: The FPM differed between seasons ($p<0.05$), having spring and summer the highest and winter the lowest values of FPM. TSP also differed between seasons ($p<0.05$), having autumn the highest and winter the lowest values of TSP. There was no difference between seasons for TPM ($p=0.31$) and FI ($p=0.21$). The Intra Class Correlation (ICC), that described the variability caused by bull and season, was 18% for bull and 5% for the season in FPM; 30% for bull and 5% for the season in TPM and 24% for bull and 4.5% for the season in FI.

Conclusions: We concluded that even though the season and the bull affect fresh sperm motility and total sperm production, the bull was an important variable for fresh and frozen thaw motility and also freezability index parameters in the beef crossbreed bulls studied. So, determining the reasons for that variations is crucial for an early bull selection.

Keywords: Bovine, season, semen characteristics.

RB-P09

Cryoconservation program in Blanca Cacereña breed

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The CENSYRA of Badajoz (Animal Selection and Reproduction Center) aims are the conservation, improvement and development of livestock breeds. The FAO (Food and Agriculture Organization of the United Nations) considers that conservation of animal genetic resources may be undertaken for a number of reasons. In the context of domestic animal diversity, ex situ conservation means conservation away from the habitat and production systems where the resource developed. This category includes both the maintenance of live animals and cryoconservation. Cryoconservation is the collection and deep-freezing of semen, ova, embryos or tissues for potential future use in breeding or regenerating animals.

Objectives: The present research describes the cryoconservation program in Blanca Cacereña breed ("CrioBlanCa"). The main objective of this program is provide the possibility of breed recreating if they are lost as the result of a calamity. Storage of germplasm for this purpose is for long term, and does not involve frequent use of the stored material or necessitate regular updating of the collection. A second purpose is to

support in vivo conservation. Frozen semen and embryos can be used to minimize inbreeding and genetic drift in small managed populations; the combination of live animals and cryopreserved germplasm can be a powerful tool in conservation.

The "CrioBlanCa" program is based on FAO recommendations whit the following goals:

- Breed reconstruction using semen. When semen is cryoconserved, the principal method for reconstituting a breed or population is through backcrossing. FAO estimates a number of semen doses required to reconstitute a breed of cattle with 150 founder females with different pregnancy rate. CrioBlanCa program sets the following goals: 25 doses from 50 males with, at least, 40% pregnancy rate for using in 150 founder males.
- Beed reconstruction using embryos. Biologically, the embryo offers the complete genetic complement of the breed. FAO estimates the number of embryos that need to be cryopreserved in order to obtain a reconstructed population of 25 breeding males and 25 females, depending on the survival of the embryo from thawing to birth and the subsequent survival of the animal from birth to breeding age. CrioBlanCa program sets the following goals: 150 embryos from, at least, 25 donor females, with 60% embryo survival (thawing to birth) and 90% offspring survival (birth to breeding age).

FAO also established that, in order to minimize the risks of loss, the genetic material must be located in two different locations: a main bank, and a security reserve collection. The main bank is the Animal Germplasm Bank of CENSYRA of Badajoz and the duplicate is located in the National Bank of Animal Germplasm (BNGA) in the CENSYRA of Colmenar Viejo (Madrid). Therefore, the objectives discussed above are doubled by two (25000 doses and 300 embryos).

Material and methods: The semen and embryos are obtained in CENSYRA´s reproduction centre according the legal requirements. The semen is obtained by artificial vagina or electroejaculation and cryopreserved following the recommendations of the regulations in force. The fertility rate is estimated in accordance with the seminal quality parameters. Embryos are obtained through multiovulation and embryo transfer (MOET). The embryo survival and offspring survival rate is calculated in an estimated way according to the embryonic quality parameters.

Candidate donors are selected through their genealogy under genetic distance between individuals criteria.

Results: Currently, 66% of the goal "stored doses for Blanca Cacereña breed reconstruction", has been reached, because there are 825 doses of 33 donor males (25 doses per animal) stored in the main Bank (CENSYRA of Badajoz), and a copy of them (825 doses) are in the BNGA. In total, there are 1.650 doses.

Actually, the goal "embryos stored for reconstruction of the breed", has been reached in 30.66%, because there are 92 embryos of 16 donor females stored only in the CENSYRA of Badajoz. 58 embryos from at least 9 donors are necessary to fulfill the objective and in addition, another 150 embryos would have to be obtained to send to the BNGA.

Conclusion: To met the objetive is necesary firstly, to send a copy of availables embryios to BNGA and secondly, to obtain 208 embryos from the maximum number of female donors (for example, 20). 5 years is the proposed period for complete the objectives.

Keywords: Breed, conservation, semen, embryos, Blanca Cacereña.

RB-P10

Effect of a recombinant glycoprotein with eCG- like activity on fertility of beef cattle timed inseminated using a 5-day CoSynch protocol

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Objectives: The objective was to compare pregnancy per timed AI (TAI) in *Bos taurus* beef cattle synchronized with a 5-d CoSynch plus progesterone protocol and receiving or not a glycoprotein with eCG-like activity (reCG).

Material & Methods: All procedures were conducted in accordance with the guidelines of the Committee for the Care and Use of Experimental Animals (N° 229/2021 CICUAE - CERBAS, for its Spanish acronym). Angus cows [n=809 (nulliparous: n=376, primiparous: n=159 and multiparous: n=274)] from 6 herds were submitted at random in each farm to either a 5-d progesterone CoSynch with reCG (reCG; n=404) or without reCG (Control; n=405). The day of the TAI was considered as Day 0 of the experiment. On Day -8, body condition score (BCS, scale 1-9) was recorded and all animals received an initial injection of 100 µg GnRH analog (Gonadoreline acetate, 2 mL, im, Prolusyn®, Syntex) and a 1.0 g of progesterone intravaginal device (DIB®, Syntex), on Day -3, 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 nulliparous cows and 400 IU (2 mL, im, reCG, PCT/EP2019/073277, Syntex) in primiparous and multiparous cows of reCG or remained as untreated controls. Also, a subset of animals (n=710, reCG=355 and control=355) were painted on the sacrum to the tail head (Ce-Lamark®) and on Day 0, the degree of color loss was evaluated for determination of the expression of estrus (percentage of color loss ≥ 30% was considered as showed estrus). On Day -2 (72 h after DIB removal), animals received a second dose of sodium closprostenol and finally on Day 0, received 100 µg of gonadoreline acetate in concurrent with TAI. Pregnancy diagnosis was

evaluated by ultrasonography with a 5 MHz transrectal linear probe on approximately Day 30 (range, 27 to 35 d) after TAI. Data was analyzed using SAS (Statistical Analysis System). Optimal BCS was considered in nulliparous cows ≥6 and in primiparous and multiparous cows ≥5 and was observed that 51.5% of animals were below the optimal BCS at Day -8. Baseline comparisons were established evaluating the distribution of cows in both treatment group using a Chi-square test (Proc Freq, SAS system®). The effect of treatment group (reCG vs. control), herd (1, 2, 3, 4, 5 and 6), parity (nulliparous, primiparous and multiparous), BCS (<optimum vs. ≥optimum) and expression of estrus (showed estrus vs. not showed estrus) on pregnancy per TAI was determined by univariate analysis with Chi-square test. The association between treatment group (reCG vs. control), expression of estrus (showed estrus vs. not showed estrus) and their interactions on pregnancy per TAI were analyzed by multiple logistic regression models (Proc Logistic, SAS system®) using the backward elimination procedure. The variables included in the models were treatment group, parity, BCS and expression of estrus.

Results: There was no difference in the distribution of cows by herd (P=0.97), parity (P=0.73) and BCS (P=0.23) in both treatment groups. The proportion of animals that showed estrus was greater in reCG than the control group [268/355 (75.5%) vs 243/355 (68.4%), P=0.04]. Pregnancy per TAI was greater in reCG than control group [229/404, (56.7%) vs 201/405, (49.6%), P=0.04]. Pregnancy per TAI was not affected by herd (P=0.12), parity (P=0.13), BCS (P=0.90) or manifestation of estrus (P=0.13). In the multivariable analysis, pregnancy per TAI was also greater in reCG than control group (P=0.001) and there was also an interaction between treatment group and manifestation of estrus (P=0.002). While there was no difference on treatment in pregnancy per TAI in animals that showed estrus (reCG=55.6%; control=54.7%), reCG presented almost 2-fold increase in pregnancy per TAI in animals that did not show estrus (reCG=63.2%; control=37.5%).

Conclusions: In conclusion, reCG addition in a 5-d CoSynch plus progesterone protocol increased the TAI pregnancy rate in *Bos Taurus* beef cattle, especially in animals that did not show estrous.

Keywords: reCG, 5-day CoSynch, FTAI.

RB-P11

Pregnancy rates in Nellore and crossbreed beef cattle with a single and multiple corpus luteum

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Objectives: In vitro fertilization and embryo transfer programs are implemented to increase the population of animals with good meat quality and high reproductive performence. One of the main limitation of these techniques is the low pregnancy rates obtained under field conditions. Previous studies reported that recipients with larger corpus luteum (CL) and high levels of plasma progesterone have higher pregnancy rates than cows with smaller CL. We have previously demonstrated that ablation of the dominant preovulatory follicle resulted in double ovulaton and development of two corpus luteum. However, wether double ovulations results in higher rates of pregnacy has not yet evaluated. The objective of the present study was to evaluate the pregnancy rates of beef breed Nellore and crossbreed recipients and weather recipients with more than one corpus luteum (CL) has higher pregnancy rates than recipients with a single CL.

Materials & Methods: A total of fourty multiparous cows were synchronized with progesterone implants 0.5 g on Day 8 progesterone implants have been removed and prostaglandin F2alpha intramuscularly injected. Between Days 7 and Day 9 of progesterone implant, animals were injected with hCG 1500 IU or FSH 5 IU to induce ovulation of more than one follicle. Immature oocytes were collected from donnors, then oocytes were matured and fertilized in vitro. Excellent quality in vitro fertilized embryos were transfered into the uterine lumen of recipients seven days after estrus. The number and size of the CL were recorded before embryo transfer procedure for further analysis. Experimental animals were divided as follows: Group A: recipients with single CL, Group B: recipients with two or more CL. Two embryos were transfered into one horn or in opposit uterine forns after confirmation of functional CL. Pregnancy dianosis was performed on Day 30 and Day 90 post-fertilization.

Results: Recipients with 2 or more CL at the time of embryo tranfer have higher pregnancy rates than those recipients with single CL.

Conclusion: Overall results suggest that pregnancy rates is positively affected by number of CL. Double gestations were obtained by embryo transfer into a single or separated uterine horns.

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Keywords: Bovine, Embryo Transfer.

RB-P12

Testosterone and sperm quality of purebred Wagyu bulls under field conditions in Spain

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Objectives: Semen characteristics are influenced by breed and are of crucial importance for the success of artificial insemination (AI). Semen cryopreservation is the most common technology in AI programs since enables genetic material from superior bulls to be stored for an unlimited period and its availability without time or geographical constraints. However, freezing-thawing procedures negatively affect the survival of the sperm cells even using the best available technique and is consistently dependent on the individual. Consequently the success of AI depends on semen quality which must be evaluated in detail for each single bull. Therefore, the aim of this study was to carry out a thorough qualitative characterization of ejaculates obtained by electroejaculation from purebred wagyu sires selected as donors for a farm germplasm bank, under field conditions.

Material & Methods: A total of 36 semen samples were obtained by electroejaculation (Electrojac 6®, Neogen, USA) from 4 selected purebred wagyu bulls, using the manual program, with the application of electrical stimuli according to the bull's response. The scrotal circumference was measured and blood samples were taken for further plasma testosterone concentration assessment. Ejaculates were immediately transported to the own farm laboratory in a portable incubator and stored in a water bath at 37◦C for evaluation. Semen quality was assessed by volume (Vol.; mL), sperm concentration determined by photometer (SpermaCue, Minitub®, Germany; [x10^6]/mL), total number of sperm per ejaculate (TSx10^6), mass motility (MM), individual motility (%IM), quality of sperm movement in a scale 0-5 (Q), morphological normal sperm (%N), intact acrosomes (%NAR), functional integrity of the sperm membrane (hypoosmotic swelling test; %HOST), sperm viability (live-dead, eosin-nigrosin vital stain; %V) and the live-acrosome intact sperm (triple stain eosin-nigrosin-Giemsa; %LAI). Differences in seminal parameters among bulls were analysed by One-way ANOVA using IBM SPSS® Statistics v. 25.0 (IBM Corp., New York, USA).

Results: Values of semen quality obtained in purebred wagyu sires were acceptable for the vast majority of seminal parameters and bulls evaluated and, in general, slightly lower than the results previously described for other beef breeds when using electroejaculation for semen collection under field conditions. Mean plasma testosterone concentration was 12.87±6.29 ng/mL and ranged from 14.94±6.44 (bull-C) to 6.37±5.61 ng/mL in the youngest one (bull-D; P=0.06). Scrotal circumference differed significantly among bulls (P<0.001) but no differences on the quantitative seminal parameters were found: Vol (7.9±3.31 mL); [x10^6]/mL (676.58±519.52 x10^6sperm/mL) and TSx10^6 (5665.07±5295.52 x10^6sperm). Regarding qualitative parameters, no differences in MM or Q were found among bulls (means 3.49±0.87 and 4.07±0.37, respectively), however, %IM was significantly lower (P=0.01) in the oldest bull (bull-A; 62.5±23.83%) than in the other bulls (%IM>80%). The percentage of normal spermatozoa was numerically higher in the oldest sire (bull-A; 81.1±8.45%) than in bull-B (67.39±19.18%) or bulls C and D (>70%), but without statistical significance. Other qualitative sperm parameters did not differ significantly among bulls although means ranged from 64.95±26.11% (bull B) to 82.36±19.01% (bull-C) for %NAR, from 73.54±17.15% (bull-C) to 87.68±10.04% (bull B) for %HOST, from 70.04±20.6% (bull B) to 79.27±7.91% (bull-D) for %V and from 62.75±24.77% (bull-A) to 78.44±12.75% (bull-C) for %LAI. This lack of significant differences may be due to the large variability in the parameters.

Conclusions: The results provide important information regarding the semen quality of purebred wagyu bulls under Spanish livestock production conditions and reveal differences on semen quality among sires, which highlights the need to select the animals that will act as semen donors for the germplasm bank according to these characteristics, together with the phenotypic and genetic-genomic selection.

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Keywords: Bull semen, Wagyu breed, Sperm quality.

RB-P13

Effect of extender used on casa motility results of Wagyu semen obtained by electroejaculation under field conditions in Spain

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Objectives: Computer-Assisted Sperm Analysis (CASA) has become the gold standard tool for evaluating sperm motility and kinetic patterns because it provides objective data for thousands of sperm tracks. However, CASA results are affected by many factors including extender used for samples dilution, therefore the standardization of analytical practices is a fundamental requirement. For this reason, we evaluated the effect of two commercial extenders (Andromed® and Bioxcell™), after semen samples dilution, on the motility and kinetic results of purebred wagyu semen obtained by electroejaculation, using CASA.

Material & Methods: Ejaculates from four purebred wagyu sires were obtained by electroejaculation on the farm using an Electrojac VI® electroejaculator (Neogen, USA) in 3 sessions (12 ejaculates). Semen samples were immediately transported to the own farm laboratory in a portable incubator, stored in a water bath at 37ºC for further evaluation and cryopreservation, and split in two aliquots. One aliquot was diluted with Andromed® (Minitüb, Germany) and the other with Bioxcell™ (IMV Technologies, L'Aigle, France) to reach a final sperm concentration of 30 x10^6 spermatozoa/mL for CASA analysis. After placing a diluted semen sample in a pre-warmed Makler chamber, kinematic analyses of each aliquot/ejaculate/bull were performed using the ISAS®v1.2 CASAMot system (Proiser, Valencia, Spain), which consisted of a video camera (C13-ON High Speed 500 fps) attached to a portable microscope (UOP UB 2000i, Proiser, Valencia, Spain) equipped with a 10 negative phase contrast objective and a heat stage. Six

different fields were captured with a minimum of 2000 sperms per analysis. For each sample, total motility (TM%); progressive sperm motility (PM%); rapid sperm subpopulation (R%); curvilinear, average path and straight-line velocities (VCL, VAP, and VSL, respectively; µm/s); the three progression ratios of linearity (LIN), straightness (STR), and wobble (WOB); the amplitude of lateral head displacement (ALH; µm) and the beat/cross frequency (BCF; Hz) were recorded and the results analysed by two-way ANOVA (IBM SPSS® Statistics v. 25.0) including the bull and the extender used.

Results: Statistical model was significant for VSL (P<0.0001), VAP (P=0.003) and BCF (P=0.011). TM%, PM% and R% were 91.15±11.34%, 35.9±13.1% and 89.24±12.9%, respectively, without statistical differences among bulls or according the extender used for these parameters. Mean VCL was 155.06±26.12 µm/s without bull or extender effects affecting this kinetic parameter either. However, bull and extender had a statistical significant effect for VSL obtaining better results with Bioxcell™ (55.43±8.04 *vs.* 49.18±10.49; P=0.013) and showing statistical differences among the bulls (P<0.0001). BCF was affected by bull and extender (P=0.011 and P=0.002, respectively), with higher BCF values with Bioxcell™ being obtained than with Andromed® (17.78±2.98 *vs.* 16.18±2.57; P=0.002). For VAP, a significant interaction between bull and extender used was detected in such a way that in the oldest bull, there were statistical differences in mean VAP values between Andromed® and Bioxcell™ (56.53±2.34 *vs.* 80.1±3.05 µm/s; P=0.029), whereas for the rest of the bulls, extender had no effect on this parameter and mean VAP value was ≥78% µm/s for all, regardless of extender used. Bull was significant for LIN (P=0.044) and the extender tended to be significant (35.98±5.42 *vs.* 32.08±5.05 for Bioxcell™ and Andromed®, respectively; P=0.056). However, for the other two indexes bull and extender had no effect (mean STR=65.0±8.22 and mean WOB=52.23±3.31). ALH results were similar among bulls and extenders used (3.01±0.55 *vs.* 3.18±0.40, for Bioxcell™ and Andromed®, respectively; P>0.05).

Conclusions: CASA parameters in ejaculates obtained from purebred Wagyu sires show that the bull, together with some preparation procedures of ejaculates for CASA analysis, like extender used for samples dilution, are the major sources of variation on sperm motion kinetics. The differences found between extenders on motion kinetic parameters, underline the need for a standardization of CASA analysis in bovine semen to compare results.

Supported by CDTI-IDI-20180254 grant.

Keywords: Bull semen, CASA analysis, Wagyu breed, Sperm motility.

RB-P14

Comparison between 5-day and 7-day progestogen-based protocols for estrus synchronization and ovulation in pasture beef cattle in Spain: fixed-time AI and subsequent natural services

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Objectives: The present study evaluates the reproductive efficiency of two fixed-time AI treatments based on progestogen devices maintained for 5 or 7 days in pasture-based beef cattle in Spain. Also, it was analyzed the influence of different factors on the mentioned treatments. A total of 609 virgin beef heifers and 1041 suckler beef cows were involved in the present study.

Materials & Methods: They were reared in 16 farms under extensive production system, located in Southern Spain. All cows showed a body condition score (BCS) above 5 points according to the scale from 1 to 9. In recently calved cows, synchronization treatments were initiated during lactation, and they were routinely weaned around 6 or 7 months after parturition. For estrus synchronization, an intravaginal device containing natural progesterone (CIDR; 1.38 g progesterone; Zoetis Animal Health) was inserted and GnRH (Gonavet Veyx 50 µg/ml Gonadoreline acetate; Veyx pharma GMBH) was i.m. administered. One day before the device removal, prostaglandine (Dinolytic 12.5 mg/ml; Dinoprost trometamol; Zoetis Animal Health) was i.m administered. Devices were maintained for 5 (CIDR-5) or 7 (CIDR-7) days, and eCG (400 IU in heifers and 500 IU in cows) was administered. CIDR-5 received another prostaglandin dose 6 hours after the first dose. Artificial insemination was carried out on 60 h and 72 h after CIDR withdrawal in heifers and cows, respectively, and 50 µg/ml GnRH was i.m. administered. Five days after insemination, bulls were housed with cows in order to promote the mounting during natural estrus after fixed-time AI. It was determined the fertility rate after AI and the overall fertility rate (including AI and natural services during the breeding season). After the estrous synchronization treatments and AI, the pregnancy diagnosis was realized on day 45-60 later. To know the fertility rate during the natural mounting, pregnancy diagnosis was carried out three months after this first contact between bulls and cows, and again at the end of the breeding season. For pregnancy diagnosis, transrectal ultrasonography was realized using a linear probe with 7.5 MHz (Imago, IMV, France). Cows were considered as pregnant when anechoic fluid and embryo or fetus was observed into the uterus.

Results: The pregnancy rate after estrus synchronization using CIDR-5 and CIDR-7 was 51.9% and 53.1%, respectively, and no significant differences were observed. The pregnancy rate obtained in suckler cows treated at short (13-77 days), medium (78-192 days) or late (193-812 days) postpartum interval did not show significant differences (54.3%, 50.0% and 53.9%, respectively), and no differences between treatments were detected. The overall pregnancy rate (considering

both the AI and mounts carried out during the reproductive season) was 82.37% and 83.68% for CIDR-5 and CIDR-7 treatments, respectively; no differences were observed between treatments. The logistic regression analyses indicated that non variables (farm, treatments, parity and cow breed) should be included in the model which explain the pregnancy rate after AI. Parity was included in the model for explaining the pregnancy rate at the return to estrus after AI. Higher total fertility was observed in cows, in contrast to heifers (84.61% vs 79.3%, p<0.05); although no differences were detected for treatments, animals treated with progestogen during 7 days reached soon the higher pregnancy rates, which it is an advantage in contrast with 5-day CIDR treatment. Those animals failing to get pregnant after fixed-time AI were analyzed in order to explore the impact of parity on the pregnancy rate; it was observed that those open cows showed significantly higher pregnancy rates than open heifers (67.21% vs 58.00%, p<0.05). Progesterone-based treatments allow the ovulation induction and time-fixed insemination, reducing the clinical monitoring in treated animals.

Conclusion: In conclusion, the reduction of CIDR treatment from 5 to 7 days offers similar pregnancy rates after AI and for the overall breeding season. When animals do not get pregnant after de AI, it was observed that those open suckler cows showed a significantly higher pregnancy rate at natural mountings that open heifers. In reference to parity, it does not influence on the fertility after AI, but this percentage was higher for sucker in comparison with heifers when the complete breeding season was analyzed.

Keywords: Beef cattle. fixed-time IA, progestogen, synchronization.

RB-P15

Effect of supplementation with vitamins and minerals at the beginning of a fixed timed AI protocol on fertility of sucking beef cows

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Objective: Study the effect of supplementation with vitamins and minerals at the beginning of a fixed timed AI (FTAI) protocol on the fertility of suckling beef cows.

Material and methods: Estrus was synchronized in sucking beef cows (n=350) with ≥2 parturitions in Cordoba, Argentina. At the beginning of the FTAI protocol, cows were body condition scored (BCS, 1-5 scale; 1=emaciated-5=obese), and transrectal ultrasonography was performed to determine ovarian cyclicity (CYCL; cycling=presence of CL, superficial anovulation=follicles ≥10 m; deep anovulation=follicles<10 mm). In addition, all animals had administered a vaccine for reproductive disease (Bioabortogen H® [BoHV type 1, BVDV type 1 and 2, *Campylobacter fetus fetus, Campylobacter fetus venerealis, Histtophilus somni,* and *Leptospira interrogans Pomona pomona*], Biogénesis Bagó, Argentina). Cows with even tag number were assigned to the treatment group (TRT, n=190) and were administered a vitamin A and E supplement (vitamin A palmitate 175 mg, vitamin E acetate, 250 mg; Adaptador® VIT, Biogénesis Bagó), and a copper, zinc, manganese, and sodium mineral supplement (copper edetate 50 mg, zinc edetate 200 mg, manganese edetate 50 mg, Sodium selenite 25 mg; Adaptador® MIN, Biogénesis Bagó). Cows with odd tag numbers were assigned to the control group (CON, n=162) and were not administered a vitamin and mineral supplement. All cows were started in an estrus synchronization and FTAI protocol and received on day (d) 0 an intravaginal progesterone releasing devise (DIV) insert (0.5 g P_4, Cronipres®, Biogénesis Bagó), and estradiol benzoate (2mg; Bioestrogen®, Biogénesis Bagó). On d7, the DIV insert was removed and al cows were administered estradiol cypionate (CE, 1mg; Croni-Cip®, Biogénesis Bagó), D-cloprostenol (PGF, 150 mg, Enzaprost DC®, Biogénesis Bagó), and equine chorionic gonadotropin (eCG, 400 UI; Ecegon®, Biogénesis Bagó). In addition, at d7, all cows were tail painted (Celo Test® Biotay, Philbro Animal Health). All cows with >50% of tail paint removed were FTAI at 48-52 h of DIV insert removed, and those not detected in heat were administered buserelin acetate (GnRH, 8 ug; Gonaxal®; Biogénesis Bagó) to induce ovulation. All FTAI were done by three technicians (TECH) with the semen of two bulls (BULL). Cows were exposed to bulls for natural service at 10 days post-FTAI. The pregnancy rate to FTAI was determined 65 d after FTAI by transrectal ultrasonography (Esaote Tringa L, Genoa, Italy). Pregnancies resulting from FTAI (PREGAI) were distinguished from natural services pregnancies based on embryo/fetal size (PREGNS; TPREG= PREGAI + PREGNS). Data were analyzed with logistic regression models that included the effects of TRT, CYCL, TECH, BULL, GnRH, and BCS (≥2.75 vs. <2.75).

Results: The BCS was similar between groups (3,21±0,27; P>0,38). At the start of the protocol, 78.41% of cows were cycling (P>0.41). The PREGAI tended to be higher in the TRT than in the CON group (54.50 % [103/189] vs. 44.72% [72/161], P=0.06), and TPREG was higher in the TRT than in the CON group (75.13 % [142/189] vs. 62.73% [101/161], P=0.01). The PREGAI and TPREG were similar for TECH, BULL, CYCL, and cows that received or did not receive a GnRH to induce ovulation at TFAI (P=0.52, P=0.27, P=0.98, P=0.48).

Conclusion: Treatment of beef cows with a vitamin and mineral supplement at the beginning of an FTAI protocol improved the pregnancy rate by 12.4%.

Keywords: Beef, vitamin supplement, mineral supplement, fertility.

RB-P16

Comparison among color-Doppler ultrasonography and conventional transrectal B-mode ultrasonography of corpus luteum, and serum PSPB to diagnose early pregnancy in beef heifers.

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The main objective of this study was to compare the accuracy of transrectal color-Doppler ultrasonography (CD-TRUS) of the corpus luteum (CL) on Day 21 post timed artificial insemination (TAI) with conventional transrectal B-mode ultrasonography (B-TRUS) of the CL to detect pregnancy in Hereford, Angus and crossbred heifers. Furthermore, the accuracy of testing serum pregnancy-specific Protein-B (PSPB) on Day 21 and 23 post-TAI to diagnose pregnancy was evaluated. Thirty-eight cyclic heifers 24-month-old, in moderate to good body condition score (3.25 to 5, scale 1 to 5) received a progesterone intravaginal device (0.5 g progesterone) on Day -8, a luteolytic dose of prostaglandin and device removal on Day -3, and GnRH and TAI on Day 0. On Day 16 post-TAI all heifers received a second progesterone device for resynchronization. In order to increase the accuracy of ultrasonography to diagnose pregnancy on Day 21 post-TAI, estrus detection was performed between Day 1 and 16 post-TAI. On Day 21 post-TAI, progesterone device was removed and CD-TRUS and B-TRUS were conducted to determine pregnancy according to previously described criteria (Siqueira et al., 2013). In addition, on Day 21 post-TAI, blood samples from the coccygeal plexus were collected. Fifteen open heifers (diagnosed by CD-TRUS, B-TRUS or estrus expression) received a luteolytic dose of prostaglandin and were TAI three days later. On 23 pregnant heifers at Day 21 post-TAI, B-TRUS of the uterus (B-TRUS 25) was conducted on Day 25 post-TAI to confirm pregnancy according to previously described criteria (Kastelic et al., 1988). In addition, in pregnant heifers at Day 21, blood samples from the coccygeal plexus were collected on Days 23 and 25 post-TAI. Serum was extracted and PSPB concentrations were determined using a quantitative ELISA [BioPRYN®, BioTracking Inc., Moscow, ID, USA]. Pregnancy status based on PSPB was determined following the manufacturer's recommendations. Agreement between pregnancy diagnose based on CD-TRUS and B-TRUS was evaluated. In pregnant heifers at Day 21, agreement between PSPB on Day 21 or Day 23 and PSPB on Day 25 post-TAI were determined. CD-TRUS detected 19 pregnant heifers and B-TRUS detected 23 pregnant heifers. Strong agreement was found between both techniques (Spearman $\rho = 0.801$; $P < 0.001$). PSPB on Day 21 post-TAI detected 24 open heifers, 7 pregnant, and 7 needed to be rechecked. In 23 pregnant heifers at Day 21 post-TAI, PSPB on Day 23 post-TAI detected 8 open heifers and confirmed pregnancy on 15 heifers, 14 of them previously identified as pregnant or needed to recheck with PSPB on Day 21 post-TAI. PSPB on Day 25-Post TAI detected the same 8 open and 15 pregnant heifers. Considering assertiveness in diagnosing pregnancy by PSPB on Day 21 post-TAI resulted in a moderate agreement with PSPB on Day 25 post-TAI (Spearman $\rho = 0.483$; $P = 0.020$). PSPB on Day 23 post-TAI resulted in total agreement with PSPB on Day 25 post-TAI ($P < 0.001$). B-TRUS 25 detected 9 open and 14 pregnant heifers. In conclusion, CD-TRUS is a better predictor of pregnant heifers than B-TRUS on Day 21 post-TAI. PSPB on Day 23 post-TAI is totally reliable to diagnose pregnancy in crossbred beef heifers.

Keywords: Pregnancy-specific Protein-B, Pregnancy, color-Doppler, Ultrasonography, Beef Heifers.

RB-P17

Some tools to chose a good sire: Young bulls of Bruna dels Pirineus beef testing station

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Objectives: A standard goal for beef cattle production is one calf per mature cow and year. There are several factors influencing on this goal, and the reproductive performance of the bull is one of them very important one. The evaluation of the bull reproductive aptitude allows to predict its breeding performance and should allow to identify those bulls that are genetically superior, even in those herds where natural service is used as the reproductive management system. Taking this into account, the Catalan Federation of the *Bruna dels Pirineus* beef cattle breed (FEBRUPI) has a testing station (Bellestar, Lleida, Spain) where veterinaries from the Veterinary School of the Autonomous University of Barcelona (Bellaterra, Spain) perform the reproductive assessment of young bulls.

Materials and methods: This study was carried out on field data from the testing station of the *Bruna dels Pirineus* breed, an autochthonous beef cattle breed located in the mountainous areas of Catalonia (northeastern Spain). The *Bruna dels Pirineus* is a medium-sized cattle breed with an approximate census of 13,000 active breeding cows in the herd book. This breed is reared under extensive conditions to produce beef calves.

Raw reproductive data from 693 purebred young bulls from 33 testing series were recorded between 2003 and 2021. The final data set for reproduction traits consisted on 445 young bulls sampled, autumn-winter and spring-summer. They were weaned calves between 5 and 8 months old, with known and registered genealogy.

At the end of each test series, we carried out a visual examination of the reproductive system to identify morphological anomalies, an analysis of the seminal quality by collecting an ejaculate from each individual by electro-ejaculation, and the measurement of the scrotal circumference. This measure was taken with using a tape around the scrotal area on the largest diameter, and with a precision of 0.5 cm.

Results: As a whole, 73.8% of young bulls passed the tests and were distributed to breeding herds by auction. Remaining animals (26.2 %) were discarded because of breed standard flaws, bad temperament, or pedigree inconsistencies, among other.

The results of the reproductive analyses showed that evaluation was carried out at 11.9 months [6.9-17.4], with a mean live weight of 461 kg [261-696] and a mean growth of 1.33 kg/d (±0.01) during the testing period. Scrotal circumference was 34.2 cm [27-43] and seminal traits evidenced a mean viability of 59.3 % with 37.8% of total abnormality in sperm cells.

The scrotal circumference has been reported to have moderate-high heritability (0.41-0.57) (Bourdon and Brinks, 1986, Lunstra et al., 1988, Kealey et al., 2006), suggesting that a rapid genetic improvement could be obtained for this trait by selection. According to Moser et al. (1996) and Siddiqui et al. (2008), males with a larger scrotal circumference are capable of impregnating more cows in less time than bulls with smaller scrotal circumference they transmit this merit to their male offspring and better reproductive behavior to their female offspring.

Along these years, 203 tested bulls generated 13,459 offspring in 113 herds. Calving ease scores highlighted a total of 98.40% of normal births, 1.14% of calvings with strong help from the stockbreeder, and 0.46% of calving with veterinarian assistance.

Conclusions: Individual evaluations test of young bulls would serve to identify those future sires that will improve reproduction traits and its outcomes, specially calving ease, they being important to obtain better economic benefits in beef cattle herds.

Keywords: Young bulls, testing station, reproductive traits.

RB-P18

Results of fixed time insemination in the Retinta breed, during the period 2009-2021

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Introduction: The connection between livestock, allows to assess males in different livestock and thus increase the reliability of the genetic evaluation. This connection can be made through the transfer of bulls between farmers (with obvious biosecurity problems), or through artificial insemination.

Objectives: The goal of this study was to relate the percentage of pregnancy obtained after fixed time insemination in beef cattle, with various factors to verify its possible influence on the result of the fixed time insemination.

Materials & Methods: Fixed-time insemination data between 2009 and 2019 in 23 different livestock farms in 993 animals have been analyzed.

All animals were Retinta breed. The cattle ranches were between Andalusia and Extremadura. The pregnancy diagnosis was performed by ultrasound at 35 days post-insemination. The proportion between adult cows and heifers is close to 50%. The treatment used for the synchronization of ovulation and artificial insemination had a duration of 8 days (the program was: Day 0 = insertion of progesterone implant (1.38 gr, CIDR® Zoetis), and administration of GnRH (250 µgr, Fertagyl®, MSD); Day 5 = CIDR® Removal and PG (25 mg I.M, Dinolytic®, Zoetis), PMSG (500 UI Foligon®, MSD); Day 6: PG (25 mg, Dinolytic®); Day 8: all animals were inseminated with frozen semen and received GnRH dose.

Results: The percentage of pregnancy obtained during the last 12 years has varied from a minimum of 15.5%, in 2009, to 75.7%, in 2019. Throughout this period, different conditions that may influence the result of artificial insemination, have been compiled and have been related to the percentage of pregnancy obtained.:

1. Age: A higher percentage of pregnancy (+ 6%) was obtained in heifers (females under 2 years of age at the time of insemination) than in cows. In females of less than 18 months, 68% of pregnancy has been obtained, while in older cows over 9 years, only 35% has been obtained.
2. Reproductive development. Before starting treatment, a rectal ultrasound of the reproductive structures was performed. Taking into account the follicular map (number and size of the follicles) in the ovaries and the presence of corpus luteum, different results were obtained. In females with the presence of corpus luteum, an average of 65% of pregnancy was obtained, in females with follicles greater than 10 mm and without a corpus luteum, 58% of pregnancy was obtained, while in animals with follicles below 10 mm an average pregnancy percentage of 44 was obtained. The pregnancies, went down to 25% in the case of heifers older than 2 years.
3. Livestock, management and facilities. There are large differences in the percentages of pregnancy between livestock:there are livestock with a 5% pregnancy rate and others close to 80%. Animal welfare as animal health status is very important in order to obtain good results and any additional source of stress negatively influences the results: a big influence on the results is attributed to the facilities that are used to perform the gynecological visits, synchronization and artificial insemination.

Conclusion: The artificial time insemination protocol that is being used to connect Retinta cattle farms is adequate, since the pregnancy rate in recent years exceeds 70%. In addition, as a proposal for improvement, the use of heifers should preferably be considered in the connection program between livestock, continuing with the previous gynecological evaluation of the participating animals by ultrasound. The need for participating farms to meet a series of conditions in

terms of management and facilities should also be taken into account, since in addition to improving pregnancy outcomes, they must increase the safety guarantees at work of the responsible technicians. It would be advisable to identify what some of them are doing well and others are no. It is also necessary to perform preventive and diagnostic actions, apply biosecurity measures and treatments against reproductive diseases if necessary. Due to the assessment and selection of candidate animals, the pregnancy rates have been increasing over the years.

Keywords: Artificial insemination, Pregnancy rates, Beef cattle, Reproductive development, Estrous synchronization.

RB-P19

Effects of r-met-hu G-CSF on Testicular Morphometry in Prepubertal Brahman Bulls

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I**ntroduction and objectives:** Puberty is the physiological process when fertility is acquired. Precocious animals have the potential to offer longer productive lives. The aim of this study was to test the effects of the drug Granulocyte colony-stimulating factor (G-CSF; Filgrastim) on various reproductive parameters in pre-pubertal Brahman bulls, such as 1) body weight (BW); 2) scrotal circumference (SC); 3) testicular weight (TW); 4) testicular volume (TV); and 5) hematology.

Materials and methods: Experimental animals were 12 entire Brahman bulls (Age: 9 months; Average weight= 244 ± 17.6 (Kg ± SD), coefficient of variation (CV) = 7.2% and randomly sorted into 4 groups. All the animals were clinically examined before starting the study and showed no apparent pathological condition. Two animals were castrated at day 0 in order to know the initial anatomical and physiological status of the testis. The control group (T1) n= 3; was administered 1mL of saline (NaCl 0.9%), subcutaneously (s.c.). Treated groups were Treatment two (T2) n= 4; administered 1 µg/Kg BW and treatment three (T3) n= 3; 4 µg/Kg BW Filgrastim s.c. Experimental bulls received this injection scheme for 4 consecutive days starting from day 0.

To certify the efficiency of r-met-hu G-CSF, blood samples were taken from the coccygeal blood vessels from experimental animals during each of the 4 days of treatment and a final sample at day 60. Immediately after bleeding, smears were made and fixed with 95% ethanol for 10 min and stained with 6%-Giemsa in dH2O (12 hours) to perform white cell counts.

Experimental animals were castrated 60 days after the last application of Filgrastim by veterinary professionals, in compliance with *Universidad San Francisco de Quito* (USFQ) Animal Ethical Committee guidelines and approval. Weight of the bulls was estimated with a bovinometric tape calibrated for zebu cattle. Scrotal circumference was measured with a tape measure. TW and TV were carried on with a digital scale and water displacement, respectively. The measurements were made at the beginning of the experiments and once a month for 4 months. Finally, statistical analyses for hematological and morphological results were performed using statistical software IBM SPSS® for Windows v17., specifically one-way ANOVA. The level of significance was set to $P < 0.05$ and multiple comparisons were performed (Bonferroni post-hoc test) for statistically significant results.

Results: Regarding hematological parameters (white series), there were significant differences in the concentration of lymphocytes and monocytes at day 1. For lymphocytes ($p < 0.05$. T1 > T2 and T3), T1= 54.1% ± 3.0; T2= 45.3% ± 2.21; T3= 32.2% ± 6.1. For monocytes ($p < 0.05$. T2 < T3 and T1), T2= 1.48% ± 0.74; T3= 4.56% ± 1.48; T1= 6.07% ± 0.67. At day 3, there were changes in eosinophil concentration ($p < 0.05$. T1 < T2 and T3), T1= 11.5% ± 1.10; T2= 19.2% ± 5.27; T3= 20% ± 4.61. On day 60, there was a variation of the concentration of neutrophils ($p < 0,05$. T1 > T2 y T3), T1= 40.3% ± 7.47; T2= 17.0% ± 4.75; T3= 16.2% ± 0.85. On the contrary, there were no significant differences in BW, SC, TW and TV ($p > 0.05$).

Conclusions: White series changes evidenced a hematopoietic response of the bone marrow to r-met-hu G-CSF. The stability of gross parameters (BW, SC, TW, and TV) after r-met-hu G-CSF administration open the question of whether the drug may be affecting the testicular microenvironment. Future work will be needed to answer if indeed r-met-hu G-CSF accelerates spermatogenesis in treated animals by measuring parameters such as total number of spermatids per testis; daily sperm production (DSP); daily sperm production (DSP) per bull or per testis; and daily sperm production (DSP)/g of testicular parenchyma as a measure of spermatogenesis efficiency in addition to germ cell kinetics. This data will allow determining if r-met-hu G-CSF works as an inductor of sexual development in prepubertal cattle and its possible impact on reproductive function in Brahman bulls.

Keywords: Filgrastim, Puberty, Bos indicus, Hematopoietic response.

RB-P22

Pilot survey of zearalenone in the concentrates and forages fed to low and high fertile Hungarian cows

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Abstract

The objective of this investigation was to study the existence and the level of zearalenone (ZON) in concentrates and forages for cow consumption and their effect on reproduction in Hungary. A survey of ZON was performed in the samples of concentrates and forages of high fertile (45) and low fertile cows (45), concentrates and forages using ELISA. Results indicated that 45 samples those fed to low fertile cows (50.0%) were contaminated in a range from 5.1 to 476 ug/kg.

The positive samples according to ELISA analyses were then confirmed using HPLC method in combination with an immuno-affinity column for clean-up. Before measuring the samples, validation of HPLC method was done using a blank maize matrix. Detection limit based on a signal-to-noise ratio 3:1 was 3.0 ug /kg for ZON in maize samples and the linear range applicable for analyses was 5–900 ug/kg of ZON. Of 45 samples confirmed by HPLC, 36 samples were proved to be contaminated in a range from 7.1 to 601 ug /kg. Correlation analysis of positive samples using the ELISA and HPLC methods showed coefficient correlation factor of R=0.8879.

In conclusion, this present study revealed that low fertility could be attributed to contamination of concentrates and forages contaminated with ZON. This investigation also elaborated that samples' analysis using ELISA and HPLC was good correlated together.

Keywords: Zearalenone (ZON); concentrates; forages; HPLC; ELISA.

RD-P01

Superovulatory response and embryo production in holstein heifers

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Objectives: The Multiple Ovulation and Embryo Transfer (MOET) technique for bovine genetic multiplication has been used commercially since the 1960s. Using the new knowledge of reproductive physiology, such as follicular growth wave control, there was an improvement in the results, but variations in the superovulatory response and embryo production are still limitations to the greater use of this biotechnology. Even donors who respond well to superovulatory stimulation do not always produce embryos in quantity and quality. The objective of this study was to relate characteristics of the superovulatory response (SOV) of holstein heifers with total and viable embryo production submitted to Multiple Ovulation and Embryo Transfer (MOET).

Material and Methods: Data of SOV and embryo production from 309 donors, from the same farm, over a period of 12 months were used. All donors were superovulated in a same SOV protocol using Folltropin™ (Vetoquinol-Brazil), in decreasing doses. Donors were flushing by the same veterinarian. Uterine flushing was performed using DMPBS. Each uterine horn was flushing individually 6 to 7 times. The content of the uterine flushing was evaluated using a stereoscopic microscope in 20x magnification. All localized structures were transferred to another dish containing holding solution and then classified according to their development stage and quality in 40x magnification. The number of ovulations (CLs) was determined by ultrasonography (Mindray M5™-China) immediately before to flushing. A block design was used, dividing the donors according to the superovulatory response. Embryo production, embryo viability and recovery rate in donors of the different groups were compared between blocks using the SAS ProcMix protocol (SAS Software, v9.4), considering significance at 5% probability.

Results: Flushing was performed in all females with two or more ovulations (281 donors - 90.9% of total superovulated donors). In these donors the mean CLs were 9.6±5.6. A total of 6.7±3.7 embryos and 4.3±3.3 viable embryos per donor were obtained. The rate of embryonic recovery defined by total CLs divided by total recovered embryos was 62.7%. The percentage of viable embryos was 70.4%. The donors were divided into 4 groups according to SOV response. G1: 2 to 5 CL (N = 50-17.9%); G2: 6 to 10 CL (N = 97-34.6%); G3: 11 to 15 CL (N = 75-26.8%) and G4: up to 16 CL (N = 56-20.0%). The number of total embryos was 2.5 ± 1.4^{d}; 5.8 ± 2.0^{c}; 8.3 ± 3.2^{b} and 9.7 ± 4.1^{a} ($P<0.05$) and viable embryos was 1.8 ± 1.5^{c}; 4.5 ± 2.2^{b}; 5.7±3.2 and 6.2±4.2 ($P<0.05$) for groups 1, 2, 3 and 4, respectively. The recovery rate was 64.9 ± 33.4^{ab}; 73.6 ± 23.7^{a}; 64.5 ± 23.9^{ab} and 53.9 ± 23.3^{b} ($P<0.05$) and the embryo viability 61.1±41.4; 71.9±27.6; 65.3±28.7 and 60.1±28.9 ($P>0.05$) for groups 1, 2, 3 and 4 respectively. Total embryo recovery was directly related to SOV. The number of viable embryos was positively related to the SOV until the SOV response of 15 CLs per animal. From this, no relation was found. The recovery rate of donors with SOV above 16 CLs (G4) is lower than those with SOV between 6 to 10 CLs (G2). Not always a greater stimulus that promotes a greater number of ovulations is beneficial. Donors with too many ovulations may not have the good efficiency in viable embryos production. According to the literature, this may occur due to anatomical issues related to the increase in the volume of the ovary, which impairs the oocytes pick up and possible hormonal disorders related to many follicles growing simultaneously.

Conclusion: It is concluded that donors that present very large responses to SOV, above 16 CLs, are less efficient in relation to the process, and it may be necessary to reduce the superovulatory stimulus for these animals in a next MOET procedure.

Acknowledgment: Vetoquinol, Biotran, Fapemig, Unifenas, CNPq and CAPES.

Keywords: Corpus luteum, embryo production, superovulation.

RD-P02

Risk and protective factors of milk components on dairy herds days open in northern region of Antioquia-Colombia

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Objectives: The aim of this study was to estimate the amount of milk yield, fat concentration, somatic cell count, milk ureic nitrogen and lactose, and to determine the probability of being risk factors to increase days open (DO) in dairy cows.

Materials and methods: Productive and reproductive data were obtained from a total of 794 primiparous and multiparous dairy cows between 2013 and 2015 from 9 herds allocated in the northen region of Antioquia between 1.800 and 2.900 m.a.s.l, Colombia. Dairy herds used an intensive rotational system of grazing of approximately 40 and 45 days. Botanical composition of forrages included *Cenchrus clandestinus* (Hochst. Ex Chiov.) and at milking (morning and afternoon) concentrate supplement with 16 and 18% of protein was supplied. Productive collected data included milk yield (kg/day), fat (%), Somatic Cell Count (SCC) in tank (cells/ml) and Milk Ureic Nitrogen (MUN mg/dL) and reproductive data recorded was days open (days). The analysis was performed using a logistic multivariate binomial model in which the risk factors had the probability to increase up to 70, 80, 90, 100, 110, 120, 130 and 160 of DO after calving. The statistic model included the effects of the milk components evaluated on the dependent variable days open.

Results: Average milk concentration of somatic cell count and milk ureic nitrogen increased with DO, whereas milk yield and lactose decreased in the assessed periods. Fat content was overall stable. Results demonstrated that all milk compo-

nents significantly affected categorized groups of DO, being somatic cell count and milk ureic nitrogen, risk factors on DO indicating that the chance to enlarge DO of each group was higher with any increase in these factors; fat concentration, milk yield and lactose were protective factors against the DO groups, which means that any elevation in their concentration on milk, lowers the probability of the cows to extend the DO over each categorized group.

Conclusions: Desirable shorten DO after calving are significantly related to milk yield, lactose and fat concentration in milk. Somatic cell count and milk ureic nitrogen should be recorded and controlled frequently in order to maintain/enhance the reproductive behavior.

Keywords: Milk composition, dairy cow postpartum, lactose, somatic cell count, milk ureic nitrogen.

RD-P03

Blood lactate concentration as a diagnostic tool for survival of cow and calf and further fertility after Caesarean section with and without uterine torsion

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Objectives: In case of a uterine torsion, the rotation of the uterus leads to vascular congestion and low tissue perfusion. This can lead to edema, but also bleeding or even tissue necrosis, which in turn leads to a deterioration in the prognosis for the survival and fertility of the cow. Currently anamnesis and clinical examination play the most important role for the prediction of prognosis. Low tissue perfusion causes anaerobic glycolysis, which results in L-lactate production. It is described that blood lactate concentration correlates with prognosis in horses with colic, in dogs with gastric torsion and cows with displaced abomasum or uterine torsion. The aim of this work was to investigate the significance of blood L-lactate of cows intra partum as a prognostic factor for the survival of cow and calf as well as for further fertility of the cow.

Materials & Methods: Retrospectively, the data of 193 cows with Caesarean section were analysed. By negative selection 100 of the 193 cows had a uterine torsion on arrival at the clinic. The data collected included the breed, age, number of births, general condition, cause of dystocia and degree of rotation of the uterine torsion. L-lactate was measured in venous blood using bloodgasanalyser RapidlabTM 248 (Siemens Medical Solutions Diagnostics). Furthermore, it was determined how long the cows had lived after birth or a new pregnancy had occurred and whether the calf was dead or alive at birth. The data were analysed using chi-square-, t- or Mann-Whitney U and Kruskal-Wallis tests and Receiver Operating Characteristic (ROC) analysis were carried out. A p-value of ≤ 0.05 was considered as significant.

Results: The mean lactate concentration of cows with uterine torsion of 3.5 mmol/L was not significantly different from that of animals with other causes of dystocia (3.8 mmol/L). In the animals with uterine torsion, the degree of rotation had no influence on the lactate concentration. However, there was a significant difference between the lactate concentration of animals still alive after half a year (3.1 mmol/L) and those that were dead at that time (4.8 mmol/L; p=0.05). However, the area under the ROC curve with 0.63 showed that the classifier lactate concentration has only a very limited effect on discrimination. At the optimal classifier threshold of 3.85 mmol/L, the specificity was only 0.72 and the sensitivity 0.28. To achieve a specificity of 0.9, a threshold of 7.5 mmol/L was set. The sensitivity dropped to 0.21 and in the Chi-square test, the p-value was 0.08. When using a limit value from the literature of 6.5 mmol/L, the test was not significant. In contrast, there was a significant difference between the lactate concentrations of animals with good or bad general condition (good 3.1 mmol/L versus bad 6.0 mmol/L; $p < 0.01$). A significant difference in the survival rates of the animals was also observed on the basis of general condition ($p < 0.05$). After six months, 72 % of the animals with good general condition were still alive, while only 41 % of the animals with poor general condition were alive.

Neither the lactate concentration nor the general condition of the mother had an influence on the perinatal mortality of the fetuses.

Conclusion: Even if a correlation between lactate concentration and cow survival after a uterine torsion could be proven, an equally good prediction seems to be possible on the basis of clinical examination. The lactate concentration does not seem to be caused by the rotation of the uterus alone. Thus, there was no difference between uterine torsion and other causes of dystocia. In addition, the duration of the uterine torsion and obstetric measures just ahead of measurement could also have an influence on lactate concentration.

Keywords: Cattle, torsio uteri, lactate, prognosis.

RD-P04

Injectable progesterone on timed artificial insemination protocols in dairy cows

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Objectives: Low circulating progesterone (P4) concentrations before and after AI have been implicated as having a negative effect on conception rate, especially in high producing dairy cows In addition, exposure to insufficient P4 concentrations during the development of the ovulatory follicle affects fertility. In this situation, is possible a premature maturation of the ovulatory follicle, leading to ovulation of an 'aged' oocyte of poor quality and compromised subsequent embryo development. The study aimed to evaluate the effects of Progecio™ (Agener União–Brazil), an injectable progesterone product, with concentration of 70mg/mL, in the alteration circulating P4 and of follicular development during a timed artificial insemina-

tion (TAI) protocol in crossbred dairy cows.

Materials and methods: Forty crossbred dairy cows (Girolando breed), from 40 to 90 days postpartum, with body condition score (BCS) between 2,5-4.0 (1-5 scale), daily production above 25 liters, primiparous or multiparous were homogeneously distributed among the experimental groups according to the days postpartum, BCS and size of the largest follicle. The endogenous P4 was blocked by P4 intravaginal device (Primer™ Agener União-Brazil) insertion in D-7 and two doses of 0,5mg of cloprostenol (Estron™ Agener União-Brazil) IM, one of D-7 and the other in D-2. The P4 device was removed in D-1 and all animals were included in a timed artificial insemination protocol (TAI) in D0. The TAI protocol was: D0: P4 intravaginal device insertion and 2mg of estradiol benzoate (RicBe™ Agener União-Brazil); D8: removal of P4 device and 0,5mg cloprostenol IM; D9: 50µg of Lecirelina IM (TecRelin™ Agener União-Brazil) and D10: TAI. On D0 the cows were randomized into G1: 5mL of Progecio® (N = 20) or saline (N=20), all treatments applied via intramuscular. Blood samples were taken to P4 measure on D0, D+2, D+4, D+8 and D+10. Ultrasound evaluation (Mindary M5–China) were made on D0, D+2, D+8 and D+10 for follicle and corpus luteum measure. Progesterone measurements were performed by electrochemiluminescence (ECL), using (Elecsys ™-Roche-Germany). The means of P4 concentration and follicle diameter were compared between the treated and control groups, using analysis of variance (ANOVA). All tests were considered at the 5% significance level and were performed using the SAS Program (SAS Software, v9.4).

Results: The protocol for blocking endogenous P4 was efficient. The cows that were included in the study presented P4 concentrations considered baseline (below 0.3mg / mL). Thus, it can be considered that the circulating concentrations of this steroid, during the study, were due to the P4 device used in the protocol and the Progecio™. The average P4 concentrations were 0.16 ± 0.09^{a} and 0.15 ± 0.07^{a}; 3.22 ± 0.97^{a} and 2.32 ± 0.79^{b}; 2.05 ± 0.31^{a} and 1.72 ± 0.70^{b}; 1.41 ± 0.63^{a} and 1.27 ± 0.40^{a}; 0.15 ± 0.08^{a} and 0.14 ± 0.06^{a} ($P<0.05$) in D0, D+2, D+4, D+8 and D+10 for the G1 and G2 respectively. Progecio® provided higher P4 concentrations ($P <0.05$) up to D+4. In both treatments at D+10, TAI day, P4 concentrations were at baseline. The animal metabolizes the P4 applied during the protocol, therefore, there is no residual P4 at the time of IATF, which could interfere in the ovulation rate. There was no difference ($P> 0.05$) in the average follicle diameter between treatments on any of the evaluation days. This situation indicates that treatment with Progecio™ does not negatively affect the pattern of follicular development.

Conclusions: It was concluded that the application of a single dose, intramuscularly of 350mg od Progesterone (5mL of Progecio™), at the beginning of a TAI protocol in dairy cows leads to a higher concentration of progesterone during the four days of the protocol. The concentrations of progesterone provided by the treatment do not interfere with the normal follicular development of cows.

Acknowledgment: Biotran, Agener União, Fapemig, CNPq, and CAPES.

Keywords: Girolando cows, reprodution, reproductive efficiency.

RD-P05

The association between BHB, NEFA and glucose with progesterone and milk yield duting postpartum in Jersey cows

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Several studies have studied the association between blood metabolites during the early postpartum, milk yield and progesterone in Holstein cows; however, few research trials have been reported within the Jersey breed. The objective of this study was to determine the association between plasma concentration of beta-hydroxy-butyrate (BHB), non-esterified fatty acids (NEFA) and glucose at first week postpartum and plasma progesterone and milk yield up to 28 days postpartum in Jersey cows. The study was conducted in a Jersey commercial dairy farm from the state of Missouri, USA. Cows were milked twice a day with an ME 305 of 7,064 kg. Cows were fed a TMR and were inseminated after estrus detection. The herd had a 21-day annualized pregnancy rate of 36%. Selected cows (n=147) were subjected to a blood sample collection at week 1, 2, 3 and 4 postpartum. Body condition score at calving, parity and milk production up to the 4th week of lactation were recorded. Plasma BHB, NEFA and glucose were determined by approved standard methodologies only from the sample collected at week 1. Plasma progesterone was determined by RIA, weekly up to week 4 postpartum. Milk yield was recorded daily and then averaged weekly up to 4 weeks postpartum. The median value for blood metabolites at week 1 was used as a cut-off to classify the animal as having either low or high concentration of the respective metabolite (BHB, NEFA, glucose). For each metabolite, a mixed model for repeated measures was developed to compare the curves of milk production and progesterone until week 4 postpartum. Main effect was group, defined as the concentration of each metabolite (high vs low based on the cut-off value). In addition, the model considered BCS at calving and parity (primiparous vs multiparous). The interaction between group by week was the most important effect in the models, because it compares the parallelism of curves for progesterone and milk yield through time. For milk yield, cows with low BHB at week 1 postpartum produced less milk at week 1 postpartum ($P=0.001$), but similar production at week 2, 3, and 4 than cows with high BHB. Cows with low NEFA at week 1 postpartum produced less milk at week 1 postpartum ($P=0.006$), but similar production at week 2, 3, and 4 than cows with high NEFA. However, cows with low glucose at week 1 postpartum was associated with more milk yield at week 1 postpartum ($P=0.018$), but similar production at week 2, 3, and 4 than cows with high glucose. For the concentration of progesterone, there were no differences during the 4 weeks between cows with high and low BHB and NEFA ($P > 0.05$); however, cows with high glucose at week 1 had greater concentrations of progesterone at week 4 than cows with low glucose (2.29 vs 3.03 ng/ml, $P=0.009$). In conclusion, cows with low levels of NEFA and BHB and high levels of glucose at week 1 postpartum were associated with lower milk yield only at week 1 postpartum. Only cows with high concen-

trations of glucose at week 1 postpartum were associated with higher levels of progesterone at week 4 postpartum.

Keywords: BHB, NEFA, glucose, Jersey, Progesterone.

RD-P06

Differences in cervical mucus characteristics between repeat-breeder and fertile cows. Preliminary study

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Cervical mucus (CM) is produced by secreting cells on the cervical epithelium. Physical or rheological characteristics of CM vary throughout the cycle as a result of fluctuations in oestrogen and progesterone levels. During estrous, under the influence of high estradiol concentrations, production of CM is particularly abundant which contributes to maintain the vaginal tract moist and lubricated, and plays an important role in sperm selection and transport. Variations in CM characteristics have been linked with the time of ovulation (Layek et al., 2013) and fertility (Lim et al., 2014).

In contrast, under progesterone influence, CM is secreted in less quantity, more dense and viscous, and it acts as a barrier against the entry of microorganisms to the uterus.

Objective: The aim of this study was to compare CM characteristics between fertile cows and cows with repeat-breeder syndrome to determine a potential involvement of CM in this syndrome.

Materials and methods: Thirty-one Holstein cows were evaluated: 13 fertile cows (FC) and 18 repeat-breeder cows (RBC). Repeat breeder cows were considered those that had calved at least once, without detectable disturbances in their genital tract and that needed 3 or more inseminations to become pregnant. Cervical mucus was collected on the day of artificial insemination (AI), after a Ovsynch protocol. To obtain these samples, both perinaeum and vulva were cleaned with a dry paper. After that, a gloved hand was introduced in the vagina to collect a sample, which was subsequently introduced in a 50 ml test tube. Finally, all samples were transported in refrigeration to the laboratory within 1 hour.

The following CM characteristics were evaluated:

1. Quantity, which was classified in 3 categories: absent, moderate and copious. This evaluation was performed during sample collection.
2. Consistency, which was classified in 3 categories: dense, intermediate and fluid .
3. Spinnbarkeit: 2-3 drops were placed on the thumb and squashed with the index finger. Next, both fingers were slowly separated until the mucus broke and this length was measured with a ruler.
4. pH: use of a pH meter.
5. Cellularity: a drop of CM was smeared on a slide and stained with Diff Quick. In samples with epithelial cells and/or leucocytes, a total of 150-200 nucleated cells were counted and the percentage of polymorphonuclear neutrophils (PMN) calculated.
6. Crystallization pattern: a drop of mucus was smeared on a slide and allowed to dry. Afterwards, it was observed under the microscope at magnification of 10x/20x. Crystallization patterns were classified as described by Odeblad (1994).

Data were analysed using the SPSS 20.0 program. Significant values were considered when $p<0.05$. A Chi^2test was used to evaluate volume, consistency and crystallization pattern; one-factor ANOVA and ROC curves were performed to evaluate Spinnbarkeit, pH and cellularity.

Results: Cervical mucus characteristics were not different for FC and RBC. Percentage of PMN in CM tended to be higher ($p=0.074$) in RBC than in FC (53.71±37.82% vs 27.35±40.79 %). ROC curves indicated that cellularity was the characteristic of CM most related to fertility, presenting an area under the curve of 0.705 ($p<0.01$), it a cut-off point of 15%PMN, sensitivity and specificity were 78% and 69.2%, respectively.

Conclusion: It was concluded that the only parameter related to repeat-breeder syndrome is the presence of a high number of PMN in the CM.

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Keywords: Cattle, Infertility.

RD-P07

Can image analysis be used to predict bovine foetal body weight, calving time and risk of dystocia?

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Objectives: There is currently a growing interest in and research about prediction of calving time. However, most of this work does not focus on dystocia, which is of greater interest to farmers. The most common cause, foeto-maternal disproportion (FMD) is due to a disproportionately large foetal body weight. To date it has not been possible to predict foetal body weight, and hence, FMD accurately. In this study it was hypothesized that the profile of abdominal contractions during

calving (which are visible to a farmer) might be related to foetal body weight. Hence, the objective of this proof-of-concept study was to investigate the relationship between abdominal contractions and calf birth weight using an automated computer vision solution.

Material And Methods: The study was carried out between 27th of January 2020 and 28th of February 2020 on a Teagasc dairy research farm in Cork, Ireland. During this period 90 cows calved in a straw-bedded, group calving pen (approximately 75 m^2). Pregnant cows were moved into this area when close to calving, with 1 to 6 cows present at any one time. A camera was installed in the roof of the calving facility and cable-linked to a processing unit. The camera vision system used a standard CMOS image sensor and processor to monitor each individual animal when present in the area under observation. Uniquely, the HerdEye vision system non-invasively detected abdominal contractions during calving of multiple animals simultaneously. The time of calving (calf expulsion) was retrieved from the video files and the details of the dam, calf and calving from the farm records.

Results: Data from a total of 72 calves was collected with 19 calvings unmonitored due to connectivity issues. For this preliminary first communication from this research, a subset of 5 cows was selected which represented a range of dam parities (1-6), calf birth weights (23-40 kg) and included two births where assistance was provided and one set of twins to represent typical calvings encountered by farmers. The data in Table 1 show the characteristics of the dams and their calves, the calving assistance and the results automatically collected by the HerdEye vision system. Abdominal contractions (n=20-45/calving) were first detected within 45 minutes of calf expulsion. There appears to be a positive correlation between the weight of the calf and number of detected contractions as well as the time the first abdominal contractions were detected before birth. It is interesting to note that the abdominal contractions for the twin calves (No. 97 & 98) were only registered 2 minutes before the birth of the first calf (No. 97) with calf No. 98 born 39 minutes later. The cow manifested abdominal contractions for only 4 minutes prior to the birth of the second calf. Contraction data were collected in a group calving area and as a result some contractions were not recorded due to occlusions by one or more of the other cows present. This is a study limitation but also represents the environment in which the majority of calvings occur on modern large dairy farms internationally.

Conclusions: In this novel research study a computer vision solution was used to monitor the abdominal contractions of multiple calving cows simultaneously. An initial assessment showed that heavier calves had an earlier onset of, and greater number of, abdominal contractions. Further data analysis is required to confirm these findings. In future work to validate the system it will be used to monitor isolated cows to reduce occlusion caused by other animals.

Keywords: Calving prediction, automated computer vision solution, HerdEye.

RD-P08

An evaluation of timing of AI with frozen sex-sorted semen in lactating dairy cows in pasture-based seasonal-calving herds

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Objectives: The objective was to use ovulation synchronization with a PRID-Ovsynch protocol for timed artificial insemination (TAI), to evaluate the effect of timing of insemination (16 h or 22 h after the second GnRH) with frozen sex-sorted semen on fertility performance in pasture-based seasonal calving herds.

Materials and Methods: Ejaculates from three Holstein-Friesian bulls that were resident at a sex-sorting laboratory were used. Ejaculates were split and processed to provide frozen sex-sorted semen at 4 x 10^6 sperm per straw (SexedUltra-4M; SS) and frozen conventional semen at 15 x 10^6 sperm per straw (CONV). A pre-breeding trans-rectal ultrasound scan was conducted on all cows, and only those with good uterine health status were enrolled on the trial.

Table 1. Details of five calvings monitored by the HerdEye vision system

Calf No.	Dam No.	Dam parity (No.)	Calf Sex	Calf birth weight (Kg)	Interval between first detected contraction and birth (mins)	Total detected contractions (No.)	Median interval between groups of contractions (mins)	Calving assistance type
98	7796	6	Male	23	40	18	4.75	None
97	7796	6	Female	23.5	2	18	4.75	None
27	403	1	Male	30	13	21	1.82	Hand pull
29	427	1	Female	33.5	44	25	1.94	Hand pull
33	23	3	Female	37	35	42	3.94	None
81	8937	3	Male	40	42	43	2.51	None

A modified PRID-Ovsynch protocol was used for estrous synchronization: on day 0 all cows received progesterone-releasing intravaginal device (PRID Delta®, Ceva Sante Animale, Libourne, France), and an injection of GnRH analogue (Cystoreline®/Ovarelin®, Ceva), on day 7 a luteolytic dose of prostaglandin F2α (PGF) (Enzaprost T®, Ceva) was administered and 24h after a second injection of (PGF) was given and the PRID was removed, all the cows 32h after PRID removal received a second GnRH injection. TAI occurred 16 h after the second GnRH injection for cows assigned to CONV and 16 h sexed semen (SS-16) treatment, cows in (SS-22) received TAI with sexed semen 22 h following the second GnRH injection. Pregnancy diagnosis was conducted by ultrasound scanning 35-40 d after FTAI (n = 2175 records available for analysis). The GLIMMIX procedure in SAS were used to examine effects on pregnancy per artificial insemination (P/AI). Fixed effects included treatment (n=3), bull (n=3), treatment by bull interaction, parity (n=4), DIM category (n=3) and treatment by DIM category, with herd (n=24) included as a random effect.

Results: P/AI was greater for CONV compared to both SS-16 and SS-22 (61.0%, 49.3%, and 51.3%, respectively), and the SS treatments did not differ from each other (relative P/AI for SS-16 and SS-22 vs. CONV were 80.8% and 83.9%, respectively). There were significant bull and treatment by bull interaction effects. The study also identified marked herd to herd variation (the relative P/AI for the combined SS treatments vs. CONV ranged from 50.9% to 116.8%). The best third of herds achieved a mean relative P/AI of 101.5% (range = 93.9% to 116.8%), indicating that P/AI equivalent to CONV is achievable with SS.

Conversely, the lower third of herds achieved a mean relative P/AI of 67.4% (range = 50.9% to 76.9%). Interestingly, the third of herds with the poorest relative P/AI had greater P/AI with CONV than the third of herds with the best relative P/AI, indicating a satisfactory response to the synchronization protocol on the induction of a fertile ovulation event in these herds.

Conclusions: The use of PRID-Ovsynch protocol for FTAI allowed for satisfactory P/AI varying between 60% for CONV to ~50% for SS. The use of SS resulted in lower P/AI compared with CONV semen regardless of timing of AI. Marked variation existed between herds, however, with one third of herds achieving P/AI results equal to CONV. Identification of factors responsible for the large herd to herd variation in P/AI with SS, warrant further research.

Keywords: Sex-sorted sperm, synchronization, fixed time AI, seasonal calving.

RD-P09

Evaluation of an investigative model in dairy herds with high calf perinatal mortality rates in Switzerland

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Objectives: In Switzerland, farmers and practicing veterinarians perceive an increasing rate of perinatal mortality (PM). However, only one study was carried out in Switzerland on PM, over a decade ago, which reported an increasing PM rate from 1.8% to 2.8% (2005-2007). The study neither examined calves nor their dams nor were any further investigations undertaken. The objective of this study was to evaluate an investigative model which encompassed the risk factors, incidence, timing and causes of PM (0-48h) on high risk dairy farms (PM of >5% in the previous year) in Switzerland.

Material & Methods: This pilot-study was carried out on 47 predominantly Holstein PM calves from 21 dairy farms, between September 2016 and January 2018. Gross pathological examinations of calves and placentae as well as histopathological examinations of internal organs and placental tissue were performed. Further investigations included microbiological examinations: broad-spectrum bacterial and fungal culture, detection of *Chlamydia abortus*, *Coxiella burnetii*, pathogenic *Leptospira* spp. and *Neospora caninum* by real-time PCR (qPCR) and of bovine viral diarrhoea virus (BVDV) by Ag-ELISA. Maternal blood samples were used for serology of bovine herpesvirus 1 (BHV-1), *Brucella abortus*, *Chlamydia abortus*, *Coxiella burnetii* and nine pathogenic leptospiral serovars and the evaluation of trace element status. A questionnaire was completed with the farmer, which included general farm characteristics and case-related data. Inbreeding coefficients were calculated for pure-bred matings. Data on the PM rate (0 to 48h) in the recruited herds during the period of study and the parity of the dam, calving age, breed of the dam and calf, number of calves, and degree of calving assistance (slight, severe, caesarean section, no answer) were retrieved from the national database (www.agate.ch). In addition, national-level data on PM rate and dam breed were retrieved from the national database for 2016 to 2018.

Results: At the farm-level, the PM rate was 10.0% (5.3-28.2%) and at the cow-level, 11.5%. These values, from high-risk farms, were approximately five-times higher than the contemporary national bovine PM rate (2.3%) in Switzerland. The risk factors associated with these high PM rates were the self-selection of high risk herds, the high proportion of primiparae in these herds (45%) and the evidence of widespread pathogenic infections on these farms (exposure: 67% of herds,

53% of dams; infection: 57% of herds, 45% of calves). The majority (68.1%) of calves died intrapartum. The most commonly diagnosed initiating/ultimate cause of death (UCOD) was infection (34%) of which *Coxiella burnetii* was the most frequently detected pathogen, by antigen. The most frequently diagnosed proximate cause of death (PCOD) was asphyxia (44.7%), though multiple PCOD was also common (21.3%).

Conclusion: This study was the first detailed investigation of bovine PM in Switzerland. Infectious causes were diagnosed more frequently than expected. While the findings from these high PM Swiss herds may have limited external validity, the investigative model adopted and the detailed research methodologies employed can be replicated and re-evaluated, respectively, in future studies on PM internationally.

Keywords: Perinatal mortality, infection, Coxiella burnetii, necropsy, Switzerland.

RD-P10

Retrospective evaluation of the incidence of later embryo death and early fetal death in high production dairy cows in the Canton of Fribourg (CH)

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Introduction: Late embryonic death is a serious problem in the breeding of high production dairy cattle. Losing a gestation between 30-60 days of pregnancy costs about 600 € to the breeder and we speak of an average incidence of 12-13%.

Objectives: 1st goal: to verify whether late embryonic death is a problem in the district of the canton of Fribourg 2.nd objective: to verify if there is a temporal distribution of the incidence of late embryonic death, which can be related to possible environmental causes.

Materials & Methods: The trial has been conducted in 58 Holstein dairy farms located in the canton of Fribourg (French Switzerland), on a plateau about 600 meters above sea level. A total of 2,500 cows in milk were tested; all the cows were high genetic merit cows (average production 33 L/day). The trial developed between January 2015 and December 2018. The diagnosis of pregnancy was made between 28-35 days of gestation, with an ultraportable ultrasound system (Imago IMV Imaging, Aigle France), with a linear probe of 5.0-7.5 MhZ. A second pregnancy check was carried out after the 55th day of gestation and during this second check the fetal sex was systematically carried out. Embryonic death at the 1st pregnancy test was diagnosed on the basis of the presence of an embryo, but in the absence of a heartbeat. The diagnosis of early fetal death at the 2nd preg-check was made on the basis of the absence of a heartbeat, or on the basis of the essence of the fetus.

Results: The late embryonic death rate was 7.37%, 7.67%, 6.95% and 6.27% respectively in 2015-16-17-18. By analyzing the trend of the incidence of embryonic death per month/year, it is possible to notice the same trend in the four years, being able to identify three peaks of embryonic death, which are repeated in all four years covered by the observation. A peak in March-April-May; a peak in July-August and a peak in September-October. The embryo death rate in the three distinct periods was as follows: 6.57%, 10.12%, 10.37% (2015); 7.57%, 9.45%, 10.98% (2016); 6.33%, 9.65%, 9.75% (2017); 6.61%, 8.03%, 8.73% (2018).

Conclusions: On the basis of the data obtained, it can be said that embryonic death is present in the canton Fribourg, but it does not represent a particularly worrying problem, considering the average value of embryonic death in similar farms in other Countries/Regions. It is possible to make hypotheses to explain the three embryonic death peaks during the year: the first and third, coincide with the beginning and end of grazing. During the same periods there are constant increases in the urea values in the milk. The summer peak can be related to heat stress: between 2015-2018, during the months of July and August, the average temperature in the region ranged between 13º-24ºC, with a humidity of 64%. This leads to a temperature humidity index (THI) above 72, or already in an important risk zone.

Greater attention to nutritional management at the beginning and end of grazing, as an incisive contrast to heat stress, could reduce the incidence of late embryonic death.

Keywords: Embryo death, Ultrasonography, Incidence.

RD-P11

Is twin pregnancy, calving and pregnancy loss predictable by serum pregnancy specific protein B concentration (PSPB) 28-35 days after AI in dairy cows?

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Objectives: Double ovulation occurs more frequently in multiparous cows with high milk production than in primiparous cows and the rate of twin pregnancy/calving is worldwide increasing. Diagnosis of twin pregnancy is possible by ultrasound at the time of early pregnancy examination (28-34 d after AI). Pregnancy proteins are also well known indicators of gestation. The risk of pregnancy loss during the first trimester of gestation for cows carrying twins is three to nine times higher than for cows carrying singletons. PSPB is a good indicator not only for pregnancy but pregnancy loss as well.

Materials and methods: The aims of this study were a) collecting calving data in some Hungarian Holstein-Friesian herds (n=7300) to compare PSPB serum concentration (measured 29-35 days post insemination) in twin and singleton calving cows (Trial 1) and b) to check the predictive value

of PSPB serum concentration for twin pregnancy and pregnancy loss in high producing Spanish Holstein-Friesian cows (n=98; Trial 2).

Results: Our results showed almost 7 % twin calving rate. Although it is a common belief that hormonal treatments are major causes of twin pregnancies, our data do not support this hypotheses. The only exception is the single PGF injection, which significantly increased twin calving. No effect of milk production on the risk of twin pregnancy was found and twin pregnancy increased with parity. AI bull, bull's sire, bull's grandfather and the cow's father affected also twin calving (p≤0.02). We found much higher frequency of twin calving in cows diagnosed pregnant with more than 3 ng/ml serum PSPB concentrationsat 29-35 days after insemination. In Trial 2, non-significant but well-marked differences were found between PSPB serum concentration of singleton and twin pregnant samples (2.1 and 2.9 ng/ml) at different bleeding times.

Conclusions: Although lower pregnancy loss rate (9.4 %) was recorded in twin pregnant than singleton pregnant (55.9 %) cows, no question that twin pregnancy is higher risk factor for terminating pregnancy in cattle.Probably the low size of the study population and the effects of milk production on PSPB values may explain this lack of significance. Our results provide further possibilites for evaluating cattle twin pregnancies through serum protein parameters.

Keywords: Ultrasound pregnancy diagnosis, early foetal period, dairy cattle, PSPB.

RD-P12

Impact of the intramammary infections on reproductive parameter in cows

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Objectives: The mammary gland inflammation is the most common and the most expensive disease of dairy cows. It causes loss of milk production and its sanitary quality, as well as fertility problems. It is the cause of oestrus cycle dysfunctions, delayed appearance of the first post-partum heat, embryonic mortality, as well as deterioration of fertility rates in cattle herd. Reproductive dysfunctions can be seen mainly in cows which manifest acute, clinical inflammations.

Materials and methods: The experiment lasted for one year on a farm of 500 dairy, holstein-fresian cows, kept in loose housing system with the average yield of 9000 litres of milk throughout 305 days-long lactation. Cows classified for the research were examined up to 45 days after artificial insemination. Both clinical and laboratory exams of mammary gland, milk and reproductive organs were performed. The animals were divided into two groups: I group – the experimental group, II group – the control group. The experimental group consisted of 56 cows, 20 of which suffered from subclinical mastitis, 10 cows with the acute mastitis and 26 with the chronic form of the mammary gland inflammation. The II goup – the control one, consited of 16 cows which did not suffer from mastitis. In both groups the following rates were calculated: the conception rate, the pregnancy index and the intrapregnancy intervals. Furthermore, in both groups the microbiological and cytological milk tests were performed.

Results: The pregnancy rate among cows with mastitis clinica was 3,03, for cows with mastitis subclinica 2,1 and 1,9 for cows with healthy mammary gland. The longest intrapregnancy intervals had cows with mastitis clinica – 139,06 days, cows with mastitis subclinica – 105,95 days and 101,6 days for healthy cows. Conception rate was the highest among healthy cows – 25,00%, 20,00% among cows with mastitis subclinica and 5,88% among cows with mastitis clinica. The cultures of milk from the cows with mastits allowed to isolate the following bacteria: Staph. xylosus (31,25 %), Staph. Ureus (25,00 %), Strep. uberis (12,5 %), CNS (12,5 %), E. Coli (9,3 %). Among cows with healthy mammary gland (the control group) changes in the gland were not found, neither was the increase of somatic cell count (SCC). Cows with mastitis subclinica had increased SCC (150 000 – 350 000/ml of milk). Among cows with mastitis clinica changes in the gland tissue were found and the SCC was high (over 400 000/ml of milk).

Conclusion: The clinical forms of mastitis, both clinical and and subclinical, have negative influence on the embryonic stages of pregnancy in cattle and lower cows'.

Keywords: Mastitis, udder, reproductive parameter.

RD-P13

Assessment of reproductive performance of adamawa gudali cattle using methanol leaves extract of *Ficus sur* and PGF2α as synchronizing agent

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Objectives: The primary factor affecting the profitability of animal breeding enterprise is reproduction and the most powerful tool at the breeder's disposal to significantly impact reproductive performance is estrus synchronization. Estrus synchronization optimizes labor and time, facilitates breeding and improves the ease of using artificial insemination in cattle. The study was conducted to assess phytochemistry of *Ficus sur* leaves and the estrus response and conception rates of Adamawa Gudali breed using the plant extract compared to synthetic PGF2α.

Materials and Methods: The research was conducted at the Teaching and Research Farm of School of Agriculture, Modibbo Adama University, Yola, Nigeria. Thirty healthy cows aged between 4 and 6 years were used. The study lasted for a period of 3 months. The animals were sub divided into two groups of 15 each. Group 1 animals were each administered with 20 ml of the plant extract (*Ficus sur*) intramuscularly while group 2 animals were individually administered with 2

ml of PGF2α. The heat period was detected using KaMar heat mount detector. About 35-40 days after the synchronization, the animals were palpated *per rectum* to ascertain their pregnancy status.

The fresh *Ficus sur* leaves were harvested from Sebore Farm, Mayo Belwa, Adamawa State, Nigeria and identified. The leaves were washed, shade-dried and grounded into fine powder. The powder was extracted using 95% methanol. The residue of the powder was screened for phytochemical properties. The concentration required for the dose to be administered was then calculated.

Results: The phytochemical analysis of *Ficus sur* leaves revealed the presence of alkaloid, carbohydrate, tannins, flavonoid, resins, saponins, steroid and terpenoid. Alkaloids have antibacterial effect, tannins have antibacterial, anti-inflammatory, anti-oxidant, anti-convulsant, anti-tumor and accelerate blood clothing. Flavonoids own a number of pharmacological activities such as anti-ulcer, spasmolytic, anti-depressant, anti-bacterial, anti-hypertensive, anti-diabetic, anti-inflammatory, anti-cancer, antioxidant, anticonvulsant, sedative, cardioprotective and hepatoprotective activities, whereas steroids stimulate estrus which showed the therapeutic effects of the plant and its role in estrus synchronization.

All the 15 animals administered with the methanol extracts of *Ficus sur* leaves showed estrus with variation in time of onset. The result showed 6, 3, 3 and 3 animals came in heat after 24hrs, 36hrs, 72hrs and 120 hrs respectively. Thirteen of the 15 (86.7%) animals conceived after natural mating. The estrus response and conception rates indicate the effectiveness of *Ficus sur* leaves extract in synchronization.

However, of the 15 cows administered with PGF2α, 6 came in estrus 36 hrs after synchronization, 4 after 60 hrs and another 2 after 8 day. The remaining 3 cows didn't come in heat. The estrus response rate was 80%. Six of the 12 cows (50%) that came in to heat were diagnosed pregnant after natural mating.

Conclusions: The study showed the *Ficus sur* leave extract is a good synchronizing agent with remarkable estrus response and conception rates than PGF2α. The extract has bioactive ingredients such as steroid that is capable of inducing heat with the ability to regulate the female reproductive cycle. The variation in the time of onset of estrus exhibited by the animals may be due to disparity in stages of follicular and luteal developments at the time of administration of the leaves extract. The methanol leave extract of the *Ficus sur* is economical and should be used as a substitute of PGF2α for estrus synchronization in cattle.

Keywords: Reproductive performance, Adamawa Gudali, Ficus sur, Synchronizing agent.

RD-P14

Positive long-term effect of increased days open on milk production during that lactation and the following lactation in Holstein dairy cows

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Objective: Emphasis on early conception after calving has long been a target as an important step for high productivity in dairy cattle. The objective of this study was to determine if cows that conceived early after calving (low days open [DOPEN]) did indeed have better production compared to cows that conceived later (high DOPEN) in a long-term study encompassing two consecutive lactations.

Materials and Methods: This was a longitudinal observational study based on data collected at 6 dairy farms in the US and Canada ranging in herd size between 300 and 3,000 milking cows. Milk production was automatically measured every milking, every day with milk meters that identified each cow based on a pedometer acting as RFID (afimilk®, Israel).

Cows had to have at least 150 DIM in their third lactation to be eligible for the study, and no more than 7 lactations. Milk production was added over 2 lactations: the lactation when DOPEN were measured and the following lactation. This total production was then divided by the total days elapsed from the initial calving to the end of the next lactation, including days dry, to account for the possibility of an extended dry period in cows with late conception. To account for inter-cow and inter-herd variability in milk production, average milk production in the initial lactation for each cow and herd were included as independent variables, as well as lactation number, days dry and following lactation DOPEN. Data was analyzed by multiple regression, both using DOPEN as a continuous variable and in categories: early (<90 days open [DOPEN]), average (90-120 DOEPN) or late conception (>120 DOPEN). The outcome variable was average milk production throughout two consecutive lactations.

Culling risk in the following lactation was also evaluated by DOPEN in the previous lactation.

Results: A total of 3911 cows were enrolled in the study. Longer DOPEN resulted in higher overall milk production in two consecutive lactations. When evaluated in categories, cows with average DOPEN produced 0.90±0.24 Lbs/day of milk more than cows in the early DOPEN category, while cows with late conception produced 1.58±0.23 Lbs/day of milk more than cows in the early DOPEN category. This production is to be multiplied by the average length of 665±85 days of production in two consecutive lactations (including days dry).

When evaluated as a continuous variable, each additional DOPEN past 50 DIM resulted in 0.016±0.002 Lbs/day extra milk production throughout two consecutive lactations.

There was a farm effect for 4 of the 6 farms, but there was no difference in culling risk.

Conclusion: The effect of DOPEN is not limited to the lactation during which it is measured, there are important productive implications in the following lactation. When evaluating the effect of reproductive interventions, it is important to take

into account that the cows are to be part of the herd for longer than just the studied lactation, and that effects that may seem positive in the short term, may have detrimental long-term implications. Farmers rely on sustained milk production by the same cows over several years. This study shows that cows that get pregnant later in lactation had higher milk production, both in that lactation and the following lactation.

Keywords: Days open, milk production, long-term effects, short-term, culling.

RD-P15

Early estrus after calving results in improved reproduction in Holstein dairy cows

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Objective: It is known that follicles that have grown during prolonged presence of progesterone are more fertile than those that have had little progesterone exposure. The objective of this study was to determine if cows that showed estrus early after calving conceived earlier than cows that had their first heat later in the lactation, closer to the time of first insemination.

Materials and Methods: This was a longitudinal observational study based on data collected at 2 dairy farms in the US with similar reproductive management. First insemination is based on a synchronization protocol (TAI) targeting minimum of 70-72 DIM at first insemination. All cows are fitted with pedometers (afimilk®, Israel) at the time of calving, and activity data is used to determine estrus starting on day 9 after calving, based on thousands of observations that showed this is s common starting day for many cows. Cows were determined to be in estrus when their activity deviated at least 80% from their daily baseline. Activity peaks that coincided with pen changes or hoof trimming were not considered estrus, unless they lasted for 2-3 milking sessions.

Days to first estrus were recorded for each cow. If cows had not shown natural estrus before TAI, days to first estrus was recorded as the day of TAI. Days open and services per conception were used as outcome variables to evaluate reproduction.

Results: A total of 4,010 cows were enrolled in the study. Both farms had similar distributions of days to first heat and average days open. Multivariate regression analysis showed that for each additional 10 days to 1st expressed estrus after calving, days open (DOPEN) increased by 1.54±0.21 days. Another way of expressing this is that for each missed estrus after calving, the first of which is expected around 10 DIM, days open increased by 2.78±0.45 days. There was a farm effect on Farm1, which overall had 11 more DOPEN than Farm 2.

Conclusion: Typically, little attention is paid to recording estrus before the voluntary waiting period (VWP). The results of this study show that reproductive health can be monitored very early after calving, and therefore early interventions can be implemented. The next step is to determine if inducing estrus in naturally acyclic cows can improve reproduction.

Keywords: Estrus, activity monitoring, anestrus, days open, reproductive health.

RD-P16

Vaginal temperature at artificial insemination correlates negatively with the conception rate of dairy cows

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Objectives: To evaluate possible relationships between vaginal and rectal temperatures at artificial insemination (AI) and corpus luteum (CL) size (mm) 7 days post-AI on the conception rate in high producing dairy cows. Moreover, to investigate a possible correlation of both temperatures with heat stress conditions determined with the environmental maximum temperature humidity index (THI).

Materials and methods: This study was performed on a commercial dairy herd of 200 Holstein-Friesian lactating dairy cows in Lleida from December 2018 to December 2019. Cows were fed complete rations and milked two times daily to give a mean annual milk production for cow of 9,550 kg. Only healthy cows free of detectable reproductive disorders and free of clinical diseases during the study period (days -5 to 7 of insemination) were included. Exclusion criteria were the following disorders: mastitis, lameness, digestive disorders and pathological abnormalities of the reproductive tract detectable by ultrasonography. Cows were synchronized for fixed-time insemination (FTAI). Cows were synchronized with a progesterone-releasing intravaginal device (PRID-DELTA; CEVA, Spain) plus Dephereline (100µg gonadorelin acetate [6-D-Phe] Gonavet Veyx, Ecuphar, Spain) upon PRID insertion. The PRID was left in place for five days, and these animals were also given cloprostenol (500 µg; PGF Veyx Forte, Ecuphar, Spain) on PRID removal. Twenty-four hours and thirty-six hours later, the cows received a second cloprostenol dose and a second Dephereline dose, respectively, and were artificial inseminated (AI) 12 hours later. A combination of ultrasonography and manual rectal palpation was used to confirm a cow in oestrus and determine the follicular size as pre-ovulatory. Vaginal and rectal temperatures were measured were with a Thermometer 1312-EN-01. Immediately before temperature measurements, the vulva and the perineal region of the cow were washed with a disinfectant solution and the transducer introduced 10 cm deeply into the vulva and rectum. Cows were AI before temperature measurements with proved bulls. Seven days post-AI the number and size of corpora lutea were registered. Pregnancy was diagnosed by ultrasonography on day 28 post-AI. The following data were registered for each animal: parturition and AI dates; parity (primiparous versus multiparous); follicular and luteal size (mm) determined by ultrasonography at the time of AI and 7 days later; vaginal and rectal temperatures (ºC) at AI and 7 days later; maximum THI the day of AI, insem-

inating bull and pregnancy after FTAI. All statistical analyses were performed using SPSS computer package, version 11.5 (SPSS Inc., Chicago, IL, USA).

Results: The final study population consisted of 88 monovular lactating cows. Mean Fertility during the study period was 34.1%. Mean size (±SD) of preovulatory follicle at AI and CL 7 days post-AI were 18.0 mm (±2.7) and 23.3 mm (±5.6), respectively. Statistical analysis determined that a maximum THI at AI were negatively correlated with the pre-ovulatory follicular size (P= 0.045). Follicular size of the pre-ovulatory follicle was positively correlated with the CL size formed 7 days after AI (P= 0.04). Based on the odds ratio, one ºC increase vaginal temperature at day of AI yielded a 0.37-fold decrease (0.15-0.91) in the risk of positive pregnancy diagnosis (P=0.03). No other variable affected conception rate.

Conclusions: Vaginal temperature correlates negatively with the conception rate on high producing dairy cattle. Increased THI diminished follicular size and subsequent CL size.

Keywords: Artificial insemination (AI); corpus luteum (CL); temperature humidity index (THI); vaginal temperature.

RD-P17

Two cloprostenol doses before artificial insemination protocol was related to an increased corpus luteum size besides an increased pregnancy rate in postpartum dairy cows

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Objectives: To determine whether the addition of cloprostenol at Days 30-36 (pp1) and 44-50 (pp2) postpartum could improve luteal function and pregnancy rates of high producing dairy cows independently of the ovarian structures at pp1.

Materials and methods: This study was performed on a commercial Holstein-Friesian dairy herd in northeastern Spain. During the study period (January 2018-January 2019), the mean number of lactating cows in the herd was 895 and mean annual milk production was 11,250 kg per cow. In postpartum checks, the following puerperal diseases were treated until resolved or until culling: signs of injury to the genital area, metabolic diseases, retained placenta (fetal membranes retained longer than 12 h after parturition), or primary metritis. Cows with a retained placenta or primary metritis were always treated with oxytetracycline boluses introduced into the uterus. At Day 30-36 postpartum cows were randomly assigned to the Control (n=45; no treatment) or PG group (n=90; 0500 µg; PGF Veyx Forte, Ecuphar,Spain). Cows that were at PG group received a further dose of cloprostenol at Day 44-50 postpartum. At days 51-57 postpartum all cows were treated with a progesterone-releasing intravaginal device (PRID) (PRID-DELTA; CEVA Salud Animal, Spain) plus Dephereline (100µg gonadorelin acetate [6-D-Phe] Gonavet Veyx, Ecuphar,, Spain) upon PRID insertion. The PRID was left in place for 5 d, and these animals were also given cloprostenol on PRID removal. Twenty-four h and 36 h later, the cows received a second cloprostenol dose and a second Dephereline dose, respectively, and were inseminated 50-56 h after PRID removal (fixed time artificial insemination-FTAI). Ovarian follicular structures larger than 10 mm in diameter and the absence or presence of one or more corpus luteum (CL) at least 10 mm in diameter were assessed by ultrasonography at pp1 and pp2. CL size was taken as the mean of two measurements approximating the greatest length and width. Pregnancy rate was defined as the percentage of cows that became pregnant at FTAI determined by ultrasonography on Day 28 post-AI out of the total number of cows in the corresponding group. The following data were recorded for each animal: parturition and treatment dates; parity (primiparous versus multiparous); placenta retention; metritis; treatment (Control vs PG); season of treatment (cool-October to April- warm: May to September); luteal and follicular size at pp1 and pp2 (mm); number of CL at pp1 and pp2; inseminating bull and pregnancy after FTAI. All statistical analyses were performed using SPSS computer package, version 11.5 (SPSS Inc., Chicago, IL, USA).

Results: Of the 135 animals, 38 (28.1%), 93 (68.9%) and 4 (29.6%) cows presented no CL, one CL or two CL at pp1, respectively. At pp2, 32 (23.7%), 89 (66.0%) and 10 (7.4%) cows presented no CL, one CL or two CL, respectively. Statistical analyses determine that cows in the PG group has bigger CL at pp2 than control animals (P=0.03). Mean size (±SD) of CL were 20.7mm (±4.0) in the Control group compared to 23.9mm (±4.9) of animals in PG group. Treatment did not affect CL number at pp2. Based on the odds ratio, one mm increase of CL at pp2 yielded a 1.15-fold increase (1.05-1.3) in the risk of positive pregnancy diagnosis (P=0.03). No other variable affected the pregnancy rate.

Conclusions: PG treatment at days 30-36 and 44-50 days postpartum increased luteal size at the second dose of PG. This increase was positively related to fertility of high producing dairy cows undergoing a FTAI protocol.

Keywords: Luteal size, pregnancy rate, postpartum.

RD-P18

Effect of equine chorionic gonadotrophin (eCG) in 7-day GnRH plus progesterone device protocol on TAI pregnancy rate in dairy cows in Argentina

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Objective: Was to evaluate the effect of equine chorionic gonadotrophin (eCG) on pregnancy rate per timed AI (TAI) in dairy cattle synchronized with the 7-d GnRH plus intravaginal progesterone releasing device protocol (DIV) in Argentina.

Material and Methods: The experiment was conducted in a dairy farm in the west of Buenos Aires Province (Argen-

tina) where 805 Holstein cows that were milked twice a day and had a rolling herd average of 9176 liters. Reproductive management included a voluntary waiting period of 50 days and a breeding program that combined AI at detected estrus and TAI. From April to August 2021 all cows were treated with the same 7-d GnRH plus DIV program: d0, buserelin acetate (10.5 µg, im, Gonaxal®, Biogénesis-Bagó) and progesterone DIV insertion (1.0 g; DIB®, Zoetis); d7, DIV removal, sodium cloprostenol (500 ug, im, Ciclase DL®, Zoetis), and were tail-head painted (Fil®, GEA); and d8, sodium cloprostenol (500 ug). On d7, cows with even tag number received eCG (TRT; 400 IU, im, Novormon®, Zoetis; n=391) and cows with odd tag number remained as untreated control (CON; n=414). From d7 to d10, cows were detected in heat and cows that show estrous (>75% paint loss; n=654) were inseminated; and cows that did not display estrous received buserelin acetate (10.5 µg, im) and were inseminated at 72 h after device removal (d10; n=151). Pregnancy diagnosis was performed by ultrasonography at d32 Response variable was pregnancy rate (PR) and explanatory variables were treatment (TRT), days in milk (DIM), body condition score (BCS), milk production (MILKP), parity (PAR), number of AI (NAI), AI technician (TECH), bull (BULL), estrous manifestation (HEAT) and their interactions. Statistical analysis was performed by multiple logistic regression using PROC GLIMMIX from SAS® 9.4 (SAS Institute Inc., 2013).

Results: The BCS (mean ± SE, scale 1-5) was 2.90±0.01, the LAC 1.64±0.03, DIM 136.14±4.27, NAI 2.70±0.10 and a MILKP of 30.15±0.25 l/cow/day. The TAI PR was 27.58% (222/805) and was similar between eCG and control (28.13% [110/391] vs. 27.05% [112/414], P=0.90). Estrous manifestation was 81.24% (653/805) and the PR was higher in cows that showed estrous than non-estrous cows treated with GnRH (29.97% [196/654] vs. 17.23% [26/151]; P=0.004). While in 1 AI cows eCG treatment tended to increase the PR (32.70% [69/211] vs. 26.92% [56/208]), in cows with ≥2 AI PR was not affected (22.78% [41/180] vs. 27.18% [56/206]; eCG TRT by NAI interaction; P=0.06). Primiparous cows tended to have a higher PR than multiparous cows (29.20% [146/500] vs. 24.92% [97/386]); P=0.06). The PR was not affected by the bull or the inseminator (P=0.10; P=0.97).

Conclusions: Cows that showed estrous in a 7-dy GnRH plus a intravaginal progesterone releasing device had greater PR, and eCG treatment tended to increased PR in first AI cows but not in cows with second or greater AI.

Keywords: eCG, GnRH, TAI, pregnancy.

RD-P19

In vitro produced frozen Angus embryos has similar conception rate than in vitro produced fresh embryos in high producing dairy cattle

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Objectives: To compare conception rate (CR) between in vitro produced (IVP) fresh and IVP Angus frozen embryos. Moreover, to analyze whether uterus size of receptor cows affect subsequent CR following embryo transfer (ET) in high producing dairy cows.

Material and methods: Study population consisted in five experimental groups of 32, 28, 21, 25 and 24 Holstein-Friesian lactating dairy cows from five different farms, respectively. The experiment was conducted in Lleida from October 2020 to November 2021. Conception rate (positive pregnancies related to ET) did not differ between experimental farms, and, for that reason, data were analyzed only considering differences between IVP fresh *vs*. frozen embryos.

Ovaries were collected from slaughtered cows from Angus breed and transported in a zyploc bag at 35 ºC. Cumulus−oocyte complexes (COCs) were IVM in 500 µL of IVF-Bioscience commercial media under Nidoil. COCs matured during 24 h at 38.7 °C, 5% CO2, and high humidity. In vitro fertilization (IVF) was performed using commercial frozen/thawed sperm dose from Angus breed. Oocytes and sperm were incubated for 18−20 h at 38.7 °C and 5% CO2 humidified atmosphere. After IVF, COCs were denuded using 125 µM capillary and in vitro culture (IVC). Presumptive zygotes were cultured in 100 µL of pre-equilibrated IVC-Bioscience media under mineral oil at 38.7 °C, 5% CO2, 5% O2, 90% N2, and saturated humidity. Embryos were cultured in groups until Day-7. Excellent and good-quality morulae and early blastocysts were selected. On day 7 post insemination, those selected embryos were slow frozen in 1.5 M ethylene glycol (EG) containing 0.1 M sucrose. Freezing rate was -0.5 ºC/min from -6 ºC (seeding temperature) to -32 ºC.

Cows were synchronized for ET using a progesterone-releasing intravaginal device (PRID-DELTA, containing 1.55 g of progesterone (P4); CEVA, Spain). The PRID was left for 5 days, and cows were also given prostaglandin (25 µg i.m.; Enzaprost T; CEVA, Spain) on PRID removal. Then, 24 and 60 h later, cows received a second prostaglandin dose and a GnRH dose (100 µg gonadorelin i.m.; Cystoreline; CEVA, Spain), respectively. Six days after last GnRH dose, ovulation was determined by the presence of at least one corpus luteum (CL) by ultrasonography and a second GnRH dose was administrated. Finally, 12 hours later cows were submitted to deep uterine unicornual ET ipsilateral to CL. During ET uterus size was evaluated (1: pelvic cavity; 2: between pelvic and abdominal cavity; 3: abdominal cavity). The pregnancy diagnosis was made 21 days after ET and 14 days later, the pregnancy was confirmed. All statistical analyses were performed using SPSS computer package, version 11.5 (SPSS Inc., Chicago, IL, USA).

Results: The final study population resulted in 130 ETs: 81 IVP frozen embryos and 49 IVP fresh embryos. There was no significant relationship between IVP ET frozen and fresh embryos CR (Pearson's Chi-squared = 0,53). Moreover, CR of frozen embryos was not affected by uterus size (Pearson's Chi-squared = 0,58).

Conclusions: IVP fresh and frozen embryos had similar conception rate in high producing dairy cattle. Uterus size did not affect CR of IVP froze embryo. Thus, frozen IVP embryos could be routinely transferred in commercial farms without compromising fertility.

Keywords: Bovine, embryo transfer, in vitro fertilization, conception rate.

RD-P20

Can ovarian follicle size predict the optimal time for mating as defined by an Automated Monitoring System in dairy grazing farms?

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Objectives: Evaluation of heat detection performance on farm has long relied on retrospective data analysis, or expensive serial progesterone testing. The objective of this study was to assess whether the size of ovarian follicle, measured by rectal ultrasonography, can be used to predict optimal breeding time as identified by an Automated Monitoring System (AMS; Allflex® Livestock Intelligence™). This system continuously collects data and, through a proprietary algorithm, converts the data into usable outcomes, such as identification of animals in heat and suggestion of an optimal breeding window. The breeding window displays the potential for successful insemination and conception at various time points leading up to ovulation. It is presented as a 26-hour countdown, with peak heat (height of oestrus related behaviors) at 26 hours after the start of countdown, and the end of the breeding window at 4 hours (-4h) after the peak heat. Previous Allflex internal data have identified an optimal conception period to be between 23 hours and 8 hours after the start of the countdown.

Materials & Methods: Seven dairy grazing farms equipped with the monitoring system and located in the Waimate region (South Island of New Zealand), were enrolled into the study in October 2020. All animals with a heat event recorded by the system that were in the breeding window (countdown position of between 26 hours and -4 hours) at the time of scanning (n=336) were included. Ultrasound examination of the ovarian structures was performed using an Easi-Scan Curve BCF scanner on an 8cm setting. Both ovaries were examined, and the diameter of the largest follicle was measured with up to 1mm accuracy. Information was recorded in a date and time stamped Excel sheet, and a report was exported from the monitoring system at the end of the scanning session to capture the breeding window of each cow. Animals with a follicle size > 25mm were excluded.

The largest follicle size was then related to the time in the breeding window. Animals were defined as being within an optimal breeding window if they were between 26 and 8 hours in the window countdown. Optimal follicle size was calculated based on the Youden's Index. Sensitivity, specificity, predictive values, and likelihood ratios were calculated. Predictive values indicated the greatest proportion of animals identified within the optimal breeding window based on the ovarian follicle size.

Results: Of the 336 cows enrolled in the study, 7 were excluded due to follicular size above 25mm, leaving 329 animals with follicular size and breeding window data available for analysis. Of these 329 animals, 199 (60.4%), were within the optimal breeding window (countdown position of 26 to 8 hours) while the remaining ones were within the wider, non-optimal breeding window (8 to -4 hours). For the Youden index analysis of the optimal and non-optimal groups, at the optimal follicular size cut-off of 15mm, the sensitivity was 0.93, specificity 0.61, positive predictive value 0.81 and a negative predictive value 0.76. Animals with a follicle size ≤ 15mm had a 9.3 times greater risk of not being in the optimal breeding window. Animals with a follicle size of > 15mm had a 2.4 times greater risk of being in the optimal breeding window.

Conclusion: In the presented study settings, animals defined by an AMS to be in the optimal breeding window were with a high level of confidence identified as having optimal follicular size for successful conception. This finding further confirms the suitability of the AMS used in this study as a tool to improve reproductive success in grazing farms. In cases where such a AMS may not be present, assessment of the dominant follicle size at ultrasound examination can aid in monitoring of heat detection accuracy in real time. Moreover, data shows that animals with a follicle size ≤ 15mm can be with high level of confidence considered to be outside of the optimal breeding window. Therefore, in the absence of an automated heat monitoring system, ovarian follicle scanning shows promise as a herd-level tool to identify farms where sup-optimal heat detection may be contributing to poor reproductive performance and allows changes to be implemented on farm at the start of the breeding season.

Keywords: Heat detection, ultrasonography, breeding-time, Monitoring System, breeding season.

RD-P21

Reproductive performance of dairy cows supplemented with a low dose of bypass omega 3 fatty acids

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Objectives: The aim of this study was to evaluate the effect of supplementation with a low dose of bypass omega 3 fatty acids (FA), on reproductive performance of a dairy herd.

Materials and methods: Ninety-one adult Holstein cows were enrolled in the study, they were individually identified and had free access to drinking water. Animals were housed under standard production conditions, where the environmental conditions were controlled through fans. Cows received a production total mixed ration (TMR) supplemented with 250 g/animal/day of HIFLAX® (HFLX), equivalent to 32.5 g of omega 3 FA/animal/d. Cows consumed this ration at least for 2 weeks prior to artificial insemination (AI) and the product was supplied for 8 weeks. Cows that did not consume omega 3 fatty acids prior to artificial insemination were considered as CONTROL group (CTRL).

The reproductive data collected were the number of inseminated cows, number of previous calvings (PM), number of inseminations per cow, days open, and number of cows pregnant. Days open are defined as the time between the calving date and the fertilizing AI. Milk yield and composition were measured at a farm level. The study was an *on-off* trial. Data were analyzed using a mixed-effects model with treatment and number of calving as fixed effects, and the random effect of the cow within the treatment.

Results: It was observed that the pregnancy rate per AI in each experimental group was significantly different (P = 0.004). CTRL cows had a pregnancy rate of 26.8%, while cows that were supplemented with omega 3 fatty acids (HFLX) prior to insemination, obtained a pregnancy rate of 50.3%. When analyzing the first 3 artificial inseminations, HFLX cows had numerically higher pregnancy rate in all of them. However, in the 2nd AI this difference showed a tendency (P = 0.10), and in the 3rd AI, was significantly different (P = 0.05) among treatments. Regarding the total number of AIs required to achieve a pregnancy, significant differences (P = 0.003) were observed between the two treatments. Cows that were not supplemented with omega-3 (CTRL) required on average 3.15 AIs to achieve a pregnancy, while cows consuming HIFLAX® required only 1.5 AIs. The days open had a tendency (P = 0.10) to be different between both groups. In the case of cows supplemented with omega 3 fatty acids (HFLX) this was 100 days on average, however, in non-supplemented cows (CTRL) this time lasted 169 days. Milk yield (kg/cow/d) means were not different (P = 0.9) among the experimental groups. Milk fat (%) had no differences (P = 0.2) among treatments, and milk protein (%) neither (P = 0.8).

Conclusion: Supplementation with omega-3 fatty acids (HIFLAX®) significantly improved the pregnancy rate. This meant that these cows got pregnant earlier, requiring fewer inseminations, and ultimately having fewer days open than cows that did not receive these fatty acids. Milk yield and composition were not affected by omega 3 supplementation.

Keywords: Omega-3, fertility, pregnancy.

RD-P22

Determining the optimal characteristics of the corpus luteum as the key to satisfactory pregnancy results in embryo recipient cows

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Objectives: The effectiveness of embryo transfer (ET) - despite the development of assisted reproductive technics - has not improved in recent years. This results in a constant search for other factors influencing the final pregnancy rate after ET. One of them, often underestimated, is the correct clinical evaluation of the corpus luteum (CL) found in the recipient immediately before ET. The aim of the study was to determine the range of corpus luteum sizes at which the introduction of the embryo into the uterus provides the highest chances of pregnancy.

Material and methods: The analysis included the results of pregnancy of embryo recipients heifers of the Polish Holstein-Friesian breed (N = 282), obtained in the years 2020-2022. The ET was preceded by an ultrasound examination (iScan, Dramiński, Poland), in which:

1) the form of the corpus luteum (cavitary/homogenous) was determined, and

2) the image of CLs widest cross-section was saved in the camera's memory.

Then, based on the ultrasonogram, the surface area of the luteal tissue was measured. In the case of the cavitary CL - the cross-sectional area was reduced by the cross-sectional area of the cavity. Before ET, the embryo development stage (blastocyst/morula) and its quality (excellent - 1, good - 2, poor - 3) were determined. For each of the recipients, the time of Cassou's pistol passage through the cervix for embryo deposition was also determined. The obtained results were analyzed using the STATISTICA 7.1 software and, depending on the type of data, with the Chi² and MIR tests.

Results: Embryo transfer was successful in 38% (107/282). The pregnancy rate was higher in the recipients with the cavitary CL compared to the recipients with the homogenous CL - 45% and 34%, respectively (P<0.05). The cavity inside the CL was found in 107 cows, while 175 CLs had a homogenous structure. The widest cross-sectional area of the average CL was 333.4±124.1 mm² (in the range between 88-848 mm²). Based on the statistical analysis, three groups of CLs were distinguished: Group I - small (<270 mm²), Group II - medium (in the range between 271-452 mm²) and Group III - large (>452 mm²). The percentage of recipients with small, medium and large CLs, regardless of their morphological form, was 36.5%, 47.9%, and 15.6%, respectively. The percentage of recipients with a large CL was significantly lower than with small and medium CLs (P<0.05). At the same time, in the small-sized CL group, a significantly more homogenous CL was found (68%; P<0.05). For the remaining groups, these differences were not significant. The groups described above (I-III) differed in the size of the CL cross-sectional area (212±48 mm², 360±56 mm², 536±78 mm², respectively for Groups I, II, and III; P<0.05). Simultaneously, the other assessed parameters were irrelevant: the quality of the embryo (2.03±0.74, 2.02±0.73, and 1.98 ± 0.82, respectively, for Groups I, II and III; P>0.05), and the time of passage through the cervix for embryo deposition (41.9±30.3 s, 45.0±37.3 s, 45.4±26.7 s, for Groups I, II and III, respectively; P>0.05). In heifers with small and large CLs, the pregnancy rate (31.7% and 31.1%, respectively) was significantly lower (P<0.05) than in heifers with a medium CL (45.4%), and was not related to the stage of embryo development. Concurrently, in the group of recipients whose CL was of medium size, the percentage of pregnancies after blastocyst transfer (57.5%) was significantly higher (P<0.05) than that of morula (40.2%).

Conclusions: Our results suggest that the assessment of the widest cross-sectional area of the CL is one of the important elements in the selection of a recipient in cows. The prognostic significance of this parameter is supported by the possibility of assessing the surface area before making a de-

cision to transfer the embryo. It is also worth pointing out that significantly more pregnancies were found after transferring the embryo to recipients with a cavitary CL. This may indicate a higher production potential of the cavitary form of the CL compared to its homogenous counterpart.

Keywords: Embryo transfer, pregnancy rate, corpus luteum, recipients, heifers.

RD-P23

Detection of *Coxiella burnetii* by PCR in tank milk from cattle herds with reproductive problems in France

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Objectives: Q fever is a ubiquitous disease in cattle, sheep and goat farms. In France, a study has shown that nearly 30% of cattle herds have cattle seropositive for Q fever (Gache *et al.,* 2017). The diagnosis of Q fever remains a daily challenge for practitioners (Viavoice 2020 study, Perceptions of farmers and animal and human health professionals on Q fever). While diagnostic methods during an abortive episode have progressed, combining PCR and serological analyses, screening for Q fever without abortions remains uncommon. A first step in herds with reproductive problems may be to identify if *Coxiella burnetii* is actively circulating. The objective of this study was thus to evaluate the frequency of detection of *Coxiella burnetii* by PCR applied to tank milk, in dairy cattle herds for which reproductive performance is disappointing.

Materials and methods: Two methods of animal recruitment were implemented, each corresponding to a different entry route for triggering investigations when reproductive troubles are suspected.

Firstly, recruitment via veterinarians was implemented. Eligible dairy cattle herds had to present both a clinical suspicion of Q fever "excluding abortion" (abnormal incidence of metritis, retained placentas, premature and/or weak calves) as well as an alert on reproductive performance: rate of renewal, success rate at first artificial insemination (AI), number of cows and heifers with more than 3 AI, objectified in particular by figures compared to the first quartile of the breed concerned on the Reproscope website: http://164.177.30.205/pentaho/api/repos/:public:reproscope:accueil_reproscope.wcdf/generatedContent?&userid=public_user&password=_Repr0sc0pe_Pu). Knowledge of farms has allowed to refute the involvement of most of the other classic factors negatively impacting reproduction (e.g., feeding, management, building, other health problems). Thus 288 farms were recruited between June 2020 and December 2021.

At the same time, recruitment was implemented by analyzing the annual reproduction reports, resulting from performance checks in September 2021. Farms located in Pays de la Loire, France were targeted for poor reproductive performance. To limit bias, only Holstein breed herds of more than 30 animals, with a barn average between 8500 and 9500 kg/L/year were considered. Eligible herds had to have 3 degraded reproduction criteria (success rate at first AI, rate of cows with more than 3AI and percentage of late returns in heat according to the annual reproduction report as of March 31, 2021). 136 farms among this population were targeted.

The analysis method was the same in both cases: real-time PCR on bulk tank milk.

Results: In the first population, 46% of the 288 farms investigated by veterinarians had a positive PCR, attesting an active circulation of the bacterium on the farm, and with a probability that Q fever was the cause of the poor reproductive performances observed. On these farms, the continuation of the investigations to confirm a suspicion of *Coxiella bunetii* involvement may be relevant (for example with individual serological surveys on cows with reproductive problems).

Of the 136 farms with reproductive problems targeted during the performance check, 21.3% were positive.

Conclusion: Whether on simple criteria (clinical, macroscopic analysis of reproductive performance), or detailed analysis of reproductive performance over a season, *Coxiella burnetii* was detected by PCR on bulk tank milk in 1 farm out of 5 to 1 farm out of 2, depending on the recruitment method of the farms.

A bulk tank milk PCR can be a good first orientation analysis to judge the relevance of further investigations to confirm the involvment of *Coxiella burnetii* in reproductive disorders in dairy cattle.

Keywords: Coxiella burnetii, Q fever, reproduction, epidemiology.

RD-P24

Postpartum events associated with the uterine health of dairy cows during the voluntary waiting period

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The objective of the study was to establish associations between type of calving, postpartum puerperal examination, vaginal discharge and the absence of clinical endometritis in dairy cows during a 45-day voluntary waiting period.

Materials and methods: Eighty three Holstein cows were selected (50 primiparous and 33 multiparous) belonging to a dairy located in Saturnino María Laspiur, Argentina from 9/20/21 to 11/15/21. Twenty-one days before delivery, they entered there were located in a pen where they were constantly monitored and assisted the delivery if it was required. Cows that calved without assistance were considered Normal calving, while cows that received mild to moderate assistance were considered Assisted calving. The puerperal control was

performed at 6.9 ± 2.7 days postpartum (range 2 to 12 days) where cows with an abnormally enlarged uterus and a fetid, watery reddish-brown discharge, were considered with Metritis, while cows that did not presented this condition, were counted as Healthy. Cows with Metritis were treated with an Antipyretic Antibiotic for three days and, in the case that they did not respond to the previous treatment, a long-acting injectable Antibiotic. At 32.7±5.9 days postpartum (range 23 to 43 days) they were examined with a Metrickeck® and classified into 4 categories according to vaginal discharge: 0=clear or transparent mucus, 1=mucus containing whitish flocs of pus, 2=discharge containing ≤50% mucopurulent material, 3=discharge containing >50% purulent material. Scores 0 and 1 were considered as Mucosal Discharges, while scores ≥2 were considered as Purulent Discharges. After the vaginal check-up, a transrectal ultrasound was performed to detect uteri without content or images compatible with purulent endometrial content, which were considered as Clinical Endometritis. To analyze the categorical data, a multiple correspondence analysis was used while, to establish the associations between type of calving, category of puerperal examination, vaginal discharge, and Clinical Endometritis, chi-square tests were performed and the Phi coefficient was calculated to determine the grade of association between the variables evaluated.

Results: The 84.1% of calvings were Normal, 59.8% of the cows were considered healthy during the puerperal examination, 56.7% presented mucous vaginal discharge and 72.3% of the cows did not present Clinical Endometritis. In the analysis of associations of the uterine health during their postpartum period, it was determined that the first two components represent 71.0% of the variability of the data, therefore it is an adequate representation. Uterine health and disease were different. In the case of the cows that had a "Normal" calving, they were characterized by being healthy during the puerperal examination, presented Mucous discharge and did not present Clinical Endometritis. In the opposite case, it is observed that cows that had calving "Assistance", were diagnosed with Metritis, presented Purulent discharges and were diagnosed with Clinical Endometritis. No associations were established between type of calving with puerperal examination (P=0.2756, Phi=0.12) and between type of delivery with clinical endometritis (P=0.3623, Phi=0.10) but significant slight associations were found between type of delivery and vaginal discharge (P=0.04, Phi=0.22) (Pett, M., 2015) where, 61% of the cows that had a "Normal" calving presented a mucous vaginal discharge which, in "Assisted" calving it was only 31%. Associations were determined between the puerperal examination and vaginal discharge (P=0.0339, Phi=0.24) which were strong between vaginal discharge and Clinical Endometritis (P=0.0001, Phi = 0.71). The 66% of healthy cows during the puerperal examination, presented a mucous vaginal discharge and 44% in the cows with Metritis. On the other hand, 100% of the cows with mucosal vaginal discharge were not diagnosed with Clinical Endometritis while, only 36% of the cases with purulent discharge, were healthy.

Conclusions: It is concluded that the uterine health of cows during the voluntary waiting period is associated with "Normal" calvings, a healthy puerperal diagnosis, a mucous vaginal discharge and the absence of Clinical Endometritis. Puerperal disease and clinical endometritis were not associated with the type of calving, whereas puerperal examination at week postpartum and vaginal discharge and vaginal discharge with clinical endometritis at 30 days postpartum was closely associated. These associations made it possible to identify cows with affected uterine conditions during an early puerperal examination that should be monitored during the voluntary waiting period to minimize their potential negative impact on future fertility.

Keywords: Uterine health, dairy cows, voluntary waiting period.

RD-P25

Effect of metritis on postpartum ovarian cyclicity of primiparous dairy cows

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Introduction: Uterine diseases cause infertility, not only by compromising the endometrium but also by affecting ovarian function. High systemic inflammations during the early postpartum period is negatively associated with the ovulation of the first dominant postpartum follicle in dairy cows. The objective of the study was to determine if the postpartum cyclicity of primiparous dairy is affected and therefore the voluntary waiting period, by the presence of metritis during the first 2 weeks postpartum.

Materials and methods: Seventy three (n=73) Holstein primiparous cows were selected from a dairy located in Saturnino María Laspiur, Córdoba, Argentina from 9/20/21 to 11/8/21. The puerperal examination was performed at 7.6 ± 3.8 days postpartum (range 2 to 21 days) where cows with an abnormally enlarged uterus and a fetid watery reddish-brown discharge were considered with Metritis and, those without this condition, Healthy or Normal. Cows with Metritis were treated with Antipyretic Antibiotic (Duvamycin 5,000,000) for three days and long-acting injectable Antibiotic (Oxytretracycline) in the case that they did not respond to the previous treatment. At 35.0 ± 6.9 days postpartum (range 23 to 48 days) a transrectal ultrasound examination was performed to detect the presence of follicles and / or corpus luteum in the ovaries; Cows with the presence of a corpus luteum in one of the ovaries were considered as Cyclic, while cows with an absence of uterine tonicity and the presence of only follicles in the ovaries were considered in Anestrus. The Mann-Whitney Test was performed to determine the similarity in the postpartum days when the Normal and Metritis cows were diagnosed and checked. Chi square tests were performed to establish the association between uterine disease and postpartum cyclicity.

Results: The days on which the puerperal exams (P = 0.2363) and postpartum cyclicity (P = 0.9423) were performed were similar between groups. The 83.3% and the 80.6% of

Normal and Metritis cows respectively, had Normal calvings (P> 0.05). An association was established between the puerperal examination and postpartum cyclicity (P = 0.0472). At 35.0 ± 6.9 days postpartum, 68.2% (30/44) of the Normal cows presented a corpus luteum, while 55.2% (16/29) of the cows with Metritis were in anestrus.

Conclusions: It is concluded that there is an association between puerperal Metritis and cyclicity in primiparous dairy cows, producing a delay in cyclicity of more than 20% before 40 days postpartum in cows that were treated during the immediate postpartum period.

Keywords: Metritis, postpartum ovarian cyclicity, primiparous dairy cows.

RD-P26

Treatment with intramuscular minerals one month before FTAI improve body condition score in first-service Girolando heifers

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This study aims to evaluate the effects of intramuscular mineral supplementation (Fosfosal®) on body condition score in first service Girolando heifers submitted to FTAI. Our hypothesis is that the intramuscular treatment with minerals thirty days before the FTAI protocol improve heifers body condition score (BCS). Five hundred and twenty-three Girolando heifers (n=523) with an average age of 16,0±6,0 months were weighed (average of 364,0±35,4kg) and evaluated for body condition score (average of 3,19±0,40) on Day -30. At that time, the heifers were divided into two groups according to the weight and the BCS to receive the treatment with 10 ml of intramuscular minerals (Fosfosal®; n = 261) or placebo (n = 262). Thirty days later, heifers were reevaluated for BCS on the first day of the FTAI (D0). For statistical analysis, a 2 x 3 factorial design was used according to the treatment (Fosfosal® or Placebo) and the weight on Day -30 divided into tercis [low (less than 350 kg), intermediate (between 351 and 379 kg) and high (more than 380 kg)]. The analyzed variables were the mean BCS at D0 (mean ±SEM) and the BCS *delta* (ΔBCS; mean ±SEM), i.e., the difference between BCS on D0 and D -30. There was no interaction (P > 0.05) between the treatments (Fofosal® or placebo) and weight classes (low, intermediate and high) for the BCS on D0, thus, only the main effects will be presented. Heifers that received treatment with intramuscular minerals (Fosfosal®) showed higher mean BCS on D0 (P = 0.01) when compared to heifers in the placebo group (3.23±0.03 vs 3.18±0.03, respectively). However, there was an interaction (P = 0.04) between the treatments and weight classes for ΔBCS. Heifers treated with intramuscular minerals (Fosfosal®) improved ΔBCS, especially in lower (3.15±0.03 vs 3.10±0.02, respectively) and intermediate (3.22±0.03 vs 3.16±0.03, respectively) heifers, while heifers classified as higher, variations were negative (-0.08±0.03 vs -0.07±0.03, respectively). The BCS at the beginning of the FTAI protocol was higher in heifers that received intramuscular minerals (Fosfosal®), especially in those classified as low and intermediate weight.

Keywords: FTAI, Girolando, BCS, intramuscular minerals.

RD-P27

Risk factors and causes of abortion in dairy herds – has the infectious profile changed?

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Objective: Since the commencement of BVD eradication in 2013, dairy herd expansion following EU milk quota abolition in 2015, increased use of vaccines and improved herd fertility, the profile of abortion in Irish dairy herds may have changed. Hence, the objective of this longitudinal study was to monitor both the risk factors for, and the causes of, abortions in Irish dairy herds since 2015.

Materials and methods: A whole herd, prospective, active surveillance model was used to investigate all recorded abortions (<260 days) in 40 Munster (southern) dairy herds over seven years. A text-and-collect service was provided. In total 210 foetuses (and 29 placentas) were examined.

Results: ***Characteristics of abortion***

Abortions occurred between September and May, with a peak in January (breeding April-July; calving January-April) This profile corresponded to abortions from the fifth to ninth month of gestation, with a peak in the ninth month. These foetuses varied in weight from <1kg to 34kg, averaging 17kg. While the majority of abortions occurred in cows, some 33% were in first calvers. Most cows were mated to dairy sires with 30% to beef sires. The majority of affected cows were bred by AI, with 25% served by natural service bulls. The majority of cows were in good body condition (3-3.5), with some 10% <3 and 5% >3.5. There were more female (55%) than male foetuses (45%), with a high proportion of multiple foetuses (nearly 20%). The majority (~66%) of foetuses were moderately or badly decomposed. In 95% of cases, the foetus was reported as dead at birth/found dead (though necropsy examination showed some 15% of calves had breathed). Very few cows (~15%) were noticed with signs of abortion; restless, mounting other cows, swollen udder, vaginal discharge on cow/in cubicles. In some (~10%) cases, the foetus was malpresented; 30% of abortions were assisted.

Causes of abortion

A clinico-pathological diagnosis was made in half of all abortions using the history, the necropsy and the laboratory findings (Table 1). The two most important causes were infections (~30%) and lethal congenital defects (~10%). The

most commonly detected primary pathogens were *Trueperella pyogenes* (11%), *Leptospira* spp. (5%; PCR), *Listeria monocytogenes* (4%), *Neospora caninum* (4%; PCR) and *Bacillus licheniformis* (3%). The majority of congenital defects affected multiple body systems, likely due to *de novo* mutations. In the majority (~60%) of cases of no diagnosis, the foetus was decomposed.

Table 1. Summary of diagnostic findings in 210 aborted foetuses

Diagnostic group	Subcategory (No.)	No.	%
Infection[a]	Single (52), coinfection (4), dam (4)	60	28.6
Congenital defect	Individual defect (9), multiple defects (8)	17	8.1
Other COD[b]	Maternal stress/trauma, twin-twin syndrome	17	8.1
Multiple COD	Infectious and non-infectious	13	6.2
Diagnosis not reached	Fresh/mild (42), moderate/marked (61) foetal decomposition	103	49.0
Total		210	100

[a]Includes primary pathogens only (secondary pathogens were detected in 32 other foetuses); [b]COD = cause of death.

Conclusions: As expected, the majority of reported abortions occurred in the last trimester (larger foetus and housed cows, so easier to observe) but the peak in January (>40% of which were after Jan 15th, so probably during the calving season) is perhaps surprising. Abortions tend to be associated with the autumn in spring-calving herds. This novel finding and the high assistance rate at abortion (30%) indicates that farmers need to take personal and herd health biosecurity precautions, even during the calving season. This is particularly relevant, as imminent signs of abortion were not observed in most (85%) cases. Most diagnosed abortions were due to sporadic pathogens carried by cows or in their environment. Key preventive measures include maintenance of herd immunity through nutrition and good farm hygiene (sporadic infections), effective prevention and therapy of metritis/endometritis/mastitis (*T. pyogenes*), feeding well-preserved silage (*L. monocytogenes, B. licheniformis*, fungi), preventing canid access to placentae/dead calves (*Neospora*) and vaccination (*Leptospira, Salmonella, BVD, IBR*). Surprisingly, salmonellae were not detected; this may reflect high vaccination rates and the success of the BVD eradication programme. The low placental submission rate (~15%) needs to be improved to increase the diagnosis rate.

It is concluded from this new research that in a cattle population with widespread vaccination and ongoing BVD eradication, most abortions may now be caused by sporadic rather than endemic infections.

Keywords: Dairy, Abortion, Causes, Infection, Defects.

RD-P28

Best practice recommondation for calving management: a survey from veterinary subject matter experts and non-experts

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Objective: Optimal calving management enhances the chances for a good start into new lactation for the mother and a healthy start into life for the calf. The objective of this study was to filter best practice recommendation for calving management practices for dairy cows. Special focus was on reliable signs of onset of parturition and if / when to move cows into a maternity box.

Material and Methods: We designed a questionnaire with 18 questions on imminent signs of parturition (SIP), definitions of stages of calving, information on influencing factors during calving, technical devices, and management of dystocia. Questionnaire was sent to either subject matter experts (SME, n = 80) or given to veterinary practitioners (non-SME) at a workshop for dairy specialists (n = 24). SME were defined as authors of studies on "Calving Management OR Calvit eng Prediction" in peer-reviewed journals.

Results: We received forty seven (23 SME and 24 non-SME) questionnaires suitable for data analysis. Most respondents (89.7%) agreed, that it is beneficial for successful calving management to differentiate between stage I and II of parturition. Respondents named 12 signs of imminent parturition (for stage I and II); “restlessness” and “visibility of fetal parts in vulva” were most frequent on the list of SME and non-SME. There was no consensus of respondents on the right time to move the cow to the maternity pen, recommodations varied from one to over 21 days before expected calving date. Next to half of the respondents (45.7%) recommended a 6-hour observation interval for prepartum cows in the maternity pen.

Conclusion: This study identified a strong consensus on the SIP and how and when to observe cows prior to parturition. SMEs and non-SMEs provided broadly similar recommendations.

Keywords: Calving, management, experts, survey.

RD-P29

The accuracy of color-Doppler ultrasonography of the corpus luteum on Day 22 post artificial insemination to detect pregnancy in Holstein heifers

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The objective of this study was to evaluate the efficacy of Doppler ultrasonography of the corpus luteum on Day 22 post timed artificial insemination (TAI) to detect pregnancy in Holstein heifers. Eighty-nine Holstein heifers 14-month-old received a progesterone intravaginal device (0.5 g progesterone) on Day -8, a luteolytic dose of prostaglandin and device removal on Day -3 and GnRH and TAI (sexed semen) on Day 0. On Day 22 post-TAI, transrectal color-Doppler ultrasonography of the CL was conducted to evaluate the blood irrigation of CL and heifers were considered open or pregnant according to previously described criteria (Siqueira et al., 2013). On Day 25, pregnancy was determined by transrectal color ultrasonography of the uterus according to previously described criteria (Kastelic et al., 1988). On Day 22 and 25 blood samples from the coccygeal plexus were collected and serum pregnancy-specific Protein-B (PSPB) concentrations were determined using a quantitative ELISA [BioPRYN®, BioTracking Inc., ID, USA]. Pregnancy based on PSPB was determined following the manufacturer's recommendations. Specificity, sensitivity, positive and negative predictive value for color-Doppler ultrasonography of CL on Day 22, serum concentration of PSPB on Day 22 and ultrasonography of the uterus on Day 25, were determined using the serum concentration of PSPB on Day 25 as gold standard. Serum PSPB concentration on Day 25 detected 42 pregnant and 47 open heifers. Transrectal ultrasonography of the uterus on Day 25 detected 43 pregnant and 46 open heifers. Transrectal color-Doppler ultrasonography of the CL on Day 22 detected 56 pregnant and 33 open heifers. Serum PSPB concentration on Day 22 detected 20 pregnant and 69 open heifers. The specificity, sensitivity, positive predictive value and negative predictive value were 97.9%, 100%, 97.7%, and 100%, respectively for transrectal ultrasonography of the uterus on Day 25, 100%, 47.6%, 100%, and 68.1%, respectively, for serum PSPB concentration on Day 22 and 70.2%, 100%, 75%, and 100%, respectively, for transrectal color-Doppler ultrasonography of the CL on Day 22. In conclusion, serum PSPB concentration does not increase enough 22 days post-TAI to detect pregnancy and color-Doppler ultrasonography is a useful technique to detect non-pregnancy 22 days post-TAI, but it has a low specificity (29.8 % false positives) for detection of pregnancy.

Keywords: Pregnancy-specific Protein-B, Pregnancy, Color-Doppler, Ultrasonography, Holstein heifers.

RD-P30

Ovarian dynamics. Progesterone profiles and conception rate of a TAI protocol using prostaglandins and 17b estradiol as presinc treatments

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Introduction: Ovulation synchronization protocols that use a combination of estrogens, progesterone and prostaglandin (PG) present dispersion in the resumption of the follicular wave, follicular growth with low progesterone, incomplete luteolysis after PG, differences in the size of the dominant follicle and timing and ovulation failures. The use of pre synchronization treatments had minimized these inconveniences. The objectives of the study were to evaluate the ovarian dynamics, the progesterone profiles and the conception rate of a new protocol of synchronization of ovulation in relation to a conventional protocol of synchronization of the ovulation based on estrogens and progesterone.

Materials and methods: The study was carried out at the EEA INTA Rafaela dairy farm, Argentina from 5/4/20 to 10/10/20.

Two synchronization protocols were compared: Modified Conventional (MC) and Fertility Protocol (FP). The MC consisted of a 0.7 g of P4 intravaginal device (ID) insertion and 2 mg IM of estradiol benzoate (day -9). Upon withdrawal of the ID (day -2), 0.15 g IM of D+cloprostenol (PG), 1 mg IM of estradiol cypionate and tail painting were applied. A second PG was injected 24 hours later (day -1). The FP "pre sinc" treatment consisted on the IM injection of 0.15 g PG on day-12, 2 mg of 17 B estradiol on day -9. On day -6, 0.7 g of P4 IDs were inserted and then removed on day -2 together with PG injection and tail painting. Twenty four hours later (day -1), a second dose of PG and 1 mg IM of estradiol benzoate were injected. On day 0, 60 hours after the ID was removed, TAI was performed in both groups, considering to be "in heat" and "not in heat" according to the total or none removal of the tail paint respectively. To the last ones, 0.0105 g of buserelin acetate (GnRH) was applied. Ovarian dynamics was performed in 25 Holstein cows (FP (n=8) and MC (n=17)) with 57.2±3.3 days postpartum with 2.64±0.26 body condition score (1-5) and 31.8±6.0 L/d of milk, which were transrectal ultrasound examined on days -12 and -9 to determine ovulation rate at the beginning of the protocol (OR) while, to establish the sizes of the corpus luteum (CL) and of the preovulatory dominant follicle (PDFPO) at the time of DI removal, it was performed on day -2 and, to determine the size of the ovulatory dominant follicle (ODF) at fixed time artificial insemination (TAI), on day 0. Additionally, at the beginning of the protocol and at the time of ID removal, blood was extracted with EDTA to determine progesterone(P4) concentration by Chemiluminescence. Conception rate (CR) was analized in another group of 173 Holstein cows (FP (n=106) and MC (n=67)) with 81.6±57.3 days postpartum and 28.8±7.5 L/d of milk. To establish differences between protocols in the PDFPO, PDF and CL, ANOVA was performed. To detect differences in P4, the Mann-Whitney U

test was performed for independent samples and differences in proportions to compare the OR and the conception of the protocols.

Results: The OR was 100% and 23.5% for FP and MC respectively (P= 0.000458). The PDFPO (13.27±2.82 mm FP vs. 15.87±5.76 mm MC), the CL (23.28±4.87 mm FP vs. 22.84±8.33 mm MC) and the ODF (15.73±3.67 mm FP vs. 14.23±2.23 mm MC) were similar between protocols (P>0.05). The P4 at the beginning of the protocol was 0.61±0.37 ng/mL in FP and 9.30±2.89 ng/mL in MC (P=0, 0006), while at ID withdrawal it was 4.30±2.87 ng/mL and 1.23±1.70 ng/mL for FP in MC respectively (P=0.0056). The conception of the FP was 43.4% (47/106) and 22.4% (15/67) in MC (P=0.00352).

Conclusions: The new fertility protocol induced a higher rate of ovulation at the start of treatment, increased the concentration of progesterone at the time of device removal and duplicated the conception rate of dairy cows. Even though the several steps, this suggests to put focus on the development of pre synchronization protocols in the dairy cows that ensure higher conception rates than the traditional ones.

Keywords: Ovarian dynamics, progesterone profiles, conception rate, prostaglandins, 17B estradiol.

RD-P31

Association between anogenital distance and specific body measurements in Holstein-Friesian heifers - preliminary results

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Objectives: Anogenital distance (AGD) is a sexually dimorphic trait that is believed to be a biomarker of prenatal hormonal exposure. In females, it is defined as the distance between the center of the anus and either the dorsal commissure of the vulva (AGDv) or clitorial base (AGDc). Studies in humans, rodents, and rabbits have shown that in females, a longer AGD is associated with reduced reproductive performance. In the little research that has so far been published on AGD in cattle, promising results to use this measurement as an indicator for female reproductive success have been mentioned (Gobikrushanth et al., 2017, 2019; Carrelli et al. 2021). To the best of our knowledge, there however is no bovine study available in which the AGDv and the correlation between both AGDs and body morphometrics have been determined. The principal objective of the present study was to generate normative data of both AGDs in nulliparous Holstein heifers at reproductive age and to determine their association with other body measurements that are known indicators of heifer growth and development.

Material and methods: Dairy farms were selected based on their willingness to collaborate and on their availability of accurate data on herd and animal level. All available nulliparous heifers between 12 and 15 months of age were included in the study. AGDs (AGDv and AGDc) were measured using digital callipers (mm). Heart girth (HG), height at the hip (HH) and ultrasonographic back fat thickness (BFT) were measured using standardized procedures, to assess the overall size and development of the heifers. Statistics were performed in R studio version 3.3.6. Normality of the variables was assessed using histograms and Shapiro-Wilk tests. Pearson correlation coefficients were calculated to measure the correlation between individual body measurements.

Results: From February till October 2021, we measured 512 Holstein Friesian heifers on 22 Belgian and Dutch dairy farms. Animals were on average 13.6 ± 1.11 months old. Heart girth (176 ± 7.3 cm), HH (138 ± 4.1 cm), AGDv (62.9 ± 7.99 mm) and AGDc (108.0 ± 9.39 mm) were normally distributed, while BFT was slightly skewed (14 ± 3.7 mm). The most significant correlation found was between HH and HG (r=0.55, P<0.001), followed by the correlation between HG and BFT (r=0.42, P<0.001). None of the correlations calculated between the overall body measurements and AGDs were significant. However, BFT was slightly correlated with AGDc (r=0.11, P=0.014) and tended to be correlated with AGDv (r=0.08, P=0.074). The correlation between AGDv and AGDc was 0.50 (P<0.001).

Herd has a significant effect on both AGDs, so it was included as a random effect in all models. Age of the heifer had a significant influence on HH, HG and BFT with all measurements being greater in older heifers (P<0.001). In contrast, AGDv and AGDc were not affected by the age of the heifer.

Conclusion: Results suggest no strong correlations between AGDs and body measurements. That is important information that we will include in our future studies about the potential association between AGDs and reproductive performance. Age is not significantly associated with AGDv nor AGDc. The latter might indicate that the AGD is largely determined prenatally and less influenced by the overall body growth and development of the heifer.

Keywords: Anogenital distance, dairy heifer, hip height, heart girth, back fat thickness.

RD-P32

Effect of type of anticoagulant, transportation time, and glucose supplementation to the culture medium on circulating neutrophil viability and functionality in dairy cattle

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Objectives: There is a state of immune dysfunction in the peripartum of dairy cows, which may lead them to the development of infectious diseases. White blood cells, specifically polymorphonuclear neutrophils (PMN), play a vital role in the innate immune response in the early postpartum. Thus, in dairy research, *in vitro* assessment of innate immune function is commonly evaluated by the flow cytometric assessment of phagocytosis (P) and oxidative burst (OB) of circulating PMNs. However, some common methodological aspects may affect the outcome of the procedure. We hypothesize that the presence of sugar in the anticoagulant of the blood collection tubes (e.g., citric acid, trisodium citrate, and dextrose (ACD) coated tubes), the transportation time, and the glucose concentration in the culture medium in which the PMN function is performed, influence the PMN viability and functionality. Our objectives were to compare the circulating PMN viability, P, and OB of blood samples collected with ACD or ethylene diamine tetra-acetic acid (EDTA) coated tubes and stored for 0, 3, 6, 9, and 12 h at 4ºC (to mimic transportation). Moreover, we evaluated the effect of the presence of glucose (7.2 mM versus no glucose) in the culture medium in which the PMN functions were performed.

Material & methods: Coccygeal blood samples were collected from 3 nulliparous Holstein heifers (each animal 10 blood samples, 5 ACD, and 5 EDTA) in 5 biological replicates. Blood samples were gently mixed and placed in ice (4ºC) for 0, 3, 6, 9, and 12 h, to mimic transportation. At each time point, PMN were isolated and immunolabeled using a specific bovine granulocyte marker (CH138A), and viability (viable, apoptotic, or necrotic) was determined using a tricolor staining. Phagocytosis was assessed by co-incubating PMN with fluorescent beads and activated normal cow serum. Oxidative burst was analyzed by co-incubating PMN with 2′,7′-dichlorodihydrofluorescein diacetate, and phorbol 12-myristate 13-acetate. Functionality tests were run in duplicate in cell culture medium without glutamine supplemented with glucose (7.2 mM) or without glucose. Viability and functionality were evaluated via flow cytometry with 10,000 events in the area of interest. The association of PMN viability and functionality with ACD or EDTA, incubation times, and presence of glucose (or not) in the cell culture medium were fitted in linear regression models in R.

Results: The percentage of viable PMN at 0 h were 92 ± 4.6% for ACD and 93 ± 4.6% for EDTA and it decreased to 78 ± 4.6% for ACD and 79.6 ± 4.6% for EDTA after 6 h of storage ($P < 0.01$). After 6 h of storage, the percentage of apoptosis and necrosis was higher than 0 h for ACD and EDTA ($P < 0.05$). The type of anticoagulant (ACD or EDTA) did not have an effect on PMN viability parameters at any time point ($P > 0.05$). In EDTA tubes, storage time did not influence P ($P > 0.05$). However, in ACD tubes, the percentage of PMN that engulfed beads and the P fluorescence intensity (FI) were lower at 0 h compared to ≥ 3 h storage time ($P < 0.05$). Oxidative burst FI was not different between ACD and EDTA at any time point ($P > 0.05$). Interestingly, OB FI was 2-fold higher when it was performed in cell culture medium supplemented with glucose compared to no glucose supplementation ($P < 0.001$).

Conclusion: This study shows that the anticoagulant used to collect blood samples did not influence circulating PMN viability. Results showed that samples should not be stored (or transported) for more than 3 h. Most cell culture mediums have supraphysiological concentrations of glucose in their composition. We demonstrated that 7.2 mM glucose, a common glucose concentration found in cell culture medium, drastically increased the *in vitro* OB capacity of PMN. Importantly, this improvement may be artificial and mask impaired neutrophil function *in vivo.*

Keywords: Bovine, neutrophil, flow cytometry, glucose, anticoagulants.

RD-P33

Effect of estrus expression during the VWP on reproductive performance of dairy cows managed with programs that prioritized insemination at detected estrus or TAI

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Objectives: Our objective was to evaluate the association between estrous expression as determined by automated estrus alerts (AEA) during the voluntary waiting period (AEA-VWP) and the reproductive performance of dairy cows managed with programs for first service that prioritized AI at detected estrus (AIE) and used timed AI (TAI) for cows not AIE or managed with a program that used all-TAI after synchronization of ovulation and an extended voluntary waiting period (VWP).

Material & Methods: Lactating Holstein cows (n=849) from a dairy farm were fitted with a neck-attached sensor tag (Heatime Pro+, Allflex). Automated estrus alerts were recorded from 15 to 49 days in milk (DIM) to retrospectively generate groups of cows with at least one (AEA-VWP) or no AEA (NoAEA-VWP) during the VWP. At 6 ± 3 DIM, cows were blocked by parity and semen used for first AI (Holstein vs. beef) and then randomly assigned to predominant AIE (P-AIE; n=408) or an all-TAI (A-TAI; n=441) treatment. Cows in P-AIE were eligible for AIE for either 28 or 14 d after a 50 d VWP if in the AEA-VWP (n=248) or NoAEA-VWP (n=160) group. Cows not AIE were TAI after Ovsynch with progesterone supplementation. Cows in A-TAI received TAI at 76±3 DIM after a Double-Ovsynch protocol. Binary data were analyzed by logistic regression and time to pregnancy with Cox's regression. Models included estrus group during the VWP as fixed effect and reproductive treatment, parity, type of semen, season of calving or AI, calving features, health disorder occurrence, and predicted transmitting ability for daughter pregnancy rate (DPR) as confounders as appropriate.

Results: The overall proportion of cows with AEA-VWP did not differ (P=0.72) for the A-TAI (63.7%) and P-AIE (66.3%) treatment and did not differ (P=0.28) for primiparous (62.5%) and multiparous (57.9%) cows. Calving season (P=0.28), calving features (P=0.11), DPR (P=0.99) and health disorder occurrence up to 14 DIM (P=0.11) had no effect on the proportion of cows with AEA-VWP.

Within the P-AIE treatment, 88.2 and 43.7% of the cows were AIE out of the group with or without AEA-VWP.

Overall, first service pregnancies per AI (**P/AI**) were greater (*P*<0.01) for cows in the AEA-VWP (43.6%) than the NoAEA-VWP (27.5%) group and were greater (*P*=0.01) for the A-TAI (41.0%) than the P-AIE (29.8%) treatment. For the P-AIE treatment, P/AI were greater (P<0.01) for cows in the AEA-VWP (39.1%) than the NoAEA-VWP (20.4%) group. For the A-TAI treatment, P/AI were also greater (*P*<0.01) for cows in the AEA-VWP (48.8%) than the NoAEA-VWP (35.3%) group. Overall, P/AI were greater for primiparous cows (*P*=0.04), cows AI with Holstein semen (*P*=0.02), and during the cool season (*P*=0.04).

The hazard ratio for time to pregnancy was greater (*P*<0.01) for the AEA-VWP than the NoAEA-VWP group when all cows (HR=1.6; 95%CI 1.3-2.1), cows in the P-AIE (HR=1.6; 95%CI 1.2-2.3) and cows in the A-TAI (HR=1.7; 95%CI 1.2-2.2) treatment only were compared. For all cows combined, median days to pregnancy were 90 and 130 for the AEA-VWP and NoAEA-VWP group, respectively. For the P-AIE treatment, median days to pregnancy were 90 and 123 for the AEA-VWP and NoAEA-VWP group, respectively. For the A-TAI treatment, median days to pregnancy were 79 and 137 for the AEA-VWP and NoAEA-VWP group, respectively. Time to pregnancy did not differ (*P*=0.80) between the P-AIE and A-TAI treatment (HR=0.8; 95%CI 0.8-1.3). Primiparous cows had a greater (*P*<0.01) hazard of pregnancy than multiparous cows. The proportion of cows pregnant at 150 DIM was greater for the AEA-VWP than the No-AEA-VWP group when all cows (76.7% vs 59.9%; P<0.001), cows in the P-AIE (73.3% vs 65.8%; P=0.01) and cows in the A-TAI (80.4% vs 60.3%; P<0.01) treatment only were compared. There was no difference (*P*=0.13) between the P-AIE (65.5%) and A-TAI (72.5%) treatment. More (*P*<0.01) primiparous (76.6%) than multiparous (60.9%) cows were pregnant at 150 DIM.

Conclusion: We conclude that estrous expression during the VWP was associated with improved reproductive performance of dairy cows submitted to first service with programs that prioritized AIE or all timed AI. Therefore, detection of estrus based on automated estrus alerts during the voluntary waiting period might be used as a predictor of reproductive performance and to generate subgroups of cows for implementation of targeted reproductive management strategies.

Keywords: Dairy cow, voluntary waiting period, timed artificial insemination, automated estrus detection.

RD-P34

Protocols for in vivo bovine embryo production using sexed semen

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Objectives: Even though it has experienced great evolution in recent years, in vitro embryo production (PIVE) still cannot achieve, due to scale and logistic reasons, to many properties. Mainly some dairy herds did not use PIVE for this reason also did not use the in vivo production for the bad results observed with sexed semen. In recent years however, there have been developments in superovulation (SOV) protocols and in the quality of sexed semen. Figueiredo et al. (Animal Reproduction, 15, 3:142, 2018) reported good results in the in vivo embryo production with sexed semen, but with the insemination of donors in a protocol with estrus detection. Although it is a great evolution, the need for estrus detection is still a limitation because the probability of inseminations at inadequate periods. The objective of this study was to verify the efficiency of sexed semen to MOET and compare the efficiency of two protocols for donor insemination at fixed time (FT) to produce bovine embryos in vivo with sexed semen.

Materials and methods: Twenty Girolando (Gyr x Holtein) heifer donors, from a commercial dairy farm, located in the Southern region of Minas Gerais state, Brazil. This location had an altitude of 854 meters and a CW climate, according to Köppen classification. Donors with 16 and 52 months old, were used in a crossover scheme, where all the animals participated in both treatments. Females were superovulated using 180 mg Folltropin™ (Vetoquinol-Brazil) in a decreasing eight-dose protocol. The superovulation protocol was as follows: D0 - progesterone device insert; D1 - 2 mg of estradiol benzoate IM, D5 to D8 - Folltropin every 12 hours. In D7 the afternoon 0.5 mg of cloprostenol was applied and in D8 in the morning the device was removed. In D9, donors were divided into two groups: G2AI (N = 23), received 0.05 mg of Gonadorelin at 2pm and was inseminated in D10 at 7am and 7pm. G3AI (N = 23) received 0.05 mg of Gonadorelin at 7am and was inseminated at D9 at 7pm and at 10 at 7am and 7pm. All inseminations were made using sexed semen from Holstein bull (ABS Pecplan-Brazil). Uterine flushings were made by the same technician, seven days after the first artificial insemination, using DMPBS™ (Reprodux-Brazil) on D16. The ovaries were evaluated by ultrasonography (Mindray - M5™-China) on the day of flushing to measure the corpora lutea (CL). The number of CLs and embryonic production was compared using Anova at 5% probability (SAS Software, v9.4).

Results: The number of CLs on the day of collection was 14.2+6.4 and 12.4+6.2 (P>0.05) for G2IA and G3IA. The mean total embryos were 8.7+4.6 and 9.9+7.9 (P<0.05) and viable of 5.6+3.2 and 6.9+4.0 (P<0.05) for G2IA and G3IA. These results show that there is no difference in SOV, indicating that the design study used was correct, since this response to SOV has great individual variation. However, even with a similar ovarian response, G3IA donors produced more total and via-

ble embryos, indicating that an insemination protocol with an additional insemination has better results.

Conclusion: In conclusion, it is possible to efficiently use sexed semen for production of bovine embryos by MOET. The protocol of SOV using three doses of sexed semen to produce bovine embryos in vivo is superior to that using only two doses.

Acknowledgments: Vetoquinol, ABS Pecplan, IMV, Reprodux, Biotran, CNPq and CAPES.

Keywords: Girolando, MOET, superovulation, uterine flushing.

RD-P35

Progesterone profiles and standardization of the electrochemiluminescence test during natural and synchronized estrous cycles in dairy cattle in the Ecuadorian highlands (*Preliminary report*)

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Objectives: The objectives of this present study were to monitor progesterone (P4) profiles during a natural estrous cycle (NEC) and during estrous synchronization (ES) and to standardize the electrochemiluminescence test in order to measure the concentration of this hormone in dairy cattle (*Bos taurus*) in the Ecuadorian highlands.

Materials and Methods: In total, 15 dairy cows (*Bos taurus*) were divided into three groups in the Ecuadorian Andes at 3,500 m.a.s.l. (meters above sea level). Prostaglandin group (PG; n = 5): The animals received a luteolytic dose of prostaglandin F2α (25 mg; Dinoprost; Lutalyse; Zoetis SA Ecuador) after confirming the presence of the corpus luteum by rectal palpation. Blood samples were taken every two days from the day of application of the PGF2α (approximately 45 days postpartum) until the completion of the estrous cycle. Heifer group (HE; n = 6; over 370 kg): Animals that had already begun puberty were sampled in the same way as the PG group. Control group (CO; n = 4): Adult cows of approximately 45 days postpartum (pp) were also sampled every two days but they were not applied PGF2α. In order to measure progesterone levels in the serum samples, the electrochemiluminescence test was the method of choice.

Results: As expected, the PG group showed a decrease in serum P4 levels after the application of the luteolytic injection, demonstrating lysis of the corpus luteum. The average at the start of the investigation was 12.09 ng/mL, then it decreased to 0.46 ng/mL. At the end of the investigation, it increased to 10.40 ng/mL. In the HE group, two animals did not enter puberty before 370 kg, so they did not participate in the experiment. The remaining four animals started puberty before 370 kg and showed a progesterone curve similar to the PG group, with slight differences in the values obtained. The average at the beginning was 18.25 ng/mL and 0.92 ng/mL at the lower end of the curve; and 6.64 ng/mL at the end of the investigation. This indicates that the animals were at the end of their first estrous cycle, with the possible presence of a corpus luteum when the blood samples were taken. Based on this, it is suggested that the lack of weight gain may mark a delay in the onset of puberty. On the other hand, CO group produced a progesterone curve typical of a normal estrous cycle. At the starting point of the curve the average was 0.35 ng / mL; 12.69 ng / mL at the highest point; and 7.16 ng / mL at the end of the investigation. Interestingly, the animals at this altitude (3,500 m.a.s.l.) do not change their reproductive physiology, suggesting that dairy cattle can follow a natural reproductive course regardless of altitude. The electrochemiluminescence test was essential because of its specificity and sensitivity, which allows us to have confidence in its results.

Conclusions: In conclusion, serum progesterone levels are consistent with normal reproductive physiology and are directly related to the presence of the corpus luteum. At high altitudes, the progesterone curve does not vary from the values obtained closer to sea level. The electrochemiluminescence method proves to be reliable during the quantification of hormone levels due to its high specificity and sensitivity.

Keywords: Progesterone, Electrochemiluminescence, Higlands.

RD-P36

Antibodies against leptospirosis in dairy cows and reproductive parameters

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Objective: To terminate the presence of antibodies against Leptospirosis in dairy cows and their effect in interval between deliveries (IP), services by conception (S / C) and open days (DA).

Material and methods: Blood samples were taken from the coccygeal vein through the technique of Ojeda et al., (2016), 30 cows of the Holstein breed from a production unit located in the State of Hidalgo, Mexico with a history of abortions, mummifications and embryo reabsorption. The samples were analyzed in the Leptospira laboratory of the Autonomous Metropolitan University-Xochimilco, using the microscopic agglutination technique, which consists of mixing the serum to be

studied with leptospires of different serotypes, and then evaluating the degree of agglutination in the microscope (Méndez et al., 2013). Sera were considered positive that at 1: 100 dilution or higher, showed 50% agglutination or disappearance of cells from the field to observation with the dark field microscope. The bacterium that was used was 9 serovars of L. interrogans (Icterohaemorrhagiae, Pyrogenes, Grippotyphosa, Canicola, Pomona, Hardjio, Wolffi, Tarassovi, Bratislava) (Moles et al., 2002). Its relationship with the following reproductive parameters was determined: IP, S / C and DA. The results were analyzed by non-parametric statistics.

Results: In the analysis of the 30 serological samples, 73% (22/30) of positive bovines were found, at one or more serovars of Leptospira interrogans. In the positive sera, three Leptospira interrogans serovars were found: Hardjio, Tarassovi and Hardjio H89, 14% of the animals showed positivity at the three serovars, 32% at two serovars and 55% at one serotype. However, the results suggest an active infection by Tarassovi serovariety since it was found in all cases.

Conclusions: The presence of antibodies against leptospirosis indicates that the condition is present in the production unit of this study, which explains the problems in the reproductive parameters studied IP, S / C and DA.

Keywords: Leptospirosis, dairy cows, reproductive parameters.

RD-P38

Relationship between lochia characteristics and subsequent development of endometritis in postpartum dairy cows

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Objectives: Endometritis is usually diagnosed at 5 or more weeks postpartum in dairy cows. However, earlier identification of high-risk animals would be beneficial to improve subsequent reproductive performance. Therefore, we focused on lochia as a potentially useful indicator of intrauterine environment during the puerperal period. The objective of the present study was to clarify the relationship between characteristics of lochia with particular reference to its color, pro-inflammatory cytokine expression and pathogenic bacteria, and subsequent development of endometritis in postpartum dairy cows.

Material & Methods: Lochia samples were taken on days 2, 5, 9, and 16 days postpartum (pp) and endometrial smear samples were collected at week 5 pp from 74 Holstein-Friesian cows in a dairy farm. Blood samples were collected from the jugular vein and rectal temperature was recorded at all sampling time points. Lochia samples were photographed with a digital camera, and the pictures were analyzed by RGB colorimetry. In RGB analysis, R, G, and B values were expressed in three-dimensional space, and the square root value of the sum of three (R, G, and B) square values was named as white index (0 to 441 with 0 being black and 441 being white). Also, the square root value of the sum of three (R - 255, G, and B) square values was named as red index (0 to 441 with 0 being red). The median of red index was set as the threshold, and the lower half was defined as Red group and the higher half was defined as Non-red group. The cows were divided into two groups according to whether any treatment for parturient disorders was given (Treated; n = 16) or not (Non-treated; n = 58). In Non-treated group, expressions of pro-inflammatory cytokine mRNAs in lochia and endometrium were analyzed, and the DNA copies of pathogenic bacteria (Escherichia coli, Trueperella pyogenes, Prevotella spp, Fusobacterium necrophorum, Fusobacterium nucleatum) were determined on days 9, 16 and week 5 pp by real-time PCR. As the rectal temperature was higher than 39.3 degree Celsius on day 2, the cow was diagnosed with having puerperal fever. In endometrial cytology at week 5 pp, 6 (%) was set as a cut-off point for a diagnosis of endometritis. Pregnancy diagnosis was conducted by rectal palpation 45 to 55 days after first service pp.

Results: The median of red index was 206.5. Eight of 31 Red group (25.8%) and one of 31 Non-red group (3.2%) were diagnosed with having postpartum fever. PMN% in Red group (5.7 ± 1.3; mean ± SEM) was higher than that in Non-red group (2.5 ± 0.4) ($P < 0.05$). A moderate positive correlation ($r = 0.518$, $P < 0.05$) was observed between white index and PMN% at day 16 pp. White index of lochia at days 5 and 16 pp was associated with the degree of inflammation in the endometrium at week 5 pp. All coordinates of the color of lochia formed a surface on a three-dimension space (B = G) that included black (0, 0, 0), white (255, 255, 255), and red (255, 0, 0). Formula of the surface was calculated as $B = -0.09R + 0.95G + 0.96$. Cows with endometritis had a higher ($P < 0.05$) white index at day 16 pp compared to that in cow without endometritis at week 5 pp. Cows with endometritis had a lower white index ($P < 0.05$) compared with cows without endometritis at day 5 pp. Expressions of IL-1α, IL-1β, and IL-8 in cows with endometritis were lower at day 5 pp but higher ($P <0.05$) at week 5 pp than those in cows without endometritis. Expressions of IL-1α* and IL-8** tended to increase (* $P = 0.06$, ** $P = 0.09$), and expression of IL-1β increased ($P < 0.05$) by day 9 pp in cows that conceived to first service. In bacteriology, F. necrophorum increased from day 9 to day 16 and E. coli increased from day 16 to week 5 pp.

Conclusion: White index of lochia and expressions of pro-inflammatory cytokines days 5 to 16 pp may serve as indicators for evaluating the risk of postpartum fever, endometritis and infertility in postpartum dairy cows.

Keywords: Cytology, Dairy cows, Endometritis, Lochia, Pro-inflammatory cytokines.

SP-P01

The longevity of Nelore cows in grazing system of the Bolivian tropics

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The objective of the study to evaluate the longevity of Nelore cows in a grazing system of the Bolivian tropics. For the research study, retrospective data were used corresponding to the period between 2005 and 2019, belonging to the San Juan de Yapacaní Integral Agricultural Cooperative (CAISY) located in the Japanese Communities San Juan, Santa Cruz, Bolivia (16 ° 59 ′ 0 ″ south latitude, 63 ° 58 ′ 0 ″ west longitude). The Japanese communities is located at 286 m.a.s.l. and it has a tropical climate, with significant rains in most months of the year and a short dry season with little effect on the general climate. The average annual temperature is 24.3 ° C in San Juan Japanese communities with average rainfall of 1805 mm. The data corresponding to 289 Nelore cows, primiparous and multiparous, were used, with a total of 800 calvings. Primiparous cows calve between the months of May and July of each year, while the rest calve between the months of July and September. Weaning occurs between seventh and eighth months in two or three stages depending on the body condition and general condition. Gynecological control is performed routinely, at weaning, by a technical advisor, as well as health. The feeding of the rodeo was grazing managed under intensive conditions, on 82 hectares with cultivated pastures Brachiaria decumbens (10 to 14 t / ha / year of DM), Brachiaria humidicola (8 to 12 t / ha / year of DM), Brachiaria dictyoneura (7 at 9 t / ha / year of DM), Cynodon dactylon (10 to 20 t / ha / year of DM) and Panicum maximum cv mombaza (20 to 28 t / ha / year of DM). The first artificial insemination (AI) is performed to the cows in October, then the second AI in early December. At the end of December, a natural service is made to cows that are still empty. The averages and standard deviations of the variables were obtained, Longevity (L): Discard or death date - date of birth in days, Age at first calving (AFC): Age of first calving (date of birth - date of first calving) in months, calving-calving interval (CCI): the calving-calving interval (date of calving - the date of the last previous calving) in days, Live Weight (LW) in kg. For the variable number of median deliveries and ranges, Number of calving (NC): The number of births (∑ of the deliveries of each cow). The survival curve for herd data was calculated, using the non-parametric method of Kaplan-Meier. The results show that the L of the herd had an average value of 74.8 months, this indicator is not used by the literature, so the value obtained presents an antecedent of the same, no longer has values of reference. The value used is productive life that takes into account the days from the first calving to its discard without taking into account the stage of breeding and rearing. The value of AFC is above those found in Nelore cattle in the Parabano farm (Cordillera Province, Santa Cruz Department) where the age at first birth was 35.6 ± 8.8 months. The value of CCI was 15.2 months. The LW found are lower than those mentioned by Lopes of 555 ± 71 kg in adult cows. The low NC found coincides with those reported by other authors of 2.4 births, which would imply an annual replacement of 50%, this value is far from being the most biologically and economically efficient for the system, since to have 20% Replacement cows should have at least five deliveries. These results could be a reflection of the requirements established in the different conditions of herd management, environmental conditions and nutritional aspects. The survival curve obtained from Kaplan-Meier for the herd evaluated. The probability of cows that would remain alive in days from birth to discard or death is shown. The risk of discard obtained during the initial days of L is high, showing that at 2282 days only 50% of the cows arrive. It is concluded that the longevity of Nelore cows in the studied grazing system of the Bolivian tropics is below what is required to maintain an annular replacement of 18-20%.

Keywords: Longevity, Nelore cows, grazing system.

SP-P02

Progression of the length of life of cows Nelore cows of the Bolivian tropics

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Analyzing longevity through survival analysis allows us to detect genetic differences between animals in traits other than production, such as health, fertility, conformation and old age. The objective was to evaluate the behaviors of cows in terms of ages of beginning and end of their productive life in Nelore cows in the same grazing system of the Bolivian tropics. For the research work retrospective data were used corresponding to the period between 1987 and 2019 belonging to the Cooperativa Agropecuaria Integral San Juan de Yapacaní (CAISY) located in the Japanese communities San Juan (16 ° 59 ′ 0 ″ south latitude, 63 ° 58 ′ 0 ″ west longitude) and the Technological Center on Agriculture and Livestock in Bolivia (CETABOL) in the Japanese communities Okinawa. (17 ° 13 ′ 12 ″ south latitude, 62 ° 53 ′ 39 ″ west longitude) Santa Cruz, Bolivia. Data corresponding to 800 calvings of the Caisy group (CA) and 3734 calvings of the CETABOL (CE) group of Nelore cows were used, with a total of 4534 calvings. Primiparous cows birth between the months of May and July of each year, while the rest did so between July and September. Weaning occurs between seven and eight months in two or three stages depending on the body condition and general condition. Gynecological control is performed routinely, at weaning, as well as health. The feeding of the herd was grazing managed in intensive conditions with cultivated pastures of Brachiaria decumbens (10 to 14 t / ha / year of DM), Brachiaria humidicola (8 to 12 t / ha / year of DM), Brachiaria dictyoneura (7 to 9 t / ha / year of DM), Cynodon dactylon (10 to 20 t / ha / year of DM) and Panicum maximum cv mombaza (20 to 28 t / ha / year of

DM). As a descriptive indicator of longevity, the life of each cow was calculated: Longevity = ∑ [date of birth - date of discard or death], in days. For the analysis of the progression of the proportion of females of each of the two groups in relation to the duration of the productive life, the Kaplan-Meier technique used for the calculation of survival curves was applied. The behavior of both groups was compared with the log-rank test (Mantel-Cox). The average length of life of both groups was 2264 days (6.12 years). Survival curves for the end of the productive life event for each group showed that there was a 50% probability that the cows of the EC group end their life before 2100 days of life and those of the CA group end their life before 2600 days old. The average length of life of the CA group was 2554 days (7 years) and the EC group was 2044 days (5.6 years). The differences between survival functions for the different groups were statistically significant (p≤0.0001). The greater probability of permanence of the Nelore cows of the CA group compared to the cows of the CE group could be attributed to better management and food that allowed a length productive life, and also to the production objectives of each cooperative. It is concluded that the Nelore cows of the CA group are more likely to complete their productive life at an older age than the cows of the CE Group in grazing systems of the Bolivian tropics.

Keywords: Progression, Life of cows Nelore, Bolivian tropics.

SP-P03

From a healthy calf to a performing cow: a case-control study

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Objectives: As well as for any enterprise, the main target of the bovine dairy industry is economic sustainability. In other words, the core objective of a dairy operation is to reach an amount of milk production that helps to meet its goals. A dairy herd must be considered as an integrated, productive unit. Starting from newborn calves to milking cows, the health status of the animals is pivotal at obtaining satisfactory economic results. Neonatal calf diarrhoea (NCD) is considered the primary pathology involving newborn calves. Conversely, respiratory disease is the leading cause of losses after 60 days of life. This study aimed to assess the impact of NCDs on health, growth and milk productivity in a selected population of dairy cattle.

Materials & Methods: A case-control study was carried out in 300 newborn calves from 5 large dairy cow operations located in the Po Valley (Italy). The animals were split out in two groups: group A (cases) included 150 animals experiencing severe (needing antibiotic therapy) neonatal diarrhoea and group B (controls) included 150 calves without a clinical sign of neonatal enteritis. Weight at the born, 6 and 15 months of life besides the amount of milk during the whole lactation were collected. Also, the mortality rate and prevalence of respiratory disease episodes, involving single animals and requiring anti-inflammatory-antibiotic treatments, were recorded.

Results: The comparison of group A (cases) with group B (controls) showed: mean weight at the born 47.6±4.2 vs 47.2±3.8kg; at six months of life 182.5±32 vs 198.5±23kg, at 15 months 360.82±32.4 vs 379.4±21kg and average milk production of 11,720±425 vs 12,480±346kg. Group A suffered a mean loss of 760kg of milk per cow, equivalent to about €350-450, depending on the milk productive destination. The calculation did not take into account losses from mortality and costs for therapy of respiratory disease episodes. Besides, the mortality rate and prevalence of respiratory disease were respectively 9% and 31% in group A vs 5% and 21% in group B. In particular, 45% vs 21% of respiratory disease cases showed relapsing character.

Conclusion: The complex of obtained data supports the thesis that neonatal enteritis harms the weight gain during grow period and on milk production too. Regarding the pathogenesis of NCD, failure of passive transfer (FPT) plays an important role in favouring viral, bacteria and parasite infections and related pathogenic effect. A concentration of IgG <1000 mg/100 ml of blood serum collected from calves 3-10 days of life is considered the index of FPT status. The phenomenon is widespread in dairy cattle. A previous study carried out on newborn calves serum samples from 254 Italian Friesian herds with high milk production (≥11000kg/cow/lactation), pointed out an FPT mean prevalence of 28%. Colostrum samples from animals of the same herds showed a 17% prevalence of low-quality colostrum. Parity did not significantly affect colostrum quality. In this study, animals with previous diarrhoea showed a higher prevalence of the respiratory disease in their lifetime. Impairment of enteric barrier triggers a microbial (mainly bacteria) translocation from gut to the bloodstream and then to different organs, lung included. Isolation of E. coli enteropathogenic strains from the lung of calves experienced neonatal diarrhoea credits of value that pathogenetic hypothesis. Furthermore, the anatomy of the bovine lung (8 lobes, absence of interalveolar pores and presence of interlobular septs) hampers the microbial clearance, promoting the persistence of silent foci of infection that can reactivate long life causing relapsing respiratory episodes. If severe, NCD affects health, growth and production of dairy cattle, the control of the disease is the first step to support the sustainability of the business. Following the mantra “is better to prevent than cure”, vaccination of dam during the dry period is included in the protocols to cope with neonatal enteric disorders. If an FPT status persists, even in the presence of active maternal immunization, prevalence and seriousness of the disease often persist as well. To avoid that, it’s pivotal to detect the origin of FPT in the herd, setting up possible solutions.

Keywords: Calf, neonatal calf diarrhea, bovine respiratory disease.

SP-P04

Estimation of the environmental impact of CLAS vials in comparison with glass vials using a life-cycle assessment approach

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Objectives: Ceva Santé Animale has developed a new type of plastic vials called CLAS® which is intended for its injectable products in replacement of the traditional glass vial. On the field, many users acknowledge the superiority of the CLAS vials because it is lighter, more resistant and ergonomic in comparison with glass vials. As the environmental impact of CLAS vials has not been previously assessed, a life-cycle assessment (LCA) was undertaken to determine the level of environmental impact of these new type of vials compared to the glass vials.

Material and methods: A complete life-cycle assessment (LCA) was conducted by an independent laboratory (APESA, Pau) to compare the environmental impacts of glass and CLAS vials. LCA is a methodology for assessing environmental impacts associated with all the stages of the life-cycle of a commercial product (from cradle to grave). Environmental impacts were assessed from raw material extraction and processing, through the product's manufacture, distribution and use, to the recycling or final disposal of the materials composing it. The following criteria were taken into account when calculating the environmental impacts: potential for climate change, freshwater eutrophisation, fine particles emission, depletion of fossil-fuel resources, toxicity or carcinogenicity for humans, ecotoxicity, acidification, water depletion. In addition, the IMPACT 2002+ life cycle impact assessment methodology was used to estimate the overall environmental impact (Jolliet, 2003). The IMPACT 2002+ life cycle impact assessment methodology proposes a feasible implementation of a combined midpoint/ damage approach, linking all types of life cycle inventory results (elementary flows and other interventions) via 14 midpoint categories to four damage categories (human health, ecosystem quality, resources, climate change). Finally, an external expert reviewed the compliance of the LCA with the requirements of ISO 14040 standards.

Results: Following this LCA, CLAS vials were found to have a lesser environmental impact than traditional glass vials (-33%). In particular, CLAS vials were associated with a marked reduction in the potential consequences for human health and ecosystems (-50%,), a significant reduction on the depletion of resources (-23%) mainly due to the reduction in electricity consumption and a small reduction in the potential for global warming, but of the same order than in the initial assessment of potential impacts (-14%). Methodology used in this LCA was found compliant with the requirements of ISO 14040 standards.

Conclusion: According to the results from this life-cycle assessment, CLAS vials have considerably less environmental impacts compared to traditional glass vials.

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Keywords: Environmental impact, glass vials, life-cycle assessment, CLAS.

SP-P05

Effects of age at calving and calving season on milk yield and quality traits in Holstein dairy heifers in Greece.

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Objectives: The objectives of the study were to assess milk yield (MY) and milk quality traits (MQT) and investigate the effects of age at calving and calving season in Holstein heifers in Greece over the last 17 years.

Material & Methods: A total of 60 dairy cattle farms from six counties in Greece were enrolled in the study. Physiological and production traits were retrieved for 5,249 heifers from the data base of Holstein Association of Greece (years 2003 to 2019). Records included farm ID, ear tag, calving date, age at calving, total milk yield in 305-days of lactation (305d-MY), as well as daily milk- (DMY), fat- (DFY), protein- (DPY), lactose yield (DLY) and somatic cell counts (SCC) in the first stage of lactation (15 to 40 days post-calving). Descriptives (mean±standard error) and analytical statistics were calculated using SPSS v23. Namely, univariate analysis of covariance (UNIANCOVA) was used to estimate the fixed effects and the estimated marginal means (EMM) of calving month (12 levels; January to December) and calving year (17 levels; from 2003 to 2019), the random effect of farm (60 levels) and the effect of age at calving (covariate), on 305d-MY, DMY, DFY, DPY, DLY and the logarithm of SCC (LogSCC).

Results: The average 305d-MY in the studied heifer population was 9,898±27.9 kg and DMY was 29.1±0.10 kg. DFY, DPY and DLY were 1.19±0.006, 0.91±0.003 and 1.43±0.005 kg, respectively. Mean LogSCC was 2.02±0.009. A positive and statistically significant effect of age at calving was observed for all the studied traits, at the 0.001 level, with the exception of LogSCC (P=0.420). In detail, one-month increase in calving age was associated with a 55 and 0,165 kg increase on 305d-MY and DMY, respectively, and with a 13, 4 and 8 g increase on DFY, DPY and DLY, respectively. Calving season had a statistically significant effect on all the studied traits (P<0.001); heifers calved in December had the highest EMM for 305d-MY (9,233±135.61 kg), DMY (28.7±0.57 kg), DFY (1.23±0.031 kg), DPY (0.94±0.018 kg) and DLY (1.42±0.031 kg). On the contrary, the lowest EMM for 305d-MY was found in heifers calved in May (7,754±146.9 kg) and the lowest values of DMY (25.3±0.77 kg), DFY (0.99±0.043 kg), DPY (0.81±0.026 kg) and DLY (1.24±0.044) were observed in July. The highest values of LogSCC were observed for heifers calved from May to July (between 2.12±0.055 and 2.23±0.073) and the lowest for heifers calved from August to December (between 1.93±0.055 and 2.01±0.052). With the exception of DFY, calving year had a significant effect on all the studied traits, whereas, the overall

random effect of farm was statistically significant in all cases (P<0.001).

Conclusion: In heifers, MY and MQT are improving by the age at calving, however, the improvement is not adequate to justify a later beginning of their reproductive life. Calving season significantly affects all the studied traits, with calving during autumn and early winter being associated with an increased productive potential regarding 305d-MY, DFY and DPY and decreased SCC in milk. On the other hand, calvings during spring are associated with decreased 305d-MY but increased DMY, DLY and LogSCC in the first stage of lactation in heifers. In summer (July), decreased 305d-MY, DMY, DFY, DPY, DLY and increased LogSCC are observed in heifers in Greece.

Acknowledgements: The authors acknowledge Holstein Association of Greece for its collaboration.

Keywords: Heifers, age at calving, calving season, milk yield, milk quality.

SP-P06

A Cow–Calf Farming System Fully Adapted to Elevation and Harsh Conditions in Andorra (Europe).

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Objectives: This work describes the project of the Bruna d'Andorra (BA), a local cow breed (begin year 2000) from Andorra. The main goal of this strategic project of national interest is to achieve a homogeneous bovine population of a typical local breed from Andorra, well adapted to the mountain pastures of the country, and able to maximize the use of natural resources in a sustainable way and in an area with adverse environmental conditions. This system of cattle production includes the breeding, the fattening of calves, obtaining replacement animals, the slaughter of animals, and the marketing of their high-quality meat in the Andorran territory.

The main aim of this work is to reflect the evolution of BA population, meat productive performance, and main reproductive data that have been achieved during this 21-year period (2000–2020). A second objective is to explain the plans for the future of the project as a highly sustainable and environmentally friendly farming system.

Materials and methods: The BA breed is genetically very closely related to the Bruna dels Pirineus (BP), an autochthonous beef breed located in the mountainous areas of Catalonia (Northeastern Spain) and Parda Alpina (PA), an autochthonous beef breed located in the mountainous areas of Aragon (Northeastern Spain). The BP and PA breeds originated from the cross of native cattle with imported old-type Brown Swiss individuals during the first decades of the 20th century.

The BA genetic improvement program focused its efforts on maximizing the strengths of the breed (adaptability to the environment, acceptable productive yields, and good maternal fitness) and minimizing the weak, unwanted, or risky points (gene of Mh, inbreeding, and aggressiveness). The genetic selection of the included blood sampling, anatomical evaluation and measurements, productive, carcass quality data and breed standards.

The data used for this descriptive study were from official sources, with more than 20,000 data points from 47 farms. Data under study are: census (females > 12 months old, bulls, and animals in the fattening units), fattening performance (body weight—BW—at birth (kg), BW at slaughter (kg), average daily gain (ADG) (g/days of life, % meat yield (Kg carcass/kg BW × 100-), carcass quality based on muscular profile (SEUROP rating), and reproductive performance (age at first calving, average number of calving, calving ease, interval calving–calving). Data compiled are from pure BA and BA x Limousine breeds.

The cow–calf BA extensive system is thoroughly described and productive and reproductive performance, compiled over 21 years (2000–2020), has been analyzed by years with the Chi-square test or ANOVA to compare proportions or means, respectively, and regression analysis was used to decipher evolution across years.

Results: The data available cover a total of 21 years (2000–2020). During this period, the yearly population of bovines older than 12 months (cows, bulls, and replacements) ranged between 1008 and 1390 bovines. The data analyzed were from 14,128 calvings and 9982 slaughtered males and females, including pure BA and BAxL bovines.

The results show a population with a census large and stable enough to avoid inbreeding. Moreover, a sustained and significant improvement of the productive performance (ADG in crossbred and meat yield for males and females of both, pure and crossbred), better carcass quality and better maternal fitness (anatomy and behavior, the eradication of muscle hypertrophy in BA bulls, and a reduction of BW at birth – from >41.0 kg at begin of the program to always <40.0 kg nowadays for a better calving ease) has been observed.

There were no significant differences between the years regarding to reproductive performance parameters such as the age at first calving, the average number of parturitions per cow and interval calving–calving.

Conclusion: The work concludes that local breeds can achieve sustainable animal production, especially when farmers, public administration and commercial circuits in the area agree to cooperate on such projects. A low inbreeding risk, population, and maternal aptitude in the BA breed seem guaranteed. The study also concludes that the BA cow breed can still improve in meat and reproductive performance.

Keywords: Beef cattle, extensive production, local breed, Bruna d'Andorra.

SP-P07

Measuring the sustainability of dairy production at the farm gate: the PEF initiative

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Objectives: The European Commission's Product Environmental Footprint (PEF) initiative, is working towards harmonising a methodology to calculate and help communicating environmental footprint of products. This study aims to assess the environmental impact of the dairy value chain in Catalonia, north-eastern Spain, with scope cradle to farm gate, in order to test the suitability of PEF and dairy-specific PEF Category Rules (PEFCRs) guidelines to our production systems.

Material and methods: The environmental impact of cow milk was assessed at three farms located in Catalonia. As stated in the PEFCR for dairy, the study was performed following the four standard phases by the LCA methodology (ISO-14040, 2006). The functional unit was 1 tonne of fat protein converted (FPCM) milk. The scope of the study was cradle to farm gate. A questionnaire was designed to collect primary data from each farm. Datasets used for secondary data (e.g.: electricity mix) were adapted to local conditions. Emissions were calculated following Tier II from IPCC (2019) and European Environmental Agency (EMEP/EEA, 2019). Regarding system multifunctionality, the allocation method by Nemecek & Thoma (2020) was followed. All sixteen impact categories listed in the dairy specific PEFCR guidelines were assessed.

Results: Table 1 shows the results from a selection of impact categories for each farm. Carbon footprint (CC) ranged between 1,320 and 2,150 kg CO_2 eq tonne^{-1} FPCM milk at farm gate, being the benchmark value 1,530 kg CO_2 eq tonne^{-1} including processing and distribution (European Commission, 2018). Regarding variability across the studied farms, CC varied up to 830 kg CO_2 eq per tonne of FPCM milk across farms (Table 1). When performing an LCA from liquid milk at the industry gate, one challenge would be how to account for the impact that variability at the farm level can have on results. The most accurate way would be to perform a weighted measure, what would require a major effort. Another solution would be to have local databases of farms, initiative that should be promoted, together with the use of labels that show the range in which the impact varies, rather than absolute values, to account for the primary sector variability in agrifood products.

Conclusions: This study shows some of the challenges when utilising the PEF methodology in real-world applications to assess environmental impact of agri-food products: how to capture the agriculture-inherent variability in final product results. We propose to promote work towards having representative pictures of farms at regional level. Nevertheless, for a complete assessment of how variability at farm level could be determinant in the product environmental footprint results, the whole value chain should be considered.

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Keywords: milk, livestock farming, LCA, sustainability, supply chain.

Table 1. Results of the environmental impact assessment for a selection of categories by tonne of FPCM cow milk produced. BMR: ratio between live weight of sold animals and FPCM. The characterised benchmark values also include processing and distribution.

	Units	*Farm 1*	*Farm 2*	*Farm 3*	*Characterised benchmark values*
BMR	%	2.2	3.4	9.47	N/A
Climate change	kg CO_2 eq	1.32E+03	1.47E+03	2.15E+03	1.53E+03
Acidification	mol H+ eq	5,19E+00	8,23E+00	7,90E+00	1.25E+01
Eutrophication, freshwater	kg P eq	1,70E-01	1,80E-01	2,79E-01	1.04E-01
Eutrophication, marine	kg N eq	4,15E+00	7,88E+00	8,54E+00	3.75E+00
Eutrophication, terrestrial	mol N eq	2,85E+01	5,43E+01	6,13E+01	5.34E+01
Land use	Pt	6,10E+04	1,27E+05	1,46E+05	1.51E+05
Water use	m^3 depriv.	3,69E+03	6,24E+03	7,25E+03	3.11E+02

SP-P08

Development of a NIRS calibration equation for in situ analysis of green pasture

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Objectives: To develop a rapid method of analysing the quality of grass consumed by grazing beef cattle in order to optimise the administration of concentrate feed according to the characteristics of the grass at each time of the year, using NIRS equipment in the laboratory and in situ, using portable NIRS equipment.

Materials and methods: The experimental phase was developed between May 2018 and November 2021, in six suckler cow farms in and around Castilla y León, representing different ecosystems. In each farm, a pasture sample was taken every 45 days for chemical and NIRS analysis.

The constituents analyzed were moisture (%), crude protein (%), crude fiber (%), neutral detergent fiber (%), acid detergent fiber (%), lignin (%) and ash (%).

The grass sampling was carried out using two different techniques: random sampling following the classical methodology and targeted sampling using GPS collars, placed on animals from three of the herds, to obtain their most frequent locations, as sampling points.

All pasture samples, a total of 550, were analysed by the same instrument DS2500 (FOSS)-NIRS (near-infrared spectroscopy) in order to obtain their spectra in a range from 400nm to 2200nm at constant intervals of 0.5nm. Some of them (350) were also analysed by the Portable X-NIR-(Dinámica Générale).

The samples were not subjected to any kind of pre-treatment, following the same analysis protocol:

- 4 sub-samples were taken and scanned separately.
- Selection of spectra.
- Chemical analysis of the selected samples.
- Calibration and validation of the regression equations.

ISI Nova and Mosaic software (from FOSS) were used to collect the spectral data. For the development of the equations, different mathematical treatments and cross-validation were tested with the WINISI IV software (Infrasoft International), regression models were generated using MPLS (Modified Partial Least Squares), combining different mathematical treatments and light scattering correction using SNV (Standard Normal Variate) and Detrend mathematical techniques.

The statistics used for the selection of the best calibration equations were SEC, SEVC, R^2, r^2, SEP, RSQ, RPD and RER.

R^2(RSQ): coefficient of determination; SEC(SEP): calibration/validation standard error; SECV: cross-validation standard error; (1-VR) r^2: determination coefficient, RPD: Ratio between the standard deviation of the reference data for the validation group and the SEP. RER: Relationship between the range in the reference data for the validation group and the SEP.

Results and Discussion: The evaluation of the accuracy and reliability of the obtained equations would need further discussion, but in general terms the results obtained indicate calibrations with r^2 values higher than 0.9 with are perfect for quantitative predictions. The lower r^2 values remain high (0.8-0.9), except for lignin (0.76) in the portable X-NIR.

These models also presented RPD greater than 3, a good indicator of the predictive capacity and robustness of the model, except for lignin and crude ash in the DS2500 NIR (2.43 and 2.42 respectively) and for lignin in the Portable X-NIR (2.12).

Therefore, the calibration models developed on both equipments give reliable and fast predictions in the laboratory and on the farm, so they can be used to evaluate the nutritional quality of a pasture area and adapt the complementary feeding in a short period of time (hours).

As a result of this work, a nutritional advice tool has been developed for extensive cattle farmers.

Keywords: Pasture, NIRS, extensive, cattle.

SP-P09

Association of antibiotic therapy expenditure in clinical mastitis and genomic prediction for mastitis in European Holstein cows

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Objective: The objective of this study was to determine the association of genomic predictions of wellness traits - in this case, mastitis - with the incidence of mastitis and, in particular, on antibiotic use, using a large European data set from Holstein cows in commercial herds.

Material & Methods: Data from 10 different farms in the United Kingdom (4 farms), the Netherlands (3 farms) and Italy (3 farms) were used for this preliminary analysis. In total, the dataset included 3987 lactations from 2482 cows from 2015-2021. Genomic data of young calves and heifers were compared with accurate records of the occurrence of clinical mastitis and treatments administered, which were obtained from the animals' farm documentation in herd management systems. The Clarifide Plus genomic evaluation (Zoetis, USA) was used to estimate the genetic risk of mastitis occurrence. The animals were divided into two groups (≤100 STA (Standardized Transmitting Abilities), >100 STA) based on the genomic evaluation of the mastitis trait. Statistical analysis was performed with generalized linear mixed models using genetic evaluation group and lactation number and the interaction of these two variables as fixed effects; and country, herd and animals within the herd as random effects.

The target variables examined were incidence of mastitis, days under antibiotic treatment, and days under withdrawal time per lactation.

Results: Mastitis STA group was significantly associated with all outcome variables. On average (estimated means), animals in the >100 STA group had lower incidence of mastitis (18.6% for >100 STA, 26.7% for ≤100 STA; P<0.001), fewer days under treatment (0.50 days for >100 STA, 0.89 days for ≤100 STA; P<0.001), and fewer days under withdrawal time per lactation (0.81 days for >100 STA, 1.15 for ≤100 STA; P<0.001).

Conclusion: These results indicate that genomic data of young calves and heifers can be used to effectively predict future antibiotic use. Reducing mastitis incidence through direct genetic selection represents a compelling opportunity for dairy farmers to reduce antibiotic use due to mastitis and thus improve herd health and profitability when combined with modern management.

Keywords: Mastitis, genomic prediction, antibiotic treatment, antibiotic doses, Europe.

SP-P12

Milk production evaluation of family dairy farms in Pontal do Paranapanema, State of São Paulo, Brazil

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Objectives: Milk production evaluation of family dairy farms from Pontal do Paranapanema region.

Material and methods: Eight rural communities from the Pontal do Paranapanema region were selected to evaluated the milk production from this region. It was visiting approximately 80% of the milk producing properties of each community, 27 family dairy properties were visited in Porto Maria, municipality of Rosana, SP; 19 family dairy farms in Rancho Grande, municipality of Euclides da Cunha, SP; 29 milk producing properties in Bom Pastor, municipality of Sandovalina, SP; 21 family dairy farms in Floresta Fernandes, municipality of Presidente Bernardes, SP; 4 family dairy farms in Asa Branca, municipality of Presidente Bernardes, SP; 19 family dairy farms in Santa Rosa 1, Mirante do Paranapanema, SP; 32 family dairy farms in São Paulo, municipality of Presidente Epitácio, SP; 8 family dairy farms in Yapinari, Ribeirão dos Índios, SP). A questionnaire was carried out for each of the family producers, to learn about the characteristics of the productions.

Results: 159 properties were visited, and the following results were obtained: 74.84% (119/159) of properties in this region have between 16 and 24 hectares. 67.30% (107/159) has an average of 40 animals, most of them are milk production properties 91.19% (145/159) and has an average of 30 cows per property. 96.85% (154/159) has a milk production below 100 liters daily. 53.46% (85/159) perform manual milking and 46.5% (74/159) perform mechanical milking (bucket at the foot). 83.64% of the properties perform only one milking a day. 81.13% (129/159) stimulates the milk to descend with the presence of a calf, 84.27% (134/159) does not clean the teats before milking (pre-dipping), 86,16% (137/159) do not perform a test to diagnose clinical mastitis in cows. 83.64% (133/159) do not perform the CMT test, 93.08% (148/159) didn´t use mastitis treatment without knowing the cause or etiological agents of mastitis. 86.79% (138/159) didn´t dispense the milk after mastitis treatment, 36.95% (51/138) provide this milk for pigs, 26.81% (37/138) supplies this milk to the dogs on the farm, 18.84% (26/138) supplies this milk to the calves and 17.39% (24/138) throws the milk into the sewer. 81.13% (129/159) of the properties store milk in gallons after milking. 88.67% (141/159) of the properties carry out natural breeding as animal reproduction.

Conclusion: It concluded that this region should be more monitored and has a possibility to improve your production if applicate better technology. The development of this region after the settlements politics is one reality but exist lack information between them about how is the better practice to produce milk with quality. The future of this producers will depend on politics and investments in education and sanity of this animals. Support programs for these producers is necessary to improve their benefits.

Keywords: Bovine, cattle, calves, hygiene, technology.

SR-P01

Efficacy of condensed tannin as an alternative anthelmintic in small ruminants raised in Thailand

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Objective: Internal parasitism has been considered as a major life threatening and predominant cause of economic loss of small ruminant farming in Thailand. Traditional deworming by chemical anthelmintics was applied as a major disease control measure. Low efficacy and resistance of chemical anthelmintics are recently a major concerns for farmers. Alternative anthelmintics, particularly the organic substance, condensed tannin have been of interest to replace chemical anthelmintics. Thus this study aims to evaluate efficacy of a condensed tannin extract on reducing fecal gastrointestinal nematode egg count in sheep and dairy goats under Thai farming condition.

Materials and methods: Animals: Thirty-nine goats in a dairy goat farm and thirty-six sheep in a sheep farm were included in this study. Animals were randomly assigned into 3 groups. Group 1 received a single subcutaneous injection of ivermectin and a dietary condensed tannin supplement for 30 days; group 2 received a dietary condensed tannin; and group 3 received ivermectin. Ivermectin was used at the dosage of 0.2 mg/kg. The dietary condensed tannin supplement was used at the dosage of 15 mg/head/day in young animals and 30 mg/head/day in adult animals. Fecal samples were collected before deworming and at 14, 28, 42 and 56 days after deworming.

Laboratory analysis: Modified McMaster technique with the detection limit of 50 eggs per gram was performed to determine FEC within 2 days after sampling. Saturated NaCl was used as a flotation fluid for the test.

Data analysis: Descriptive statistics were performed to examine FECs before and after treatment. FECs were transformed by adding one and then taking natural logarithm for further statistical analyses. A mixed linear regression model using group, time and interaction between group and time as fixed effects, animal as a random effect was built. The unstructured time correlation matrix was included in the model. Data of sheep and goat were analyzed separately. P-value < 0.05 was considered statistically significant for all inferential statistical analyses.

Results: Sheep: Most sheep in group 2 had quite stable FECs along the 8-week study period. There was no specific trend in the other 2 groups. At D14, the means FEC were lower than the means at D0 in all three groups. On the contrary, the means FEC at D56 in all groups were higher than the means at D0 . However, there was no statistical difference among groups and times of sampling based on inferential statistics ($p > 0.05$).

Goats: FEC in each goat along the study period was varied, regardless of group which it belonged to. The means FEC at D14 and D28 were lower than the means at D0 in all three groups. The model indicated a significant difference of FECs among 5 sampling times ($p = 0.002$). The further multiple comparisons with Bonferroni adjustment revealed that the mean FEC at D14 tended to be lower than that of D0 ($p = 0.06$), while no tendency was found from other comparisons ($p > 0.10$). Group had no significant effect on FEC ($p = 0.399$).

Conclusions: Dietary condensed tannin extract and ivermectin at the dosage used in this study could not significantly reduce gastrointestinal nematode egg excretion in both sheep and dairy goats. Then the further study of factors may influence to anthelminthic effect of condensed tannin need to be clarified.

Keywords: Condensed tannin, anthelmintic, small ruminants, Thailand.

SR-P02

Diversification in sheep production systems in the Central zone of Chile

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Within the traditional ovine areas in Chile are rainfed areas of the Central zone. The sheep farms in this sector correspond to farms of small or medium size, managed in semi-extensive or extensive production systems, in many cases, considered as agroecosystems belonging to the Peasant Family Farming. Although it is true, the ovine activity plays a fundamental role, it does not develop in isolation, but in a complementary way with other agricultural activities. The factors that justify this combination of activities respond to economic, social, and environmental reasons. A survey was conducted on a sample of 52 farms for the aim of characterizing the diversification of the sheep production systems in the Central zone of Chile and associate it with the family and farms characteristics. The survey consisted of 42 questions related to the family composition, the diversity of animal and plant species, the perspectives of incorporation of new activities to the farm, among others. The analysis of the results considered the obtaining of descriptive statistics and the determination of relations between variables through the development of contingency tables and Chi-square tests. As a result, it was found that the average age of those responsible for the farms was 65 years old. The farms had on average a flock of 114 animals, maintained in an area of 55 ha. About the diversification in the 94% of the farms, it is possible to find another agricultural activity besides the sheep production, being among the main the production of cattle, goats, fowls, pigs, production of forest trees and fruit and vegetables. Thus, the average of activities carried out per farm is 3.0, and within them, 2.3 activities are related to livestock production. Self-consumption is one of the objectives of animal production, so, for example, in the case of sheep production, this occurs in 71% of farms. The sheep production, only in the 7.7% of the farms corresponds to the unique source of income, representing in average 20% of the income. This situation highlights the importance of diversification from the economic point of view, which is reaffirmed by the fact that 50% of the farmers have incorporated an activity to their

farm in the last ten years and with the expectation of a 40% of farmers incorporate a new activity in the medium term. The environmental aspect of diversification is observed in the use of by-products for the closing of cycles, for example, 62% of the producers that accumulate guano, use it for the fertilization of grasslands and 25% for the fertilization of crops. The diversification of activities depends on several factors, many of which are not easily detectable. Thus, it was not possible to find significant associations between the number of activities and gender, age of the operator, the number of members of the family or farm size.

Keywords: Sheep, small farmers, rainfed.

SR-P03

Effect of ambient temperature and humidity on blood metabolic profile and cortisol level in two different sheep breeds

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Objectives: Climatic variations directly affect the animals, changing their physiology. Quantitative and morphological changes in blood cells are associated with heat stress. Apparently in sheep, hyperthermia conditions during prolonged periods reduce blood metabolite levels related to the energetic metabolism and increase metabolite concentrations. The aim of the study were to determine the effect of temperature and humidity index (THI) and breed on biochemical blood parameters and cortisol level.

Materials and methods: This experiment was conducted from 2018 December till 2019 December. Two sheep breeds from two different farms were chosen for this study: Merino (n=19), Lithuanian blackhead (n=19).For the study sheep (n=38) were selected according to those: adult female sheep (3 years old), not pregnant during the whole investigation, clinical healthy and without any signs of diseases after a precise clinical examination. Means of body weight of the animals in the groups were roughly equal (on average 35 kg). The animals were kept in similar conditions in a loose housing system and were fed a feed ration throughout the year at the same time balanced according to their physiological needs. The climate of the country is transitional between the maritime type of Western Europe and the continental type found farther east, characterized by warm, dry summers and fairly severe winters. Average ambient temperature during experimental year was 10.50 ± 1° C, relative humidity 77.60 ± 2%. Blood samples were taken one time per month at 7:00 h to 8:00 h after overnight fasting from the identical animals. The values determined were calcium (Ca), phosphorus (P), magnesium (Mg), copper (Cu), zinc (Zn), iron (Fe), urea, total proteins, (TP), glucose (Gl), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), total bilirubin (TBIL), albumin (Alb), creatinine (Crea), lactate dehydrogenase (LDH) and cortisol. According to the test date, subgroups were created by breed (2 breeds) and THI (3 classes). The daily temperature-humidity index (THI) values were calculated using the equation (a) by Marai et al. (2007). THI _ T-(0.31- 0.0031× RH) × (T-14.4) (a), where T is the dry-bulb temperature and RH is the relative humidity. The average daily values of temperature and relative humidity were exploited to calculate the daily values of THI that were analysed in this study. Normal distributions for all blood indicators were assessed by the Kolmogorov-Smirnov test. The results of statistical analysis were expressed as the mean±standard error. The linear relationship between biochemical blood indicators and cortisol were evaluated using Pearson's correlation. The one-way ANOVA test was used to assess the influence of breed and THI level, on blood parameters. A probability of less than 0.05 was considered as being significant (P <0.05). This work followed all ethical principles involving animals in research. Study approval number – PK014606.

Results: The analysis showed that the breed had a statistically significant effect on many blood parameters, with the exception of the concentration of Fe, Mg, P and cortisol. The Lithuanian native breed had significantly higher levels of urea (7.48±0.32 mmol/l), Crea (108.27±1.48 µmol/l), Ca (2.57±0.02 mmol/l), TP (72.25±0.67 g/l) (P <0.001) and a lower levels of TBIL (2.23±0.05 µmol/l) and LDH (1067.15±21.87 U/l) (P <0.001) compared to the Merino breed. Studies have shown that THI 1 prevailed in Lithuanian native breed records, THI 2 – in Merino breed. The Merino breed had 1.3 times less TI 3 records than the Lithuanian native breed. THI 1 class consisted of 181 records (48.66%), THI 2-160 (43.01%), and THI 3-31 records of animals (8.33%). THI levels had a statistically significant effect on blood urea, Gl, Crea, TP, TBIL, Cu, Zn, cortisol (P ≤ 0.001) and LDH (P = 0.022). Blood cortisol statistically significant correlated with urea, Alb (P<0.001), P (P=0.002), Crea (P=0.034) and TP (P=0.037).

Conclusions: According our results we can conclude, that the response to temperature is dependent on animal breed and enviromental conditions. The Lithuanian native breed had significantly higher levels of urea, Crea, Ca, TP (P <0.001) and a lower levels of TBIL and LDH (P <0.001) compared to the Merino breed. It was found that heat stress had a statistically significant effect on blood urea, Gl, Crea, TP, TBIL, Cu, Zn, cortisol (P ≤ 0.001) and LDH (P = 0.022).

Keywords: Sheep. heat stress. temperature-humidity index. cortisol. biochemistry.

SR-P04

Efficacy of two vaccinations for contagious pustular dermatitis (scabby mouth) in sheep in New Zealand

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Objective: This field study aimed at assessing the efficacy of two commercially available vaccination products against

contagious pustular dermatitis (CPD, scabby mouth or orf) in New Zealand conditions.

Material & Methods: On day 0, twenty lambs were selected to be randomly vaccinated with either the commercial CPD live vaccine PC (PHENAX CLASSIC, Virbac New Zealand Ltd, Hamilton, NZ) using the manufacturer's applicator, or SPG (SCABIGARD, Zoetis New Zealand Ltd, Aukland, NZ) using a palm-grip applicator. Successful vaccination produces a 2-5mm wide, whitish, thickened or raised area of scab along the line of the scratches within 7 to 10 days. Seven days later the lambs were visually assessed for response to vaccination and categorised as primary, secondary, intermediary or nil. The completeness of the response was assessed by measuring the total length of the visible response with a ruler. Any response less than 40 mm in total length was deemed to be incomplete. Due to a high percentage of incomplete responses on day 7 for SPG lambs the protocol was amended to include a third group of twenty lambs which received Scabigard, applied with a V-Grip applicator (SVG) to be started on day 41. On day 41, the PC and SPG lambs were allocated into one of two sub-groups of ten lambs each. Each sub-group was then given a challenge vaccination of either PC or SPG. Seven days later (day 48) the lambs were visually assessed for response and response completeness to the challenge vaccination as for day 7.

Results: There was no difference between PC and SPG (p=0.98) in the percentage of lambs showing a primary response to the initial vaccine when examined 7 days later. PC lambs had a 15 times greater odds of complete response (95%CI OR [3.50;81.9]) in comparison to SPG lambs. There was no difference in primary response completeness between SPG and SVG lambs seven days after the respective initial vaccination (p=1). There was no difference in the number of secondary or intermediate responses to challenging vaccination on Day 48 between PC or SPG lambs, regardless of challenge vaccine on day 41 (p=0.18). All responses to the challenge vaccine were incomplete.

Conclusion: Both vaccines created a primary immune response to initial vaccination and had a reduced response to a challenge vaccination. Nevertheless, PC produced a significantly higher percentage of complete initial vaccination responses than the other vaccine, applied with either a palm-grip or v-grip applicator. Application of scabby mouth vaccines must be done according to label recommendations and the response to vaccination visually assessed in several live lambs to check efficacy.

Keywords: Contagious pustular dermatitis, sheep, vaccination.

SR-P05

Study on the effect of vaccination with an inactivated vaccine against biofilm-forming *Staphylococcus* spp. in Assaf sheep

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Objectives: Immunisation against the major causative agents of mastitis is a strategy which is rarely used to reduce somatic cell counts (SCC) and production losses associated with such infections in dairy sheep in Castile and León (Spain). The objectives of this study were to:

1. Quantify the effect on SCC of an inactivated vaccine against biofilm-forming *Staphylococcus* spp.
2. Compare milk production in vaccinated vs. unvaccinated lactating ewes.

The results, which will be used to assess this control strategy in flocks of dairy sheep, will be of interest to both technicians and breeders.

Materials and methods: This study was conducted in 2019, in a flock of Assaf dairy sheep with 2,361 female breeding animals located in Torres del Carrizal (Zamora, Sapain), part of the ASSAFE breeding program and therefore subject to qualitative and quantitative tests, based on a total of 1,847 dairy production controls and milk composition analyses in 415 ewes.

In order to study the effects of vaccination on logSCC and production rate, pregnant ewes with a due date between July and August 2019 were selected and divided into two groups: control and treated. Multiparous ewes were selected based on available production data. The groups formed were homogenous in terms of lactation number, milk yield, logSCC in the last tests pre-drying off, and average logSCC in the 24 pre-drying tests.

The vaccination protocol of the treated group was as described in the summary of product characteristics of the vaccine. The animals received a 2 ml dose of the vaccine on 8 June 2019, five weeks before the expected lambing date, and a second dose of 2 ml three weeks later, on 29 June 2019.

Statistical analysis for comparison of the means of both groups was performed using the Student's t-test of the *SPSS Statistics* software package.

Results: The average logSCC in the vaccinated group was found to be significantly lower than that of the control group, indicating the positive impact of the vaccine against biofilm-forming *Staphylococcus* spp. on reducing subclinical mastitis in this breed of sheep.

There were no significant differences in the duration of lactation between the two groups; as regards milk yield during lactation, only one result approximating a statistical trend was observed despite the 13-litre difference in milk yield. This can be explained by the high variability of this production index due to the large number of factors that influence it. However, when studying primiparous and multiparous ewes separately, significant differences ($p < 0.05$) in milk yield were observed between the treated primiparous group (325.5 litres) and the control (or

untreated) primiparous group (284.1 litres).

During the study, we analysed the six milk tests conducted during the lactation period that followed the lambing for which the animals were vaccinated. For every milk test, the percentage of animals with a SCC <500,000 cells/mL was higher in the treated group than in the control group. In contrast, the percentage of animals with a SCC >1,000,000 was consistently lower in the treated group than in the control group.

We also analysed the geometric mean of the individual SCC obtained in the six milk tests performed after lambing in both groups. All tests showed lower values in the vaccinated group.

Finally, based on the average daily yield of the animals in each group, it was found that the vaccinated group followed a parallel lactation curve, but with a greater daily yield than the control group throughout the lactation period. While the differences were not statistically significant overall, as mentioned previously, the difference was statistically significant for primiparous ewes.

Conclusions: Vaccination against biofilm-forming *Staphylococcus* spp. was associated with a significant reduction in the logSCC of all ewes, and a likewise significant increase in the milk yield of primiparous ewes.

Keywords: Mastitis, vaccination, SCC, staphylococcus, yield.

SR-P06

Effective use of acupuncture to reduce acute inflammation and pain after failure of meloxicam and fentanyl treatment in a dairy goat

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Objective: To determine the effect of the acupuncture technique "circling the dragon" for the treatment of acute inflammation with facial nerve paralysis after failure of treatment with a fentanyl patch and meloxicam.

Materials and Methods: A 2-year-old Saanen dairy goat was presented for deferred hospitalization after initial evaluation and treatment at the veterinary school hospital, due to cost concerns. The goat had been diagnosed with acute masseter inflammation with local lymphadenopathy and middle ear infection. Diagnostic tests performed at the veterinary school hospital included CBC, chemistry panel, lymph node aspirate and a computerized tomography (CT) scan to identify the culprit lesion.

After diagnosis, the goat was discharged with anti-inflammatory and pain medication (meloxicam and a fentanyl patch) and antibiotics (systemic procaine penicillin and ceftiofur, and local ear drops).

The swelling was not better after 4 days, and the goat was not eating properly, at which point acupuncture was administered using the technique "circling the dragon" using 8 needles (34 g x 1") at 3 days intervals for 1 total of 4 treatments.

Results: The only clinicopathologic change found in blood tests was a slight alkalemia (pH=7.51, normal 7.35-7.45). All other values were within normal limits. The lymph node aspirate showed mixed bacteria and evidence of necrosis. The CT scan revealed severe myositis on the left side of the head, with middle ear infection and extensive local lymphadenopathy.

After the first acupuncture treatment the goat started eating better and swelling decreased by 80% within 48 hours and 100% within 72 hours. A second treatment was administered because the goat became anorectic again. Immediately after treatment, the goat ate better for another 3 days and then stopped eating again. This pattern repeated itself twice more, for a total of 4 acupuncture treatments, at which point it was decided to euthanize the goat.

Necropsy revealed a distal fracture on the left tympanohyoid process of the hyoid bone with complete transverse fracture of the left bulla. Both bones showed evidence of necrosis, which explained the recurring relapse of symptoms.

Conclusion: Acupuncture proved to be an effective treatment of pain and inflammation in this goat even after treatment failure of Western therapy with powerful medication (fentanyl) and in the presence of massive bone damage to the inner ear and hyoid apparatus to allow the goat to eat and ruminate for up to 3 days without any other treatment. Acupuncture should be considered, if not as sole therapy, at least as an adjunct therapy to Western medications to help improve pain management and to palliate harmful inflammation to help in the overall healing process and animal wellbeing.

Keywords: Acupuncture, goat, fracture, pain management, anorexia.

SR-P07

Influence of humic acids as a food additive on haemonchosis of lambs

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Objective: The objective was to evaluate the effects of commercially available humic acids (HA) upon lamb haemonchosis and growth.

Materials and Methods: The study protocol was approved by the ethical committee of the Pontifical Catholic University of Paraná (PUCPR) under number 01707. The experiment was carried out in the sheep unit of the Experimental Farm Gralha Azul of PUCPR, at Fazenda Rio Grande, Brazil. 20 crossbred Texel x Ile de France lambs, male and females, weaned at approximately 60 days of age and uniform weights were included in the experiment. Exclusion criteria were: parasitemia level

≥ 10000 eggs/g, hematocrit ≤ 18 and weight loss measured weekly.

During the experiment, all animals had access to pasture throughout the day to be naturally infected by gastrointestinal parasites and the diet was supplemented during late afternoon and night in stable with hay, corn silage and balanced concentrate based on 1% of body live weight, maintaining 18% of crude protein in dry matter offered once a day. Previous studies demonstrated that *Haemochus contortus* was the most prevalent gastrointestinal parasite in the flock. The lambs were separated into two groups: control group (CT) without humic acids administration; treatment group (TG), with HA (Omnia KS100®) mixed together with the concentrate at a dose of 0.5 g HA/kg body weight daily. When stabled, the lambs were divided into smaller groups of two, to avoid excessive food competition. The total duration of the project was 60 days, in which weekly and fortnightly analyzes were carried out. Weekly the weight and Famacha® were evaluated and biweekly fecal egg counts (FEC – log transformed), hematocrit, plasma protein concentration (PPC) by refractometry, glutaraldehyde test, and with ultrasound (Sonoscape, using a linear 7.5 MHz probe) the rib eye area (REA) and subcutaneous fat thickness (SCFT). Data were analyzed using ANOVA-procedure using Statgraphic Centurion v.18, Stat-point Technologies Inc. Warrenton, Virginia, USA.

Results: Three lambs (1 CG; 2 TG) were excluded using previous described criteria. No significant differences ($p > 0.05$) between groups were observed for weight gain (CG: 32.1±0.69 Kg; TG: 30.6±0.74 kg); LogFEC (CG: 1.83±0.13 *Strongylides* eggs/g; TG: 1.91±0.15 *Strongylides* eggs/g; CG: 0.13±0.07 *Strongyloides* eggs/g; TG: 0.17±0.07 *Strongyloides* eggs/g), hematocrit (CG: 32.45±0.49%; TG: 33.22±0.47%), PPC (CG: 6.35±0.059 g/dL; TG: 6.43±0.056 g/dL), REA (CG: 7.04±0.22 cm^2; TG: 7.13±0.24 cm^2), SCFT (CG: 3.89±0.12 mm; TG: 3.91±0.13 mm).

Conclusion: These results indicate that treatment with 0.5 g/kg/day of HA of Omnia KS100® during 60 days didn´t change the evaluated parameters, therefore it cannot be used as a preventive nor control method against gastrointestinal parasitosis in lambs. It also did not affect weight gain or growth of lambs. As other HA are described as beneficial and nothing harmful was noted, future studies should analyze HA of different sources for its potential benefits.

Acknowledgment:To CAPES, Fundação Araucária and CNPq for the scholarship received by the first and second authors

Keywords: Haemonchus contortus, ovine, parasitism, humic acids.

SR-P08

Case study: Efficacy of a vaccine against staphylococcal mastitis in dairy sheep and goat farms based on field experience in Greece

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Objectives: The Greek small ruminants sector leads sheep and goat milk production in the European Union[1], being one of the most important livestock sectors in Greece[2]. Mastitis in small ruminants is the most important health problem and the cause of the greatest economic losses in dairy farms, being associated with reduction in milk production and quality[3]. Nevertheless, different strategies could be used for mastitis control and prevention, such as correct management, antibiotic treatments and/or vaccination. In this context, the aim of this study was to evaluate the efficacy of a commercial vaccine against staphylococcal mastitis in dairy small ruminant farms under field conditions in Greece.

Materials and methods: Four farms in different regions of Greece with similar characteristics (management and milking parlour) and suspected of mastitis problems were selected for a longitudinal study to evaluate the efficacy of a commercial vaccine against staphylococcal mastitis (VIMCO®, HIPRA) through the comparison of two consecutive milking years. All flocks were vaccinated during the second year of the study, following manufacturer instructions. It is to be noted that all animals were housed in the same conditions in both years in order to avoid possible environmental effects during the study.

For the evaluation of efficacy, different parameters of milk production and health status of the flocks were provided by the farmers, such as milk production at tank level, milk production per animal, clinical mastitis cases and deaths caused by mastitis. Furthermore, the milk production data allowed the estimation of global economic gain per animal.

Results: Farms were selected from different regions of Greece (West Macedonia, Central Macedonia, Central Greece and Peloponnese) with flocks of between 145 and 330 animals. A similar number of animals in milking was reported in both campaigns. All births were around October and November in both campaigns, so the environmental conditions were similar in all herds.

Farms showed an improvement of milking parameters evaluated at the end of the second year that all flocks were vaccinated. Specifically, the results showed an increase in milk production per animal of more than 20 litres in the second year. Similarly, vaccinated flocks showed an improvement in health status presenting an absence of animal deaths associated with mastitis and a reduction of between 34.3% and 84.6% in mild mastitis cases. Finally, farmers evaluated the improvement in the milking parameters as an increment in the global economic gain of between €13 and 21 per animal.

Conclusions: This study assessed production and healthy parameters for two consecutive years before and after the administration of a commercial staphylococcal mastitis vaccine (VIMCO®) in all dairy ovine and caprine flocks from the European market leader in this agricultural production sector.

Selected farms are representative of the dairy small ruminant farms in the country based on average size and general management[1], and are appropriate candidates for vaccination with commercial staphylococcal mastitis vaccine with different clinical mastitis prevalence scenarios (4.6% - 15.2%).

All flocks showed similar results regarding the benefit of vaccination in all parameters evaluated, although the data collected was not enough to perform a statistical analysis. Furthermore, these results are similar to those previously published by Vasileiou *et al*. 2019[4] regarding the reduction of clinical staphylococcal mastitis, as well as the effects on production parameters.

Farmers involved in the study recognized the improvement of productive parameters and health status of their flocks after vaccination, hence, most of them will keep using this commercial vaccine in their flocks in the following years. Finally, it is noted that mastitis is a multifactorial disease, and vaccination should therefore be combined with other management measures to improve mastitis prevention and control.

Acknowledgment: Thank you to all the farmers involved in the study.

Keywords: Sheep, goat, mastitis, vaccination, staphylococci.

SR-P09

Characterization and typology of sheep farms in isolated areas in Southern Chile

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A survey was developed and applied to 173 farmers in the commune of Palena and Futaleufú to characterize and identify groups of family sheep farms located in isolated areas of temperate climate in the X Region (Southern Chile). These geographically isolated communes, due to the lack of roads and commercial airports, are faced with extra difficulties during their production process. The survey consisted of a total of 121 questions and was conducted during visits to each of the farms. Descriptive statistics were used to characterize farms. For the development of the typology, multivariate analysis methodologies were used, including principal component analysis, cluster analysis, and discriminant analysis. For the comparison between groups, variance analysis and contingency tables were used. The average size of farms was 133 ± 165 ha, with a flock of 66 ± 60 sheep. 65% of farms are managed by men, whose average age is 57 ± 13 years. The average sheep stocking rate was 1.2 ± 1.4 sheep/ha; however, in 50% of the farms, it had less than 0.8 sheep/ha. In addition to sheep production, 97% of the farmers perform another activity, among which were the production of cattle (86%), pigs and poultry (31%), orchard (34%), wood and/or firewood (26%), crafts (23%) and tourism (17%). Through the analysis of principal components (PC), five principal components were obtained, which together accounted for 68.6% of the variance of the data. The KMO coefficient was 0.7, indicating the sampling adequacy. PC1 was related to dimensional variables, PC2 and PC3 with variables related to grazing feeding and supplement use, PC4 was associated with variables related to sheep mortality and PC5 to specificity in sheep production within of farm. The cluster analysis allowed the definition of 4 farms groups. The discriminant analysis correctly classified 97.1% of the farms. A first difference in the groups was the size of the farm, so GII was the one with the smallest land surface (23.4 ± 21.4 ha) ($p < 0.05$). GII also has the highest sheep stocking rate (3.1 ± 2.2 sheep/ha) and the highest percentage of the surface dedicated to sheep production (90%). The rest of the groups do not show differences in the size of the flock or farm surface, presenting flocks between 60.9 ± 42.5 sheep (GIII) at 80.5 ± 89 sheep (GIV) and land surfaces of 136.6 ± 120.8 ha (GI) at 176.8 ± 186.4 ha (GIV). The sheep stocking rate, as well as the proportion of the land surface destined for sheep production, are lower than in GII ($p < 0.05$). The differences between these three groups (GI, GIII, and GIV) arise around the suplementary feed.Thus, 100% of the farms in groups II and III carry out supplementation with hay, a percentage that drops to 4% and 34% in groups I and IV, respectively. In the GIV, only 2.5% of farms carry out supplementation with concentrate, which is consistent with the fact that this group has the lowest sheep stocking rate (0.7 ± 0.6 sheep/ha). 65% of the farmers report owning breed animals, the main ones being Suffolk and Corriedale with a presence in 42% and 29% of the farms, respectively. However, in 91% of farms, the flocks correspond to crossbreeds. Within the identified groups, it is possible to detect differences in the managed breeds ($p < 0.05$), so in GII and GIII, the percentage of farms with Suffolk and Corriedale breeds reaches 46% respectively. Concerning the way of commercialization of lambs, 80% of the producers do it directly on the farm; however, in the GIV, this percentage drops to 60%, due to the increase in sales in nearby cities (18%) and local fairs (7%). This study allowed us to identify the variables that define types of farmers, and given the context of farm isolation, visualize the marketing and genetic improvement problems they face.

Keywords: Sheep, typology, isolated areas.

SR-P10

Serologic and molecular detection of small ruminant lentiviruses in blood and semen of rams

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Small ruminant lentiviruses (SRLV) belong to the family Retroviridae and include various genotypes that frequently cross the species barrier between sheep and goats, causing a cosmopolitan infection that affects small ruminant production

bringing about economic losses. The success of the control programs depends on precise and detailed knowledge of the diagnosis, and the routes of disease transmission.

Objective: The aim of this study was to detect rams infected by SRLV using serological and molecular techniques in blood and semen.

Material & Methods: A non-probabilistic sampling of 73 males over seven months of age, from different herds in the states of Mexico and Hidalgo, was carried out. Blood was collected by puncturing the jugular vein and sperm was collected from rams by electroejaculation or using an artificial vagina. The samples were cold-preserved and transported to the Virology Laboratory. Blood was centrifuged to obtain blood plasma (BP) and peripheral blood leukocytes (PBL). Seminal fluid (SF) and seminal cells (SC) were obtained from semen. BP and SF were used for serologic detection using an Indirect Enzyme-Linked Immunosorbent Assay based on the use of the recombinant protein p16 (iELISA-p16) derived from sequences of a SRLV genotype B. DNA of PBL and SC was extracted to detect the proviral DNA by nested Polymerase Chain Reaction (nPCR) that amplifies a partial region of the *gag* gene of a SRLV genotype A. The products obtained from the second round of nPCR were loaded on agarose gels and electrophoresis was performed, then stained with ethidium bromide and observed using a transilluminator.

Results: Five males were positive for iELISA-p16 in BP and no sample of SF was positive. *Gag* region amplifications were found in PBL and SC of 41 rams: 23 were positive in PBL and 32 in SC, of which 14 rams were positive in both samples. Three nPCR positive rams were also in iELISA-p16. Two of the five seropositive males coincided with the PCR results for both samples (PBL and SC) and one of them coincided with a positive result for nPCR in SC. In total, of 73 rams, 43 tested positive using one or both diagnostic techniques. These differences in detection between the iELISA-p16 test and the nPCR may be due to the infective genotype in the studied animals.

Conclusions: Detection of rams infected with SRLV was more efficient through the use of nPCR in blood and semen. The presence of antibodies against SRLV was identified using an iELISA-p16 test only in blood plasma. This study highlights the value of semen as a sample in the diagnosis of SRLV, demonstrates the importance of using more than one diagnostic method that includes the genotype / antigen prevalent in the regions, and emphasizes the need to conduct further research on the importance of transmission through semen. Acknowledgements**:** Projects: PAPIIT IT201217 and SAGARPA-CONACYT N°. 291311.

Keywords: Small Ruminant Lentivirus, semen, Polymerase Chain Reaction, Serologic Tests.

SR-P11

Mastitis vaccination in sheep: which vaccination protocols ensure optimal efficacy?

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Objectives: Mastitis is the most common disease and the cause of the greatest economic losses for the ovine dairy sector; therefore, there are various strategies for the mastitis prevention[1]. Mastitis vaccines usually have clear protocol recommendations to ensure optimal efficacy. However, dairy sheep field conditions do not always enable a total protocol compliance due to the production system characteristics. These, usually affect the intervals between doses or between booster and lambing. Thus, the aim of this study was to evaluate the impact of different vaccination protocols with regard to vaccination efficacy in field conditions.

Materials and methods: A comparative, double-blind, controlled trial was conducted during the lactation period (6 milking controls) in 6 dairy farms. The farm inclusion criteria comprised milk recording data availability, intra-mammary infections aetiological adequacy with vaccine valence and moderately high tank somatic cell counts (SCC) before vaccination (annual mean 1-1.3x10^6 cell/ml).

In each flock, ewes were vaccinated with a commercial vaccine against staphylococcal mastitis (VIMCO®, HIPRA) following different protocols: group V (recommended protocol: 21±3 days between doses and 10 to 45 days from booster to lambing), group V1 (slightly shorter interval between doses: 14-18 days), group V2 (markedly shorter interval between doses: < 14 days), group V3 (longer interval between doses: > 24 days), group V4 (shorter interval from booster to lambing: < 10 days) and group V5 (longer interval from booster to lambing: > 45 days; and longer interval between doses).

For the evaluation of efficacy, milk production and individual SCC were recorded. Individual SCC were used to estimate SCC kinetics, mean SCC, and subclinical mastitis incidence (threshold of 250,000 cell/ml). The logarithmic score for SCC was defined as Ali and Shook (1980)[2]. Data were analysed using an ANOVA model of linear regression with the farm and ewes' parity as random effects. Post-hoc Dunnett's test was performed, comparing all groups against group V.

Results: A total of 1206 recorded ewes were monitored monthly. The distribution of the ewes was as follows: 44.9% group V, 13.8% group V1, 15.3% group V2, 12.7% group V3, 5.0% group V4 and 8.2% group V5. Highlight, the group V5 ewes lambed 3 months after the bulk of the flock; thus 3 milking controls only were available.

All groups compared to the reference protocol (group V) showed significant worse results for some of the evaluated parameters, except for group V1 and V4 showing non-significant differences with group V. No significant differences in mean milk production were observed between the groups in the first 3 records, while group V was significantly lower than group V3

with regard to annual milk yield analysis. Nevertheless, the analysis at each milking record showed a significantly higher production in group V when compared to group V2 (2nd and 3rd record) and group V5 (3rd record). The comparative analysis between group V and V3 showed significantly higher and lower values for group V3 in 1st record and 3rd record, respectively.

The annual mean SCC was significantly lower in group V than in group V2 (+0.55 SCC log score). The analysis of the first 3 records showed a significantly higher mean SSC in group V2, V3 and V5 when compared to group V. This was seen with regard to SCC kinetics with a statistical difference being observed in the 2nd (group V vs group V5) and 3rd record (group V vs group V2, V3 and V5). Finally, the and subclinical mastitis incidence was significantly higher in the first 3 records in group V3 and V5, although these statistical differences were not observed in all periods.

Conclusion: For the various criteria subject to analysis, the worst results were identified in group V2, followed by group V3 and V5. The recommended protocol was significantly superior to these groups for milk production, mean SCC and subclinical mastitis incidences. Therefore, compliance with the recommended intervals – mainly the interval between the two doses (21±3 days) – is necessary to optimise vaccine efficacy. Regarding the recommendation of ensuring at least 14 days from the booster to lambing, the results obtained in these 6 flocks did not show statistical differences in group V4 compared to group V; however, this recommendation should be followed in order to avoid immunization during the late pregnancy immunodepression period.

Keywords: Mastitis prevention, dairy sheep, vaccine efficacy, vaccination protocols.

SR-P12

Small ruminant *Coxiella burnetii* abortions epizootiological description and simultaneous bulk tank milk serosurvey evidence the emerging features of Q fever in central Portugal

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Objectives: Q fever is a worldwide zoonotic infectious disease caused by *Coxiella burnetii* and sheep and goats are known to be the main reservoir for human infection. In these animals, *C. burnetii* infection can result in epizootic abortions, often linked to vast bacterial shedding in matrices such as birth fluids and placentas, which increases the risk of disease spread. The present study describes the epidemiological and laboratory findings of *C. burnetii* epizooties affecting sheep and goat flocks, also providing data from a bulk-tank milk serosurvey to assess *C. burnetii* circulation in a population of sheep living in close contact to the human population in Central Portugal.

Materials and Methods: Two small ruminant abortion outbreaks were investigated using questionnaires and samples were tested by real-time PCR targeting the *C. burnetii* genome. The bulk-tank milk serosurvey was performed on milk samples collected in 2015 and 2016, using a commercial Enzyme linked immunosorbent assay.

Results: *C. burnetii* DNA was identified in tissues of the resulting abortions by qPCR. As for the serological survey, 10.2% (95%CI: 4.5-19.2) of the 78 bulk tank milk samples collected in 2015 showed to be positive for *C. burnetii* IgG. The same farms were visited and sampled in 2016 and 25.6% (95%CI: 16.4-36.8) were positive. This increase showed to be statistically significant (p = 0.020).

Conclusion: Seen together, *C. burnetii* outbreaks in small ruminants and increasing bulk tank milk *C. burnetii* IgG samples are strongly suggestive of Q fever emergence in Central Portugal. Measures on animal health and on disease spread control to the human population should be considered.

Keywords: Small ruminant, abortions, Q fever, Portugal.

SR-P13

A longitudinal sero-epidemiological approach to ascertain the circulation of Coxiella burnetii in selected sheep of Portugal

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Objectives: Q fever is a worldwide zoonotic disease caused by *Coxiella burnetii,* a small, Gram-negative, nonmotile, obligate intracellular bacterium with a high infectivity ca-

pacity. In sheep, abortions are considered the most important clinical presentations of Q fever, mostly occurring with no preceding clinical symptoms at the end of pregnancy. In the present study a prospective serosurvey was performed to study *C. burnetii* circulation in a population of sheep in the central region of Portugal.

Materials and Methods: Blood was drawn in 2015 and 2016 from a representative sample of 168 sheep, and tested for IgG anti-*C. burnetii* by a commercially available enzyme-linked immunosorbent assay. Doubtful samples were retested and if again resulting doubtful, considered as negative. Data obtained from the sera screening were used to calculate population and geographic (municipality)-specific seroprevalence values.

Results: In 2015, 7.7% (13/168) animals tested positive for IgG anti-*C. burnetii* while in 2016, 17.3% (29/168) tested positive. The seropositivity difference between 2015 and 2016 was statistically significant (p = 0.008) which shows and increase in anti-*C. burnetii* seroprevalence.

Conclusions: This is the first study in Portugal providing prospective data on the serological status of a sheep cohort of Portugal and showing an increase in the occurrence of Q fever. There is the need to provide clearer understanding of Q fever epidemiology in Portugal, ideally by implementing monitoring programs on sentinel herds.

Keywords: Qfever, serosurvey, sheep, Portugal.

SR-P14

Ultimate causes and timing of neonatal lamb mortality – a prospective necropsy study

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Introduction: Causes of lamb mortality may be defined as ultimate (initiating) or proximate (final), the former (e.g. traumatic lambing assistance) meaning the precipitating cause and the latter the terminal cause (e.g. major complex polytrauma). Only by identifying the preventable and non-preventable ultimate causes of mortality can farmers, vets and advisors reduce lamb mortality.

Objective: The objective of the current study was to determine the ultimate causes of neonatal lamb mortality in mid-season, grass-based systems of prime lamb production.

Material and methods: The population at risk for this prospective observational study was 3 flocks (1,100 ewes and 1,855 full term lambs) lambing at the Teagasc Research Centre, Athenry in Ireland. Flock 1 consisted of three ewe genotypes; Belclare, Suffolk x Belclare and >75% Suffolk. Flock 2 consisted of 4 ewe genotypes; Belclare x Suffolk, Belclare x Texel, Suffolk x Cheviot and Suffolk x Texel. The ewes in these two flocks were joined with Charolais rams. Flock 3 consisted of purebred Belclare, Suffolk or Texel ewes from New Zealand or Ireland. The three flocks were housed in early December and lambed indoors. Flocks 1 and 3 were shorn in December and Flock 2 was shorn in May. All ewes were offered grass silage *ad-libitum* during the housing period. Concentrates were offered according to silage feed value and expected litter size during the final 6 weeks of gestation. Ewes were turned out to pasture within 3 days of lambing. All full-term foetuses and lambs which died within a week of birth (n=172) were submitted for necropsy examination to the National Veterinary Laboratory Service (DAFM). All lambs (dead or alive) were tagged and weighed at birth. A recording form containing the dam, lamb, lambing and mortality details was submitted with each carcass. A time-of-death was assigned to each lamb based on the submission form details and the results of the necropsy; 0h (stillborn; did not breath), Day 1 (breathed and died within 24h) and Day 2 to 7. Where a cause of death could be assigned these were aggregated into 5 main groups (accident, congenital defect, dystocia, infection and other) and the remaining cases were categorised as diagnosis not reached (DNR). Cause of death was analysed as a multinomial variable using logistic regression with time-of-death category as the explanatory variable. The analysis was fitted using the Logistic procedure in SAS 9.4.

Results: The mean neonatal lamb mortality rate was 9.3% (8.8-9.6% by flock). As the mortality rate did not differ between flocks, the overall dataset was analysed. In total, 113, 22 and 37 lambs died at 0h, Day1 and Days 2-7, respectively. Infection was the main cause of mortality, accounting for 37.7% of deaths. Lambs dying at birth or Day 2-7 were more likely to die due to infection ($p < 0.05$) than lambs dying in Day 1. Dystocia was the second most common cause of mortality. Lambs dying at birth were more likely ($p<0.05$) to die due to dystocia than those dying thereafter. Infection and dystocia combined accounted for 52.8% of all neonatal lamb mortality. Accidents were the third most common cause of mortality and they occurred equally in all three time-of-death groups. The cause of mortality was not diagnosed for 28.5% of lambs; these were predominantly stillborn.

Conclusion: The majority of neonatal losses occurred at birth. Infection and dystocia were the most common causes of death overall. These causes of death are potentially preventable. The high DNR rate, particularly in stillbirths, requires further research.

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Keywords: Lamb, mortality, cause of death, necropsy.

SR-P15

Reproductive results comparing two estrus synchronization devices under anoestrus conditions in a commercial meat sheep farm

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Objective: Producing lambs throughout the year, especially in the second half when prices are better, is a fundamental pillar for the profitability and sustainability of meat sheep farms. To achieve this, planning and controlling the reproduction of the herd is needed. In addition, reproductive planning allows for a better distribution of farm labor, food resources and other agricultural tasks. To achieve this, inducing and synchronizing female estrus, according to the availability of rams, is needed to achieve the highest number of pregnancies per batch. The objective was to compare the fertility obtained with two different estrus synchronization devices: intravaginal progestagen-impregnated sponges (Chronogest® 20mg, MSD Animal Health) and progesterone-loaded silicon-based devices (CDIR® Ovis: Controlled Intravaginal Drug Release, Zoetis) under field conditions.

Material and methods: The study was carried in a Merino sheep farm located in the province of Cáceres (Spain). Reproduction management include three mating periods per year.

A total of 300 females participated in the synchronization study performed in April, thus under anestrous climatic conditions. The sheep were divided into 4 mating batches (one batch per week) with an average of 75 sheep included per batch. The selection of females for its inclusion in the trial was based on 1) Birth-breeding Interval >60 days, first breeding lambs were not included and 2) Body Condition: animals with a body condition below 2.25 were not included

The sheep were blindly and randomly allocated to one of the study groups: Chronogest® 20mg-CHG group (CHG) or CIDR® Ovis (CIDR). Randomization was stratified based on body condition, in order to ensure that it was similar between both groups and avoid possible interference in the fertility outcome.

To improve the reproductive response and the ovulation rate of the animals, all sheep and rams were subjected to an energy flushing, starting 15 days before the beginning of the mating period, consisting on 300 grams per day of maintenance concentrate (15% crude protein) as a complement to grazing.

Both progesterone devices were removed after 14 days. After the removal of the devices, a dose of 440 IU PMSG-eCG (Foligon®, MSD Animal Health) was applied to the sheep of both groups.

Fertility was calculated based on the ultrasonographic scanning for pregnancy diagnosis for each batch and each study group.

The data were analyzed using ANOVA for the comparison of means using the statistical program IBM SPSS Statistics, version 25.

Results and discussion: The results of the study show that it is possible to achieve high fertility results (measured as percentage of pregnant sheep confirmed by ultrasound 40 days after ram's removal) under anoestrus conditions in Merino meat sheep, by using an estrus synchronization protocol. Thus, minimizing anestrus seasonality effect and improving farm profitability. The best average overall fertility results were achieved in the CHG group (92%), with better homogeneity on the four batches for CHG group (min 83.8- max 97.4% fertility) than in the CIDR group (min 70,3- max 92,1%). These results endorse the data previously published by Abecia et al. (2011), who indicated that progestogens with Fluorogestone acetate (such as Chronogest® 20mg) are 20 times more potent than progesterone-based devices, thereby achieving better synchronization and fertility of sheep females.

Conclusions: Results show both P4 devices achieved very good fertility results despite sheep were synchronized at anestrus period. Synchronization with Chronogest® 20mg tended to be significantly better ($p < 0.10$) than treatment with CIDR® Ovis, both at batch and at overall level.

Keywords: Sheep, reproduction, fertility, synchronization, anestrus.

SR-P16

Tannin concentrate ration during postpartum period did not affect reproductive performance in Rasa Aragonesa ewes

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Objectives: To evaluate the effects of tannin concentrated ration on reproductive parameters of rasa aragonesa ewes such as postpartum uterine involution, return to ovarian activity and subsequent fertility.

Material and Methods: The experiment was conducted between April and December 2019 in the experimental facilities in Centro de Investigación y Tecnología Agroalimentaria (CITA) de Aragón (Zaragoza, Spain). The ewes (rasa aragonesa breed) had between 3 and 12 years old, nursed a single lamb and were submitted to a different feeding programme: fresh sainfoin (*onobrychis viciifolia*) (SAINFOIN, n= 10) and fresh sainfoin+PEG (polyethylene-glycol "PEG") (SAINFOIN+PEG, n=10). Sainfoin was offered *ad libitum* and the nutritive value was 21.3 ± 0.1% dry matter (DM), 10.6 ± 0.2% crude protein, 38.1 ± 0.5% neutral detergent fibre and 27.0 ± 0.4 acid detergent fibre. In addition, all ewes were fed with a supplement of barley (200g/day). The dietary condensed tannins (CT) content in sainfoin was 45.2 ± 2.1 g sainfoin-eq/kg DM. Ewes from the SAINFOIN+PEG group had a chelate in diet (PEG) to capture the CT from fresh sainfoin. PEG 4000 was administered daily by mouth at a dose of 50 g PEG/100

ml of water. Ewes were weekly examined by transvaginal ultrasonography (7.5 MHz transducer; EXAPAD, imv-imaging) and vaginoscope at 10±2 (V1), 17±2 (V2), 24±2 (V3) and 31±2 (V4) days postpartum. Twelve and 13 weeks after parturition blood samples were collected for progesterone determination (Plasma/Serum ELISA, Ridgeway Science, St. Briavels, Gloucestershire, UK). Progesterone levels >1ng/ml were considered as presence of luteal activity. Three months after parturition 2 rams were introduced in the flock for natural breeding during 45 days (n=19, one sheep was excluded due to unknown reasons). Subsequent parturition was recorded for lambing rate and prolificacy analysis. The following data was recorded for each animal: vaginal discharge (loquia, purulent discharge and no discharge), intrauterine fluid (IUF- presence vs absence) and ovarian structures in V1, V2, V3 and V4, feeding programme (SAINFOIN vs SAINFOIN+PEG), data of calving, plasma progesterone levels, lambing rate and prolificacy. Data were statistically analysed by Fisher´s exact test using SPSS 18.0 (SPSS, Chicago, USA). Statistically significant difference was set at 95%.

Results: Ewes from both groups showed an adequate uterine involution at day 31±2 d postpartum. On V2 all animals examined presented IUF while 7 and 8 animals from SAINFOIN and SAINFOIN+PEG groups, respectively, had lochia discharge or even no discharge. On V3, 80% of the ewes in both groups presented IUF, while only one animal (10%) from SAINFOIN group had purulent vaginal discharge. Sixteen ewes on V4 did not present IUF [9/16 (56.3%) vs 7/16(43.7%), animals from SAINFOIN and SAINFOIN+PEG groups; p>0.05] and no vaginal discharge was detect in that visit. On V4, 14 ewes have had an ovarian follicles or corpus luteum at least in one postpartum examinations determined by ultrasonography [8/10 (57.1%) vs 6/10(42.9%), ewes from SAINFOIN and SAINFOIN+PEG groups; p>0.05].

Nineteen sheep were finally submitted to the rams and 15 ewes lambed after breeding (78.9%). Eleven of 14 ewes that had follicles or corpus luteum at least in one postpartum examinations and 4 of the 5 females with no ovarian structures in the postpartum examinations did lamb (78.6% vs 80%, respectively; p>0.05). Regarding to high plasma P4 levels, 90% (10/11) of the ewes with luteal activity and 62.5% (5/8) of the dams without luteal activity at 3 months after parturition did lamb (p>0.05). No effect of diet was detected on lambing rate [7/10 (70%) vs 8/9(88.9%), ewes from SAINFOIN and SAINFOIN+PEG groups; p>0.05]. Eleven ewes had multiple lambing (73.3%). No effect of diet was detected on prolificacy [4/7 (57.1%) vs 8/9(88.9%), ewes from SAINFOIN and SAINFOIN+PEG groups; p>0.05].

Conclusions: The results showed no beneficious effect of tannin concentrated ration on postpartum involution nor subsequent fertility or prolificacy. However, further studies with larger sample size are needed to evaluate the possible effects of tannin concentrated ration on reproductive performance.

Keywords: Tannin concentrate, ewe, postpartum, reproduction.

SR-P17

Udder health indicators and their relationship with somatic cell count, and milk electrical conductivity and refractive index in dairy ewes

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Objectives: The objective of the study was to assess the relationship between udder health indicators (UHI) and somatic cell count (SCC), electrical conductivity (MEC) and refractive index (RIM) of milk in dairy ewes.

Material & Methods: A total of 501 purebred adult milking ewes of four breeds, from ten intensive dairy sheep farms in Greece were enrolled in the study. UHI were recorded and milk samples were collected once a day the first month of milking period. Udder health assessment was performed for each individual animal by a single, trained observer, before the milking. It included six UHI, namely, the occurrence or not of clinical mastitis (CM), udder skin lesions (USL), udder abscesses (UAB), mammary cysts (MC), udder papillomas (UP) and udder asymmetry (UAS). Milk samples were collected during the milking, using ICAR (International Committee of Animal Recording)-approved equipment (Waikato Milkmeter, InterAg, Hamilton, New Zealand) and protocols. In these samples, SCCs were measured using Fossomatic™ FC, whereas RIM and MEC were measured by a handheld refractometer (RHB-32ATC, according to the brix scale) and a portable conductometer (EZDO 7200) at 20°C. Body condition score was recorded using a five-degree scale (1= emaciated, 5= obese). SPSS v23 was used for the statistical analyses which included descriptive statistics (mean±standard deviation and frequency) and analysis of covariance as described below:

$$Y_{ijklmno} = \mu + F_i + M_j + S_k + A_l + C_m + P_n + S_o + a_1 \times BCS + e_{ijklmno}$$

where, $Y_{ijklmno}$= dependent variables (LogSCC= logarithm of SCC, MEC and RIM), μ= overall mean, F_i= random effect of farm (i= 10 levels), M_j= fixed effect of CM (j= 2 levels), S_k= fixed effect of USL (k= 2 levels), A_l= fixed effect of UAB (l= 2 levels), C_m= fixed effect of MC (m= 2 levels), P_n= fixed effect of UP (n= 2 levels), S_o= fixed effect of UAS (o=2 levels), a_1= regression coefficient of BCS, e= error term.

Results: The average values of SCC, MEC, RIM and BCS were *ca.* $763\times10^3\pm1{,}282.2\times10^3$ cells/ml, 1764±418.9 μS/cm, 13.7±1.00 brix and 2.5±0.39, respectively. The most prevalent udder pathologies/abnormalities were USL (45.9%, 230/501) and UAS (23.8%, 119/501), followed by MC (15%, 75/501), UAB (13.2%, 66/501) UP (5.4%, 27/501) and CM (1.8%, 9/501). The effects of BCS and USL on LogSCC were statistically significant (P=0.001 and P=0.002, respectively). A 1-unit increase of BCS was associated with a 0.26 increase in LogSCC, whereas the presence of USL was associated with a 0.18 decrease in LogSCC. The occurrence of UAS was associated with a 47.6 μS/cm increase in MEC (P=0.044) and a 0.185 brix decrease in RIM (P=0.042), whereas, the occurrence of UP was associated with a 0.36 brix increase in RIM (P=0.041). No other significant effects were observed.

Conclusion: The relationship between BCS and skin lesions and the SCC could be attributed to the confounding ef-

fect of milk yield, which was not measured in our study. Udder asymmetry is associated with increased MEC and decreased RIM values, whereas udder papillomas increase the RIM of sheep milk. Potential mechanisms justifying these findings need to be further investigated.

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Keywords: Dairy ewes, udder health indicators, somatic cell count, electrical conductivity, refractive index.

SR-P18

Effect of pregnancy on clinical and laboratory parameters of Texel ewes

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Objectives: This study aimed to investigate the metabolic changes caused by pregnancy on clinical and laboratory parameters of Texel ewes.

Material & Methods: This study was approved by the Ethics Committee on the Use of Animals of Santa Catarina State University. Fifteen healthy Texel ewes from only one property located at São José do Cerrito/SC, Brazil, were submitted to artificial insemination and pregnancy was confirmed 30 days after by ultrasonography. The ewes were kept in oat and ryegrass pasture and were also fed with corn and soy concentrate. Each animal was evaluated before pregnancy (T0) and at 140 days of pregnancy (T140), which characterized the third trimester of pregnancy. Blood samples were collected to perform complete blood count, serum biochemistry (urea, creatinine, aspartate aminotransferase-AST, gamma-glutamyltransferase-GGT, cholesterol, and triglyceride) and serum malondialdehyde as lipid peroxidation index. In addition, physical examination and urinalysis were obtained. Parametric data were initially evaluated by the one-way analysis of variance test, and non-parametric data were analyzed using the Kruskal-Wallis test. When significant, the data were compared by Tukey's multiple comparison test. For all tests, the level of significance was 5%.

Results: The heart rate (T0: 99.9±13.5 beats per minute, T140: 96.5±16.3 beats per minute, P=0.548), respiratory rate (T0: 67.3±19.7 breaths per minute, T140: 74.3±17.2 breaths per minute, P=0.313) and rectal temperature (T0: 39.47±0.20ºC, T140: 39.45±0.23ºC, P=0.802) did not show a significant difference. Animals showed tachypnea at both times, probably because of restraint stress and not by respiratory disease or other conditions. The complete blood count was normal and there was no significant difference in erythrocyte count (T0: 9.2±1.8 x10^6/µL, T140: 8.2±1.6 x10^6/µL, P=0.127), hemoglobin concentration (T0=11.0±1.7 g/dL, T140=11.4±1.4 g/dL, P=0.572), packed cell volume (T0=29.4±3.8%, T140=27.7±4.0%, P=0.818), and plasma fibrinogen (T0=226.7±128.0 mg/dL, T140=333.3±158.9 mg/dL). In addition, there was no difference for the leukocyte (T0=8,767±3,148/µL, T140=8,457±3,883/µL, P=0.812), neutrophil (T0=3,770±1,609/µL, T140=4,594±2,386/µL, P=0.283) and lymphocyte count (T0=4,199±1,792/µL, T140=3,912±1,486/µL, P=0.636). There was a significant (P=0.015) decrease of serum urea concentration from before pregnancy (T0=41.8±10.7 mg/dL) to the final of pregnancy (T140=33.1±7.2 mg/dL), but all values were in the normal limit. However, serum creatinine concentration remained unchanged (T0=0.97±0.22 mg/dL, T140=0.90±0.10 mg/dL, P=0.279), as well as activity of AST (T0=100.4±41.8 UI/L, T140=95.9±37.2 UI/L, P=0.758) and GGT (T0=39.9±8.3 UI/L, T140=44.2±8.1 UI/L, P=0.160). There was a significant increase of serum triglyceride (T0=4.0 (P25=3.0, P75=5.0) mg/dL, T140=9.0 (P25=8.0, P75=14.0), P<0.001) and cholesterol (T0=36.6±11.4 mg/dL, T140=62.5±10.9 mg/dL, P<0.001), probably because of an increase in lipid synthesis and mobilization by the liver for incremental energy in late pregnancy. Nevertheless, there were no ketone bodies found in urine. Serum malondialdehyde increased slightly from 0.84±0.46 µmol/L before pregnancy (T0) to 1.14±0.61 µmol/L at the late pregnancy (T140), however with no significance (P=0.177), suggesting pregnancy can cause an increment in oxidative metabolism.

Conclusion: It was concluded that pregnancy in Texel ewes caused physiological metabolic changes related to lipid mobilization and a slight change in oxidative metabolism.

Keywords: Sheep, hematology, serum biochemistry, oxidative metabolism, pregnancy.

SR-P19

Effect of Egg Yolk and Removal of Seminal Fluid on Semen Cryopreservation in Norduz Goat

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Objectives: Norduz goat is a breed of relatively high milk yield, resistance disease, preweaning viability and better growth performance. Genetic improvements of farm animals rely on intensive use of a few high quality males either for natural mating or for use in artificial insemination programs. Many countries have already started artificial insemination in goats, although its commercial application is not extensive. However, the main factor limiting more widespread use of frozen semen in caprine reproduction is the reduction of sperm viability during freezing processes. The aim of this study was to investigate the effects of seminal fluid and different of egg yolk (%5- %10) concentrations added to extender on post-thawed Norduz goat semen quality.

Materials and methods: A total number of 20 ejaculates

were collected with an artificial vagina from 5 mature Norduz male goats (3 and 4 years of age) twice a week, during the breeding season twice a week. Each ejaculate were divided into four equal aliquots. The samples were then diluted 1:9 (v: v) in washing solution, centrifuged at 600 g for 10 min at room temperature and the supernatant discarded for sperm washing process. Fresh and washed samples then were diluted with skim milk containing %5 egg yolk and %10 egg yolk, respectively. Extended samples were loaded into 0.25 ml straws, equilibrated at that 4 C for 2h, frozen in nitrogen vapour for 10 min and plunged into liquid nitrogen. Samples were assessed for motility and acrosome integrity upon collection and after freezing and thawing.

Results: Progressive motility and acrosome membrane integrity of frozen–thawed semen diluted in 10% egg yolk were significantly higher than those diluted in 5% egg yolk regardless of the effects of washing. When washing was not performed before freezing, progressive motility and acrosome membrane integrity were significantly higher than the other groups. Sperm motility values of extender containing 5% egg yolk were significantly lower in washed semen compared with the other groups. The percentage of abnormal sperm in extender containing 5% egg yolk were significantly higher compared with theother grups.

Conclusions: It is recommended that a skim milk extender with 10% egg yolk should be used for non-washed semen during breeding season in Norduz goat. In addition to this, egg yolk ratio and centrifugation are important factors for successful cryopreservation of Norduz goat spermatozoa.

Keywords: Acrosome, centrifugation, cryopreservation, egg yolk, goat semen.

SR-P20

Prevalence of Endoparasites in Austria depending on management factors and their impact on clinical parameters

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Objectives: This work should give an overview about the prevalence of endoparasites in small ruminants depending on management factors and their impact on clinical parameters in Austria.

Material and methods and results: Gastrointestinal nematodes

In Austria prevalence is reported to be 65.6% in lambs (Lambacher et al., 2019), 73.80% in goats and 62.18% (Schoiswohl et al., 2021) and 78.80% in sheep (Schoiswohl et al., 2017b) and also in sheep up to 94.70% before and 100.00% after pasturing (Schoiswohl et al., 2017a).

Liver fluke

In the literature the prevalence of *Fasciola hepatica* (*F. hepatica*) in sheep in Austria is reported to be between 0 (Tix, 2012; Schoiswohl et al., 2017b; Lambacher et al., 2002) and 9.7% (Schoiswohl et al., 2021) and 14.10% (Schoiswohl et al., 2018). The economic damage caused by *Dicrocoelium dentricum* (*D. dentricum*) is much lower but infestation is of great importance worldwide (Khanjaria et al., 2014). In three previous studies in Austria the prevalence of *D. dentricum* in sheep is reported to be between 0.06% (Schoiswohl et al., 2017b),14.68% (Lambacher et al., 2020) and 74.80% (Schoiswohl et al., 2021).

Lungworm

In Austria neither Lambacher et al (2020) nor Schoiswohl et al (2017ab) nor Feichtenschlager et al (2014) were able to detect large lungworms in small ruminants. The prevalence for Protostrongiliedes in Austria has been reported to be 2.30% (Lambacher et al., 2020), 18.80% (Feichtenschlager et al., 2014) and 54.20% (Schoiswohl et al., 2017b).

Management factors and clinical parameters

Sheep which were infected with *F. hepatica* had significantly lower epg-values (p= < 0.001). There was a statistical significance correlation between epg and BCS (p=0.030) but no correlation between either *D. dentriticum* infection (p=0.734), *F. hepatica* infection (p=0.702) or infection with Protostrongylides (p=0.231) and BCS (Schoiswohl et al., 2021). In Schoiswohl et al. (2017b), significant correlations between BCS and epg were not found in sheep, but in goats. There was no correlation between faecal consistency and epg (p=0.082) as well as infection with *F. hepatica* (p=0.300) or *D. dentriticum* (p=0.077) (Schoiswohl et al., 2021). A significant correlation between consistency of the faeces and the epg was reported by Schoiswohl et al. (2017b) in goats, while in this study the correlation was not significant and this accords with the results of Schoiswohl et al. (2017b) in sheep. There was a significant correlation between infection with Protostrongylides and the consistency of the faeces; Protostrongylide-positive animals had higher faeces consistency than Protostrongylides-negative sheep (p=0.040) (Schoiswohl et al., 2021). No significant difference between colour of the conjunctiva and the epg (p=0.176) or an infection with Protostrongylides (p=0.206), *F. hepatica* (p= 0.766) or *D. dentriticum* (p=0.345) was detected (Schoiswohl et al., 2021). A significant difference between lung auscultation and the detection of Protostrongylides was detected and Protostrongylide-positive animals showed significant more often moderate hash lung sounds (p= 0.021) (Schoiswohl et al., 2021). There was no significant correlation between conventional and biological management in relation to epg- values (p=0.134) but there is a slight trend that conventional farmed animals had lower epg-values, but this was not significant (Schoiswohl et al., 2021). There was also no significant correlation between the herd size (p= 0.500) and epg values. Full-time farms had significantly higher epg-values than smallholders (p= 0.017) (Schoiswohl et al., 2021). There was no significant correlation in epg- values between sheep which were kept together with sheep from other farms during the summer and sheep which were kept without sheep from other farms (p= 0.434) (Schoiswohl et al., 2021). There was also no significant correlation when wild animals were observed at the pasture (p= 0.777) (Schoiswohl et al., 2021). Sheep which were kept on wetland meadows had significantly higher epg-values than sheep which were grazed on normal meadows (p=0.041) (Schoiswohl et al., 2021). Neither mowing nor mulching (p= 0.906) nor fertilization (p= 0.575) had any significant impact on epg- values (Schoiswohl et al., 2021).

None of these management factors had any significant influence of the presence of Protostrongylides, *F. hepatica* or *D. dentriticum* (Schoiswohl et al., 2021).

Conclusion: All grazing animals are at risk from infections with endoparasites with substantial morbidity as a possible consequence.

Keywords: Fasciola hepatica, Dicrocoelium dentricum, Protostrongiliedes.

SR-P21

Diagnosis and treatment of diseases of the teeth and jaws of small ruminants

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Objectives: In recent years there has been a large increase in numbers of small ruminants presented to veterinary practices, which reflects the numbers now reared as pets. However, only a few published studies have dealt with their dental and jaw diseases. We conducted a retrospective study to determine how to improve diagnosis and treat these oral diseases in small ruminants.

Materials and methods: Data on the tooth and jaw diseases of five domestic sheep (*Ovis gmelini aries*) and 16 domestic goats (*Capra aegagrus hircus*) collected between 2006 and 2017 were analysed retrospectively. Clinical findings were evaluated with regard to species, age, sex, reason for presentation, body condition, appetite, diagnosis and type and duration of therapy. They comprised the dental patients from a total of 1,669 small ruminants (784 sheep and 885 goats) presented to the University Clinic for Ruminants during the investigation period. Where necessary, radiographs and other diagnostic imaging techniques were employed.

Results: The records of 21 animals, 16 goats and five sheep with oral problems from a total population of 1,669 referrals were examined. In 18 cases radiographic examinations were performed, which is well proven to be an important tool for diagnosis and treatment. The body condition was also taken into consideration. Since the average age of the animals was 12 years, this also appears to be an important prediction criterion for oral disease. Our data demonstrates an incidence of periodontal diseases in 66.7%, as well as neoplasia (28.6%) and endodontic diseases (23.8%). A combination of antibiotic and a non-steroidal anti-inflammatory drug is recommended for successful therapy. While specific antibiotics should be selected on a case basis, carprofen has proven to be effective in small ruminants for pain management.

Conclusion: In conclusion, the dental health of sheep and goats is important for their welfare and productivity. Dental examinations on a regular base and timely interventions at the earliest signs of disease are the best prophylaxis. When dental changes become apparent, a thoroughly examination is recommended and radiographs should be taken to ensure the appropriateness of therapy, to minimize the risk of recrudescence and determine whether tooth extractions are warranted.

Keywords: Body condition, welfare, prophylaxis.

SR-P22

Neurological signs in a flock of rams caused by "salt poisoning"

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Objectives: On the 27th of October, a clinical case was referred to the Ruminant Clinical Service of the University of Zaragoza from the Basque Country (Spain). The affected animals came from a sheep flock with 60 ewes and 40 rams and showed neurological clinical signs. The case started in August when 15 rams began to show neurological clinical signs.

Material and methods: During the clinical examination performed at the farm, the animals showed wobble and experienced a certain degree of obnubilation and ataxia, although they were alert and with a good appetite.

Although most of the animals were grazing, twenty of the rams were permanently stabled as they were used for sports and cultural activities in the area. Fifteen of these housed showed neurological clinical signs. The housed animals were fed with 1.5 kg sugar beet pulp which was hydrated with 2 litres of water each day*, ad libitum* hay and beans. In addition, they had intermittent access to salt blocks. However, they did not have *ad libitum* water supply,-drinking only the water added to the sugar beet pulp. Once a day, they were taken outside with a rope for exercise.

One of the affected rams was referred to the Ruminant Clinical Service of the University of Zaragoza to observe its evolution and perform the necessary ancillary tests to emit the final diagnosis. A complete clinical and neurological examination, haematology, CT scan and cerebrospinal fluid sampling and culture were carried out.

Results: At the ruminant service, the animal was fed 800 gr per day of compound feed and straw ad libitum. In addition, he was given water at will, taking care of the first few days so that he did not drink too much at once.

Haematology showed a high hematocrit above the upper limit. The mean corpuscular value (MCV) was also high, while the mean corpuscular hemoglobin concentration (MCHC) was below normal values.

Clinical examination did not show any other clinical signs, presenting a good body score (3.5/5) and a perfect state of alertness. Neurological examination showed no damage to the peripheral nervous system or spinal cord. However, it presented ataxia, dorsal medial strabismus of the left eye, and sometimes the animal became drowsy and lost consciousness (reticular formation in the brainstem), which are signs of intracranial damage.

On the other hand, there was no growth of any microor-

ganism in the cerebrospinal fluid analysis, and in the computed tomography, no lesion was observed.

Considering the neurological signs, a differential diagnosis was developed, and the different possibilities were classified in pathology categories following the VITAMIN D initials (Vascular, Inflammation/Infection, Trauma, Congenital abnormalities, Metabolic, Idiopathic, Neoplasia/Nutritional, Degenerative). The main suspicion was a metabolic and degenerative process associated with water deprivation. It was suspected that the animals presented salt poisoning. This type of intoxication may be caused by a restriction in water consumption, which can be accentuated as the concentration of salt in the diet increases. Augmented plasma sodium levels may reduce the cellular size as intracellular liquid content is taken out via osmosis. This process is particularly dangerous in cerebral cells, as irreversible neurological damage may occur.

During his stay in the service, the animal clearly improved its clinical signs, these almost completely disappearing, so that finally, the male was returned to his farm of origin.

Serial haematology showed a progressive improvement in blood parameters since the patient's arrival at the hospital.

Conclusions: Early detection of the clinical symptoms shown in salt poisoning is crucial as treatment is effective before permanent brain damage happens. In advanced cases where neurological symptoms have appeared, the main objective will be to perform a slow and controlled rehydration: water consumption per hour should be smaller or equal to 0.5% of the animal's weight during 2 to 3 days.

The farmer was recommended to administer water ad libitum to all the animals, and within a few weeks, he informed us that the clinical signs had disappeared in most of the rams.

Keywords: Ovine, neurological, flock, rams.

SR-P23

Effect of Trace Mineral Injections Before Mating and Lambing on Conception Rates, Branding Percentages, Lamb Weights and Economic benefits in Diverse Farms in Victoria, Australia

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Objectives: Optimum trace mineral (TM) status in ruminants is essential for fertility and the survival of the offspring by preventing oxidative stress and improving immunity. The benefits of injectable TM (ITM) supplementation in the lead up to high demand periods, such as pregnancy and calving, has been demonstrated in cattle. However, scarce data are currently available in sheep. The objective of this field study was to evaluate the effect of ITM supplementation pre-mating and pre-lambing to ewes on conception rates, ewe and lamb survival and farm profitability.

Material & Methods: This study was conducted in five commercial farms across Southeast Victoria, Australia, between September 2018 and November 2019 where TM status in ewes was within normal ranges before mating. Mix breeds ewes (n=1484) were randomly allocated to receive either nil treatment (Control) or two injections of an ITM product containing zinc (40 mg/mL), manganese (10 mg/mL), selenium (3 mg/mL), and copper (10 mg/ mL); 0.2 mL per 10 kg BW (Multimin® plus Copper for Sheep, Virbac Australia Pty Ltd) 30 days before the start of mating and 30 days before the start of lambing. Approximately 90 days after mating, pregnancy status and conception rate were determined by ultrasound. The branding rate was determined approximately four weeks after the end of lambing, and lamb weights were determined at weaning (12 weeks after the end of lambing). Weaning weight data were compared using parametric ANOVA (both within and across all farms) and Spotfire S+ while conception, branding and weaning rates and ewe mortality were compared (within and across farms) using the Test Based Method and MedCalc. Significance was defined as $P < 0.05$.

Results: ITM treatment did not affect the conception rate. Across all farms, the average conception rate was 156 ± 11.0 % ($P > 0.05$). The branding rate of treated ewes was 9 % higher than non-treated ewes (95 % Confidence Interval 3 - 21 %). Lambs born to treated ewes were heavier at weaning than lambs born to non-treated ewes (2.31 kg; $P < 0.001$). Overall, there was between 0.75 and 4.27 kg benefit to weaning weight with ITM treatment. Although not significant, ewe mortality across farms was 1.3 % lower in the treated group than in the control group. On average, ewes treated with ITM pre-mating and pre-lambing produced more and heavier lambs that represent an approximate extra AUD$2,793/100 ewes net benefit for the producer.

Conclusion: These results demonstrate that increasing TM status in ewes using an ITM supplement before mating and lambing can improve lamb survival, lamb weight at weaning and farm profitability. These results help to understand TM supplementation for animal health and performance beyond the treatment of clinical deficiencies. Furthermore, we have demonstrated the economic benefits of a strategic TM supplementation in the lead up to high demand periods in sheep.

Keywords: Trace minerals, sheep, reproduction, economic benefits.

SR-P24

Live yeast supplementation during last month of gestation improves ewes ruminal microbiota, oxidative status, colostrum quality and offspring immune competence

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Objectives: In dairy ruminant system, peripartum is a highly challenging period as drastic physiological, hormonal and nutritional changes occur. Negative Energy Balance is commonly observed and results in an increase of lipid mobilization and reactive oxygen species production, threatening immune and metabolic status of the animal thus impairing health and performances. Colostrogenesis occurs at the end of gestation and colostrum quality is of paramount importance in the management of optimal growth and infectious disease prevention in early life. This study aimed at investigating the effect of live yeast supplementation during the last month of gestation on ewe's ruminal microbiota, oxidative status, colostrum quality and impact on offspring immune status.

Material and methods: Twenty-eight gestating ewes carrying two fetuses were homogeneously balanced in supplemented (SC) or control (C) groups according to body weight, age, body condition score and parity. The SC group received the live yeast product *Saccharomyces cerevisiae* CNCM I-1077 (Levucell SC TITAN) included in the concentrate at 8 x 10^9 CFU per day per individual from 1 month before expected lambing date up to parturition. Ruminal and blood samples were collected before supplementation (BS), few days before parturition (Pa) and 2 weeks post-partum (PP). Ruminal microbial populations (bacteria and eukaryotes) were analyzed through high-throughput DNA sequencing and fermentative activities were assessed through volatile fatty acids (VFA) production. Glutathione peroxidase (GPx) and Malondialdehyde (MDA) were measured in ewes blood. At parturition, lamb birth weight was recorded. Lambs remained with their mother for 12h to ensure a first colostrum intake and were then separated and fed with milk replacer for the rest of the experimental period. Colostrum was collected aseptically by manual milking over the first 72h to measure nutrient, oligosaccharides (OS) and IgG concentrations. Immune passive transfer was evaluated through IgG measurement in lamb serum during the first 55 days of life. Ewe's data were tested according to linear mixed model with repetitions considering BS values of each parameter as the baseline. Colostrum data were also tested according to linear mixed model with repetitions. Statistical significance was declared at $p < 0.05$.

Results: Parturition was associated with a significant shift in ruminal microbiota composition. A transient decrease of alpha diversity ($p < 0.01$) was observed at Pa. A decrease of ruminal functional populations linked with parturition was observed in both groups for Fibrobacteres and Neocallimastigomycota phyla and among protozoal population. Overall, SC supplementation was shown to alleviate the impact of parturition. In C group, a stronger decrease of Fibrobacteres was observed (- 38 %), while it was more stable in SC group (-8 %), although the difference was non-significant ($p > 0.05$). A greater stability of protozoal genera such as *Isotricha*, *Eudiplodinium* and *Entodinium* was observed in SC group over time ($p < 0.05$). This greater stability of several microbial populations was associated to higher fermentative activities in SC group as total VFA and Acetate concentrations were significantly more stable over time vs Control ($p < 0.05$).

SC supplementation was also shown to mitigate oxidative status of the gestating ewes with a lower MDA production (lipid peroxidation biomarker, $p < 0.01$) and higher GPx concentration (antioxidant enzyme, +29% vs +6%, $p = 0.19$) in SC vs C groups.

An increase of bioactive molecule levels in the colostrum of SC-supplemented animals was measured, with a significant higher concentration of IgG and oligosaccharides ($p < 0.05$). Immune passive transfer was increased by SC supplementation as lambs born from SC ewes presented significantly higher IgG in their serum up to 7d after lambing. Finally, lambs from SC ewes were 7.2 % (+ 270g on average) heavier than those born from C ewes.

Conclusion: Parturition has significant impacts on the female metabolism, digestive microbiota and oxidative status. SC supplementation alleviated these negative impacts through stabilization of key functional microbial populations and mitigation of metabolic and oxidative status. It is likely that better management of energy and metabolism requirements during peripartum through SC supplementation significantly improved colostrum bioactive molecule levels which benefited neonate immunity, potentially enhancing its survival early life and further health and performances.

Keywords: Gestating ewes, colostrum, parturition, immunity, microbiota.

SR-P25

Influence of a probiotic supplementation on selected blood biochemical and parasitological parameters of lambs

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Objectives: This study aimed to investigate the effects of a probiotic (DBR Sacch®) containing six different bacterial strains and the yeast *Saccharomyces cerevisiae* upon blood biochemistry and parasitological parameters in lambs.

Materials and methods: Forty-two weaned Texel and Ile de France crossbred lambs aged 60.0±5.2 days and body weight of 22.8±3.2 kg were randomly allocated into three groups (n=14 lambs). The control group (CG) received the basal diet without feed supplement; the treated group 1g (T1G) received the same basal diet supplemented with the probiotic mixed into the feed at a dose of 1 g/lamb/day; the treated group 5g (T5G) received the same basal diet supplemented with the probiotic mixed to the feed at a dose of 5 g/lamb/day. The experimental period was 84 days. Every two weeks the following parameters were evaluated: haematocrit, total protein, albumin, globulin, fibrinogen, plasma protein, faecal egg count (FEC) and diarrhea score (score 0 = normal consistency - well-formed and slightly moist dung to score 4 - watery consistency). 20 lambs were slaughtered for histological evaluation of the rumen and abomasal wall, and to recover

and count abomasal nematodes. Data were analyzed using ANOVA and Tukey's test (p≤0.05).

Results: The lambs in the T1G (609±1000) and T5G groups (813±1363) had the lowest FEC (p=0.05) compared to the CG (958±1739) however, the recovery values of larval and adult nematodes showed no differences (p>0.05) between CG (4404.3±2814.0), T1G (3580.0±3491.8) and T5G (2840.0±1084.2). Evaluating the incidence of diarrhea, the T1G and T5G groups had a higher prevalence of score 0, with respectively 35% and 39% (p=0.05), while the CG group presented 22%. All the other evaluated parameters demonstrated no significant difference (p>0.05) between the groups.

Conclusions: The probiotic had a beneficial effect upon gastrointestinal health of weaned lambs through a decrease in parasitic infection and an improvement of fecal consistency, without influencing the evaluated haematological, other parasitological (recovery of abomasal nematodes) and histological parameters of the lambs.

Keywords: Sheep, Animal health, Gastrointestinal nematode, Food additive.

SR-P26

Johne's disease infection in Korean black goat *(Capra hircus aegagrus)*: A case report

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Objectives: *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is the etiologic agent of paratuberculosis in ruminants, commonly known as Johne's disease (JD). JD in cattle commonly showed watery diarrhea and weight loss, while the disease could affect insidiously in small ruminants. In Korea, there are numerous native Korean black goats (*Capra hircus coreanae*) which are the only breed that is indigenous to Korea. The study aimed to investigate the pathological characteristics of the JD infected Korean black goat case. The results could help veterinarians to accurately diagnose JD in Korean black goats, thereby preventing the MAP spread within herd level.

Materials and Methods: The goat (1-year-old) showed gradually decreased weight and loss of appetite, and showed diarrhea before death. The MAP specific antibody titer was analyzed immediately after the goat died by a commercial ELISA kit. After death, we collected nine organs, including mesenteric lymph node, lung, ilium, duodenum, colon, rectum, liver, cecum, and jejunum aseptically from the necropsied goat. The ISMav PCR assay was performed to detect MAP from all collected tissue samples. The samples were sectioned and subjected to hematoxylin & eosin and acid fast staining.

Results: The goat showed hyperaemia of lung and intestine, swelling in gastrohepatic lymph node, hemorrhagic lymphadenitis in the mesenteric lymph node, and watery feces in intestine. The MAP antibody titer was 158.9 (S/P%), and antigen-positive from all collected tissues. The inflammatory cell infiltration was observed in lung, ileum, colon, liver, and jejunum. The lung of case showed pulmonary edema, and the villi fusion and microabscess was observed in mucosa layer in jejunum. Moreover, the mild lymphoid depletion was detected in cecal lymph node. All the tissue samples were given positive reaction for the PCR assay. The ilium, colon, liver, and cecal lymph node was acid fast positive.

Conclusion: This case report suggested that the JD infected goat died suddenly and showed mild pathological lesions. This detailed knowledge of pathological lesions could help the practical veterinarians to diagnosis of MAP in the field, which would contribute the minimizing its impact.

Keywords: Johne's disease, MAP, goat, case report.

SR-P27

Biochemical and minerals profile in White shorthaired goats with subclinical mastitis during postpartum period

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Objectives: The purpose of the study was to evaluate basic biochemical and mineral parameters in White shorthaired goats with subclinical mastitis during postpartum period.

Material and methods: Total of 57 milk and blood samples were collected from random 19 clinically healthy White shorthaired goats. Blood and milk samples were taken in three separate occasions: first 3-6 DIM – days in milk, second 12-14 DIM and third 29 – 31 DIM. After mikrobiology cultivation of milk samples overall 11 goats with positive results thus subclinical mastitis were identified. In those animals, the total protein (TP), albumin, globulin, urea, aspartate aminotransferase (AST), creatine kinase (CK), gamma glutamyl transferase (GMT), lactate dehydrogenase (LD), triacylglycerol (TAG), beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEMK), zinc and selenium were measured in blood. We used statistics to compare 3 sampling among each other.

Results: The mean TP was significantly lower (68,67 g/l) in the first sampling compared to the second (P<0,01) and third (P<0,05) sampling. A significant increase in the serum level of TP (75,59g/l) was observed in second sampling compared to first (P<0,01) and significant increase in the serum level of TP (73,94g/l) was observed in third sampling compared to first (P<0,01). The mean serum concentration of TP (75,59g/l) in second sampling was not significantly higher than in third sampling (P<0,05). The highest albumin concentrations (36,13g/l) were measured in third sampling and were significantly higher compared to first (P<0,001), and second sampling (P<0,01). Increased albumin values were observed during the whole monitored postpartum period. The highest values of globulin (43,50g/l) were measured in second sampling and they were significantly higher compared to first (P<0,05) and third sampling (P<0,05). The highest levels of TAG (0,20 mmol/l) were

measured in second sampling and were significantly higher than first (P<0,05) and third sampling (P<0,001). The highest mean NEFA concentration (0,43 mmol/l) was recorded in the first sampling and was significantly higher (P<0,01) compared to second sampling. GMT values were above reference range during whole monitored postpartum period. The highest mean GMT concentration (1,04 µkat/l) was measured in the third sampling and was significantly higher (P<0,01) compared to the first and second sampling (P<0,001). A significant decrease in level of LD (3,53 µkat/l) was founded in third sampling compared to the first (P<0,001) and second sampling (P<0,001). The activity of AST was highest (1,92 µkat/l) in first sampling and was significantly higher compared to third sampling (P<0,05). The activity of AST was not significantly higher (1,69µkat/l) in second sampling compared to third . The highest level of CK (4,43 µkat/l) was observed in second sampling, and was significantly higher compared to the first (P<0,05) and third sampling (P<0,05). Increased zinc values were observed during the whole monitored postpartum period. The highest mean zinc concentration (9,55µmol/l) were recorded in third sampling and they were significantly higher compared to first (P<0,01) and second sampling (P<0,001). The values of BHB and selenium did not show any significant changes during postpartum period. Differences in the urea serum levels were not significant during the whole monitored postpartum periods.

Conclusions: The presented results show that blood biochemical profile in postpartum period is variable in goats with subclinical mastitis. In subclinical mastitis cases are observed important metabolic changes during 4 weeks post partum period.

Keywords: Mastitis, goats, blood, milk.

SR-P28

Urea and creatinine dosage by serum biochemistry and blood gas cartridges in healthy sheep: Comparison of results

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Objectives: The objective of this work was to compare the results of urea and BUN (blood urea nitrogen) and creatinine dosage methodologies in sheep. The justification for this study is related to the greater application of blood gas in buiatric routine and the existence of these variables in cartridges of portable devices. Therefore, we sought to study the correlation of serum biochemistry values with the data obtained in blood gas analysis in order to verify if the results can be analyzed by the same reference interval proposed for the species.

Materials and Methods: Sixty pure healthy origin-bred Dorper ewes were used, raised under semi-intensive management, from two to five years old, with an average weight of 60 kg, from Araí & Zumbi farm, located in Pardinho, São Paulo State, Brazil. The sheep were released in the morning and were kept on pasture (13.5% Crude Protein - CP) during the day (Vaquero® grass - *Cynodon dactylon*). At the end of the afternoon, the animals were kept in collective pens of 12m² in a masonry shed, with rice straw bedding, where they were fed with concentrate for maintenance 0.5kg/animal (20.9% CP) and 1.0kg of corn silage animal (8.3% CP). Blood samples were collected by jugular vein puncture, in tubes without anticoagulant, under vacuum. After centrifugation to obtain the serum, aliquots were stored at -20°C. In a single time, the samples were thawed and analyzed in a spectrophotometer (Cobas Mira Plus - Roche®) using commercial kits, using the following methods: enzymatic and colorimetric, to determine the urea concentration; kinetic and colorimetric, for creatinine determination. One milliliter polyethylene syringes with sodium heparin attached to a 30x8mm needle were also collected for blood gas analysis in a portable device (I-STAT®, Abbott Laboratories) immediately after collection. The CHEM8+ cartridge was used to measure: sodium, potassium, chloride, ionized calcium, BUN, glucose, creatinine and hematocrit and was calculated hemoglobin and anion gap. To correct the values, the rectal temperature was measured at the time of collection, from conception time to 48 hours postpartum, in nine moments, and hence, 540 samples were analyzed. The urea (17.12 to 42.80mg/dL) and creatinine (1.2 to 1.9mg/dL) values were compared to the normal range for the species proposed by Kaneko et al. (2008). In the statistical analysis, the data were submitted to the Shapiro-Wilk test to verify the normality of the residues and the removal of outliers was performed based on the r-of student. The comparison between treatments (analytical methods) was performed using the SAS PROC MIXED. Correlation analysis was performed using PROC CORR, and the equation was obtained from PROC REG.

Results: It was observed that the mean urea measured by serum biochemistry (36.55 ± 10.07mg/dL), as well as the BUN (13.57 ± 4.58mg/dL) remained within the reference standard for sheep, since to obtain the urea value, it was multiplied by 2.1428. The mean of serum creatinine (1.03 ± 0.16mg/dL) by the conventional biochemical method was in the lower borderline range, while the measurement of blood gas cartridge underestimated the values (0.86 ± 0.15mg/dL). This fact corroborated with the correlation factor, which was strong for urea (r = 0.7714) and for creatinine it was low (r = 0.4668).

Conclusions: The portable blood gas cartridge used in this work can be applied to measure BUN in sheep, relative to serum urea. However, for creatinine measurement, the equipment may underestimate the values, therefore, it may mask an increase in the serum level of this metabolite, which, in turn,

may not demonstrate a subtle change in renal function. Therefore, it is suggested to carry out studies in animals with renal impairment in order to verify if the cartridge will be sensitive to azotemia. In addition, it is worth mentioning that, in addition to clinical signs, other tests can help in the diagnosis of kidney disease, such as urinalysis and imaging tests.

Keywords: Blood urea nitrogen, clinical pathology, kidney function, healthy ewes, small ruminants.

SR-P29

Decreased serum leptin in dairy goats affected by pregnancy toxemia

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Objective: Pregnancy toxemia (PT) is one of the most important metabolic diseases that affect small ruminants worldwide and data related to leptin concentration are scarce in the literature with dairy goats. The objective was to quantify the serum concentrations of leptin and biomarkers of energy and endocrine metabolism in goats affected with and without PT.

Material and methods: A total of 31 goats were evaluated, pregnant, primiparous and multiparous, crossbred or pure of the Saanen, Pardo Alpina, Alpina Americana and Togenburg dairy breeds, with an average milk production of around 3 kg/day, with an average weight of 50 kg, body condition score during the gestation and lactation period between 3 and 3.5, raised intensively, submitted to a diet composed of sugar cane (Saccharum sp), sugarcane bagasse, palm (Opuntia tuna L. Mill), corn bran, wheat bran, and cottonseed meal, whose proportions in the diet varied with the availability of these foods on each property, in addition to mineralized salt and water ad libitum. The animals were treated at the Clínica de Bovinos, Campus Garanhuns/UFRPE, and clinically examined. The goats were divided into two experimental groups, with goats diagnosed with pregnancy toxemia (G1; n=20) and the other consisting of healthy animals (G2; n=11). Blood samples were collected by venipuncture of the jugular vein, using a vacuum tube needle (25x0.8 mm), in tubes containing the anticoagulant sodium fluoride and in tubes without anticoagulants. After centrifugation, the samples were conditioned in Eppendorf tubes and kept in an ultra-freezer (−80°C) for further laboratory processing. The following determinations were performed on the blood serum: NEFA (RANDOX Laboratories Ltd); β-OHB (RANBUT RANDOX Laboratories Ltd), and the analysis were determined in an automatic biochemical analyzer (Labmax 240 Premium, Labtest Diagnóstica S.A., Brazil). Serum concentrations of insulin and cortisol were measured using an enzyme-linked immunosorbent assay by electrochemiluminescence and Beckman Couter Access II system (Beckman Couter Inc., Fullerton, CA, USA). Serum leptin concentration was determined by radioimmunoassay at the Department of Animal Science, University of Missouri, Columbia, USA. The data were submitted to the t-student test at the 5% probability level.

Results: Lower concentration of leptin was observed in goats with PT (p=0.0040). Higher concentrations of Cortisol (p=0.0412), NEFA (p=0.0070) and BETA (p=0.0300) were recorded in goats with PT. With regard to insulin, even though there was no statistical difference (p=0.2040), a lower serum concentration was observed in the group with PT in relation to the group of healthy goats. Sequence of means and lower and upper limits of the parameters in their respective groups: Control Group [Leptin (ng/ml) = 8.10 (4.75; 18.44), Insulin (pmol/L) = 24.36 (14.65; 33.68), Cortisol (nmol/L) = 61.75 (18.31; 156.3), NEFA (mmol/L) = 0.53 (0.3; 0.8), BHB (mmol/L) = 0.45 (0.36; 0.56)]. TP group [Leptin (ng/ml) = 5.03 (2.40; 8.60), Insulin (pmol/L) = 14.28 (1.24; 52.71), Cortisol (nmol/L) = 106.41 (16.98; 389.3), NEFA (mmol/L) = 1.09 (0.42; 1.78), BHB (mmol/L) = 3.64 (0.72; 9.39)].

Conclusion: In view of the findings obtained in this work, we can conclude that the knowledge of leptin dynamics in goats with PT is of paramount importance, as it provides information on the lipid mobilization suffered in the transition period, and its interface with metabolic and hormonal indicators, stating that leptin can be used as an important biomarker in metabolic disorder related to negative energy balance in dairy goats.

Keywords: Ruminants, metabolic diseases, diagnosis, hormone, pregnancy.

SR-P30

The role of urinary N-acetyl-β-D-glucosaminidase for prediction of renal damage in copper-poisoned sheep

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Objectives: Evaluate the activity of N-acetyl-β-D-glucosaminidase (NAG) in sheep experimentally poisoned with cop-

per and treated with Ammonium Tetrathiomolybdate.

Method: Ten Santa Inês sheep, male, aged approximately six months and with an average body weight of 35 kg were used in an experimental Cu poisoning. The experiment was carried out with two experimental groups of five animals each: G1-Control (0.9% NaCl solution as placebo) and G2- [Ammonium tetrathiomolybdate (ATTM) for four days (3.4 g/kg body weight)]. The animals received orally a solution of Cu sulfate pentahydrate. The animals initially received doses of 3 mg Cu/kg/BW/d during the first week and every week if no toxicity occurred, the dose was increased by more 3 mg Cu, yielding the box hemoglobinuria (CH=hemolytic crisis), when the administration was completely interrupted. Blood and urine samples were collected for laboratory analysis (Serum Cu; Activity of urinary NAG; Endogenous creatinine clearance rate; Urinary NAG activity; Urinary excretion index of Cu and NAG; Urinary index of Cu and NAG, and Total excretion of Cu and NAG) in different times (-30d, -7d, HC and 2d after HC). The serum Cu concentration was determined using a colorimetric method with a commercial kit after deproteinization of the sample, with the addition of bathocuproine sulfonate as a specific reagent for Cu. The readings were made on a digital spectrophotometer. The activity of urinary NAG was determined using a commercial kit Boehringer Mannheim. An analysis of variance was done and the treatment means were compared by the Tukey test.

Results: For both groups, lower serum concentrations of Cu and different urinary indexes of Cu and NAG (Urinary NAG activity, Urinary excretion index of Cu, Urinary excretion index of NAG, Urinary index of Cu, Urinary index of NAG, Total excretion of Cu and Total excretion of NAG) were observed in the pre-hemolytic phase when compared to the hemolytic crisis phase, the opposite occurring with the endogenous creatinine clearance rate, according to the averages of the parameters and their respective observation times for G1: [Serum Cu (-30d=15.70b; -7d=21.75b; HC=70.02ª; 2d=77.98a); Urinary NAG activity (-30d=0.90b; -7d=3.82b; HC=142.60a; 2d=295.00a); Endogenous creatinine clearance rate (-30d=10.70a; -7d=3.39b; HC=0.22c; 2d=0.34c); Urinary excretion index of Cu (-30d=2.37c; -7d=96.23b; HC=370.84a; 2d=360.84a); Urinary excretion index of NAG (-30d=0.24c; -7d=2.40c; HC=51.34b; 2d=162.34a); Urinary index of Cu (-30d=1.36c; -7d=56.32b; HC=204.44ª; 2d=207.69a); Urinary index of NAG (-30d=1.60d; -7d=15.88c; HC=449.93b; 2d=1086.54a); Total excretion of Cu (-30d=15.15b; -7d=105.18a; HC=153.64a; 2d=149.12a) and Total excretion of NAG (-30d=17.40c; -7d=50.59c; HC=241.56b; 2d=594.19a)], and for G2: [Serum Cu (-30d=15.92c; -7d=22.55c; HC=70.59a; 2d=45.28b); Urinary NAG activity (-30d=0.88c; -7d=3.46c; HC=136.20a; 2d=79.40b); Endogenous creatinine clearance rate (-30d=9.63a; -7d=4.12b; HC=0.29c; 2d=0.41c); Urinary excretion index of Cu (-30d=2.76c; -7d=29.36b; HC=343.62a; 2d=59.04b); Urinary excretion index of NAG (-30d=0.26b; -7d=2.22b; HC=53.06a; 2d=28.60a); Urinary index of Cu (-30d=1.59c; -7d=16.96b; HC=203.42a; 2d=34.24b); Urinary index of NAG (-30d=1.69d; -7d=14.99c; HC=424.65a; 2d=188.20b); Total excretion of Cu (-30d=17.21b; -7d=85.17a; HC=155.03a; 2d=33.19b) and Total excretion of NAG (-30d=17.38c; -7d=46.72c; HC=249.34a; 2d=157.81b)]. For G1 animals, the averages observed 48 hours after the hemolytic crisis were higher than those observed in HC for the respective indices: Urinary excretion index of NAG, Urinary index of NAG and Total excretion of NAG, while for the other indices and serum Cu concentration the means were similar. For G2 animals, these differences were observed in the following parameters: Serum Cu, Urinary NAG activity, Urinary excretion index of Cu, Urinary index of Cu, Urinary index of NAG, Total excretion of Cu and Total excretion of NAG. With the exception of the endogenous creatinine clearance rate, a significant difference (p<.0001) between the groups was recorded for the other parameters, in which lower means were observed in the animals of the ATTM group. Strong positive correlation occurred between serum Cu and Urinary NAG activity (r=0.70; p<.0001).

Conclusions: The assessment of urinary NAG could be considered as a useful marker in prediction on renal function in Cu-poisoning in sheep, which is significantly high and undergoes rapid and significant change with the increase in serum Cu. The NAG can be considered as a further criterion in the diagnosis of urinary tract disorders in Cu poisoning.

Keywords: Renal Diseases, Renal injury marker, Small Ruminants, Intoxication, Minerals.

SR-P31

Effect of strategic supplementation of dietary by-pass linolenic acid on fertility and milk quality in Sarda ewes

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Polyunsaturated fatty acids (PUFA) are well known for their beneficial role in different body systems and their function in mammals. Their use as feed additive has been investigated with the aim of improving the reproductive and productive performance in different livestock species but their impact on fertility in dairy sheep is scarcely documented. Therefore, the aim of the present study was to assess whether the supplementation of by-pass α-linolenic acid (ALA) can increase fertility and prolificacy in pluriparous Sarda ewes at the end of lactation. A secondary objective was to evaluate the effect of such supplementation on milk production and its composition. Forty-eight ewes were assigned to the following groups: Control group (CTR; N=24), fed with a control diet without ALA; and treatment group (ALA; N=24), fed with a diet supplemented with ALA (10.8 g/ewe/day). Both diets had similar crude protein and energy level and were offered for 39 days (-21 to +18 day after artificial insemination). The trial included an adaptation period (7 days) followed by regular supplementation (32 days) period. The ewes of CTR and ALA groups were homogeneous for Age, Body Condition Score (BCS), Body Weight (BW), Milk Production (MP) and Days in Milk. Milk production was measured at days -28, -1, +10 and +19. Milk samples were collected to determine the content of fatty acids. Also, BW and BCS were recorded at days -28, -21, +2 and 19. Estrous synchronization was induced in all the ewes using intravaginal sponge

(45mg fluorgestone acetate) for 12 days and equine chorionic gonadotropin (350 UI/ewe) at the end of synchronization period. Fifty-five hours after pessaries withdrawal all ewes were inseminated using cervical route and fresh semen. At day 11 after insemination transrectal ultrasonographic scanning was done to determine the presence, number, and area of luteal tissue. Blood samples were obtained at days +11 and +18 after insemination to determinate progesterone levels in plasma. Pregnancy was diagnosed by ultrasound scanning at days +21 and 28. Fertility rate, prolificacy and total reproductive wastage of pregnant ewes (number of CL from pregnant ewe - lambs born/ewe) were also recorded. Statistical differences were established at P< 0.05. There was no difference between groups in terms of ovulation rate, progesterone levels in plasma, fertility rate, prolificacy and total reproductive wastage. However, total area of luteal tissue was higher in ALA group (1.69 ± 0.12 vs 1.20 ± 0.11 cm^2; P < 0.01;) as well as in pregnant ewes from ALA group (1.81 ± 0.16 vs 1.15 ± 0.13 cm^2; P < 0.01). There were no differences between groups for BW, BCS and MP. However, there were differences in milk fatty acid composition. The percentage of PUFA n-3 was higher in ALA group at days -2 (3.28 ± 0.05 vs 3.11 ± 0.05 %; P< 0.05) and +19 (3.12 ± 0.06 vs 1.41 ± 0.06 %; P< 0.001). Results obtained demonstrates that ALA supplementation exerts a positive role in corpus luteum size at the onset of peri-implantation period as well as on milk fatty acids profile in Sarda dairy ewes.

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Keywords: PUFASn-3, Ewe, Reproduction, Milk.

SR-P32

Role of small ruminants in semi-intensive systems as a reservoir of q fever: preliminary study in the eastern peninsular

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Q fever is a zoonosis produced by *Coxiella burnetii* (Cb) with an important relevance in the ruminant sector. Many wild and domestic ungulates can serve as reservoir of this infection (Ruiz-Fons et al., 2007). Q fever has been largely focused on herds managed in intensive systems. Nevertheless, there are few results of infection in grazing herds and their possible interaction with wild ungulates (Wu). This study is based on the hypothesis of the possible underestimated role of Wu as a reservoir of Q fever, as well as their role in the transmission of this infection to grazing domestic ruminants. The objective of this work is to study the possible infection of Cb in flocks of sheep and goats subjected to semi-intensive systems, as well as in Wu, which share pastures with them in the east of the Iberian Peninsula.

For this purpose, a serological and molecular study has been carried out to determine evidences of the presence of Cb through different routes of excretion in small ruminants from 3 sheep herds, 1 goat herd and 1 mixed sheep-goat herd located in the provinces of Albacete, Castellón, Alicante and Valencia. Furthermore, samples obtained from a population of 18 Wu were also include in this study after being hunted. None of the domestic herds were vaccinated against Cb. A total of 52 domestic ruminants (37 sheep and 15 goats) were sampled during the first week postpartum. Vaginal swab, faeces, and blood samples were obtained from each small ruminant. In addition, a pool of milk was taken from each farm. A total of 18 Wu were sampled during the hunting season (January-February 2022): 6 red deer (*Cervus elaphus*), 4 mouflons (*Ovis aries*), 2 Iberian ibex (*Capra pyrenaica*), 6 wild boars (*Sus scrofa*). The following samples were taken from each Wu: nasal swab, vaginal/preputial swab, faeces, blood, spleen, liver and lung tissue. The serological diagnosis was made with blood samples in domestic ruminants (*Coxiella burnetii* Monoscreen Ab-ELISA. BIO-X DIAGNOSTICS K 298/2) and for Wu (ID screen Q fever indirect multispecies ID-VET FQS-MS_ES-2P). A real time q-PCR (*Coxiella burnetii* monodose DTEC-qPCR with internal control, GPS Genetic Analysis Strategies) was applied for the molecular identification of Cb.

A total of 287 samples were processed. Of these samples, 70 were serologies (blood) and 217 samples were analysed for q-PCR (70 faeces; 18 nasal samples; 8 preputial swabs; 62 vaginal swabs; 5 milk pool samples; 18 spleen samples; 18 liver samples; 18 lung samples). The presence of Cb was not detected in any of these analysed samples by q-PCR for domestic herds or Wu. Regarding seroprevalence, 3 of 5 (60%) herds of small ruminants were seropositive. Specifically, 18.92% of the sheep (7/37 sheep) and 6.67% of the goats (1/15 goat) were positive for serology. On the one hand, a 15.38% total seropositivity in this study in domestic small ruminants (8/52) is related to data published by Ruiz-Fons et al., 2010, showing positive serological results (54%) in semi-extensive grazing sheep in northern peninsular regions. Moreover, in the Basque Country (Northern Spain), domestic ruminants in semi-extensive systems have been suggested as a potential source of infection for wildlife due to faecal contamination of pastures (Astobiza et al., 2011). On the other hand, there is a lack of scientific evidence of Q fever infection in Wu in the east of Spain. So, this preliminary study suggests that Wu did not act as a reservoir of Cb in this region of Iberian Peninsula. In this sense, the circulation of Cb in grazing herds is possibly undervalued. Although it is necessary to continue with this study, perhaps it would be necessary to allocate resources in order to implement or reinforce control and prevention measures against Q fever in semi-intensive systems. This study was supported by the project from Spanish Ministry of Science and Innovation of UCH-CEU (PID2020-119462RA-I00/AEI/10.13039/501100011033) and I+D+i project UCH-CEU/Ceva Santé Animale (IP: A. Gómez-Martín).

Keywords: Wildlife, small ruminants, Q fever, Coxiella buerntii.

SR-P33

Relationship between somatic cell count, California mastitis test and microbiological culture in dairy goat subclinical mastitis diagnosis

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Introduction: Goat milk production has shown significant growth in recent years. Mastitis, as occurs in cattle, is one of the most prevalent diseases in goats and it is responsible for great economic losses. The diagnosis of mastitis in goats is usually tested using dairy cows parameters, which often leads to incorrect interpretations. The objective of this work is to compare 3 different diagnostic methods to detect subclinical mastitis.

Materials and methods: Two hundred goats, located in 3 farms of Río Negro and Chubut provinces, Argentina, varying from 2 to ≥ 5 years of age, with different numbers of lactation and born goatling, and never before machine-milked were used. Animals were Angora, Criolla, and their crossbreeds (angora for criollo). They were raised extensively mainly for family consumption, with a mixed production (hair, meat, and milk). Individual samples of each udder half were tested by California Mastitis Test (CMT) considering 0 for negative reactions, 1 for weakly positive, 2 for positive, and 3 for strongly positive samples. Pool milk samples from both udder halves were used for the somatic cell count (SCC) in animals with suspicion of subclinical mastitis. SCC was carried out using the Prescott-Breed technique. For the microbiological identification samples were individually collected from each mammary half, and transported in sterile 15 ml falcon tubes, after discarding the first jets of milk, antisepsis of the teat was made with 70% alcohol. Milk samples were frozen and sent to the laboratory. Bacterial isolation was performed on plates with CHROMagar™ Staph aureus (CSA) chromogenic medium. They were incubated for 48 hours at 37°C. According to CSA guidelines, *S. aureus* colonies appear pink to mauve, while SCNs appear turquoise blue and colourless.

The relationship between the variables SCC, CMT, and bacteriological culture results was analyzed using linear correlation (Pearson) and simple linear regression. On the other hand, the associations between CMT with SCC, CMT with bacteriological culture results, and SCC with bacteriological culture were evaluated using the Chi-Square Test. For the indicated analyses, the statistical package Statgraphics Centurión XVII 112 was used.

Results: Fifty-five (27.5%) goats considered to be suspected of subclinical mastitis by CMT were selected of the total 200 animals tested. From all CMT-positive mammary half sampled, 37.2% (41/110) were negative for bacterial culture on CHROMagar™ Staph aureus chromogenic medium. CMT grades 1, 2 and 3 showed weak correlation with SCC/mL (r= 0.22; p= 0.02) and R^2 0.26 (p= 0.0067). However, no association was observed between CMT and SCC/mL (X^2 = 0.9150), neither between CMT and bacterial culture (p=0.5371) nor between SCC/mL and bacterial culture (p= 0.6021). The mean CCS/mL was 4.4 x 10^{11} (SD= 4.9 x 10^{11}, range 1 x 10^5 - 2.3 x 10^{11}) in milk samples with bacteriological culture positive, and in negative udder halves, the mean CCS/mL was 3.8 x 10^{11} (D= 4.3 x 10^{11}, range 2 x 10^5 - 1.8 x 10^{11}).

Conclusion: In extensive goat production, the positive CMT is not always an indicator of infections in the mammary gland which differs from dairy farms. In goats, the SCC depends on different variables (parturition number, lactation number, lactation stage, stress). A number of different cell types contribute to the SCC. Polymorphonuclear neutrophilic leukocytes (PMN) are the most abundant cell type in goat milk and constitute 45–74% of the SCC in uninfected glands and 71–86% in infected glands. Macrophages and lymphocytes are less abundant and the fraction of these cells decreases with infection. About 6% of the cells in uninfected goat milk are epithelial in origin. The function of PMN is to ingest and kill invading mastitis pathogens. It is therefore surprising that these cells are present in high numbers in milk from apparently uninfected animals. Other studies showed that PMN increases during lactation and explained this late-lactation rise with the presence of higher chemotactic activity in non-mastitic, late-lactation milk. Briefly, this phenomenon is non-pathological and could play a physiologic regulatory role in mammary gland involution. So, we can conclude that bacterial isolation is the gold key and the method of choice for the definitive diagnosis of subclinical mastitis in this species. Bacterial identification guarantees the rational use of antimicrobials in the treatment of subclinical mastitis in goats.

Keywords: Goats, mastitis, CCS, MCT, Staphylococcus spp.

SR-P34

Impact of Protein Supplement on Reproduction of Tswana Goats in Semi-Arid Area of North West Province of South Africa

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This study evaluated the impact of protein supplements on the reproduction performance of female Tswana goats. Twenty-four female weaner goats 3 months old and 10.56±1.28 kg body weights were used. Animals were grouped into three treatments of eight in a randomized block design according to live weight, supplemented with concentrate mixtures consisting of 10% of maize, grass and soybean meal, informed based on their weight (3.5% of body weight) as treatment group 1 to 3. Observations on reproduction performances were made by following planned standard procedures. The results obtained showed that protein supplementation diet significantly increases the weight, the % of kid production, their body weight at

birth. In addition, Goats that received high concentrations of protein gave birth to twins as compared to others. The study revealed that protein supplementation effectively influences twinning by 50%. Finally, the survival rate of kids correlated with protein supplementation to the extent that the highest survival rate was observed among kids born from animals supplemented compared to control treatment.

Keywords: Body weight, Dietary, Progesterone, Serum, Twinning.

SR-P41

Atypical case of a puerperal paresis in sheep

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Objective: The Ruminant Clinical Service of the University of Zaragoza (SCRUM) received an ewe with postpartum paresis in the hind limbs. A complete clinical examination was performed with especial emphasis on the neurological exam in order to achieve a definitive diagnosis of the final cause of the paresis.

Materials and methods: A six-year-old sheep came from a meat farm located in the province of Zaragoza, Spain, where there were 2000 ewes and 150 rams. At arrival to the SCRUM, we were informed that ewe went through a dystocic birth 21 days ago and since then she was prostrated.

The animal was subjected to the same protocol as all animals referred to the service: clinical examination, hematology, ancillary tests, such as ultrasonography or thermography, and *post mortem* examination after humanitarian sacrifice. If necessary, samples are taken for microbiological, molecular and histopathological analysis. In addition, based on the clinical signs of the animal, an exhaustive neurological examination can be carried out by our neurologist.

Results: The only relevant data that was seen in the clinical examination was the paresis of the hind limbs. For that reason, the neurologist proceeded to perform the neurological examination.

The ewe was alert and vigilant and its mental state was normal, but the position in station was abnormal with the hind limbs flexed and the fore limbs delayed. In addition, when she was able to walk, the pelvic member was under the belly and she moved the posterior limbs doing small jumps. All the postural reactions were fine, except the posterior member jump, where the animal showed signs of weakness. As the sheep could do adduction and abduction movements without expressing abnormal gestures, it was dismissed an injury in the obturator nerve, usually associated with dystocia, especially in mares.

Concerning the spinal reflexes, the animal showed left patellar hyporeflexia, that was still more remarkable on the right side, where it was almost areflexia. The patellar reflex serves to study the functionality of the femoral nerve, therefore, the absence of this reflex could indicate that this nerve was affected.

The most relevant muscles of the hind limb were also palpated and bilateral quadriceps´ atrophy and hamstrings´ hypertrophy were detected. These findings explain why the animal was unable to stand, since the quadriceps are the muscles that support the weight of the animal's posterior third. In addition, this allowed us to rule out the affection of the sciatic nerve because the hamstrings had tone. Finally, cranial nerves reflexes were also studied and no abnormalities were found, thus discarding intracranial pathology.

After the complete neurological examination, it was possible to conclude that the animal presented a bilateral injury located between L4-L6, more specifically in the femoral and the saphenous nerve. This second nerve was also suspected because the ewe did not have sensitivity in the medial part of the knee.

At the *post mortem* examination, a muscular dissection of the pelvic limb was performed and it was observed that iliopsoas muscle had not lesion, dismissing the lesion in the spinal cord, and both, anterior rectum and vastus lateral, were atrophied and the two Sartorius muscles that are saphenous territory were decreased. Samples of the affected muscles were taken for a histopathological study that showed signs of local inflammation that confirmed all the previous findings.

Conclusions: In this case, the lesion of the femoral nerve could be confirmed after a dystocic delivery in sheep. This fact has never been described in the literature, always associating dystocia with lesions of the obturator nerve as occurs in mares and bovine.

Keywords: Paresis, dystocic birth, femoral nerve.

SR-P47

Evaluation of protein and total sugars by Brix refractometry of milk from Saanen, Nubian, Alpine and Toggenburg goats during 135 days of lactation

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Milk is the most important goat product on the market, it allows the production of a variety of derivatives including cheeses of high commercial value, its nutritional characteristics allow the use of elderly people with gastric problems and children who are allergic to bovine milk. However, some breeds demonstrate variation in the milk composition, making cheese production harder. The objective of this work was to evaluate the amount of total protein (g / dL) and total sugars (% ° Brix, where for each 1 °Brix we have 1 g of lactose in 100 g of solution) in the milk of different breeds of goats. To measure the amount of total sugars and proteins, the present study evaluated the milk of goats of four breeds using the MISCO PA202 Palm Abbe Digital refractometer, seven females

per breed: Toggenburg, Alpine, Saanen and Nubian. Before milk collections, a physical examination of the mammary gland was performed, followed by a dark-bottomed mug test and the California Mastitis Test (CMT) so that animals with abnormal glands were excluded from the study. Then the samples were collected and the sugars and total proteins were evaluated by Brix refractometry on days 15, 30, 45, 60, 77, 90, 105, 120 and 135 of lactation. Statistical analysis was performed using the GraphPad Prism® program, version 6.0, the normality of the distribution of results was verified, using the Kolmogorov-Smirnov test. For the evaluation of the differences between the averages of the results obtained between the races and between the times, ANOVA 2-way analysis of variance tests with Bonferroni's posttest (for data with normal distribution) and Kruskal-Wallis with posttest were performed Dunn's (for data that did not show a normal distribution). For all results, analyzes with P≤0.05 were considered significant. When comparing the right breast halves between the breeds, we observed a difference between Toggenburg in relation to the other breeds, presenting an average Brix lower than the others (P <0.0001). The same was observed for the left breast halves (P <0.0001), where the biggest difference was between Toggenburg and Nubian breeds, although there was also a difference between the Saanen and Nubian breeds at 90 days of lactation, with the Nubian breed having the highest average Brix degree at all times of collection. When analyzing the total protein values, we observed a behavior similar to that obtained in the analysis of the percentage of ° Brix, when comparing the total protein values in the right breasts, we observed a lower value in Toggenburg animals compared to other breeds (P <0.0001). The same behavior was observed for the left breasts (P <0.0001), with the biggest difference between Toggenburg and Nubian, but there was also a difference between the Saanen and Nubian breeds at 90 days of lactation, with the Nubian breed being the breed that had the highest average total protein at all times of collection. All animals received the same food (24% protein twice a day, grass (*Panicum maximum*) and legume (*Gliricidia sepium*) and mineral salt at will). The lactose content in milk can be influenced by the amount of concentrate available in the diet and is directly related to the volume produced. Although in the present work there was no control of milk production, the lower lactose levels of the Toggenburg breed are not related to the volume of milk produced, as they had a higher volume compared to Nubian goats. These lower sugar and protein values in the Toggenburg breed were also observed by other authors, as well as the highest values in the Nubian breed (Sung et al., 1999; Madureira at al., 2017). Producers have been using the milk of Nubian goats combined with Saanen and Alpine goats' milk to increase cheese production, as they ally the greater production of total solids in the Nubians with the greater volume in the Saanen and Alpines.

Keywords: Brix, goat milk, total protein, milk sugar, breeds.

SR-P48

Caprine blood cells' dynamic from 15 days old to adult age

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A high mortality rate of newborns often occurs in goat breeding systems, meaning significant losses in the first days of life. A basic accessible and effective method of diagnosis and prophylaxis that ensures the animals' health is the blood count. The objective of this paper is to study hematological and total protein values of healthy goats aged 15, 30, 60, 90 days, and adults in the state of São Paulo. 75 male and female Saanen animals considered healthy in the physical examination were evaluated: 15 kids from each age group mentioned and 15 adults, housed in Jacareí City, state of São Paulo. At birth, goats received cow colostrum in 2 feedings and at 7 days old they begun to ingest tifton hay. At 15 days old, commercial concentrate for calf was introduced. Milk was delivered until 30 days old and weaning occurred at 40 to 45 days old. The animals were first dewormed at 15 days old and then at 60 days old. Blood was collected from jugular vein and placed in EDTA tubes which were refrigerated and sent to the laboratory, in order to perform erythrogram and leukogram manually. The obtained data were subjected to variance analysis by the ANOVA test and to comparison by the Tukey test at 5% of significance. There was a decrease in erythrocytes, MCH and haematocrit at 30 days old, and at 60, which is recovered and then stabilized remaining stable until adulthood. The animals showed thrombocytopenia up to 60 days old, and at 90 – when the highest mean is observed. At 60 days old, goats showed an increase in total leukocytes: segmented and monocytes. From 30 to 60 days old, they showed a decrease in lymphocytes and eosinophils: recovered at 90 days old and reaching the highest mean in adulthood. Also at 60 days old, the animals had the lowest lymphocyte mean, which increases at 90 days old; while the eosinophil count revealed a gradual increase up to 60 days old and a sudden drop at 90, coinciding with the decrease in total leukocytes and the increase in monocytes. This study was able to elucidate the goat kids hematological oscillations through these animals' age monitoring.

Keywords: Goat; immunity; baby goat; hemogram.

SR-P49

In-vivo efficacy of aqueous and alcoholic extracts of *Hedera helix* (IVY plant) against fasciolosis in sheep

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Objectives: Fasciolosis is a common disease of ruminants in many countries in the temperate climates and often causes

severe economic losses. This study was carried out to evaluate the *in-vivo* efficacy of aqueous and methanolic extracts of *Hedera helix* against fasciolosis in sheep.

Material & Methods: For this purpose, 30 sheep suffering from fasciolosis were divided into 5 groups (A-E). Sheep in groups A and B were given single dose of aqueous extract of *H. helix* at concentrations of 1.13 gm/kg and 2.25 gm/kg body weight per oral, respectively. Animals in groups C and D were treated with methanolic extracts of *H. helix* at 1.13 gm/kg and 2.25 gm/kg body weight per oral, respectively. Sheep in E group were given Triclabendazole at 10mg/kg body weight per oral. Fecal samples from each animal ware collected at day 0 (pre-treatment) and then at days 4, 7 and 14 (post-treatment) for EPG count.

Results: During *in-vivo* experiment, all the treatments showed significant reduction in EPG. The reduction in EPG was 20% by aqueous extract when given at 1.13 gm/kg while it was 40.47 % at 2.25 gm/kg. EPG count was further decreased to 45.24 % at day 7. Reduction in EPG by methanolic extract at dose rate of 1.13 gm/kg at day 4 was 29.54 %. At day 7 post treatment, the reduction in EPG was 40.09 % and at day 14 the reduction was 43.18%. Reduction in EPG by methanolic extract at 2.25 gm/kg at day 4 was 56.09 %. At day 7 post treatment the reduction of EPG was 64.85 % and at day 14 post treatment the reduction was 68.29 %. The positive control group treated with Triclabendazole showed the maximum reduction of 97.5 % in EPG.

Conclusion: It was concluded that extracts of *H. helix* have the anthelmintic efficacy against liver fluke infestation in sheep.

Keywords: IVY, Sheep, liver fluke, EPG.

SU-P01

Dental extraction allowed normal feeding and rumination in a Holstein dairy cow with multiple broken teeth

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Objective: Dairy cows, as ruminants, need full use of their teeth to be able to ruminate the feed. The objective of this case report was to determine if dental extraction of broken teeth would prove sufficient to allow a Holstein cow to regain complete use of its mouth for feeding and rumination.

Materials and Methods: A 2-year-old Holstein cow was presented for depression, anorexia and excessive salivation at 9 DIM (days in milk). On physical exam it was determined that she had multiple broken teeth in the left mandible. Diagnostic determination of which teeth were affected was by palpation. Extraction of the broken teeth found was performed under standing sedation. Radiographic images were obtained to confirm the complete extraction of affected teeth. No other treatment aside from a non-steroidal anti-inflammatory medication was provided.

Results: One adult incisor and one deciduous incisor, plus one deciduous premolar on the left mandible were broken across the neck at the level of the gingival margin. One adult premolar was completely fractured transversely through the crown, bifurcating the mesial and distal roots. Radiographic evaluation post-extraction revealed no other broken teeth and complete removal of the fractured ones.

The cow started eating the next day and recovered uneventfully, to complete a normal first lactation at about 90% of the average of her herd mates. She conceived from her first insemination.

Conclusion: Careful palpation and manipulation of all teeth under sedation may be sufficient to identify broken teeth that can cause decrease feed intake and rumination, thereby affecting the health of the animal. Complete healing is possible with simple extraction of broken teeth, even in ruminants that require extra use of their molars to masticate, ruminate, and thrive.

Keywords: Teeth, dental, extraction, rumination.

SU-P02

Entropion in Brahman bull: Case report

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The objective of this study is to report the modified Holtz-Celsus technique in case of entropion in a Brahman bull. A six-year-old Brahman bull,, weighing 700 kilos, with a history of bilateral tearing 20 days ago, was examined at the Veterinary Hospital of Uberaba. On ophthalmological examination it was possible to diagnose bilateral inversion of the lower eyelids, characterizing entropion. After performing the fluorescein test, bilateral corneal ulcer was found as a result of entropion. Bilateral blepharoplasty was indicated, using the modified Holtz-Celsus technique. After water and food fasting for 24 hours, the animal was sedated with xylazine and submitted to the right lateral decubitus. Local anesthesia infiltrative was performed, administering lidocaine on both eyelid margins. It was not necessary to shave the hair and antisepsis was performed using a dilution of 10-milliliter of povidine iodine in 1000-milliliter of saline solution. We started the surgical procedure with the excision of a skin flap in the shape of a half moon, located two-millimeter from the eyelid margin. We perfomed frehandly cutaneous excision with approximately two-centimeter width, on the upper and lower eyelid, bilateral. We removed a small strip of orbital muscle aiming to create a greater internal scar and to reduce the intensity of the skin stretch in the postoperative. The skin suture was simple interrupted, using 5-0 silk suture, two-millimeter apart, without the need for subcutaneous suture. We administered in the postoperative period, systemic treatment with enrofloxacin every 72 hours, totaling three applications and flunixin meglumine, for three days, both drugs intramuscularly. For the treatment of corneal ulcers, saline solution, EDTA (ethylenediaminetetraacetic acid) and chloramphenicol eye ointment were used. After 15 days the suture was removed and in 21 days the cornea was completely healed. The surgical treatment allowed the correction of the eyelid inversion, preventing the occurrence of corneal ulcers and tearing. We concluded that the modified Holtz-Celsus technique was efficient for the treatment of bilateral entropion in Brahman bull.

Keywords: Bovine, eyelid, Holtz-Celsus, ulcer.

SU-P03

Osteosynthesis of open metatarsal fracture in Girolanda calf: Case report

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The objective is to report osteosynthesis using the external fixation in open fracture metatarsus in calf. A six-month old female Girolando calf, weighing 150 kilos, was examined at the Veterinary Hospital of Uberaba, with a severe lameness in the left pelvic limb of traumatic origin, with a six-hour evolution. We observed during physical examination the instability on the proximal third of the metatarsus, with bone exposure in a wound of the plantar region of the limb. On the radiographic

examination, it was possible to diagnose a transverse fracture in the proximal third of the left third metatarsal. We submitted the animal to sedation with xylazine, anesthetic induction with ketamine and midazolam and anesthetic maintenance with isoflurane to perform the surgery. As the closed reduction of fractured bone was not possible it was chosen open reduction. We made a 20-centimeter half-moon incision in the skin of the dorsal metatarsus affected. The common extensor was removed to the side to identify the focus of the fracture. After alignment of the fracture focus, three four-milimiter pins with a central screw thread were inserted transcortical and in parallel in each bone fragment. We fixed the pins externally by stem and acrylic resin. The subcutaneous tissue was sutured with simple continuous suture using absorbable monofilament suture 0 and the skin was closed using a Wolff suture interrupted with mononylon 0 suture with plastic tubes. We administered in the postoperative, ceftiofur, gentamicin and meloxicam for seven days. A daily dressing was performed with 0.5% alcoholic chlorhexidine until the suture was removed and the wound was completely healed. Bone healing occurred after 45 days, with a new surgical intervention using the same anesthetic protocol, to remove the pins. The calf showed excellent evolution, did not present a lameness after hospital discharge and was destined for the dairy production system. We conclude that an osteosynthesis technique using external fixation, in addition to the low cost and easy execution, presents satisfactory results in open fracture metatarsus in calves.

Keywords: Bovine, external fixation, third metatarsal, trauma.

SU-P04

Therapy of an open luxation of the fetlock joint in a Holstein Friesian heifer

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Objectives: This case report describes therapy and outcome of an open luxation of the fetlock joint in a Holstein Friesian heifer.

Case report: A two-year-old Holstein Friesian heifer was submitted to the clinic; the animal was pregnant in the 5th week. The patient showed an open luxation of the fetlock joint at the right hind limb accompanied by an extended, infected wound at the lateral metatarsus. Clinical, radiographic and ultrasonographic examination revealed that flexor and extensor tendons of the toe and the digital flexor tendon sheath, respectively, were intact. The surface of the limb distal of the fetlock joint was warm and the skin showed no divergent colour. Sensibility of the toe was preserved. The owner insists in treatment of the animal despite a very poor prognosis. Therapy consisted in wound revision and arthrodesis of the fetlock joint by resection of the joint surfaces with a surgical drill. After surgery immobilisation of the fetlock joint was achieved by a synthetic resin cast reaching up over the tarsal joint with iron reinforcement. Weight bearing of the claw was prevented by a metal rod support at the distal end of the cast. Additionally, a parenteral antibiosis and NSAIDs were administered.

Outcome: The synthetic cast was fenestrated above the wounds to gain access and wound care was undertaken every two to three days. Due to progressive osteolysis another resection of altered bone was necessary 25 and 33 days after arthrodesis. Ten weeks after initial surgery a bony sequestrum (10 x 3 cm) was removed at the distolateral metatarsus. All wounds were completely closed 4.5 months post operationem. During weight bearing of the affected limb the toe showed hyperextension. Sensibility of the distal limb was not affected. The fetlock joint was clinically not stable, radiographic examination revealed a partially ossification of the former joint space. The heifer was released from the clinic 7 months after surgery with a moderate lameness and a supportive bandage. During telephone consultation the owner reported that the cow gave birth to a healthy calf but died two days after calving because of unknown reasons.

Conclusions: Important aspects when performing arthrodesis are the complete resection of altered tissue and an adequate immobilisation of the joint after surgery. The reasons for an incomplete ossification of the fetlock joint could be that immobilisation with a synthetic resin cast was not sufficient. Furthermore, since a large amount of bone had to be resected in this heifer, a wide gap had to be filled with new bone formation. In conclusion, therapy of an open luxation of the fetlock joint were costly and time-consuming and is only suitable for very valuable animals.

Keywords: Athrodesis, arthritis, sequestrum.

SU-P05

Complete eversion of bladder in periparturient nelore cow

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The partial or complete bladder eversion is a poor prognosis rare condition in cows, commonly associated with the intense tenesmus observed at the periparuriente time. As most of the cases described in the literature result in death of the animal, the aim of the present report is to describe a successful case of complete bladder eversion in a multiparous cow. A 14-year-old, 1000 kg, Nellore cow at 280 days of pregnancy with body condition score of 5 out of 5 was referred to the Veterinary Hospital of the School of Veterinary Medicine and Animal Science - UNESP, Brazil with a 24-hour bladder prolapse. At clinical evaluation, a complete exteriorization of the bladder (22 cm) through the vulvar vestibule, tachypnea (52 mpm), intense tenesmus, congestion and thickening of the bladder wall and small residual urine volume were detected. Initially, intercoccygeal epidural anesthesia (C1-C2) was performed

with an association of lidocaine and morphine, followed by 20 min of cryotherapy with ice in the exteriorized segment, thus obtaining 50% reduction in the total volume everted. The eversion was partially reduced through manual reduction after 40 minutes of attempts. The everted portion was repositioned, remaining 10 cm of remaining segment that could not be reduced. Subsequently, five liters of carboxymethylcellulose was administered thought the urethral orifice into the bladder, in order creat a gravitational to help reduce bladder eversion. The cow was maintained with a human gastric tube number 16 intravesically. In addition to constant monitoring of eversion and fetal condition, frequent antisepsis of segment with potassium permanganate solution was also performed. The eversion was fully reduced after 12 hours of local treatment. As at that time secondary subclinical ketosis and hypocalcemia were detected (β-hydroxybutyric acid: 1.2 mmol/l; total calcium: 5.8 mg/dL), in addition to antibiotic therapy (5mg/kg ciprofloxacin once daily i.m) for cystitis, a solution of glucose, calcium and propylene glycol was administered (Idem..). In order to monitor the labor, parturition was induced (20 mg dexamethasone i.m). Then, after 24 hours a healthy 52 kg calf was delivered and the placenta expelled 26 h after calving. Cow and calf were discharged on the sixth day of hospitalization. The antibiotic therapy was kept during 15 days of treatment. In contrast the cases described in the literature, the present case was successful, with absence of relapses and secondary complications, after the treatment instituted, considering the animal's follow-up.

Keywords: Everted bladder, cattle, pregnant cow, cystitis.

SU-P06

Occurrences of spastic paresis in Korean Native Cattle (Hanwoo) and application of tenotomy in the field

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Objectives: Bovine spastic paresis is assumed to occur mainly at the age of 3-8 months after birth due to genetic factors and is characterized by muscular cramps with stiffness and constant pain of hind limbs unilaterally or bilaterally. When a similar symptom appears in cattle over 1 year of age, it is classified as a bovine spastic syndrome. The occurrence of bovine spastic paresis in most breeds has been reported, but there has been no report of such spastic paresis in Korean Native Cattle (Hanwoo). This case study is the first report on the diagnosis, treatment, and prognosis of spastic paresis in Hanwoo. Also, although bovine spastic paresis has been presumed to be caused by genetic factors, one case was an acquired form in which the symptoms appeared after trauma caused by an accident.

Materials and methods: Two calves, 2-month old and 4-month old respectively, were referred to the Farm Animal Medical Teaching Hospital, Seoul National University for lameness. In the 4-month-old calf, lameness developed abruptly unrelated to any accidents, and a local veterinarian suspected spastic paralysis and decided to refer for diagnosis and treatment. There was no musculoskeletal disorder in the X-ray, and the calf was diagnosed as spastic paresis based on the characterized symptoms and sudden onset of lameness. A two-month-old calf showed lameness with the hyperextension and pendulum-like movement of a left hind limb after the fence-related accident. Another local veterinarian performed the cast, but the symptoms did not improve and the calf was referred. The X-ray revealed lesions near the calcaneus and calcaneal tuber, and the muscle damage and regeneration were presumed to have occurred, resulting in the classification as acquired spastic paresis. In both calves, surgical treatment was indicated to relieve the symptom of spastic paresis. Surgical treatments for spastic paresis include neurectomy, requiring special pieces of equipment such as an electric stimulator, and tenotomy, more aggressive but not needed any special tools. Since there are restrictions on the provision and operation of such equipment in the field, tenotomy was performed. To prevent infection after surgery, preoperative antibiotics and anti-inflammatory drugs were administered intramuscularly for 3 days. Before surgery, sedation was performed by intramuscular injection of xylazine (0.2mg/kg), and the surgical site was anesthetized using 2% lidocaine. An incision was made on the upper lateral side of the point of the hock, to access the tendon. Partial tenotomy of the gastrocnemius muscle was performed following incision of the tendon sheath and confirmation of tendon stiffness with bending and straightening up the fetlock. After the surgery, the cast was applied from tiptoe up to a part of the tibia to prevent muscle rupture due to excessive weight bearing on the remaining tendon and was removed 3 weeks later. The prognosis was evaluated in 3 weeks and 6 months after surgery.

Results: In both calves, improvement of lameness was shown following the cast removal after 3 weeks post-operation. Also, the stiffness of the hind limb was relieved. Although pus on the incision line was confirmed after casting removal in the calf with acquired spastic paresis, it was recovered after repeated washing and disinfection. Both calves did not show any other post-operative sequelae, and there was no other disorder or relapse of lameness when confirmed 6 months after the surgery.

Conclusions: Bovine spastic paresis has been reported to occur at 3-8 months after birth by genetic factors. But, if a problem occurs near the calcaneus and calcaneal tuber due to not genetic factors but any accidents, the same symptom as spastic paresis can be shown and be recovered to a normal state by the tenotomy with treatment for bovine spastic paresis. This study is the first report of spastic paresis and acquired spastic paresis due to trauma in Hanwoo. It is significant in that spastic paresis was corrected by partial tenotomy that can be applied without any special equipment at the field.

Keywords: Spastic paresis, Korean Native Cattle, Tenotomy.

SU-P07

Castration of a high-producing dairy cow in late lactation, while correcting a left displaced abomasum

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Objectives: The objective of this proposal is to describe a simple castration technique that can be performed simultaneously to the displaced abomasum surgery in last lactation cows and the benefits it entails.

This technique does not involve added cost and will only take a few minutes longer for the surgery. In this way, in addition to solving the displaced abomasum we obtain a castrated animal with added value.

Material and methods: *Material:*

- Surgical material and sutures.

- Xylazine.

- Procaine hydrochloride.

- 530x8mm polyamide flange.

- Chlorhexidine.

-Guillotine nail clippers

Methodology:

We proceed to sedation of the animal with Xylazine.

We shave and infiltrate in inverted L with 50ml of procaine hydrochloride.

We disinfect the field with a chlorhexidine solution.

After performing the laparotomy on the right side, we proceed with the strangulation of the ovarian pedicle of the ovary on that side, which is easily accessible, by using a flange. We must cut the remaining piece of flange with a guillotine nail clipper.

Then we perform the same operation on the other ovary.

Both ovaries can be easily reached because the cut we make to resolve the displaced abomasum is bigger than that needed for a castrating surgery.

Next, we resolve the displaced abomasum by relocating it and fixing the pylorus to the abdominal wall with the right paralumbar omentopexy technique.

Results: The post-surgical evolution of the animals subjected to this simultaneous surgery is not different from that of those that are operated of just abomasum displacement.

The post-operative therapy is the same as in a displaced abomasum surgery.

The material used only differs in the use of two flanges and nail clippers.

Conclusions: Castration by laparotomy in cows is completely harmless and straightforward, compatible with any other intervention that involves the abdominal cavity. It is a surgery that can be interesting in the ordinary tasks of the field clinician , due to its low cost and the following benefits:

Medical: we suppress the reproductive role of the animal so that it can dedicate all its energy to recovering from its pathology.

Reproductive: ovarian surgery allows us to perform either bilateral or unilateral ovarian atresia and ovariectomy ; unilateral atresia and ovariectomy can be useful in processes such as granulosa tumors, cystic ovaries, etc and therefore it offers a surgical approach to treating certain processes that cause infertility.

Productive: surgical castration produces longer lactation periods, increased weight gain, and avobe all the suppression of oestrus associated problems such as traumatic injuries caused by the process of mounting other cows.

Keywords: Surgery, abomasum, castration.

SU-P08

Application of Acupuncture to Astasia Cow after Parturition

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Objectives: Astasia cow after parturition called 'Downer' cow syndrome is a relatively common complication caused by various reasons, including metabolic disorder, infection, toxicosis, and neurological trauma. Multi-modal therapy has been indicated, whereas most cows with neurological damage causes are considered to slaughter due to the nonresponsive to the therapy and economic issue. This case report is conducted to report the outcome of acupuncture treatment for astasia cow suspected neurological damage after parturition.

Material and Methods: 3-year-old female, primiparity Korean native cow was referred with astasia after parturition. Two weeks before referring, the cow had a history of a caesarian section through the left abdominal flank approach. After surgery, right hind limb hemiplegia was presented, and there was no response to medical treatments, including dexamethasone and calcium injection in a local animal hospital.

Results: On the physical examination, astasia by right hind limb hemiplegia with non-weight bearing was revealed, and knuckling was also presented in the affected limb. Other physical examination results were in the normal range, and no reasonable results on clinicopathological tests. Based on the history and physical examination, sciatic or femoral nerve injury caused by dystocia was suspected, and consequently, acupuncture treatment was decided. Acupuncture points were selected with GB30, GB31, GB44, ST45, SP6, and KID1 for the dry needle. Electroacupuncture at the following pairs of points for 15 minutes using 20Hz and 80Hz; BL31, BL32, BL33, BL34, and BL54 with bilateral pair; ST36-GB29 and BL36-ST34 with four pairs, was performed. After one day, the cow was able to stand, and an assisted ambulatory training exercise using a hip lifter was started for rehabilitation. After one week, acupuncture therapy was repeated, and the cow was able to walk spontaneously with a stiff gait.

Conclusions: Acupuncture has been widely used as a complementary treatment option in veterinary medicine to produce analgesic effects, normalize physiologic functions, and treat neurological disorders. Although the evidence-based scientific results are scarce for acupuncture efficacy, according to this case, acupuncture could be considered along with other treatment options in astasia cows suffering from neurological damage after parturition.

Keywords: Acupuncture, Astasia, Parturition, Downer Cow Syndrome, Cow.

SU-P09

Surgical resolution of a tibial fracture, in a calf, using LCP plates

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Summary: Traumatological problems affecting the extremities of bovine animals usually take place sporadically due to abrupt falls or run over (Baxter and Wallace 1991) or byobstetric manoeuvres related to the care of delivery (Nuss and Col 2011). The tibia is the most compromised bone structure, it represents more than 50%of all fractures involving the long bones of the pelvic limbs and with a survivalrate higher than horses ($p<0.01$) (Crawford and Fretz 1985). However some animals with compromised health status must be removed from the heard, because they represent a bad prognostic, both productive and reproductive (Gangl and Col 2006)

Also the cost of treatments either through the application of reduction techniques closed by compression and external immobilization with plasters or combinations with Thomas coaption trays (Adams and Fessler 1996), Walker trays and Spica bandages (Baird and Adams 2014) have yielded variable results in young bovines. On the other hand, in adult animals osteosynthesis by external skeletal fixation apparatus is considered an ideal method and is the most used technique in the reduction of fracture in the species (Adams 1985).

Currently, in our environment, the decision to institute a surgical treatment is likely, taken into account the productive value of Angus PC(pure controlled) and Hereford PR(pure registered), Holstein and even pedigree sheep breeders, in which the surgical results obtained, amply justify such treatment in rural conditions.The cost of that surgical treatments are similar in other countries (Adam and Fessler 1996 USD 410-500) .

The post-operative complications they may present are also easily controllable (pain management and infection, reducing locomotive dysfunctions as disuse atrophy). The cost generated by the surgery can be recovered in the medium (30 days) or long term (60 days) and preserving the animal's life (Vitoria-Gasteiz 1999).

To date more than 18 surgical treatments (osteosynthesis) were performed in field conditions in different agricultural establishments in the Province of Buenos Aires.

The present work shows the valid surgical treatment for an Angus/Hereford cross calf of 80Kg that presents a fracture of the tibia in the posterior right limb by traumatic origin. It was treated by the trauma service for rumiants of the Hospital Escuela UBA.

Materials and Methods: Implants used:

Locked plate LC-LCP (Locking Compression Plate) 4.5mmm with locking screws. The advantage in using a plate in the form of a bridge pillar is that it reduces the contact between the bone and the plate. The plate architecture causes a homogeneous deformation and greater resistance to torsion and flexion, improving its technical qualities. Another interesting feature is that it has mixed compression and locking holes that allows it to be adapted to all types of osteosynthesis.

Anesthetics Protocol

Pre medication.

Xylacine 2% 0.1mg/kg IM was used combined with Tramadol at a rate of 3mg/Kg SC.

Regional blockade of the Ischaetic Nerve and Femoral Nerve was carried out with Lidocaine 2%, ultrasound guided with 90/12 needles.the

Surgical technique

First phase.

Surgical approach with fracture ends, the perimedullary hematoma of the complete fracture is observed in green rod.

Second phase.

Fracturary hematoma toilette, reduction of bones lines and aligment of ends.

Third phase.

LCP plate is placed in the fracturary defect, where the position of the self-tapping screws is observed at both ends of the tibial diaphysis (6 screws were placed).

Fourth phase.

Closure of the tibia fascia and skin with absorbable suture (Vicryl N° 0) a continuous pattern Reverdin type.

Post-surgical treatment

Tramadol 2mg/kg during 3 days.

Dexamethasone phosphate 0.3mg/kg during 3 days.

Penicillin-Streptomycin 20,000 UI/kg during 3 days.

Results: It was possible to reduce, align and stabilize the fracture. The radiological evolution was very satisfactory and without complications with full recovery of the functionality of the operated limb.

The results obtain through the use of these plates allow the restoration of functionality earlier than with alternative pos-surgical osteosynthesis and required a minimum of care in comparison with other models (e.g. K/E or intramedullary nails).

Keywords: Traumatological, osteosynthesis, Surgical, calf.

SU-P10

Regional block of a tibial fracture in a calf

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Introduction: Regional blocks in ruminants are currently a great tool as they help, in first instance to pain management and in second instance to anaesthetic management, lowering the doses, improving the anesthetic recovery and having low-impact on patient's internal environment while anesthetic procedures are being made, as they take part of what we call multimodal anesthesia/ analgesia. Currently regional blocks are being use specially in podal, head and abdomen pathologies, in this case a block has been made for a tibial fracture in an Angus mestizo calf.

Materials and methods: To carried out the block it was used a neuro localizer with the help of an ultrasound scanner, achieving to block the ischiatic and femoral nerve. To conduct the procedure, the patient's sedation was made with medetomidine 5 microgram/ kg intravenous way. The approach for each nerve was, for the femoral nerve an iliac approach, and for the sciatic nerve an inguinal approach. To that end it was used bupivacaine at 0.025%. using 0.2 ml/kg for each block. The blocks were carried out using an ultrasound guide which helped the procedure. The maintenance was executed with an isofluorane mask and propofol drip.

Results: Observing that the anesthetic plane maintenance was only made with isofluorane and propofol, we estímate the block results were as expected since there was no need to performe analgesic rescue throught out the surgery turning out to be very satisfactory.

Conclusions: The application of ultrasound guided blocks in an anesthetic protocol states a change in this species anesthetic paradigms and that we can work with maintenance drugs reduction so that, in first instance we can have a low impact in the patient's internal environment and in second instance, the no need of intra-surgical use of analgesic drug. This will result in the incorporation of the technique in a not too distant future to all anesthetic procedures.

Keywords: Anaesthetic,management,fracture, calf.

TE-P01

Characterisation of final year students´research output in Lusofona university 2013-2018

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The objectives of this study were to understand trends of farm-animal related research outputs in the Faculty of Veterinary Medicine (FMV) of University Lusofona in Lisbon.

Topics of all students that completed the degree between 2013 and 2018 were analysed. The areas of research were split into: Small Animal Clinics, Equine Clinics, Farm Animal Clinics, Food Safety & Technology and Others. Frequencies of number of dissertations written in Farm Animal clinics were analysed along the years between 2013 and 2018, and split into different species: Bovine, Small Ruminants, Swine and Poultry. The number of scientific publications were calculated based on online search on PubMed by the name of the Dissertation's author (student).

The number of students writing their dissertation related to farm animals was 15%, between 2013 and 2018 in the FMV-University Lusofona. From these, 81% did their research work on Clinics, 9% on Food safety and the remaining 10% split their work on other areas. Species-wise, 67% of the dissertations were on cattle, 10% on small ruminants, 14% on swine, and 5% on poultry. No papers were published in peer-reviewed journals as a result of these research activities.

These results show that there is a significant potential for farm animal research outputs in the Faculty, however there are limitations to publishing that need to be studied and addressed.

Keywords: Teaching, research output, farm animals.

TE-P02

Facilitating behavioural change of veterinary professionals and their farmers via virtual avatar communication training.

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Objective: The agricultural industry faces many challenges due to internal and external pressures over the past years. These challenges have direct impacts on the role of veterinary professionals and require the continuous development of knowledge and skills, especially on communication and behavioural change. Research has shown that communication skills play a vital part in delivering advice with impact, and yet many veterinary professionals are lacking in this department.

Material & Methods: Training communication skills can be very successful, provided that participants actively practice their newly acquired skills and receive feedback. The Veterinary DialogueTrainer (VDT) makes this possible, by creating a safe environment for one to practice consultancy conversations with a virtual avatar. Improving the effectiveness and impact on these conversations and resulting in both a higher client and job satisfaction. In comparison to traditional role plays, the VDT is more effective and time efficient when training larger groups and can be accessed worldwide.

The VDT is like a computer game, which simulates and assesses interactions between people, for example between a veterinary professional and a farmer. The VDT can be played on any device and allows participants to actively practice their skills in a realistic and safe environment. Various simulations can be played and re-played, allowing for numerous challenging situations and topics to be practiced. Data is collected from all the plays which allows progress to be objectively measured and monitored. The data gives insight into player choices and highlights were extra knowledge and training is necessary.

Results: Early results of application of the VDT in education and training of veterinary professionals and veterinary students show both a positive experience by participants as well as positive results, as participants are able to improve their scores by playing various simulations.

Conclusion: Preliminary results are promising with the VDT possibly being the important link to translating learnt communication skills into practice. Facilitating behavioural change of both veterinary professionals and their farmers. Resulting in better adherence to veterinary advices and increased health and wellbeing of farm animals.

Keywords: Communication, virtual, behaviour change, veterinary professionals, avatar.

TE-P03

Bovine ovarian phantoms - an important part of transrectal palpation teaching to veterinary students

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Objectives: Transrectal clinical examination of the reproductive tract in cows is an essential part of future veterinarians' training. Current difficulties in animals access and European sanitary restrictions limit the possibility of learning on a large number of cows. In order to increase the practical skills of future vets with the least possible disruption to animal welfare, ready-made phantoms equipped with limited elements (uterus, ovaries) are used. Unfortunately, the properties of those structures do not always reflect the physical properties of organs they replace. The aim of this study was to create silicone phantoms of ovaries (SPO), based on physical parameters and structures of real ovaries, which can serve as a good tool for future vets' training.

Material and methods: Ovaries (N=80) from multiparous cows at different stages of the estrous cycle, were used to

create SPO. In the initial phase of the experiment, cuffs were made for silicone casting of the ovaries. The post-mortem obtained ovaries were cleaned of connective tissue and blood, then filled with a two-component silicone compound (Kuposil Rebound 30 KR30). The molds prepared in this way were set aside for 24 h until the silicone was completely dry. After that time, the ovaries were removed and the molds were cleaned. The next step was to measure:

1. the turgidity of the ovarian parenchyma, wall, and corpus luteum (CL) at different stages of development,
2. previously prepared silicone casts of different hardness (binary preparation KR 15, 25, 27.5, and 30).

The elasticity was evaluated using a bond tester (Nordson Dage Bondtester 4000 Plus) capable of non-destructive force testing. During the measurements, the device, with the use of a special pressure plate, applied a compressive force to the structure, while recording the plate displacement. This enables the calculation of the sample modulus of elasticity. The compressive force value always ended at 0.24945 N, with the contact time oscillating between 3 to 6 seconds, depending on the elasticity of the structure. The results obtained were collated so that the fragments of the ovary and its structures corresponded to a particular type of silicone. Silicone masses were then prepared for the parenchyma, wall, and CL consistent with the previously performed measurements. The obtained ovarian parenchyma mass was colored with Silc-Pig Flesh-Tone dye of flesh color. The CL was injected with silicone of yellow to orange color depending on the predetermined phase of the estrous cycle. All the SPO components were introduced into prepared molds and removed after 24 h. Ovarian follicles imitations in SPO were obtained by introducing air with the use of a syringe.

Results: The values of the distance from the beginning of pressure by the plate to the end of the measurement were in the range: 66.254-107.42 µm for the CL, 241.63-300.98 µm for the ovarian parenchyma, and 145.84-163.02 µm for its wall. Analogous measurements were performed on ovarian casts. The SPO stipes for types 15, 25, 27.5, and 30 were 725.67-766.44 µm, 319.47-420.94 µm, 143.93-176.55 µm, and 102.35-121.81 µm, respectively. The casts prepared in this way faithfully recreated ovarian appearance, shape, and texture.

Conclusions: By matching the turgidity of ovaries and their functional structures to the turgidity of silicone, it is possible to create realistic copies of those ovaries. A large number of differentiated SPOs may replace animals in many situations related to ovarian diagnosis during transrectal palpation studying. Therefore, the created SPOs are an excellent tool for teaching students as well as improving the practical skills of veterinarians. Work is currently underway to make a silicone uterus model.

Keywords: Phantoms, ovaries, cattle, veterinary students.

TE-P04

Cattle palpation skills of veterinary students consulting their experience and motivation

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Objectives: Transrectal examination of the female reproductive organ is one of the obligatory skills that every veterinarian should possess. Rectal palpation is currently the fastest, cheapest, and easiest technique to accurately assess the condition of the uterus and ovaries. However, it is hard to assess the actual level of rectal examination correctness among veterinary medicine students. The purpose of the research was to measure veterinary medicine students' skills of rectal examination consulting their experience and motivation.

Material and methods: The research was conducted among 4th-year veterinary medicine students of Nicolaus Copernicus University in Torun, Poland. Five sessions (4 training sessions and 5th to control results) of rectal examination training were run from October to November 2021. Sessions took place on milk cattle farms near Torun in agreement with farms' owners. In the research 21 veterinary medicine students took part. They were divided into 3 groups according to their experience and motivation. The first (I) group included highly motivated students, which means those who planned to work with farm animals in the future, they were raised on cattle farms and had experience in rectal examination (N=2). The second (II) group included highly motivated students (willing to work in the future with farm animals) but inexperienced in rectal examination (N=4). The last (III) group included low motivated students who did not want to treat large animals in the future, and without experience in rectal examination (N=15). Furthermore, first and second group students had trained palpative rate on ovarian phantoms before.

Students had to:

1. find a uterus and a cervix and rate their size and consistency,
2. find and rate the size of a right ovary and a left ovary.

Time and correctness of palpation of every training and testing session were noted. Examination time was measured since entering a hand in the cow's rectum to find every organ of the reproductive system. Only multiparous cows were examined.

Results: During training sessions students from group I correctly recognized 75% of required female reproductive system structures, students from group II 43.7% ($P<0.05$), and students from group III 28.3% ($P<0.05$). After training in the testing session, the correctness increased to 100% in group I, to 87.5% ($P<0.05$) in group II and to 75% ($P<0.05$) in group III. In the testing session compared to the training sessions, the correctness of rating a uterus size increased from 50% to 100% in group I, from 46.7% to 100% in group II ($P<0.05$), and from 61.7% to 100% in group III ($P<0.05$). In the testing session, compared to training sessions the correctness of rating uterus consistency increased from 12.5% to 50% in group I ($P<0.05$), from 20% to 87.5% in group II ($P<0.05$), and from 28.3% to 53.1% in group III ($P<0.05$). In the testing session compared to training sessions, the correctness rating of the

left ovary size increased from 25% to 75% in group I (P<0.05), from 13.3% to 62.5% in group II (P<0.05), and from 3.3% to 28.1% in group III (P<0.05). Correctness of rating the right ovary size increased from 8.3% to 31.2% in group III (P<0.05), whereas in groups I and II the increase was not noticed. The average time needed to finish a rectal examination totaled: 108 s, 284 s, 250 s in training sessions, and 52 s, 149 s, 158 s in testing sessions in groups I, II, and III, respectively.

Conclusions: The most motivated and experienced students proved the highest cattle palpation skills. Although the relevant increase in correctness of transrectal examination was noticed in every-student group, the increase in correctness of the right ovary size rating was noticed in group III only. The research needs to be continued.

Keywords: Cattle, rectal palpation, skills, veterinary medicine students.

TE-P05

Rickettsia massiliae circulation in sheep and attached ticks, Portugal, 2021

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Objectives: The aim of this study was to investigate rickettsiae infection in ticks and their sheep host from 27 farms of four districts of central Portugal, in order to clarify the role of the sheep host in the maintenance and transmission of these zoonotic agents.

Materials and methods: Between March and May 2021, EDTA blood samples (n=100) of healthy grazing sheep and their ticks (n=100, one tick per animal) were collected during a herd health program in Central Portugal and tested for the presence of *rickettsiae* by PCR targeting the *omp*B gene, and positives were retested for *omp*A gene, followed by bidirectional sequencing for genetic characterization. The *omp*A gene sequences identified in this study and representative sequences for the *R. massiliae* obtained from GenBank were used for the phylogenetic analysis.

Results: From a total of 100 paired ticks and host sheep, Rickettsia was detected in 62 *Rhipicephalus sanguineus* ticks and 35 grazing sheep blood samples, according to the *omp*B screening. Sequencing and BLAST analysis of *omp*A products showed the presence of *R. massiliae* sequences, with 100% identity with *R. massiliae* sequences obtained from Pakistan and Argentina.

Conclusions: Although no animal presented clinical signs and the relevance of rickettsial infection in sheep seems to be minimal, the circulation of *R. massiliae* in domestic cycle, close to human environment, increases the risk of transmitting this infection to humans. These findings suggest sheep as important reservoirs maintaining *rickettsiae* infection.

UH-P01

The use of cabergoline at dry-off reduced SCC and increased milk production in a commercial dairy herd in Brazil

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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 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). 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 somatic cell count and 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 effect of one single administration of cabergoline at dry-off (Velactis®, Ceva Santé Animale, Libourne, France) on the somatic cell counts (SCC) and milk production of high yielding dairy cows during the subsequent lactation. Our hypothesis was that by reducing intramammary infections we would reduce SCC and therefore reduce the negative impact of mastitis on milk production.

Materials and methods: The trial was conducted from April 2017 to September 2018, and involved 326 Holstein- Frisian dairy cows from one modern cross-ventilated commercial dairy farm in São Paulo State. Cows were enrolled in the trial if their daily milk production was equal or more than 25 kg the day before dry-off. All cows were dried off abruptly and randomly assigned to one of two groups: cabergoline group received a single intramuscular injection of 5.6 mg of cabergoline (n = 168) after last milking and the control group (n=158) was left untreated. Individual SCC (log), milk production (Kg), presence of subclinical mastitis (SSC > 200 000 cells/mL) in the day before dry- off and dry period length were recorded for all cows. Cow's individual SCC (log) and milk production were recorded monthly and daily, respectively, from day 7 to day 112 post-partum. Statistical analysis was performed using SAS 9.3.

Results: At dry-off, average SCC (1.86 vs. 1.87), presence of subclinical mastitis (19.6% vs. 21.5%), milk production (30.4 Kg vs. 29.7 Kg) or dry period length (45.5 days vs. 45.6 days) did not differ between cabergoline and control groups, respectively (P > 0.10). In the subsequent lactation, during the study period, cows treated with cabergoline presented lower log SCC compared with non-treated cows (1.57 vs. 1.77, P < 0.01). The average milk production per cow during the study period was significantly higher in cabergoline group compared to the control group (4954 kg vs. 4801 Kg, P < 0.01).

Conclusions: It is the first time that the impact of cabergoline on subsequent lactation milk production and SCC is evaluated in Brazilian commercial farms. Our data provide evidence that a single injection of cabergoline significatively reduced SCC and increased milk production in the following lactation. This is of particular interest under tropical climate where cows are undergoing severe heat stress and consequently greater SCC levels.

Keywords: Cabergoline, Milk Production, Dry off, Somatic cell counts.

UH-P02

The value of regular dynamic milk testing in practice: special findings in a case with E. coli mastitis

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Objectives: This case report discusses the value of regular dynamic testing of milking machines in veterinary practice. Bacteriological findings and data analyses pointed towards environmental mastitis in cows without high cell counts. Dynamic testing helped identify parlour specific problems, and in addition separate issues were also recognised in solving this outbreak of clinical mastitis.

Materials and methods: The herd under investigation consisted of 53 Holstein Friesian dairy cows with an average milk yield of 9,854 kg milk/year. Cows are grazed on pasture from April until October and in winter, the animals were kept indoors on a ration of grass- and maize silage, concentrates and minerals. Dry cows were kept indoors all year round on a ration of grass- and maize silage and dry cow minerals. Cows were housed in a cubicle barn with rubber mats. Sawdust was brought in the cubicles twice a day. The milking parlour was a 2 times four herringbone.

The farm was officially leptospirosis, BVDV and BHV1 free, had no salmonellosis and paratuberculosis the past 5 years. The bulk tank cell count of the last two years was on average between 28,000 and 69,000 cells and an incidence of 10% clinical mastitis cases per year. Dry cow treatment was done selectively: in cows below 50,000 and heifers below 150,000 cells only teat sealants were used. The animals above these thresholds got double treatment (antibiotics and teat sealants). The last year the new infection rate was 3% and the cure rate was a 100%.

Results: In 2019 the farmer identified 6 cases of clinical mastitis in June. It were all cows of parity 4 and over, the animals had no history of high cell count and varied in lactation stage. Bacteriological examination revealed two negative results, three E. coli and one mixed growth.

A mastitis investigation including dynamic milk testing was performed. The cows were in good body condition and very clean; hygiene score > 95% of the cows had score 1. Blood testing in end of lactation cows showed no deficiency in minerals and trace elements.

The milking parlour was very clean and the clusters were changed every 6 months (recommend every 250 days).

During milking routine first all 4 cows were pre dipped with hydrogen peroxide (Oxyfoam©) and after 30 seconds cleaned with one paper towel per cow. The time between pre-cleaning and cluster attachment was correct between 60-90 seconds. Gloves were worn and cleaned in-between cows. After milking the teats were dipped with iodine based dip (Blockade©).

The cows were milked properly, no bimodal release was seen. The clusters were well positioned. The pulsation rate was 60 cycles per minute. The pulsation ratio was consistent during the first two cows measured, but afterwards there was a big variation in the pulsation ratio between the cows measured on both sides of the milking parlour. This was an indication of decreased function of the pulsator, even though during static testing no abnormalities in pulsator function were seen. Closer examination of the machinery showed that the printed circuit boards (PCB's) were melted down.

Intervention and follow up: The PCB's were replaced and after one month another dynamic testing was performed. The pulsation ratio was now consistent throughout the whole milking but there was another additional problem identified. One cluster could not keep its vacuum (only 26-33kPa). After searching for problems in the machinery the only thing that was found was stray voltage caused by improperly earthed metal panels. After consulting an electrician the problem was solved even though a link between the two was never found. No severe mastitis case was seen.

Conclusions: On this farm regular testing and data analysis did not reveal the cause of the mastitis. Somatic cell count levels and bacteriological results pointed us in the direction of environmental mastitis. However, the hygiene of the cows was very good, the housing is clean and immunity of the cows correct, leading to examining the milking process in more detail. Dynamic testing is not a popular service amongst our clients. As we could carry out a dynamic testing ourselves, the farmer was more inclined to have the parlour tested. We were therefore able to add extra value and to solve this case of mastitis outbreak.

Keywords: Dynamic milk testing, E. coli mastitis, Value in practice.

UH-P03

The effect of chronical cupper accumulation on a high yielding dairy farm – a case of severe mastitis outbreak

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Objectives: This case report discusses the effect cupper accumulation on immunity of a high yielding dairy farm. A summer outbreak of E coli mastitis occurred in 2018 in mid lactation cows. Some diagnostics was done, hygiene adaptations were implemented (shaving of udders, higher frequency of manure removal and increase of bedding material). Also mastitis vaccination was started. As the improvement was not satisfactory extra diagnostics was done. A chronic cupper accumulation was found in the ration.

Materials and methods: The herd under investigation consisted of 87 Holstein Friesian dairy cows with an average milk yield of 11,142 kg milk/year. Cows were housed in a cubicle barn with rubber mats and partly rubber floor. Sawdust was brought in the cubicles twice a day. Two Delaval robots were installed in March 2017. In summer time cows grazed on pasture from April until October. In wintertime, the animals were kept indoors on a ration of grass- and maize silage, concentrates and minerals. Dry cows were kept in the same stable on a ration of grass- and maize silage and dry cow minerals.

The farm was officially leptospirosis, BVDV and BHV - 1 free and the cows were vaccinated for BVD and IBR according the leaflet. The bulk tank cell count before the outbreak was between the 178-216.000 cells and normally the farm had an average of mastitis around 22-40% per year in the last 5 years. Dry cow treatment was done selectively with the threshold below 50,000 in cows and below 150,000 cells in heifers.

Results: The onset of severe mastitis started in spring 2018. Bacteriological examination of 14 animals in mid lactation (March/April/May) revealed in 50% E. coli. Almost all cows did not regain milk production and had to be slaughtered.

A herd investigation was performed. The cows were in good body condition but cleanliness was moderate (hygiene score 2 or 3). Teat end score was good. The faeces consistency of lactation cows was varying. In milk recording no evidence of ketosis or ruminal acidosis was seen. Ruminal fluid had normal pH and active ruminal protozoa and bacteria. Blood testing in end of lactation cows showed no deficiency in minerals and trace elements.

Hygiene adaptations such as higher frequency of manure removal and shaving of udders were implemented. The mixing of the ration was increased, to decrease selection of the cows. The amount of bedding material was increased and mastitis vaccinations started.

During summer and autumn the problems decreased but still 6 animals had severe mastitis and the farmer was not satisfied with the results. Blood testing of 5 cows between 200-300 days in lactation all 5 cows revealed elevated liver enzymes (AST, GLDH and Y-GT). Liver biopsy of 3 of those 5 cows exposed elevated cupper values (2 even in toxic range). Searching for the source of the copper, the drinking water was tested ok. The cupper in the total ration was calculated and levels of 20.6-21.4 mg/kg DM were found (normal range for dairy cattle in lactation: 11-12 mg/kg DM). The nutritionist dropped the cupper levels in the concentrate.

Intervention and follow up: A new calculated ration with less cupper was fed from March 2019 (levels of 10-11 mg/kg DM in total ration). The farmer kept his focus on hygiene of his cows and barn and vaccination was continued. In 2019 a total of 30 mastitis cases occurred in 21 animals, but no animal had to be removed and milk production the mastitis cases returned. Hopefully the effect on bulk milk cell count and the amount of mastitis will be more visible in 2020 as it takes 2-3 months to lower the cupper levels in the liver.

Conclusions: In this case the immune function of the liver against bacteria causing mastitis was impaired. When regular testing and data analysis did not reveal the problem of the mid lactation coli-mastitis, we had to dig deeper in to the function of the immune system of the cow with the focus on the liver. Other causes of liver damage such as accumulation of fat, liv-

er fluke or plants were ruled out. Biopsy of the liver with the results of cupper accumulation was the key role in this case.

Keywords: Cupper accumulation, Mastitis in high yielding farm.

UH-P04

Treatment of clinical mastitis in the dairy cow. Is there a benefit of combining the intramammary and parenteral routes?

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Introduction: In dairy operations, mastitis control is the leading cause of antibiotic use. Even though the treatment of intramammary infections (IMI) should root in the solid background of pharmacodynamic and pharmacokinetic knowledge, guided by a parsimonious use of antimicrobials, on-farm treatment follows empirical rules. It relies a lot on combinations of drugs, mainly antibiotics. The objective of this study was to assess the relevance of combining the intramammary route and the intramuscular route, in four different therapeutic regimens.

Material & Methods: From June 2016 to June 2018, 339 cases of clinical mastitis were identified in two dairy farms of the customer base of the Veterinary Faculty of UNESP, Campus of Botucatu, San Pablo (SP), Brazil. An experienced veterinarian performed the clinical examination of all diseased animals and collected a milk sample for milk culture. Animals were treated (d0) and followed up for 21 days. Samples were also collected on d7 and d14 post-treatment. Cows with IMI were deemed bacteriologically cured when the pathogen isolated on d0 was no longer present on d7 and d14. Since the epidemiological status was well established and regarded as stable, the experiment was carried out in two consecutive phases. From 2016 June to 2017 March, affected cows (n=169) were given intramammary infusions and were randomly assigned to either group A (n=80; Cefalexin monohydrate 100 mg, Neomycin sulfate 100 mg, Prednisolone 10 mg, b.i.d., 2 consecutive days), or group C (n=89; Cefalexin monohydrate 200 mg, b.i.d., 2 consecutive days) (RILEXINE 200 NP, RILEXINE 200, Virvac do Brasil). New cases enrolled between 2017 September and 2018 June (n=170), were treated the same way but they were also dosed with cefalexin (monohydrate), 10mg/kg BW, IM, s.i.d., 3 days) (RILEXINE 150, Virbac do Brasil). Animals were assigned to group B (n=90; similar to group A+injections) or group D (n=80; similar to group B+injections). Chi-squared test was used to assess the difference across groups (P<0.50, significant; 0.5<P<0.10, tendency).

Results: Among the four groups, and over the study period, no difference (P=0.19) was detected in the prevalence of the different pathogens. *Escherichia coli* was the most prevalent causative agent. To that extent, 71.7% were environmental pathogens with 35.1% of coliform bacteria, and 28.3% were contagious pathogens, of which 13.9% were *Staphylococcus aureus*. Calculated bacteriological cure rates were 73.8, 69.7, 83.3 y 70.0% for groups A, C, B and D, respectively (P=0.13). Regarding *S. aureus*, the bacteriological cure rates were 54.5 (6/11), 46.2 (6/13), 66.7 (6/9) y 58.3% (7/12) for groups A, C, B and D, respectively (n.s.).

Conclusion: The different treatment regimens resulted in economically sound and similar cure rates, even though numerical differences were detected across groups. However, the treatment of an IMI in group C required 800mg of antibiotic whereas group B animals (500kg) received 15.800mg for clinical mastitis of the same kind with an exposition of non-targeted microbiota to cefalexin and neomycin. The combination of an intramammary infusion and injection of antibiotic must be decided on an individual basis. The potential benefit of treating *S. aureus* IMI with intramammary infusions and injections of cefalexin deserves to be further investigated.

Keywords: Mastitis, cefalexin, antibiotic stewardship.

UH-P05

Evaluation of an on-farm pathogen identification kit on a large dairy farm for the use in clinical mastitis

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Objectives: The aim of this study was to evaluate a commercially available pathogen identification kit (VétoRapid®, Vétoquinol) for detection of mastitis pathogens in dairy cows.

Material and Methods: Part 1: Field study

The study was carried out on a large dairy farm in Slovakia (apx. 2,500 Holstein dairy cows). Cows were examined for signs of clinical mastitis in the rotatory milking parlor by trained farm staff and separated into a mastitis group. Subsequently, the cows underwent clinical examination and mastitis was classified based on a mastitis severity score in mild (abnormal milk), moderate (abnormal milk + altered mammary gland appearance) and severe (abnormal milk + fever ≥ 39.0 °C) mastitis by a veterinarian. In an on-farm laboratory, aseptically collected quarter milk samples were plated out on Columbia agar containing 5% sheep blood (Oxoid, Wesel, Germany) for routine bacteriological examination (referend method) and on the tri-plate chromogenic agar VétoRapid® (Vétoquinol, Lure, FR). Growth pattern on both agar plates were evaluated after incubation at 37 °C for 24 hours. The VétoRapid® test was evaluated according to manufacturer's specifications.

On the first level, the tri-plate differentiates between staphylococci (modified mannitol-salt agar), streptococci (modified Edwards agar) and gram-negative bacteria on the three selective media. On a second level, bacteria can be discriminated based on colony morphology and hemolytic patterns as follows: coagulase negative staphylococci (CNS) and *Staphylococcus (S.) aureus*, α-, *β*- and non-hemolytic streptococci,

esculin-positive and esculin-negative streptococci, *E. coli* and other coliforms.

Inoculated Columbia agar plates (reference method) were sent to the diagnostic laboratory of the University Clinic for Ruminants of the University of Veterinary Medicine, Vienna, Austria and diagnosed according to the guideline of the National Mastitis Council (NMC 2004).

Part 2: Laboratory evaluation

In the second part, defined mastitis causing streptococci (n=40), staphylococci (n=38) and gram-negative bacteria (n=40) were plated out on VétoRapid®. Diagnostic accuracy was calculated based on the number of correctly identified bacteria. Pathogen growth on the tri-plate was assessed by following the manufacturer's specifications.

Statistical analysis

Validation criteria for VétoRapid® (sensitivity, specificity, positive and negative predictive value, Cohen's Kappa) were calculated in two steps depending on the level of differentiation (first and second level) based on the results gained from the reference method.

Results: Part 1: Field study

In total 259 collected samples originating from cows with clinical mastitis were used for calculation of test validity criteria. The reference method resulted in no-growth 42.9% (n=111), staphylococci 10.8% (28), streptococci 27.4% (71), coliforms 15.4% (40), yeasts 1.2% (3), *Trueperella (T.) pyogenes* 1.5% (6). More precise differentiation revealed in *E. coli* (38/40) and *Klebsiella spp.* (2/40). *S. aureus* was identified in 19 of the 28 staphylococci positive samples; nine were classified as CNS. In total 45/71 of the streptococci were classified as *Sc. uberis*, 2/71 group B streptococci, 16/71 group C streptococci, 4/71 group D streptococci and 4/71 as *Streptococcus* species in the reference method. Test sensitivity (SE), specificity (SP), positive (PPV) and negative (NPV) predictive values based on the differentiation between staphylococci, streptococci and gram-negative (first level) were as follows: SE=90.5%; SP=89.2%; PPV=91.8%; NPV=87.6%; Cohen's Kappa=0.86. Calculating the diagnostic values based on the results from a more precise (second level) differentiation; validity criteria decreased as follows: SE=89.5%; SP=79.2%; PPV=82.2%; NPV=87.6%; Cohen's Kappa=0.78.

Part 2: Laboratory evaluation

Diagnostic accuracy was 0.73 for staphylococci, 0.92 for streptococci and 0.96 for gram-negative bacteria. Especially the differentiation between CNS and *S. aureus* was insufficient; only 52.1% (12/23) were correctly identified as CNS.

Conclusion: VétoRapid® showed high diagnostic values for the differentiation of streptococci, staphylococci and gram-negative bacteria (first level). The more precise differentiation between *S. aureus* and CNS was error-prone (second level). The differentiation is linked to the fermentation of mannitol. Some CNS species may also ferment mannitol; some *S. aureus* showed a delayed (after 48 hours) mannitol fermentation leading to false-positive and false-negative results. Additionally, some mastitis causing pathogens (e.g. yeasts, *T. pyogenes*) were not detected by the on-farm culturing system and lead to false-negative results. Keeping in mind the limitations of the test kit; VétoRapid® is a suitable tool for the identification of mastitis pathogens and can be used on-farm for the implementation of culture-based treatment protocols. Nevertheless, standard laboratory procedures are still indispensable for diagnostic interventions on a herd-level.

Keywords: Bacteriological examination, rapid pathogen detection, targeted treatment, sensitivity, specificity.

UH-P06

Treatment of acute Mastitis with Prednisolone. II Modulation of myeloid effector cells

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Objectives: Treatment of clinical mastitis not only requires the use of antibiotics but also a therapeutic interference with inflammatory processes. Non-steroidal anti-inflammatory drugs and glucocorticoids should both inhibit overshooting immune mechanisms and induce mechanisms favoring the resolution phase of an inflammatory response. Since myeloid cells (granulocytes, monocytes, macrophages) are key players in this aspect, the objective of this study was the characterization of modulating effects of prednisolone for bovine myeloid cells.

Material & Methods: Leukocytes, granulocytes and mononuclear cells (MNC) were obtained from heparinized bovine venous blood after density gradient centrifugation and hypotonic lysis of contaminating erythrocytes. Monocytes were separated by magnetic-activated cell sorting and differentiated in vitro into macrophages (MPh) under polarizing conditions in the presence or absence of prednisolone (PRED, 10 – 1000 ng/ml) resulting in PRED-MPh and CON-MPh. Granulocytes (GRAN) were incubated in vitro with PRED (0.5 - 5 µg/ml). Their viability, phagocytic capacity for fluorochrome-labelled bacteria (bacteria:cells = 20:1, 50:1, 100:1) and generation of reactive oxygen species was assessed flow cytometrically. The expression of surface markers (CD163, CD204, CD11b, MHC class II) on monocytes and macrophages was assessed flow cytometrically using marker-specific monoclonal antibodies. CON-MPh and PRED-MPh were cultured with autologous MNC (MNC:MPh = 5:1) and stimulated for 48 h with Concanavalin A (5 µg/ml) to assess the lymphocytes proliferative response by flow cytometry.

Results: Viability and function (generation of ROS, phagocytosis of bacteria) of GRAN remained completely unaffected by PRED. Numbers of viable MPh after 24h or 48 h in vitro remained unaffected by PRED ($p > 0.05$). Presence of PRED during the first 24 h of the in vitro differentiation of monocytes into macrophages resulted in a pronounced and significant upregulation of CD163 expression density (3-fold, $p < 0.01$), a downregulation of CD204 (2 fold, $p < 0.05$) and MHC class II molecules (3-fold, $p < 0.01$). ConA-stimulated lymphocytes in the presence of PRED-MPh displayed a significant lower size (forward scatter, $p = 0.0009$) compared to lymphocytes stimulated in the presence of CON-MPh.

Conclusions: Prednisolone does not act at the level of immigrated neutrophilic granulocytes but modulates the phenotype and function of bovine macrophages. The selective PRED-induced inhibition of MHC class II and CD204 expression in parallel with the significant upregulation of CD163 on differentiating MPh is reminiscent of anti-inflammatory and/or regulatory MPh. The latter is substantiated by the inhibitory function of these macrophages for lymphocytes. Thus, PRED both inhibits the generation of inflammatory MPH and favors the preferential induction of anti-inflammatory MPh. The findings. Such immune enhancing functions of glucocorticoids may be induced and utilized in a more rational way in the metaphylaxis and therapy of bovine clinical mastitis.

Keywords: Mastitis, Prednisolone, Granulocytes, Monocytes, Macrophages.

UH-P07

Evaluation of thermal imaging for detection of subclinical mastitis at the quarter level in cows

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Objective: The aim of this study was to investigate the efficiency of imaging of udder surface temperature using a thermal camera as a possible tool for the diagnosis of subclinical mastitis at the udder quarter level in dairy cows under field conditions.

Material & Methods: The presented study was conducted on 2 different farms in the same geographic area in March 2019. Thirty-three and 6 cows of farm 1 and farm 2 without any clinical disease were included this study, respectively. Thermographic images were collected using a commercial handheld infrared thermal camera (FLIR E5, FLIR) from 72 quarters in the milking parlor. The images of quarters were taken from a lateral and medial approach with a 90-degree angle for fore and rear quarters, respectively. Sterile milk samples were collected and transported within a cold-chain for counting of somatic cells by BactoCount IBCm (Bentley Instruments Inc., MN, USA) and bacteriological examination to serve as a gold standard for calculation of the sensitivity and specificity of thermal imaging. All laboratory analyses were performed within 4 hours after the sampling. A threshold of a SCC of 200 x 10^3 cells/mL was used to differentiate a sub-clinically infected quarter (above threshold) from a healthy quarter (below threshold).

Results: Mean udder surface temperatures were similar (p=0.676) between quarters < 200k cells/ml (34.33 ± 0.23 °C) and quarters > 200k cells/ml (34.51 ± 0.31 °C). Infected (34.52 ± 0.24°C) and non-infected quarters (34.14 ± 0.29°C) had similar skin surface temperature (p=0.311). Sensitivity and specificity of thermal imaging of infected and uninfected quarters was 69.23% and 46.34%, respectively (p=0.294; AUC=0.575), compared to bacteriological examination (gold standard).

Conclusion: Udder skin temperature was not associated with the SCC threshold nor with bacteriological isolation suggesting that measurement of skin surface temperature using a thermal camera may not be effective for the diagnosis of subclinical mastitis. Evaluation of repeated measures within a period of time would be useful to increase specificity and sensitivity.

Keywords: Diagnostics, Subclinical mastitis, Dairy Cattle.

UH-P08

The effect of automatic cluster remover settings on milking performance, udder health, and teat condition

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Objectives: Since their advent in 1970, automatic cluster removers (ACRs) have fostered profitability in dairy operations by increasing the automation of the milking process, decreasing labor costs, and improving parlor efficiency. The basic concept of ACRs is that they utilize the intrinsic nature of the milk flow curve to determine the optimal termination point of an individual milking. Removal of the milking unit is initiated once the milk flow drops below a preset threshold. Early studies showed that an increase in the cluster remover take-off milk flow threshold from 0.2 to 0.4 kg/min led to a reduction in milking time without adversely affecting milk production. These initial studies were followed by work from researchers around the globe investigating different ACR settings in conventional milking systems, pasture-based systems, and confinement and pasture-based automatic milking systems. The majority of these studies supported the results of the earlier works. The threshold values under investigation ranged between 0.2 and 0.82 kg/min. Recent developments in milking machine settings in the industry support an increased threshold, and a cluster remover take-off milk flow threshold of up to 1 kg/min has been suggested. This trend was further supported by a recent field study on a commercial dairy farm in Michigan that applied a threshold of 1.1 kg/min, as well as studies from our own group on farms that applied a threshold of 1.3 kg/min. To our knowledge, there are no studies investigating the effect of such high ACR settings on milking performance, teat condition, and udder health. The objectives were to study the effect of 2 different automatic cluster remover settings on 1) milking characteristics, 2) milk component yields, 3) teat tissue condition, and 4) udder health.

Materials and Methods: In a randomized field trial, Holstein cows (n = 689) from 1 commercial dairy farm with a thrice-daily milking schedule were allocated to 2 treatment groups. Treatment consisted of a cluster remover take-off milk flow threshold of 1.2 (ACR1.2) or 0.8 kg/min (ACR0.8) for 57

d. Milking characteristics (milk yield, MY; and milking unit-on time, MUOT) were obtained with electronic on-farm milk meters. Composite milk samples were collected and analyzed for fat, protein, lactose, and somatic cell count. Machine milking induced short- and long-term changes to the teat tissue condition were assessed visually.

Results: General linear mixed models demonstrated differences in MUOT, whereas no meaningful differences in MY were detected. Milk yield (least squares means, 95% CI) was 11.3 (10.9-11.8) and 11.3 (10.8-11.8) kg/milking in groups ACR1.2 and ACR0.8. The effect of treatment on MUOT was modified by parity. Milking unit-on time in 1st, 2nd, and ≥ 3rd lactation cows, respectively, was 260.7 (252.0-269.4), 257.8 (247.4-268.1), and 260.2 (252.6-267.9) s/milking in group ACR1.2; and 273.7 (264.9-282.5), 279.1 (269.4-288.8), and 295.7 (287.9-303.6) s/milking in group ACR0.8. We detected no meaningful differences in milk component yields or linear somatic cell score. Least squares means in groups ACR1.2 and ACR0.8, respectively, were milk fat yield, 0.41 (0.40-0.44) and 0.42 (0.40-0.44) kg/milking; milk protein yield, 0.36 (0.35-0.37) and 0.37 (0.36-0.374) kg/milking; milk lactose yield, 0.61 (0.60-0.63) and 0.63 (0.61-0.64) kg/milking, and linear somatic cell score, 1.9 (1.8-2.0) and 1.9 (1.8-2.0). A generalized linear mixed model revealed an effect of treatment on machine milking induced short-term changes. The odds of short-term changes to the teat tissue were lower for cows in group ACR1.2 [odds ratio (95% CI) = 0.78 (0.63-0.96)]. No meaningful differences were detected in machine milking induced long-term changes between treatment groups.

Conclusion: Increasing cluster remover take-off milk flow threshold from 0.8 to 1.2 kg/min decreased individual milking duration and alleviated machine milking induced short-term changes to the teat tissue without adversely affecting milking performance or somatic cell count. Future studies are warranted to investigate the effect on milk production and udder health over a whole lactation period.

Keywords: Well-being, bovine, mastitis, parlor, teat.

UH-P09

Efficacy of antimicrobial peptides (AMPs) against major bovine mastitis pathogens

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Objectives*:* Mastitis accounts for the highest antimicrobial usage in dairy farming. During intramammary infections, endogenous AMPs are highly upregulated in mammary tissues, however little is known about their efficacy against mastitis pathogens. The aim of the study was to analyze the effect of relevant recombinant AMPs on bacterial growth of major mastitis pathogen isolates *in vitro*.

Material & Methods*:* By means of a time kill assay, *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus)* and *Streptococcus uberis (Sc. uberis)* were incubated in the presence and absence of different concentrations of AMPs. Bacterial growth in LB medium was detected photometrically (OD 600 nm) every 30 min over a time period of 6 h. Commerciably available recombinant human S100A8/A9, recombinant bovine S100A9, recombinant bubaline LAP (lingual antimicrobial peptide), recombinant bovine CCL20 and recombinant human SLPI (secretory leukocyte protease inhibitor) were tested.

Results*:* The AMP S100A9 and the antimicrobial chemokine CCL20 significantly (P<0.05) inhibited growth of *S. aureus* and *Sc. uberis*, whereas the heterodimer S100A8/A9 only inhibited *E. coli* growth (P<0.05) and partly *Sc. uberis* growth at comparably high concentrations. In contrast to earlier reported data on recombinant bovine LAP, recombinant bubaline LAP had no inhibitory effect on all tested pathogens. The same accounted for recombinant human SLPI. However high concentrations of CCL20, LAP and SLPI enhanced *E. coli* growth in LB medium *in vitro*.

Conclusion*:* In conclusion, AMPs show antimicrobial activity against major bovine mastitis pathogens. However effects are pathogen-specific and depend on concentration and the recombinant peptide target species. Triggering the expression of endogenous AMPs may be a future approach to battle intramammary infections more effectively and to reduce the amount of antimicrobial drug usage in the dairy industry.

Keywords: Defensin, S100-proteins, S. uberis, S. aureus, E. coli.

UH-P10

Susceptibility to lincomycin and neomycin of Staphylococcus spp isolated from bovine mastitis

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Objectives: In bovine mammary health diagnostic in 2018 at the Regional Association for Animal Health and Identification (Ciney, Belgium) E.Coli and S.Uberis were the most prevalent, with respectivly 33,9% and 25,2% but one fifth of the cases involved Staph spp: S.aureus (11,4%) and CNS (9,3%).

Our objective was to determine the resistance and/or the susceptibility to lincomycin, neomycin or a combination lincomycin and neomycin of staphylococci isolated from cattle with mastitis.

Material & Methods: From July to October 2018, 44 strains of Staphylococcus aureus and 52 strains of Staphylococcus coagulase-negative were isolated for bacteriological diagnosis of mastitis at the Regional Association for Animal Health and Identification, Ciney, Belgium. An antibiotic susceptibility test on Mueller Hinton (I2A, Montpellier, France) was performed on each of these strains according to the AFNOR UN 47-107 version 2012 standard. The interpretation follows the recommendations of the CA SFM Veterinary CA of 2018. The sensitivity of these strains to lincomycin (Lincomycin disc 15µg, I2A, Montpellier), neomycin (Neomycin disc 30UI, I2A, Montpellier) and Lincomycin/neomycin combination (Lincomy-

cin disc/neomycin 75µg, Oxoid, USA) was evaluated on these bases. The strains were classified as resistant to lincomycin for diameters less than 17 mm and sensitive for diameters equal to or greater than 21 mm. For neomycin, strains were classified as resistant for diameters less than 15 mm and sensitive for diameters equal to or greater than 17 mm.

Results: All strains have a diameter greater than 17 mm around the neomycin disc and are therefore interpreted as sensitive. 18.8% of the selected strains are resistant to lincomycin and have a diameter of less than 17 mm. Around the Linco-Neo disc, all strains have a diameter greater than 27mm.

Conclusion: Neomycin is an aminoglycoside antibiotic that has a broad spectrum of activity against Gram-positive bacteria, including Staphylococcus species. Neomycin binds to the 30S subunit of the bacterial ribosome, resulting in malformation of the ribosomal protein binding. At high concentrations, aminoglycosides also damage the cell membrane of bacteria. They are therefore generally considered to have both bacteriostatic and bactericidal properties.

Lincomycin is a lincosaminide antibiotic with specific activity against gram-positive bacteria, particularly Staphylococcus and Streptococcus species. Lincomycin binds to the 50S subunit of the bacterial ribosome, inhibiting protein synthesis in the cell. It is generally considered to be a bacteriostatic compound.

Based on the results of this study, we can state that Staphylococcus spp isolated in Belgian farms shows susceptibility to Neomycin and to the combination Lincomycin/Neomycin.

Keywords: Staphylococus spp, lincomycin, neomycin.

UH-P11

Preliminary results for ranking risk factors for dairy cow mastitis using expert knowledge elicitation

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Objectives: Mastitis is one of the most common diseases affecting dairy cows and can cause a decrease in dairy cow welfare, economic loss and substantial overuse of antimicrobials. It is a multifactorial disease affected by a variety of factors, which makes its prevention, management and control difficult. Despite extensive dairy cow mastitis literature, ranking the different and numerous risk factors affecting the disease onset and clearly classifying herds according to mastitis risk is imprecise.

The objective of this preliminary study was to select and characterize, using expert knowledge elicitation (EKE), the most important risk factors that could lead to subclinical (SM) and clinical mastitis (CM) on dairy farms with loose housing, medium to high production levels, and machine milking. The ultimate goal of this study is to develop a herd classification system based on mastitis risk that will scientifically support and assist dairy owners.

Material & Methods: Based on the mastitis control program recommended by the National Mastitis Council (https://www.nmconline.org/resources) and the latest literature, a focus group selected 72 management and environmental risk factors critical for the onset and spread of mastitis in dairy cows. These risk factors were divided into 8 points: 1) Milking equipment; 2) Milking procedures; 3) Environmental hygiene and comfort; 4) Dry cow management; 5) Biosecurity management; 6) Therapeutic choice and treatment administration during lactation; 7) Stockperson training/management and 8) Record keeping (milk data and mastitis cases).

Forty Italians, experienced in the field of mastitis, participated in the study. A modified Delphi technique, consisting of 2 successive rounds, was used to gather their opinions.

The participants were asked to score the 72 risk factors on a scale from 0 to 10 (least to most important), in relation to the potential ability to cause SM and/or CM. Four types of mastitis were considered: SM and CM caused by environmental bacteria, SM and CM caused by contagious bacteria.

Results: Presently, only the first round of the Delphi process has been completed. Thirty-seven of the participants completed the survey and their responses were available for preliminary analysis.

The breakdown of the group was as follows: 16 dairy practitioners/consultants; 1 dairy practitioner/dairy farm owner; 5 academic professors or researchers; 7 veterinarians working for private companies (pharmaceutical, animal feed or dairy industries); and 8 employed in public veterinary services.

For both SM and CM caused by environmental bacterial, the most important risk factors identified were ones dealing with point 6 (mean score 7.35 for SM and 7.58 for CM); point 8 (mean score 6.97 for SM and 6.62 for CM) and point 7 (mean score 6.44 for both SM and CM).

For SM caused by contagious bacteria, the most important risk factors were ones dealing with point 8 (mean score 7.68), point 7 (mean score 6.94) and point 5 (mean score 6.79).

For CM caused by contagious bacteria, risk factors included in point 8 were found to be the most important (mean score 6.49), followed by point 6 (mean score 6.42) and point 7 (mean score 6.39).

Conclusion: The preliminary results underscored the importance, for both environmental and contagious SM and CM, of correct record keeping of milk and mastitis data (somatic cell count, laboratory tests results, treatment records, etc.), adequate training/management of staff, especially milk-

ers (training on milking routine and mastitis diagnosis, correct information flow between milkers, farmers and veterinarians, etc.) and correct therapeutic choices and treatment administration (antibiogram results, proper hygiene in the administration of the therapies, etc.).

At the end of the Delphi process, the results of the EKE will be validated on-farm and will be used to define a list of measures to be included in a protocol for ranking dairy farms according to their mastitis risk. The system will identify farms with high or low-risk profiles and be useful for surveillance purposes and effective mastitis risk management.

This work was supported by the Italian Ministry of Health, grant RiskMast - PRC 2017006.

Keywords: Dairy cow, mastitis, expert knowledge elicitation, risk assessment.

UH-P12

Efficacy of the prudent use of benzylpenicillin (Ubropen® 600 mg) and Meloxicam (Metacam® 40 mg / ml) in the treatment of clinical mastitis. Preliminary results

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Introduction and objectives: The prudent use of antibiotics leads to using reduced spectrum antibiotics in clinical mastitis (CM) to avoid generating environmental pressure for the induction of resistances. Penicillins are the antibiotic group of choice in cases of CM caused by susceptible Gram + bacteria. When CM occurs we are forced to treat even before knowing the result of specific antibiograms. Therefore, the indication of antibiotics is justified by the knowledge of the etiological agents of mastitis and the effectiveness of penicillin in these farms. The objective of this study, still ongoing, was to determine the prevalence of the pathogens causing CM of grades 1, 2 and 3, and to evaluate the clinical cure rate of the treatment with intramammary benzylpenicillin (Ubropen® 600 mg intramammary suspension for lactating cows, Vetcare OY) and Meloxicam (Metacam® 40 mg / ml solution for injection for cattle and horses. Boehringer Ingelheim Vetmedica GmBH).

Materials and methods: These are preliminary results of a descriptive, prospective, randomized and multicenter study, with six veterinary groups involved that have submitted 54 cases of CM from 14 farms. The inclusion criteria were CM of different severity classified in grades 1, 2 or 3, affecting a single quarter, of cows without prior mastitis in that lactation or during the last 3 months of the previous lactation in the case of cows with <60 days in milk (DIM), and which had not received any antibiotic treatment during the last 30d before inclusion. Once a case of CM was detected, a milk sample was taken for etiological identification and antibiogram (Interprofessional Milk Association of Catalonia, ALLIC, Spain). Immediately afterwards, the first benzylpenicillin cannula (UBROPEN®) was applied for a total of 3 or 5 (according to the veterinarian's criteria) and a single dose of Meloxicam (Metacam® 40 mg / ml) injected. Data recorded were cow identification, DIM, severity of mastitis, date of inclusion, if clinical cure five days later after the application of the last antibiotic cannula and date of cure. If the animal did not cure, it received an alternative treatment.

Results: Average DIM were 135 ± 92.11d and average calvings 2.4 ± 1.2 (1-5). A total of 54 cases were complete; of these 37 samples induced growth, and 29 resulted in specific isolation and identification. A total of 68.8% of the cases were treated with three tubes and the rest with five. Mastitis incidence with severity 1, 2 and 3 were 56.3, 41.7 and 2.1%, respectively. A total of 27.1% were in front right quarters, 33.3% front left, 14.6% rear right and 25% rear left. A total of 58.6% of cases were caused by Gram+ bacteria, with 62.5 and 58.3% of CM cases being grades 1 and 2, respectively. One single case of severity grade 3 was caused by Gram negative bacteria. Prevalence data obtained in the isolates so far are consistent with the available literature. The cure rate after the treatment with benzylpenicillin and Meloxicam was 79.2% when all cases were included and 82.4% for cases of mastitis caused by Gram +. Accordingly to the severity of mastitis the efficacy of clinical cure was 74.1% in grade 1, and 90% in grade 2 CMs. The clinical cure rate of cases treated with three doses of antibiotics was 81% and 73.3% for CM treated with 5 cannulas. Only two relapses were observed, both grade 1, caused by benzylpenicillin-sensitive germs and both in front quarters. A logistic regression test did not detect any significantly factor associated with efficacy. Only the factor “farm” showed a statistical tendency to influence the cure rate (P=0.08). Regarding the laboratorial sensibility to benzylpenicillin, 28.6% were resistant to penicillin (*Strep. gallolyticus*, *Staph. aureus*, CNS and *Strep. uberis*) and two cases showed intermediate sensitivity (both *Strep. uberis*). All cases came from different farms from different Spanish regions. Regarding the mastitis cases caused by *Streptococcus uberis* a 62.5% of cases were benzylpenicillin-sensitive and 12.5% resistant. The rest were intermediate.

Conclusions: The study is still ongoing but the preliminary results on clinical efficacy (around 80%) and laboratory sensitivity (around 70%) are satisfactory and consistent with the current literature.

Keywords: Penicillin, Bencil Penicillin, Meloxicam, Mastitis, Prudent use.

UH-P13

Effect of Introduction of Multivalent Mastitis Vaccine in Cows with High Detection Rate of Significant Mastitis Pathogens

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Purpose: Despite the application and introduction of various preventive and treatment measures in order to control mastitis, the losses attributed to mastitis still dominate the dairy industry. In this study, we introduced a multivalent mastitis vaccine (STARTVAC® HIPRA) to a Holstein dairy herd with an increased number of bacterial isolation and a high rate of detection of significant bacteria suspected of clinical mastitis.

Materials and Methods: This study was conducted at a dairy farm with 115 Holstein cows in the Ishikari region of Hokkaido, Japan. A multivalent vaccine containing E. coli J5 strain and Staphylococcus aureus as active ingredients (STARTVAC® HIPRA) was administered according to the manufacturer's recommendations (45 days and 10 days before delivery and 52 days after delivery, total of three injections). The total of 140 cows was vaccinated from late July 2018 to October 2019. The quarter milk samples from all cows at the farm was sampled and tested for bacteria isolation at the time of vaccine introduction (July 2018 and December 2019). In addition, the disease incidence rate, total bacteria count in the milk, number of significant mastitis pathogens in the milk, Somatic Cells Count and the daily milk production per animal were recorded and compared with those from 2014 to 2017 (before the introduction of the vaccine). The production parameters after the introduction of the mastitis vaccine were compared in individual cows with the previous 2 lactation periods using the Dairy Herd Improvement data. Statistical analysis was carried out with the Student's t test, Mann-Whitney U test, and Fisher's exact test using statistical software (R, version 3.5.1).

Results: Detection rate in cows with significant bacteria at the time of introduction of the vaccine at December 2019 was 95.0% (94/99) and 61.4% (51/83), respectively, ($p < 0.01$, 95% CI: 4.15 to 40.65). Detection rate in quarters with significant bacteria was 67.9% (258/380) and 28.7% (94/328), respectively, ($p < 0.01$, 95% CI: 3.76 to 7.36) Both parameters were significantly improved. Average annual disease incidence decreased to 315 cases after the introduction of the vaccine from 340 cases before introduction. The number of lactation diseases also decreased to 102 from 117 cases prior to vaccination. Average Somatic Cell Count in milk decreased to 122,000 from 184,000 prior to vaccination. Milk production per cow per day increased to 33.4 kg from 33.0 kg. Those parameters improved after the introduction of the vaccine. The number of quarters of isolated Streptococcus spp. decreased most (67.8 to 16) followed by Staphylococcus aureus (17 to 10), E. coli (56.8 to 32) and CNS (22.5 to 19) after introduction of the vaccine. Significant improvement was observed in the milk-to-fat ratio and milk protein ratio during the current lactation period versus the previous two periods in the Dairy Herd Improvement data.

Conclusions: By introducing the mastitis vaccine (STARTVAC®, HIPRA), improvement in lactation-related parameters, such as the number of cows and quarters, isolated significant bacteria, diseases incidence rates, the number of lactation-related diseases, somatic cell count in milk, the amount of milk produced per cow per day, the number of quarters infected with mastitis causing bacteria and the percentage of milk fat and milk protein were observed. The results of this study demonstrate the efficacy against mastitis of a multivalent vaccine (STARTVAC®, HIPRA), which contains Escherichia coli J5 strain and Staphylococcus aureus and CNS bacteria as active ingredients, in cattle herds with a high detection rate of significant mastitis pathogens.

Keywords: Mastitis, vaccine, Escherichia coli, Staphylococcus aureus.

UH-P14

Peracetic acid effectively kills contagious mastitis pathogens in milking liners

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Objective: Two of the most important contagious mastitis pathogens in dairy farms include *Staphylococcus aureus* and *Streptococcus agalactiae*, and the primary method of transmission is from cow to cow during milking. While teat skin sanitation before and after milking reduces significantly the microbial contamination on teat skin, it is also very important that the milking machine cluster surface that will be in contact with skin is free of microorganisms. Microbial load of milking clusters after milking depends on microorganisms shed from infected mammary glands, but also from the microflora of teat skin and skin lesions. Flushing and sanitation of milking clusters post milking is a common practice in farms that suffer from outbreaks of contagious mastitis or those that want to maintain these pathogen populations in control. This study evaluated the efficacy of Peradis (DeLaval), a peracetic acid solution, on liners exposed to high concentrations of *S. aureus* and *S. agalactiae* in a lab environment.

Materials and Methods: In separate experiments, new DeLaval VMS milking liners were immersed in a 10^8 CFU/mL broth of *S. aureus* (ATCC 6538) or *S. agalactiae* (ATCC 27956) for a period of 30 min. Afterwards, the inside of the liners were flushed for 4 seconds with either 0.8% Peradis (PAA) or 300 ppm $CaCO_3$ (HW) at a rate of 13.7 mL/second. After 11 seconds extra contact time, the inside of liners (5 per treatment) was swabbed (soaked in D/E neutralizer) and plated immediately in Tryptic Soy Broth (MPN). As a positive control (POS), liners exposed to the broth, but receiving no treatment were also swabbed. Plates were incubated at 36°C and enumerated after 48 hours. Log bacterial reduction was calculated by the difference between the positive control and the treatment. European Norms (EN) standards for surface tests of this type re-

quire a 4 log reduction (LR) for a test product to pass the test.

Results: For *S. agalactiae*, log recovery was 6.8 ± 0.7 (POS), 1.6 ± 0.0 (PAA), and 4.1 ± 0.4 (HW). For *S. aureus*, log recovery was 7.0 ± 0.5 (POS), 2.0 ± 0.9 (PAA), and 4.6 ± 0.9 (HW). Only PAA passed the test for both pathogens with >4 LR at a total 15 second contact time.

Conclusion: Management of contagious mastitis pathogens in dairy farms requires control of bacterial contamination both on teat skin (by using teat disinfectants) and milking surfaces (e.g. use of disinfectants on liners). Under demanding milking conditions, where time saving is critical for increasing cow throughput without adversely affecting udder health, liner disinfection with Peradis proved to be a reliable method for controlling *S. aureus* and *S. agalactiae* on liners.

Keywords: Disinfection, liners, Staph aureus, Strep agalactiae, contagious.

UH-P15

Dynamics of four Streptococcus uberis clinical mastitis outbreaks by using RAPD analysis

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Objectives: *Streptococcus uberis* is recognised as one of the microorganisms causing frequently clinical cases of bovine mastitis. It is classified as an environmental pathogen detected especially in bedding material, on milking parlour and in the surroundings of cattle housing. However, in the past (Zadoks et al., 2005; Leelahapongsathon et al., 2016), thanks to the use of molecular typing methods, such as pulsed-field gel electrophoresis (PFGE), outbreaks of *Str. uberis* mastitis have been described as the likely result of contagious transmission. These clonal outbreaks indicated a cow-to cow transmission of this microorganism. The aim of this study was to apply the random amplified polymorphic DNA (RAPD) PCR technique to evaluate the clonal dynamics of four *Str. uberis* clinical mastitis outbreaks happened in Northern Italy.

Materials and methods: A total of 47 isolates were collected from the quarter clinical mastitis milk samples in four different farms located in the North of Italy. The samples were collected aseptically and the isolation of *Str. uberis* strains was performed according to colony morphology, esculin splitting, catalase testing (National Mastitis Council, 2017), the API 20 Strep (bioMérieux) and confirmed by using the MALDI-TOF and a log (score) >2.0. Genomic DNA was extracted from pure cultures, as previously described (Cremonesi et al., 2006), without a pre-treatment step. All the isolates were then typed and compared by using five different primers for *Str. uberis* RAPD analysis: ERIC1 (5'-ATGTAAGCTCCTGGGGATTCAC-3'; Schmitt-Van de Leemput et. al, 2007); M13 (5′-GAGGGTGGCGGTTCT-3′); D11344 (5'-AGTGAATTCGCGGTCAGATGCCA-3'); D8635 (5'-GAGCGGCCAAAGGGAGCAGAC-3') and OPAA10 (5'-GGACTACCAGGGTATCTAAT-3'). RAPD-PCRs were performed as previously described (Morandi et al., 2016).

Results: The results obtained revealed the presence of a dominant profile circulating in each herd, but different among herds confirming the wide variety of *Str. uberis* profiles spreading in dairy cattle in Italy. In the first farm, RAPD-PCR assay by using D11344 and D8635 primers showed the presence of four different profiles with a main profile (54% of the isolates) and a minority one (23%). In the second farm, primers D11344, D8635 and ERIC1 classified 77% of the isolates as a clonal profile circulating. By using these three primers, a same RAPD pattern was also showed by 66% of the isolates within each of the remaining two farms. The remaining two primers (M13 and OPAA10) used in this study revealed a worst clusterization between RAPD types.

Conclusions: Among the primers used in this study, ERIC1, D11344 and D8635 revealed a better association between the RAPD types. RAPD-PCR is a useful technique for distinguishing strains within species in epidemiological studies, more specifically for investigating the source of *Str. uberis* mastitis outbreaks, thereby contributing to better management of *Str. uberis* mastitis in dairy herds.

Keywords: Streptococcus uberis, clinical mastitis, outbreak, RAPD.

UH-P16

A milk line sampling system to detect milk line contamination: a case investigation from the State of Georgia, United States

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The objective of this case investigation was to determine the effectiveness of a milk line sampling device to detect bacteria either coming from a group of milked cows or from the milking line that is potentially contaminated. Two groups of 16 cows each were individually sampled for a composite of the 4 quarters right before milking. The first group was sampled by an inexperienced person with gloved hands. The second group was sampled by an experienced veterinarian using a standard milking sampling protocol and gloved hands to minimize the contamination of collected samples. Before beginning milking, a line sampling device (QualiTru Sampling System, Oakdale, Minnesota 55128, USA) was set up in the parlor. The system consisted of a stainless-steel port connected to the milking line. A sterile septum was placed in the port. Then a sterile 18-G needle with a line connected to a sterile bag was inserted in the septum. Before reaching the sterile bag,

the line passed through a portable peristaltic pump that allow a frequency flow sampling over the entire process run. The pump was set up for a volume of sampling of 60 ml of milk per minute. Individual composite samples from each cow and milk line pooled sample were refrigerated and then transported directly to the Tifton Veterinary Diagnostic and Investigational Laboratory at the Tifton campus, University of Georgia. After arrival, both types of milk samples (individual cow and pooled milk line) were cultured for isolation and identification of aerobic bacteria on Blood Agar and MacConkey Agar, and for the detection of mycoplasma on Mycoplasma Selective Agar by conventional approved methodologies. First group of cows sampled by the inexperienced employee showed that cow 1, 2, 13 and 15 were negative to any bacterial isolation. The following cows were positive to: cow 3 Enterococcus spp., cow 4 Arthrobacter like spp., and Brevibacterium spp., cow 5 Streptococcus spp., and Arthrobacter like spp., cow 6 Arthrobacter like spp., and Brevibacterium spp., cow 7 Arthrobacter like spp., and Prototheca, cow 8 Corynebacterium spp., and Pseudomonas spp., cow 9 Acinetobacter baumannii, cow 10 Leifsonia aquatic, cow 11 Aerococcus viridans, and Corynebacterium spp., cow 12 Arthrobacter like spp., cow 14 Lactobacillus spp., and Prototheca, and cow 16 Candida zeylanoides, and Candida rugose. Pooled sample from the milk line sampling device was positive to Acinetobacter baumannii, Enterobacter sakazakii, Escherichia coli, and Klebsiella pneumonia. Second group of cows sampled by the experienced veterinarian showed that cow 1, 2, 3, 4, 6, 7, 8, and 10 to 16 were negative to bacterial isolation. Cow 5 was positive to coagulase negative Staphylococcus and cow 9 to Prototheca. However, the pooled sample from the milk line sampling device was positive to non-hemolytic Streptococcus, Klebsiella pneumoniae ssp pneumoniae, Acinetobacter spp., Klebsiella ssp. ozaenae, Enterococcus durans, and Streptococcus bovis. These results revealed that none of the bacteria isolated from the sampling device were present in the individual cow cultures, suggesting that the milk line was highly contaminated with biological material from milk and several environmental bacteria. As was expected, group 1 showed several contaminated individual samples because of the inexperienced sampler, but none of those bacteria appeared in the composite milk line device sample. In the group 2, most of the cows were negative, indicating a good sample protocol carried out by the experienced veterinarian. Interestingly, the composite milk line sample was positive to several environmental bacteria that were not found from the individual cultures. This pilot study illustrated the effectiveness of a milk line device sample to detect microbial contamination of the milk line that would warrant the need to periodically flush and disinfect the milk line at regular intervals with certified stronger products in addition to the normal flushing and disinfection carried out at the end of each milking.

Keywords: Milk line sampling device, dairy, contamination, United States.

UH-P17

A milk line sampling system to detect mastitis-causing organisms: a case investigation from an Argentinian dairy herd

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The objective of this case investigation was to determine the effectiveness of a milk line sampling device to detect mastitis-related bacteria from a group of individual milk cultured cows with high somatic cell counts. On January 20, 2020 (sampling 1), and on February 10, 2020 (sampling 2) during the morning milking, 6 cows per group with a history of high somatic cell counts and/or clinical mastitis belonging to 5 different lots with approximately 70 cows each were sampled for milk cultures. Before beginning milking, a line sampling device (QualiTru Sampling System, Oakdale, Minnesota 55128, USA) was set up in the parlor. The system consisted of a stainless-steel port connected to the milking line. A sterile septum was placed in the port. Then a sterile 18-G needle with a line connected to a sterile bag was inserted in the septum. Before reaching the sterile bag, the line passed through a portable peristaltic pump that allow a frequency flow sampling over the entire process run. The pump was set up for a volume of sampling of 60 ml of milk per minute. Individual cows' composite samples and milk line pooled sample were refrigerated and then transported directly to a commercial certified mastitis lab in Argentina. After arrival, samples were cultured for aerobic bacterial isolation and mycoplasma using standard approved methodologies. Sampling 1 showed that in group 1 one cow was positive to Prototheca and another cow to Streptococcus uberis and the sample device was positive to Staph aureus. In group 2, one cow was positive to Sthap aureus, other was positive to Staph spp., other to Strep dysgalactiae and other to Strep uberis. The device was positive to Staph spp. In group 3, two cows were positive to Sthap aureus, and 3 cows were positive to Strep uberis. The device was positive to Staph aureus. In group 4 one cows was positive to Strep uberis and another to Strep dysg. The device was positive to Strep spp. In group 5, 2 cows were positive to E. coli and one cow Strep dysg. The device was positive to Strep spp. These results showed that 4 out of the 5-milk line sampling were able to detect at least the same bacteria genus detected in the individual cultures. However, in group 1, Prototheca and Strep uberis were not detected by the device. Sampling 2 showed that in group 1 one cow was positive to Streptococcus uberis and another cow to Staph spp. The sample device was positive to Strep uberis, E. coli and SCN. In group 2, one cow was positive to Staph spp. The device was positive to Staph aureus, SCN, Strep. uberis, and E. coli. In group 3, 6 cows were positive to Sthap spp., one cow to Strep. uberis and one cow to E. coli. However, the device was positive only to Strep uberis and E. coli, without detecting any Staph. spp. In group 4, only one cow was positive to Strep uberis. The device was positive to Strep uberis, E. coli, and SCN. In group 5, two cows were positive to Strep uberis, and one cow to Staph spp. The device was positive

to Strep. uberis and E. coli., without detecting the Staph spp. In group 6, one cow was positive to Staph aureus, two cows to Strep uberis and two cows to Staph spp. However, the device was only positive to Strep uberis and E. coli. These results showed that 6 out of the 6-milk line sampling device were able to detect at least the same bacteria genus detected in the individual cultures. However, in 3 groups, the device was not able to detect Staph. spp. that were cultured from individual cows. It is concluded that in general the milk line sampling device is effective in detecting mastitis-causing microorganism shed by positive cows, but there were some false negative results linked to Staph spp., perhaps due to the effect of dilution and/or lack of accuracy of lab techniques. It is concluded that milk line sampling device can be used as an effective epidemiological surveillance tool for mastitis within different milking groups.

Keywords: Milk line sampling device, dairy, mastitis, bacteria, Argentina.

UH-P18

Investigation of subclinical bovine mastitis by MALDI-TOF MS

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Objectives: Little is known on the general prevalence of mastitis pathogens in cows with elevated somatic cell count (SCC) in Denmark. Therefore, a pilot study was set up to examine the mastitis pathogens in quarter milk samples from cows expressing elevated SCC during the last three consecutive monthly samplings.

In the battle against bacterial infections, an important challenge is to identify the infectious agent as quickly and accurately as possible. Historically, techniques for identifying bacteria rely on laborious biochemical tests all of which are to some extent prone to misidentification. Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) is a well-known and versatile method which has been applied to bacterial species identification mainly for human pathogens. During the last decade, MALDI-TOF MS has evolved into the veterinary field of bovine mastitis as well. Therefore, the current project aims to describe the variety of cultivable pathogens found in cows with elevated SCC.

Materials and methods: Originating from 505 Danish dairy cows expressing an elevated somatic cell count (SCC > 200000 cells/mL) distributed on 19 farms, 2000 quarter milk samples were tested for potential bacterial growth. Due to non-productive quarters, 4% (20/505) of the cows were sampled only on 3 quarters. At DTU 10 µl of the milk sample was plated on calf-blod agar (KMA, Herlev, Denmark) and incubated O/N. Subsequently the plates were examined after 24 hours and in case of no or slow growth after 48 hours. Followed by visual examination, colonies were subcultured for analysis by MALDI-TOF MS. The colonies were identified by direct diposition on the target plate according to Bizzini et al. (2010) combined with overlay of 70% formic acid prior to matrix deposition using the BDAL database combined with the VET-DTU database for veterinary spectra and Staphlycocci (Mahmood et al. 2018 & Nonnemann et al. 2019). The identification by MALDI-TOF rely on a log score between 0 and 3. Log score 0-1.69: No ID, Log score 1.70-1.99: Genus level, Log score 2.00-3.00: Species level. In case of no growth the procedure was repeated with 100 µl milk.

Results: Nine percent (180/2000) of the quarters were considered contaminated with > 2 types of bacteria in the sample. Three percent (61/2000) displayed a log score below 1.69 which was considered "No identificaiton by MALDI-TOF", the remaining 97% of the samples wre identified to the species level. In total 76.6% (387/505) Non aureus staphylococci were found, mostly as the second agent. Fifty-three percent (268/505) *Corynebacterium* sp. of which 22% (111/505) *Corynebacterium bovis*, 12.3% *Staphylococcus aureus*, 8.9% *Streptococcus uberis*, 6.1% *Staphylococcus microti*, 3% (15/505) *Candida* sp., 2.7% (14/505) *Streptococcus dysgalactiae*, 0.8% (4/505) *Escherichia coli*, 0.6% (3/505) *Streptococcus agalactiae*, and 0.4% (2/505) *Klebsiella* sp. Amongst the remaing 14.9% (75/505), the isolates identified were *Acinetobacter* sp., *Aerococcus* sp., *Psychrobacter* sp, *Micrococcus* sp, *Kocuria* sp and *Trueperella pyogenes*. Escherichia coli

Conclusions: Despite that the study only focuses on cows with elevated somatic cell count and subclinical mastitis, we identified Staphylococcus aureus in 78 cases (quarters) ~ 62 cows and E. coli in four quarters ~ 4 cows. Since S. aureus is considered a major pathogen it is alarming that 12.3 % of the cows are infected and may be considered *S. aureus* carriers.

Our study indicated that application of culture-based diagnostics in combination with MALDI-TOF MS might reach a considerable number of undiscovered subclinical and clinical mastitis-diagnoses. This study is based on 19 farms so far. However, the pilot is part of a larger study including 100 Danish farms and a prolonged sampling period. Results are so far confirming the findings in the presented pilot study. Moreover, MALDI-TOF MS confirmed that *S. microti* isolates is present in Danish dairy cows, a bacterium that so far only have reported in Polish dairy (Krol et al. 2016).

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Keywords: Subclinical mastitis, MALDI-TOF.

UH-P19

Udder prepping benchmarks in automatic milking systems

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Objective: Risk factors affecting the health status of a mammary gland are well known and documented. In terms of mastitis prevention, the prepping of teats prior to milking is pivotal for decreasing the chances of intramammary infections (IMI) and thus ensure milk quality. In conventional milking, the application of the pre-milking routine steps: forestrip, disinfect, wipe and disinfect after milking, rely on the milker to perform them adequately in order to guarantee success. Milkers are also able to identify cows that require extra attention. In the DeLaval VMS™ milking system (Classic and V300 models) and DeLaval AMR™ (Automatic Milking Rotary), milkers' responsibilities are performed by the robot. This consists of a robust prepping system that combines cleaning, forestripping and drying steps using a cleaner cup, and disinfection of teats pre- (where allowed) and post-milking by a spray system in the robotic arm. Refinement of machine settings and constant monitoring are key points to achieve milk quality standards, so this paper proposes benchmarks, focusing on udder hygiene aspects of udder prep, to monitor performance in VMS farms.

Material and Methods: Different experiments were conducted to evaluate teat cleaning and disinfection during udder prep on VMS farms under several conditions. Altogether, over 1,500 teats and 8 VMS barns in USA and Belgium were evaluated. Experiment 1, cows were evaluated for udder hygiene score using a 1 (clean) to 4 (very dirty) category system. Experiment 2 and 3, teat cleanliness before milking was evaluated using a teat skin dirtiness score system. Experiment 4, cows were scored after spraying for hit rate (number of teats with a droplet of teat disinfectant at the teat end) and teat coverage assessed by an imaginary division of a teat in four quadrants: upper front, lower front, upper back, and lower back. Teats were then evaluated as the percentage of teats with ≥ 50% area covered by the teat disinfectant.

Results: Results from experiment 1 indicated that the udder hygiene score (UHS) was an accurate predictor for teat cleanliness. Cows with very dirty udders (score 4) were 4 times more likely (95% CI=1.2 - 16.2) to have 3 or 4 dirty teats compared to cows with very clean (score 1) udders. On average 46% of cows in the herds had UHS ≥ 3 demonstrating that barn and cow hygiene are some prevailing challenges in VMS barns. Experiments 2 and 3 demonstrated that the use of a cleaning solution on the teat cleaner cup (DeLaval PureFlow™) can greatly improve teat cleaning compared to the use of water alone. Results showed that dirty teats (scores 3 and 4) cleaned with a teat cleaning solution containing surfactants were ~4.5 times more likely to be clean (scores 1 and 2) after prep than using water alone. In Experiment 3, 94% teats enrolled had scores of 1 and 2 after cleaning with the soap compared to 78% teats when only water was used. These experiments demonstrated that barn cleanliness and cubicle hygiene management are important for VMS, as very dirty teats can be a challenge to the robot when only using water in the teat preparation phase, making necessary the use of a teat cleaner solution to mitigate this effect. As for teat disinfection, the V300 spray system relies on a camera system (DeLaval InSight™) that detects teat position before delivering teat disinfectant. This ensures accuracy in teat disinfectant application which is critical to ensure good teat condition and prevent IMI. Experiment 4 demonstrated an acceptable performance of the V300 used at light spray setting when 7 products were tested, with 97-100% teat end hit rate and 83-98% of teats with ≥ 50% area covered by the teat disinfectant.

Conclusions: Successful milk harvesting in VMS requires good combination of equipment, settings and management. Monitoring equipment performance is essential to ensure consistent milking routine. A VMS farm should target ≥ 75% cows in a barn with UHS 1 and 2. For udder prep, ≥ 95% teats with a score 1 and 2 should be possible when using a cleaning solution in a VMS. Proposed benchmarks for teat disinfection using the V300 are ≥ 97% hit rate and >75% of teats with ≥50% area covered by the teat disinfectant.

Keywords: Automatic milking system, benchmark, udder hygiene, teat disinfection.

UH-P20

The use of genomics data to accurately predict mastitis events in UK dairy herds

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Objectives: CLARIFIDE Plus is the genomic test from Zoetis that enables farmers to accurately predict an animal's genetic potential using their DNA. The results contained within CLARIFIDE Plus provide producers with predictions for wellness, health, production, fertility, longevity, type, and milk component traits, as well as parentage.

To demonstrate the effectiveness of CLARIFIDE Plus Wellness Traits in UK Holsteins, a retrospective demonstration study was performed using Zoetis Wellness Traits and lactation records (i.e. mastitis) collected from on farm herd management software.

Material & methods: Health record data was collected from on herd management software (e.g. Dairy Comp 305, Uniform Agri, etc.). Genomically enhanced standardised transmitting abilities (STA) for the wellness traits were used to assign cows to genetic groups of relative risk (Worst 33%, Middle 33% and Best 33%) within herd. For CLARIFIDE Plus Wellness Traits, STAs of 100 represent average relative risk for the corresponding health event with larger values communicating lower relative risk. Statistical analysis was conducted using a general linear mixed model (Proc GLIMMIX) in SAS 9.4 (SAS Inc., Cary, NC, USA).

Results

Table 1. Average Standardised transmitting ability means, disease incidence and estimated disease cost per 100 cows					
Mastitis - Genetic group	**Number**	**Mean STA**	**Disease incidence**	**P-value**	**Disease cost per 100 cows[3] (£)**
Worst 33%	197	95	22.34	0.0259	4025
Middle 33%	201	100	12.52		2256
Best 33%	186	105	11.31		2038

Conclusion: Genomic testing of dairy animals enables dairy farmers to accurately predict future performance of their livestock. The ability of the CLARIFIDE Plus wellness traits to effectively predict subsequent health events has been evaluated in both the US[1,2] and European Holsteins, including the Holsteins in the UK. The results of this UK study demonstrate that there is a significant difference in the incidence of mastitis between the best 33% and worst 33% as ranked by MAST_STA (Mastitis Standardised Transmitting Ability). Contrasting the incidence of mastitis in the best 33% with the incidence of mastitis in the worst 33% was associated with a 49% difference in incidence of mastitis, representing a savings of almost £2000 per 100 cows.

These findings support our hypothesis that greater MAST_STA values would be associated with a lower incidence of clinical mastitis compared to animals with a lower MAST_STA value. This demonstrates the effectiveness of CLARIFIDE Plus MAST_STA in predicting mastitis events in commercial UK dairy herds. The results of this study are consistent with findings from other demonstration studies we have conducted globally. These findings highlight the importance of predicting health events as a means of increasing the profitability of dairy cows within the herd.

In conclusion, this study provides UK dairy producers with a compelling opportunity to proactively reduce the incidence of health events in their herd through direct selection of replacement animals for lower relative risk of mastitis. Including direct selection for the wellness traits to a farm's genetics program using CLARIFIDE Plus allows producers to select and breed replacement animals for greater wellness, health, production, fertility, longevity, milk components, and type.

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Keywords: Clarifide, genomics, mastitis, prevention.

UH-P21

Antimicrobial susceptibility of environmental Streptococci isolated from bovine mastitis cases in 2019 in northeastern Italy

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Objectives: Environmental streptococci and coliforms are the most common mastitis agents isolated in many countries in dairy cows. Antimicrobial treatment is recommended in clinical mastitis due to environmental streptococci; therefore "in vitro" antimicrobial susceptibility test data are a valuable tool to direct bovine practitioners for a conscious, effective and prudent drug usage. The aim of the study is to share data on antimicrobial susceptibility test results of *Streptococcus uberis, Streptococcus dysgalactiae* and *Enterococcus* spp. strains isolated from clinical mastitis cases in northeastern Italy in 2019.

Material and methods: In 2019, bacteriological culture was performed on 14,027 bovine milk samples collected from clinical mastitis cases. Bacteria identification was based on their phenotypic and biochemical properties according to National Mastitis Council recommended procedures. Respectively, 581 *Streptococcus uberis*, 140 *Streptococcus dysgalactiae* and 155 *Enterococcus* spp. strains were tested by minimum inhibitory concentration (MIC) method for the evaluation of their susceptibility to the following antibiotics: ampicillin, amoxicillin/clavulanic acid, cefazolin, cefoperazone, cefquinome, erythromycin, gentamycin, enrofloxacin, oxacillin, lincomycin, spiramycin, penicillin, trimethoprim/sulfamethoxazole and rifampin. Results were interpreted as sensitive (S), intermediate (I) and resistant (R), according to the breakpoints set by international guidelines and literature references.

Results: Excluding "no growth" or contaminated samples, 7,628 samples tested positive at the bacteriological culture. The overall amount of environmental streptococci isolated was 2,299 (30.1%), thus divided: 1,558 *Streptococcus uberis (*20.4%), 466 *Enterococcus* spp. *(*6.1%) and 275 *Streptococcus dysgalactiae* strains (3.6%). Other bacteria identified included: 2,356 non-*aureus Staphylococcus* spp. (30.9%), 1,058 *Staphylococcus aureus* (13.9%), 607 coliforms (8%), 161 *Streptococcus agalactiae* (2.1%). Environmental streptococci submitted to MIC test showed the highest proportion of susceptible strains (media = 98.9%) to the combination amoxicillin/clavulanic acid, followed by ampicillin (98%), penicillin (93%) and trimethoprim/sulfamethoxazole (91.4%). Lincomycin displayed the smallest proportion of susceptible isolated (42%) with a MIC_{90} above the highest concentration tested (> 4 µg/mL) for each species group. *Streptococcus dysgalactiae* displayed the greatest susceptibility to the tested antimicrobials ranging from 64.3% for lincomycin to 100% for amoxicillin/clavulanic acid, ampicillin and cefquinome. Isolates of *Streptococcus uberis* displayed higher MIC_{90} values for almost all antimicrobials than *Streptococcus dysgalactiae* and 54.7% of *Streptococcus uberis* strains (n=318) were resistant to oxacillin (MIC_{90} = 2 µg/mL). Proportion of susceptible strains to erythromycin was equal to 69.3% of the total number of streptococci tested, with the highest percentage of resistance in *Enterococcus* spp. isolates (intrinsically resistant) displaying

a MIC_{90} value above the highest concentration tested (> 8 µg/mL) in each species group. Fluoroquinolones and rifampicin displayed respectively a proportion of 84.0% and 77.0% of susceptible strains. Due to their intrinsic resistance patterns and the adoption of different breakpoint criteria compared to *Streptococcus uberis* and *Streptococcus dysgalactiae*, *Enterococcus* spp. strains showed higher proportion of resistance to the tested antimicrobial molecules with MIC_{90} values always higher than the other streptococci species.

Conclusion: A significant proportion of the samples collected from clinical bovine mastitis cases showed the growth of environmental streptococci, with *Streptococcus uberis* as the major pathogen representing this group. Data collected highlighted in these bacteria species "in vitro" high susceptibility level to beta-lactams, trimethoprim/sulfamethoxazole and fluoroquinolones, but with significant difference among the different bacteria species. Hence, the need to achieve a correct diagnosis of the pathogen involved in mastitis beyond the group level to avoid treatment failure and occurrence of antimicrobial resistance.

Keywords: Antimicrobial susceptibility, Environmental streptococci, Streptococcus uberis, mastitis, Dairy cow.

UH-P22

The effect of selective dry cow therapy on new intramammary infections and the bacterial diversity of quarter milk

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Selective dry cow therapy could be an alternative for reducing the use of antibiotics in healthy cows. The present study aimed to evaluate the effect of teat sealant on healthy cows at the drying-off period on the bacterial diversity of quarter and the new intramammary infection (NIMI) after the postpartum. One dairy herd was selected based on (i) the adoption of selective dry cow therapy protocol, (ii) having data recording systems in place and, (iii) routine diagnostic of clinical mastitis program. All selected Holstein cows (n = 80, parity ≤ 3 and a milk yield average of 36.5 kg/cow/day) were randomly assigned to two groups at the drying-off period (step 1): a) healthy (with four quarters without isolation of mastitis-causing agents, no history of clinical mastitis and, the last three monthly SCC < 200×10^3 cells/mL), which were only treated with 2.6 g bismuth subnitrate internal teat sealant (n = 28); and b) subclinical mastitis (culture-positive in at least one of the quarters and at least one out of the three monthly SCC > 200×10^3 cells/mL), which were treated with 250 mg cephalonium and teat sealant (n = 52). A total of 626 sterile milk samples were collected at the mammary quarter level (step 2) on the day of drying-off and between 7-15 d postpartum for microbiological culture (MC) analyses. Based on the results of MC, the quarter was considered: a) healthy: when there was no isolation of microorganisms in all MC analyses; b) cured: when it presented a positive isolation result at dry-off and postpartum result was negative, or the MC result after calving was different from the one at dry-off; c) NIMI: when there was no isolation at dry-off and there was an isolation after calving or when there was isolation in the postpartum sampling of microorganisms different than the agent isolated at dry-off; and d) uncured: when there was isolation of the same agent at dry-off and at postpartum. The identification of pathogens was performed at the species level using MALDI-TOF MS. Quarter milk samples from healthy (n = 38) and cured cows (n = 38) had the DNA amplified with the V4 primer oligonucleotides of the 16S rRNA gene. New generation sequencing (NGS) was performed using MiSeq (Illumina) equipment. Statistical analyses for bacterial diversity were performed using R with phyloseq 1.22.3 and vegan 2.5-3 packages. The effect of teat sealant at risk of NIMI was analyzed by PROC GLIMMIX, using herd and cow as random effects to consider the correlations of cows within-herd and mammary quarters within the cow. Based on the results of MC, 240 quarters out of 313 were considered healthy, 38 cured, 31 NIMI and 4 uncured. The microorganisms most frequently isolated at drying off was non-aureus Staphylococci (52.4%), *Corynebacterium* spp. (19%), *E. coli* (16.6%), *Prototheca* spp. (4.8%), *S. dysgalactiae* (2.4%), *S. uberis* (2.4%) and *T. pyogenes* (2.4%). The bacterial diversity using NGS was similar when compared both healthy and cured quarters submitted to both drying-off protocols. The Firmicutes was the most abundant phylum, followed by Proteobacteria, Actinobacteria, and Bacteriodetes. The estimated NIIM risk by logistic regression analysis was 10 cases per 100 quarters at risk using antibiotic and teat sealant, and 7 when using only teat sealant. Healthy cows treated with selective dry cow therapy protocol using only teat sealant presented a lower risk of NIIM and no alteration of bacterial diversity in comparison to cows treated with antibiotics, which suggests that the use of dry cow therapy should be recommended only for cows with a history of subclinical or clinical mastitis. The authors acknowledge CAPES/PNPD and FAPESP for the scholarship (Proc. nº. 2017/26997-2).

Keywords: Milk quality; bacterial diversity; drying-off therapy of healthy cow; no antibiotic-treated; teat sealant.

UH-P24

Udder Health parameters evolution in Spain for the last 20 years

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Objectives: Somatic Cell Count (scc) has been used for years to monitor the udder health of dairy cows and most farmers and veterinarians base their decisions on these data. But

a lack of consistency when different veterinarians or different Milk Record Associations calculate basic parameters to monitor udder health has been clearly identified. Then, comparisons on udder health between farms or different areas becomes not reliable. That restricts the benchmarking benefit.

ANEMBE (Asociacion Nacional de Especialistas en Medicina Bovina de España) Milk Quality Committee and CONAFE (Confederación Nacional de Asociaciones de Frisona Española), as a responsible of milk recording database in Spain, have been working during the last year in harmonizing the definition of 13 parameters of milk quality calculated by using monthly test day somatic cell information and making them available online for farmers and milk quality technicians.

The objective of this poster is to present the result of this collaboration and to discuss the observed trend of the calculated average by year of the 13 parameters throughout the last 20 years in Spanish dairy herds.

Materials and methods:

About 1,749,228 test-days of milk recording collected between 2000 and 2019 in Spanish dairy herds have been used to calculate milk quality parameters for each test day control and each herd. Weighted averages by year for all indices have been calculated at herd level, at Autonomous Community level and at National level. Calculated parameters are:

1. % cows less than 200 scc
2. % first lactation cows less than 100 scc
3. Average SCC
4. Average Liner Score (LS)
5. New Infection Risk (NI) between two consecutive tests
6. Cure Risk between two consecutive tests
7. % cows less than 200 scc in two consecutive tests
8. % cows more than 200 scc in two consecutive tests (chronic cows)
9. New Infection Risk during dry off period
10. Cure Risk during dry off period
11. Corrected Cure Risk during dry off period (including New Infection Risk)
12. % first lactation cows less than 100 scc at first test after calving
13. % adult (=> 2nd lactation) cows less than 200 scc at first test after calving

Results: Results show a clear improving for all milk quality parameters analyzed along this last 20 years. As an example, % of cows less than 200 scc moves from a 64,6% in year 2000 to a 76,3% in 2019. Average Linear Score moves from 3,28 in 2000 to a 2,71 in 2019. Or New Infection Risk between 2 consecutive tests moves from 18,0% in 2000 to a 11,8% in 2019.

YEAR	%scc<200	Av LS	NI Risk	Cure Risk	NI at dry off	Cure at dry off
2000	64,6%	3,28	18,0	27,4	26,7	47,2
2001	65,4%	3,29	17,5	28,3	38,2	37,6
2002	66,1%	3,29	17,1	28,3	39,6	37,3
2003	65,9%	3,28	17,3	28,7	39,3	51,4
2004	66,7%	3,27	17,0	29,2	17,8	58,0
2005	68,5%	3,19	16,1	30,4	19,8	61,7
2006	68,8%	3,21	15,9	30,3	18,4	78,4
2007	68,2%	3,22	16,0	29,7	18,5	82,8
2008	67,6%	3,25	16,2	28,7	18,4	65,0
2009	67,5%	3,27	16,1	28,5	18,7	57,8
2010	68,6%	3,21	15,6	29,4	17,6	61,7
2011	69,6%	3,14	15,0	29,7	20,4	62,6
2012	71,4%	3,05	14,2	30,8	22,9	61,0
2013	72,1%	3,01	13,8	31,2	19,3	65,7
2014	72,6%	3,00	13,5	31,5	13,3	74,7
2015	73,8%	2,91	13,0	32,4	13,9	65,3
2016	75,0%	2,82	12,5	33,1	17,9	66,0
2017	75,7%	2,77	12,1	33,7	16,9	71,4
2018	75,9%	2,77	12,2	34,3	16,0	66,6
2019	76,3%	2,71	11,8	34,4	18,3	60,6

Conclusions: Udder health in Spain has been improving constantly for the last 20 years as shown in the results. Dairy farmers, veterinarians, consultants, dairy factories and consumer demands has made this change and this trend possible. Spain still has a long way for improvement, especially on antibiotic reduction. Continuous improving udder health will lead to use less antibiotics and produce a better milk.

Keywords: Udder health.

UH-P25

Investigation of subclinical mastitis and the role of non-aureus staphylococci in a dairy farm with chronically elevated bulk tank milk somatic cell count

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Objective: The objective was twofold: a) to retrospectively evaluate subclinical mastitis (SCM) in a dairy farm with persistently elevated bulk tank milk somatic cell count (SCC), and b) to investigate the role of non-aureus staphylococci (NAS)

among cows with chronic SCM.

Materials and methods: The study was performed in a dairy farm with a history of chronically elevated bulk tank milk SCC. The farm had 120 milking Holstein cows, with a mean 305-days milk yield of 10,588 kilograms, milked twice daily and housed in a free stall barn. The study consisted of two parts. In the first part, a retrospective evaluation of SCM was performed, based on monthly individual and bulk tank milk SCC reports, provided by the dairy herd improvement (DHI) program, from 2015 until 2018. SCC cut-off used to determine SCM was preset in the DHI at 250,000 cells/mL. Cows were grouped in the DHI reports by lactation number (1st, 2nd, ≥3rd), stage of lactation (0-60, 61-120, 121-180, ≥181 days in milk) and herd as a whole. SCM prevalence was calculated as the percentage of cows over the cut-off in a monthly report, per herd, per lactation number and per stage of lactation. Chronic SCM prevalence was calculated as the percentage of cows over the cut-off in ≥2 sequent monthly reports, per herd, per lactation number and per stage of lactation. Finally, a dynamic group of chronically high SCC cows was created. In the second part of the study, the farm was visited on a monthly basis during 2019 by the same team of veterinarians to clinically assess the aforementioned dynamic group and collect milk samples. Microbiological cultures from these samples were performed in the laboratory to investigate the presence of bacterial pathogens. Monthly DHI reports were available for continuous monitoring of this dynamic group throughout 2019. Cows with SCC below cut-off in a new monthly recording were removed from the dynamic group and new cows exceeding the cut-off in ≥2 sequent recordings entered the dynamic group.

Results: The retrospective analysis (2015-2018) revealed an overall SCM prevalence of 46%. SCM prevalence in ≥3rd lactation cows was 60% with 87% of them being chronic cases. SCM prevalence was noticeably high from 1st lactation, affecting 18% of the cows. Half of the cows at late lactation (≥181 days in milk) had SCM and almost all of them were chronic cases. The incidence rate of new infections at early lactation (0-60 days in milk) was 4 times higher than later stages. On average, 8 out of 120 milking cows were responsible for more than 50% of somatic cells in the bulk tank milk in each monthly recording in 2019. Approximately 60% of these cows remained high somatic cells' contributors for ≥2 months and 87% were chronic cases of SCM. Among these cows 59% were at late lactation and 65% were in ≥3rd lactation. Overall, 52 cows were monitored in the chronically high SCC dynamic group, either for a single month or more. After a year of monthly milk cultures, this dynamic group was identified as major-pathogens free (Staph. aureus, Str. uberis, Str. agalactiae and Str. dysgalactiae). Non-aureus staphylococci (Staph. chromogenes, Staph. hyicus, Staph. warneri, Staph. epidermidis) were involved in 44% of chronically high SCC cows, either solely (26%) or in mixed infections (18%) with other pathogens (mainly, Corynebacterium bovis, E. coli and Bacillus licheniformis). Infections caused solely by the latter pathogens were 30%. In 26% of all samples no pathogen was isolated.

Conclusions: High somatic cell contributors in bulk tank milk were mainly cows with chronic SCM being in ≥3rd lactation and at late lactation stage. NAS were involved in the majority of chronic SCM cases. In compliance with literature, the presence of NAS in these cows seems to play an inhibiting role against major pathogens.

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Keywords: Non-aureus staphylococci, subclinical mastitis, somatic cell count, dairy cows.

UH-P26

Case study – Efficacy of vaccination program of against clinical mastitis at a well-managed dairy farm in Poland

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Objectives: Clinical mastitis caused by environmental pathogens seems to be one of the greatest problems in the dairy industry, even at well-managed dairy farms. It leads to economic losses connected to milk yield decrease, therapy, withdrawal period and high Somatic Cell Count (SCC). In Poland, the cost of clinical mastitis is estimated for 1075 Polish Zlotys (PLN) at about €250. Preventing environmental clinical mastitis is also based on proper hygiene during the milking process and the cow's environment, as well as vaccination programs limiting the incidence of disease. The aim of this study is to evaluate the effect of a synchronized vaccination program with two vaccines for a mastitis prevention (STARTVAC® and UBAC®, HIPRA) on the prevalence of clinical mastitis and milk parameters at a well-managed dairy farm in Poland.

Materials and methods: The study was conducted on a dairy farm of 150 lactating cows, milked twice a day in a fishbone parlour, kept in free stall barns on rubber mats and concrete slats. For the past few years, the herd has been vaccinated with a polyvalent vaccine for Coagulase Negative Staphylococcus (CNS), *Staphylococcus aureus (Staph. aureus), Escherichia coli (E. coli)* and coliforms (STARTVAC®, HIPRA); vaccination was administered according to the product label (42 and 10 day prior parturition (PP) and 51 days after parturition (AP)). The average SCC for 2018 was 181,000 cells/mL. Despite this, in 2018, there was an increase in the incidence of clinical mastitis, so a PCR examination of clinical cases and bulk milk tank was performed (Uddercheck®, HIPRA). In connection with the test result showing *Streptococcus uberis* (Strep. uberis) as the main source of the problem, the vaccination program of synchronized use of both vaccines (STARTVAC® and UBAC®, HIPRA)) was introduced on September 2018. Due to the specific characteristics of the farm, the vaccination protocol was adapted by farm veterinarians and vaccinations were performed 60 and 21 days PP and 21 days AP. The data about incidence and time of occurrence of clinical mastitis and milk yield were collected from January 2018 until December 2019. All analyzed values were com-

pared between 2018 and 2019.

Results: The received parameters are presented in the table below:

Analyzed value / year	2018	2019
Clinical mastitis cases (overall)	**27**	**21**
Clinical mastitis cases in first 90 days of lactation	**19**	**10**
Milk yield (day average; kg)	**32.563**	**33.345**

The comparison of data between 2018 and 2019 revealed a 22% reduction in clinical mastitis during whole period and a 47% reduction in first 90 days of lactation. Daily milk production increased in 2019 by 0.782 kg, which resulted in a 285 kg annual increase.

Conclusions: Vaccination programs targeted at specific pathogens are an excellent tool to minimize the impact of environmental bacteria on udder health and the incidence of clinical cases, especially in such a critical period as the beginning of lactation, and thus play an important role in the economy of a modern dairy farm. The vaccination with a subunit vaccine for *Strep. uberis* (UBAC®, HIPRA)) is useful tool in udder health management and could also have a positive impact on well-managed dairy farms.

Keywords: Dairy, vaccination, mastitis, prevention.

UH-P27

Genetic variability between *S. AUREUS* isolates and differences in epidemiology and control of subclinical mastitis in a subset of low and high SCC Irish herds

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Objectives: *Staphylococcus aureus* is the main causal agent of mastitis in domestic ruminants worldwide. Intramammary infections produced by *S. aureus*, although frequently subclinical, may lead to huge economic losses. There is limited data currently available on the impact of genetic variability within *Staph aureus* isolates on the cure rates following routine treatments, and on the ability of common mastitis control strategies to limit the spread of contagious mastitis within infected herds. This study aimed to isolate *S. aureus* from cows infected with subclinical mastitis in 10 control and 10 problem herds to establish if *S. aureus* strains affecting low SCC herds were similar to those affecting high SCC herds. One objective was to determine if some isolates were non-bovine-adapted strains, which would have obvious implications for control measures. A second objective was to assess the impact of genetic variability between *S. aureus* isolates on the epidemiology of contagious mastitis in the study herds.

Material & methods: Mastitis investigation visits were carried out in 10 high SCC problem herds and 10 low SCC control herds where *S. aureus* was previously identified. Milk samples from study herds were collected from 20% of first lactation and 20% of multiparous cows. Target animals were selected based on historical milk records and CMT testing. Standard microbiological methods recommended by the *National Mastitis Council* were used for identification of *Staphylococcus aureus*. In brief, milk was cultured on blood agar, isolates were single colony purified and identified based on colony morphology, haemolysis, Gram stain, catalase and coagulase test and growth on Mannitol Salt agar. *S. aureus* isolates were finally confirmed by PCR amplification of the *aroA* gene. In addition, antimicrobial susceptibility of isolates was evaluated using the disk diffusion method, in accordance with Clinical and Laboratory Standards Institute guidelines (CLSI, 2007). To obtain genomic data, isolates were cultured overnight in TSB and genomic DNA was extracted using the PurElute Bacterial Genomic Kit (Edge Biosystems, MD, USA) following the manufacturer's guidelines and diluted in 100 µl of deionized water. DNA concentration was determined using a Nanodrop spectrophotometer at a wavelength of 260 nm, and genomic DNA was purified and sent for Whole Genome Sequencing.

Results: 180 isolates have been now sent for whole genome sequencing and genomic data will be available soon. Clonal diversity of the sequenced isolates will be examined by Multi-Locus Sequence Typing (MLST) in order to determine sequence types (STs). The eBURST algorithm will be used to identify groups of related *S. aureus* genotypes and all isolates will be compared with all entries of bovine origin in the MLST database. In order to assess the response to routine mastitis control protocols implemented on each study farm, the following epidemiological parameters will be assessed: (i) the percentage of recent infections will inform about the level of on-going spread of mastitis within these herds and may highlight variation in the ability of different strains to lead to new infections in the face of preventive measures within the milking routine, as well as their abilities to bypass the innate physical and immune defences of exposed cows; (ii) the percentage of persistent infections over time may highlight strain variations with respect to cure rates resulting in the build-up of significant sources of infection within each herd; (iii) the dry cow cure rate will be used to indicate the response of *S. aureus* strains isolated to various dry cow antibiotic therapies used on these farms.

Conclusions: Although data analysis is still in progress, we believe output from this study will build awareness in relation to the responsible use of antimicrobials and also lead to tailored therapeutic protocols to control mastitis in dairy herds. Data on the impact of genetic variability between *S. aureus* isolates on the epidemiology of contagious mastitis in affected herds will be hugely valuable information in an environment where it is critical to optimise therapeutic practices and culling decisions on farm.

Acknowledgements: This study has received funding from the Irish Research Council and the EU Horizon 2020 Research & Innovation programme through the CAROLINE Cofund scheme, under the Marie Skłodowska-Curie grant agreement No.713279.

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Keywords: Staphylococcus aureus, mastitis, genomics, epidemiology.

UH-P28

Case Study - Effect of the administration of two vaccines against mastitis on some key udder health indicators in a dairy cattle herd in Galicia

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Objective: To evaluate the effect of the implementation of an immunization programme involving two vaccines against bovine *Escherichia coli (E. coli),* coliform, Coagulase-Negative Staphylococci (CNS), *Staphylococcus aureus (Staph. aureus)* and *Streptococcus uberis (Strep. uberis*) mastitis (START-VAC® and UBAC®, HIPRA) on the main indicators of subclinical mastitis: prevalence of infection, incidence of new infections, cure rate and percentage of cows with chronic mastitis.

Material and methods: The study was conducted in a dairy cattle herd in the province of A Coruña (Spain), in a family farm with about 70 milking cows housed in freestalls and fed with a Unifeed system. Milking was performed twice daily in a 20-point rear milking parlour. The farm is attached to the official AFICOR milk testing agency and receives monthly production and individual analytical data, including somatic cell counts.

For several years, the farm had had a significant mastitis problem due to the high incidence of clinical cases and, most of all, the high somatic cell count (SCC) in the milk tank. The aetiology of the individual cases of mastitis, diagnosed by culture and laboratory identification techniques, was very diverse. Thus, between August 2016 and May 2019, a total of 143 samples were analysed from individual teats, 105 of which were positive for growth of one or more pathogens. The species identified were predominantly from the cow's immediate environment, *Strep. uberis* being the most common (accounting for 45% of all identifications). Found in 18% of positive samples, *Staph. aureus*, which is typically contagious, was also significantly present.

The immunization programme, which was adapted for this particular farm under veterinary supervision, included two vaccines: a polyvalent vaccine (STARTVAC®, HIPRA) which protects against *Staph. aureus*, *E. coli* and coliform bacteria and CNS, and a subunit vaccine (UBAC®, HIPRA) indicated for prevention of *Strep. uberis* mastitis. In both cases, a blanket vaccination regimen was designed which included lactating cows, dry cows and heifers in the last third of gestation.

(STARTVAC®, HIPRA): primary vaccination with two doses given 21 days apart and a booster every 4 months.

(UBAC®, HIPRA): primary vaccination with three doses given 30 days apart and a booster every 6 months.

In order to assess the effect of vaccination on subclinical mastitis, the average monthly indices in the year preceding the start of the programme were compared with those of the year that the vaccines were administered. A cow was considered to have an intramammary infection when the SCC provided by the milk testing agency was greater than 200,000/ml.

Results: The average prevalence of infection, which was 35% in the year prior to vaccination, decreased to 27.3% in the following year. The monthly incidence of new infections fell from 23.9% to 16.7%, while the cure rate increased from 21.8% to 28.2%. Finally, the average percentage of cows with chronic mastitis decreased from 19.2% to 15.8%.

Conclusion: After vaccination to prevent mastitis (START-VAC® and UBAC ®, HIPRA) in a herd with cases of subclinical mastitis of diverse aetiology, a positive improvement was observed in all indicators used to monitor levels of intramammary infection. Thus, vaccination successfully improved udder health, a determining factor for productivity and economic profitability in the herd.

Keywords: Dairy, vaccination, mastitis, prevention.

UH-P29

Detection of multiple *Mycoplasma* species in different sample types collected from dairy herds with mycoplasma mastitis

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Objectives: Mycoplasma species have a worldwide distribution causing mastitis, pneumonia, otitis, reproductive disorders and many other infectious diseases in dairy cattle. Interaction of mycoplasmas with their hosts can vary from primary or opportunistic pathogens to commensals. Mycoplasma can be found in the mucous membranes of healthy cows and stress conditions may allow the same organism to enter other body sites and systemically disseminate causing different infections in the same animal. As mycoplasma organisms can be highly contagious and therapy-resistant, prevention and control are crucial. Mycoplasmas cause the greatest economic loss to dairy farmers since animals identified as positive are culled. *Mycoplasma bovis* is the most relevant species for higher incidence and severe pathogenicity but other species of mycoplasma can be isolated from dairy cattle. The aim of this study was to evaluate mycoplasma shedding in four New York State (NY) dairy herds having positive cases of mycoplasma mastitis.

Materials and methods: On four dairy farms in NY State, we collected samples from cows within one week of their first diagnosis of mycoplasma mastitis. From these farms, we also selected an equal or greater number of cows previously reported as Mycoplasma negative and untested calves. In total,

we chose 16 positive cows, 18 negative cows and 21 calves. From each group of cows, we collected milk samples from each quarter, vaginal and nasal swabs; nose and ear tract samples were taken from each calf and bedding samples were taken from each farm. All samples were tested by standard microbiological procedures for mycoplasma detection. For positive results, the isolates were submitted for molecular speciation by PCR and sanger-sequencing. Milk samples were also tested by a real-time PCR assay specific for the detection of Mycoplasma species in milk samples.

Results: The results obtained showed that 8 of the 16 positive cows had inconsistent mycoplasma shedding. Milk samples collected from those animals at a second time point resulted as mycoplasma negative by culture and 4 cases also resulted negative by real-time PCR.

From the 18 negative cows, we found 1 new infection that could be also associated with intermittent shedding. The intermittent shedding was not associated with a specific mycoplasma species but applied to multiple species which also included *M. bovis*.

In the group of positive cows, 13% of the nasal swabs resulted as mycoplasma positive with 50% of those identified as *M. bovis*, the other 50% as *M. bovirhinis*. Within this group, only one cow showed a positive vaginal swab, identified as *M. bovigenitalium*. In the group of negative cows, 17% of nasal swabs resulted as positive and all were identified as *M. bovirhinis*.

For the calf group, 81% of the nasal swabs and 10% of the ear swabs resulted in mycoplasma positive. For the nasal swabs, the mycoplasma species identification appears to be farm-related. From two farms 100% of the positive samples identified as *M. bovis*. From the other two farms 100% of the positive samples identified as *M. bovirhinis*. Only one farm had calves with positive ear swabs and these were all identified as *M. bovis* which was also found in the noses of calves and cows and in milk samples from that same farm. 50% of bedding samples resulted mycoplasma positive by cultures but all were identified as *Acholeplasma laidlawii* by sequencing.

Conclusions: Based on our results, it is shown that mycoplasma mastitis can occur with intermittent shedding that seems to not be species-related. In dairy herds, mycoplasma species can be present in different specimen types obtained from multiple body sites. Mycoplasma has been found in the nose cavities of a large group of animals whether they were part of a positive or negative cow group. *M.bovis* was only found in the nasal swabs of cows with previous or ongoing mycoplasma mastitis. All other animals were positive for *M. bovirhinis* which seems to be frequently present in the nose tracts of healthy cows and calves.

Our results from bedding samples showed that *Acholeplasma laidlawii*, *often* mistaken for Mycoplasma species in culture, could represent an environmental contaminant.

The incorporation of molecular mycoplasma speciation to support routine bacteriological screening provides epidemiologic information about mycoplasma disease and may form management strategies that could lead to a viable alternative to slaughtering.

Keywords: Mycoplasma.

UH-P30

Prevalence of Mastitis Pathogens in Southern Germany – Observations of a stratified random sample

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Objectives: The objective of this study was to describe the prevalence of mastitis pathogens in Bavaria, Southern Germany.

Materials & Methods: In the fall of 2017 a list of all Bavarian dairy farms - excluding farms shipping less than 200L milk per day - was split into quarters based on milk shipped. For each stratum, randomly selected herds, were contacted with the aim to recruit 40 herds per stratum. The herds were visited once by technicians of the Bavarian Animal Health Services (TGD) to collect aseptic quarter milk samples of all lactating dairy cows as well as CMT scores. Mastitis pathogens were identified by standard culture methods or MALDI TOF at the accredited milk quality laboratory of the TGD. Cows without microbiological growth in all four quarter milk samples were considered uninfected. Cows were considered infected with a pathogen, if that pathogen was isolated from one of their quarters. The within-herd prevalence (WiP, median) refers to cow level prevalence within farms that tested positive for that pathogen.

Results: In the end 152 herds were visited between late fall 2017 and June 2018. The majority of dairy farms milked only Fleckvieh cows (>75% farms). Quarter milk samples of 6,988 cows were collected from, on average, 38 cows per farm (Range: 9-250 cows). The majority of samples showed no-growth (89%) and were also coagulase-negative Staphylococci (CNS) negative (79%). The most commonly isolated pathogens were CNS (5%), *Staphylococcus aureus* (3%), *Streptococcus uberis* (1%) and *Streptococcus dysgalactiae* (1%). Besides no-growth (30%), *Staphylococcus aureus* (14%), and *Streptococcus uberis* (12%) were the most commonly isolated pathogens of quarters with clinical mastitis. *Streptococcus canis* was isolated from 13% of clinical quarters. However, this was due to one herd that contributed almost all clinical quarters with *Streptococcus canis*. On the day of the visit: 3-teated cows or cows with clinical mastitis were found on 38% (WiP: 3%) and 47% (WiP: 3%) of farms, respectively. CNS were present on most farms (90%, WiP:13%). *Staphylococcus aureus* was found on 71% of farms (WiP: 6%). *Streptococcus uberis* was found on 57% of farms (WiP: 4%) and *Streptococcus agalactiae* was present on 3% of farms (WiP: 11%). Very few farms had cows with *Escherichia coli* (10%, WiP: 3%), *Klebsiella ssp.* (2%, WiP: <1%), or other gram-negative mastitis pathogens (14%, WiP: 3%).

Conclusion: This is the first time that an unbiased prevalence of mastitis pathogens on Bavarian dairy farms was determined. The majority of quarters were CMT negative and showed no-growth. However, *Staphylococcus aureus* and *Streptococcus uberis we*re the most commonly isolated mastitis pathogens. Therefore, control programs should continue to focus on known on-farm risk factors to prevent the new infections with these pathogens.

Keywords: Mastitis, pathogens, Germany, cows.

UH-P31

Effect of milking machine and cow management improvement on teat end condition

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Objective: Teat end closure is mammary's gland first defense mechanism against new intramammary infections (IMI). Poor milking machine regulation is one of the most important causes of teat end damage, thus having a direct influence on teats health.

The objective of this investigation was to evaluate the impact of improvements made on cow management, milking parlor, and milking machine settings on the teat end condition.

Material e methods: This was a 17-months observational study done in one dairy farm in Minas Gerais, Brazil with 180 Holsteins cows, housed in a compost barn system. Cows were milked in 8x8 low milk line parlor, averaging 38 kg/cow/day. From Oct 2017 to Mar 2019 monthly visits were performed by Evoluir team to assess milking machine performance and milking routine.

Milking machine performance evaluation was made using a VADIA "Vacuum Diagnostics" Biocontrol equipment in at least 10 cows per visit. After assessment using the test "Milk Time Test" averages of each evaluated parameter were recorded and compared between period and month of evaluation. Parameters evaluated were; machine on time, total average claw vacuum, overmilking time, and average flow rate. The milking routine was also evaluated, and consisted of pre-dipping, strip, wipe, attach and post dipping, in a territorial system, with 4 cows per milker and average of 135 seconds of prep lag time. To improve cow comfort, some adjustments were made to the stalls with the inclusion of a waiting area with separation gates. To avoid overmilking, milk flow rate switch-point was continuously raised at a rate of 100 g/ml, from 300 g/ml on the beginning of the study, until 650 g/ml.

Finally, cows were evaluated for teat end condition every three months during the study period by the same evaluator, using a 4 point score system, where scores 3 and 4 were considered as having hyperkeratosis. The prevalence of hyperkeratosis of the cows in lactation was measured for each evaluation and compared between months.

Results: After changes were applied to the milking system, machine on time decreased from 04:42 minutes on the first month of evaluation to 03:15 minutes at the end; representing a reduction of 30%. Overmilking time decreased from 1:32 minutes to 00:50 minutes. It was observed an increase of 21.5% on average milk flow rates during the study period, moving from 2,45 L/min to 3,12 L/min at the beginning and end of the study respectively.

After decreasing work vacuum settings from 44 kpa to 42 kpa, average claw vacuum also decreased from 38,9 to 36,8 kpa leading to a more gentle milking process. Teat end hyperkeratosis prevalence (score 3 and 4) steadily decreased during the trial from 15% (105/688) in Jun 2018, 13% (45/668) in Set 2018, 16% (104/676) in Dez 2018 to 9% (58/600) in Mar 2019. The changes on milking machine settings and cow management were applied during this period.

Conclusion: The investigation concluded that reducing milking on time by changing milking settings in milk flow rate switch point, and ensuring the cow comfort during the milking, had a positive impact on reducing teat end hyperkeratosis.

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Keywords: Mastitis, Milkmachine, Teatcondition.

UH-P32

Statistical model to predict bulk tank SCC variations when key parameter are changed at 272 farms in Galicia

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Objectives: Somatic cells are the most essential factors naturally present in milk and somatic cell count (SCC) is used as an indicator of monitoring mastitis incidence in the herd and also to assess milk quality. In addition, bulk tank SCC is frequently used to determine quality payments to dairy producers. The SCC is directly related to obtaining maximum milk production from individual cows and a lower SCC indicates better animal health, since somatic cells originate only from inside the animal's udder.

The objective of this studio is to predict how much bulk tank SCC vary when some changes are made at each farm, according to the recommendations of SERAGRO veterinarians, in order to help in the decision-making process.

Material and methods: Bulk tank SCC data were collected daily for 54 months (January 2015 until June 2019), from 341 Galicia dairy farms. SERAGRO veterinarians collected monthly data from management (general, feeding, milking, drying), facilities (milk parlour and farm), mastitis vaccination with a polyvalent vaccine (STARTVAC®, HIPRA) and herd characteristics (individual lactation number and milking days) by farm, adding all 109 variables together.

From the 109 initial variables collected by SERAGRO veterinarians, most of them were removed by technical criteria (milk quality control vets), significance, collinearity between variables measured through clustering analysis and Spearman correlation. Finally, at this point, 30 variables were selected.

Farms with missing values in more than 5 variables were removed from the study, while if they were missing fewer than 5, these parameters were statistically imputed with a customised algorithm. The final number of farms in the study was 272.

After these steps, a linear regression model with farm as a random variable was built. Through an automatic procedure named Stepwise, 12 of the 30 variables were selected as the most important to explain bulk SCC tank in the studied farms. Among these factors, vaccination for mastitis with a polyvalent vaccine (STARTVAC®, HIPRA) was included.

Results

Type of removal system (automatic or manual), removal quality (right/overmilking), lactation excluded average of first lactation, % of first lactation cows, farm size, bedding materials (organic, inorganic), vaccination (STARTVAC®, HIPRA), vacuum work, static test, cow hygiene, month and regulator were the selected explanatory variables that predict the logarithm bulk SCC tank (bSCC) at SERAGRO farms. The coefficient of determination (R^2) of this regression model was 0.61.

Due to the fact that "farm" was selected as a random variable, each farm will have its own intercept in order to know how bSCC would vary after changing any of the previous selected explanatory variables at each farm.

Conclusions: As we can see in this analysis, bSCC can vary due to many factors. The predictive model developed for SERAGRO is a useful decision-making tool. With this model, vets and farmers can understand the impact of bSCC when making any decision in advance. These studies also show that vaccination for mastitis plays a key role in controlling SCC.

Keywords: Dairy, vaccination, mastitis, prevention.

UH-P33

Cause of intramammary infection in free-stall barns milked with automatic or conventional milking system

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Objectives: Automatic milking has several differences to conventional milking and may alter the distribution of bacteria that cause intra-mammary infection (IMI). The same milking-unit milks approximately 50 to 60 cows before getting washed, and applying milking order is not straightforward. The aim of this study was to see if the distribution of bacteria that cause IMI differed in herds milked with different milking systems.

Materials and methods: We gathered data from 49 free-stall barns in Finland. We used all results of individual quarter mastitis samples sent to Valio laboratory during 2011. Samples were analyzed with PCR. In total we had 4285 quarter samples from 2542 cows. A diagnosis for every quarter was made, and only quarters with definitive diagnosis were considered. This meant that only results with adequate amount of bacterial DNA (++ and +++) as a main finding were used (omitting – and + results). Samples with more than three bacteria were excluded. Only the first finding of a quarter with a particular bacterium was taken into account. Data consisted of 1528 quarters with samples from 17 AMS farms and 2757 quarters with samples from 32 farms with parlour milking. In total 2653 diagnosis were made and used in the analysis.

The herds were visited in winter 2012. Visits included a structured interview about management and grouping of cows, observations of the cows, and measures of the barn. Herd health recordings were provided by the Agricultural Data Processing Centre Ltd. Data were analyzed with linear regression model using Stata/MP 16.0.

Results: Data consisted of 11 different bacteria, including koagulase-negative staphylococcai (CNS), *Str. uberis, Str. dysgalactiae, S. aureus, E. coli, Trueperella pyogenes, Str. agalactiae, Corynebacterium bovis, Enterococcus spp., Klebsiella,* and *Serratia marcescens*, mentioned in the order of prevalence. CNS was found from 839 quarters (31,6%). From the quarters milked with AMS 43% of the quarters had CNS, compared to 25% of quarters milked in milking parlor. Streptococcai caused a problem in some farms: *Str. uberis* at farms with milking parlour (20 vs. 11%) and *Str. agalactiae* at farms with automatic milking (4 vs. 1%). Proportion of CNS correlated negatively with proportions of *T. pyogenes, E.coli, S. aureus, Str. uberis* and *Str. dysgalactiae*. According to a regression model for the proportion of CNS of all bacteria, increased proportion of CNS was associated with AMS and cleaner udder, and nearly associated with slatted floor (P=0,067) and softer bed (p=0,057). CNS were also associated with severe hock lesions, if milking system was left out of the model.

Conclusions: Automatic milking was associated with increased proportion of CNS IMI, compared to conventional milking, where bigger proportion of IMI seemed to be caused by major bacteria. However, more milk samples, considering herd size, were taken in AMS farms. CNS was associated with cleaner udders, softer beds and slatted floors, which could indicate that dirtier udders, solid floors and harder beds might harbor environmental and more pathogenic bacteria than CNS instead.

Keywords: Mastitis, intramammary infection, CNS, automatic milking.

UH-P34

Cross-linking and analysis of DHIA milk recording data with bacteriological milk results for implementation of information-based udder health management in dairy farms

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Objectives: Sustainable treatment of bovine mastitis and dry cow therapy requires additional information prior to drug administration in order to reduce antibiotics in dairy herd health management. Therapy plans should not only be based

on culturing results completed by resistance pattern. Moreover, additional information like DHI test data which must be cross-linked to lab data without excessive workload, should be considered. These complex data structures for systematic dairy udder health improvement was restricted to national projects so far.

Often somatic cell counts (SCC) from the last milk recording are considered whether blanket dry cow treatment (DCT) is provided or not. But single data sets are of reduced value for udder health estimation. Long-term analysis of SCC data combined with regular culturing of single cows can provide cow files and hints at prognostic evaluation for antimicrobial treatment.

In order to connect all available cow data to an informational file in a time saving manner, it must be stressed that IT solutions are needed for effective data handling, visualization, and decision making.

Materials and methods: The veterinarians of the private enterprise bovicare (Potsdam, Germany) has developed a couple of IT tools during the last years which are aiming on data connection of various sources (lab database, DHIA data, herd health management programs). These tools provide integrated herd and single cow data, visualize data to statistical tables and figures, and serve as support while giving advice to farmers and farm veterinarians. By using these overviews, the tools help saving time and clarify the high amount of aggregated data for the farmer for monitoring the herd, as well as figuring out sources for reduced udder health on farm level.

Results: The core tool is represented by a database storing all monthly milk recordings of the participating farms. These data can be downloaded to a frontend, are matched with all culturing data and automatically aggregated to herd lists and a summarizing report which can be commented by the bovicare vets, additionally. The frontend includes milk recording data over 12 month and lab data over 24 months, respectively.

Herd lists contain current DHIA data and current culturing data within the same row.

A second list contains cows showing remarkable SCC differences (threshold 100 cells per ml) compared to the previous month (new infection lactation, NIL) or to the last milk recording prior to dry off (new infection transit, NIT). Cows not cured during dry period (NCD) and new infections in heifers (NIH) are listed as well.

Prognostic evaluation especially for a sustainable DCT (chance for cure during dry period) is provided by a score visualized in a Traffic Light System with four phases. Each milk recording with SCC 200k per ml and more, and SCC 700k per ml and more, a cow's value increases by 1 and 2 points, respectively, always summarizing the last three recordings. State 'Green' is given in cows showing SCC <200k per ml during the last three milk recordings (0 points). Traffic Light 'Yellow', 'Orange' and 'Red' is represented by values of 1 to 2, 3 to 4, and 5 to 6, respectively. The prognostic cure chance remarkably decreases from 'Green' to 'Red'.

A list of complete culturing results in chronological order of all cows figures out the course of infection or cure over a long time.

Another tool analyses bacteriological and resistance pattern herd data to ensure overview over feasible antimicrobials by certain pathogens, as a basis for treatment protocols in clinical mastitis to fill the gap between occurrence of clinical cases and lab result.

Conclusion: The presented tools for advisors, farm veterinarians and farmers are suitable, to aggregate the pool of available information about udder health state of single cows and the complete herd. The package helps to refine advice in order to improve success of therapies of clinical mastitis and subclinical cases of contagious micro-organisms, as well. Furthermore, it supports decisions whether cows have to be dried off using antimicrobials or not. Long-term aspects of cow's health values help to provide cull lists containing chronically affected cows. All these data are in accordance to the worldwide growing demand of saving antibiotics in herd health management of dairy cows.

Keywords: Udder health, dry cow treatment, culturing, herd monitoring.

UH-P35

Milking routine and quality of chilled raw milk produced in Southwest Paraná

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The Southwest region of Paraná has a dairy basin with high production, but there are some difficulties related to management that may compromise the quality of the milk produced. The goal of the present work is to understand the milking routine and assess the quality of the milk produced in the Southwest Paraná region through the application of a questionnaire and laboratory analysis. Sixty-nine producers from six municipalities were interviewed and milk samples from the bulk cooler were collected. The samples were submitted to somatic cell count (SCC), total bacterial count (TBC), and microbiological examination. The questionnaire included questions about the milking routine adopted on each farm. The samples for SCC and TBC were collected in 50 mL bottles containing bronopol and azidiol, packed in an isothermal box and sent to the laboratory of the Paranaense Association of Holstein Breed Cattle Producers. Milk samples for microbiological examination were collected aseptically in sterile 4 mL bottles with a sterile pipette, packed in isothermic boxes, sent to the Qualileite laboratory (FMVZ/USP) and submitted to mass spectrometry test (MALDI-TOF). The data obtained was tabulated and statistically analyzed. According to the questionnaire, 82.6% (57/69) of the farmers washed the teats with water, 95.6% (66/69) did not use disposable gloves, 81,2% (59/69) did not use mug of black background for clinical mastitis diagnosis, 85.5% (59/69) did not use pre-dipping, 81.2% (56/69) used the same towel

on all animals and 73.9% (51/69) did not use post-dipping. Laboratory results of SCC showed that 47.8% of the properties presented values above 500,001 cells/mL and 65.2% presented TBC above 300,001 colony forming units/mL. In the bacteriological examination, eighteen genera of bacteria and eighty-one species were isolated. Bacteria of the genus *Macrococcus* spp. were highlighted (23.5% - 19/81). After that, *Staphylococcus* spp. (18.5% - 15/81), *Lactococcus* spp. (16% - 13/69), *Enterococcus* spp. (7,4% - 6/81) and *Acinetobacter* spp. (6,2% - 5/81) were isolated. With the results, it is possible to conclude that the region presents management problems during the milking line, because it has a deficiency in the quality of the milk produced. This information was confirmed by the values obtained in the SCC and TBC tests showing that the majority of producers do not meet the quality standards required by the normative instruction in force. In addition, we proved that the bacteria isolated from chilled raw milk are microorganisms present in the environment and/or udder of the animal and agents that cause contagious mastitis, easily reducible with correct management and hygienization. We emphasize the need to adopt quality technical assistance to guide producers on milking line management, in order to minimize SCC, TBC, and milk contamination.

Keywords: Mammary gland, questionnaire, milk quality, MALDI-TOF.

UH-P36

Embolic pneumonia associated with udder cleft dermatitis in dutch dairy cows

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Objectives: Udder cleft dermatitis (UCD; also *bovine ulcerative mammary dermatitis* or *foul udder*) is an inflammation of the udder skin and is most often located between the frontquarters and at the transition of the frontquarters and the abdominal wall. Cows with UCD may have increased risk of clinical mastitis, and associations between UCD and digital dermatitis have been suggested. The lesions can impair animal welfare, milk production, and milk quality and can lead to death and premature culling. Thus preventive measures are warranted, but in daily practice UCD is not always adequately detected by farmers and their cattle veterinarians. The objective of this study was to investigate the prevalence of cows with complicated UCD on postmortem examination, associated with embolic pneumonia as most likely cause of death.

Material and methods: GD Animal Health registered veterinary pathologists diagnosed 39 dairy cows from different Dutch dairy herds with (mild to severe) UCD from 1 January 2019 – 31 December 2019 based on postmortem external examination in the necropsy room for mammalian species. Histological examination and microscopy, as well as additional bacteriology testing was carried out in the GD Animal Health laboratory. The pathology department is part of the GD Animal Health laboratory, which is ISO 17025 accredited for a large number of laboratory tests and based on these accredited procedures the postmortem examinations were performed. Photographs were taken from the relevant lesions.

Results: From 20 of the 39 dairy cows (51%) with UCD on postmortem external examination in 2019 it is most likely that a complicated UCD was the fatal cause, due to the fact that no other causal factor for death was identified by the veterinary pathologists. Based on histology from the udder skin, a necrotic inflammation of the subcutaneous abdominal (milk) vein was observed with many bacteria involved, besides an ulcerative dermatitis of the skin. A comparable inflammation of the blood vessels in the lungs, with extension to the surrounding tissue, was observed, which is typical for embolic pneumonia. Bacteriological culture of the lung tissue revealed 7 times *Helcococcus* spp., twelve times *Trueperella pyogenes*, and in two cases a mixed infection of these bacteria was isolated. In some cases *E. coli*, streptococci, and/or anaerobic bacteria were cultured from the diseased lung tissue. All the isolated bacteria can be considered as opportunistic bacteria.

Interestingly, in all of these 20 cases, the clinical signs during life of these cows (that were reported by the veterinarian on the accompanying form) were fever, respiratory distress, milk drop and death, despite antimicrobial treatment. In only one case the history taken by the veterinarian reported UCD.

From 19 of the 39 dairy cows (49%) with UCD on postmortem external examination in 2019 passed the necropsy room with uncomplicated UCD. In these cows ulcerative dermatitis was described, although there was no related embolic pneumonia observed, but other most likely causes that lead to death of these cows.

Conclusion: 51% of the postmortem examination of cows resulted in the diagnosis of embolic pneumonia associated with UCD. In these cases, UCD lesions, which were apparently not important enough for the farmers and the veterinarians to report (or not even observed), are thought to have been the initiating source of infection. It is crucial that both farmers and veterinarians are made more aware of the possible severe consequences of udder cleft lesions and that they adopt strategies to allow early detection of UCD so that interventions can be initiated as early as possible.

Keywords: Udder Cleft Dermatitis, Embolic pneumonia, Postmortem examination.

UH-P37

Evaluation of milk cellularity for mastitis detection in primiparous and multiparous Lacaune ewes and distribution *Staphylococcus* non *aureus* in milk and environment by mass spectrometry

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Mastitis is inflammation of the mammary gland and has multiple etiologies. Its early diagnosis minimizes economic losses and improves animal welfare. *Staphylococcus* non *aureus* (SNA) microorganisms are the most pathogens associated with mastitis in small ruminants and their differentiation is important to evaluate the impact on the udder. The objective of the present study was to evaluate the accuracy of the California Mastitis Test (CMT), Differential Somatic Cell Count (DSCC) and Microscopic Somatic Cell Count (MSCC) tests for the diagnosis of subclinical mastitis in primiparous and multiparous Lacaune ewes, as well as to identify the distribution of *Staphylococcus* non *aureus* in the environment, teat apex and milk by mass spectrometry (MALDI-TOF). Twenty primiparous and 30 multiparous Lacaune ewes were used and milk collections were performed on the day of calving (M1), one day after calving (M2), 3 days (M3), 7 days (M4), 15 days (M5) and 30 days after calving (M6). The CMT was evaluated by the intensity of the reaction. In the DSCC the leukocytes were differentiated into neutrophils, macrophages, lymphocytes, basophils and eosinophils. The MSCC was performed according to the method proposed by Prescott & Breed (1910) and modified for colostrum samples, in M1 and M2, which were diluted in a 1:1 ratio with phosphate buffered saline solution. The leukocytes were counted in 100 fields differentiating polymorphonuclear and mononuclear leukocytes. Both slides, DSCC and MSCC, were stained with May Grunwald-Giensa. MALDI-TOF analyses were performed on Bruker Autoflex Smart Beam III equipment. The FlexControl 3.3 software was used to obtain the mass spectra acquired within a range (m / z) from 2,000 to 20,000. *Staphylococcus* non *aureus* were the most frequently isolated microorganisms. *S. simulans* showing the highest frequency of isolation in the environment (44.44%) and in multiparous milk (19.33%). *S. xylosus* was isolated in large quantities in the apex of the primiparous (45.6%) and multiparous (27.3%) teat, primiparous (25%) and multiparous (16%) milk and environment (16.4%), characterizing an environmental microorganism. *S. sciuri* was more frequent in the environment (20.77%) with little frequency in teats and milk. *S. saprophyticus* had a high frequency of isolation in the apex of the teat of primiparous (16.6%) and multiparous (20.8%) but without being isolated in milk and can be classified as associated with the apex of the teat. *S. equorum* and *S. chromogenes* were more frequently isolated in milk, 13.4% and 7.7% in milk of primiparous and 8% and 12.6% in milk of multiparous respectively. Multiparous ewes showed higher frequency and probability of isolation of pathogens than primiparous, especially in M1, M2 and M3. The MSCC showed moderate to high positive correlation with CMT (0.629 to 0.770) and low to moderate positive correlation with neutrophils (0.279 to 0.524). The multiparous ewes showed higher cell values of polymorphonuclear leukocytes, neutrophils and lymphocytes than primiparous ewes. The healthy animals, both primiparous and multiparous, had higher percentages of lymphocytes when compared to infected animals. MSCC proved to be the best indirect test with better predictive values (0.33 to 0.49) than CMT (0.25 to 0.33) along with neutrophil counts at the six collected moments. Thus we showed that Lacaune multiparous ewes have a higher incidence of mastitis during lactation than primiparous ewes. *Staphylococcus* non *aureus* were the microorganisms most frequently isolated from milk and are distributed in the apex of the teat and environment of the animals. The CMT did not prove to be a good indicator of inflammation during lactation and the MSCC proved to be a test with better predictive values. Lymphocytes predominated in healthy mammary glands in this study. The MSCC was the best indirect test for subclinical mastitis in Lacaune ewes.

Keywords: Milk, ewes, Maldi-Tof, diagnosis.

UH-P38

Performance study with VetoSlide: Comparison study between VetoSlide and Columbia agar plates with sheep blood

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Objective: The aim of this study is to compare the performance of the mastitis rapid test VetoSlide® to conventional Columbia agar plates with sheep blood used in the diagnosis of mastitis.

VetoSlide® is a culture system for the differential detection of Gram-positive and Gram-negative bacteria from milk samples. It consists of a two-sided carrier with a selective culture medium for Gram-positive pathogens (reddish) on one side and one for Gram-negative pathogens (greenish) on the other side. The VetoSlide is dipped into a freshly taken milk sample and then aerobically incubated for 18 - 24 hours (up to max. 48 hours) at 37°C.

Study concept: The object of investigation are 15 bacterial pathogens from the strain collection of the Clinic for Cloven-Hoofed-Animals (see Table 1), which have caused acute mastitis.

From each of these pathogens, a stock solution in Ringer's solution with a McFarland of 0.5 was prepared. The pathogen concentration was determined in a preliminary test using a dilution series and plating out the dilution stages on blood agar. From this, the necessary amount of stock solution was calculated, which had to be transferred to 30 mL of milk so that it contained 10^6 CFU/mL. Based on this working solution, a decadal dilution series was prepared in milk over a further 5 stages, so that in the end 30 mL of milk with a pathogen concentration of 10^6 - 10^1 CFU/mL was available from each pathogen. Condensed milk (4% fat) was used as the sample matrix, as it was similar to mastitis milk in terms of consistency and fat content.

Two VetoSlide sample carriers were inserted into each working solution. In addition, 10 µL were smeared on each of two Columbia agar plates. These were then incubated in an aerobic atmosphere at 35 - 37 °C for 18-24 hours. The visible colony growth on each agar surface was recorded. The plates were then incubated for a further 24 hours and the growth assessment was repeated. Growth was evaluated by a semi-quantitative determination of the live bacterial count: one colony corresponded to a concentration of 10^2 CFU/mL, 10 colonies to 10^3 CFU/mL, 100 colonies to 10^4 CFU/mL and more than 100 colonies to > 10^4 CFU/mL. For the evaluation

of the VetoSlide sample carrier, the measurand is the visible colony growth per dilution after maximum 48 hours incubation. The lowest pathogen concentration per species is determined as the detection limit. The semi-quantitative determination of the bacterial count via the smear of the dilution on the Columbia agar plates serves as a comparative value.

Results and evaluation: All pathogens under consideration can be reliably detected by smearing on Columbia agar plates with sheep blood. By counting the colonies on the blood agar plates the actual pathogen concentration could be checked. It agrees with the theoretical pathogen density. The pathogens were reliably detected on Columbia blood agar from pathogen concentrations of 10^1 to 10^4 CFU/mL after an incubation period of 24 hours. After prolonging the incubation period for another 24 hours, the detection limit for Trueperella pyogenes and Corynebacterium bovis dropped from 10^4 to 10^1 and 10^2 CFU/mL respectively.

Compared to Columbia blood agar, the VetoSlide often has the same or a lower detection limit. The individual detection limits can be found in Table 1. VetoSlide enables a reliable differentiation between Gram-positive and Gram-negative pathogens after 24 hours. Only Enterobacter cloacae showed growth on both sides of the carrier.

Keywords: Mastitis, Diagnostic, On-farm, bacteria.

UH-P39

Statistical analysis of 33 Dutch farms of several udder health KPIs and antibiotic usage before and after using a polyvalent mastitis vaccine

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Objectives: Mastitis is frequent and costly disease in dairy cattle. It has negative impact on animal welfare, milk quality as well increased use of antibiotics. The objective of this poster is to analyse the effect of polyvalent mastitis vaccine (STARTVAC®) against Staphylococcus aureus (S. aureus), non S. aureus Staphylococci (NAS), Escherichia coli (E. coli) and coliforms vaccination in commercial dairy farms in Netherlands.

Material and methods: 33 commercial dairy farms were vaccinated with polyvalent mastitis vaccine (STARTVAC®) for 12 months. Then two periods were compared (12 months before and 12 months after the vaccination) using 17 different Key Performance Indicators (KPIs): number of cows, age of cows, milk production, % of fat and protein, BSK (standardized milk production value for each cow to the 50th day of the third lactation period), bulk tank somatic cell count (bSCC), % cows with high SCC, % of new cows with high SCC, number of cows treated of mastitis, number of mastitis treatment, Defined Daily Dose for Animal (ADDD) at farm level, ADDD of mastitis injectors, ADDD for dry cow therapy, % of culled cows, number of dead cows and cell count of culled cows. Due to complexity and number of data a statistical model of paired t-test for normal distribution data and Wilcoxon paired test for non normal distribution data have been used.

Results: Statistical analysis revealed that polyvalent mastitis vaccine (STARTVAC®) had a positive effect in all measured KPIs. However significant improvements have been observed in increased milk production (BSK +2.79%), lower bSCC (-5.41%), lower % of cows with high SCC (-8.98%), lower % of new cows with high SCC (-12.58%), less cows treated with for mastitis (-33.52%), less mastitis treatments (-39.84%), lower ADDD at farm level (-17.63%), less ADDD mastitis injectors (-38.36%) and positive economical return in corelation with mastitis reduction +1816€.

Conclusions: Along with other management practices to mastitis, vaccination with polyvalent mastitis vaccine (STARTVAC®) in these farm is having a positive impact contributing to improved udder health, improved milk production and decrease of antibiotic use resulting in high return on investment (ROI) 3.09:1.

Keywords: Mastitis, vaccine.

UH-P40

Statistical analysis of 7 dairy farms with Automatic Milking System (AMS) in France before and after the use of polyvalent mastitis vaccine

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Objectives: AMS provide solid data related to milk quality thus the efficacy of a polyvalent vaccine against Staphylococcus aureus (Staph. aureus), non S. aureus Staphylococci (NAS), Escherichia coli (E. coli) and coliforms (STARTVAC®) 7 commercial dairy farms in France has been analysed.

Material and methods: AMS farms ensure automated and consistent milking routines especially within the same brand. 7 commercial dairy farms with 565 cows were vaccinated with polyvalent mastitis vaccine (STARTVAC®) for 12 months. According to the specific characteristics of each farm and under veterinary supervision, the protocol used was: 3 injections 3 weeks apart in lactating cows and dry cows, followed by a booster every 3 months. Then three periods were compared: period 0 (8 months before vaccination), period 1 (12 months within vaccination) and period 2 (6 months after the vaccination) using 4 major Key Performance Indicators (KPIs): bulk tank somatic cell count (bSCC), mastitis rates and treatments, milk production and % of discarded milk. Due to complexity and number of data a statistical model of…has been used. The calculation (ANOVA test) test was performed with farm as random effect and herd age as factor. Differences for milk production, discarded milk and somatic cell count were significant between the three periods.

Results: In period 0, the average individual cell count for all cows was 178,000 cells/ml and the average daily production per cow was 28.2 kg. In period 1, the average individual cell count was 167,000 cells/ml, with daily production of 29.3 kg per cow, and in period 2, it was 162,000 cells/ml and 29.9

kg per cow. Decrease in the average individual cell count over the first year continues throughout the next 6 months of the second year of vaccination. This decrease is correlated with an increase in average daily individual milk production. The quantity of discarded milk affects the amount of milk delivered. It represents 4.6% of the milk produced in period 0, it fell to 4.17% in period 1, then to 4.12 % in period 2. The mastitis rate in period 0 was 139%. In periods 1 and 2, it was 117% and 71%, respectively. There is a significant decrease in treatment rate over the first year of vaccination, which seems to accelerate during the first 6 months of the second year of vaccination.

Moreover two other parameters were monitored: the average number of lactations and the average age of the herd, which would potentially increase if the rate of culling due to mastitis decreases. The average number of lactations decreased continually over the first two periods, going from 2.440 to 2.429 lactations per cow, for this reason herd age was considered in the model. It was only from period 2 onwards that the trend began to reverse (2.431). Moreover, the average age of the herd followed the same trend (period 0: 4.56 years; period 1: 4.46 years; period 2: 4.49 years). We can assume that culling due to chronic mastitis continues during the first year of vaccination and then, once the herd is "clean" of chronic animals, we can appreciate the effect of vaccination helping to preserve healthy cows and therefore the ageing of the herd.

Conclusions: This study confirms the preventive value of mastitis vaccination as an aid to controlling cell count and reducing mastitis treatment. This study also aims to show farmers that preventive tools such as vaccination contribute positively to the economy of the farm by optimising the cows' productive capacities, limiting the amount of discarded milk and reducing the rate of culling due to mastitis. Last but not least vaccination with polyvalent mastitis vaccine (STARTVAC®) resulted with high return on investment (ROI) 5.4:1.

Keywords: Mastitis, vaccine, AMS.

UH-P41

Field experience: vaccination against *Streptococcus uberis* experience in a dairy farm in Italy

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Objectives: Holistic control programs are much needed to control mastitis in dairy herds, as mastitis is one of the most frequent and costly disease. Over the years, *Streptococcus uberis* (*Strep. uberis*) have been one of the most prevalent pathogens.

UBAC® (HIPRA) is a subunit vaccine indicated for active immunization of healthy cows and heifers to reduce the incidence of clinical intramammary *Strep. uberis* infections, to reduce the somatic cell count (SCC) in *Strep. uberis* positive quarter milk samples and to reduce milk production losses caused by *Strep. uberis* intramammary infections.

The study is prospective with an historical control. The individual productive parameters were recorded from each cow vaccinated. The historical control is constituted by the productive performance records of the cows before vaccination.

Material and Methods: The study was conducted in a commercial dairy farm of 210 lactating cows on average, situated in the Po Valley, in the Province of Parma in the Parmigiano Reggiano cheese production consortium. Due to the specific characteristics of the farm, the veterinarian team responsible agreed to vaccinate all animals in a blanket protocol including lactating and dry cows, and late gestation heifers with a booster every 6 months after the primary immunization. The vaccination started in July 2020. Clinical data, SCC from bulk tank (bSCC) and individual (iSSC), among other data were compiled in an Excel file (Microsoft®) for subsequent statistical analysis.

The etiological diagnosis of mastitis was confirmed with official analyses by the Zooprophylactic Institute of Lombardy and Emilia Romagna. Animals that were culled or died for reasons other than mastitis were excluded from the final analysis.

Results: The results obtained showed an improvement over the vaccination period, when compared to the previously annual homologous period (Table 1, showing main result in %).

Table 1: Main results of vaccination (%). LS: linear Score (LS), Infected animals (Inf). treatments (Treatm).

Milk prod	bSCC	iSCC	LS	Inf	Culled	Treatm
+4	-25,2	-22,2	-6,4	-9.8	-19 (7 cows saved)	-7.8

In the economic analysis, the vaccine showed a ROI: 15,35:1. Saving a total of €60.040,34/200 cows/year.

Conclusion: This study showed that the use of this subunit vaccine against *Strep. uberis* mastitis (UBAC®, HIPRA) induces production and health improvements that significantly increase the profitability of the farm when compared to the previous homologous period. These results showed that the use of this subunit mastitis vaccine is an important tool for holistic *Strep. uberis* control in dairy herds.

Keywords: Mastitis, treatment reduction, clinical, vaccination.

UH-P42

Case Report: Usage of subunit Streptococcus uberis vaccine on a commercial farm in Southern Germany

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Objectives: Mastitis control programs are frequently used in dairy production. Mastitis is still one of the most frequently occurring and costly diseases in modern dairy farms (Halasa

et al., 2007), with *Streptococcus uberis* (*Strep. uberis*) being one of the most prevalent causing agents (Bradley et al., 2007; Ruegg et al., 2015).

Mastitis vaccines are widely used in some countries, showing significant results in the reduction in the severity of clinical signs and duration of infection of environmental mastitis (Wilson et al., 2009). The goal of this study was to evaluate vaccination against *Strep uberis* mastitis (UBAC®, HIPRA).

Material & methods: The study was conducted in a commercial dairy farm of 180 lactating cows on average, situated in the southern of Germany. Due to the specific characteristics of the farm, the veterinarian team responsible agreed to the vaccination of all animals in a blanket protocol: primovaccination 3 times (4 weeks apart) including lactating and dry cows. A first booster was set approximately 5 months in a blanket way after primary immunization. Vaccination started in December 2019. Amount of antibiotic intramammary tubes, Somatic Cell Count (SCC) and other data were compiled in an Excel file (Microsoft®) for subsequent statistical analysis.

Mastitis diagnosis was confirmed by microbiology at an accredited lab.

Results: The results obtained showed a reduction in intramammary tubes used to treat mastitis compared to the previously annual homologous period (reduction of 40%). The number of used tubes was reduced from 1,032 to 618 tubes. As the average treatment was around 5 tubes per mastitis case before vaccination started, this meant a reduction of about 82 mastitis cases the year when vaccination was applied. With regard to the average bulk tank SCC, there was a reduction of around 30,000 SCC (323,000SCC/ml to 291,000SCC/ml).

In the economic analysis, the vaccine showed a gain of €2.15 on investment (€5,728 for the doses in the first year), assuming the reduction of 82 clinical mastitis cases and estimating a cost of €150 per case (€12,300 on reduction of clinical *Strep. uberis* cases). These results are only regarding the economic saving related to treatments, not counting the extra milk sold and extra milk produced.

Conclusions: This study showed that the use of this subunit vaccine for *Strep. uberis* mastitis (UBAC®, HIPRA) with the adapted protocol according veterinarian team reduced the incidence of clinical cases in the period following vaccination when compared to the previous homologous period. These results showed that the use of this subunit mastitis vaccine is an important additional tool for controlling *Strep. uberis* in dairy herds.

Keywords: Mastitis, treatment reduction, clinical, vaccination, uberis.

UH-P43

Case Report: Usage of subunit Streptococcus uberis vaccine on a commercial farm in the north of Germany

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Objectives: Mastitis control programs are frequently used in dairy production. Mastitis is still one of the most frequently occurring and costly diseases in modern dairy farms (Halasa et al., 2007), with *Streptococcus uberis* (*Strep. uberis*) being one of the most prevalent causing agents (Bradley et al., 2007; Ruegg et al., 2015).

Mastitis vaccines are widely used in some countries, showing significant results in the reduction in the severity of clinical signs and duration of infection of environmental mastitis (Wilson et al., 2009). The goal of this study was to evaluate vaccination against *Strep. uberis* mastitis (UBAC®, HIPRA).

Material and Methods: The study was conducted in a commercial dairy farm of 390 lactating cows on average, situated in the north of Germany. Due to the specific characteristics of the farm, the veterinarian team responsible agreed on the vaccination of all animals in a blanket protocol: primovaccination 3 times (4 weeks apart) including lactating and dry cows with a booster every 6 months in a blanket way after the primary immunization. Vaccination started in October 2019. Amount of antibiotic intramammary tubes, Somatic Cell Count (SCC) and other data were compiled in an Excel file (Microsoft®) for subsequent analysis.

Mastitis diagnosis was confirmed before the start of vaccination, with 49% of mastitis cases caused by *Strep. uberis.*

Results: The results obtained showed a reduction in antibiotic intramammary tubes compared to the previous annual homologous period (reduction of 27,8%). The number of tubes used was reduced from 4,008 to 2,894 tubes. As the normal treatment was around 8 tubes per mastitis case, this means a reduction of around 139 mastitis cases. With the average bulk tank SCC (bSCC), there was a reduction of about 70,000 SCC (from 314,000 SCC/ml to 246,000 SCC/ml).

In the economic analysis, the vaccine showed a gain of €1.74 on investment (€12,000 for the doses in first year), assuming the reduction of 139 clinical mastitis cases and estimating a cost of €150 per case (€20,887 on reduction of clinical *Strep. uberis* cases). These results only consider the economic saving related to treatments, not the increase in milk sold and produced.

Conclusion: This study showed that the use of this subunit vaccine for *Strep. uberis* mastitis (UBAC®, HIPRA) with the adapted protocol according veterinarian team reduced the incidence of clinical cases in the period following vaccination when compared to the previous homologous period. These results showed that the use of this subunit mastitis vaccine is an important additional tool for *Strep. uberis* control in dairy herds. And showed its profitability.

Keywords: Mastitis, treatment reduction, clinical, vaccination, uberis.

UH-P44

Regular milking within natural suckling behavior interval minimizes differential somatic cell count in dairy cows milked by automatic milking systems

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Objectives: Somatic cell count (SCC) is a reliable and approved parameter for the estimation of udder health. The main cell types regarding somatic cells in the udder are lymphocytes, macrophages, and polymorph nuclear leucocytes (PMN). The differential somatic cell count (DSCC) represents the proportion of lymphocytes and PMN to total SCC, the remaining percentages to SCC are macrophages. So far, the effects of milk yield, season, parity, milking frequency, days in milk, and major pathogens on the DSCC are already described. A further known effect on udder health and SCC is the milking interval (MI). On farms with automatic milking systems (AMS) the MI can vary for each cow compared to conventional milking systems. Regarding DSCC and SCC, cows milked by AMS systems showed higher values compared to cows milked by conventional milking systems. Therefore, the aim of this study was to evaluate the effect of MI on DSCC.

Materials and methods: Data from 27 dairy herds from Germany including 6,500 dairy cows and 43,229 recordings were evaluated. The data resulting from milk yield performance testing were collected between January to December 2020. All herds used automatic milking systems combined with free cow traffic. Milk yield performance testing data was recorded 11-times per year on each farm and included the DSCC measured using the FOSS method described by Damm et al. (2017). Date and time from each milking at the farms were available and used to calculate each individual MI between milkings. MI ranged from 1 h minimum to 24 h maximum. Data of milking interval >24 h were excluded. Means were compared using Wilcoxon test. P-values were Bonferroni adjusted; the threshold for significance was set after adjusting to $\alpha < 0.05$. A linear mixed model was used to estimate the effect on DSCC including MI, milk yield, lactation, days in milk, and season as fixed effects and herd, individual cow, and residuals as random effects.

Results: Mean MI was 10.6 h (±0.04 h SE). MI of ≤4 h resulted in the highest DSCC (52.3 ±1.0%). The DSCC decreased significantly for cows showing a MI >4 and ≤6 h (39.0 ±0.6%) and had its minimum between MI >6 and ≤8 h (37.9 ±0.4%). MI between >8 - ≤10 h resulted in a DSCC of 40.5% (±0.4%). The DSCC increased for MI >10 - ≤12 h and for >12 h MI (42.8 ±0.4%; 46.6 ±0.3%, respectively; all P-values < 0.001). Therefore, the most frequently milked cows showed higher DSCC compared to cows between 4 and 8 hours MI. Considering natural behavior, the suckling interval of calves from their mothers ranges between 4 to 6 times per day, which results in a MI of 6 to 4 hours, representing the MI of the second lowest DSCC found in this dataset.

The standard deviation of the MI (MI_{SD}) expresses the irregularity of milkings. Data evaluation showed the lower the MI_{SD}, the lower the DSCC. For MI_{SD} ≤2 h the DSCC was lowest (38.8 ±0.7%), compared to MI_{SD} >2 - ≤4 h (41.0 ±0.5%), MI_{SD} >4 - ≤6 h (43.2 ±0.7%), and MI_{SD} >6 h (48.1 ±1.1%). Irregular milking is also known to impair udder health and increase the SCC of cows.

Conclusions: Milking interval between 4 to 8 hours minimizes DSCC, which aims the natural MI of suckling calves. A more regular milking interval in AMS farms could reduce DSCC and therefore improve udder health. AMS farms should strive their management and settings of the AMS to encourage cows to visit the AMS more regularly.

Acknowledgement: We kindly acknowledge the QNET-ICS GmbH, Erfurt, Germany, for providing the dataset of DSCC values and milk yield recording data for this study.

Keywords: Aiming natural suckling behavior interval and regular milking minimize differential somatic cell count in dairy cows in automatic milking systems.

UH-P45

Reduction of SCC, new IMI, CM and Milk Production in Heifers with and without the use of an Internal Teat Sealant

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Objectives: Intramammary infections (IMI) are common in dairy heifers, and these can negatively affect future milk production. Bismuth subnitrate-based internal teat sealants (ITS) have been used to prevent new IMIs in heifers and dairy cows by creating a physical barrier within the teat that prevents pathogens from entering the gland. Several studies show that the use of ITSs in heifers 35 to 75 days before calving reduces new IMI, somatic cell counts (SCC), and the risk of clinical mastitis (CM) in future lactation. The aim of this work was to establish the reduction of new IMI, SCC, CM, and milk production in heifers with and without the use of a new ITS.

Material & Methods: The study was conducted in a commercial farm in the province of Buenos Aires, Argentina, with 700 Holstein cows. Forty-four heifers (176 quarters) were selected for the study. Half of them (n=22 /88 quarters) were given a new ITS 35 to 45 days before calving (treated group=TG). The rest of the heifers (n=22/ 88 quarters) did not receive any treatment (control group =CG). The ITS used in the study was **Cloxacum Sell®** (4g) (Laboratory Calier of Argentina, S.A.), which contains 65% of bismuth subnitrate, nisin and natamycin as bactericide and antifungal, respectively. The new ITS also has a dye that allows a better visualization of its presence and elimination at the time of calving. The udders of the selected heifers presented correct implantation and conformation, the teats did not present lesions and their conformation was normal. After 7 to 10 days after calving, milk samples were taken for isolation and identification of mastitis pathogens, following the indications of the NMC (National Mastitis Council, 2017 a - b). At 45 days postpartum, SCC was performed using electronic equipment. Cases of clinical mastitis were recorded, and milk samples were taken for bacteriological analysis. On the results obtained, an analysis of variance (ANOVA, for a $P \leq 0.05$) was performed. In this model, the effect of treatment on the response variable with and without the use of ITS was studied. The test unit was heifers for SCC, CM and milk production, and quarters for isolation of mastitis pathogens.

Results: The mean of the SCC, at 45 days postpartum,

were 144,864 cells/mL for the TG and 280,455 cells/mL for the CG. On the other hand, heifers with linear score (LS) greater than 4, were in TG 18.19% (4/22), while for CG, these values were 59.09% (13/22), with significant differences for SCC between TG vs. CG (P=0.001015). There were no cases of CM in the first 30 days postpartum in the TG, while in the CG, 2 episodes of CM were recorded (2/22) which corresponds to 9.09 % in heifers and to 3.41 % of the quarters (3/88). Regarding milk production, the mean lbs/day of TG vs. CG was 74, 91 lbs/day and 69, 05 lbs/day, respectively, no significant differences were found between the groups (P= 0.142838). The % of quarters with positive isolation for TG vs. CG, was 9.09 % (5/88) and 23.86% (21/88), respectively. The predominant microbiota in the isolates of both groups were CNS, Corynebacterium spp., yeasts and Bacillus spp. On the other hand, the ITS plug was present until first milking after calving in 87.5% (77/88) of the quarters.

Conclusions: Based on the results obtained, the use in heifers of the new ITS *Cloxacum sell*®, reduced NIIM and SCC.

Keywords: Internal Teat Sealant, SCC, Heifers.

UH-P46

Retained placenta as a potential reservoir of milk pathogens in dairy cows

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Objectives: This study aimed to evaluate the presence of the main milk pathogens in dairy cows with retained placenta.

Materials and methods: Uterine fluid samples were taken from 15 cows presenting retained placenta between 24 and 48 h after calving (12 dairy farms in Porto region, Portugal, 2021). Each cow was simultaneously sampled for conventional bacterial culture and RT-PCR: *Staphylococcus* spp. (including coagulase-negative staphylococci and blaZ gene), *Staphylococcus aureus*, *Staphylococcus* ß lactamase, *Streptococcus uberis*, *Streptococcus dysgalatiae*, *Streptococcus agalatiae*, *Escherichia coli*, *Enterococcus* spp., *Klebsiella oxytoca*/*Klebsiella pneumoniae*, *Serratia marcescens*, *Mycoplasma* spp., *Mycoplasma bovis*, *Corynebacterium bovis*, *Arcanobacterium pyogenes*/*Peptoniphilus indolicus*, Prototeca and Fungi). Cows were classified according to the parity (primiparous vs. multiparous) and absence or presence of fever (rectal temperature ≥39.5 ºC and toxemia signs).

Results: Despite fungi (92.9%; 95% interval confidence: 59.0,99.2%), *E. coli* (93.3%; 95% IC: 70.2,98.9%), *Staphylococcus* spp. (93.3%; 95% IC: 70.2,98.9%), *A. pyogenes*/*P. indolicus* (80.0%; 95% IC: 54.8,93.0%), *Streptococcus uberis* (78.6%; 95% IC: 52.4,92.4%) and *Streptococcus dysgalatiae* 57.1% (95% IC: 32.6,78.6%) were observed in more than half of the cows using RT-PCR methodology. *Mycoplasma* spp. was more likely to found in multiparous (66.7%) than primiparous (33.3%; p<0.05) cows. Fever was more frequently detected in cows presenting *Strep. uberis* in uterine fluid (63.6 vs. 33.4%; n=14, one sample was inconclusive; p<0.05). The blaZ gene was identified in 28.6% (95% IC: 11.7,54.7%). Except for E. coli (Cohen's kappa coefficient = 0.64), the agreement between RT-PCR and bacterial culture was slight or fair for *Strep. dysgalatiae* (κ=0.11), Enterococcus spp. (κ=0.15) and *Strep. uberis* (κ=0.34). No significant associations (P>0.05) between parity or fever and the presence of milk pathogens were observed according to the conventional bacterial culture method.

Conclusions: These findings suggest that the uterine secretions from cows with retained placenta can be a relevant contamination source of milk pathogens in the environment and udder skin surface. Specific care on cows with retained placenta should be addressed in udder health management programs of dairy herds.

Keywords: Mastitis pathogens, reservoir, postpartum cows.

UH-P47

Microorganisms and risk factors associated with bovine mastitis in Andalusia, Spain

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Objectives: Mastitis in dairy cattle is a globally widespread infectious disease that is responsible for large economic losses for the dairy industry due to lower milk yield and quality. In Andalusia, Córdoba is the main province dedicated to dairy cattle, gathering around 60% of dairy cows census in the last years.

The objectives of this work were to identify the microorganisms isolated from clinical and subclinical mastitis milk samples from cattle from Córdoba (Andalusia, Spain) and to evaluate their correlation with the environmental and management parameters in the farm of origin.

Materials and methods: A total of 477 bovine clinical and subclinical mastitis milk samples belonging to 103 farms (Valle de los Pedroches region, Córdoba, Andalusia, Spain) were taken and analysed between April 2020 and February 2021.

From theses samples, 484 isolates were obtained and identified by automatized VITEK® system. Once identified, isolates were classified into 8 groups (*Enterobacteriaceae*, gram negative non-*enterobacteriaceae*, streptococci, staphylococci, enterococci, corynebacteria, yeast and other microorganisms not included in the previous categories).

At the same time, an epidemiological survey was conduct-

ed in all farms to assess the exposure of affected animals to environmental and management risk factors, including bovine farm size; type, material and m^2 of bed; supplements added to the bed; bed plowing; rotavapor; bedding frequency; total replacement of the bed; use of separator and selective dry cow therapy.

Results: Isolates were identified and taxonomically classified as: streptococci (34%), *Enterobacteriaceae* (22%), enterococci (14%), staphylococci (12%), corynebacteria (6%), yeasts (3%), gram negative non-enterobacteriaceae (2%) and other microorganisms (7%). *Escherichia coli* (18.59%), *Streptococcus uberis* (18.38%) and *Streptococcus dysgalactiae* (12.19%) were the species most frequently identified. Among species included in the "other microorganisms" group, it highlights *Aerococcus viridans* (2.69%) and *Lactococus lactis* (2.06%), species that can be mistakenly identified without specific techniques and then being erroneously included in *Streptococcus spp* or *Enterococcus spp* group.

After analysing the epidemiological data, the following risk factors were detected as significant ($p \leq 0.05$) regarding some of the groups mentioned above: bovine farms with more than 100 animals (bovine farm size) and bed plowing are related to the isolation of *Enterobacteriaceae*; and complete replacement of the bed twice a year, to the isolation of enterococci. At the same time, protective factors resulted: the use or manure bed and bed plowing are associated with the isolation of group of other microorganisms; and biweekly or monthly frequency of bedding, complete annual replacement of the bed and plowing, with isolation of streptococci. Selective dry cow therapy was not identified as a risk or protective factor against the isolation of any microbial group.

Conclusions: The main microbial groups isolated from clinical and subclinical bovine mastitis milk samples from farms in Córdoba (Andalusia, Spain) were streptococci (*S. uberis*, *S. dysgalactiae*), *Enterobacteriaceae* (*E. coli*) and enterococci.

Management factors related to the number of animals (farm size), bedding frequency and replacement or bed plowing had a statistically significant impact on the isolation of some microbial groups.

Keywords: Mastitis, risk factors, microorganims, streptococci.

UH-P48

Microbial group classification by NIRS analysis of milk for the appropriate choice of bovine mastitis antibiotic treatment

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Objectives: Bovine mastitis is a multifactorial process considered the most prevalent infectious disease in dairy cattle, being responsible of huge economic losses in dairy sector. A correct diagnosis of the responsible agent of bovine mastitis becomes essential nowadays, as it determines the subsequent treatment. In general, the use of antibiotics is only recommended in those mastitis cases where Gram-positive microorganisms are involved. The current diagnostic methods are mainly based on microbiological culture, which requires specific laboratory expendables, trained personnel, and 24-48 hours to obtain results.

In order to reduce the use of antibiotics and antimicrobial resistance, the current regulation promotes the employment of rapid diagnostic tests to identify microorganisms involved in bovine mastitis using antibiotics only when necessary, avoiding it prophylactic and unjustified use. Against this background, the objective of the present study was to assess milk analysis through near infrared spectroscopy (NIRS) as a rapid and reliable methodology to classify bovine mastitis according to the microbial group involved to choose an appropriate treatment.

Materials & methods: 57 bovine milk samples without macroscopic alteration coming from animals with mastitis were studied. The microorganism species was identified through culture and VITEK® system, distinguishing 3 categories: Gram positive (G+), Gram negative (G-) and samples without growth (SC). Milk samples were oven-dried using DESIR method and were analysed using a NIRS system FOSS-NIRSystems 6500. A discriminant model was designed with software WINISI IV to classify milk samples according to the spectral information, using Discriminate by Maximum X-Residuals algorithm.

Results: The resulting model achieved a classification success of 91,2%. While all G- and SC samples (12 and 20, respectively) were correctly classified, 2 and 3 out of 25 G+ samples were misclassified in G- and SC groups, respectively. The misclassification of samples may be related to the involved microorganism variability, since G+ group includes a high number of gender and species, giving rise to spectral differences which difficult the model classification.

Conclusions: This preliminary study highlights NIRS technology capacity to achieve a rapid and simple classification of the microbial group involved in bovine mastitis and so to choose an appropriate treatment.

Keywords: NIRS, Mastitis, antibiotics.

UH-P49

A bulk-tank somatic-cell count below 200,000 indicates less burden with mastitis pathogens in the herd

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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) <50,000. Up to now, there is no scientific support that this low bulk tank threshold is indicative of better udder health in individual cows. The objective of this study was to show that a bulk tank SCC of <200,000 is an indicator for better udder health and milk quality in the herd, compared to a higher SCC.

Materials and methods: Five consecutive samplings were performed in four-month intervals on eight Austrian dairy farms. The farms were grouped into low SCC farms (LSCC; >200,000 cells/ml in <10% of tests, n=4) and high SCC farms (HSCC; >200,000 cells/ml in >10% of the tests, n=4), according to their bulk-tank SCC during the observation period. None of the herds ever exceeded the 400,000 cells threshold for shipment. Milk samples were collected along the milking process (16 composite-milk samples (CM), milk filter (MF), separator milk (SE), bulk-tank milk (TAM)). Milk samples were analyzed for mastitis-causing pathogens using PathoProof Complete-16 Mastitis qPCR Assays (Thermo Fischer Scientific, AT), and the results were expressed as bacterial cell equivalent (BCE) per mL. The CM and TAM were analyzed for SCC and TAM for aerobic mesophilic count (AMC) per mL. Statistical analysis was performed using SPSS (v 27), with LSCC and HSCC as fixed effects and the BCE of the targeted pathogens and AMC as dependent variables for the entire dataset and grouped for sample type. Correlations between SCC and BCE and AMC were analyzed using the Spearman correlation coefficient.

Results: HSCC had higher AMC in the bulk tank than LSCC (23.000 vs. 14.000/mL; *P*<0.001), with a low positive correlation between bulk-tank SCC and AMC (r=0.3, *P*<0.001). The major mastitis pathogens *Trueperella pyogenes* and *Staphylococcus aureus* were only detected in HSCC (in 1.6 and 1.3% of the samples; *P*<0.05). The major pathogens *Escherichia coli*, *Streptococcus dysgalactiae, and Streptococcus uberis* were higher in total and in the CM in HSCC compared to LSCC (*P*<0.01). The minor pathogenic coagulase-negative Staphylococci (CNS) were higher in total and for all sample types in HSCC (*P*<0.01). *Enterococcus* sp. was similar in total, in MF, SE, and TAM for LSCC and HSCC (*P*=0.66) but was higher in HSCC in CM (*P*<0.01). For all mentioned pathogens, the percentage of positive samples was higher in HSCC. The BCE for the minor pathogen *Corynebacterium bovis* was higher in total and for all sample types in LSCC herds (*P*<0.038) but had 36.8% positive samples, less than HSCC (75.3%). The Staphylococcal *blaZ* gene was higher in HSCC in the CM samples (*P*<0.005), with 18.2% of the CNS positive samples being positive for *blaZ*, and 13.4% in LSCC. The correlation analysis with SCC/mL revealed only a low positive correlation in the CM and TAM for *C. bovis* (r=0.3; r=0.36) and *S. uberis* (r=0.33; r=0.43) and moderate positive correlation in the CM for CNS (r=0.57) (*P*<0.02).

Conclusion: The study showed that a bulk-tank SCC threshold of <200,000 indicates less occurrence of both major and minor mastitis pathogens on associated farms. A lower burden with mastitis pathogens suggests fewer disease cases, reflecting higher cow health and welfare on these farms. Farms with a lower SCC also had lower AMC in the bulk-tank milk, indicating better milking hygiene and raw milk quality. Potential human pathogens such as *E. coli* and *S. aureus* were detected in lower numbers in low SCC herds, which implies a higher level of food safety. The lower abundance of *blaZ* suggests a lower risk for antibiotic resistance in LSCC herds, which is important in the One Health concept. The use of a lower SCC threshold for best-grade milk is supported by these data in terms of cow health and food safety.

Keywords: Major mastitis pathogens, minor mastitis pathogens, blaZ gene, qPCR.

UH-P50

Comparison of physical properties of 6 different teat sealants on the US market

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Objectives: Internal teat sealants are being increasingly used to prevent new intramammary infections in the dry period of dairy cows and in the last years, many new products have been introduced on the market. Although all of them contain 4 grams of ointment with 2.4 - 2.6g (60 - 65%) of bismuth subnitrate, different formulations and physical properties may lead to differences in convenience of application and risk for iatrogenic new infections. The objective of the study was to compare the practical physical properties of 6 different brands of teat sealants available currently on the US market.

Materials and methods: Six teat sealants of different brands were compared using 2 or 3 batches for each product, evaluating each batch 3 times: Boviblock® (2 batches, n=6, MWI Animal Health), Mastishield® (2 batches, n=6, Aspen Veterinary Resources), U-Seal® (3 batches, n=9, Durvet Animal Health Products), Lockout® (3 batches, n=9, Boehringer Ingelheim), Orbeseal® (3 batches, n=9, Zoetis) and Shutout® (3 batches, n=9, Merck Animal Health, Madison, NJ, USA).

The study was carried out at Merck Animal Health's, Madison, NJ, USA R&D laboratory facility using a gliding force apparatus to measure the force required to empty the tube when the plunger was pushed at a constant speed. Syringeability was defined as the amount of force, in Newtons (N), needed to push the plunger to the end-position. Plunger stroke distance was defined as the maximum distance, in millimeters (mm), done when pushing the plunger. Product viscosity, expressed in Pascal-seconds (Pa·s), is related to the force required to push the plunger and was measured using a rheometer.

In addition, the amount of air in the syringe was judged by stratifying in 3 relative categories: large, medium or small. Furthermore, the following parameters were assessed: presence of dual cap (yes/no), and if yes, effort of breakable cap removal (easy/difficult) and evenness of breakable cap edge (smooth/rough).

Results: Average viscosity (in Pa·s) was for Boviblock: 360 ± 6; Mastishield: 392 ± 23; U-Seal: 1217 ± 375; Lockout: 575 ± 125; Orbeseal: 434 ± 329; Shutout: 446 ± 65. Average syringeability (in N) was for Boviblock: 23,1; Mastishield: 22,2; U-Seal: 49,3; Lockout: 35,8; Orbeseal: 22,2; Shutout: 29,0. Average plunger stroke distance (in mm) and amount of air in syringe category was for Boviblock: 30,1, medium; Mastish-

ield: 30,0, medium; U-Seal: 19,7, small; Lockout: 17,9, small: Orbeseal: 48,2, large; Shutout: 23,2, small. Availability of the dual tip option, effort of breakable cap removal and evenness of the cap edge after breaking was for Boviblock: yes, difficult, rough; Mastishield: yes, difficult, rough; U-Seal: no, N/A, N/A; Lockout: no, N/A, N/A; Orbeseal: no, N/A, N/A; Shutout: yes, easy, smooth.

Conclusions: The 6 different brands of teat sealants varied substantially in their convenience of application and physical properties. Out of evaluated teat sealant tubes, Boviblock, Mastishield, and Shout combined favorably easy syringeability with the presence of dual cap option allowing for partial insertion. On the other hand, U-Seal, Lockout and Shutout tubes offered decreased plunger stroke distance and reduced air pockets within the syringe. The short tip was easy to break and resulted in smooth edge of the tip only in case of Shoutout.

Convenience of application is mainly determined by viscosity of the content, syringeability, plunger stroke distance and presence of air in the tube and can be important in large herds where large amounts of tubes need to be applied in a short period. To what extend application convenience influences the risk for new intramammary infection is not known and should be a focus of further research.

In contrast, partial insertion of tubes has been shown to reduce new infection rate likely by avoiding damage to the keratin plug and should be considered as a requisite for the use of teat sealants. However, for successful partial insertion, the short tip should be easy to break and have a smooth surface to minimize contamination of the tip.

Keywords: Teat sealant, udder health, diary cows.

UH-P51

Economic impact of subclinical mastitis on milk performances of dairy cows in Northwestern Spain

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Objectives: The objective of this study was to process data from a sample of a dairy herd improvement (DHI) organisation of Galicia (Spain) in order to detect economic differences between milk production tests of healthy cows (<200 x somatic cell count) compared to the milk production tests of the population (all data) and cows with milk production test during subclinical mastitis (>200 x somatic cell count [SCC]).

Material & Methods: The mathematical representation of milk production against time represents one of the most successful applications of mathematical modelling. Parametric models are those that found large applications to fit the lactation curves. Wood's model was the most popular parametric model. Peak yield and persistency describe the shape of the curve. The cost of milk production depends largely on lactation persistency. The unexpected drop in production after the peak increases the cost of production.

This study tends to measure the effect of somatic cell count on the shape of the lactation curve and the estimation of the classical parameters of the curve by comparing the data sets on a 305-day lactation basis according to three groups of data coming from the period: '01-01-2019' to '01-01-2022':

- Group 1: Tests from the whole population (1273139 data – 100 %),
- Group 2: Tests from cows with SCC< 200.000 (990307 data – 77.78 %),
- Group 3: Tests from cows with SCC>200.000 (282832 data – 22.22 %).

We´ve used the Wood model (Wood, 1967 – Nature, Vol 216, October 14 1967) to draw the lactation curve on every group and the following parameters of every lactation curve for the comparison:

1. Production at peak (L),
2. Persistency beyond the peak (%),
3. Accumulated production over standard 305-day lactation (L),
4. Average daily milk yield (L/day).

Results: The results are described as follows:

1. Group 1 (total of the population): Produced 10637 L (34.88 L/day) in a 305-day lactation with a monthly persistency of 3.84% having a peak on week 9.
2. Group 2 (healthier cows): Produced 10784 L (35.36 L/day) in a 305-day lactation with a monthly persistency of 3.52% having a peak on week 9.
3. Group 3 (subclinical mastitis): Produced 10123 L (33.19 L/day) in a 305-day lactation with a monthly persistency of 4.52% having a peak on week 10.

Statistically, we have analyzed the regression of the logarithm of the day and the logarithm of the milk test in both groups to be able to compare the parameters obtained. The parameters (a+bx+cx1) have a P value of 0 in both case (Highly significant), and they never overlap taking into consideration the lower and upper limits of the 95% confidence interval which means both distributions are clearly different.

Conclusion:

- There is a clear difference in litres produced per 305 days lactation of cows with subclinical mastitis compared to the total population:
  - -514 L between Total population and >200 x SCC which represents -1.69 L/cow/day
  - 0.68% worse persistency for cows with >200x SCC
  - Also, there is a difference of 1 week on the peak of lactation.
- There is a clear difference in litres produced per 305 days lactation of healthier cows (<200x SCC) compared to the total population:
  - +147 L between Total population and <200 x SCC

which represents +0.48 L/cow/day
    - 0.32% better persistency for cows with <200x SCC
    - There is no difference in the peak of lactation (same week).
- There is a clear difference in litres produced per 305 days lactation of healthier cows (<200x SCC) compared to cows with subclinical mastitis:
    - +661 L between >200x SCC and <200 x SCC which represents +2.17 L/cow/day
    - +1% better persistency for cows with <200x SCC
    - Also there is a difference of 1 week on the peak of lactation
- Comparaison of lactation curves drawn by the parametric model of Wood can measure the differences between groups of animals quantifying economic differences.
- Mastitis causes a lot of economic losses that can be measured as drug expenses or milk withdrawn from sales but the hidden cost caused by a lack of production can be measured in a group of animals and customized in a particular farm (with a particular lactation curve shape and percentage of animals with mastitis).

Keywords: Subclinical mastitis, milk yield, economics.

UH-P52

Effect of duration of intramammary treatment with amoxicillin clavulanic acid on cure rate of bovine clinical mastitis

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Objectives: Mastitis is the most common reason for use of antimicrobials in dairy cows. Reported cure rates of treatment differ between studies and countries depending on the severity of the disease, the antimicrobial substance and route of administration used, duration of the course, and characteristics of the causing pathogen. In addition, management factors and immune response of the cow affect the treatment response. For these reasons it is necessary to study the effect of mastitis treatments using different protocols and in different countries. The aim of our study was to explore cure rate of a commonly used amoxicillin-clavulanic acid intramammary treatment in clinical bovine mastitis in Uruguay. We compared cure rates after treatment with 3 or 8 intramammary tubes administered at a 12 hour interval.

Material and methods: Dairy cows with clinical mastitis from 10 commercial farms were enrolled in the study. All farms milked twice a day. Cows were randomly allocated into treatment groups (A and B) based on the cow's ID number (even and uneven). Group A was treated with three intramammary tubes of amoxicillin-clavulanic acid (Clavamox® Zoetis) at 12 hour intervals (short treatment as per manufacturer's recommendation) and group B with 8 intramammary tubes of the same product at 12 hour intervals (extended treatment). The history of the cow was recorded and an initial milk sample taken from the mastitic quarter before treatment. Two follow-up milk samples were collected three and four weeks post treatment. The treated quarter was defined as cured if both follow-up samples were bacteriologically negative or the species isolated differed from that in the initial milk sample. The final study group consisted of 171 cows and 174 quarters with mastitis.

The majority of the cows were from one herd (n=114), the other nine herds enrolled from one to 31 cows. 96 % of the cows were Holstein or crossbred Holstein (n=160). The parity of the cows was divided as follows: 9.7% first parity, 29.1% second parity, 29.1% third and 32.1% fourth or more parity. Information of parity was missing in 6 cows. Median of days in milk (DIM) on the first day of treatment was 63.5 DIM. The data of DIM was missing from 15 cows.

The chi-squared test was used to determine whether there was a difference between the short and the extended treatment.

Results: The most common bacterial species isolated in the treated quarter was *Streptococcus uberis* (74/174; 42.5%). No statistical difference between cure rates of treatments with different duration was found (p=0.96). With short treatment (group A), 65.9% (58/88) and with extended treatment (group B), 66.3% (57/86) of the quarters were cured, the overall cure rate being 66.1%. The clinical treatment failures, i.e. cases in which the treatment had to be changed, occurred in both groups: in group A 9.1% (8/88) of the quarters and in group B 7.0% (6/86) of the quarters. A new infection was detected in 23.9% (21/88) and in 23.3% (20/86) of the quarters in group A and B, respectively.

Conclusions: No difference between the different treatment lengths was detected. It can be concluded that in conditions such as in the present study it is not necessary to extend treatment of mastitis to longer than 3 intramammary tubes at 12 hour intervals. As almost half of the quarters were infected with *Str. uberis*, our results mainly apply to this pathogen. In Uruguay no narrow spectrum beta-lactam intramammary products are available and we had to use this broad spectrum product. A narrow spectrum betalactam would be appropriate for most pathogens isolated in the study.

Keywords: mastitis treatment, amoxicillin clavulanic acid, antibiotics.

UH-P53

Prevention of naturally occurring intramammary infections by application of a new teat disinfectant after milking - a randomised non-inferiority trial

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Objectives: The aim of this study was to conduct a positive controlled field study under natural exposure conditions to test the efficacy of a newly developed chlorine dioxide-based disinfectant (EX) for non-inferiority compared to an already established chlorine dioxide-based teat disinfectant (PC)). The newly developed EX product features longer visible marking of the dipped teats, easier handling and lower drip losses compared to established products with the same disinfection mechanisms. Thus, it is sufficient to mix the EX product from the two components every 14 days rather than daily. In *in-vitro* tests (required by the Italian Ministry of Health/ in the European Union) it has been shown that by applying the EX product, a killing of the original bacterial count is achieved at a level of 5 logarithmic levels for Staphylococcus aureus, Streptococcus uberis and Escherichia coli (EN 1656), and more than 4 logarithmic levels for Candida albicans (EN 1657). This is true on day 1 after activation as well as 1 week and 2 weeks later.

Material & Methods: The primary outcome variable of the preliminary data analysis was the frequency of new intramammary infections. Furthermore, practical aspects of the application and the effect of the treatment on the condition of the teats were determined. About 200 Holstein cows in early to mid-lactation stages from a dairy farm near Padua, Italy were randomly assigned to one of two groups after blocking by parity. Over a 13-week period between September and December 2021, the two groups were treated with either the experimental product (EX) or the positive control product (PC) after each milking.

All individual quarters of the experimental cows were sampled weekly to determine infection status. Teat condition was assessed in trial week 1, 5 and 9. Mixed logistic regression was used to analyze the effect of treatment on the incidence of new glandular quarter infections. For the non-inferiority analysis, the upper limit of the 95 % confidence interval for the difference in new infection rate between the two treatments (EX - PC) had to be to the left of the critical value d (0.035) to conclude that EX was non-inferior to PC in terms of the risk of new infections.

Results: The results showed that the incidence of new infections in the quarters treated with EX (1.0%) was not different from that in the udder quarters treated with PC (1.0%). No overall difference was found between the treatments in terms of teat condition either. Since the upper limit of the 95 % confidence interval of the new IMI rate difference was smaller than the predefined non-inferiority limit, it was concluded that the experimental product was non-inferior compared to the positive control.

Conclusion: Thus, the chlorine dioxide-based teat disinfectant investigated in this study can be considered an effective post-milking teat disinfectant and safe, as the product did not negatively affect skin condition.

Keywords: Mastitis, new intramammary infection, teat disinfectant, post dip, non-inferiority.

UH-P54

Estimation of the economic impact of different dry-cow therapy protocols

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Intramammary treatments are responsible for more than 50% of antimicrobial use in dairy production, 50% of which are used for dry-off treatment. Compared to blanket treatment, selective dry-cow therapy (SDCT) is a method to significantly reduce the antimicrobial use at dry-off. The main objective of our study is to evaluate the optimal SDCT alternatives via a multicriteria approach including farmer's income, workload, and antimicrobial use on farm for different udder health profile. The main assumption is that SDCT is economically advantageous compared to blanket therapy except in situations of poor udder health (high prevalence of intramammary infections (IMI)).

A stochastic simulation model of dairy farming (DairyHealthSim©) was used to simulate herd dynamics, reproduction management, production, antibiotic consumption, culling decisions, and health event management. The simulation model was coupled to an economic optimization framework. The result is a bioeconomic model with a holistic approach to dairy farming.

A specific module (DHS-Mastitis) for the simulation of clinical and subclinical mastitis and different SDCT alternatives has been developed. IMI are simulated for each quarter using baseline risks for each etiology (*S. aureus*, *S. uberis*, *E. coli*, *Klebsiella* spp., non-aureus staphyloccoci and "strep-like" pathogens). Risk of each of these etiologies is associated with udder health management practices in the barn and during milking and associated to SDCT at dry off. For contagious mastitis, the calculated risk considers contamination between quarters of a cow and between cows in the herd.

Each infected quarter transits between different states: subclinical, clinical (mild, moderate, or severe). Each status determines the effects on the quantity and quality of milk produced by the quarter, the conception rate of the infected cow and the treatment to be applied by the farmer or veterinarian. A chronic status is given to quarters that have been infected for at least six weeks. A quarter with a chronic infection has a lower cure rate. Different scenarios for the baseline infectious risk were defined, they reflect the global cleanliness and care taken to prevent infections in the farm (time spent, material expenditures and infection relative risks). It resulted in five different scenarios for the risks due to premises and five for those due to the milking procedure. Twenty-five SDCT protocol scenarios were also defined, which will allow to compare the economic profitability of different SCC thresholds allowing the selection of animals to be treated (from 50,000 to 200,000 c/mL), the use of bacteriology alone or simultaneously with the use of SCCs as well as the use of internal teat sealants or not. In total, 625 scenarios were simulated and coupled with an economic model to define the optimal strategy considering the resource constraints of the farm.

Preliminary results show a higher profitability of SDCT

compared to blanket therapy except in case of poor udder health where IMI are not under control. Since no additional economic cost is associated to SCC use, it appeared to be the most cost-effective method for SDCT, followed by combined methods and finally bacteriology alone. Within a given baseline risk scenario, bacteriology-based methods resulted in a greater reduction in clinical mastitis incidence, quarters' infections duration and antimicrobial consumption compared to SCC alone.

Analyses are ongoing to evaluate the effect of scenarios on animals' welfare and to evaluate an economic scenario of subsidy for bacteriology analyses on farm.

Keywords: Dry-cow therapy, mastitis, health economy.

UH-P60

Selective dry-cow therapy as a tool to reduce antimicrobial usage in dairy cows in Italian herds

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Objectives: The application of selective dry cow therapy (SDCT) is one of the measures suggested to reduce the use of antibiotics. The aim of the study was to verify the application of a standardized protocol for SDCT and to assess the cure rate (CR) and new infection rate (NIR) and to identify risk factors affecting both CR and NIR.

Material & Methods: The study considered 516 dairy cows (2064 quarters) from five dairy herds. These cows were treated with antimicrobials when SCC was >100.000 cells/ml at the end of first lactation and >200.000 cells/ml when they are pluriparous. These thresholds were defined by our previous published study on over 45.000 records.

Quarter milk samples were collected 2 times within 10 d before drying-off (DO) and 2 time within 15 days after calving (AC). On all these samples were bacteriologically analysed and bacteria recovered were identified by Vitek™ system. Two different antimicrobial treatment were applied (cefalonium 250 mg. Cepravin, MSD, Italy -A and cefalexin 504.7 mg Rilexine HL, Virbac Italy- B) in cows with SCC over the specific thresholds. Statistical analysis was performed by the appropriate procedure of SPSS 26 (IBM, USA).

A true IMI (TIMI) was defined when a quarter has a major pathogens IMI in one of the two duplicate samplings (before DO or AC) or it has the same bacteria in both duplicate samples. A latent IMI (LIMI) was defined when only one of the duplicate samples (before DO or AC) was positive for a minor pathogen. CR was defined based on the absence of IMI or the presence of an IMI with a different etiological agent after calving, compared to LIMI and TIMI at DO. NIR was defines when negative quarters at DO, showed an IMI after calving, or when a LIMI caused by different bacteria was observed AC, in LIMI quarters at DO.

Results: The results of bacteriological analyses showed as 999 quarters (48.4%) were bacteriological negative at both samples taken before drying-off. 531 positive quarters (25.7%) harboured a TIMI, 506 (24.5%) a LIMI and 28 (1.4%) were blind. Since the protocol is based only on SCC before DO, both healthy and infected cows were included in the three group of treatment (negative, A and B).

Based on the health status at DO and by treatments the results showed as among negative cows the NIR was in the range 28.3-53.4% with a significant lower rate for untreated animals and a significant higher rate for cows receiving treatment A. Among true IMI the no cure rate was in the range 25.5-35.1% without significant differences among treatments.

The results of logistic analysis on 5 risk factors (parity, drying-off length, teat sealant application, health status at drying-off and treatment) showed as cows with 2 parities have a lower probability to be cured (O.R. 0.716) when compared with primiparous cows as well as drying-off period shorter than 45 d was associated to a significant decrease in cure rate (O.R. 0.590) when compared with drying-off of 46-75 d. Teat sealant application was significantly associated to a higher cure rate (O.R. 3.3). Heath status and treatment were not significantly associated to cure rate.

When risk factors for NIR were considered cows with ≥3 parities showed a significant lower risk of new IMI, when compared to primiparous ones. Drying-off length shorter or longer than 46-75 d were associated to a higher NIR, as well as the absence of teat sealant application. Both the presence of a TIMI or LIMI at drying off were associated to an increase of NIR (odds ratio respectively of 5.8 and 4.0). The treatment A was significantly associated to a higher frequency of new IMI when compared to no treatment, while this latter one was not different from treatment B in terms of preventive efficacy.

Conclusion - SDCT showed to be applicable without significant negative effects on health status of cows. However, some of the factors considered showed to be significantly associated to CR and NIR. Particularly, a significant positive effect was observed for primiparous cows, teat sealant application, drying-off length 46-75d, healthy status at drying-off and type of antimicrobial treatment. These risk factors should be considered to apply an efficient monitoring protocol after calving.

Keywords: Mastitis, dry-cow, selective therapy, antimicrobials.

UH-P61

Differential cell count in individual cow milk as a tool to investigate udder health status

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Objective: The progressive decrease of mean SCC in dairy herds worldwide is affecting SCC accuracy as a subclinical mastitis marker. This evidence supports studies aiming to apply differential cell count (DSCC) as a tool to identify mastitis. Recently availability of high-throughput milk analysers, able to perform a partial DSCC on milk, allowed designing studies under field conditions involving large sample sizes, and aiming to define health status and mastitis risks in individual milk samples. We report the results of two studies performed aiming a) to define the threshold levels to predict cow health status in Italian dairy herds and b) to investigate DSCC as potential marker for an early detection of intramammary infections (IMI)and for changes of milk secretion in cows with <50.000 cells/ml.

Material & Methods: Milk tests were performed by certified methods currently applied Regional Breeders Association of Lombardy (ARAL). Milk analyses were carried out on Fossomatic 7C (Foss A/S, Hillerød, DK). The instrument assesses, SCC, DCC, proportion (%) of protein, fat, lactose and casein and other parameters not considered in this study. Cellular data (DSCC) are expressed as proportion (%) of PMNs and LYM in the sample, whereas its complement to 100% represent MAC proportion. Individual milk samples were analysed using Mastit M4BDF kit (DNA Diagnostic A/S, Risskov, DK). Statistical analysis was performed by the appropriate procedure of SPSS 26 (IBM, USA).

Results: The first study considered 4,386 milk test records from four dairy herds with different size, management and milking management. DSCC data were analysed by ROC procedure. This procedure allows identifying the threshold giving the highest accuracy and the highest combined value for sensitivity and specificity, among all the possible thresholds. Among the different ways used to classify milk samples, the analysis applied to days in milk (DIM) showed the highest mean values for sensitivity plus specificity, and the value for accuracy (AUC) was the highest observed. The thresholds identified were 66.3% (AUC 0.907), 69.2% (AUC=0.919) and 69.3% (AUC 0.822) respectively for cows with 100 DIM, 102-200 DIM and >200 DIM. These thresholds are now currently applied nationwide by Italian Breeder Association. Moreover, these thresholds (THs), when combined with SCC in the same sample showed allowed us to define four different classes: cows not at risk (SCC<200.000 cells/ml and DSCC< THs), cows with subclinical mastitis (SCC >200.000 cells/ml and DSCC>THs), cows at risk for subclinical mastitis (SCC<200.000 cells/ml and DSCC>THs) and cows at risk to be chronic (SCC>200.000 cells/ml and DSCC<THs).

The second study was focused on samples <50.000 cells/ml, values that are not usually reported by the instrument. We were able to retrieve, thanks to the cooperation with the producer of the instrument (Foss A/S, Hillerød, DK), and to design a study aimed to evaluate DSCC as potential marker for an early detection of intramammary infections (IMI) and changes of milk secretion. Overall, 3,022 cow milk test records performed in 2018 from 24 dairy herds located in Lombardy region were considered, 901 of them have SCC ≤50,000 cells/ml, representing our final study sample. Overall, 20.75% of the samples (187) were positive at qPCR. However, the health status did not show any significant effects on DSCC variability. Indeed, mean values for the different pathogens were the following: healthy cows 49.61±0.80%, CNS 48.95±1.46, and major pathogens 48.50±1.47. Whereas, the analysis of the influence of DSCC on milk fat, protein and casein showed a significant decrease of the three components as DSCC increase, whereas there was a significant increase of lactose proportion as DSCC increased. Indeed, samples with DSCC> 60.9% showed a significant decrease in fat% (4.16%), protein% (3.39%), casein% (2.60%) and a significant increase in lactose % (4.86%) when compared with samples with lower DSCC proportion. Therefore, DSCC in very low SCC cows may be suggested as a marker to identity early stage of inflammation and/or changes in milk composition, as a result of alteration in milk secretion mechanism.

Conclusions: The availability of this new high-throughput milk analyzer opens the way to a series of new studies aiming to improve the performances of standardized and automatized milk analyses. This will improve the quality and usefulness of information supplied to dairy farmers, and it will help to increase the levels of herd management and efficiency.

Keywords: Somatic cell count. differential somatic cell count, subclinical mastitis, milk composition.

UH-P62

Cross-sectional study of subclinical mastitis in intensively-reared dairy ewes and determination of ewe-related risk factors

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Objective: The objective of the study was twofold; (i) to estimate the prevalence of subclinical mastitis of intensively reared dairy ewes, at early lactation, along with the cumulative incidence and incidence rate, throughout the milking period and (ii) to investigate which groups of ewes are most at-risk.

Material and methods: The study was carried out in four commercial farms of purebred Greek Chios dairy sheep. A total of six hundred and nine (609) ewes in their 1st or 2nd lactation were used. Test-day milk yield was recorded for each ewe and individual milk samples were collected in monthly intervals for the first five months of the milking period. Each sample was subjected to cytological examination using California Mastitis Test (CMT). Milk samples were also analyzed to determine somatic cell count (SCC) and total viable count (TVC). Incidence of clinical mastitis was also recorded at the same time. Samples with both SCC ≥ 500,000/ml and CMT ≥ 2 were set as a threshold indicating a higher risk of subclinical mastitis; samples from ewes with clinical signs of mastitis were rejected. To investigate the presence of underlying pathogens, milk samples matching the latter criteria were subjected to bacteriological examination. Data analyses were performed using R version 3.5.1 and statistical package "lme4". Generalised mixed linear models based on the maximum likelihood were built to assess the fixed effects of test-day milk yield, age of ewe and lactation stage on the status of being at a higher risk

of subclinical mastitis. The random effects of ewe and farm were also estimated. For the analyses, test-day milk yield records were distributed into four levels and lactation stage was represented by months in milking.

Results: Prevalence of subclinical mastitis at early lactation was 14.8% for the total population, while within farm prevalence ranged from 2.6% to 25.3%. Cumulative incidence and incidence rate throughout the milking period were 13.5% and 0.071 cases/ewe/month, respectively. Within farm ranges of cumulative incidence and incidence rate were 5.8 - 20.5% and 0.03 – 0.15 cases/ewe/month, respectively. The total number of records subjected to data analyses amounted to 1,815; a total of 293 (16.1%) of which, were at a higher risk of subclinical mastitis. Of all milk samples that were subjected to bacteriological examination, 59.9% was found positive. In most of the cases (68.6%) Coagulase-Negative Staphylococci were isolated. The second most common species of bacteria was *Staphylococcus aureus* (20.0%). In lower frequencies Streptococcus spp. (7.6%) and Pasteurella spp. (3.8%) were also isolated. The status of being at a higher risk of subclinical mastitis was significantly affected by all of the parameters tested (P<0.05). Specifically, increase of age by one year rendered ewes 1.9 times more prone to subclinical mastitis (P<0.05). Furthermore, ewes producing less than 1.4 kilograms of milk daily were 2.4 to 4.6 times more likely to develop the condition compared to high-yielders (P<0.05). A small effect of lactation stage was also observed; in the first month of the milking period (right after weaning), ewes were 3.4 times more susceptible to subclinical mastitis compared to the fifth month (P<0.05).

Conclusions: The results revealed high prevalence, cumulative incidence and incidence rate of subclinical mastitis for Chios ewes. Older ewes with low milk yield and ewes in the first month post weaning were more prone to subclinical mastitis.

Keywords: dairy sheep, subclinical mastitis, risk factors, Chios breed.

UH-P67

Combating multidrug-resistant *Staphylococcus aureus* with extracts of *Eucalyptus globulus* and *Calotropis procera,* and their role in modulation of beta lactam drug resistance

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This study was carried out to determine the efficacy of *Eucalyptus globulus* and *Calotropis procera* against multidrug-resistant *Staphylococcus aureus* (MDRSA) and in modulating beta lactam drug resistance. Aqueous and ethanolic extracts of leaves of both plants were tested by well diffusion and broth dilution method against MDRSA isolated from mastitic camel milk. Effectiveness of cefotaxime and ampicillin was tested alone and in combination with several concentrations of the plant extracts. Treatment with ethanolic extracts of both plants produced significantly ($p < 0.05$) higher zones of inhibition than did aqueous extracts. The ethanolic extracts combined with cefotaxime showed significant differences ($p < 0.05$) from the combination of cefotaxime with aqueous extracts of each plant. Similar response was noted in case of ampicillin combination with ethanolic and aqueous extract of each plant. The modulation factor for both drugs combined with all concentrations of ethanolic extracts of both plants was less than 0.5, indicating strong synergy. The minimum inhibitory concentration (MIC) of the ethanolic extract was significantly lower than that in aqueous extracts of either plant ($p < 0.05$). it was concluded that MIC of ampicillin was reduced when combined with ethanolic extracts of *C. procera* at higher tested concentrations and with all ethanolic concentrations of *E. globulus*. The modulation factor was >2, indicating a synergistic response.

Keywords: Drug resistance, Staphylococcus aureus, E. globulus, C. procera, beta lactam.

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