

## AH-01

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

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

**Keywords:** *Neospora caninum*, abortion, cattle, outbreak.

## AH-02

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

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**Objectives:** Infectious disease represents one of the biggest problems facing dairy producers today. This is largely due to the high economic costs associated with disease outbreaks. A prompt and accurate diagnosis is important to minimise the

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

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

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

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



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

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

### AH-03

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

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

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

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

ITIH4 assay showed linearity until 4 mg/mL. Limit of detection was established in 0,003 mg/mL. No hook effect was

observed with ITIH4 concentration up to 9 mg/mL. Coefficients of variation were always below 4% for within-run and between day determinations. There was no interference by the presence of hemoglobin (20 g/L), triglycerides (10g/L) or bilirubin (150 mg/L).

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

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

### AH-04

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

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

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

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

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

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

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

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

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

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

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**Keywords:** Dairy calf mortality.

## AH-05

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

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

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

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

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



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

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

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

#### AH-06

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

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

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

The median (range) reduction of IgA concentrations was

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

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

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

#### AH-07

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

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

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



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

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

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

**Keywords:** Calves, Weaning, ruminal fluid pH.

## AH-08

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

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

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

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



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

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

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

## AH-09

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

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

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

Holstein cows between 4 and 30 DIM were flagged by the system if they had 3 consecutive milkings with a fat:prot ratio>1.4 and then confirmed by testing a venous blood sample for BHBA (beta-hydroxybutyrate acid) with a cow-side device (Precision Xtra™, Abbott, US). Cows were assigned to one of

four treatment groups, stratified by lactation group (1<sup>st</sup> lact and 2+ lact). Treatment groups were (A) 2 L of red wine the first day followed by 1 L/day for 2 days, (B) 300 mL/day of propylene glycol for 3 days, (C) 2 L of ethanol the first day, followed by 1 L/day for 2 days and (D) on-farm drench protocol that provided 300 ml of propylene glycol in 20 L of water with a proprietary blend of electrolytes. A follow-up blood sample was tested the day after the last treatment (day 4).

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

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

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

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

## AH-10

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

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

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



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

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

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

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

**Keywords:** Beef, Calves, Calf health, FPT.

## AH-11

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

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

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

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

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



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

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

#### AH-12

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

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

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

**Materials and methods:** The sale data were retrieved from the general database managed by Québec beef producers (Producteurs bovins du Québec) from the 6 auction markets in activity during the study period. Specific data analysed were: the distance traveled from then farm of origin to the auction market, the season, the year, the breed (Holstein, cross-beef or non-Holstein dairy), gender, and calves' weight.

The dependent variable was defined as a calf sold <0.1 CAD/lb (0.22 CAD/kg) which represented 30 024 calves (2.06% of the dataset) during the study period. Multivariable logistic regression models were developed using relevant interactions between independent variables as previously studied in beef feedlot calves.

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

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

**Keywords:** Dairy, veal, calves, multivariable.

#### AH-13

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

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

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



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

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

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

**Keywords:** bacterial therapeutics, cattle, respiratory microbiota, tulathromycin, bovine respiratory disease.

#### AH-14

### Prepartum magnesium butyrate supplementation improves the health and performance of dairy cows

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**Objectives:** In the dry period, the size of the rumen papillae decrease by more than 50%. After provision of a high energy lactation ration, it takes 50-60 days for the rumen papillae to fully redevelop. As a consequence, during early lactation the ruminal absorption of volatile fatty acids (VFA) and nutrients is limited. Rumen papillae redevelopment is stimulated by VFA, with butyrate being the most effective due to its ability to increase cell proliferation and to inhibit cell apoptosis. Butyrate also increases epithelial blood flow, magnesium absorption and VFA transport in the rumen wall. Furthermore, relative to parturition, butyrate supplementation has been demonstrated to improve colostrum composition in pigs. We hypothesized that dietary magnesium butyrate supplementation during the peripartum period of dairy cows would increase colostrum quality and improve the health and performance of dairy cows.

**Materials & Methods:** Two hundred-and-nineteen healthy multiparous Holstein-Friesian cows were blocked by expected calving date and then randomly assigned to treatment and control groups. For the cows in the treatment group (n=108), magnesium butyrate (Rumen-Ready®, Palital Feed Additives B.V.) was supplemented to the pre-calving total mixed ration (TMR) during the three-week close-up period. This resulted in

cows receiving 150 g of Rumen-Ready® per day until calving. Rumen-Ready® contains 70% magnesium butyrate encapsulated in a fat matrix, and the magnesium butyrate is completely released and solubilized within the rumen. The pre-calving TMR of the control group (n=111) had no supplementation. At partum, cows were monitored in terms of calving ease, colostrum quality and quantity. The daily milk yield of the cows up to 70 days postpartum was recorded. Body condition and lameness of the cows were scored weekly during the trial. Fertility-related parameters of the cows were assessed: days to first heat, services per conception and late embryonic/early fetal mortality rate. The health status of the cows was determined until 70 days postpartum by a trained veterinarian who was blinded to the treatment groups. Significant differences, relative to the control group, were declared at  $P < 0.05$ .

**Results:** Magnesium butyrate supplementation was associated with a significant decrease in the calving assistance rate and incidence of retained placenta. Colostrum volume and the total yield of IgG, protein and lactose were significantly higher in the treatment group. Supplemented cows had significantly higher milk yield during the first three weeks of lactation, and a significantly higher body condition score 3-9 weeks after calving. In terms of reproductive performance, the number of services per conception, the late embryonic/early fetal mortality rate and the number of days to first heat were significantly decreased by magnesium butyrate supplementation.

**Conclusion:** Supplementation of magnesium butyrate, in the form of Rumen-Ready®, significantly improved the colostrum, calving ease, early lactation performance, body condition, health and fertility of dairy cows. These wide-ranging benefits are underpinned by the positive stimulation of the rumen during the close-up period, and the provision of a readily available source of magnesium.

**Keywords:** Lactation, dry period, rumen, fertility, colostrum.

#### AH-16

### Identification of potential predictive biomarkers and molecular mechanisms contributing to BRD-associated mortality in post-weaned beef cattle

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**Objectives:** Bovine respiratory disease (BRD) is a multifactorial disease complex possibly exacerbated by inflammatory responses to pathogenic agents. Diagnosis is commonly based on non-specific clinical signs, which lack sensitivity.



Elucidating early genomic mechanisms involved in BRD could lead to improved diagnostics. Moreover, early identification of cattle at increased risk for death might support interventions that decrease mortality. Our objective was to determine whether whole blood transcriptomes of post-weaned beef cattle identify predictive biomarkers and biological processes associated with BRD mortality.

**Materials and Methods:** Blood was collected at arrival from six cattle that ultimately developed BRD within the following 14 days. Replicates were placed into two cohorts based on BRD-attributed mortality (n=3 ALIVE; n=3 DEAD). Blood RNA was sequenced into 80 million 150bp paired-end reads per sample via an Illumina HiSeq 3000. RNA-seq was performed with a HISAT2/Stringtie/edgeR pipeline, utilizing the ARS-UCD1.2 assembly for alignment. Differentially expressed genes (DEGs) identified between groups (FDR <0.10) were modeled in WebGestalt, Reactome, GLAD4U, and STRING to identify GO-biological process (GO-BP) terms, pathways, disease phenotypes, and predicted interactions.

**Results:** 69 DEGs were identified between cohorts. GO-BP terms and pathways indicated increased expression of type I interferon-associated and antiviral genes in DEAD (FDR<0.05). Disease phenotyping of DEGs increased in DEAD indicated antiviral mechanisms (FDR<0.05). STRING analysis of DEGs predicted co-expression of interferon-associated gene products (CI>0.900).

**Conclusions:** RNA-seq and downstream analysis of whole blood collected at arrival demonstrated significant increases in type I interferon-associated genes in animals that succumbed to BRD, indicating that pro-inflammatory mechanisms at arrival are associated with BRD outcomes.

**Keywords:** Bovine, blood, interferon, transcriptome, gene expression.

## AH-17

### Effect of live yeast supplementation on growth and health of pre-weaned dairy calves

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**Objective:** High growth rates of calves during pre-weaning period are associated with better performance later in life. However, this period is also characterized by high morbidity and mortality. Hence, the objective here was to investigate the effect of an oral probiotic supplement containing the live yeast strain *Saccharomyces cerevisiae boulardii* CNCM I-1079, on performance and health of pre-weaned dairy calves.

**Materials and methods:** Forty-one Holstein calves born from late September to mid-November 2019, in a medium-size (390 milking cows) dairy farm in Greece were enrolled in the study. All calves received the colostrum from the dam within

2-4 h of birth and transitioned to milk replacer (MR) at day 3 of age. A blood sample was collected at 2-3 days of age to assess the transfer of passive immunity using a Brix refractometer. Calves were randomly assigned in two experimental groups, designated as Test (T) and Control (C), stratified by dam's parity, gender and serum Brix (%) value. Calves were individually housed in elevated stalls and were offered daily 7L of MR, divided in 2 equal meals, in buckets, at a dilution rate of 0.125kg /L, providing 4.6 Mcal metabolizable energy (ME) / kg of dry matter (DM) (21.5% crude protein and 18% fat). They had *ad-libitum* access to water and were offered pelleted starter (6.72 Mcal ME/kg DM) after week 1 and chopped alfalfa hay after week 6 of age. T calves received 5g of live yeast at a dosage of  $2 \times 10^9$  cfu/day, diluted in the MR for the whole experiment. Weaning was done on week 9 of age (day 60) with a gradual reduction of MR volume the previous 2 weeks. Calves' bodyweight (BW) was measured with a portable livestock scale at birth and week 9 (weaning). Average daily gain (ADG) was calculated for the whole nursery period. Starter and hay refusals were weighted weekly. Feed efficiency (FE), as total ME intake divided by BW gain, was calculated. Respiratory and fecal scores (RS and FS respectively) were assessed weekly by the same evaluator. Univariate regressions were performed to assess the effect of treatment on ADG and FE. The effect of treatment on pelleted starter and hay intake were assessed with linear mixed models. Repeated measures logistic regressions were used to assess RS and FS, using binary variables (0: scores 0 and 1; 1: scores 2 and 3). Statistical analysis was performed using IBM SPSS v.25.

**Results:** The initial BW (mean  $\pm$ SD) was  $41.2 \pm 3.9$  kg and  $41.9 \pm 4.0$  kg for T and C calves, respectively ( $P=0.797$ ), while mean BW at weaning was  $64.3 \pm 15.3$  kg and  $61.0 \pm 7.0$  kg for T and C calves, respectively ( $P=0.103$ ). One T calf and one C calf died from acute bloat and pneumonia, respectively, and their data regarding ADG and FE were excluded from the analysis. Three out of 21 C calves (vs. 0/20 T calves) were decided by the farmer to be weaned 2 weeks later than scheduled due to poor performance. High overall morbidity was recorded during the study. Ninety percent of T calves and 95% of C calves were at least once assigned a FS 2 or 3, while 80% of T calves and 67% of C calves were at least once assigned a RS 2 or 3. Week-to-week starter and hay intakes did not differ between groups. On average, total starter and hay consumptions were 23.0 kg ( $\pm 10.2$ ) and 1.9 kg ( $\pm 0.7$ ), respectively for C calves, and 25.7 kg ( $\pm 8.8$ ) and 1.7 kg ( $\pm 0.7$ ), respectively for T ones. T calves tended to have higher ADG than C ones (estimated marginal means: 0.361 kg/d vs. 0.299 kg/d, respectively;  $P=0.066$ ). T calves had a numerically better overall FE of about 0.9 Mcal ME/kg BW gain compared to C ones. The FS was not affected by the treatment while the T calves had about 1.3 more respiratory afflictions than controls ( $P=0.048$ ) during the experiment.

**Conclusions:** Pre-weaned calves fed MR supplemented with *Saccharomyces cerevisiae boulardii* CNCM I-1079 tended to grow with higher rates than control calves despite the higher respiratory afflictions compared to untreated ones. This was more evident during the second month of life, probably due to better nutrient digestibility and better feed efficiency utilization.

**Keywords:** Probiotic, milk replacer, calf, growth, health.

## AH-18

**Impacts of bronchopneumonia in dairy calves: results from a systematic review and meta-analysis**

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**Objective:** Bronchopneumonia (BP), also known as enzootic calf pneumonia, is one of the most common disease in dairy calves. Surprisingly, impacts of BP on health and performance of dairy calves have not been recently reviewed and summarized. This information is nevertheless crucial to increase awareness of BP's significance and thus increase adoption of management practices known to reduce BP prevalence.

The objective of this work was to quantify impacts of BP occurring during the first 12 months of life in dairy calves on subsequent risk of mortality and average daily gain (ADG).

**Materials and methods:** A systematic review and meta-analysis of the scientific literature was performed following PRISMA guidelines. PubMed and CAB Abstract databases were screened for relevant studies that were published in English (until Feb 3<sup>rd</sup>, 2020). Only studies reporting naturally occurring BP cases from birth to 12 months of age in dairy calves in Europe and North America were considered eligible. Both observational studies and randomized controlled trials were included. Random effect meta-analysis models were used to quantify impact of BP on outcomes of interest.

**Results:** From an initial pool of 525 studies, 13 studies reported impact of BP on growth and 10 studies reported mortality effect. In 1 and 3 studies, respectively a numeric effect could not be included in the meta-analyses. Case definition for BP differed among studies. Odds of mortality were 2.80 higher (95% CI: 1.42-5.51; n = 9 studies) for calves with BP. Average daily gain was decreased by 67g/day (95% CI: 34-99g/day; n = 10 studies) in BP vs apparently healthy calves. Heterogeneities of the models were important but was improved when accounting for adjusted (multivariable) vs non-adjusted (raw) estimates and prevalence of disease in the study.

**Conclusions:** To the authors' best knowledge, this systematic review and meta-analysis summarized for the first time impacts of BP on mortality and ADG. Calves with BP had on average 2.8 more risk of dying and grown  $\approx$  70 g/day less than apparently healthy herd mate.

**Keywords:** Meta-analysis, bronchopneumonia, calves, dairy.

## AH-19

**Feeding pre-weaned calves with waste milk containing antibiotic residues is related to a higher incidence of diarrhea and alterations in the fecal microbiota**

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**Objectives:** Waste milk (WM) is a major by-product of the dairy industry that includes low-quality colostrum, transitional milk, milk from cows administered veterinary drugs for the treatment of mastitis and/or other diseases, milk with high somatic cell counts (SCC), and milk that cannot be marketed. The large amount of discarded WM generates environmental pollution and represents the loss of a valuable resource that may serve as a good feed source for dairy calves because of its high nutrient content. However, using WM for feeding calves might expose new-born calves to infectious agents. Moreover, residual antimicrobials may have a negative influence on the animals' gut microbiota in terms of selection of antimicrobial-resistant microorganisms as well as in terms of interference with its proper maturation, by acting on specific taxonomic groups or by inducing long-term dysbiosis. The constant antibiotic pressure exerted on the calf microbiota by these residues may interfere with its physiological development by selectively inhibiting specific phylogenetic subgroups and increasing the selection and transfer of antibiotic resistance genes to the gut microbiota. Therefore, despite the apparent economic advantages for the farmer, WM might impair the correct physiological and immunological functionality of the calf gut and favor the selection of antibiotic resistance traits. We assessed the impact of WM on the correct development of the calf intestinal microbiome by analyzing the effect on intestinal health and on the fecal microbiota of calves fed with WM in the first weeks of life.

**Materials and methods:** WM was assessed for nutritional content and antimicrobial residue concentration by mass spectrometry. The trial, carried out over 8 weeks, included 12 Italian Friesian male calves. After 3 days of colostrum, 6 calves were fed Waste Milk (WM group) and 6 calves were fed Bulk Tank Milk (BM group) for 2 weeks. Then, for 6 weeks, all 12 calves received a weaning diet of milk substitute and starter feed. Every week for the first 2 weeks, and every 2 weeks for the remaining 6, we subjected all calves to clinical examination and collected rectal swabs to investigate the fecal microbiota composition by 16S rRNA gene analysis. Research protocols were approved by the University of Milan (protocol number 78\_2018).

**Results:** The composition on WM and BM in terms of SCC, fat, protein, lactose, and microbial inhibitors content was as follows. WM: 450,000 cells/ml; 3.7%; 3.6%; 4.7%; present. BM: 284,000 cells/ml; 4.23%; 3.60%; 4.97%; absent. At the end of the trial, the two groups showed statistically significant differences in both clinical terms, including reduced weight gain and increased occurrence of diarrhea events in the WM group, and in terms of fecal microbiota composition. Almost all WM calves (5/6) developed diarrhea in the first 2 weeks (vs 1/6



BM calves). In the following 6 weeks, only 1 episode of diarrhea occurred in 1 WM calf. WM calves' body weight was significantly lower than BM calves along the trial. The 16S rRNA gene analysis indicated a sharp reduction in the fecal microbiota alpha-diversity of WM vs BM calves, most significant at Wk4 ( $p < 0.02$ ), two weeks after exposure to WM. Beta-diversity of the fecal microbiota was significant between time-points ( $p = 0.0069505$ ). As for the alpha-diversity results, significant differences were observed between WM and BM calves at Wk4 ( $p < 0.05$ ). Based on the normalized relative OTU levels, WM and BM calves showed significant differences at all time-points. At the end of the trial, Bacteroidetes, Firmicutes, and Saccharibacteria decreased while Chlamydiae increased. Significant changes were also observed in 7 classes, 8 orders, 19 families, 47 genera. Among the most relevant findings was the general decrease of beneficial taxa, like Faecalibacterium, vs an increase in other taxa and potential pathogens, including Campylobacter, Pseudomonas, and Chlamydomphila. Lactobacillus and Lachnoclostridium increased, but since the first was present in the milk substitute, its higher abundance in WM calves might indicate a lower microbiome resilience.

**Conclusion:** In conclusion, these results suggest that adding to the risk of increasing antibiotic resistance, feeding pre-weaned calves with WM is related to a higher incidence of calf diarrhea and relevant changes in the fecal microbiota composition.

**Keywords:** Calf, gut microbiome, milk, antibiotic residues, mastitis.

## AH-20

### Bovine besnoitiosis control plan: a two-year experience in an extensive herd from the dehesa agroecosystem

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Bovine besnoitiosis, caused by the parasite *Besnoitia besnoiti*, is characterized by local and systemic clinical signs. Currently, male reproductive failure is the major concern as breeding bulls may develop sterility. The disease is widely spread in Southwest Europe and is endemic at least in Spain, Portugal, France and Italy. Moreover, new outbreaks have been recently reported in Central and Northern Europe. Nowadays bovine besnoitiosis is one of the most relevant cattle diseases in extensive husbandry systems. In Spain the disease is present in mountainous areas in Central and Northeast Spain and in the dehesa agroecosystems in Southwest Spain. The lack of drugs or vaccines hampers the control, and it is a re-emergent disease in Europe. In this context, biosecurity measures coupled to an accurate diagnosis play a key role in the control and should be adapted to the facilities and management possibilities of the farm.

**Objective:** The aim of this study was to implement and monitor the efficacy of a control plan for two years (2018 and 2019) in a herd infected with *B. besnoiti* with a low fertility rate. Management measures were applied based on an exhaustive diagnostic protocol.

**Material & methods:** The herd under study is in southwestern Spain. It is fenced and covers an area of 3,000 hectares including a hunting reserve of Eurasian wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) with Mediterranean scrubland mainly composed by evergreen oak (*Quercus ilex*) and cork oak (*Quercus suber*) and 800 hectares of dehesa agroecosystem dedicated to extensive cattle farming. The census initially included 154 crossbreed cows and seven breeding bulls of the Charolais, and Limousine breeds and the farm has its own replacement. At that time the herd fertility rate was 36.7% and with a calving interval of 524 days.

Once *B. besnoiti* infection was confirmed in one male by clinical inspection and serological analysis the whole herd was serologically tested in March 2018. Initially a validated ELISA test was used, and all doubtful animals were retested by western blot as a confirmatory test. Replacement heifers were tested at one year of age. The whole herd was tested again following the above-mentioned serological protocol at the end of the study in October 2019.

**Results:** Initially the intra-herd seroprevalence was 34.97% and six out of the seven (85.7%) bulls were seropositive. Sperm quality was monitored in all bulls and a low concentration of sperm was observed in four of them as well as defects in sperm motility and morphology.

Based on the diagnosis results obtained several control measures were recommended:

- i. Infected (seropositive) from non-infected (seronegative) cows were separated and progressive selective culling of seropositive breeding bulls and cows was carried out. These seropositive animals were fattened prior to the selective culling.
- ii. Replacement cattle were tested at least twice.
- iii. Artificial insemination was implemented in the seronegative group for one year. In the meantime, new breeding bulls were entered into the herd and serologically monitored. Nowadays, reproduction management is based again in natural mating with seronegative breeding bulls.

As a result of these management measures implemented for the control of bovine besnoitiosis all animals present in the herd were seronegative to *B. besnoiti* at the end of the study, the calving interval between 2018 and 2019 decreased up to 469 days and the fertility rate increased up to 76.7%.

Based on the results obtained it is possible to design and implement effective control strategies in dehesa agroecosystems since the regular health status monitoring of new entries was carried out and the extension of these farms favour to manage the herd into two separate groups to avoid parasite transmission by direct contact. Other biosecurity measures to maintain a closed herd recommended such as vector control and the absence of contact with wildlife were not possible in this herd. The inexistence of new seropositive animals could be due to the lower frequency of vector borne cases and the low relevance of this parasitic disease in wildlife (eg. wild ru-

minants) since they do not pose a significant risk for parasite transmission to cattle.

**Conclusion:** This study represents the first successful attempt to implement and monitor the effectiveness of a control program for bovine besnoitiosis with an improvement of reproductive parameters.

**Keywords:** Bovine besnoitiosis, extensive herd, control.

## AH-21

### *Salmonella enterica* isolated from dairy cattle in Uruguay presents a diversity of antibiotic resistance genes and mobile genetic elements

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**Objectives:** The objective of this work is to study the presence and distribution of antibiotic resistance genes, plasmids and integrons in *Salmonella enterica* of bovine origin from Uruguay.

**Materials and methods:** In total 75 isolates from the collection of Plataforma de Investigación en Salud Animal-INIA-Uruguay collection were included. Forty-two isolates were obtained between 2016 and 2020 from 33 outbreaks of neonatal diarrhea and/or mortality due to bovine salmonellosis in calves that presented or succumbed to the disease with bacteriological and histopathological diagnosis. The remaining 33 isolates were obtained between 2019 and 2020 from one dairy farm with a history of bovine salmonellosis. Isolate sources included calves and cows' feces, water and food for animal consumption.

The genome of this isolates' set was obtained using Illumina MiSeq in MicrobesNG (UK). The sequence reads were uploaded to *Enterobase*. The isolates' serovar was determined using the *SISTR1+SEqSero2* scheme, and the sequence type (ST) using the *Achtman* scheme. The assembled genomes were then further analyzed using the tools *ResFinder 4.1*, *PointFinder*, *PlasmidFinder 2.1* and *IntFinder 1.0* from the *Center for Genomic Epidemiology (CGE)* for identifying acquired resistance genes, chromosomal mutations, plasmids and integrons.

**Results:** In total, 31 isolates were typed as *S. Typhimurium* (STy, 41.3%), 24 as *S. Newport* (SNw; 32.0%), 11 as *S. Anatum* (SAn; 14.6%), 6 as *S. Dublin* (SDb; 8.0%), one *S. Agona* (SAG; 1.3%); one *S. Montevideo* (SMv; 1.3%) and one as *IIIb61:i:z53* (1.3%).

For every serovar identified, only a single ST was present. Every STy isolate was classified as ST19, SNw isolates as ST45, ST64 for the SAn isolates and SDb isolates were typed as ST10. Less frequent isolates SAG, SMv and *IIIb61:i:z53* were typed as ST13, ST138 and ST430 respectively.

There was a high diversity of antibiotic resistance genes

in the isolates collection. Every isolate carries the *aac(6)-Iaa* gene that confers resistance to aminoglycosides. Within this group, *aph(3'')-Ib* and *aph(6)-Id* were also frequently present. Fifty-one isolates presented resistance genes for tetracyclines, *tet(A)* was detected in 47 isolates while four carried *tet(B)*. The *sul2* gene was present in 29 isolates conferring resistance to sulfonamides. Interestingly some genes like *aadA1*, *aadA2*, *aadA17*, *floR* and *cmIA1* that generally are present in genetic mobile elements were detected. The *bla*<sub>TEM-1B</sub> gene related to penicillin was detected only in 7 isolates of SA and ST isolates. Plasmidic *qnr* gene was carried by 5 isolates while 37 isolates had the *parC\_T57S* mutation both conferring resistance to quinolones. One SD isolated showed the mutation *acrB\_R717Q* to confer resistance to azithromycin.

Considering the presence of antibiotic resistance in all the genomes, twenty-four were multi drug resistant (MDR). This characteristic was only detected in STy, SA and SAg serotypes. Among these, one presented antibiotic resistance genes for 4 groups of antibiotics and five for 6 groups of antibiotics.

All except five isolates carried at least one plasmid. The most frequent plasmids were *IncFII(pHN7A8)*, *IncFII(S)*, *IncFIB(S)*, *Col440I* and *IncI1*. Forty-nine strains had 1 plasmid, 17 strains had 2 plasmids, and 4 strains had 3 plasmids. All STy carried plasmids and the most frequent combination of plasmids was *Col440/ IncFIB(S)*.

Two class 1 integrons were detected in the collection. *In705*, carrying *aadA1* gene, was detected in two STy isolates while *In1363*, carrying *aac(3)-IId* and *intI1*, was detected in one SAn isolate and in one STy. These 4 isolates were MDR and carried at least one plasmid.

**Conclusions:** The evaluation of the genome allowed us to detect serotypes involved in the outbreaks with STy as the most frequent. A wide distribution of MDR isolates was found in the farm's outbreaks and environment. There was a high number of isolates carrying genes that confer resistance to aminoglycosides, tetracyclines and sulfonamides, antibiotics that are widely used in veterinary practice in Uruguay. Most of the serotypes detected in the collection could have an impact on human health. This work represents one of the first studies assessing the MDR genes in *Salmonella* from dairy cattle in South America.

**Keywords:** Salmonellosis, dairy cattle, antimicrobial resistance, genome, South America.

## AH-23

### Assessing the utility of leukocyte differential cell counts for predicting morbidity, mortality and growth in a grain fed veal facility: A prospective single cohort study

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**Objectives:** Automated leukocyte differential cell counts (DCC) that can be acquired and analyzed quickly on farm may be useful to incorporate into calf risk identification protocols



at the time of arrival at a veal or dairy beef operation. The objective of this study was to assess the utility of DCC taken at the time of arrival at a grain fed veal facility and 72 hours post arrival for determining morbidity risk, mortality risk and growth during the production cycle.

**Materials and Methods:** Data were collected at a veal research facility in Ontario, Canada between June and October of 2018. A total of 240 calves were enrolled upon arrival and a subset of 160 calves were revisited 72 hours post arrival. Leukocyte differential cell counts were evaluated using the QScout BLD test for leukocyte differential cell counts (Advanced Animal Diagnostic, Morrisville, NC). At the time of arrival, all calves were screened using a standardized health scoring protocol and blood samples were collected to evaluate serum total protein (TP) and DCC. A second blood sample was collected from the subset calves 72 hours post arrival to evaluate DCC. Cox proportional hazards models were constructed for both morbidity and mortality outcomes. Mixed linear regression models were constructed for the outcome of average daily gain. Models were constructed in Stata 15 (StataCorp LP, College Station, TX).

**Results:** Results from data collected at the time of arrival suggest that TP values greater than 5.1 g/dL reduce the hazard of mortality (HR = 0.29,  $P < 0.001$ ) and a rectal temperature greater than 39.6°C was associated with an increased hazard of morbidity (HR = 1.48,  $P = 0.04$ ). Calves that were dehydrated gained less (-0.09 kg/d,  $P = 0.03$ ), however, an increased lymphocyte count was associated with calves having a higher level of growth (+0.05 kg/d,  $P = 0.02$ ). Results from DCC collected 72 hours post arrival suggest that lymphocyte counts between 4.8 and 5.8 x 10<sup>9</sup> cells/L decrease the hazard of mortality (HR = 0.22,  $P = 0.03$ ) and > 7.0 x 10<sup>9</sup> cells/L decrease the hazard of morbidity (HR = 0.56,  $P = 0.02$ ), whereas, neutrophil counts > 6.0 x 10<sup>9</sup> cells/L increased the hazard of mortality (HR = 5.2,  $P = 0.02$ ).

**Conclusions:** This study demonstrates that machine DCC at the time of arrival and 72 hours post-arrival has potential for use in calf risk identification protocols in veal and dairy beef facilities. Further work to determine the effects of time post transportation on blood leukocyte measurements in this population of calves should be performed to help determine the best time to measure blood leukocytes post arrival.

**Keywords:** Male dairy calf, risk factors, biomarker, leukocytes.

#### AH-24

### Effect of over-conditioning on the hepatic global gene expression pattern of dairy cows at the end of pregnancy

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**Objectives:** Lipolysis in the prepartum has been linked with liver dysfunction and systemic inflammation, which in turn are associated with an increased incidence of postpartum clinical disease. We hypothesize that over-conditioning is associated with excessive (basal) prepartum fat mobilization, leading to steatosis and modifications in the global gene expression pattern of the liver. Thus, the aim of this study was to evaluate the effect of over- and normal-conditioning on the differential hepatic transcriptomics profile of prepartum dairy cows.

**Materials and methods:** Ten non-lactating pregnant Holstein cows were euthanized two weeks before expected calving. Body condition score (BCS) and backfat thickness (BFT) were evaluated, and blood samples for non-esterified fatty acids (NEFA) were taken before euthanasia. After slaughtering, liver biopsy samples were collected and frozen for further triacylglycerol (TAG) concentration analysis and RNA sequencing.

**Results:** Five cows were classified as normal-conditioned (BCS = 2.5–3.5) and five as over-conditioned (BCS = 3.75–5). Regression models revealed that normal-conditioned cows presented lower BCS (3.17 ± 0.10; mean ± SEM), BFT (1.29 ± 0.29 cm), and serum NEFA (0.16 ± 0.04 mmol/L) in comparison to over-conditioned cows (4.35 ± 0.21, 3.14 ± 0.43, and 0.38 ± 0.07 for BCS, BFT, and NEFA, respectively;  $P < 0.05$ ). Hepatic TAG concentrations were not different between normal- and over-conditioned cows (4.63 ± 0.40 and 6.06 ± 0.44 mg/g of liver fresh weight, respectively;  $P > 0.05$ ). Compared to over-conditioned, normal-conditioned cows presented four up-regulated (PLAC8, COL27A1, IDO-1, and MEP) and two down-regulated genes (IGFVP-1 and CSE).

**Conclusions:** As expected, prepartum over-conditioning (based on BCS) was associated with greater BFT and basal lipolysis than normal-conditioned cows. However, hepatic TAG concentrations were similar between prepartum over- and normal-conditioned cows. This was reflected in the low number of differentially expressed genes between these groups of animals. All the differentially expressed genes were associated with protein-coding. Normalized counts and fold changes of targeted genes in KEGG pathways should be evaluated to further explore the potential effects that over-conditioning may have in the hepatic global gene expression pattern.

**Keywords:** Transition period, metabolism, lipolysis, transcriptomics.

#### AH-25

### Risk factors associated with total and pathogen-specific immunoglobulin G concentrations in western Canadian neonatal beef calves

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Inadequate transfer of immunoglobulins from dam to calf via colostrum is a challenge on cow-calf operations because it negatively affects pre-weaning calf health and growth. There is limited knowledge about risk factors for poor transfer of passive immunity (TPI) and the specific antibodies being transferred to beef calves via colostrum in the perinatal period under current production conditions in western Canada. Thus, the objective of this study was to determine factors influencing total and pathogen-specific immunoglobulin G (IgG) concentrations against selected neonatal calf diarrhea (NCD) and bovine respiratory disease (BRD) pathogens in the serum of newborn beef calves. A total of 420 serum samples were available from 1 - 7 day old beef calves born on 6 farms in Alberta, Canada. Samples were analyzed by radial immunodiffusion assay for total IgG concentration and by enzyme-linked immunosorbent assays for pathogen-specific IgG concentrations against *Escherichia coli* (*E. coli*), bovine Rotavirus (BRoV), *Cryptosporidium parvum* (*C. parvum*), Parainfluenza Virus Type 3 (PI-3), Bovine Respiratory Syncytial Virus (BRSV), and Bovine Herpesvirus-1 (BHV-1). Data collected about individual dam- and calf-level risk factors included dam parity, dam vaccination status, calving ease, birth month, calf sex, twin status, and route of colostrum consumption (i.e., nursed from dam, bottle-fed, or tube-fed). Multivariable multilevel linear and logistic regression models were built to evaluate risk factors associated with total and pathogen-specific IgG concentrations, failed transfer of passive immunity (FTPI; serum IgG < 10 g/L), and inadequate transfer of passive immunity (ITPI; serum IgG < 24 g/L). Farm was included as a random effect in all models to account for clustering at the herd level. Five percent (n = 20) of calves had FTPI and 18% (n = 75) of calves had ITPI. Calves born to heifers and those receiving colostrum intervention (i.e., fed colostrum or colostrum product by either bottle or tube) showed significantly lower total IgG concentrations (P < 0.0001). Calves that received colostrum intervention had higher odds of FTPI (Odds ratio (OR): 6.1, 95% CI: 2.0 - 18.9) and ITPI (OR: 4.8, 95% CI: 2.1-10.8). Pre-calving dam vaccination was associated (P < 0.0001) with increased IgG-concentrations against *E. coli*, BRoV, and BHV-1, whereas PI3 and BRSV-specific IgG concentrations were unaffected by dam vaccination status. Vaccine practices varied greatly within and between farms, and producer compliance with label instructions was inconsistent. This study highlights the opportunity for improvement of management protocols pertaining to dam vaccination and colostrum intervention on cow-calf operations.

**Keywords:** Antibodies, immunity, colostrum management, vaccination, beef cattle.

## AH-26

### Impact of total and pathogen-specific serum immunoglobulin G concentrations on the health and growth of western Canadian beef calves

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Neonatal calf diarrhea (NCD) and bovine respiratory disease (BRD) are the most common calfhood diseases in beef calves worldwide. Vaccination of pregnant dams against specific NCD- and BRD-associated pathogens and good colostrum management have the potential to increase the transfer of those pathogen-specific antibodies to the calf via colostrum and thus protect against disease during early life. However, little is known about the associations of pathogen-specific antibody concentrations and the risk of disease and mortality, as well as the impact of such antibody concentrations on pre-weaning growth in beef calves raised under current production conditions in western Canada. The objectives of this study were to determine the impact of total and pathogen-specific immunoglobulin G (IgG) concentrations on the odds of pre-weaning treatments and mortality, and on average daily gain (ADG). A total of 420 serum samples from 1 - 7 day old beef calves born on 6 farms in Alberta, Canada, were collected. Samples were analyzed by radial immunodiffusion assay for total IgG concentration and by enzyme-linked immunosorbent assays for pathogen-specific IgG concentrations against *Escherichia coli* (*E. coli*), bovine Rotavirus (BRoV), *Cryptosporidium parvum* (*C. parvum*), Parainfluenza Virus Type 3 (PI-3), Bovine Respiratory Syncytial Virus (BRSV), and Bovine Herpesvirus-1 (BHV-1). Data regarding pre-weaning treatments, mortality, and birth and weaning weights were collected. Multivariable multilevel logistic and linear regression models were built to evaluate associations between total and pathogen-specific IgG concentrations and the odds of pre-weaning mortality and ADG, respectively. Farm was included as a random effect in all models to account for clustering at the herd level. The impact of IgG concentrations on the odds of pre-weaning treatment was established by univariable logistic regression analysis. Receiving colostrum intervention (i.e., being fed colostrum or colostrum product by either bottle or tube) significantly increased the odds of mortality (OR: 7.4, 95% CI: 1.7-31.6). Calves with FTPI and ITPI were more likely to die (OR: 18.5, 95% CI: 3.7-93.4 and OR: 10.1, 95% CI: 2.6-40.2, respectively), calves with FTPI were more likely to receive treatment (OR 7.9, 95% CI 2.7-23.7), and calves with ITPI had lower ADG (-0.09 kg, SE: 0.03, P < 0.002). Calves that were treated in the pre-weaning period showed lower *E. coli* and BRoV-specific IgG concentrations at birth (P < 0.0001), and BRoV-specific IgG concentrations were lower in calves that died in the pre-weaning period (OR: 0.97, 95% CI: 0.95-0.99). These results suggest a protective effect of *E. coli* and BRoV-specific antibodies and highlight the importance of



increasing their transfer from dam to calf. Interestingly, neither being treated pre-weaning nor any of the pathogen-specific IgG concentrations were associated with ADG. This study highlights the need to review and refine protocols with respect to colostrum management on cow-calf operations to decrease pre-weaning treatments, calf losses and improve pre-weaning growth.

**Keywords:** Antibodies, immunity, pre-weaning infectious disease, beef cattle, vaccination.

## AH-27

### Evaluating the Efficacy of Colostrum as a Therapy for Diarrhea in Young Calves

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**Objectives:** Diarrhea is the primary cause of morbidity and mortality in dairy calves. Many cases of diarrhea in calves are treated with antimicrobials, increasing the risk of antimicrobial resistance, therefore, creating a need for alternative therapies. The objectives of this study were to evaluate the effects of feeding colostrum at the onset of diarrhea on growth and duration and severity of the disease in pre-weaned dairy calves.

**Methodology:** At a calf-raising facility in Ontario, Canada, calves were scored for fecal consistency twice daily on a scale of 0 to 3 and enrolled into the trial when they had two consecutive fecal scores of 2 (runny or spreads readily) or one fecal score of 3 (liquid consistency, splatters). Calves were then randomly allocated to receive one of three treatments: 1) Control (CON) (n = 35): eight feedings over 4 d of 2.5 L of milk replacer at a concentration of 130 g/L (26% crude protein and 17% fat), 2) Short term colostrum supplementation (STC) (n = 35): four feedings over the first 2 d of 2.5 L of a mixture of milk replacer at 65 g/L and colostrum replacer at 65 g/L (26% immunoglobulin G and 14.5% fat) followed by four feedings over 2 d of 2.5 L of milk replacer at a concentration of 130 g/L, or 3) Long term colostrum supplementation (LTC) (n = 38): eight feedings over 4 d of 2.5 L of a mixture of milk replacer at 65 g/L and colostrum replacer at 65 g/L. Serum immunoglobulin G (IgG) was determined at arrival to the facility. Body weight (BWE), days to enrollment since facility arrival (DTE) and severity of diarrhea (SDE) were recorded and a fecal sample was taken to determine the diarrhea causing pathogens (PT) present at enrollment. Daily health exams evaluating fecal consistency were performed for 28 consecutive days after enrollment and body weight was measured at d 0, 1, 2, 3, 4, 7, 14, 21, 28, 42 and 56 after enrollment.

**Results:** The median days for resolution of an abnormal fecal score was 3.5 d (range = 0.5-11.5), 2.5 d (range = 0.5-11), and 3 d (range = 0.5-7) in the CON, STC, and LTC groups, respectively.

Using a Cox proportional hazards model, it was found that

calves in LTC group had faster resolution of diarrhea compared to calves in the CON group (Hazard Ratio (HR): 2.29;  $P = 0.01$ ; 95% CI = 1.31 – 4.01). In addition, there was an association between both DTE and BWE and resolution of diarrhea, where calves who were at the facility longer prior to enrollment (HR: 2.29;  $P = 0.01$ ; 95% CI = 1.56 – 5.45) and heavier at the onset of diarrhea (HR: 2.37;  $P = 0.01$ ; 95% CI = 1.23 – 4.56), resolved diarrhea quicker. Moreover, calves with a fecal score of 3 at enrollment took longer to resolve their case of diarrhea (HR: 0.44;  $P = 0.01$ ; 95% CI = 0.28 – 0.70). The number of types of diarrhea causing pathogen also had an impact, with cases where no or one pathogen was isolated was associated with a faster resolution (HR: 3.30;  $P = 0.01$ ; 95% CI = 1.62 – 6.68). In addition to a reduced time to resolution, improved growth was observed in calves in the LTC group gaining 98 g/d more than the CON group over 56 d following enrollment (HR: 0.98;  $P = 0.04$ ; 95% CI = 0.01 – 0.19).

**Conclusion:** Supplementation of colostrum at the onset of diarrhea was associated with a reduced duration of diarrhea and improved growth in young calves. This may provide life-long benefits for calves diagnosed with a gastrointestinal disease in the pre-weaning period. Due to the preliminary nature of this study, future research is necessary to determine the most practical and effective dose, duration, and timing of this therapy.

**Keywords:** Morbidity, treatment, pre-weaned.

## AH-28

### Association between acute phase proteins and clinical signs of respiratory disease in dairy calves

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**Objectives:** Bovine respiratory disease (BRD) is a leading cause of morbidity, mortality, and antimicrobial consumption of pre-weaned dairy calves. Assessment of clinical BRD symptoms in field conditions impedes making inferences on the presence of infection and inflammation and thus sets subjective criteria for antimicrobial treatment. The acute phase response (APR) is a part of host innate immunity and the first response to infections and tissue damage, which causes significant concentration revision in acute phase proteins (APPs), e.g., haptoglobin (Hp), serum amyloid A (SAA), and fibrinogen (Fib), in the blood. APPs have poor diagnostic specificity but are considered very sensitive in detecting clinical or subclinical inflammation or infection. So far, there is limited information available about which of the respiratory disease signs better predicts the infection and inflammation of the respiratory tract.

Therefore, this study aimed to identify the possible associations between different APPs with clinical signs of respiratory disease in dairy calves under field conditions and to assess their potential as a diagnostic tool complementing current BRD diagnosis.





**Materials and Methods:** Sixteen large Estonian commercial dairy farms with >400 dairy cows were included in this study. At each farm, five clinically healthy calves and five calves with clinical BRD signs in the age range of one to ten weeks were selected. The following clinical parameters were measured in each calf: rectal temperature ( $^{\circ}\text{C}$ ; 0 = < 39.0, 1 = 39.0–39.49, 2 =  $\geq$ 39.5), respiratory rate (breaths/min; 0 = <45, 1 =  $\geq$ 45), nasal discharge (0 = no, 1 = yes), cough (0 = no cough, 1 = yes), and demeanor (0 = normal, 1 = depressed). Blood samples from the jugular vein of each calf were collected in EDTA tubes for Fib measurement and in serum tubes for SAA and Hp measurements. From one farm, six healthy calves were included, making for an overall sample size of 161 calves for SAA and Hp estimation. Fib measurements were performed from blood samples from 11 farms, making for an overall sample size of 110 calves. Serum Hp concentration was determined using an ad hoc hemoglobin-binding assay. Serum SAA concentration was measured with a commercially available ELISA kit. Plasma Fib concentration was measured using the heat precipitation method. Linear mixed-effect regression analysis with farm as random intercept was used to study the associations between the recorded clinical signs and concentrations of APPs; separate models were built for the three outcome variables Hp, SAA, and Fib. The final models were produced by backward elimination of the variables from the original models. Age was controlled in the statistical models, except for Hp, to avoid a possible confounding effect. Pearson's correlation analysis was used to identify the relationships between APPs. The modelling and the diagnostics were performed using Stata/IC 14.2. Results were interpreted as significant if  $p < 0.05$ .

**Results:** Clinical signs that were observed in calves were diarrhea (62.1%), increased rectal temperature ( $\geq$ 39.5 $^{\circ}\text{C}$ ; 36.0%), increased respiratory rate (29.2%), nasal discharge (16.8%), cough (14.3%) and depressed demeanor (5.0%). Serum SAA concentration was positively associated with increased rectal temperature of  $\geq$ 39.5 $^{\circ}\text{C}$ . Hp levels was positively associated with increased rectal temperature of  $\geq$ 39.5 $^{\circ}\text{C}$  and presence of nasal discharge. In case of Fib concentration, positive associations were found with increased rectal temperature of  $\geq$ 39.5 $^{\circ}\text{C}$ , increased respiratory rate of  $\geq$ 45 breaths/min, and calves exhibiting cough.

Fib showed a moderate positive correlation with Hp (correlation coefficient = 0.46) and SAA (correlation coefficient = 0.42) concentrations, whereas between Hp and SAA a weak positive correlation (correlation coefficient = 0.32) was found.

**Conclusions:** This study found association between serum APP concentrations and clinical signs of respiratory disease in dairy calves. Increased rectal temperature ( $\geq$ 39.5 $^{\circ}\text{C}$ ) best represented the inflammation during respiratory disease measured through elevated APPs. Calf-side tests for Fib measurement could serve as valuable diagnostic tool to make inferences about the presence of systemic inflammation and to assess underlying tissue damage, thus aiding in treatment decisions.

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**Keywords:** Acute phase proteins, respiratory disease, dairy calves.

## AH-29

### Comparison of total protein, antibody ELISA and electrophoresis as tests for failed transfer of passive immunity in calves in a Bayesian latent class analysis

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**Objective:** Failure of passive transfer (FPT), the insufficient transfer of passive maternal immunity to calves, is a leading risk factor for many calfhood diseases, mortality and growth retardation. The prevalence of FPT is generally high and highly variable between farms. Regular monitoring of the FPT status of the herd is an essential element of calf health management. Direct tests (determining immunoglobulins) and indirect tests (determining total protein as a proxy of immunoglobulins) are available, among which total protein and brix refractometry are most accessible and best validated. Radial immunodiffusion (RID) is often used as a gold standard test in North America, but likely no real gold standard tests for FPT exists. Capillary gel electrophoresis and antibody ELISA are offered in many European veterinary laboratories, but their diagnostic accuracy to diagnose FPT has hardly been evaluated. Therefore, the objective of this study was to determine the diagnostic accuracy of capillary electrophoresis and a commercial antibody ELISA for FPT diagnosis in a Bayesian latent class framework with total protein refractometry as comparative test.

**Materials and method:** In this diagnostic test study, 200 clinically healthy calves (dairy and beef) of less than one week old were sampled for blood serum by practitioners throughout Flanders (Belgium) with a maximum of five samples per farm. Subsequently, on each sample, total protein and the various serum protein fractions were determined by capillary electrophoresis (MiniCap Flex Piercing, Sebia, France), and serum immunoglobulin concentration was determined by ELISA (Bio-X Diagnostics ELISA kit for Bovine Immunoglobulin Assays, Rochefort, Belgium). Subsequently, Bayesian latent class models in the statistical freeware program Winbugs (version 1.4.3., MRC Biostatistics unit, Cambridge, United Kingdom) were used to determine the diagnostic accuracy of the three tests. For this study, an independent model was used in which both an uninformed and an informed model were run. In the first model, prior information on all parameters was set at uninformative, beta(1,1). The second model included informative priors on the prevalence FPT in Flanders (18%), and the third model included informative priors on the prevalence of FPT and the sensitivity (76%) and specificity (89%) of 51 g/L total protein as a cut-off for FPT. Priors for the sensitivity (76%) and specificity (89%) of TP 51 g/L were estimated from a study of Buczinski et al. with 95<sup>th</sup> percentile 60%. The priors were modeled using beta distribution parameters of the corresponding prior distributions (Epitools), resulting in beta(21.1824;7.3734), beta(8.6849;1.9498), and beta(2.3087;6.9619) for sensitivity, specificity, and prevalence, respectively. All models were run with 200.000 iterations.



**Results:** All models converged, and the prior and posterior densities of models 1 to 3 were very stable between models. Also, the deviance information criterion values of the three models differed minimally (<2). As the informative priors narrows the parameter uncertainty, model 3 is expected to be the most accurate. Electrophoresis showed the highest sensitivity and specificity with 98.1% (95% credibility interval (CI) = 90.0 – 99.9) and 99.3% (95% CI = 96.1 – 100), respectively. Total protein sensitivity and specificity were respectively 63.8% (95% CI = 53.2 – 73.6) and 97.0% (95% CI = 93.4 – 99.0). The sensitivity and specificity of the ELISA were 95.5% (95% CI = 88.1 – 99.0) and 74.5% (95% CI = 66.8 – 81.3), respectively.

**Conclusion:** Based on analysis of this dataset electrophoresis appears a promising, near gold standard test for FPT, which is readily available in many veterinary laboratories. Given the lower specificity of the evaluated antibody ELISA, we recommend evaluation of antibody ELISA in a similar framework given the large differences in diagnostic accuracy between tests that may exist.

**Keywords:** Maternal immunity, calf health, specificity, sensitivity.

#### AH-31

### Effect of serum total protein concentration on early life health and growth of dairy calves

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**Objective:** To assess the effect of serum total protein (STP) concentration on the early life health and growth of dairy calves. **Materials and Methods** A total of 39,619 neonatal Holstein, Jersey, and crossbred calves from 15 dairy operations. Calves arrived at a single calf-raising facility at approximately 2 days old. Each calf was weighed at facility arrival and a blood sample was obtained the next day for determination of STP concentration by refractometry. All calves were managed in a standard manner, and health events were recorded for 120 days. A subset of 3,214 calves was weighed at 120 days old, and the average daily gain (ADG) was calculated. Linear mixed models were used to assess the effect of STP concentration on specific health events. Results STP concentration was associated with the incidences of death, diarrhea, pneumonia, and whether a calf received IV fluid therapy. In general, the incidence of adverse health events decreased as STP concentration increased to 6.0 g/dL, plateaued at STP concentrations between 6.0 and 8.5 g/dL, and increased at STP concentrations > 8.5 g/dL. Although STP concentration was not associated with ADG, the ADG for Holsteins increased as STP concentration increased to 8.5 g/dL and then decreased at STP concentrations > 8.5 g/dL. **Conclusion** Results suggested that, for neonatal dairy calves, an STP concentration between 6.0 and 8.5 g/dL was optimal for health and growth,

and calves with an STP concentration < 5.0 or > 8.5 g/dL should be considered at high risk for adverse health events.

**Keywords:** colostrum, neonatal calf health, passive transfer.

#### AH-32

### Eradication of Paratuberculosis from a Dairy Farm - Following the Administration of *Mycobacterium vaccae*

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**Background:** Paratuberculosis is a chronic enteric infection, primarily of ruminants, that causes significant economic losses, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Young animals are at the highest infection risk. To date, on-farm prevalence reduction is based on protecting these animals from contact with the microorganism by hygienic measures and by removing shedding animals, a long, expensive, and partially successful process.

*Mycobacterium vaccae* has been reported to have immunomodulatory and immunotherapeutic capabilities, by activation of the cellular immune system, of primary importance in preventing paratuberculosis and its evolution to the clinical stage. We have previously demonstrated the safety of the oral administration of live *M. vaccae* to new-born calves.

**Objective:** Assessment of the impact of introducing *M. vaccae* on the prevalence of paratuberculosis in a dairy herd.

**Materials and Methods:** Since some of the heifers were found, in a previous experiment, to shed *M. vaccae* and thus expose eventual control animals to the microorganism, a case-control study was not possible. Consequently, a “before-after” experimental design was implemented. In this model, untreated animals that lived on the farm before the onset of the experiment served as negative controls. A dose of 10<sup>10</sup> CFU live *M. vaccae* was administered, by gavage, to all heifers on the farm, within 24 hours of birth and 2 weeks later. The prevalence of paratuberculosis was assessed yearly by milk ELISA (mELISA). Fifty percent of cows aged 3 years, born within 3 years before the experiment’s onset, and all the treated cows were tested, at the same age, by qPCR for MAP shedding. Management improvements that might have had a bearing on the prevalence of MAP were not implemented during the experiment’s period. Paratuberculosis positivity was not considered a reason to remove cows from the farm.

**Results:** The rate of mELISA positive cows was reduced from 6% to 0% within 3 years and remained unchanged thereafter. 48.9% of highly positive cows reverted to being mELISA negative. Interestingly these cows were not treated with *M. vaccae* but were likely to have been exposed to the microorganism from their environment. Due to the disrepair of the dairy’s fencing, shedding heifers mingled with the adult animals, thus exposing them to *M. vaccae*. The rate of qPCR positive control cows was 2.46% whereas that of the treated cows was 1%. Four years after the onset of the experiment

(October 2019) 2 control shedding cows, still on the farm, became qPCR negative. The test cow continued shedding MAP and was removed from the herd in December 2020 due to infertility. One additional test cow turned mELISA positive in February 2021 but did not shed MAP when tested in June of the same year. The cow is still in the herd.

**Conclusions:** The results of this study, seemingly unprecedented, indicate, pending further confirmation, that the introduction of live *M. vaccae* reduces paratuberculosis prevalence to the verge of eradication conceivably resulting from its immunoprophylactic and immunotherapeutic activity, in an economic, safe, and straightforward manner, even without the implementation of paratuberculosis mitigating management measures.

**Keywords:** Paratuberculosis, Dairy Herd, Live *Mycobacterium vaccae*, Eradication.

### AH-33

#### The effect of colostrum supplementation during the first 5 days of life on calf morbidity, enteric pathogens, weight gain and immunological response

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**Objectives:** The objective of this study is to investigate the preweaning health, performance, immunity, and enteric pathogen shedding in calves supplemented with colostrum from dams vaccinated against rotavirus, coronavirus and *Escherichia coli* F5 during five days after birth compared to non-supplemented calves.

**Material & methods:** On a commercial dairy farm, healthy and viable new-born calves from vaccinated (Bovilis® Rotavec Corona) dams received at least 3 liters of colostrum from their own dam in the first 24h after birth and were randomly assigned to the colostrum supplementation treatment (treatment calves) or control treatment (control calves). The colostrum supplementation treatment consisted of the addition of a liter of previously frozen colostrum (from Bovilis® Rotavec Corona vaccinated cows) to the milk replacer on the second day of life and a half a liter of previously frozen colostrum to the milk replacer on day 3, 4 and 5 of life. Control calves similarly received once daily the same amount of supplement made from skimmed milk with similar protein and fat composition as the colostrum supplement. The daily milk intake and health parameters were monitored during the first week of life. Calves were weighed at birth, on day 28, and at weaning (approx. two months of age). Average daily gain (ADG) for the different periods was calculated. Faecal samples were taken at 7, 14, and 21 days of age. Additionally, on the first day of diarrhoeal disease diagnosis a faecal sample was collected. The presence of faecal pathogens was determined using a rapid ELISA kit (rotavirus, coronavirus, *Cryptosporidia*, *Clostridia*, *E.*

*coli* F5). Faecal samples may also be evaluated for the microbiome composition when significant changes in the results will be identified. Serum samples were taken at 1, 7, 14, and 21 days of age to determine serum antibody levels to bovine coronavirus, rotavirus, and *E. coli* F5. Additionally, total serum IgG concentration was determined in the sample taken on day 1 to evaluate the transfer of passive immunity. Outcomes were analysed in multivariate statistical models controlling for calf age, passive transfer of immunity status, parity of dam and other potential confounders. In outcomes evaluating serial measures on calves, a repeated measures design was used.

**Results:** The study is ongoing. Preliminary results are presented from 19 treatment calves and 21 control calves. The passive transfer of immunity in all calves was adequate to good and similar for both the treatment and the control calves. Limited enteric and respiratory disease has not revealed any significant effect of treatment on preweaning health in the calves. The ADG in the preweaning period of the calves was above 1 kg/day and no significant difference in ADG between treatment and control calves in the first month, the second month, or the overall preweaning period was observed. The faecal samples collected on day 7, 14, and 21 of age were all negative for coronavirus and *E. coli* F5. There was a non-significant trend for more rotavirus positive samples in control calves on day 7 and 14 of age and more cryptosporidia positive samples in control calves on day 14 of age compared to treatment calf samples. Furthermore, faecal levels of clostridia on day 14 of age were non-significantly higher in control calves compared to treatment calves. The specific serum antibody levels against rotavirus, coronavirus, and *E. coli* F5 were not significantly different between treatment and control calves. It may be decided to do a faecal microbiome analysis if significant differences will be found between treatment and control group to see if prolonged colostrum administration may have a positive effect on the microbiome and be an explanation for the significant findings.

**Conclusion:** This study indicates a trend towards lower excretion of cryptosporidia and rotavirus in preweaning calves supplemented with colostrum from vaccinated dams.

**Keywords:** Prolonged colostrum feeding, calf health, immunity, enteric pathogens.

### AH-34

#### Prevalence of tail injuries in German Dairy cows

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**Objectives:** Tail injuries especially often occur in fattening bulls. Their prevalence increases, when beef cattle are housed on slatted floors. The origin of tail tip inflammation and necrosis is often reported as technopathy, irritation due to manure contact or itching followed by scratching, but also subacute rumen acidosis and laminitis seem to occur in association with tail tip injuries in fattening bulls. Several kinds of tail tip scor-



ing systems already exist, foremost for fattening cattle. These scoring systems include between 4 to 6 grades of severity, but do not differentiate between different kinds of tail alterations. In literature, prevalences measuring tail alterations in fattening bulls range from 5% up to 90%. In dairy cows, prevalences between 2.5 and 7.7 % have been reported.

Since tail alterations in dairy cows are not investigated very well, the aim of this study was to develop a scoring system, which covers different kinds of tail alterations detected in dairy cows and could be used to phenotype this trait as a first step towards animal health improvement. Secondly, histo-pathological and clinical evaluations will follow to be associated with the scoring group and grade of severity.

**Material & Methods:** Data collection started in December 2019 from a German 75 German Holstein (HOL) cows dairy herd. All cows were examined every two weeks during six months regarding any kind of tail alterations. The findings were described and photographed. Data analysis resulted in seven different kinds of tail alterations: 1. very tip of the tail, 2. ring-like, 3. scurf, 4. swelling, 5. thinning, 6. axis anomalies, and 7. verruca-like mass.

Hereinafter, prevalences for the observed tail alterations were calculated based on monthly data collection from five different dairy herds: 3 HOL herds, counting average herd sizes of 75, 300, and 1300, respectively; 2 German Fleckvieh (FV) herds, counting 60 cows, each. All cows were housed in free stall barns with conventional (HOL, FV) or automatic milking systems (FV).

In total, 4443 Dairy Cows' Tail Scores were recorded. Data preparation and analysis were performed using R version 4.1.2. Prevalences for tail alterations were calculated by dividing the number of observations within by the total number of observations of each kind of tail alteration and was given in percent. For calculating the total prevalence per breed and farm, the occurrence of at least one tail alteration counted as an observation, was divided by the total number of cows under investigation and given in percent.

**Results:** The overall prevalence for any kind of tail alteration was 88% in German Holstein and 99% in Fleckvieh cows; it varied between farms from 74% to 99%. Prevalences for HOL and FV regarding alterations of the very tip of the tail were 26% and 71%, ring-like alterations 24% and 30%, swelling 26% and 42%, scurf 55% and 60%, thinning combined with axis anomalies 16% and 21%, and verruca-like mass 10% and 21%, respectively. Number per tail ranged for ring-like alterations and thinning/axis anomalies from 1 to 5 and for verruca-like mass from 1 to 3.

**Conclusion:** During this study, high prevalences for different tail alterations in HOL and FV dairy cows were found out. The grouping of different alterations as described above can be useful to phenotype tail alterations in dairy cows. However, further investigations regarding pathogenesis, aetiology, and genetics of the observed alterations in dairy cows' tails are needed to understand their origin and impact on animal health and welfare.

**Funding:** This research was funded by the Tönnies Forschung, Rheda, Germany.

**Keywords:** tail injuries, tail necrosis, tail inflammation, tail alterations, dairy cows.

### AH-35

#### Nutritional supplementation of a multi-strains yeast fraction improves health and increases beneficial gut microbiota of pre-weaned dairy calves

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**Objectives:** High morbidity and mortality rates of calves during pre-weaning period will dramatically impact the sustainability of the farm. The reduction of the prophylactic treatments in calves production has contributed to the development of new microbial-based solutions to improve gut health and consequently production. The objective was to determine the effect on health of a multi-strains yeast fractions product (MSYF) introduced to the calves' diet during the rearing period (5-65 day of life).

**Materials and methods:** Thirty Holstein calves born from late June to mid-November 2019, in a medium-size (500 milking cows) dairy farm in Poland were enrolled in the study. All calves received the colostrum from the dam within 2 h and 2.5 L of transition milk, 2 times a day until 5 days of age. At enrollment, 6 days-old calves, were individually housed and were offered daily 8 L of MR, divided in 2 equal meals, in buckets, at a dilution rate of 0.125kg /L, providing 4.6 Mcal metabolizable energy (ME) /kg of dry matter (DM) (21.9% crude protein and 18% fat). They had *ad-libitum* access to water and pelleted starter (3.55 Mcal ME/kg DM). Calves were randomly assigned in the two experimental groups, designated as supplemented (MSYF) and Control (C), stratified by dam's parity, gender and initial BW. Supplemented calves received 0.8 g of a MSYF /kg of MR, diluted in the MR meals for the whole experiment. Fecal consistency (scores) was scored daily by the research team during the morning feeding using a 1 (firm) to 4 (watery) scale (Wenge et al., 2014). Two fecal samples were collected at time = 10 days and time = 60 days of the trial for each calf. Incidence of health disorders was recorded daily for each animal. Every abnormal health condition such as fever or bronchitis as well as every treatment (antibiotic, electrolyte therapy) was recorded.

A linear mixed model was performed to assess the impact of the supplementation on the different parameters measured. Calf was used as random effect, sex, group, and sex\*group interaction were used as fixed effects and initial BW as a covariate. Statistical analysis was performed using IBM SPSS v.25 and RStudio.

**Results:** No calves of MSYF group experienced respiratory diseases during the trial whereas 50% of the non-supplemented calves had respiratory problems ( $P = 0.034$ ). In addition, the number of diarrhea cases was lower for MSYF compared to Control group (2.2 vs. 0.9/calf respectively,  $P = 0.007$ ) as well as the duration of the diarrhea case (5.6 vs. 2.9 days/calf respectively,  $P = 0.005$ ). Consequently, a lower fecal score was reported all along the trial for MSYF compared to Control calves (1.6 vs 1.9 respectively,  $P = 0.002$ ). Along with those observations, a lower number of calves received a therapeutic treatment in MSYF group compared to Control group

(18.8 and 62.5% respectively,  $P = 0.034$ ), and the average number of therapeutic treatment used for MSYF calves was lower compared to Control calves (0.3 vs 2.7/calf respectively,  $P = 0.005$ ). Finally, less MSYF calves were supplemented with electrolytes compared to Control calves (37.5 vs. 81.3% respectively,  $P = 0.035$ ). From a fecal microbiota standpoint, MYSF supplementation in neonate calves increased milk associated bacteria (*Bifidobacterium*, *Lactobacillus*) and beneficial mucinolytic *Akkermansia* (stimulation of mucine production, and immune system) which might have improved milk metabolism compared to Control and optimize the nutrients supply from this feed.

**Conclusions:** The overall health of pre-weaned calves was significantly improved when they received daily a multi-strains yeast fractions supplementation. Consequently, less therapeutic treatment was applied to the supplemented calves. This better health could be explained by an enrichment of beneficial gut microbiota of supplemented calves likely involved in a better valorization of the milk.

**Keywords:** health, probiotic, yeast fraction, microbiota, calf.

#### AH-37

### Relationship between calf housing design and bovine respiratory disease prevalence in pre-weaned Irish dairy calves

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**Objectives:** Bovine respiratory disease (BRD) is a significant welfare and economic problem in housed dairy calves internationally. While the type of housing, the environment within and the management of that environment are significant factors influencing the prevalence of BRD, the relative importance of each of these risk factors is unclear. Hence, the objective of this study was to determine the interrelationships between housing type, housing environment, management factors and the prevalence of BRD.

**Materials and Methods:** Spring-calving dairy farms (n=64) throughout the Republic of Ireland were either randomly recruited (n= 39) or referred as BRD problem herds (n= 24) by Regional Veterinary Laboratories. Farms were visited in 2020 and in 2021; Each farm was visited twice: once one month prior to the calving season to allow for installation of temperature and relative humidity sensors in a calf pen, and a second time during the spring period when the calf house was occupied. At the second visit, housing type, environment, calf management and respiratory health were characterised. To do this, housing type was recorded and the dimensions measured, Temperature and relative humidity sensors were recovered, air samples were taken to quantify bacterial load, a survey of calf management practices was conducted, and pre-weaned

calves were examined.

To determine the herd-level prevalence of BRD in the calf house, 20 randomly selected calves between 4 and 6 weeks old were assessed in two ways. Firstly, clinical signs of BRD were assessed using the Wisconsin Respiratory Scoring System. Secondly, a thoracic ultrasound (TU) was performed by one veterinarian using a Linear 5 MHz ultrasound probe (Easy Scan Go, IMV). Each calf was assigned a TU score between 0 and 5, with 0 indicating healthy lungs, 1 indicating diffuse comet tails, 2 indicating patchy consolidation, 3 indicating lobar consolidation, 4 indicating lobar pneumonia affecting two lobes and 5 indicating pneumonia affecting more than two lobes. A score 3 or greater was considered a case of TU-BRD.

An Analysis of Variance (ANOVA) model was used to investigate the association between housing type and the herd-level prevalence of BRD as assessed by TU. As the distribution of BRD prevalence within housing type was not normal, a Kruskal-Wallis rank sum test was conducted.

**Results:** In total, 1,258 calves were examined by TU. Across all calves, the mean prevalence TU-BRD was 10%. The minimum, maximum and median prevalence TU-BRD in the 5 predominant housing types was:

1. Duo pitch (n=28) (Median Prevalence = 5%, Min = 0%, Max = 45%)
2. Round-top houses with additional lean-to roof(s) (n=12) (Median Prevalence= 15%, Min =0%, Max = 80%)
3. Mono pitch (n=10) (Median Prevalence = 7.5%, Min = 0%, Maximum = 30%)
4. Round Top (n= 9) (Median Prevalence = 0%, Min = 0%, Max = 10%)
5. Lean to (n = 5) (Median Prevalence = 0%, Min = 0%, Max = 15%)

There was a difference in the mean prevalence of TU-BRD cases between the different housing types ( $p < 0.05$ ). The epsilon squared was 0.153 which is considered a medium effect size. A post hoc analysis relieved differences between the mean prevalence of TU-BRD in round-top houses compared to round-top houses with lean-to roofs ( $p < 0.05$ ), and between lean-to houses and round-top houses with additional lean-to roofs ( $p < 0.05$ ). However, when these p values were adjusted using the Holm-Bonferroni method for multiple comparisons, these differences were no longer considered significant.

**Conclusion:** The relationship between predominant calf housing designs present on Irish dairy farms and BRD prevalence remain unclear. The wide variation in the prevalence of TU-BRD between farms could not be solely attributed to the design aspects of calf housing investigated above. Further analysis of the temperature, relative humidity and air quality data collected in this study may contribute to a better understanding of the interactions between calf housing and BRD.

**Keywords:** BRD, Housing, Environment, Thoracic Ultrasound.

**AH-38****Effect of colostrum replacer fat content on thermogenesis, calf behavior, health and growth**

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**Objective:** The objective of this study was to determine the effect of colostrum replacer fat content on thermogenesis, calf behavior and growth.

**Materials and methods:** A total of 58 Holstein female calves were assigned to Control Colostrum Replacer (CCR; colostrum replacer at 20% IgG and 22% fat) or Defatted Colostrum Replacer (DCR; colostrum replacer at 20% IgG and 5.7% fat) manufactured by SCCL (SK, Canada). Calves were tube fed CCR or DCR providing 150 g of IgG at 1 h and 100 g of IgG at 12 h after birth. At 0 (before 1st feeding), 6, 12, 18, 24, 36, 48 and 60 h after birth, vital signs [heart rate (HR), respiratory rate (RR) and rectal temperature (RT)], shivering and posture (lying or standing) were assessed, and blood samples were collected. Temperature data loggers were inserted into the vagina and accelerometers were placed in the right hind leg to evaluate lying behavior from 1<sup>st</sup> colostrum replacer feeding to 60 h after at 2- and 1-min intervals, respectively. Body weight and height were determined at birth, 90±2 and 127±6 d after birth. Disease treatment records were collected up to the end of the study. Multivariable linear or logistic regression were used for continuous and categorical data, respectively.

**Results:** Loggers' temperature readings were higher for CCR than DCR calves (38.81±0.03 vs. 38.76±0.03 °C;  $P = 0.04$ ). Serum IgG and total protein within 60 h after 1<sup>st</sup> feeding were lower for CCR calves [14.07±0.64 vs. 17.40±0.63 mg/mL ( $P < 0.001$ ); 5.04±0.07 vs. 5.30±0.07 g/dL ( $P = 0.01$ )]. Calves fed CCR had higher body weight and average daily gain by 90 d of age [96.44±2.02 vs. 89.95 kg±2.06 ( $P = 0.03$ ); 0.65±0.02 vs. 0.58±0.02 kg/d ( $P = 0.01$ )] and 127 d of age [139.75±3.27 vs. 129.14 kg±3.27 ( $P = 0.03$ ); 0.81±0.02 vs. 0.71±0.02 kg/d ( $P = 0.001$ )]. Respiratory disease incidence was double for DCR calves (Relative risk = 2.00; 95% CI = 0.86 to 4.60;  $P = 0.09$ ) (tendency). Thus, the incidence of respiratory disease was 41% for calves assigned to DCR and 21% for calves assigned to CCR. Furthermore, a total of 2 calves assigned to DCR vs. 1 calf assigned to CCR died during the study period.

Overall, no statistically significant differences were observed for vital signs (RT, HR and RR), observed (shivering and posture) and accelerometers behavior measures, height and diarrhea treatments. Average RT was 38.78 °C, HR was 150.03 beats per min, and RR was 47.67 respirations per minute. A total of 9 calves were observed shivering at 6 h, 3 at 12 h; 4 at 18 h, 1 at 24 and 60 h, and no calves were shivering at 36 and 48 h. Accelerometers' data showed that calves spent 50 h and 55 min or 86.37% of the time lying down (also calves were lying down 78.77% of the times when posture was observed) and had 42.52 standing bouts during the first 60 h after birth. However, when two influential observations defined as observations with studentized residuals above |4| SD were excluded, lying time was higher for DCR (52 h and 15 min) than for CCR calves (50 h and 39 min);  $P = 0.04$ . Finally, di-

arrhea incidence was 21% and calves averaged 97.79 cm in height at 90 d of age.

**Conclusions:** In conclusion, feeding a full fat colostrum derived colostrum replacer versus a partially defatted form had a positive effect on thermogenesis, led to higher body weight and body weight gain within 127 d of age, and may have had a positive effect on respiratory disease, despite lower serum total protein and IgG within 60 h after 1<sup>st</sup> feeding.

**Keywords:** Calves, Colostrum, Fat, Thermogenesis.

**AH-39****Effect of dry needle acupuncture to reduce rectal temperature in dairy cows using a single needle on acupuncture point GV14 (Da-zhui)**

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**Objective:** To compare the effect of a single commonly-used size hypodermic needle on acupoint GV14 (Governing Vessel 14) versus no needle for the reduction of naturally occurring fever in commercial Holstein dairy cows.


**Materials and Methods:** Animals eligible for inclusion in this study were lactating Holstein dairy cows with a rectal temperature greater than 103.0 °F that were not receiving any other treatment or cooling interventions. Sample size calculation to detect 1.0 °F difference (alpha= 5%, power= 90%) showed the need of a minimum of 10 cows, when using the same animals as their own controls.

Animals with rectal temperature ≥103.0°F (Temp0) were enrolled in the study and the control period of the study started. After 20 minutes, temperature was measured again (Temp1) and if fever persisted, an 18 G x 1.5 inch hypodermic needle was placed on acupoint GV14 for 20 (Temp2). Rectal temperature was measured again 20 minutes after needle removal (Temp3).

The difference between the average change in temperature within each cow during the control period (no needle, Temp1-Temp0), during the 20-minute trial period (GV14 needle, Temp2-Temp1) and 20 minutes post needle removal (Temp3-Temp0) was analyzed by 1-sided paired t-test with a level of significance of  $P < 0.05$ , because acupuncture on GV14 was expected to reduce fever, never to increase temperature.

The question arose during the study whether longer duration of needle placement would have any effect on the drop in temperature. Therefore, a second set of cows was enrolled, where some retained the needle for 20 minutes and others for 40 minutes. Temperature at 60 minutes post placement was compared by a 2-sided t-test for independent means with a level of significance of  $P < 0.05$ .

**Results:** In total, 11 Holstein dairy cows were enrolled in the original study. Mean temperature difference during the 20-minute control period was + 0.04 ± 0.23 °F. Mean temperature difference for the same cows during the first 20-min-



ute study period with GV14 acupuncture was  $-0.37 \pm 0.28$  °F. Overall, acupuncture on GV14 decreased temperature by  $-0.41$  °F more than no intervention ( $P < 0.001$ ).

In the subsequent, 22 Holstein dairy cows were enrolled; 11 cows had the needle placed in acupuncture point GV14 for 20 minutes and 11 cows had it placed for 40 minutes. Mean temperature difference 60 minutes after initial needle placement was  $-0.55 \pm 0.43$  °F for cows with a 20-minute placement and  $-0.08 \pm 0.53$  °F for the cows with a 40-minute placement ( $P = 0.045$ ).

**Conclusion:** Acupuncture with a commonly-used size hypodermic needle on acupoint GV14 is an effective method of reducing fever in dairy cows. The difference in temperature change between animals seems to be associated with the disease causing the fever (diagnosis) as well as the potential duration of clinical signs (acute versus chronic disease).

Acupuncture is a viable option for reduction of fever in animals where pharmaceuticals cannot be used due to withdrawal concerns, adverse reactions, or organic farming limitations. Additionally, acupuncture at GV14 can be a useful tool to help stabilize patients while further treatment options are being explored and discussed with the owner.

**Keywords:** Acupuncture, fever, cows, GV14.