Association of barn air quality parameters and lung consolidation, airway inflammation and infection in group-housed calves

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Respiratory disease remains an important cause of morbidity and mortality in calves and is associated with substantial antimicrobial consumption. Reduction of antimicrobial use is demanded to fight increasing antimicrobial resistance. This reduction can be achieved by improved diagnostics, prudent antimicrobial use and disease prevention. Assuring a healthy environment with adequate air quality in calf barns is a preventive measure of major importance. However, which air quality parameters are exactly associated with (sub)clinical pneumonia or airway inflammation in calves is basically unknown.

A cross-sectional study was performed to explore the associations of air quality parameters with lung consolidation, pulmonary inflammation and infection in group-housed calves. Sixty beef and dairy herds were visited from January – April 2017 and a total of 428 indoor group-housed calves was sampled. To assess barn air quality the following measurements were performed at calf breathing level: continuous 24-hour measurements of ammonia concentration, relative humidity and temperature and punctual measurements of air velocity, ammonia and CO₂. Clinical examination, thoracic ultrasound and broncho-alveolar lavage for bacteriological and cytological analysis of broncho-alveolar lavage fluid (BALf) was performed. Data was analysed using multivariable mixed models and cluster analysis.

Barn temperatures showed the expected diurnal variation with an average of 14.2°C (standard deviation (SD) 4.4, range 5.5 – 23.9). Average relative humidity was 68.8% (SD 8.9, range 52.2 – 91.6). Ammonia concentrations were low with an average 24-hour concentration of 1.7 ppm (SD 0.9, range 0 – 4.0). Air velocity was variable, ranging from 0 – 2.0 m/s. This parameter was analysed as binary variable based on the optimal maximum air velocity cut-off value to detect a lung consolidation of ≥1 cm in depth, which was 0.8 m/s. Lung consolidations of ≥1 cm, ≥3 cm and ≥6 cm in depth were present in 41.1% (176/428), 27.1% (116/428) and 16.1% (69/428) of the calves, respectively. Lung consolidations of all depths were positively associated with mean or maximum temperature. Ammonia exposure, in hours ≥4 ppm, was associated with lung consolidation ≥1 cm (odds ratio (OR) = 1.73; confidence interval (CI) = 1.02 – 3.07; P = 0.04). The epithelial cell percentage in BALf was also positively associated with ammonia concentration (P = 0.01). The odds of lung consolidation ≥3 cm (OR = 7.7; CI = 1.35 – 41.7; P = 0.02) and ≥6 cm (OR = 25.0; CI = 2.5 – 33.3; P = 0.007) increased with air velocity >0.8 m/s.

Cluster analysis, based on average pen temperature and relative humidity, maximal air velocity, NH₃ and CO₂ concentration and bacterial air load, resulted in four different barn climate clusters. Cluster 1 was characterised by a warm and dry climate with ammonia accumulation (n = 19). Cluster 2 represented under-ventilated barns with accumulation of air pollutants (NH₃, CO₂) and high bacterial air load (n = 8). Cluster 3 was labelled as a presumably normal climate based on low concentrations of air pollutants and average temperature and relative humidity values, combined with low air velocity (n = 25). Cluster 4 was labelled as the draught cluster, characterised by high air velocity (n = 3). In cluster 4, the draught cluster, the prevalence of lung consolidations ≥1 cm was higher (81.8%; P < 0.001) compared to the presumably normal cluster (31.6%). Also, in the warm, dry and ammonia accumulation cluster the prevalence of these consolidations was higher (54.2%; P = 0.02) compared to the normal cluster. Furthermore, in the warm, dry and ammonia cluster the prevalence of consolidations ≥3 cm (38.1%; P = 0.04) and ≥6 cm (31.4%; P = 0.01) were higher compared to the presumably normal climate cluster (18.2% and 9.1%, respectively).

Average and maximum temperature, ammonia concentration and air velocity are associated with pneumonia in group-housed calves and therefore seem to be the most valuable parameters to measure when evaluating barn climate. Cluster analysis using different parameters can aid in identification of calf barns with sufficient air quality and further improve our understanding of calf barn climatic conditions.

Keywords: Temperature, ammonia, air velocity, thoracic ultrasonography, broncho-alveolar lavage fluid.

Does Hemorrhagic Bowel Syndrome start with small mucosal erosions?

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Objectives: To describe gross and histological lesions, as well as intestinal microbiome in cases of Hemorrhagic Bowel Syndrome (HBS).

Materials & Methods: In this descriptive study, 18 cows with HBS (7 necropsied immediately following euthanasia, 11 post mortem interval >12h) were grossly and histopathologically examined post-mortem. In addition, bacteriological examination and whole metagenome nanopore sequencing of intestinal samples of affected and unaffected intestine was performed.

Results: Hemorrhagic Bowel Syndrome was characterized by a small intestinal, intramucosal hematoma with dissection of the lamina muscularis mucosa (LMM), in all animals where histological determination was possible (10/18). In 57.1% (4/7) of the fresh cases, the small intestinal mucosa proximal to the hematoma, showed 9-14 multifocal solitary or clustered small mucosal lacerations or erosions measuring 4-45mm. In 73% (37/48) of these mucosal lesions, microscopic splitting of...
the LMM comparable to the hematoma was present. Intestinal blood loss was more severe in animals with spontaneous death compared to euthanized animals (p = 0.038). Bacteriological cultivation and nanopore sequencing showed a polymicrobial population at the hematoma and unaffected intestine, with mostly mild presence of *Clostridium perfringens*. There was no infiltrative growth of fungi within the intestinal tissue.

**Conclusions:** HBS is characterized by an intramucosal hematoma which likely originates form small mucosal lesions through dissecting hemorrhage within the LMM. Fulminant intestinal blood loss is an important factor in the acute and lethal clinical course of this disease. Gross and microscopic pathology, as well as the culture and sequencing results, were not in support of involvement of bacterial or fungal agents in the pathogenesis of HBS.

**Keywords:** Jejunal hemorrhage syndrome, hematoma, intestinal, lamina muscularis mucosae, nanopore sequencing.

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**IM-03**

**Differentiating airway inflammation in calves by means of cytological profiles of bronchoalveolar lavage fluid**

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**Objective:** Bovine respiratory disease (BRD), the primary cause of antimicrobial use in youngstock, is an inflammatory disease caused by multiple infections interacting with host and environmental factors. To improve prevention of BRD a better understanding of airway inflammation is critical. Cytology of bronchoalveolar lavage fluid (BALf) offers many possibilities to characterize and diagnose different inflammatory airway diseases, both in humans and in animals. However, in calves to date no reference values for BALf cytology are available. This seriously hampers both research and practice, as identifying truly healthy (negative control) calves for respiratory diseases is difficult. Therefore, in this study the first aim was to apply unsupervised clustering in order to identify different airway inflammation profiles in BALf from calves. Second, based on identification of a healthy profile, reference values for differential cell counts in BALf as collected by non-endoscopic bronchoalveolar lavage were determined.

**Material and Methods:** A dataset with cytologic information on BALf was available. This dataset consisted of 339 group-housed calves aged between 1 and 6 months from 32 dairy farms and 30 beef farms. The differential counts in this dataset were evaluated by counting 400 nucleated cells on cytospin preparations. A k-means++-algorithm, an unsupervised clustering method, was applied on the percentage of macrophages, neutrophils, lymphocytes, eosinophils, basophils and epithelial cells. The number of clusters was first obtained by validation with 'clValid' R package, evaluation of silhouette plots and biological relevance. Visualization of cluster centers was used to identify the different clusters. Subsequently, reference values for the normal cluster were calculated with 'Reference Value Advisor'. Lastly, a classification and regression decision tree was constructed to establish the cut off-values for cluster assigning of future observations.

**Results:** The ideal number of clusters was four, based on the different validation methods and visual evaluation. In three clusters one or two cell types were more prominent relative to the others clusters. As such, a neutrophilic (n=114), eosinophilic (n=9) and a lymphocytic-basophilic (n=31) cluster were identified. One cluster did not show any prominent change in cluster center and was identified as normal (n=185). The mean and the calculated 95% interval reference values for the normal cluster were the following: 52.2% (22.7% – 84.3%) macrophages, 21.9% (1.4%–41.3%) neutrophils, 6.1% (0.3%–18.8%) lymphocytes, 0.2% (0.0%–1.5%) eosinophils, 0.0% (0.0%–0.0%) basophils, and 19.6% (1.0%–59.0%) epithelial cells. A classification and regression decision tree model used the percentage of neutrophils, basophils and eosinophils as main predictors of cluster type for future observations and provided an accuracy of 98.2%. The first cut off value used by this model was 42.0% neutrophils. If the percentage of neutrophils was lower than 42.0% a cut off value of 0.1% basophils and 1.9% eosinophils was employed. When neutrophil count was equal or above 42%, cut off values were 0.2% and 1.2%, respectively.

**Conclusion:** Unsupervised clustering provided a useful distinction of different inflammatory profiles. The main clusters were neutrophilic and normal clusters. Also, a smaller lymphocytic-basophilic and eosinophilic cluster were identified. The distinguishing of these four clusters, and identification of a presumably normal group, could contribute to novel insights in pathogenesis, diagnosis and prevention of various airway diseases in calves.

**Keywords:** Cytology, bronchoalveolar lavage, bovine respiratory disease.

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**IM-04**

**Association of ionized and total calcium in periparturient dairy cows**

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**Objectives:** Hypocalcemia in cattle is commonly diagnosed based on serum total calcium (tCa) although only ionized calcium (iCa) is metabolically active. The objectives of this study were to study the association between tCa and iCa around calving and to identify factors most influential on the iCa:tCa-ratio.

**Materials and methods:** Lab results from 30 cows enrolled in an unrelated study investigating the effect of phosphorus-deprivation during the dry period were used. Cows were either fed a dry cow diet with adequate (0.30%) or low (0.16%) phosphorus content. Blood samples were obtained...
daily from day -4 until calving, 0h, 6h, 12h, 24h and 2, 3, 4 and 7 days post-partum. Blood gas and blood biochemical analyses were conducted.

**Results:** Correlations between iCa and tCa during the first week of lactation ranged between r=0.55, P=0.002 and r=0.84, P<0.0001. The slope of the Deming regression lines varied considerably at the different sampling times. ROC analyses with a cut off value of 1.10 mmol/L for iCa conducted for each sampling time yielded a low area under the curve of 0.64 at calving that increased to 0.93 4 days post-partum. Parameters with strongest effect on the iCa:tCa ratio were plasma albumin (r²=0.58, P<0.0001), pCO₂ (r²=0.45, P=0.0003), standard-[HCO₃⁻] (r²=0.22, P=0.01), lactate (r²=0.16, P=0.04) and NEFAs (r²=0.15, P=0.05).

**Conclusion:** The association of tCa with iCa during the first week of lactation is weak and highly variable. Several blood parameters seem to affect the iCa:tCa ratio and thereby weaken the usefulness of tCa to predict iCa in particular during the first days of lactation.

**Keywords:** Ionized calcium, total calcium, parturient dairy cow.

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**IM-05**

**Gain and loss of fat depots mass in dairy Holstein cows with different body condition during the transition period and early lactation**

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**Objectives:** Dairy cows suffer from negative energy balance (NEB) during the transition and early lactation period. NEB is an outcome of nutrient demand of late gestation, onset of lactation and inadequate feed intake. Body reserves, mainly from subcutaneous (SCAT) and abdominal adipose tissues (AAT), are mobilized to compensate NEB. Previous studies found differences in accumulation and mobilization of fat, as well as functional disparities between SCAT and AAT. The aim of this study was to compare the gain and loss of SCAT and AAT depot masses in Holstein dairy cows with initially lower and higher body condition (mean body condition score (BCS): median body condition score (MBCS): 3.48 and 3.87, respectively) in the period from day (d)-42 to d70 relative to parturition.

**Materials and methods:** The study was performed at the Friedrich-Loeffler-Institute (FLI), Braunschweig, Germany. Estimated depot mass (eDM) of SCAT, AAT, retroperitoneal (RPAT), omental (OMAT) and mesenteric (MAT) adipose depot was determined by ultrasonography on d-42, 7, 28 and 70. Thirty-one pluriparous German Holstein cows were allocated into two experimental groups according to the eDM of SCAT on d-42 relative to parturition (low body condition (LBC) group: n=16, mean eDM 8.61 kg; high BC (HBC) group: n=15, mean eDM 15.6 kg). Average daily change (aDC; prepartum gain and postpartum loss) of adipose mass was calculated for the dry (DP; d-4 to d7), fresh cow (FCP; d7 to d28) and early lactation periods (ELP; d28 to d70). Additionally, dry matter intake (DMI) and lactation performance were recorded.

**Results:** Animals of the two experimental groups represented adequate and over-conditioned cows. The AAT depot had about 2 to 3 times higher mass than SCAT. Prepartum the AAT gain during the DP was greater compared to SCAT (0.33 kg/d vs. 0.14 kg/d). More fat mass was lost from AAT than SCAT depot after calving (0.23 kg/d vs. 0.14 kg/d). Cows of both groups had similar gains in AAT (0.33 kg/dg) and SCAT (0.14 kg/dg) masses during the DP. However, more adipose tissue was mobilized by HBC than LBC cows from both depots after calving (AAT: HBC vs. LBC: 0.30 vs. 0.17 kg/d; SCAT: HBC vs. LBC: 0.19 vs. 0.10 kg/d). Correlation analysis revealed that the higher was the gain of AAT mass (r²: 0.36) during DP the higher was the loss in FCP. This was not the case for SCAT. In contrast to AAT, during FCP, a more negative EB was associated with a greater loss of SCAT mass (r²: 0.18).

**Conclusions:** The results indicate that compared to SCAT the AAT depot is considerably bigger and is preferentially mobilized after parturition to compensate NEB. Correlation analysis revealed functional differences between AAT and SCAT depots. Greater knowledge of differences between AAT and SCAT depots could improve our understanding of excessive lipomobilization and its effects on health and performance of dairy cows during the transition period.

**Keywords:** Dairy cattle, adipose depot, ultrasonography, transition period, fat mobilization.

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**IM-06**

**Do unmeasured strong ions predict mortality in sick goats?**

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Increased systemic concentrations of L-lactate and unmeasured strong ions (USI) are associated with an increased risk of mortality in humans and calves suffering from various diseases. The objective of this study was to investigate whether the concentration of L-lactate and USI, on admission to hospital, are associated with mortality in sick goats.

This study used a retrospective methodology. Medical records of 153 sick goats presented to a teaching hospital from January 2017 to September 2019 were reviewed and the following data were collected: Demographics, physical examination, venous blood gas, biochemistry analysis, and outcome. The USI concentration was calculated as: USI = SID₅₀ − HCO₃⁻ − A⁻; were SID₅₀ is the strong ion difference calculated as SID₅₀ (mmol/L) = (Na⁺ + K⁺) − (Cl⁻ + L-lactate−); HCO₃⁻ is the plasma concentration of bicarbonate (mmol/L) and A⁻ is the total negative charge of the plasma proteins (TP) (mmol/L) calculated as: A⁻ (mmol/L) = [TP x 0.343]/(1 + 10²(pKa − pCO₂) / 68.1) − Na⁺ − K⁺ − Cl⁻ − H₂CO₃.
Measurements of acute phase proteins in pharyngeal swabs and serum from healthy dairy calves

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Objectives: Early recognition and treatment of respiratory disease in calves can contribute to better animal welfare and possibly, reduce the antibiotic consumption. Detection of acute phase proteins (APP) in pharyngeal fluid could be the first step in making a non-invasive calf-side test for early detection of respiratory disease. The objective of this study was therefore to investigate if it was possible to detect the APPs serum amyloid A (SAA), haptoglobin (Hp) and lipopolysaccharide binding protein (lbp) in pharyngeal swabs from calves and if so, to compare the APP concentration in pharyngeal swabs with the serum APP concentration.

Materials and methods: Eighty-four healthy dairy calves aged 3-30 days housed in nine Danish dairy herds were included. All calves were assessed free from respiratory disease by a clinical examination and thoracic ultrasonography in the herd. After the clinical examination, serum samples were collected from the jugular vein and four long cotton swabs were placed in pharynx and moved around until saturated. The swabs were stored in cryotubes with 1 ml of phosphate buffered saline (PBS) for 24 hours, then removed and the cryotubes were stored at -80° Celsius until analysis. The swabs and serum were analysed for SAA, Hp and lbp at the Veterinary Diagnostic Laboratory at University of Copenhagen. The range and mean concentration of SAA, Hp and lbp in serum and swabs were compared.

Results: The SAA concentration in serum ranged from 32.93 - 180.94 ug/ml, with a mean of 118.28 mg/L and a standard deviation (SD) of 40.56 mg/L. There was a significant association between age and serum SAA, which decreased with age. The Hp concentration in serum ranged from 45.14 - 788.75 mg/L, with a mean of 100.60 mg/L and a SD of 100.74 mg/L and the lbp concentration in serum ranged from 7.25-56.32 ng/ml, with a mean of 20.53 ng/ml and a SD of 9.30 ng/ml. In the pharyngeal swabs the SAA concentration ranged from 0.00-8 ug/ml, with a mean of 2.18 ug/ml and a SD of 1.47 ug/ml. The Hp concentration in pharynx swabs ranged from 0.00-60.83 mg/L, with a mean of 20.13 mg/L and a SD of 13.61 mg/L. The lbp concentration in pharynx swabs ranged from 0.00-47.94 ng/ml, with a mean of 6.06 ng/ml and a SD of 11.85 ng/ml.

Conclusions: This is the first time that detection of the APPs SAA, Hp and lbp in pharyngeal swabs from dairy calves had been shown. From these results reference intervals for SAA, Hp and lbp in pharynx swabs from dairy calves housed in commercial farms can be established. This can be used further to compare with SAA, Hp and lbp in samples from diseased calves to establish the levels of APP in pharyngeal swabs of calves with respiratory disease.

Keywords: Acute phase proteins, calves, pharyngeal swab.

Sepsis and other risk factors for mortality in critically ill calves

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Objective: Critical illness in calves is a problem practitioners are frequently faced with, often resulting in mortality. It can be caused by several etiologies, with sepsis amongst them. Considering the strict antimicrobial legislation in food-producing animals, it’s crucial to identify which of the critically ill calves are in need of (critically important) antibiotics. Knowledge on factors contributing to mortality in these calves can aid in better antimicrobial decision making. Therefore, the objective of this study was to identify risk factors for mortality in critically ill calves.

Material & methods: A retrospective cohort study was conducted on 230 calves presented with critical illness in our large animal clinic. Anamnestic information, as well as clinical, ultrasonographic and laboratory information was assembled. Critical clinical illness in combination with positive hemoculture (BD BACTEC™) were used as a proxy for the presence of sepsis. Multivariable logistic regression as well as classification and regression tree analysis were used to determine parameters significantly associated with mortality.

Results: In total 61.3% (141/230) of the calves died. Enteritis/diarrhea (51.7%, n=119) and pneumonia (45.7%, n=105) were most frequently detected, followed by sepsis (34.3%, n=79), neonatal respiratory distress syndrome (17.8%, n=41) and omphalitis (17.8%, n=41). Comorbidities (presence of multiple health conditions in the animal) were frequent (54.8%, n=126), but did not significantly increase the risks of mortality (P=0.79). The final multivariable regression model showed that abnormal behavior (OR=3.3; 95% CI, 1.1-10.0; P=0.03), male gender (OR=4.3; 95% CI, 1.4-13.2; P=0.01), pale mucosae (OR=10.4; 95% CI, 2.5-43.7; P=0.001), tachycardia (≥ 126 bpm) (OR=3.4; 95.0% CI, 1.1-10.8; P=0.04), acidosis (pH ≤7.18) (OR=5.3; 95% CI, 1.3 -21.9; P=0.021) and sepsis (OR=6.9; 95% CI, 1.9-25.0; P=0.003) were associated with mortality.

Keywords: Sepsis, mortality, risk factors
Cardiovascular monitoring, laboratory findings and pain score in calves undergoing guided bilateral rectus sheath block for umbilical hernia repair: a randomized clinical trial

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Objectives: Despite detection and relief of pain are gaining considerable attention in farm animals, only a few clinical studies assess the effect of the analgesia in cattle after abdominal surgery, so far. Cardiovascular findings, serum cortisol level (SCL) and blood gas analysis (BGA) outcomes, as well as the pain score (PS) assessment, are considered some of the most reliable methods of clinical monitoring to evaluated stress, fear, and pain in animals. Based on the previous statements, the current study aims to define the health and welfare of bovine calves undergoing herniorrhaphy under general field anesthesia using an ultrasound-guided rectus sheath block (RSB) by multiple clinical approaches based on a set of parameters.

Materials and methods: The study has been performed on fourteen calves randomly assigned to receive either bilateral ultrasound-guided RSB with 0.3 mL/kg of bupivacaine 0.25% and 0.15 µg/kg of dexmedetomidine (Treated group, TG) or 0.3 mL/kg of 0.9% NaCl (Control group, CG). All animals were monitored by Holter recording to define the effects on the cardiac dynamic, as well as SCL, BGA, and UNE parameters. The SCL (spontaneous blood cortisol level) was evaluated in the morning, at skin incision and EST-t, as well as at +60min, +120min. Finally, the PS was carried out at -150min pre-surgery and at +30min, +45min, +60min, +120min, +240min, +360min post-surgery. Intra- and extra-groups differences have been calculated with appropriate parametric and non-parametric tests. Probabilities <0.05 were considered statistically significant.

Results: No significant difference was observed regarding the heart rate between the two groups at any time-interval, while regarding the same parameter a significant intra-group difference has been observed for the TG (Int1=99.9±21.0 vs. Int2=92.7±22.0; P<0.05). A statistically significant difference was observed regarding the overall time both of respiratory arrhythmia (TG=4.08min vs. GC=2.51min; P=0.0001) and sinus tachycardia (TG=0.00min vs. GC=11.07min; P<0.001). A further significant difference was found regarding the SCL between the two groups at skin incision time (TG=0.45±0.08 ng/mL ±SD vs. CG=0.82±0.06; P<0.01), while none was observed regarding the BGA. Finally, calves receiving RSB exhibited significantly lower median PS (P<0.05) between the two groups at +45min (TG=1 vs. CG=4), +60min (TG=1 vs. CG=6), +120min (TG=0 vs. CG=1) and +240min (TG=0 vs. CG=1.5).

Conclusion: The current multiple clinical approaches based on a set of parameters assess the effects on the health and welfare of bovine calves undergoing herniorrhaphy under general field anesthesia after receiving an ultrasound-guided RSB bupivacaine based, for the first time. The clinical procedures seem to reveal beneficial short-term (attested by HR and SCL) and long-term effects after surgery (attested by the PS). Although as compared to the traditional analgesic techniques, the use of this ultrasound based RSB seems to give greater beneficial effects on the health and welfare of calves affected by umbilical hernia, further studies are necessary to definitively confirm the effects and to enable this innovative analgesic procedure to become a milestone in the bovine field medicine.

Keywords: Holter calves, calves health, calves welfare, rectus sheath block, umbilical hernia.

Association of gastrointestinal microbiota and anion gap acidosis in diarrheic calves

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Objectives: Diarrheic calves often develop anion gap acidosis associated with increased concentrations of unmeasured anions including D- and L-lactate. However, the mechanisms responsible for these acid-base disorders are not completely understood. The objective of this study was to investigate the fecal bacterial alterations of calves with diarrhea and to explore its relationship with changes in blood pH, bicar-
bonate, and the anion gap and the gastrointestinal microbiota of diarrheic calves.

**Materials and Methods:** Prospective case-control study. The fecal microbiota of healthy (n= 20) and diarrheic (n= 31) calves was assessed by sequencing of 16S ribosomal RNA gene amplicons. Blood gas analysis was completed using an i-Stat analyzer. Alpha diversity was assessed using the Chao-1, Inverse Simpson’s and Shannon Even indices. The microbiotal membership and structure of the fecal microbiota was compared between groups using the Jaccard and Yue & Clayton indices, respectively. LefSe analysis was used to determine differentially abundant taxa in healthy and diarrheic calves.

**Results:** Diarrheic calves had a lower pH and bicarbonate concentration and a higher anion gap than healthy calves. In healthy calves, a higher richness, evenness, and diversity were observed compared to diarrheic calves. The Jaccard and Yue & Clayton indices showed that the community membership and structure, respectively, of healthy dairy calves was significantly different than that of diarrheic calves (AMOVA < 0.01), for both comparisons. LefSe analysis showed an enrichment in obligated anaerobes (Phocaeicola, Bacteroides, Prevotella, Faecalibacterium, Butyrivibrio, Ruminococcaceae and Lachnospiraceae) in healthy calves while in diarrheic calves there was an increase in facultative anaerobes (Enterococcus, Liggilactobacillus, Lactobacillus, Gallibacterium Streptococcus, and Escherichia/Shigella) (LDA score > 3 and P < 0.05). In diarrheic calves an increased relative abundance of lactate-producing bacteria including Lactobacillus, Streptococcus, Veillonella, Liggilactobacillus and Olsenella was detected compared to their healthy counterparts (LDA score > 3 and P < 0.05).

**Conclusions:** Calf diarrhea was associated with a shift from obligated to facultative anaerobes and expansion of lactate-producing bacteria. Those changes were related to acidemia and increased concentration of unmeasured anions (L- and D-lactate), estimated using the anion gap. These results suggested that gastrointestinal microbiota could play an important role in the development of anion gap acidosis in diarrheic calves.

**Keywords:** Acidemia, D-lactate, acidosis, bicarbonate, unmeasured anions.

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**IM-11**

**Selective use of antibiotics in neonatal calf diarrhea and its influence on fecal microbiota**

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**Objective:** The role of parenteral administration of antibiotics during neonatal calf diarrhea (NCD) is still an open issue. This study investigates the efficacy of antibiotic treatment and its influence on the gut microbiota of diarrheic calves.

**Materials and methods:** After approval of the Ministry of Health (approval number 14/2018), a total of 42 calves were selected from client-owned dairy calves with NCD, admitted to the Veterinary Teaching Hospital of the University of Milan from May 2018 to May 2019. We enrolled 1-to-10-day-old Holstein Friesian calves that did not present other concurrent neonatal diseases. Upon admission, the calves were clinically examined as described in Boccardo et al. 2017. The acid-base imbalance was assessed by venous blood-gas analysis. Serum total proteins (sTP) were measured in serum by a hand refractometer, and calves presenting a value < 55 g/L were considered as being affected by ITPI. Fecal cultures and antibiotic susceptibility tests were also performed. Conventional fluid therapy was performed on dehydration degree and acid-base imbalance. After infusion, calves were randomly assigned either to the group treated with antibiotics (Group A, n = 21 calves treated with ampicillin 10 mg/kg IV q12h for 5 days), or those not treated with antibiotics (Group B, n = 21). In both groups, ITPI calves received hyperimmune plasma containing a declared total protein quantity (PlasmaLife Calf®, Il Ceppo s.r.l., Italy). The amount of hyperimmune plasma was calculated using the formula:

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\text{Plasma amount (L)} = \frac{\text{body weight (kg)} \times \text{plasma volume [8.9%]} \times \text{sTP GAP}}{\text{plasma sTP concentration}}
\]

where sTP GAP = difference between pre–transfusion sTP and desired post–transfusion sTP (60 g/L); sTP concentration = concentration of sTP in the plasma as declared by the producer.

Calves were monitored daily with complete clinical examinations for 28 days. The clinical data were recorded using a Calf Health Scoring Chart (CHSC), and the probability of sepsis was calculated. Critically ill calves (sepsis score > 60%) were treated with antibiotics based on antibiotic susceptibility tests in both groups and this event was considered a failure of therapy. Each calf was weighed weekly, and a blood and fecal sample were collected. Blood serum samples obtained by centrifugation were analyzed to measure the concentration of sTP and IgG, while fecal samples were used for microbiota analysis.

The frequencies of mortality and treatment failure between the groups were compared with a χ2 test. The difference in the average daily gain and days of diarrhea between groups was compared with a Mann – Whitney – Wilcoxon test. Statistical significance was considered for p<0.05. The fecal microbial diversity was assessed within samples (alpha diversity). All indices (alpha and beta diversity) were estimated from the complete operational taxonomic units (OTU) table at the OTU level, filtered for OTUs with more than 15 total counts distributed in at least two samples. In addition to the number of observed OTUs directly counted from the OTU table, within-sample microbial richness, diversity, and evenness were estimated using the following indices: Chao1 and ACE (abundance-based coverage estimator) for richness, and Shannon’s, Simpson’s, and Fisher’s alpha for diversity.

**Results:** Eighteen calves (42.8%) presented ITPI (nine calves in group A, and nine in group B), and receiving the plasma transfusion (average 500 ml; min 300 ml; max 1000 ml). There was no statistical difference between groups A and B in terms of mortality rate (p=0.586), failure of treatment (p=0.449), average daily gain (p=0.556), and days with diarr-
The microbiota analysis showed that the microbiota of calves treated without antibiotics was re-established earlier than calves treated with antibiotics. Alpha diversity indexes were significantly higher in calves treated without antibiotics at the second week of convalescence than those treated with antibiotics (p=0.043). Furthermore, the antimicrobial treatment was associated with a decrease in the phylum Actinobacteria (p=0.045) and the family Lachnospiraceae (p=0.042) in the fecal microbiota of calves at the second week of study.

**Conclusion:** Our data suggest that antibiotic treatment should be omitted in the treatment of NCD both in calves with an adequate transfer of passive immunity and in ITPI calves treated with hyperimmune plasma. Furthermore, microbiota re-establishment seems to be significantly faster in calves treated without antibiotics.

**Keywords:** Neonatal Calf Diarrhea, Dairy Calves, antibiotic resistance, gut microbiota.
centrations suggested in the WHO Air quality guidelines for human exposure, being 25 μg/m³ (PM_{2.5}) and 50 μg/m³ (PM_{10}), respectively (World Health Organization, 2005).

The mean EU/µg PM_{10} was 4.25 ± 5.8 (R = 0.02 – 30.3) and mean EU/m³ 200.2 ± 197.4 (R = 2.32 – 901.0). Lung consolidations with a depth of ≥1, ≥3 and ≥6 cm were present in 43.1% (146/339), 27.4% (93/339) and 15.3% (52/339) of the calves, respectively. The odds of having lung consolidations of ≥1 (OR = 13.9; CI = 3.4 – 55.6) and ≥3 cm (OR = 7.0; CI = 1.8 – 27.0) in depth increased when endotoxin concentrations exceeded 8.5 EU/µg PM_{10}, compared to when this cut-off was not exceeded. Exposure to small and fine particulate matter fractions (PM_{2.5} and PM_{1}) was associated with increased odds of consolidations with a depth of ≥1, ≥3 and ≥6 cm (OR = 10.9; CI = 1.1 – 111.1), respectively. Total nucleated cell count of broncho-alveolar lavage fluid showed a positive association with the maximum PM concentration and neutrophil percentage with the maximum PM concentration. Only Pasteurella multocida isolation was associated with average PM_{2.5} concentration, for the other major respiratory pathogens no associations were found.

This study shows that particulate matter and endotoxin concentrations could play an important role in the bovine respiratory disease complex.


Keywords: Particulate matter, endotoxin, lung consolidation, dust, pneumonia.

IM-14

One-year cross-sectional study of dermatological lesions in 433 dairy cattle in a veterinary teaching hospital

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Little literature is available on the prevalence of bovine skin diseases. The objective of this study was to describe the dermatological lesions (DL) in dairy cattle admitted at the Faculty of Veterinary Medicine (FVM), Université de Montréal, from July 1, 2018, to June 30, 2019.

A study-dedicated dermatological lexicon was first developed by the authors. Over a year, all dairy cattle admitted at the FVM were included in the study. Dairy cattle readmitted or without intergumentary examination within 48 hours of admission were excluded. DL involving feet and ear canals were also excluded. The morphological and location of the skin and the oral mucosal DL were recorded by a trained observer.

On the 610 cattle admitted at the FVM, 433 dairy cattle were included. Most of them were Holstein breed (90%) and females (98%). The mean age was 3.3 years old (+/- 2.8 years). Of these 433 cattle, skin and oral mucosal lesions were observed in 91% and 9% of the cases, respectively. Most cattle had at least one hock (55%), one carpus (47%) or one stiffe (23%) affected. Crusts (55%), callus (54%) and alopecia (51%) were the most common skin DL. Erosions (43%) and ulcers (20%) were the most common oral mucosal lesions.

This cross-sectional study highlighted the high prevalence of the DL in this specific cattle population. Considering these results, further studies would be important to determine etiologies, economic and clinical impacts of these DL.

Keywords: Dermatology, Skin lesions, Bovine.

IM-15

Evaluation of the inter-rater agreement of the clinical signs of bovine respiratory disease in veal calves

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Objectives: In veal calves, clinical signs of bovine respiratory disease (BRD) are nasal discharge, ocular discharge, dyspnea, drooping ears, cough, and temperature. Despite the existence of several clinical scoring systems, the variability of human recognition of these anomalies has never been reported. The objective of this study was therefore to assess the inter-rater agreement of BRD clinical signs in veal calves. We hypothesized that BRD clinical signs were not detected equally between veterinarians, technicians, and producers of the veal industry and that some clinical signs have a higher inter-rater agreement than others.

Methods: During 2017-2018, we prospectively recorded 524 videos of physical examination of random veal calves from 80 batches in Quebec, Canada. A researcher, not involved in the inter-rater assessment, classified each video as presence/absence of each of BRD clinical signs except rectal temperature. For each of the 5 clinical signs, 15 videos with and 15 videos without the clinical sign were randomly selected to limit kappa paradoxes. Those 30 videos were then presented in a random order to experimented rater with BRD in veal calves: 6 veterinarians, 6 technicians, and 6 producers. Raters assessed the clinical sign using a modified Wisconsin scoring system (0=absent, 1=slight, 2=moderate, 3=severe for nasal discharge, ocular discharge and dropping ears; and 0=absent, 1=moderate, 2=severe for dyspnea and elicited cough). We used the median percentage agreement (Pa), median Cohen’s kappa (K), and Gwet’s agreement coefficient 1 (AC1) to assess inter-rater agreement. Effect of scale combination was also tested to determine the optimal combination (4-scale 0/1,2/3 vs 3-scale 0/1,2,3 or 0/1/2 vs 2-scale 0,1/2,3 or 0,1/2). The differences of inter-rater agreement between veterinarians, technicians, and producers were estimated by a Wilcoxon rank-sum test (p-value < 0.05).

Results: The 2-scale combination (0.1,3/0.1,2) had a higher inter-agreement for all clinical signs. With this combination, elicited cough was the clinical sign with the highest inter-rater agreement (Pa = 0.93; K = 0.79; AC1 = 0.87) and dyspnea was the sign with the lowest inter-rater agreement.
Stage-of-lactation specific hematology reference intervals are needed for post-parturient Holstein dairy cows

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Objectives: Hematologic profile of Holstein cows has been investigated mostly during peak and mid-lactation and dry period. However, comparative studies for hematology of post-parturient Holstein dairy cows versus the other stages of lactation is scarce. Therefore, the objective of this study was to establish hematology reference intervals (RIs) from the same population of Holstein dairy cows during different production stages and estimate the percentage of cows correctly classified if they were used interchangeably.

Material and Methods: Initially, after clinical examination, 68 dry period (DP) and 87 peak-lactation [PL, between 30 and 120 days-in-milk (DIM)] healthy Holstein cows from 10 dairy herds were enrolled in the study, blood sampled once, and used to establish DP and PL RIs. Samples from each cow were collected into 3 sterile vacuum tubes: a) with anti-coagulant (K3-EDTA, for hematology), b) with 3.2% Sodium Citrate (for fibrinogen), and c) without anticoagulant [for total protein (TP) concentration]. Complete Blood Count (CBC), fibrinogen and TP concentrations were determined using ADVIA 120 hematology analyzer, QBC VetAutoread, and ATAGO T2-NE desk refractometer respectively. Relevant RIs (DP and PL) were established with the Reference Value Advisor v.2.1. freeware. Subsequently, 1,021 Holstein cows from the same 10 herds were blood-sampled twice during the immediate post-partum period (on DIM2 and DIM8). Samples were collected and analyzed as previously described. These 1,021 cows were clinically examined daily from DIM1 to DIM8 and records from those that remained clinically healthy throughout this period (247 cows) were used to establish post-partum RIs (PP2 and PP8, respectively) with the aforementioned method. Reference intervals of PP2 and PP8 were fitted to DP and PL RIs, in order to estimate the percentage of cows correctly classified.

Results: Respective CBC 95% RIs (lower – upper limit) for DP, LP, PP2 and PP8 were: RBC: (5.15-7.63), (4.51-6.98), (5.37-8.01), (5.00-7.53) x1012/L, HGB: (87.0-126.9), (71.5-113.5), (91.0-132.8), (83.1-121.0) g/L, HCT: (0.226-0.324), (0.192-0.305), (0.231-0.350), (0.215-0.315) L/L, MCV: (37.39-50.75), (35.03-47.86), (36-51), (35.7-49.7) fl., MCH: (14.34-19.38), (13.92-18.32), (13.84-19.30), (13.70-19.20) x1012/L, MCHC: (361.9-416.5), (356.4-412.6), (352.4-417.6), (348.1-417.9) g/L, WBC: (5.63-13.11), (4.56-14.02), (4.16-15.30), (4.44-17.41) x109/L, neutrophils: (1.25-6.72), (1.40-7.60), (0.86-9.77), (0.85-9.77) x109/L, lymphocytes: (2.01-5.25), (0.89-5.64), (1.43-5.77), (1.61-5.66) x109/L, neutrophils/lymphocytes (N:L) ratio: (0.38-1.80), (0.44-1.96), (0.24-2.84), (0.24-2.84) monocytes: (0.02-1.05), (0.08-1.01), (0.08-1.03), (0.06-1.36) x109/L, eosinophils: (0.08-1.55), (0.03-0.95), (0.05-0.83), (0.04-0.61) x109/L, platelets: (62.5-532.4), (22.1-805.3), (48.2-604.8), (97.5-690.1) x109/L, TP: (53.7-88.4), (5.37-8.01), (5.29-7.236), (5.60-7.884) mg/L. For PP2, percentage of cows correctly classified compared to DP and PL were: RBC: 96.2% and 71.2%, HGB: 95.8% and 61.4%; HCT: 91.6% and 79.3%; MCV: 97.5% and 91.9%; MCH: 98.7% and 85.8%; MCHC: 93.6% and 97.0%; WBC: 83.4% and 96.6%; neutrophils: 83.0% and 88.9%; lymphocytes: 88.9% and 99.0%; N:L ratio: 77.9% and 81.0%; monocytes: 77.5% and 72.5%; eosinophils: 99.5% and 96.0%; platelets: 95.7% and 95.0%; TP: 97.0% and 84.3%. For PP2, percentage of cows correctly classified compared to DP and PL were: RBC: 99.0% and 92.3%; HGB: 96.7% and 88.8%; HCT: 97.0% and 96.0%; MCV: 98.6% and 92.4%; MCH: 96.6% and 91.8%; MCHC: 91.7% and 92.2%; WBC: 84.1% and 97.1%; neutrophils: 89.7% and 88.7%; lymphocytes: 93.7% and 97.0%; N:L ratio: 83.0% and 72.5%; monocytes: 97.1% and 93.2%; eosinophils: 99.0% and 94.0%; platelets: 85.4% and 96.0%; TP: 99.0% and 97.6%; fibrinogen: 83.8% and 99.3%.

Conclusion: More than 10% of cows during the first week post-partum would be misclassified as non-healthy based on their hematology profile, if dry period and peak-lactation RIs were used. Reference intervals derived from cows in dry period or peak lactation are not suitable for fresh cows. Only RIs specially established for the first week post-partum should be used.

Keywords: Hematology reference intervals, Holstein dairy cows, post-partum period.
**IM-17**

**Use of epidural long action steroids on calving paralysis treatment**

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**Objectives:** Calving paralysis is a syndrome that involves motor and/or sensitive disability and proprioception deficit of the hind limbs. This syndrome can present in different ways with a marked abduction associated to inability to adduct the hind limbs. Cows were diagnosed with one or more neurological syndromes: sciatic, tibial paresis, obturator or femoral, based on clinical findings consistent with damage to these peripheral nerves or their nerve roots. These cases of paralysis are more common in beef cattle since they are directly related to prolonged or dystocic calving, due to a maternal-fetal disproportion. The treatment that has been commonly established for this syndrome is based on systemic steroids and NSAIDs (nonsteroidal anti-inflammatory drugs), B-Complex vitamins and physiotherapy. This conventional treatment has shown relative success, being calving paralysis a syndrome that can hardly be reversed in beef cattle. The objective of this study is to provide a new tool for the calving paralysis treatment process through the application of methylprednisolone acetate epidural route. This innovative therapy is based on human medicine studies in which use this way to treat low back pain with very few systemic side effects. Applying epidural steroids, we get them to act directly by spreading from the epidural canal to peripheral nerves, thus decreasing the perineural inflammation that has occurred, encouraging a faster recovery.

**Material and methods:** This retrospective clinical study gathers the cases of 21 beef cows with calving paralysis. All patients underwent a clinical examination and a thorough medical and musculoskeletal clinical examination including flexor-withdrawal and sensitive test, that showed recumbency and sensory incapacity of their hind limbs, so they were treated with methylprednisolone acetate (0.5mg/kg) epidural route, being able to repeat the treatment at 10-15 days if it was necessary. All epidurals were performed between 0 and 24 hours postcalving. Along with the epidural, every cow received a dose of NSAIDs. This study shows the follow-up of all these cases, until the patient is properly able to stand up or, on the contrary, the death or sacrifice of the cow.

**Results:** The treatment based in the application of methylprednisolone acetate [Depo-Moderin®] (0.5mg/kg) epidural route was successful in 72% of the patients studied, getting up on their own on different days: 60% of the cows were able to stand up on the first 2 days, 27% of the cows on the 3rd or 4th day, and the remaining 13% from the 10th day.

**Conclusions:** In calving paralysis, the longer the cow remains in decubitus position, the worse the prognosis. So, with this results, the use of epidural long action steroids is proposed as a treatment to improve the prognosis and accelerate the recovery of patients. If the patient has a good body condition and is alert, epidural long action steroids combined with physiotherapy may be effective. Further studies are necessary, increasing the number of cases to be analysed, in order to ensure with certainty the effectiveness of this treatment.

**Keywords:** Calving paralysis, epidural, methylprednisolone.

**IM-18**

**Bright blindness in a goat flock associated with fern (Pteridium aquilinum) intake**

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**Objectives:** In the month of August 2021, a clinical case of blindness in a goat flock was referred to the Ruminant Clinical Service of the University of Zaragoza by a practitioner working in the Basque Country (Spain). Affected animals belong to a pet flock of 12 Valais breed goats reared in an extensive production system. The goats were grazing in a 4-hectare field along with some animals of other species. According to the veterinarian, there was little food in the pasture, and ferns predominated, which the farmer reported that the goats ate greedily.

The practitioner referred that in June 2021, six goats were presented with nervous clinical signs. After neurological examination, he noticed almost complete blindness in all six animals.

**Material and methods:** A clinical examination of the affected animals was performed. They presented a low body condition (2/5), heart and breath rates were normal, as well as body temperature. In addition, the examination revealed mild blindness as the only neurological clinical sign. The farmer referred that goats presented a bright greenish light in the eyes at night.

One of the goats died and was referred to the Pathological Service of the Veterinary Science Faculty for the post-mortem exam. Cerebellum, encephalon and the eyes were taken for histopathology, while sterile swabs were collected for microbiology and Rt-PCR.

**Results:** A differential diagnosis was carried out based on the clinical signs and the history. Some disorders such as heavy metal or fern intoxication were taken into consideration. Likewise, listeriosis, vitamin A or B deficiency and individual causes such as retinal detachment, cataracts or an eye ulcer were included in the differential diagnosis.

The main suspicion was intoxication based on the chronic ingestion of fern (Pteridium aquilinum) referred to by the farmer. These plants contain high concentrations of ptaquiloside, a toxic component that causes stenosis in the retinal vessels damaging and vanishing the layer of rods and cones completely. This narrowness caused an increase in tapetum lucidum reflectance, which is called “bright blindness”.

Microbiological study and Rt-PCR showed negative results.
for *Listeria monocytogenes*, and thus the necropsy revealed it was not an inflammatory process but degenerative.

At the *post mortem* macroscopic examination, there were no major findings in the brain, and no signs of cerebrocortical necrosis were found under ultraviolet light. The following histopathological study only revealed representative damage in the eye: the layer of rods and cones had almost completely disappeared, and the consequent retinal degeneration reached the macula.

**Conclusions:** The ingestion of *Pteridium aquilinum* is associated with intoxication that can have an acute or chronic evolution with different clinical pictures. Acute poisoning, also called haemorrhagic fever, is more common in cattle than sheep and presents the characteristic clinical picture of a haemorrhagic syndrome. The continued intake of low doses of toxins (chronic intoxication) is associated with the appearance of bovine enzootic haematuria, gastrointestinal carcinomas, polioencephalomalacia (action of thiamine) and a particular form of the disease described in sheep known as "bright blindness". This clinical picture has not been described so far in goats.

Progressive bilateral blindness characterises this disorder due to a degeneration of the retinal neuroepithelium. The eyes glow abnormally in the gloom. There is no inflammation or opacity of the cornea. After the eye exam, the veins and arteries are narrower, and the main vessels are more separate than normal.

**Keywords:** Brigh Blindness, Fern, Goat.

**IM-19**

**A Consensus on Calf Scour – Repositioning Vets at the Centre of the Solution**


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**Objectives:** This abstract summarises the outputs of an expert group of calf health professionals, who met remotely to discuss this topic in October 2021. The objective was to provide an updated, qualitative assessment of the current challenges on farm with regards to calf scour, including diagnostic methods used and opportunities for improvement. The ultimate objective was to help vets to re-establish themselves as the go-to people for calf scour on farm.

**Material and methods:** A meeting was held on 6th October 2021, using the Zoom virtual meeting platform. Ten calf health experts from across Europe and the Middle East attended, along with 13 Boehringer Ingelheim technical managers with extensive calf health experience. Six of the experts were in the same room as their country BI technical managers. Participants engaged in a 2 hour workshop, covering 4 main areas, with interaction through the Miro whiteboard platform. Firstly, participants were asked about the rational and emotional challenges of veterinarians and farmers from calf scour. Secondly, participants considered the methods of detecting the causes of and contributors to diarrhoea, and methods of treatment. Thirdly, participants were asked to consider the strategies for dealing with these causes. And lastly, participants were asked to consider the opportunities for reducing the impact of calf diarrhoea.

**Results:** In considering the rational challenges of diarrhoea in calves for the vet and farmer, there is often poor understanding among farmers of the disease, including economic impact, and the need for prevention as well as treatment. Investigation of a disease outbreak often requires a much larger investigation to consider underlying contributing factors such as general health status, hygiene, nutrition and the transfer of passive immunity. Farmers find treatment difficult and time-consuming, therefore it needs to be straightforward, and involvement of the vet is required to help appreciate the “bigger picture”. Vaccination should have a good ROI and farmers must remember to continue vaccinating after the disease has gone.

When considering the emotional challenges of diarrhoea, the vet needs to be recognised as the expert and to feel confident in the interventions they recommend. The vet needs to feel empathy with the farmer, recognising the extra workload that disease brings to farmers, as well as sharing the decision “journey” in developing interventions. The farmer cares about their animals’ welfare, seeing healthy animals makes them feel good and they want to make sure their farm practices are the best they can be. They want more than just medicines and vaccines from their vet and need a good relationship if they are to bring the best to their farm. They want to be confident in the sustainability of their enterprise, but also have time to spend with their family now. They also want to give a good image to the public by producing good, high-quality food.

When considering the methods of identifying the causes of diarrhoea, a standard clinical examination, as well as an investigation of the overall farm environment and calf nutrition, are essential. Antigen tests (cow-side and laboratory) and other diagnostic opportunities may more specifically identify...
an infectious cause. Farm audit and investigation of colostrum management are also of value. Farmers are often reluctant to support a full farm audit owing to the cost and time required, even though non-specific factors may be more significant in some outbreaks.

On-farm opportunities to reduce the impact of calf diarrhoea focus around creating a pride in producing a high-quality, high-welfare animal, and improving education and understanding of the disease (including economic impact) by the farmer, with the provision of formalised, specific treatment protocols and improved data recording. Ensuring good colostrum management is still considered an opportunity for improving calf health, and technological opportunities to improve the monitoring of calf health are considered valuable.

Conclusions: The diverse group of calf health experts identified that there is much potential for improvement on farm with regards to calf scour. This is despite the relatively widespread use of vaccines, however the vet has, in some instances, become disconnected from this discussion. The consensus of this Calf Expert Group was that vets are ideally positioned to provide a calf health solution, combining an understanding of and interventions in management, environment and immunity of neonatal calves.

Keywords: Calf scour, diarrhoea, consensus, vaccine, veterinary engagement.