

## DI-01

**Application of texture analysis of b-mode ultrasound images for the quantification and prediction of intramuscular fat in living beef cattle: a methodological study**

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**Objectives:** Intramuscular fat (IMF) plays a key role in determination of beef meat quality, because it contributes significantly to the aroma and tenderness of the meat. However, methods to determine fat % in muscle mass rely on visual inspection or on fat extraction from meat samples, and therefore require the animals' to be slaughtered. IMF determination *in vivo* could permit early meat quality estimation, and thus lead to changes in the animals' management to influence meat composition and better meet market requests. The aim of this methodological study was the elaboration of a formula capable of IMF% prediction from real-time ultrasound (RTU) images live beef cattle.

**Material and methods:** 26 Charolaise heifers were enrolled in the study and their longissimus dorsi (LD) muscle was investigated. Ultrasound images were scanned on the animals' right side between the 12th and 13th rib with a portable ultrasound scanner (MyLabOne™, Esaote S.p.a., Genoa, Italy) equipped with a multi-frequency convex probe (SC3421, Esaote S.p.a., Genoa, Italy; 2.5 – 6.6 MHz). All scans had 4.3 MHz frequency, 15 cm depth, and 100% gain. Texture analysis of the collected scans was performed by means of a free purpose-specific software (MaZda v4.6; Technical University of Lodz, Institute of Electronics, Poland).

One week after the *in vivo* examination, the animals were slaughtered and the whole cut of the 12th rib was collected. The cut was dissected into muscles, fat and bones. The sample of LD was analyzed with centesimal extraction: IMF% was determined by extraction with petrol ether (Randall) method.

Animals were divided in 3 groups depending on their mean lipid content percentage in 100g meat, and thresholds were chosen using IMF mean  $\pm$  ½SD (Group 1 included animals with IMF below 4.24 g; Group 2 included between 4.25 g and 5.75 g; and, Group 3 included animals with IMF higher than 5.76 g).

Texture parameters were screened with a stepwise linear discriminant analysis using IMF measured by chemical extraction (IMFq) as the dependent variable, and the results of the texture analysis as explanatory variables, to identify the best combinations of high-quality variables. The aim was a parsimonious model with as few parameters as possible, to enhance stability during validation.

The differentiation efficiency of IMFq was tested by means of the receiver operating characteristic (ROC) curves. Bland-Altman analysis was performed to validate the method and to assess the agreement between IMFq and IMFpred.

**Results:** Each scan generated approximately 300 texture parameters. Among these, 6 variables were identified as predictive by the stepwise analysis and were molded into a multiple regression equation.

IMF in the samples was then predicted by means of the formula (Predicted IMF, or IMFpred), and compared to the quantified IMF (IMFq).

Among all samples, the mean IMFq extracted from the meat was 5.08  $\pm$  1.47 g, while the mean IMFpred was 5.07  $\pm$  1.35 g.

A high linear correlation between IMFq and IMFpred was found ( $r^2=0.85$ ) and results from the ROC analysis showed an Area Under the Curve (AUC) of 92%, with a sensitivity of 80% and a specificity of 93.7%, while results from the Bland-Altman plot were  $\pm$  1.96 ( $\pm$  1.11SD).

**Conclusions:** In the present study, IMF% from beef cattle LD muscle was estimated successfully and with high accuracy, using RTU, one week prior slaughter.

Application of this technology on wide-scale breeding could lead to important economic impacts. A good accuracy in IMF% prediction could permit screening of the animals that are going to be slaughtered, and see whether their fattening is optimal for the market requirements, maximizing the profits.

This technology could also permit monitoring of fattening cycles, leading to rapid estimation *in vivo* of adequacy of the diets fed to the animals. Lastly, it could be used for genetic selection, bypassing lengthy genetic progeny testing and therefore saving large amounts of time.

Further studies to validate the method both on a wider sample and on different sex and breeds are encouraged, but such technology could be a powerful heard selection tool as well as assist farmers in fattening practices.

**Ethical Standards:** All animals were slaughtered according to EU regulations (Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing).

**Keywords:** Beef Cattle, Fat Prediction, Intramuscular Fat, Texture Analysis, Ultrasonography.

## DI-02

**Practical use of thoracic ultrasonography at feedlot arrival to identify high-risk BRD batches in suckler calves**

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**Objectives:** Bovine Respiratory Disease (BRD) is the most important disease in veal industry. The assessment of lung health of suckler calves' batches at their arrival to the rearing facilities is key for decision making and implementation of prevention and metaphylactic protocols for BRD control. However, classification and identification of high-risk BRD batches (30% prevalence) continues to be a major challenge. BRD diagnosis based on clinical signs is inaccurate (61.8%



sensitivity and 62.8% specificity; White, 2009).

The use of thoracic ultrasound (US) scanning to evaluate lung health has demonstrated to be a high sensitivity and specificity tool (79.4% and 93.9%, respectively; Buczinski, 2015). A previous study in 811 veal calves using this technique at arrival (Tejero et al., 2019) showed an average of 21% moderate and 10% severe lung lesions. However, large variability was found among batches ( $P < 0.05$ ).

In high-risk BRD batches, metaphylactic treatment has the potential to reduce the number of animals infected with a susceptible bacterial pathogen, clinical or subclinical, reducing the disease challenge in the environment for non-infected animals and limiting disease spread (Nickel, 2010). Thoracic US scanning has the potential to identify high-risk BRD batches where metaphylactic treatment is justified. However, under field conditions, thoracic US scanning is time consuming. The main objective of this study was to evaluate if the selection of a representative sample of animals for thoracic US scanning provides a reasonable accuracy to identify high-risk BRD batches.

**Materials and methods:** The number of calves to be sampled per batch was calculated using free epidemiological software (<https://epitools.ausvet.com.au>). An estimated prevalence of 30% (high-risk BRD) using a confidence level of 95% and an accepted error of 10% for calves with lung lesions equal or higher than 3, using Adams and Buzsinski (2015) scoring system, was used.

To check the practical use of the sampling table created, a simulation of this sampling procedure was done in 12 batches where thoracic US was assessed in 100% of the animals. A randomization procedure (Excel, Microsoft Office) was used to select the different sampling groups, performing 10 random samples of each batch in order to measure the degree of success to predict whether the batch was a high-BRD risk one.

**Results:** The percentage of calves sampled ranged from 21% to 62% for a 300 or 50 suckler calves' batch respectively.

Our Database of 12 batches, where thoracic US was performed in a 100% of calves at arrival, was used for random selection of calves. Real batches included 12 to 51% of calves affected with score higher than 3 (moderate and severe cases). Most of the batches were high (>30% of calves with lung lesions = 42%) or medium (20-30% of calves with lung lesions = 50%) and only one (8%) was a low risk batch (<20% of lung lesions). Randomized selection of a percentage of calves following the sampling table was performed 10 times. The average results for these 10 randomizations were very similar to the real ones, and the estimated sensitivity was 96% for batches with at least a 30 % of prevalence of disease.

**Conclusions:** Thoracic US scanning of a randomized percentage of calves at arrival may be a useful technique to identify high-risk BRD batches where metaphylactic treatment is justified. This technique can be a practical tool for a more rational use of antibiotics in the veal industry.

**Keywords:** Feedlot, BRD, ultrasound, thoracic, calves.

### DI-03

#### Applications of infrared thermography as a non-invasive technique early indicator of fever in the lambs

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**Objectives:** Ovine Respiratory Complex (ORC) is one of the main causes of morbidity and mortality in sheep feedlots with high prevalence and economic repercussions (González, 2018). Early ORC diagnosis is essential to minimize the effects of the disease, related with both the productive results and welfare of the animals.

Body temperature is an objective clinical data, although its increase is not always associated with disease, as can be also a response to the high environmental temperature or exercise. However, when it is associated with other more specific clinical signs, it is very useful for early diagnosis. Indeed, studies monitoring temperature using ruminal boluses in cattle, demonstrate how the thermal increase occurs 12-136 h before the caregiver detected other more specific clinical signs of pneumonia (Timsit et al., 2011).

On the other hand, thermography is a technology with increasing use in different fields of the veterinary medicine. Thermographic cameras detect infrared radiation emitted by the surface of that body, converting it into an electrical signal that is transformed into a color image. Indeed, Schaefer y col. (2007) already demonstrate it is useful to identify early disease in cattle. However, environmental temperature at the time of thermal image can affect rectal temperature (Church et al., 2014).

Then, the purpose of this study was to evaluate the use of an infrared thermographic camera taking into account environment temperature for the detection of animals with hyperthermia in an intensive lamb herd.

**Material and methods:** This study was carried out in a lamb fattening farm located in Córdoba (Spain). Infrared temperatures were measured in a total of 2,842 multiple source commingled Merino lambs and its crosses, 60 to 120 days old, and 18 to 30 Kg live body weight. Following previous Schaefer et al. (2007 and 2012) studies in cattle, we performed an orbital eye measurement, as in other anatomical parts the presence of hair or wool can interfere with results.

The measurements were made after the passage of the lambs through the handling or sorting sleeve and in the lazarrette measuring in duplicate both rectal temperature with a conventional thermometer and eye thermal image with an infrared thermometer.

For infrared thermography the camera Testo model 870 was used, with a resolution of 160 x 120 pixels (with the possibility of increasing to 320 x 240 pixels), thermal sensitivity

<100 mK, 34° lens with fixed focus and integrated and calibrated digital camera. The image processing software was the IRSoft Software, version 4.5. While the thermometer used was the Kruuse clinical thermometer, model Veterinär-thermometer SC 12, also calibrated, with automatic stop system by sound signal and with a measurement accuracy of 0.1°C.

At measurement time, the following data were additionally recorded: date and time, farm identification, barn, pen, etc. In addition, the inside and outside barn temperature was noted. Subsequently, we calculate a new parameter: the difference between rectal and ocular temperature (DROT).

The data were analyzed using Anova one-way for the comparison of means, as well as the correlation between the variables and the multiple linear regression using the statistical program IBM SPSS Statistics, version 25.

**Results and discussion:** We evaluate the effect of barn temperature on the ocular and rectal temperature. On the other hand, we evaluate its effect on different rectal temperature strata.

The correlations between the different variables were significant. Indeed, the combined use of variables, eye temperature and barn temperature, allowed us to establish a formula predicting the rectal temperature with an excellent correlation ( $R^2 = 0.9996$ ).

$$\text{Rectal } t^a = 1.099 * \text{ocular } t^a - 0.038 * t^a \text{ nav}$$

**Conclusions:** The rectal temperature of lambs under intensive conditions is an objective clinical parameter very useful for ORC early diagnosis. Infrared thermography is a fast, automatic and non-invasive technique helpful for early identification of sick animals. And considering the barn temperature at lecture time improves its correlation with rectal temperature.

**Keywords:** Fever, thermography, lambs, environmental  $T^a$ , ORC.

#### DI-04

### Comparison between thoracic ultrasonography and visual observation for the detection of BRD in veal calves

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**Objectives:** High levels of mortality and morbidity due to bovine respiratory disease (BRD) in combination with animal welfare, reduction of antimicrobial use, and antimicrobial resistance are currently the most important challenges in the beef industry.

The detection of BRD in farms is based on respiratory clinical signs (cough, dyspnea, ear position, nasal discharge) and increased body temperature. However, the use of clinical signs to detect lung lesions associated with BRD has some limitations (Leruste et al., 2012; Sensitivity (Se) 61.8% and specificity (Sp) 62.8%; White, 2009). Recently, new tools have been developed to achieve a more accurate early diagnosis. Among them, Thoracic Ultrasonography (TUS) shows the highest ac-

curacy to detect pneumonia at farm level (Se 79.4% and Sp 93.9%; Buczinski, 2015; Se 89% and Sp 95%; Berman, 2019).

The objective of this study was to compare Thoracic US with BRD clinical diagnosis performed by two senior veterinarians both at arrival to the rearing facilities and later along the first month on fattening.

**Materials and methods:** Two different batches of a commercial veal feedlot with 62 and 45 male calves were evaluated. The day of arrival, two experienced veterinarians (observer 1;O1 and 2;O2) classified the 107 calves as “BRD” or “Healthy” based on their clinical signs. Then, an experienced veterinarian performed thoracic US scanning (TUS) and classified the calves as having or not having lung lesions using a scoring system (Adams and Buczinski, 2015). The three veterinarians performed their scoring in a blind manner. All animals were scored again by the three veterinarians 7 and 21 days after their arrival. The different scoring results were recorded to evaluate BRD prevalence and agreement between the three scoring results. The TUS score was considered as the gold standard technique.

The comparison between the use of the diagnostic techniques (Se, Sp, positive predictive value – PPV- and negative predictive value-NPV-) and observers (concordance kappa index) was performed with free epidemiological software (<https://http://www.winepi.net>) using a confidence level of 95%.

**Results:** The prevalence of lung lesions detected by TUS at arrival was 35% for batch1 and 36% for batch2 respectively. Using clinical scoring, the prevalence of BRD was different between the O1 and O2 for batch1 (O1: 45%; O2: 29%) and batch2 (O1: 47%, O2: 38%). In the first review performed 7 days after arrival, BRD diagnosed by TUS increased to 75% and 62%, respectively for batch1 and 2. BRD prevalence diagnosed by the observers was also different in batch 1 (O1: 65%; O2: 72%) and in batch 2 (O1: 53%, O2: 47%). In the examination performed 21 days after arrival, BRD prevalence diagnosed by TUS was 67% and 49%, respectively for batch1 and 2. BRD prevalence diagnosed by the observers was (O1: 60%; O2: 52%) in batch1 and (O1: 20%, O2: 27%) in batch2.

Although the average Se and NPV were quite similar for both observers (Se 63.8% and 64.9% and NPV 60.1% and 65.3%, for O1 and 2 respectively), the Sp and PPV was higher for O2 (Sp 66.0% and 79.9% and PPV 69.4 and 79.65, for O1 and 2, respectively).

Finally, the concordance among both observers was moderated (kappa = 0.598, IC95% 0.29-0.71). Indeed, one of the observers consistently presented a better detection of BRD signs than the other. However, even for this observer, 28% of BRD cases detected by thoracic US were not identified as BRD using clinical diagnosis.

**Conclusions:** The present study suggests that Thoracic Ultrasonography can be used as an on-farm technique for BRD diagnosis. However, clinical diagnosis by experienced observers may be good enough to be used as a screening technique to identify BRD calves. The results also suggest that, with training, observers may improve their Sensitivity and Specificity for a better detection of treatment of BRD calves.

**Keywords:** Ultrasonography, feedlot, BRD, diagnosis, clinical signs.



## DI-05

### Electrical impedance tomography (EIT) to evaluate peak inspiratory and expiratory flow levels and distribution of ventilation in steers

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Electrical impedance tomography (EIT) is a novel non-invasive imaging technique that allows a visual representation of the distribution of ventilation in real-time. The aim of this study was to determine if changes in lung status can be detected by changes in EIT variables in anaesthetised steers.

In this randomised cross-over study, seven anaesthetised spontaneously breathing steers lungs were exposed to atmospheric airway pressure (NoPres), or continuous positive airway pressure of 7 cmH<sub>2</sub>O (PosPres). Steers were positioned in dorsal recumbency. EIT electrodes mounted on a belt were placed around the thorax to measure impedance changes. Post induction, EIT recordings were performed at 30, 50, 70 and 90 minutes respectively. From each recording ten breaths were retrospectively analysed and pooled for the pixel with maximal inspiratory (FlowPix<sub>insp</sub>) and expiratory flow (FlowPix<sub>exp</sub>) within the EIT image and the centre of ventilation, illustrating shift of ventilation distribution along the gravitational axis, between right and left lung (CoV<sub>r-l</sub>) and ventral to dorsal lung (CoV<sub>v-d</sub>). Descriptive statistics were used to describe the difference between NoPres and PosPres and are reported as mean (95% confidence interval).

Lower FlowPix<sub>insp</sub> and FlowPix<sub>exp</sub> were measured during PosPres compared to NoPres; FlowPix<sub>insp</sub> [311 (282-340) vs 352 (315-388)] and FlowPix<sub>exp</sub> [268 (249-287) vs 313 (282-343)], respectively. The CoV<sub>r-l</sub> was lower [54 % (53-55)] in PosPres than in NoPres [61 % (58-63)] while only a small difference of 3 % was seen for CoV<sub>v-d</sub>.

EIT can be used to detect differences in lung status by flow and CoV measurements in anaesthetised cattle.

Ethical Animal Research: This study was approved by Murdoch University ethics committee R2998/17

**Keywords:** Electrical impedance tomography, Ventilation, Cattle.

## DI-06

### Preliminary analysis in the flank temperature pattern in dairy cows during pregnancy

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**Objectives:** Recent years have seen a significant increase in interest in the use of thermal imaging in veterinary diagnostics, especially in a health herd management of live-stock animals. Owing to the possibility of fast, non-invasive and automated temperature measurement on the surface of a cows' body, this technique shows great potential in predicting local changes in the tissue metabolism and local blood circulation. The abdominal area demonstrated physiological changes during late-gestation related to the increased demand for the growing uterus and more loaded abdominal wall muscles. However, there is still a need for further research to standardize measurement data in thermal imaging. Therefore, the aim of the study was evaluated if the changes in physiological activity during late pregnancy may result in abnormal thermal patterns of the flank of dairy cows.

**Materials and methods:** Thermographic images of the right flank were obtained from 54 Holstein-Friesian dairy cows from a commercial 550 head dairy farm. All cows were housed under the same environmental conditions. The pregnancy was confirmed with ultrasound examination on day 32 after insemination and one the day of imaging in 30 cows. Based on ultrasound, the cows were divided into the following groups: NP - nonpregnant (n=24); EP - early pregnancy (1<sup>st</sup> to 3<sup>th</sup> months; n=6), MP - mid pregnancy (4<sup>th</sup> to 6<sup>th</sup> months; n=6) and LP - late pregnancy (7<sup>th</sup> to 9<sup>th</sup> months; n=24: 7<sup>th</sup> Mo, n=6; 8<sup>th</sup> Mo, n=6, 9<sup>th</sup> Mo, n=6). Images were taken indoor, with ambient temperature ranging from 14.0 to 16.0°C, using infrared radiation camera (emissivity 0.99; FLIR Therma CAM E60). Temperature pattern of the flank area was evaluated using five regions of interest (ROI 1–5) representing: the area along the transverse processes of the spine (ROI 1), the cranial area of the right abdominal surface (ROI 2), the cranial area of the right abdominal surface (ROI 2), the middle area of the right abdominal surface (ROI 3), the caudal area of the right abdominal surface (ROI 4), and the stifle area (ROI 5). Each ROI was analyzed for the minimal (T<sub>min</sub>), maximal (T<sub>max</sub>), and mean (T<sub>mean</sub>) temperatures. The results (mean±SD) were compared between NP, EP, MP, 7<sup>th</sup> Mo LP, 8<sup>th</sup> Mo LP, and 9<sup>th</sup> Mo LP groups.

**Results:** The T<sub>min</sub> was higher (p<0.0001) in all five ROIs in LP (19.67°C ±1.11) than in NP (13.55°C ±3.34), EP (14.92°C ±1.69), and MP (14.88°C ±1.64). There were no differences (p>0.05) in T<sub>min</sub> between NP, EP and MP as well as between 7<sup>th</sup> Mo (18.85°C ±1.40), 8<sup>th</sup> Mo (19.14°C ±1.50), and 9<sup>th</sup> Mo (19.68°C ±1.41) of within LP group. The T<sub>max</sub> was comparable in all five ROIs, with no differences (p>0.05) between NP (26.51°C ±2.24), EP (27.39°C ±2.57), MP (24.22°C ±3.16), and LP (25.53°C ±2.29) groups, regardless of the month of preg-

nancy. The  $T_{\text{mean}}$  was higher ( $p < 0.01$ ) in ROI 1 and 5 in LP (ROI 1:  $22.35^{\circ}\text{C} \pm 0.95$ ; ROI 5:  $24.78^{\circ}\text{C} \pm 1.09$ ) than in NP (ROI 1:  $17.54^{\circ}\text{C} \pm 4.42$ ; ROI 5:  $19.27^{\circ}\text{C} \pm 4.09$ ), EP (ROI 1:  $18.96^{\circ}\text{C} \pm 1.75$ ; ROI 5:  $19.88^{\circ}\text{C} \pm 1.87$ ), and MP (ROI 1:  $19.71^{\circ}\text{C} \pm 0.08$ ; ROI 5:  $19.97^{\circ}\text{C} \pm 1.28$ ), with no similar differences ( $p > 0.05$ ) in ROI 2, ROI 3, and ROI 4.

**Conclusions:** Thermography is proposed as a promising tool in determining changes in the flank temperature pattern in dairy cows during pregnancy. An application of different regions of interest during the superficial temperature analysis allows to established the discreet changes in the mean temperatures during late pregnancy. The minimal temperature seems to be the most reliable indicator of the superficial temperature changes related to the months of pregnancy. After examining more numerous groups of cows, the external temperature of the flank may be a useful method for the presumption of pregnancy in late gestation.

**Keywords:** Thermography, cow, pregnancy diagnosis.

## DI-07

### Associations between TUS scores and mortality and productivity outcomes on Scottish dairy farms

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**Objectives:** A previously published study (1) used thoracic ultrasound (TUS) to assess farmer accuracy of diagnosing Bovine Respiratory Disease (BRD) in cattle youngstock. We found that sensitivity of farmer diagnosis of BRD was low; demonstrating that animals with lung lesions were not being identified and treated for BRD. We also found that some farmers treated animals which did not have signs of BRD on TUS, suggesting unnecessary use of antimicrobials. This study aimed to follow the same calves assessed in our previous work, to determine the effects of lung consolidation, as diagnosed by TUS, on future mortality and reproductive outcomes.

**Materials & Methods:** TUS examinations were conducted between 2019-03-13 and 2019-11-26, by a single veterinarian over 7 dairy herds. All calves on the farm between 21 and 61 days of age during these time points were scanned and scored. This included male and female calves destined for dairy or beef rearing, which ensured a larger dataset. On scoring, they were given a TUS score on a 0-6 scale (2) as well as a calf health score based on the Wisconsin calf health scoring system which was converted to a binary "normal" or "abnormal" TUS score. Further descriptions and results of this part of the study have been published previously (1).

For the current study, all calves which had a TUS examination were included in the dataset for analysis of mortality. Female dairy calves retained on the study farms were followed through to calving, for analysis of reproductive outcomes using the farms' management system and data collection software. Mixed effects logistic regression models were used to analyse

associations between TUS score and mortality status, conception within 3 serves, time to conception and the number of serves to conception. Data was only collected for animals that were retained on the farm for the purpose of entering the adult milking herd. This meant a proportion of animals were lost to follow-up as they were sold for beef to other farms not included in the study.

**Results:** The initial dataset consisted of 347 ultrasonographic examinations. Of these, 53 calves (15.3%) were classified as abnormal and 294 (84.7%) as normal. Of the 53 calves classified as abnormal, only 13 (24.5%) were treated by the farmer, however, of the 294 classified as normal, 22 (7.5%) were treated. Of the 347 animals, 18 animals died during the follow up period of weaning to conception (5.2%), and mixed effects regression model results suggested that there was a tendency for animals with an abnormal TUS score as calves to be more likely to die during this time (OR 2.33, 95% CI 0.9-6.1,  $P = 0.08$ ).

Data for pregnancy status within 3 serves were available for 149 animals, with 16/22 (72.7%) of animals with abnormal TUS status becoming pregnant and 114/127 (89.8%) of animals with normal TUS status becoming pregnant. Mixed effects regression model results suggested that abnormal TUS score was associated with lower odds of becoming pregnant within 3 serves (OR 0.32, 95% CI 0.1-1.0,  $P = 0.06$ ). No associations were found between TUS score and time to conception or the number of serves to conception.

**Conclusion:** Follow up analysis of dairy cattle which had undergone TUS between 21-61 days of age suggest a tendency for increased mortality in animals with higher abnormal TUS scores, and a tendency for decreased pregnancy rates within the first 3 serves. This reflects similar work undertaken in other countries (3,4). To the author's knowledge, this is the first study in the United Kingdom to ascertain the prevalence of subclinical BRD in pre-weaned dairy calves, and to follow these calves to adulthood to further investigate the effects of BRD on future performance.

Alongside the immediate health, welfare, and productivity impacts of clinical and subclinical respiratory disease, it is likely that subclinical BRD during the preweaning period is associated with future reproductive and mortality outcomes. The prevention of pneumonia in calves has the potential to improve health and production outcomes. For future work, we will follow these animals to the end of their first lactation to gather data on 305-day lactation yields to analyse for associations with calthood pneumonia.

**Keywords:** Thoracic ultrasound, calf, bovine respiratory disease.



## DI-08

### Dynamics of subclinical pneumonia in male dairy calves in relation to antimicrobial therapy and production outcomes

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**Objectives:** Quick thoracic ultrasonography (qTUS) is increasingly used as an on-farm method to diagnose clinical and subclinical pneumonia in dairy calves. The primary objective of this study was to describe dynamics of lung consolidation in a purchase dependent production system for male dairy calves (veal) in relation to antimicrobial therapy and respiratory diagnostic tests. In addition, the association of cured and uncured pneumonia with average daily growth (ADG) and cold carcass weight (CCW) were studied, as well as the effects of arriving with lung consolidation on the probability of developing chronic unresponsive pneumonia and reduced performance.

**Materials and methods:** A prospective cohort study was conducted involving 295 male Holstein Friesian calves, aged 14-21 days during one production cycle in a commercial veal farm. Both clinical examination and qTUS were performed on all animals upon arrival, at week 2, 3, 4, 6, 8 and 12. Pneumonia was defined as the presence of a lung consolidations  $\geq 1$  cm. Pathogen detection was done using cultures, Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) (Brüker Daltonik GmbH, Bremen, Germany) and whole genome nanopore sequencing (WGS) on non-endoscopic bronchoalveolar lavage samples (nBAL).

**Results:** Of the calves, 17.6% (52/295) arrived with a lung consolidation  $\geq 1$  cm. At the first outbreak of respiratory disease this number had risen to 30.8%. Initial therapy with tulathromycin and subsequently doxycycline appeared ineffective, resulting in a rise in the prevalence of animals with pneumonia up to 43.8% in week 4. At the start of the first outbreak in week 2, the vast majority (91.2%) of the pneumonia cases ( $n = 91$ ) were subclinical. At week 4 the outbreak became more clinical and therapy with amoxicillin resulted in a cure rate of 52.7%. Bacterial cultures and nanosequencing diagnostics on nBAL samples identified bovine respiratory syncytial virus (BRSV) and *Mycoplasma bovis* as the dominant agents in the first outbreak. The isolated *M. bovis* strain had mutations encoding for macrolide resistance. The second outbreak was characterized by a *Pasteurella multocida* superinfection and isolation of a different *M. bovis* strain. Evaluated over the complete observation period, 53.9% of the calves cured upon antimicrobial therapy. Of the 46.1% calves that were not cured ( $n = 111$ ), 29.7% (33/111) had developed chronic pneumonia. Calves with uncured or chronic pneumonia had a significantly lower ADG (992g/day  $\pm 174$  and 930g/day  $\pm 146$ , respectively) compared to calves that did not developed pneumonia (ADG = 1103 g/day  $\pm 156$ ;  $P < 0.001$ ). In contrast, calves that did fully cure had a similar ADG as calves that never developed pneumonia (1045 g/day  $\pm 139$  and 1103 g/day  $\pm 156$ , respectively;  $P = 0.09$ ). The effect of uncured pneumonia was no longer significant for cold carcass weight. Calves with lung consolidation upon arrival had a significantly lower ADG (981 g/day  $\pm 159$  vs 1045 g/day  $\pm 159$ ;  $P = 0.01$ ) and were more likely to develop

chronic pneumonia (OR=4.2; 95% CI: 2.1 – 8.6,  $P < 0.001$ ). Animals with chronic pneumonia, in turn, had a significantly lower cold carcass weight (10.3kg  $\pm 4.4$ ; CI: 1.2 – 18.9;  $P = 0.02$ ).

**Conclusion:** The prevalence of pneumonia in veal calf facilities proves to be much higher than previously expected, mainly because of its subclinical nature. Animals with pneumonia have a reduced daily growth but appear to be able to catch up to healthy animals when cure is achieved before the age of 12 months. Chronic unresponsive pneumonia is frequently encountered and the main reason for production loss. Calves that already had lung consolidation upon arrival on the veal facility had a higher risk of developing chronic unresponsive pneumonia. Both qTUS and nBAL diagnostics offer potential for better control and prevention of bovine respiratory disease in dairy calves.

**Keywords:** Bovine respiratory disease, subclinical pneumonia, *Mycoplasma bovis*, bovine respiratory syncytial virus, economics.

## DI-09

### Use of quick thoracic ultrasonography (qTUS) to guide and evaluate antimicrobial therapy in three natural outbreaks of *Mycoplasma bovis* pneumonia

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**Objectives:** Quick thoracic ultrasonography (qTUS) is an accessible on-farm method to reliably diagnose (subclinical) pneumonia in both dairy and beef calves, offering great potential for more rational antimicrobial use and better control of bovine respiratory disease. The objective of this study was to describe the application of qTUS to guide and evaluate antimicrobial therapy in three natural outbreaks of *Mycoplasma bovis* associated pneumonia in a single beef farm.

**Materials and methods:** A retrospective analysis was performed on ultrasonographical and diagnostic data collected from 3 different outbreaks of respiratory disease in the same closed commercial beef farm housing 1000 Belgian Blue beef cows. Calves at risk aged between 2 and 6 months and were housed in groups of 6 in fully separated pens. Pneumonia was defined as a lung consolidation  $\geq 1$ cm while cure was defined by re-aeration of these lesions to  $< 1$ cm in depth. In each outbreak, qTUS was used as a tool to initiate, stop or generally evaluate antimicrobial therapy. In outbreak 1, ultrasound-guided full metaphylaxis was used, meaning that all animals at risk ( $n = 130$ ) were metaphylactically treated (one injection), after which antimicrobial therapy was only prolonged in animals which still had lung consolidation. Antimicrobial treatment was stopped as soon as ultrasonographical re-aeration of the lung occurred. Outbreak 2 ( $n = 94$ ) was approached by individual treatment of animals with ultrasonographically confirmed pneumonia, without metaphylaxis. Finally, in the third outbreak ( $n = 61$ ), an ultrasound-guided targeted metaphylaxis,

## DI-01

**Application of texture analysis of b-mode ultrasound images for the quantification and prediction of intramuscular fat in living beef cattle: a methodological study**

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**Objectives:** Intramuscular fat (IMF) plays a key role in determination of beef meat quality, because it contributes significantly to the aroma and tenderness of the meat. However, methods to determine fat % in muscle mass rely on visual inspection or on fat extraction from meat samples, and therefore require the animals' to be slaughtered. IMF determination *in vivo* could permit early meat quality estimation, and thus lead to changes in the animals' management to influence meat composition and better meet market requests. The aim of this methodological study was the elaboration of a formula capable of IMF% prediction from real-time ultrasound (RTU) images live beef cattle.

**Material and methods:** 26 Charolaise heifers were enrolled in the study and their longissimus dorsi (LD) muscle was investigated. Ultrasound images were scanned on the animals' right side between the 12th and 13th rib with a portable ultrasound scanner (MyLabOne™, Esaote S.p.a., Genoa, Italy) equipped with a multi-frequency convex probe (SC3421, Esaote S.p.a., Genoa, Italy; 2.5 – 6.6 MHz). All scans had 4.3 MHz frequency, 15 cm depth, and 100% gain. Texture analysis of the collected scans was performed by means of a free purpose-specific software (MaZda v4.6; Technical University of Lodz, Institute of Electronics, Poland).

One week after the *in vivo* examination, the animals were slaughtered and the whole cut of the 12th rib was collected. The cut was dissected into muscles, fat and bones. The sample of LD was analyzed with centesimal extraction: IMF% was determined by extraction with petrol ether (Randall) method.

Animals were divided in 3 groups depending on their mean lipid content percentage in 100g meat, and thresholds were chosen using IMF mean  $\pm$  ½SD (Group 1 included animals with IMF below 4.24 g; Group 2 included between 4.25 g and 5.75 g; and, Group 3 included animals with IMF higher than 5.76 g).

Texture parameters were screened with a stepwise linear discriminant analysis using IMF measured by chemical extraction (IMFq) as the dependent variable, and the results of the texture analysis as explanatory variables, to identify the best combinations of high-quality variables. The aim was a parsimonious model with as few parameters as possible, to enhance stability during validation.

The differentiation efficiency of IMFq was tested by means of the receiver operating characteristic (ROC) curves. Bland-Altman analysis was performed to validate the method and to assess the agreement between IMFq and IMFpred.

**Results:** Each scan generated approximately 300 texture parameters. Among these, 6 variables were identified as predictive by the stepwise analysis and were molded into a multiple regression equation.

IMF in the samples was then predicted by means of the formula (Predicted IMF, or IMFpred), and compared to the quantified IMF (IMFq).

Among all samples, the mean IMFq extracted from the meat was 5.08  $\pm$  1.47 g, while the mean IMFpred was 5.07  $\pm$  1.35 g.

A high linear correlation between IMFq and IMFpred was found ( $r^2=0.85$ ) and results from the ROC analysis showed an Area Under the Curve (AUC) of 92%, with a sensitivity of 80% and a specificity of 93.7%, while results from the Bland-Altman plot were  $\pm$  1.96 ( $\pm$  1.11SD).

**Conclusions:** In the present study, IMF% from beef cattle LD muscle was estimated successfully and with high accuracy, using RTU, one week prior slaughter.

Application of this technology on wide-scale breeding could lead to important economic impacts. A good accuracy in IMF% prediction could permit screening of the animals that are going to be slaughtered, and see whether their fattening is optimal for the market requirements, maximizing the profits.

This technology could also permit monitoring of fattening cycles, leading to rapid estimation *in vivo* of adequacy of the diets fed to the animals. Lastly, it could be used for genetic selection, bypassing lengthy genetic progeny testing and therefore saving large amounts of time.

Further studies to validate the method both on a wider sample and on different sex and breeds are encouraged, but such technology could be a powerful heard selection tool as well as assist farmers in fattening practices.

**Ethical Standards:** All animals were slaughtered according to EU regulations (Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing).

**Keywords:** Beef Cattle, Fat Prediction, Intramuscular Fat, Texture Analysis, Ultrasonography.

## DI-02

**Practical use of thoracic ultrasonography at feedlot arrival to identify high-risk BRD batches in suckler calves**

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**Objectives:** Bovine Respiratory Disease (BRD) is the most important disease in veal industry. The assessment of lung health of suckler calves' batches at their arrival to the rearing facilities is key for decision making and implementation of prevention and metaphylactic protocols for BRD control. However, classification and identification of high-risk BRD batches (30% prevalence) continues to be a major challenge. BRD diagnosis based on clinical signs is inaccurate (61.8%



sensitivity and 62.8% specificity; White, 2009).

The use of thoracic ultrasound (US) scanning to evaluate lung health has demonstrated to be a high sensitivity and specificity tool (79.4% and 93.9%, respectively; Buczinski, 2015). A previous study in 811 veal calves using this technique at arrival (Tejero et al., 2019) showed an average of 21% moderate and 10% severe lung lesions. However, large variability was found among batches ( $P < 0.05$ ).

In high-risk BRD batches, metaphylactic treatment has the potential to reduce the number of animals infected with a susceptible bacterial pathogen, clinical or subclinical, reducing the disease challenge in the environment for non-infected animals and limiting disease spread (Nickel, 2010). Thoracic US scanning has the potential to identify high-risk BRD batches where metaphylactic treatment is justified. However, under field conditions, thoracic US scanning is time consuming. The main objective of this study was to evaluate if the selection of a representative sample of animals for thoracic US scanning provides a reasonable accuracy to identify high-risk BRD batches.

**Materials and methods:** The number of calves to be sampled per batch was calculated using free epidemiological software (<https://epitools.ausvet.com.au>). An estimated prevalence of 30% (high-risk BRD) using a confidence level of 95% and an accepted error of 10% for calves with lung lesions equal or higher than 3, using Adams and Buzsinski (2015) scoring system, was used.

To check the practical use of the sampling table created, a simulation of this sampling procedure was done in 12 batches where thoracic US was assessed in 100% of the animals. A randomization procedure (Excel, Microsoft Office) was used to select the different sampling groups, performing 10 random samples of each batch in order to measure the degree of success to predict whether the batch was a high-BRD risk one.

**Results:** The percentage of calves sampled ranged from 21% to 62% for a 300 or 50 suckler calves' batch respectively.

Our Database of 12 batches, where thoracic US was performed in a 100% of calves at arrival, was used for random selection of calves. Real batches included 12 to 51% of calves affected with score higher than 3 (moderate and severe cases). Most of the batches were high (>30% of calves with lung lesions = 42%) or medium (20-30% of calves with lung lesions = 50%) and only one (8%) was a low risk batch (<20% of lung lesions). Randomized selection of a percentage of calves following the sampling table was performed 10 times. The average results for these 10 randomizations were very similar to the real ones, and the estimated sensitivity was 96% for batches with at least a 30 % of prevalence of disease.

**Conclusions:** Thoracic US scanning of a randomized percentage of calves at arrival may be a useful technique to identify high-risk BRD batches where metaphylactic treatment is justified. This technique can be a practical tool for a more rational use of antibiotics in the veal industry.

**Keywords:** Feedlot, BRD, ultrasound, thoracic, calves.

### DI-03

#### Applications of infrared thermography as a non-invasive technique early indicator of fever in the lambs

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**Objectives:** Ovine Respiratory Complex (ORC) is one of the main causes of morbidity and mortality in sheep feedlots with high prevalence and economic repercussions (González, 2018). Early ORC diagnosis is essential to minimize the effects of the disease, related with both the productive results and welfare of the animals.

Body temperature is an objective clinical data, although its increase is not always associated with disease, as can be also a response to the high environmental temperature or exercise. However, when it is associated with other more specific clinical signs, it is very useful for early diagnosis. Indeed, studies monitoring temperature using ruminal boluses in cattle, demonstrate how the thermal increase occurs 12-136 h before the caregiver detected other more specific clinical signs of pneumonia (Timsit et al., 2011).

On the other hand, thermography is a technology with increasing use in different fields of the veterinary medicine. Thermographic cameras detect infrared radiation emitted by the surface of that body, converting it into an electrical signal that is transformed into a color image. Indeed, Schaefer y col. (2007) already demonstrate it is useful to identify early disease in cattle. However, environmental temperature at the time of thermal image can affect rectal temperature (Church et al., 2014).

Then, the purpose of this study was to evaluate the use of an infrared thermographic camera taking into account environment temperature for the detection of animals with hyperthermia in an intensive lamb herd.

**Material and methods:** This study was carried out in a lamb fattening farm located in Córdoba (Spain). Infrared temperatures were measured in a total of 2,842 multiple source commingled Merino lambs and its crosses, 60 to 120 days old, and 18 to 30 Kg live body weight. Following previous Schaefer et al. (2007 and 2012) studies in cattle, we performed an orbital eye measurement, as in other anatomical parts the presence of hair or wool can interfere with results.

The measurements were made after the passage of the lambs through the handling or sorting sleeve and in the lazarrette measuring in duplicate both rectal temperature with a conventional thermometer and eye thermal image with an infrared thermometer.

For infrared thermography the camera Testo model 870 was used, with a resolution of 160 x 120 pixels (with the possibility of increasing to 320 x 240 pixels), thermal sensitivity



<100 mK, 34° lens with fixed focus and integrated and calibrated digital camera. The image processing software was the IRSoft Software, version 4.5. While the thermometer used was the Kruuse clinical thermometer, model Veterinär-thermometer SC 12, also calibrated, with automatic stop system by sound signal and with a measurement accuracy of 0.1°C.

At measurement time, the following data were additionally recorded: date and time, farm identification, barn, pen, etc. In addition, the inside and outside barn temperature was noted. Subsequently, we calculate a new parameter: the difference between rectal and ocular temperature (DROT).

The data were analyzed using Anova one-way for the comparison of means, as well as the correlation between the variables and the multiple linear regression using the statistical program IBM SPSS Statistics, version 25.

**Results and discussion:** We evaluate the effect of barn temperature on the ocular and rectal temperature. On the other hand, we evaluate its effect on different rectal temperature strata.

The correlations between the different variables were significant. Indeed, the combined use of variables, eye temperature and barn temperature, allowed us to establish a formula predicting the rectal temperature with an excellent correlation ( $R^2 = 0.9996$ ).

$$\text{Rectal } t^a = 1.099 * \text{ocular } t^a - 0.038 * t^a \text{ nav}$$

**Conclusions:** The rectal temperature of lambs under intensive conditions is an objective clinical parameter very useful for ORC early diagnosis. Infrared thermography is a fast, automatic and non-invasive technique helpful for early identification of sick animals. And considering the barn temperature at lecture time improves its correlation with rectal temperature.

**Keywords:** Fever, thermography, lambs, environmental  $T^a$ , ORC.

#### DI-04

### Comparison between thoracic ultrasonography and visual observation for the detection of BRD in veal calves

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**Objectives:** High levels of mortality and morbidity due to bovine respiratory disease (BRD) in combination with animal welfare, reduction of antimicrobial use, and antimicrobial resistance are currently the most important challenges in the beef industry.

The detection of BRD in farms is based on respiratory clinical signs (cough, dyspnea, ear position, nasal discharge) and increased body temperature. However, the use of clinical signs to detect lung lesions associated with BRD has some limitations (Leruste et al., 2012; Sensitivity (Se) 61.8% and specificity (Sp) 62.8%; White, 2009). Recently, new tools have been developed to achieve a more accurate early diagnosis. Among them, Thoracic Ultrasonography (TUS) shows the highest ac-

curacy to detect pneumonia at farm level (Se 79.4% and Sp 93.9%; Buczinski, 2015; Se 89% and Sp 95%; Berman, 2019).

The objective of this study was to compare Thoracic US with BRD clinical diagnosis performed by two senior veterinarians both at arrival to the rearing facilities and later along the first month on fattening.

**Materials and methods:** Two different batches of a commercial veal feedlot with 62 and 45 male calves were evaluated. The day of arrival, two experienced veterinarians (observer 1;O1 and 2;O2) classified the 107 calves as “BRD” or “Healthy” based on their clinical signs. Then, an experienced veterinarian performed thoracic US scanning (TUS) and classified the calves as having or not having lung lesions using a scoring system (Adams and Buczinski, 2015). The three veterinarians performed their scoring in a blind manner. All animals were scored again by the three veterinarians 7 and 21 days after their arrival. The different scoring results were recorded to evaluate BRD prevalence and agreement between the three scoring results. The TUS score was considered as the gold standard technique.

The comparison between the use of the diagnostic techniques (Se, Sp, positive predictive value – PPV- and negative predictive value-NPV-) and observers (concordance kappa index) was performed with free epidemiological software (<https://http://www.winepi.net>) using a confidence level of 95%.

**Results:** The prevalence of lung lesions detected by TUS at arrival was 35% for batch1 and 36% for batch2 respectively. Using clinical scoring, the prevalence of BRD was different between the O1 and O2 for batch1 (O1: 45%; O2: 29%) and batch2 (O1: 47%, O2: 38%). In the first review performed 7 days after arrival, BRD diagnosed by TUS increased to 75% and 62%, respectively for batch1 and 2. BRD prevalence diagnosed by the observers was also different in batch 1 (O1: 65%; O2: 72%) and in batch 2 (O1: 53%, O2: 47%). In the examination performed 21 days after arrival, BRD prevalence diagnosed by TUS was 67% and 49%, respectively for batch1 and 2. BRD prevalence diagnosed by the observers was (O1: 60%; O2: 52%) in batch1 and (O1: 20%, O2: 27%) in batch2.

Although the average Se and NPV were quite similar for both observers (Se 63.8% and 64.9% and NPV 60.1% and 65.3%, for O1 and 2 respectively), the Sp and PPV was higher for O2 (Sp 66.0% and 79.9% and PPV 69.4 and 79.65, for O1 and 2, respectively).

Finally, the concordance among both observers was moderated (kappa = 0.598, IC95% 0.29-0.71). Indeed, one of the observers consistently presented a better detection of BRD signs than the other. However, even for this observer, 28% of BRD cases detected by thoracic US were not identified as BRD using clinical diagnosis.

**Conclusions:** The present study suggests that Thoracic Ultrasonography can be used as an on-farm technique for BRD diagnosis. However, clinical diagnosis by experienced observers may be good enough to be used as a screening technique to identify BRD calves. The results also suggest that, with training, observers may improve their Sensitivity and Specificity for a better detection of treatment of BRD calves.

**Keywords:** Ultrasonography, feedlot, BRD, diagnosis, clinical signs.



## DI-05

### Electrical impedance tomography (EIT) to evaluate peak inspiratory and expiratory flow levels and distribution of ventilation in steers

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Electrical impedance tomography (EIT) is a novel non-invasive imaging technique that allows a visual representation of the distribution of ventilation in real-time. The aim of this study was to determine if changes in lung status can be detected by changes in EIT variables in anaesthetised steers.

In this randomised cross-over study, seven anaesthetised spontaneously breathing steers lungs were exposed to atmospheric airway pressure (NoPres), or continuous positive airway pressure of 7 cmH<sub>2</sub>O (PosPres). Steers were positioned in dorsal recumbency. EIT electrodes mounted on a belt were placed around the thorax to measure impedance changes. Post induction, EIT recordings were performed at 30, 50, 70 and 90 minutes respectively. From each recording ten breaths were retrospectively analysed and pooled for the pixel with maximal inspiratory (FlowPix<sub>insp</sub>) and expiratory flow (FlowPix<sub>exp</sub>) within the EIT image and the centre of ventilation, illustrating shift of ventilation distribution along the gravitational axis, between right and left lung (CoV<sub>r-l</sub>) and ventral to dorsal lung (CoV<sub>v-d</sub>). Descriptive statistics were used to describe the difference between NoPres and PosPres and are reported as mean (95% confidence interval).

Lower FlowPix<sub>insp</sub> and FlowPix<sub>exp</sub> were measured during PosPres compared to NoPres; FlowPix<sub>insp</sub> [311 (282-340) vs 352 (315-388)] and FlowPix<sub>exp</sub> [268 (249-287) vs 313 (282-343)], respectively. The CoV<sub>r-l</sub> was lower [54 % (53-55)] in PosPres than in NoPres [61 % (58-63)] while only a small difference of 3 % was seen for CoV<sub>v-d</sub>.

EIT can be used to detect differences in lung status by flow and CoV measurements in anaesthetised cattle.

Ethical Animal Research: This study was approved by Murdoch University ethics committee R2998/17

**Keywords:** Electrical impedance tomography, Ventilation, Cattle.

## DI-06

### Preliminary analysis in the flank temperature pattern in dairy cows during pregnancy

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**Objectives:** Recent years have seen a significant increase in interest in the use of thermal imaging in veterinary diagnostics, especially in a health herd management of live-stock animals. Owing to the possibility of fast, non-invasive and automated temperature measurement on the surface of a cows' body, this technique shows great potential in predicting local changes in the tissue metabolism and local blood circulation. The abdominal area demonstrated physiological changes during late-gestation related to the increased demand for the growing uterus and more loaded abdominal wall muscles. However, there is still a need for further research to standardize measurement data in thermal imaging. Therefore, the aim of the study was evaluated if the changes in physiological activity during late pregnancy may result in abnormal thermal patterns of the flank of dairy cows.

**Materials and methods:** Thermographic images of the right flank were obtained from 54 Holstein-Friesian dairy cows from a commercial 550 head dairy farm. All cows were housed under the same environmental conditions. The pregnancy was confirmed with ultrasound examination on day 32 after insemination and one the day of imaging in 30 cows. Based on ultrasound, the cows were divided into the following groups: NP - nonpregnant (n=24); EP - early pregnancy (1<sup>st</sup> to 3<sup>th</sup> months; n=6), MP - mid pregnancy (4<sup>th</sup> to 6<sup>th</sup> months; n=6) and LP - late pregnancy (7<sup>th</sup> to 9<sup>th</sup> months; n=24: 7<sup>th</sup> Mo, n=6; 8<sup>th</sup> Mo, n=6, 9<sup>th</sup> Mo, n=6). Images were taken indoor, with ambient temperature ranging from 14.0 to 16.0°C, using infrared radiation camera (emissivity 0.99; FLIR Therma CAM E60). Temperature pattern of the flank area was evaluated using five regions of interest (ROI 1–5) representing: the area along the transverse processes of the spine (ROI 1), the cranial area of the right abdominal surface (ROI 2), the cranial area of the right abdominal surface (ROI 2), the middle area of the right abdominal surface (ROI 3), the caudal area of the right abdominal surface (ROI 4), and the stifle area (ROI 5). Each ROI was analyzed for the minimal (T<sub>min</sub>), maximal (T<sub>max</sub>), and mean (T<sub>mean</sub>) temperatures. The results (mean±SD) were compared between NP, EP, MP, 7<sup>th</sup> Mo LP, 8<sup>th</sup> Mo LP, and 9<sup>th</sup> Mo LP groups.

**Results:** The T<sub>min</sub> was higher (p<0.0001) in all five ROIs in LP (19.67°C ±1.11) than in NP (13.55°C ±3.34), EP (14.92°C ±1.69), and MP (14.88°C ±1.64). There were no differences (p>0.05) in T<sub>min</sub> between NP, EP and MP as well as between 7<sup>th</sup> Mo (18.85°C ±1.40), 8<sup>th</sup> Mo (19.14°C ±1.50), and 9<sup>th</sup> Mo (19.68°C ±1.41) of within LP group. The T<sub>max</sub> was comparable in all five ROIs, with no differences (p>0.05) between NP (26.51°C ±2.24), EP (27.39°C ±2.57), MP (24.22°C ±3.16), and LP (25.53°C ±2.29) groups, regardless of the month of preg-

nancy. The  $T_{\text{mean}}$  was higher ( $p < 0.01$ ) in ROI 1 and 5 in LP (ROI 1:  $22.35^{\circ}\text{C} \pm 0.95$ ; ROI 5:  $24.78^{\circ}\text{C} \pm 1.09$ ) than in NP (ROI 1:  $17.54^{\circ}\text{C} \pm 4.42$ ; ROI 5:  $19.27^{\circ}\text{C} \pm 4.09$ ), EP (ROI 1:  $18.96^{\circ}\text{C} \pm 1.75$ ; ROI 5:  $19.88^{\circ}\text{C} \pm 1.87$ ), and MP (ROI 1:  $19.71^{\circ}\text{C} \pm 0.08$ ; ROI 5:  $19.97^{\circ}\text{C} \pm 1.28$ ), with no similar differences ( $p > 0.05$ ) in ROI 2, ROI 3, and ROI 4.

**Conclusions:** Thermography is proposed as a promising tool in determining changes in the flank temperature pattern in dairy cows during pregnancy. An application of different regions of interest during the superficial temperature analysis allows to established the discreet changes in the mean temperatures during late pregnancy. The minimal temperature seems to be the most reliable indicator of the superficial temperature changes related to the months of pregnancy. After examining more numerous groups of cows, the external temperature of the flank may be a useful method for the presumption of pregnancy in late gestation.

**Keywords:** Thermography, cow, pregnancy diagnosis.

## DI-07

### Associations between TUS scores and mortality and productivity outcomes on Scottish dairy farms

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**Objectives:** A previously published study (1) used thoracic ultrasound (TUS) to assess farmer accuracy of diagnosing Bovine Respiratory Disease (BRD) in cattle youngstock. We found that sensitivity of farmer diagnosis of BRD was low; demonstrating that animals with lung lesions were not being identified and treated for BRD. We also found that some farmers treated animals which did not have signs of BRD on TUS, suggesting unnecessary use of antimicrobials. This study aimed to follow the same calves assessed in our previous work, to determine the effects of lung consolidation, as diagnosed by TUS, on future mortality and reproductive outcomes.

**Materials & Methods:** TUS examinations were conducted between 2019-03-13 and 2019-11-26, by a single veterinarian over 7 dairy herds. All calves on the farm between 21 and 61 days of age during these time points were scanned and scored. This included male and female calves destined for dairy or beef rearing, which ensured a larger dataset. On scoring, they were given a TUS score on a 0-6 scale (2) as well as a calf health score based on the Wisconsin calf health scoring system which was converted to a binary "normal" or "abnormal" TUS score. Further descriptions and results of this part of the study have been published previously (1).

For the current study, all calves which had a TUS examination were included in the dataset for analysis of mortality. Female dairy calves retained on the study farms were followed through to calving, for analysis of reproductive outcomes using the farms' management system and data collection software. Mixed effects logistic regression models were used to analyse

associations between TUS score and mortality status, conception within 3 serves, time to conception and the number of serves to conception. Data was only collected for animals that were retained on the farm for the purpose of entering the adult milking herd. This meant a proportion of animals were lost to follow-up as they were sold for beef to other farms not included in the study.

**Results:** The initial dataset consisted of 347 ultrasonographic examinations. Of these, 53 calves (15.3%) were classified as abnormal and 294 (84.7%) as normal. Of the 53 calves classified as abnormal, only 13 (24.5%) were treated by the farmer, however, of the 294 classified as normal, 22 (7.5%) were treated. Of the 347 animals, 18 animals died during the follow up period of weaning to conception (5.2%), and mixed effects regression model results suggested that there was a tendency for animals with an abnormal TUS score as calves to be more likely to die during this time (OR 2.33, 95% CI 0.9-6.1,  $P = 0.08$ ).

Data for pregnancy status within 3 serves were available for 149 animals, with 16/22 (72.7%) of animals with abnormal TUS status becoming pregnant and 114/127 (89.8%) of animals with normal TUS status becoming pregnant. Mixed effects regression model results suggested that abnormal TUS score was associated with lower odds of becoming pregnant within 3 serves (OR 0.32, 95% CI 0.1-1.0,  $P = 0.06$ ). No associations were found between TUS score and time to conception or the number of serves to conception.

**Conclusion:** Follow up analysis of dairy cattle which had undergone TUS between 21-61 days of age suggest a tendency for increased mortality in animals with higher abnormal TUS scores, and a tendency for decreased pregnancy rates within the first 3 serves. This reflects similar work undertaken in other countries (3,4). To the author's knowledge, this is the first study in the United Kingdom to ascertain the prevalence of subclinical BRD in pre-weaned dairy calves, and to follow these calves to adulthood to further investigate the effects of BRD on future performance.

Alongside the immediate health, welfare, and productivity impacts of clinical and subclinical respiratory disease, it is likely that subclinical BRD during the preweaning period is associated with future reproductive and mortality outcomes. The prevention of pneumonia in calves has the potential to improve health and production outcomes. For future work, we will follow these animals to the end of their first lactation to gather data on 305-day lactation yields to analyse for associations with calthood pneumonia.

**Keywords:** Thoracic ultrasound, calf, bovine respiratory disease.



## DI-08

### Dynamics of subclinical pneumonia in male dairy calves in relation to antimicrobial therapy and production outcomes

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**Objectives:** Quick thoracic ultrasonography (qTUS) is increasingly used as an on-farm method to diagnose clinical and subclinical pneumonia in dairy calves. The primary objective of this study was to describe dynamics of lung consolidation in a purchase dependent production system for male dairy calves (veal) in relation to antimicrobial therapy and respiratory diagnostic tests. In addition, the association of cured and uncured pneumonia with average daily growth (ADG) and cold carcass weight (CCW) were studied, as well as the effects of arriving with lung consolidation on the probability of developing chronic unresponsive pneumonia and reduced performance.

**Materials and methods:** A prospective cohort study was conducted involving 295 male Holstein Friesian calves, aged 14-21 days during one production cycle in a commercial veal farm. Both clinical examination and qTUS were performed on all animals upon arrival, at week 2, 3, 4, 6, 8 and 12. Pneumonia was defined as the presence of a lung consolidations  $\geq 1$  cm. Pathogen detection was done using cultures, Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) (Brüker Daltonik GmbH, Bremen, Germany) and whole genome nanopore sequencing (WGS) on non-endoscopic bronchoalveolar lavage samples (nBAL).

**Results:** Of the calves, 17.6% (52/295) arrived with a lung consolidation  $\geq 1$  cm. At the first outbreak of respiratory disease this number had risen to 30.8%. Initial therapy with tulathromycin and subsequently doxycycline appeared ineffective, resulting in a rise in the prevalence of animals with pneumonia up to 43.8% in week 4. At the start of the first outbreak in week 2, the vast majority (91.2%) of the pneumonia cases ( $n = 91$ ) were subclinical. At week 4 the outbreak became more clinical and therapy with amoxicillin resulted in a cure rate of 52.7%. Bacterial cultures and nanosequencing diagnostics on nBAL samples identified bovine respiratory syncytial virus (BRSV) and *Mycoplasma bovis* as the dominant agents in the first outbreak. The isolated *M. bovis* strain had mutations encoding for macrolide resistance. The second outbreak was characterized by a *Pasteurella multocida* superinfection and isolation of a different *M. bovis* strain. Evaluated over the complete observation period, 53.9% of the calves cured upon antimicrobial therapy. Of the 46.1% calves that were not cured ( $n = 111$ ), 29.7% (33/111) had developed chronic pneumonia. Calves with uncured or chronic pneumonia had a significantly lower ADG (992g/day  $\pm 174$  and 930g/day  $\pm 146$ , respectively) compared to calves that did not developed pneumonia (ADG = 1103 g/day  $\pm 156$ ;  $P < 0.001$ ). In contrast, calves that did fully cure had a similar ADG as calves that never developed pneumonia (1045 g/day  $\pm 139$  and 1103 g/day  $\pm 156$ , respectively;  $P = 0.09$ ). The effect of uncured pneumonia was no longer significant for cold carcass weight. Calves with lung consolidation upon arrival had a significantly lower ADG (981 g/day  $\pm 159$  vs 1045 g/day  $\pm 159$ ;  $P = 0.01$ ) and were more likely to develop

chronic pneumonia (OR=4.2; 95% CI: 2.1 – 8.6,  $P < 0.001$ ). Animals with chronic pneumonia, in turn, had a significantly lower cold carcass weight (10.3kg  $\pm 4.4$ ; CI: 1.2 – 18.9;  $P = 0.02$ ).

**Conclusion:** The prevalence of pneumonia in veal calf facilities proves to be much higher than previously expected, mainly because of its subclinical nature. Animals with pneumonia have a reduced daily growth but appear to be able to catch up to healthy animals when cure is achieved before the age of 12 months. Chronic unresponsive pneumonia is frequently encountered and the main reason for production loss. Calves that already had lung consolidation upon arrival on the veal facility had a higher risk of developing chronic unresponsive pneumonia. Both qTUS and nBAL diagnostics offer potential for better control and prevention of bovine respiratory disease in dairy calves.

**Keywords:** Bovine respiratory disease, subclinical pneumonia, *Mycoplasma bovis*, bovine respiratory syncytial virus, economics.

## DI-09

### Use of quick thoracic ultrasonography (qTUS) to guide and evaluate antimicrobial therapy in three natural outbreaks of *Mycoplasma bovis* pneumonia

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**Objectives:** Quick thoracic ultrasonography (qTUS) is an accessible on-farm method to reliably diagnose (subclinical) pneumonia in both dairy and beef calves, offering great potential for more rational antimicrobial use and better control of bovine respiratory disease. The objective of this study was to describe the application of qTUS to guide and evaluate antimicrobial therapy in three natural outbreaks of *Mycoplasma bovis* associated pneumonia in a single beef farm.

**Materials and methods:** A retrospective analysis was performed on ultrasonographical and diagnostic data collected from 3 different outbreaks of respiratory disease in the same closed commercial beef farm housing 1000 Belgian Blue beef cows. Calves at risk aged between 2 and 6 months and were housed in groups of 6 in fully separated pens. Pneumonia was defined as a lung consolidation  $\geq 1$ cm while cure was defined by re-aeration of these lesions to  $< 1$ cm in depth. In each outbreak, qTUS was used as a tool to initiate, stop or generally evaluate antimicrobial therapy. In outbreak 1, ultrasound-guided full metaphylaxis was used, meaning that all animals at risk ( $n = 130$ ) were metaphylactically treated (one injection), after which antimicrobial therapy was only prolonged in animals which still had lung consolidation. Antimicrobial treatment was stopped as soon as ultrasonographical re-aeration of the lung occurred. Outbreak 2 ( $n = 94$ ) was approached by individual treatment of animals with ultrasonographically confirmed pneumonia, without metaphylaxis. Finally, in the third outbreak ( $n = 61$ ), an ultrasound-guided targeted metaphylaxis,



only using group treatments in pens were 3 or more animals had consolidations  $\geq 1$ cm, was applied. Again, as was done in outbreak 1, antimicrobial treatment was discontinued when re-aeration appeared. In each outbreak, animals older than 3 months were treated with florfenicol while animals less than 3 months were treated with oxytetracycline, both administered as long acting formulation every 48 hours. Identification of primary pathogens was done by means of whole genome nanopore sequencing (WGS) on non-endoscopic bronchoalveolar lavage samples (nBAL). Strain typing of *M. bovis* and detection of possible genomic antimicrobial resistance was done by long-read nanopore sequencing.

**Results:** At the time of intervention 43.3% (55/130), 50% (47/94) and 37.7% (23/61) of the calves from outbreaks 1, 2 and 3 had a lung consolidation  $\geq 1$ cm, respectively. In the first outbreak, where full metaphylaxis was applied, cure rate after 14 days was 98.2% (54/55). no new cases of pneumonia were detected, resulting in a total proportion of healthy calves of 99.2% (129/130) after the outbreak. In outbreak 2, over the 21-day follow up period 24 new cases of pneumonia were detected, resulting in a total of 75.5% (71/94) pneumonia within the herd. Cure rates were 61.7% (29/47) and 79.2% (19/24) for the initial and new pneumonia cases, respectively (total cure= 67.6 % (48/71)). Finally, in outbreak 3, incidence of pneumonia started at 37.7% (23/61). After a 14 day follow up, the combination of targeted metaphylaxis and individual treatment resulted in a total cure rate of 78.9% (30/38). Of the initial cases, 78.2% (18/23) was cured. 15 new cases were detected in this outbreak, of which 80% (12/15) was cured at the end of observation. Cure rate was significantly lower in outbreaks 2 and 3, compared to 1 ( $P < 0.01$ ). In outbreak 1, 2 and 3, *M. bovis* was detected by WGS and strains were classified as belonging to Belgian *M. bovis* genomic clusters IV, I and I, respectively. In the third outbreak the coronavirus was also detected. Evaluated over the outbreak, in outbreaks 1, 2 and 3, a number of animal daily dosages (ADD) per animal of 3.0, 8.0 and 5.3 ADD/animal were necessary to control the outbreak, respectively. For comparison a standard metaphylaxis with tulathromycin stands for 7 ADDs/animal.

**Conclusion:** The qTUS technique showed potential as a reliable and practically feasible method both for detection of pneumonia and therapy evaluation in larger herds. Large differences in cure rate between the outbreaks were observed which may be related to co-infection, timing of the initiation of antimicrobial therapy, strain difference or the use of metaphylaxis. Especially the latter is a point of interest given that both ultrasound-guided metaphylaxis (full or targeted) resulted in lower antimicrobial use compared to individual treatment.

**Keywords:** Bovine respiratory disease, *Mycoplasma bovis*, lung ultrasound, antimicrobial reduction.

## DI-10

### Lessons learned from 1 year of quick thoracic ultrasonography (qTUS) training for practitioners to improve pneumonia diagnosis in calves

Bart Pardon, Stan Jourquin, Thomas Lowie, Florian Debruyne, Laurens Chantillon, Mathilde L. Pas, Wouter Van Mol, Justine Clinquart, Jade Bokma.

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**Objectives:** Thoracic ultrasonography (TUS) has become the key on-farm tool for bovine respiratory disease management (BRD), in particular to rationalize antimicrobial use. Despite growing interest to implement TUS on farm, many practitioners hesitate. Uncertainty about image recognition and scanning technique, as well as concerns on time investment are the main reasons given. Quick thoracic ultrasonography (qTUS) stands for a standardized lung scanning technique making TUS of a large number of calves in limited time possible. The objective of this study was to describe diagnostic performance of novice operators after a qTUS training course.

**Materials & Methods:** All Flemish veterinarians were contacted to volunteer for a project aiming at bringing qTUS to practice as a diagnostic aid in BRD management. Participants needed to complete an online course consisting of 4 modules (Module 1: positioning of TUS among other methods for pneumonia diagnosis; Module 2: ultrasound image recognition including common pitfalls; Module 3: qTUS scanning technique and Module 4: qTUS applications in practice). The training included 4 series of exercises with feedback, two on recognition of images, and 2 on recognition of full qTUS scan image loops. In addition, practical training sessions with experienced qTUS operators were organized at the discretion of the participant to decide how many to follow. Candidates were encouraged to scan in their own practice as well. Monthly online contact moments were organized between the trainers and trainees. To finalize the exam a practical test was organized. Three test occasions (sessions) were organized. Each participant needed to scan 23 (session 1 and 2) or 18 (session 3) calves and communicated to a recorder whether a lung consolidation of  $\geq 1$  cm was present. Performing other aspects of clinical examination was not allowed. Session 1 and 2 were done in a commercial veal calf farm with animals aged 8 (group housing) and 6 weeks (individual housing), respectively. Session 3 was done in a dairy farm on calves aged between 2 weeks to 4 months (individually housed until week 5). Total scanning time and information on intensity of online learning and self-training was collected. Diagnostic accuracy of the participants was compared with an experienced TUS operator.

**Results:** In February 2021, 45 veterinarians started with the online training, of which 34 had taken the certification test by January 2022. The prevalence of calves with lung consolidations  $\geq 1$  cm ranged between 39.1% and 75.0% depending on which calves the participants scanned. Overall diagnostic accuracy (Acc) was 72.3% (standard deviation (SD)= 10.8%; range (R)= 52.2%-95.7%), mean sensitivity (Se) was 64.4% (SD= 26.6; R= 0-100) and mean specificity (Sp) was 70.6% (SD= 17.7; R= 25-100). Average Se (45.0%) was significantly lower in session 3 compared to session 1 (74.4%) and 2



only using group treatments in pens were 3 or more animals had consolidations  $\geq 1$ cm, was applied. Again, as was done in outbreak 1, antimicrobial treatment was discontinued when re-aeration appeared. In each outbreak, animals older than 3 months were treated with florfenicol while animals less than 3 months were treated with oxytetracycline, both administered as long acting formulation every 48 hours. Identification of primary pathogens was done by means of whole genome nanopore sequencing (WGS) on non-endoscopic bronchoalveolar lavage samples (nBAL). Strain typing of *M. bovis* and detection of possible genomic antimicrobial resistance was done by long-read nanopore sequencing.

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## DI-10

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(73.0%) ( $P < 0.05$ ). In contrast, average Sp (60.6%) was significantly lower in session 2, compared to session 1 (80.5%) and 3 (78.9%) ( $P < 0.05$ ). Participants that followed more than one practical training session had on average a 21% higher Se compared to persons attending a single training ( $P < 0.05$ ). Also the number of calves scanned in the own practice before the test trended towards a higher Se ( $P = 0.08$ ). Gender, years graduated, hours invested in the online course and speed of scanning did not affect Se. Sp was not influenced by any of the factors mentioned above. The required scanning time per calf (including capture and fixation of the animal) was 4.7 min. (SD= 1.1; R= 1.7-6.5 min.) on average. After the course 60% of the candidates declared to feel skilled enough to use qTUS in practice, but this was not significantly associated with diagnostic performance.

**Conclusions:** With the qTUS training program (online + live trainings) in most novice operators a reasonable Se and Sp could be reached. However, variation in Se and Sp is substantial and may be influenced by age of animals and housing/scanning conditions. Increasing the number of practical training sessions significantly improved Se. Adequate training and continued self-assessment are necessary to sustainably integrate qTUS in the highly variable conditions in daily practice.

**Keywords:** Thoracic ultrasonography; bovine respiratory disease; diagnostics; education; practice.