

## SR-01

**Pseudotuberculosis, how to find the enemy hidden inside doors**

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**Objectives:** The actual prevalence of CLA (caseous lymphadenitis) in small ruminant flocks is underestimated in many countries and continues to spread without data and information about its real economic impact. The difficulty in the accurate identification of the causative agent in internal sub-clinical cases allows the disease to spread within and between flocks. This research intends to assess the utility of an ELISA (enzyme-linked immunosorbent assay) test in the detection of internal subclinical cases of CLA in farms and to simultaneously add data on the seroprevalence of the disease in Portugal.

**Materials & Methods:** In this research, we selected a field population of 82 flocks of small ruminants (55 sheep and 27 goat flocks). The procedure was carried out on the farm and in a regional slaughterhouse in the province of Alentejo in southern Portugal. Sera randomly collected from 756 small ruminants (approximately 10 samples per holding), 70% sheep (528/756) and 30% goats (228/756) were screened for antibodies against *Corynebacterium pseudotuberculosis* using the ELISA technique based on a recombinant phospholipase D (ELITEST CLA # CK105A®). The animals showing internal lesions (n = 58) were sampled and a conventional PCR assay was made for the identification of *C. pseudotuberculosis* in pyogranulomatous lesions and for the cross-sectional study to assess the performance of the ELISA test. The ELISA assay results were subjected to frequency analysis (prevalence of the disease). The same method was applied to the data regarding the species, productive aptitude, production regime, and dimension (size) of the herd. The performance of the ELISA test was evaluated by association tests, namely,  $\chi^2$  (Chi-square – Fischer's Exact Test) with a 95% confidence interval. The statistical analysis was performed with SPSS (Statistical Package for Social Sciences) 22.

**Results:** In this investigation, we found a prevalence of CLA of 34% (258/756), with the ELISA test showing a low specificity, 78% (35/45) and high sensitivity, 100% (13/13). The descriptive analysis of the results suggests that the proportion of disease higher in goats ( $\chi^2$  (1, N = 756) = 30.773,  $p < 0.01$ ). Regarding productive aptitude, dairy farms appear to be more susceptible to CLA ( $\chi^2$  (1, N = 756) = 17.458,  $p < 0.01$ ); the same propensity appears to exist regarding the production regime, with the farms in the non-extensive regime (intensive and semi-extensive, approximately 6% of the total sample) showing an increased propensity for the disease ( $\chi^2$  (2, N = 756) = 22.561,  $p < 0.01$ ). When the results of the ELISA are analyzed regarding the size of the farm, the data are also

statistically significant. This data suggests that farms without the reported disease have an average of 85 animals, whereas farms with confirmed disease have an average of 171 animals ( $p < 0.001$ ). This observation appears to mean that CLA is more prevalent in medium to large farms (50 - 200 animals and > 200 animals). By performing a herd-level analysis (considering an infected farm with at least one animal positive for the ELISA), of the 82 farms in the study, 66 had at least one positive animal, leading to a value of 80% (66/82) of affected holdings. The performance of the ELISA test technique (ELITEST CLA # CK105A®) was evaluated by determining the positive predictive value (PPV) and negative predictive value (NPV). In this analysis, the ELISA test allowed the detection of 57% of subclinical animals, which was confirmed by the *post-mortem* examination and conventional PCR assay performed in pyogranulomatous lesions. In the group of animals in which both tests were performed (PCR and ELISA) (n = 58), 57% of the animals positive for the ELISA test were positive for the PCR assay (13/23), and all the negatives, 100%, in the ELISA test were negative for the PCR assay (35/35).

**Conclusion:** This is the first prevalence study of CL in small ruminants carried out in Portugal. This research clarifies an actual problem and pointed out the importance of CLA in small ruminant herds in Portugal showing that CLA is a prevalent disease in small ruminants, with 34% seroprevalence. Finally seems to demonstrate that the ELISA test is a good diagnostic tool for use in CLA eradication programmes, as can detect a valid proportion of subclinically infected animals.

**Keywords:** Caseous lymphadenitis, ELISA, Portugal, seroprevalence, small ruminants.

## SR-02

**Prevalence of visceral Caseous Lymphadenitis (CLA) in sheep herds of Aragón, Spain**

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**Objectives:** Caseous lymphadenitis (CLA) is an infectious-contagious pathology caused by *Corynebacterium pseudotuberculosis* biovar ovis which appears frequently in herds. When this agent affects small ruminants, it can develop a visceral clinical form that mostly affects adult animals, or a superficial clinical form, which appears more frequently in young animals. However, its subclinical appearance, especially of the visceral form, makes it difficult to diagnose it in clinical practice.

Thus, although this pathology is widespread, many times reminds underdiagnosed and is not easily identified as a cause of loss of productivity in the sheep flocks.

The main objective of the present survey was to study the relevance of Caseous Lymphadenitis as a cause of early cull-



ingin sheep herds of Aragón region, Spain, and to analyze the main clinical presentations of the disease.

**Material and methods:** The Ruminant Clinical Service (SCRUM) of the Veterinary Faculty of Zaragoza, Spain, receives animals from the Faculty's area of influence which can be culling animals belonging to collaborating farms or clinical cases referred by veterinarians. In the present survey, 483 culling animals received during the years 2017, 2018 and 2019 were analyzed.

All the received animals were subjected to the same protocol. After arrival, a rigorous clinical examination was performed and blood samples were taken to carry out a blood count test. Subsequently, depending on the preliminary diagnoses, necessary ancillary tests, such as ultrasonography, thermography or computed tomography, were carried out. Finally, all the animals were humanely sacrificed to conclude with the pathological examination. Then samples of lesions and affected organs were taken for microbiological, biomolecular and histopathological analysis. The isolation of the causative agent was carried out in the laboratories: EXOPOL diagnóstico y autovacunas S.L. and in the Agroalimentary Laboratory of the Government of Aragón and histopathology was performed at the Pathological Clinical Service of the Veterinary Faculty of Zaragoza.

The animals included in the present study as positives to CLA were those diagnosed observing compatible lesions at necropsy and subsequently with etiological confirmation.

Finally, all the data were recorded in computer programs, such as Microsoft Office Excel 2010 and IBM Statistics Base 22.0, which allowed the statistical study of that data.

**Results:** The results show that 31.06% of the animals analysed showed CLA lesions (150/483) that were subsequently confirmed by *C. pseudotuberculosis* isolation.

Attending to the clinical form of presentation, 74.67% of the animals suffered the visceral clinical form, while only 21.33% were affected by the superficial form of the disease. The remaining 4.00% of the animals showed both clinical presentations simultaneously. That result was expected due to the average age of the studied animals that was  $5.90 \pm 1.73$  years.

However, the most relevant data of this study was that obtained from the analysis of CLA as cause of early culling in sheep. Eighty-two animals out of the 150 animals diagnosed with CLA (54.67%) presented this pathology as the sole cause of culling, which could justify the loss of productivity. Moreover, when these 82 animals were analyzed regarding the 483 culling studied sheep, it was showed that 16.98% of the culling animals were discarded by this pathology as the sole cause of culling, demonstrating the importance of this disease as a cause of culling in sheep herds. The visceral clinical form was the main presentation of the disease, accounting 14.70% of the total, while the superficial clinical form was only de 2.18%.

Regarding the visceral clinical form, the primary location was that related to the respiratory system, with affection of mediastinic lymphnode, lungs or both simultaneously. Likewise, the concurrent affection of several organs, such as liver-lung liver-kidney, or other variants appeared in a significant number of animals.

**Conclusions:** It can be concluded that caseous lymphadenitis is a relevant diseaseproducing loss of productivity and

early culling in sheep in Aragón region. The visceral form was the most frequently found, this being of great importance due to the difficulty of the clinical diagnosis of this presentation of the disease, which leads to the disease being frequently underdiagnosed.

**Keywords:** Caseous Lymphadenitis, sheep, visceral, prevalence.

### SR-03

#### Evaluation of the effect of a topical wound anaesthesia formulation on concentration of serum amyloid A in lambs where the tail was excised surgically

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**Objectives:** Tail-docking is a routine global husbandry procedure conducted in lambs to reduce myiasis risk and improve fertility. It is often performed without pain relief, although use of general anaesthesia has been advocated, at an exorbitant cost to farmers. There is an urgent need to find a practical and affordable approach acceptable to farmers that avoids or markedly reduces animal suffering. This study evaluated the effect of a topical anaesthetic 'spray-on' wound management formulation containing the local anaesthetics lignocaine and bupivacaine, plus cetramide and adrenalin, in a gel matrix (Tri-Solfen®, Bayer Animal Health, Gordon, NSW, Australia). Impacts of treatments on concentration of the major acute phase protein serum amyloid A (SAA) in lambs, where the tail was excised surgically, with and without use of general anaesthesia, are reported.

**Material & Methods:** This study was approved by the Ethical Commission of the University of Zaragoza. Forty-four 45-day-old Rasa Aragonesa female lambs with similar weights in a farm in Zaragoza (Spain) were recruited for this study and divided into 4 equal cohorts (n=11): in Group A the tail was excised using a scalpel without using anaesthesia or any additional treatment; in Group B the tail was surgically excised under general anaesthesia; in Group C the tail was removed with a scalpel without prior anaesthesia and treated immediately with Tri-Solfen®; in Group D the tail was surgically excised under general anaesthesia and treatment with Tri-Solfen® applied before suturing of peri-wound skin to close the wound. Blood samples were collected (and sera were obtained and stored at -20 °C until analysed) prior to tail docking (T1), and at 5hr (T2) and 48hr (T3) intervals, plus 7 days later (T4). The concentration of SAA was assessed using a solid phase sandwich ELISA kit (PHASE TM Serum Amyloid A Assay, Tridelata Development Ltd., Maynooth, Ireland). Before statistical analysis, a logarithmic transformation (10log) was used to normalize SAA concentration. Statistical analysis were performed using IBM

SPSS statistics version 26 (2019) software (IBM, Armonk, NY, USA). For each procedure (with or without anaesthesia), a two-way mixed ANOVA was run to understand the effects of treatment (with or without Tri-Solfen®) and time (T1 to T4) on SAA concentration.

**Results:** In cohorts tail-docked under general anaesthesia (B & D), SAA concentrations increased from T1 to T2, achieving maximum values at T3 and decreasing by T4, with T3 significantly higher ( $p < 0.01$ ) than at T1, T2 and T4. These findings are consistent with previously reported significant elevations of SAA 48 hours after a noxious stimulus. The cohort tail-docked with Tri-Solfen® (D) had consistently lower SAA levels than the cohort without this wound treatment (B), although statistically significant differences were not determined between these cohorts ( $p = 0.604$ ). In cohorts tail-docked without general anaesthesia (A & C), SAA concentrations of the untreated group (A) displayed similar trends to Groups B and D. However, the SAA concentrations in the Tri-Solfen® treated group (C) failed to achieve the maximum concentrations at T3 and were similar to T1 and T2, decreasing by T4. These results appear to indicate that treatment with Tri-Solfen® avoided the elevation of SAA concentrations that is expected 48 hours after the surgery. Although statistically significant differences were not detected between lambs treated and non-treated with Tri-Solfen® ( $p = 0.913$ ), this is considered most likely due to insufficient sample size of each cohort.

**Conclusions:** These results and clinical evidence of pain relief presented elsewhere, are encouraging, suggesting that surgical tail-docking without general anaesthesia but wounds are immediately sprayed with Tri-Solfen®, is an affordable and efficacious method of conducting the procedure, although use of larger sample sizes for SAA estimates is recommended to confirm these findings.

**Keywords:** Lambs, tail-docking, welfare, topical anaesthesia, serum amyloid A.

## SR-04

### Effect of a topical wound anaesthesia formulation on the cortisol responses of lambs undergoing tail docking

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**Objectives:** Tail-docking is a routine global husbandry procedure conducted in lambs to reduce myiasis risk and improve fertility. It is often performed without pain relief, although use of general anaesthesia has been advocated, at an exorbitant cost to farmers. There is an urgent need to find a practical and affordable approach acceptable to farmers that avoids or markedly reduces animal suffering. This study evaluated the

effect of a topical anaesthetic 'spray-on' wound management formulation containing the local anaesthetics lignocaine and bupivacaine, plus cetramide and adrenalin, in a gel matrix (Tri-Solfen®, Bayer Animal Health, Gordon, NSW, Australia). Impacts of treatments on serum cortisol concentrations in lambs, where the tail was excised surgically, with and without use of general anaesthesia, are reported.

**Material & Methods:** This study was approved by the Ethical Commission of the University of Zaragoza. Forty-four 45-day-old Rasa Aragonesa female lambs with similar weights in a farm in Zaragoza (Spain) were recruited for this study and divided into 4 equal cohorts ( $n = 11$ ): in Group A the tail was excised using a scalpel without using anaesthesia or any additional treatment; in Group B the tail was surgically excised under general anaesthesia; in Group C the tail was removed with a scalpel without prior anaesthesia and treated immediately with Tri-Solfen®; in Group D the tail was surgically excised under general anaesthesia and treatment with Tri-Solfen® applied before suturing of peri-wound skin to close the wound. Blood samples were collected prior to tail docking (T0), and at 30 min (T1), 5hr (T2) and 48hr (T3) intervals post-tail excision, and sera were obtained and stored at  $-20\text{ }^{\circ}\text{C}$  until analysed. Serum cortisol concentrations were determined using a competitive ELISA assay (Salivary Cortisol ELISA SLV-2930, DRG Diagnostics, Marburg, Germany). A logarithmic transformation ( $10\log$ ) was used to normalize serum cortisol concentration before statistical analysis, which were performed using IBM SPSS statistics version 26 (2019) software (IBM, Armonk, NY, USA). For each procedure (with or without use of general anaesthesia), a two-way mixed ANOVA was run to understand the effects of treatment (with or without Tri-Solfen®) and time (T0 to T3) on serum cortisol concentration.

**Results:** In cohorts tail-docked under general anaesthesia (B & D), serum cortisol concentration did not change significantly over time ( $p = 0.250$ ). Moreover, serum cortisol concentration did not differ significantly between the cohort treated (D) or not treated with Tri-Solfen® (B) ( $p = 0.919$ ). In cohorts tail-docked without general anaesthesia (A & C), serum cortisol concentrations peaked at 30 min post tail removing and decreased thereafter, being T1 value significantly higher than T0, T2 and T3 values ( $p < 0.001$ ). However, in the Tri-Solfen® treated group (C) serum cortisol concentrations at T1 and T2 were lower than in the untreated group (A), although statistically significant differences were not detected between the groups ( $p = 0.162$ ). These results seem to indicate that treatment with Tri-Solfen® reduced the cortisol response elicited after the surgery without general anaesthesia. Low statistical power due to small sample size could have prevented from finding significant differences between A and C cohorts. The elevated cortisol response in lambs tail-docked without general anaesthesia likely reflects a combination of factors, including the effect of handling, restrain and potentially haemorrhage, in addition to pain.

**Conclusions:** These results, and clinical evidence of pain relief presented elsewhere, are encouraging, suggesting that surgical tail-docking without general anaesthesia but where wounds are immediately sprayed with Tri-Solfen®, is an affordable and efficacious method of conducting the procedure, although use of larger sample sizes for serum cortisol estimates is recommended to confirm these findings.



**Keywords:** Lambs, tail-docking, welfare, topical anaesthesia, cortisol.

### SR-05

#### Predicting energy balance in pre-partum dairy ewes by ultrasound measurements of backfat and *longissimus dorsi* thickness

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**Objective:** The objective of this study was to investigate the association of ultrasound measurements of backfat thickness (BFT) and *longissimus dorsi* muscle thickness (LDT) with energy balance in pre-partum dairy ewes. The aim is to identify practical, on-field applicable and non-invasive methods to assess energy status.

**Material and methods:** The study was conducted in four commercial dairy sheep flocks, each keeping one of the four main breeds reared in Greece. Fifty purebred ewes were pre-selected from each flock, based on ultrasound pregnancy diagnosis at day 60 after ram introduction; a total of 177 ewes (Chios, n=46; Frizarta, n=40; Lacaune, n=42 and Assaf, n=49), 3 to 5 years old, that remained pregnant and clinically healthy until lambing, were enrolled in the analysis. Body condition score (BCS) was assessed by palpation in the lumbar region and ultrasound measurements of BFT and LDT thickness were performed using a 5-MHz linear transducer, both at -30 days (-30d) and -15 days (-15d) prepartum. The probe was placed perpendicular to the vertebral column between the transverse processes of the 3<sup>rd</sup> and 4<sup>th</sup> lumbar vertebrae. Each time, three measurements were performed and the mean value (in mm) was calculated. The sum of BFT and LDT (TOTAL\_T), the LDT: BFT ratio (RATIO\_T) on each timepoint and the difference in BFT ( $\Delta$ \_BFT) and LDT ( $\Delta$ \_LDT) between the two measurements (-30d minus -15d) were also calculated. A blood sample was collected at -15d from each ewe and serum was separated within 2h of collection by centrifugation (15 min  $\times$  2500g) and stored at -20°C until analysis. Beta-Hydroxybutyrate (BHB) and non esterified fatty acids (NEFA) were measured with an automated chemistry analyzer (Vitalab flexor E., Vital Scientific N.V., Netherlands), using commercially available kits (BHB: Ben biochem. enterprise, Milano, Italy; NEFA: Randox Laboratories Limited, UK). Negative energy balance status was defined as serum BHB >0.8 mmol/L or NEFA >0.3 mmol/L and treated as a binary variable. The receiver operating characteristic (ROC) analysis was used to define thresholds for fat and muscle reserves and mobilization parameters to predict elevated BHB or NEFA status. All variables with a significant area under the ROC curve were then entered in a binary logistic regression as categorical variables (using the thresholds obtained from the ROC curves) to assess the as-

sociation of fat and muscle reserves and mobilization parameters with BHB and NEFA status.

**Results:**  $\Delta$ \_BFT and TOTAL\_T(-15d) were significant predictors of BHB status ( $R^2=0.272$ ). Ewes with a BFT loss had 6.7 times ( $P<0.001$ ) increased probability to have serum BHB >0.8 mmol/L; probability increased quadratically as  $\Delta$ \_BFT increased ( $R^2=0.486$ ;  $P<0.001$ ). Moreover, ewes with TOTAL\_T(-15d)  $\leq 25.6$  mm, which corresponds to an average BCS of 2.25-2.50, had 5.3 times ( $P<0.001$ ) increased probability to have serum BHB >0.8 mmol/L; probability decreased quadratically as TOTAL\_T(-15d) increased ( $R^2=0.425$ ;  $P<0.001$ ).  $\Delta$ \_BFT and BFT(-30d) were significant predictors of NEFA status ( $R^2=0.316$ ). Ewes with  $\Delta$ \_BFT >0.65 mm had 3.2 times ( $P=0.002$ ) increased probability to have serum NEFA >0.3 mmol/L; probability increased linearly as  $\Delta$ \_BFT increased ( $R^2=0.441$ ;  $P<0.001$ ). Moreover, ewes with BFT(-30d) >6.86 mm, which corresponds to an average BCS of 2.75-3.00, had 6.7 times ( $P<0.001$ ) increased probability to have serum NEFA >0.3 mmol/L; probability increased quadratically as BFT(-30d) increased ( $R^2=0.915$ ;  $P<0.001$ ).

**Conclusion:** Ultrasound measurements of BFT and LDT during the last month of gestation can predict quite accurately the energy status of dairy ewes. As expected, ewes that start losing fat a month before lambing and with a poor BCS at -15d are at higher risk to enter in negative energy balance. Moreover, ewes with a high BCS during the last month of gestation are at greater risk of mobilizing fat reserves pre-partum. Ultrasonography, a non-invasive technique, appears useful in monitoring energy balance during the critical pre-partum period.

**Keywords:** Energy balance, dairy sheep, ultrasound.

### SR-07

#### Q fever in sheep: Long-term control of infection by vaccination of gimmers

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**Introduction:** Small ruminants are regarded as a major source of human infection with *Coxiella burnetii* (Cb). In summer 2012 a Q fever outbreak was observed in a sheep flock with 800 adult ewes. End of 2012 we got involved in this case in order to implement a control program. At that time, we assumed that most of the sheep were already infected or immune. Yearly primary vaccination of gimmers (replacement ewes) with Coxevac<sup>TM</sup> (Ceva Santé Animale) has been introduced as a long-term measure. The vaccine is not licensed for sheep; therefore, a monitoring of vaccination was implemented. This case-report describes the long-term effect of a primary vaccination of gimmers until 2020.

**Methods:** Gimmers were vaccinated twice three weeks

apart (primary vaccination), no further revaccination was performed.

The following groups were ear-tagged for control purposes, animals were randomly selected:

1. 20-30 vaccinated gimmers each year (VG13, 14, 15 etc.),
2. As a positive control 30 ewes were vaccinated in 2013 (VE13).
3. Each year 30 unvaccinated gimmers were selected into a group of sentinels (S). Additionally, gimmers before first vaccination were included. After seroconversion animals were removed from the group.

Direct pathogen monitoring was performed by PCR-testing of vaginal swabs collected hours after parturition and nasal swabs. Indirect monitoring was based on blood samples from sentinels which were tested for seroconversion.

The immune response (PhI/PhII-antibodies, IFN- $\gamma$ -Recall Assay (RA)) was assessed before and after primary vaccination. The ratio of titers (PhI, PhII) or IFN- $\gamma$ -reactivity was assessed as n-fold (nx) increase. Quantitative PCR, PhI- and PhII-antibody tests and IFN- $\gamma$ -RA were performed as previously described (Böttcher et al., 2013; Boettcher et al., 2017).

Animals which had been primary vaccinated in 2013 (n=6 i.e. VG13 and VE13), 2014 (n=3), 2015 (n=10), 2016 (n=11) and 2017 (n=10) were once revaccinated in 2018 and the immune response was assessed before and 3 weeks after revaccination.

Data were analysed by MedCalc Statistical Software version 19.1.3 (MedCalc Software bv, Ostend, Belgium; <https://www.medcalc.org>; 2019). Groups were compared by Kruskal-Wallis-test.

**Results:** From November 2012 until February 2014 the rate of positive vaginal and nasal swabs was 78/268 and 67/263, respectively. The mean pathogen load in positive samples was  $10^{2.6}$  and  $10^{1.6}$  Cb per vaginal and nasal swab, respectively. Thereafter swabs were tested negative, the numbers of analysed vaginal/nasal swabs per year were: 2014: 69/62, 2015: 68/39, 2016: 105/42, 2017: 158/40, 2018: 86/40, 2019: 49/- and 2020(Jan): 97/-.

Percentages of annual seroconversion (PhII-titer >100) in sentinels were: 2013: 16.8% (n=95); 2014: 25% (n=52); 2015: 5.6% (n=53); 2016: 1.3% (n=76); 2017: 2.6% (n=77); 2018: 0% (n=105) and 2019: 0% (n=95).

Groups VE13 and VG13 responded well after vaccination. E.g., VG13 showed an n-fold increase of PhI-, PhII-titers and IFN- $\gamma$ -reactivity of 116x, 168x and 6x, respectively. In contrast, gimmers born after shedding had ceased (VG15-19) showed only weak immune responses after vaccination. The n-fold increase of PhI-, PhII-titers and IFN- $\gamma$ -reactivity e.g. in VG16 was only 1x, 2.5x, 2.7x, respectively.

Available animals in groups VE13, VG13-17 were revaccinated once in 2018. In all groups a similar strong n-fold increase of PhI-, PhII-titers and IFN- $\gamma$ -reactivity was observed after revaccination (e.g., VG16: PhI-titer (11x), PhII-titer (38x) IFN- $\gamma$  (33x - in this case IFN- $\gamma$  was determined as pg/ml).

**Conclusions:** Two years after an outbreak *C. burnetii* was still detected in vaginal and nasal swabs, however, pathogen load was very low. Although seroconversion in sentinels

was even detected until 2017, it did not result in shedding at parturition. Vaccination preferentially boosted an existing immunity, because gimmers born since 2015 showed only a weak immune response after vaccination. However, despite this weak immune response these animals showed a strong immune response after a single revaccination even three years after primary vaccination. Consequently, primary vaccination of gimmers is a cost-efficient long-term measure to control *C. burnetii* in sheep. We do not know if it protects from infection, however, at least in case of urgency revaccination of such a sheep flock would rapidly increase the herd-level immunity.

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**Keywords:** Coxiella, vaccination, sheep, immunity.

#### SR-08

### Influence of housing and management on claw health of Swiss dairy goats

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**Objectives:** Dairy goats are commonly housed on deep bedding without continuous access to a hard ground, at least during winter periods. Due to the lack of claw horn wear on abrasive surfaces, goats have a high prevalence of overgrown wall horn, even if trimmed several times a year (Hill et al., 1997; Kofler, 2016). Overgrown wall horn can result in claw lesions and lameness, impaired locomotion behavior and welfare, as well as reduced milk yield (Christodoulouopoulos, 2009). However, only scarce literature is available on the characteristics and health of goat claws. Moreover, in contrast to dairy cows, claw trimming in goats is less organized and little is known about the quality of claw trimming on Swiss farms. The aim of this study was to obtain greater knowledge on the characteristics and health of goat claws, and on the influence of the overgrown wall horn on the goats locomotion behavior.

**Material & Methods:** Data was collected on 28 dairy goat farms all over Switzerland during two identical farm visits in autumn 2018 and spring 2019. During each visit, the same veterinarian recorded claw condition (claw length, claw width, overgrown claw horn, abnormal claw forms) and claw lesions (e.g. toe ulcer, foreign body, white line lesion) of 12 randomly selected goats during routine claw trimming. All findings have been documented for each animal on a "claw card". In case of



a suspicion for an infectious claw disease (particularly *Dichelobacter nodosus* or *Treponema spp.*), swab samples were taken for further examination. Locomotion activity of the 12 goats was recorded over a period of three days, once just before and once two weeks after the claw trimming. Additionally, the farmers completed a questionnaire on housing conditions and management practices on their farms in order to identify potential risk factors that may contribute to claw problems.

**Results:** Preliminary, descriptive results (final results available by summer): In total 336 goats were included in the study. In spring and autumn all goats showed at least two feet with overgrown claw horn. 0.72% of the examined claws showed no overgrown claw horn, 58.31% showed moderate overgrown claw horn and 40.97% showed severe overgrown claw horn. The severity of overgrowth was greater in spring than in autumn. Animals that had been grazing in alpine regions during the summer time, showed less overgrown claw horn. Claws with severe overgrown claw horn showed more hematomas than the ones with moderate and no overgrown claw horn. Suspicious infected claws were checked for *Dichelobacter nodosus* and *Treponema sp.* with interdigital swabs, all were tested negative.

**Conclusions:** Claw health, different trimming regimes, the influence of overgrown wall horn on claw lesions, the goats locomotion activity, and potential risk factors for overgrown wall horn were evaluated and compared between autumn and spring. Final results from this study will provide important information for dairy goat farmers, contribute to prevent claw problems, and thus promote goat welfare.

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**Keywords:** Goats, claw health, housing, locomotion activity, animal welfare.

#### SR-09

### Vaccinating pregnant ewes with an iron regulated protein (IRP) vaccine could be a suitable strategy to control ovine respiratory complex

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**Objectives:** Ovine Respiratory Complex (ORC) is the leading cause of death in all types of sheep farms, times of the year, regions, and breeds. It is one of the main causes

of morbidity and mortality in lambs with high prevalence and economic consequences (González, 2018). Pneumonia in sheep is a disease complex involving interactions between host factors (immunological and physiological conditions) and different infectious agents (viruses, bacteria and mycoplasmas). *Mannheimia haemolytica* is a key bacterium that causes lung damage whilst viruses and mycoplasmas are considered predisposing agents for the disease. Indeed, *M. haemolytica* causes serious outbreaks of acute pneumonia in neonatal, weaned and growing lambs, as well as calves and goat kids (Ackermann and Borgden, 2000). Very young lambs are often affected, with very short time to protect them through active immunization (Lacasta et al, 2008).

Inactivated vaccines based on the iron regulated proteins (IRP) of *M. haemolytica* have been shown to induce a protective immune response (over 80%) from 6 weeks after the first dose of primary vaccination (Lacasta et al., 2008), and offer cross-protection to serotypes not included in the vaccine (Gilmour et al, 1991). The concept of prenatal vaccination to increase maternal immunity in newborn lambs during the first weeks of life is currently accepted for the prevention of neonatal diarrhea; however, for ORC, very limited information is available.

Therefore, the purpose of this study was to evaluate the titers of specific antibodies against *M. haemolytica* in the colostrum of vaccinated dams and their passive transfer in lambs.

**Materials & Methods:** A blinded and randomized study was performed in a Spanish dairy sheep farm with 4,300 Lacaune sheep. Two hundred pregnant ewes were randomly allocated to one of two experimental groups: Vaccinated (Ovilis® Ovipast, MSD Animal Health) and Negative Control (PBS administration). The first 59 lambing ewes were followed (31 control and 28 vaccinated) with a collection of serum and colostrum samples within 6 hours after lambing. At least 200ml of colostrum was collected to feed the lambs with a nasopharyngeal tube within 6h after birth, followed by a second administration of the same amount 6–12h after birth. Subsequently, a sample of lamb's serum was collected at 24–48 hours of life and at the age of 4–5 weeks (just before weaning).

To monitor the immune response, the titer of specific antibodies against *M. haemolytica* was quantified in the serum and colostrum samples at the Centre for Diagnostic Services in Boxmeer (MSD Animal Health, NL) using an in-house ELISA test for *M. haemolytica* IgG. Additionally, colostrum quality and passive transfer in lambs were monitored with a Brix refractometer.

A non-parametric means comparison test was used (Wilcoxon and Kruskal-Wallis test) to compare the antibody titers between experimental groups (vaccinated versus control) in both colostrum and serum with  $p=0.05$ .

**Results:** At lambing, the *M. haemolytica* antibody levels in vaccinated ewe's serum were significantly increased versus the control group ( $p<0.0001$ ). Although no significant difference in brix-measured colostrum quality was found among groups ( $p>0.05$ ), the specific level of *M. haemolytica* antibodies in the colostrum significantly increased in the vaccinated group versus the control one ( $p<0.0001$ ). Moreover, the level of antibodies present in the colostrum was correlated with the titer in the ewe's serum suggesting a transfer of serum antibodies to colostrum.



Finally, no significant differences were observed in degree of passive transfer between the lambs of the two groups (born to vaccinated versus control ewes) as measured with Brix refractometer in lamb serum at 24-48 hours of life ( $p > 0.05$ ). This result suggests that the passive antibody transfer was similar between experimental groups. The serum of lambs from vaccinated dams had significantly higher level of antibodies against *M. haemolytica* than control ones ( $p < 0.0001$ ). Moreover, at weaning this difference was still significant ( $p < 0.001$ ) and showing a correlation with the initial level of antibodies in the lambs ( $p < 0.0001$ ). An interesting point to emphasize is the correlation between the *Mannheimia* antibody titers at dam level (serum at lambing and colostrum) and lamb level (serum after colostrum intake and at weaning).

**Conclusion:** Vaccination of pregnant ewes with an iron regulated protein (IRP) vaccine (Ovilis® Ovipast) is a suitable strategy to provide maternally derived immunity as an aid to control ovine respiratory complex in young lambs.

**Keywords:** Ewes, ovine respiratory complex, pneumonia, vaccination, lambs.

## SR-10

### Microbial agents associated with infectious keratoconjunctivitis in Swedish reindeer

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**Background:** Infectious keratoconjunctivitis (IKC) is a multi-factorial, transmissible and severe ocular disease that affects ruminants worldwide. The disease in semi-domesticated Eurasian reindeer (*Rangifer tarandus tarandus*) was first reported for more than 100 years ago. Both isolated cases and outbreaks occur, mainly during the colder period of the year, and primarily affecting calves and yearlings. Intensified herding interventions (increased handling, transport, and supplementary feeding of reindeer), raising the stress level of the animals, may contribute to the present increase in appearance of IKC in Sápmi. Previous studies indicate that cervid herpesvirus 2 (CvHV2) works as a primary causative agent. In addition, the bacterium *Chlamydia pecorum* was isolated for the first time during an outbreak of IKC in 2016 in a Swedish herd of reindeer, but its role in developing of IKC remains unclear. Many different bacteria have been isolated from reindeer with IKC, such as *Moraxella bovoculi*, *Escherichia coli* and *Staphylococcus* spp. which all may play a role in the progression of disease. Further investigations, including prevalence, pathogenesis, and risk factors for IKC in semidomesticated reindeer is therefore needed.

**Objectives:** The objective was to study the presence of potential pathogens in samples from reindeer in Sweden with and without clinical signs of IKC and examine which possible secondary bacterial ocular infections followed the suspected primary pathogens CvHV2 and *Chlamydiaceae*.

**Materials and Methods:** Herds ( $n=37$ ) with ongoing outbreaks of IKC in Swedish reindeer 2019-2021 were selected for participation in the study. A referral form was developed and implemented to gather information on clinical signs from affected reindeer. Conjunctival eye swabs (eSwab, Italy) were collected from reindeer with ( $n=170$ ) and without ( $n=30$ ) clinical signs of IKC, sent to the National Veterinary Institute in Sweden (SVA), and analyzed for the presence of CvHV2 and *Chlamydiaceae* with real-time PCR. In addition, routine general aerobic bacteriological culturing was performed. No specific ethical permission was needed since the animals were sampled as part of routine veterinary investigations for medical reasons prior to treatment and for clinical evaluation and with the approval of the owners.

**Results:** The preliminary results revealed a proportion of 24.3% for CvHV2 (45/185) and 33.5% for *Chlamydiaceae* (59/176). Only 4.6% (8/174) of the samples tested positive for both CvHV2 and *Chlamydiaceae* simultaneously, whereas 50.0% (87/174) of the samples tested negative for CvHV2 and *Chlamydiaceae*.

Bacterial cultivation from 186 samples revealed bacterial growth in 91.9% (171/186) with no specific infection in 43.0% (80/186) of the samples. The most frequent isolated bacteria were *Pseudomonas aeruginosa* and *Staphylococcus aureus* cultivated in 11.8% (22/186) and 11.3% (21/186) of the samples, respectively. *Moraxella* spp. and *Klebsiella pneumoniae* were revealed in 8.6% (16/186) and 8.1% (15/186) of the samples. Mixed flora was revealed in 81.7% of the samples (152/186). Data are to be investigated with regards to bacterial growth in reindeer with and without clinical signs.

In reindeer with clinical signs of IKC ( $n=170$ ), there was an association between the presence of *Pseudomonas aeruginosa* and CvHV2, compared to *Chlamydiaceae*. On the contrary, *Klebsiella pneumoniae* and *Moraxella bovoculi* were associated with the presence of *Chlamydiaceae* and not CvHV2. Finally, *Staphylococcus aureus* was more common in samples that tested positive for *Chlamydiaceae* than for CvHV2 and among samples tested negative for both CvHV2 and *Chlamydiaceae*. The most common signs among infected reindeer, reported in 31% of the herds, were epiphora (clear eye flow), leading to a wet cheek, followed by shedding of pus and red mucosa/conjunctivitis, reported in 21% and 15% of the herds, respectively. Data will be complemented prior to the conference and only preliminary results are presented here.

**Conclusions:** In this study we hypothesized that CvHV2 and *Chlamydiaceae* act as primary pathogens of IKC in reindeer. Preliminary results support CvHV2 to be the primary cause since it was only isolated in reindeer with clinical signs, which was not the case for *Chlamydiaceae*. In addition, secondary bacterial infections seem to differ depending on previous findings of CvHV2 or *Chlamydiaceae*, which could be of use when deciding on treatment strategy and to improve our understanding of the pathogenesis and risk factors. However, to conclude whether detected *Chlamydiaceae* in general was preceded by CvHV2 requires further investigation.



**Keywords:** Cervid herpes virus 2, Chlamydiaceae, bacteria, eye disease, reindeer.

### SR-11

#### Flock sensitivity and specificity of pooled fecal qPCR and pooled serum ELISA for screening ovine paratuberculosis

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**Objectives:** The aim of our study was to evaluate the flock sensitivity and specificity of fecal qPCR and serum ELISA using pooled samples for screening paratuberculosis in French sheep.

**Materials and methods:** Using individual feces with low or high qPCR Ct values from ewes sampled in 14 infected flocks, a total of 555 pools of size 5, 10 and 20 were created by diluting individual materials in negative feces and analysed using a commercial IS900 qPCR kit. The relative performances of pooled serum ELISA analysis were evaluated based on the analysis of 181 different pools of size 5 and 10, composed of individual serum samples of various individual S/P values. Finally, a simulation study was carried out to evaluate the performances of 16 screening strategies at flock level, with varying pool size (5 to 20) and number (5 to 60).

**Results:** For pools of size 5, 10 or 20, individual fecal samples with low Ct values were invariably detected. Conversely fecal samples with high Ct values were associated with a lower detection rate in both pools of size 5 (87.0% to 90.0%), 10 (63.0% to 70.7%) and 20 (46.7% to 60.0%). After lowering the decision threshold to 25% and 15% for serum pools of size 5 and 10 respectively, the pooled serum ELISA relative sensitivity ranged between 62.2% and 100.0% depending on the composition of the pools.

At the flock level the use of pooled serum ELISA led to false positive detection rates ranging between 37.6% and 91.8% in paratuberculosis free flocks and prevents its further use in that context. For infection prevalence  $\leq$  5%, the flock sensitivity based on pooled fecal qPCR ranged between 39.0% (5 pools of size 10) and 99.9% (300 sampled individuals, with pools of size 5, 10 or 20), and was always above 93% when the infection prevalence was greater or equal to 15%.

**Conclusion:** We conclude that pooled-fecal qPCR but not pooled-serum ELISA could be a useful tool to detect sheep flocks infected with paratuberculosis.

**Keywords:** Paratuberculosis, pooled samples, faecal qPCR, Serum ELISA, flock sensitivity and specificity.

### SR-12

#### Diagnostic accuracy of a digital Brix refractometer for assessing colostrum quality and failure of passive immune transfer in neonatal lambs

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**Objectives:** This study aims at assessing the diagnostic performances of a digital Brix refractometer (Hanna HI 96801) for the evaluation of colostrum quality and failure of transfer of passive immunity (FTPI) in sheep.

**Materials and methods:** Colostrum samples from 330 meat ewes were collected within 6 hours after lambing and frozen immediately until analysis. The immunoglobulins G1 (IgG1) concentration was assessed by radial immunodiffusion (RID). The colostrum total fat content was assessed by the Gerber method. In addition, 230 plasmas were collected in 2- to 4-day old lambs and their IgG1 concentration measured by RID. All colostrum and plasma samples were assessed by the digital Brix refractometers. Test sensitivity (Se) and specificity (Sp) were calculated using RID as the reference standard with failure of passive immune transfer (FTPI) defined as IgG < 10.0 g/L. The IgG < 50 g/L threshold was used to mark poor quality colostrum. Receiver operating characteristics curves were created and optimal cut-offs values for each refractometer were selected based on the maximization of the Youden's J statistic.

**Results:** The Pearson correlation coefficient between RID and %Brix results on lamb plasma was high ( $r=0.91$ ). The RID and %Brix results on colostrum samples were also highly correlated ( $r=0.79$ ). A significant influence of the colostrum fat concentration on the %Brix value was evidenced, with an increase of 10 g/L of fat leading to an average 0.5% increase on the Brix scale. For the detection of colostrum with IgG1 concentration lower than 50g/L, the optimal decision threshold value was 24.0 % Brix (Se=87%; Sp=91%) for the Hanna Brix refractometer. For the detection of FTPI in lambs (plasma IgG1 < 10 g/L) the optimal decision threshold was 8.3% Brix (Se=97% and Sp=87%).

**Conclusion:** Brix refractometry appeared to provide reasonably accurate results for the assessment of colostrum quality and FTPI in sheep. The optimal decision cut-offs were similar to those established in cattle. For on-farm use, a digital Brix refractometer can be a valuable and inexpensive tool to help producers and veterinary practitioners in their lamb health management programs.

**Keywords:** Colostrum, transfer of passive immunity, digital refractometer, sensitivity, specificity.



## SR-13

**Detection of *Corynebacterium pseudotuberculosis* as an etiologic agent causing polyarthritis in lambs**

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**Objectives:** Polyarthritis is one of the most important pathologies in lambs, both in lactation and during fattening period, due to the implications that this pathology has in the economy of the farm, as it produces growth losses and an increase in mortality. Polyarthritis can be caused by some different microorganisms such as *Streptococcus dysgalactiae*, *Erysipelothrix rhusiopathiae*, *Trueperella pyogenes*, etc.

*Corynebacterium pseudotuberculosis* is the etiologic agent of the caseous lymphadenitis (CLA) which usually affects small ruminants. The most frequent locations of the pyogranulomas caused by *C. pseudotuberculosis* are in superficial lymph nodes, in the superficial clinical form of the disease, or in deep lymph nodes such as mediastinic and internal organs, in visceral clinical form.

In this paper is described the first presentation of *C. pseudotuberculosis* as microorganism responsible for polyarthritis in feedlot lambs.

**Materials and methods:** The Ruminant Clinical Service (SCRUM) of the Veterinary Faculty of Zaragoza, Spain, receives animals from the Faculty's area of influence which can be culling animal belonging to collaborating farms or clinical cases referred by veterinarians. Two fattening lambs coming from two different clinical cases of polyarthritis were referred to the SCRUM during 2019 in order to reach a final diagnosis of the cause of the outbreak.

Both animals were subjected to the same protocol of all the animals received at the service. They were submitted to a rigorous clinical examination and blood samples were taken to carry out a blood count test. Subsequently, thermography and arthrocentesis of the affected joints were made in both animals. In addition, a computed tomography (CT) was also performed in one of the animals. Finally, the lambs were humanely sacrificed to conclude with a complete pathological examination with sampling of affected joints. Samples obtained from arthrocentesis and *post-mortem* study were quickly submitted to the laboratory in order to perform a microbiological and biomolecular analysis. The isolation of the causative agent was carried out in the laboratory EXOPOL diagnóstico y autovacunas S.L.

**Results:** The clinical examination of the lambs showed that one of the lambs was affected in the tarsal joint of the right hindlimb and the other in the tarsal joint of the left hindlimb. The animals were lame and reluctant to move and they also showed a clear stunted growth. The affected joints were hot, swollen and painful.

Hematology revealed a clear leukocytosis with neutrophilia in both animals. These parameters can be indicative of an

acute bacterial infection. Likewise, thermography showed an increase in the temperature of the joint of the affected limbs in comparison with the unaffected joints. Finally, computed tomography (CT) scan performed in one of the lambs revealed an important enlargement of the popliteal lymph node of the affected limb that partially collapsed the adjacent vein, which hinders the venous return.

Pathological findings confirmed an important purulent arthritis in the affected joints in both lambs.

In Both analyzed animals a massive and pure isolation of *Corynebacterium pseudotuberculosis* was obtained in the affected joints, in both samples, those of arthrocentesis and those taken after necropsy.

**Conclusions:** It can be concluded that *Corynebacterium pseudotuberculosis* can cause polyarthritis in lambs, therefore, this microorganism should be included in the list of microorganisms associated with this disorder in lambs. However, the reason why *C. pseudotuberculosis* is able to migrate to the joint and produce this pathology is still unknown.

**Keywords:** *Corynebacterium pseudotuberculosis*, polyarthritis, lambs, arthrocentesis.

## SR-14

**Major concerns of the small ruminant farmer on St. Kitts**

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**Introduction:** Small ruminants make up a large percentage of livestock on St. Kitts but their numbers are declining, estimated to be less than 10,000 head. Because many of the small ruminant farmers of St. Kitts do not own pasture, their herds and or flocks will typically be allowed free range. This complicates production and disease management practices that could help provide improved sustainability. Small ruminant productivity can be increased but only after understanding, addressing and correcting the major issues and concerns of the small ruminant producer on St. Kitts.

**Objectives:** 1) Investigate the concerns and management practices of the St. Kitts small ruminant producer and 2) Prioritize and provide possible solutions.

**Materials and Methods:** Fifty small ruminant producers were solicited via telephone or in person to participate in this study. A questionnaire was administered on site to the producers to better comprehend herd and flock management practices. Information was gathered regarding general farm statistics, nutrition, marketing, reproduction, disease occurrence, disease treatment and or prevention methods and facilities. Producers were asked to list their top three major concerns. Producers were also asked to list the top 3 major disease concerns.

**Results:** The results indicate that predation by dogs af-



ected 50% of small ruminant producers. The second most common issues (36%) were genetic improvement and obtaining medications for both treatment and prevention. The fourth most common issue was theft, 30% of farms. Other major issues were nutrition (26%), general disease issues (24%), fencing/pens (24%), internal parasites (22%), external parasites, predominately ticks (22%) and availability of land/pasture (22%). Other issues that were listed < 10% of the time were, dermatophilus, livestock transport, herd health, housing, wild fires, trauma (hit by bus) and marketing. Specific diseases were not among the top four concerns but the primary specific disease concerns were internal parasites (76%), ticks (72%) and dermatophilus (36%). Other diseases or clinical conditions mentioned as concerns (< 20% of the time) were upper respiratory diseases (nasal bots), abortion, contagious ecthyma, mange, spiders, foot issues, tetanus, sudden death and frothy mouth.

**Conclusion:** The major issues affecting the small ruminant farmers were unexpected as disease issues were not in the top four (e. g. internal parasites). Efforts directed toward decreasing dog predation and theft would benefit the small ruminant farmer. Genetic improvement may be increased by importation of select sheep and goats to the island. Making medications easily, economically and readily available would benefit the St. Kitts small ruminant producer.

**Keywords:** Sheep, goat, problems, survey.

## SR-15

### Assessment of the impact of a biofilm-embedded bacteria-based vaccine against *Staphylococcus* in suckler flock ewes on lambs' development and mammary gland score

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**Objectives:** To evaluate the effect of a vaccine against staphylococcal mastitis in suckler flock ewes by assessing lambs' growth and mammary gland score in vaccinated and non-vaccinated ewes. The used vaccine (VIMCO®, Hipra) included an antigen based on a bacterin of *Staphylococcus aureus* strain, expressing the exopolysaccharide poly-N-acetylglucosamine (PNAG), which is involved in biofilm formation by these bacteria. It was hypothesized that the vaccine would reduce incidence of subclinical mastitis and thereby improve suckler lambs growth.

**Material and methods:** 200 pregnant Ile-de-France ewes were divided in two similar groups based on their parity and age. One group underwent vaccination against mastitis by 2 intramuscular vaccine (VIMCO®) injections at 5 and 2 weeks before lambing (Vacc), the second group served as non-vaccinated control (non Vacc). Their suckling lambs were weighed at birth, at 48 hours of age and at weaning, allowing to calculate daily weight gain (DWG) and age at weaning. Cases of clinically detectable mastitis were recorded. At weaning, each

ewe underwent mammary gland inspection. A mammary gland score taking into account retromammary lymph nodes (normally sized versus hypertrophy), left-right gland asymmetry and presence or absence of nodules was established for each ewe. Data were analyzed by ANOVA in order to assess the effect of vaccination on lambs' weight at 48 hours of age, weight at weaning, DWG at weaning, clinical mastitis incidence and mammary gland score. A p-value below 0.05 was considered as significant. Data from ewes whose lambs died or needed continuous bottle feeding were withdrawn from analysis, leading to 96 non-vaccinated ewes aged of 3.77±1.78 years with 158 lambs and to 93 vaccinated ewes aged of 3.76±1.66 years with 146 lambs.

**Results:** While weight at birth did not differ between groups (Non Vacc: 4.72±0.95 kg versus Vacc: 4.81±0.77 kg, p = 0.95), weight at 48 hours after birth was significantly higher in lambs raised by vaccinated ewes (Non Vacc: 5.03±0.95 kg versus Vacc: 5.25±0.89 kg, p < 0.05). At weaning around 74 days of life, weight and DWG were significantly higher in lambs suckling vaccinated ewes (weight : Non Vacc: 29.39±4.78 kg versus Vacc: 30.63±4.39 kg, p < 0.05; DWG : Non Vacc: 0.34±0.06 kg/day versus Vacc: 0.35±0.05 kg/day, p < 0.05). Mortality of newborn lambs equaled 4.5 % and did not differ between groups. Regarding udder evaluation of vaccinated and non-vaccinated ewes, the mammary gland score did not differ between groups (Non Vacc: 1.19±0.73 versus Vacc: 1.18±0.81, p = 0.89). In-tramammary small-sized nodules were detected in 14% of non-vaccinated ewes and in 15% of vaccinated ewes whereas minor udder asymmetry occurred in 40% of non-vaccinated and in 38% of vaccinated ewes. Clinical mastitis occurred in 1 non-vaccinated and 2 vaccinated ewes.

**Conclusions:** The low prevalence of clinical mastitis in the flock made not possible to assess the impact on detectable signs of mastitis. However, suckler lambs' growth increased by 4% as demonstrated by increased DWG and weight at weaning in lambs raised by vaccinated ewes.

**Keywords:** Mastitis, vaccine, suckler lambs, daily weight gain.

## SR-16

### Chronic Coenurosis in sheep: Spontaneous remission of clinical signs and role of CT and MRI in the diagnosis and follow-up

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Coenurosis is a condition caused by *Coenurus cerebralis*, the larval stage of *Taenia multiceps*, and causes enormous economic losses in husbandry production. *C. cerebralis* mainly develops in the central nervous system of sheep and other ungulates whereas *T. multiceps* inhabits the small intestine of wild and domestic canids, being the dog the main definitive host. Proper diagnosis of *Coenurus cerebralis* infection is

challenging and although epidemiological and clinical studies are useful to guide the diagnosis, a post-mortem examination is decisive to confirm the suspicion. In alive animals, a presumptive diagnosis is usually made in the light of the neurological examination, but clinical signs observed are usually non-specific. In the present study, a case of spontaneous clinical remission of chronic cerebral coenurosis is presented for the first time and the role of computed tomography (CT) and magnetic resonance imaging (MRI) in the diagnosis and follow up is studied.

Five animals presenting neurological clinical signs were referred to the Clinical Ruminant Service (SCRUM) of the University of Zaragoza. All animals were adult young sheep (1-3 years old) and they belong to a meat sheep flock reared in a semi-intensive production system. No treatments were applied to the animals prior to its reference to the SCRUM. Exhaustive clinical examination of the five animals was performed, making special emphasis on neurological examination. Although a huge variety of neurological clinical signs were observed, the most common symptoms were circling, frequent bleating, separation from the flock and visual impairment. A CT scan of the five affected animals was performed with a Bivro CT scan of two slides (General Electric Healthcare). CT imaging revealed the presence of single fluid-filled cystic structures that occupied 40-55% of the cranial cavity, comprising and atrophying the adjacent encephalic structures. In the light of clinical signs and CT images, diagnosis of chronic coenurosis was established. Four of the five animals were humanely sacrificed and the diagnosis was confirmed at the postmortem evaluation, where big intracranial parasitic cyst with multiple protoscolices were found associated with severe focally extensive pressure atrophy of the brain.

As one of the animals was pregnant, it was hospitalized until it raised the lamb. During this time, that lasted 7 months, clinical signs remitted spontaneously in the absence of any treatment application. To clarify this outstanding clinical finding both, CT and MRI (Hitachi AIRIS Mate 0.2T, Blue Star E&E) exams were performed. This second CT revealed a marked retraction of the parasitic cyst. In addition, a severe increase in tisular density was found in contrast with the fluid-filled Coenurus cyst showed in the first CT. MRI confirmed the location of the Coenurus and helped to further characterize the morphological abnormalities in the cranial cavity. Interestingly, MRI revealed that the area previously occupied by the parasitic cyst had been replaced by encephalic tissue and a mass of undefined consistence. After weaning, the ewe was humanely sacrificed. Post-mortem examination of the brain showed focally extensive polioencephalomalacia localized in the right occipital lobe. At cut section, shrunken remnants of the parasitic cyst were found as a nodular greyish mass, which distorted the white matter, expanding and compressing adjacent structures. Tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin wax for histopathological evaluation. Microscopically, degenerate coenurus were characterized by a disrupted 300µm thick hyaline tegument, partially mineralized and filled by granular acellular eosinophilic refringent material and multiple round basophilic structures. The parasite cyst was surrounded by moderate edema and a rim of abundant foamy macrophages, eosinophils, lymphocytes and plasma cells. The final diagnosis of chronic coenurosis with cyst collapse and larval death was assessed.

In this report, CT and MRI revealed outstanding results in the detection of the parasitic cysts. Moreover, valuable information of the surrounding nervous structures was obtained. In regards of these results, both techniques can be proposed as the gold standard for the diagnosis of the chronic phase of this condition. Furthermore, CT and MRI can become key tools for the diagnosis of ovine encephalic affections and the development of new therapeutical approaches in sheep. To date, this is the first report of the imaging follow-up of spontaneous clinical remission, suggesting the role of optimal management in the outcome of the chronically affected animals.

**Keywords:** Ovine, coenurosis, computed tomography, magnetic resonance imaging.

## SR-17

### A topical anaesthetic wound formulation diminishes pain responses of lambs at tail-docking

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**Objectives:** Tail-docking is a routine global husbandry procedure conducted in lambs to reduce myiasis risk and improve fertility. It is often performed without pain relief, although use of general anaesthesia has been advocated, at an exorbitant and prohibitive cost to farmers. There is an urgent need for a practical and affordable approach acceptable to farmers that markedly reduces animal suffering. We evaluated use of a topical anaesthetic 'spray-on' wound management formulation containing the local anaesthetics lignocaine and bupivacaine, plus cetramide and adrenalin, in a gel matrix (Tri-Solfen®, Bayer Animal Health, Gordon, NSW, Australia). Impacts of treatments on pain response in lambs, where the tail was excised surgically, with and without use of general anaesthesia, are reported.

**Materials and methods:** This study was approved by the Ethical Commission of the University of Zaragoza. Female Rasa Aragonesa 45 day-old lambs (n = 44) with similar weights in a farm in Zaragoza (Spain) were recruited for the study. The 4 equal cohorts (n=11) of lambs with: the tail excised with a scalpel without local or general anaesthesia and without subsequent stitching (Group A); the tail excised under general and local anaesthesia with stitching to close the wound (Group B); the tail excised with a scalpel without prior anaesthesia and without stitching but treated immediately with Tri-Solfen® (Group C); the tail excised under general and local anaesthesia with stitching to close the wound following immediate treatment of the wound with Tri-Solfen® (Group D).

Pain-related behaviour was assessed using a numerical rating scale (NRS) developed previously. A trained scientist blinded to treatment observed the lambs immediately (B0),



2.5h (B1) and 5h (B2) after tail docking. Individual lambs were ascribed an NRS score between 0 and 3, where: 0 = no pain-related behaviour; 1 = mildly abnormal posture, gait or behaviour, including mild kyphosis without hyperextension of hindlegs, ventral recumbency with hindlegs partially extended or mild stiffening of gait without overt limping or leg dragging; 2 = moderately abnormal posture, gait or behaviour, including 'statue standing' with head down and prominent kyphosis, moderate stiffening or slowing of gait or hyperextension and/or abduction of hindlegs, ventral recumbency with hindlegs fully extended; 3 = displaying severely abnormal posture, gait or behaviour, including marked agitation with twisting or writhing, high frequency of postural change from lying to kneeling or standing, distressed vocalisation, lateral or prostrate lying, kneeling, dog sitting or tremors, shaking or lip curling. Data were analyzed using the Mann-Whitney U test.

**Results:** In cohorts tail-docked under general anaesthesia (B & D) the mean of the NRS pain score at B0 was 2.364 for untreated animals and 2.045 for animals treated with Tri-Solfen® ( $p > 0.05$ ). However, in cohorts tail-docked without general anaesthesia (A & C), the mean of the NRS pain score at B0 was lower, at 1.273 for untreated animals and 0.682 for treated lambs ( $p = 0.013$ ). The differences between the two methods of tail docking at B0, regardless of whether the lambs were treated or not with Tri-solfen®, also showed highly significant differences ( $p = 0.004$ ). The group not undergoing surgical stitching suffered less pain immediately after the procedure, especially if treated with Tri-solfen®. These results accord with those previously published but where tails were removed by a hot-iron (Lomax et al. 2010).

By 2.5hrs after tail docking (B1), the NRS scores had begun to equalize, although differences were still observed between treatments. In cohorts without Tri-solfen® (A & B) the mean of the pain response was 1.136 and 1.091, whereas in cohorts treated with Tri-solfen® (C & D) the mean was 0.864 and 0.818, respectively ( $p > 0.05$ ). Finally, at 5 hours (B2) most of the animals, independent of the group they belonged to, no longer displaying pain behaviours.

**Conclusions:** These findings and additional clinical evidence of pain relief presented elsewhere are encouraging, suggesting that surgical tail-docking without general anaesthesia but where wounds are immediately sprayed with Tri-Solfen® is an affordable method of alleviating pain in lambs undergoing tail docking.

**Keywords:** Sheep, topical anaesthesia, pain, tail-docking.

## SR-18

### Effect of a topical anaesthetic formulation on wound healing after tail-docking in lambs

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**Objectives:** Tail-docking is a routine global husbandry procedure conducted in lambs to reduce myiasis risk and improve fertility. It is often performed without pain relief, although use of general anaesthesia has been advocated, at an exorbitant cost to farmers. There is an urgent need to find a practical and affordable approach acceptable to farmers that avoids or markedly reduces animal suffering. This study evaluated the effect of a topical anaesthetic 'spray-on' wound management formulation containing the local anaesthetics lignocaine and bupivacaine, plus cetramide and adrenalin, in a gel matrix (Tri-Solfen®, Bayer Animal Health, Gordon, NSW, Australia). Impacts of treatments on wound healing in lambs, where the tail was excised surgically, with and without use of general anaesthesia, are reported.

**Materials and methods:** This study was approved by the Ethical Commission of the University of Zaragoza. Female Rasa Aragonesa 45 day-old lambs ( $n = 44$ ) with similar weights in an intensively-housed farm in Zaragoza (Spain) were recruited for the study. The 4 equal cohorts ( $n=11$ ) of lambs with: the tail excised with a scalpel without local or general anaesthesia and without subsequent stitching (Group A); the tail excised under general and local anaesthesia with stitching to close the wound (Group B); the tail excised with a scalpel without prior anaesthesia and without stitching but treated immediately with Tri-Solfen® (Group C); the tail excised under general and local anaesthesia with stitching to close the wound following immediate treatment of the wound with Tri-Solfen® (Group D). Following the procedure, the animals were examined daily for 15 days, with lesions photographed. This enabled analysis of wound healing after tail-docking and the detection of secondary infections. Data were analyzed using survival analysis (Kaplan-Meier method and Breslow test), evaluating the days until the wound is found infected.

**Results:** In the period analyzed, 73% (16 of 22) of lambs in the two cohorts not undergoing surgical stitching (A & C) and 91% (20 of 22) of lambs in the two cohort undergoing surgical stitching (B & D), developed signs of wound infection. Further, the mean of days on which the animals showed no signs of wound infection was significantly later in the two groups not undergoing surgical stitching (A:10.2 & C:11.5) versus those where stitching occurred (B:5.2 & D:4.6). The median, being the day when half of the lambs displayed infection and half did not, was: A:2 days; B: 3 days; C: 4 days; and D: 1 day. Although only significant differences were found when comparing the two methods, with and without stitching, among those treated with Tri-Solfen®, it was observed that the best results was in cohort group C, being lambs whose tail was excised without stitching and the wounds were treated with Tri-Solfen®.

**Conclusions:** These results and those presented elsewhere are encouraging, suggesting that surgical tail-docking without general anaesthesia but where wounds are immediately sprayed with Tri-Solfen® is an affordable and efficacious method of conducting the procedure, since this topical anaesthesia wound treatment formulation reduces infections to a certain extent, hastening wound healing.

**Keywords:** Sheep, topical anaesthesia, wound healing, tail-docking.

## SR-19

**Study of the use of bronchoalveolar lavage as a live diagnostic method to detect clinical pulmonary Maedi Visna**

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**Objectives:** Maedi-Visna virus (MVV) is a lentivirus that infects, mainly, sheep older than two years, causing a multi-systemic and slow progressive syndrome, inducing a chronic inflammation of the lung, mammary gland, central nervous system and joints. It causes relevant economic losses worldwide and due to the lack of vaccines and treatments, a suitable diagnosis is decisive to develop control programs.

The main objective of this survey was to evaluate the usefulness of the molecular study of bronchoalveolar lavages using PCR techniques to improve the live diagnosis of the pulmonary form of Maedi Visna disease (MV). In order to investigate the efficacy of this diagnostic method, the MV compatible lung lesions found after the necropsy of the studied animals were analyzed and lung tissue samples were also collected to perform a molecular diagnosis of the MVV.

**Material and methods:** One hundred and fifty-five culling sheep coming from four collaborating farms were analyzed at the Ruminant Clinic Service of the University of Zaragoza. All the animals were subjected to the same protocol: complete clinical examination with special emphasis on the respiratory system was performed and a blood sample was collected to carry out a MVV serological analysis. Subsequently, prior to the animals being euthanized, a bronchoalveolar lavage was accomplished to obtain a sample of the lower respiratory tract in order to be analyzed by PCR. Finally, post-mortem pathological study was carried out at the Pathological Service of the Veterinary Faculty of Zaragoza and lung tissue samples were taken for molecular studies. Samples obtained in bronchoalveolar lavages and post-mortem collections (portion of lung parenchyma) were analyzed in EXOPOL diagnóstico y autovacunas S.L. by quantitative PCR (qPCR). The specific EXOone Maedi Visna - CAEV oneMIX kit developed by the EXOPOL laboratory was used. The samples were considered positive when the Cq value (quantification cycles) was  $\leq 38$ .

The data were recorded in SPSS STATISTICS 22.0. computer program and the WinEpi web platform was used to estimate sensitivity and specificity values and predictive values.

**Results:** Lungs with compatible MV lesions (12.25%) were those that presented these four lesions: lung enlargement, general grayish discoloration, grey subpleural dots and a significant increase in mediastinal lymph nodes size. Lungs suspicious of having MV (22.60%) were considered to be those with 2 or 3 of these lesions and lungs not compatible (65.15%) those with 1 or none compatible lesions. The main lesion found was lung enlargement (43.90%), followed by the discoloration (32.90%), increased size of mediastinal lymph nodes (23.90%) and grey subpleural dots (12.25%).

Regarding bronchoalveolar lavages, 16.10% of the analyzed animals were MVV-positive by PCR. Therefore, 77.80%

of animals with MV-compatible lesions and 20.60% of the animals classified as suspected gave a positive PCR result for MVV in bronchoalveolar fluid. Only 3.10% of the lungs classified as non-compatible were MVV-positive in bronchoalveolar lavage. The results of positive predictive value (PPV) and negative predictive value (NPV) related to the presence or absence of macroscopic lesion have a value of 82.40% and 96.00%, respectively.

On the other hand, 29.73% of the lung parenchyma analyzed tested positive by PCR. In this case, 89.50% of the lungs with lesions compatible with MV, 37.10% of the suspicious and 14.90% of the non-compatible ones showed positive results in parenchyma. Thus, PPV was 54.80% and NPV was 97.60%. These results could suggest that the PCR test of lung parenchyma also detects infected animals that do not show obvious MV macroscopic lung lesions.

**Conclusions:** Bronchoalveolar lavage is revealed as a suitable technique, little invasive and innovative, for the *in vivo* diagnosis of the pulmonary form of Maedi Visna disease, with adequate predictive values both positive and negative.

**Keywords:** Bronchoalveolar, lavage, diagnose, pulmonary, Maedi.

## SR-20

**Evaluation of different reproductive management protocols to advance pregnancy onset in sarda ewe-lambs at their first reproductive season**

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**Objectives:** For a successful dairy production Mediterranean dairy sheep breeding aims to initiate the mating season in late spring for adult ewes with lambing in late autumn. The ewe-lambs born in autumn are usually mated during the reproductive season of the following year (late August – December). This scenario imposes a different management strategy for the ewe-lambs which respond poorly to the male effect and tend to have poorer response to synchronization protocols when compared to adult ewes. Thus, the reproductive management of ewe-lambs relies mostly on natural mating, where young ewes are kept with the males in a single flock. This practice leads to a long lambing season with the bulk of the ewe-lambs lambing in March-April (pregnancy onset October-November). In addition, ewe-lamb's reproductive and productive performance are lower compared to adult ewes.

The present study aimed to assess if the ram effect alone or associated with a GnRH administration, are able to advance pregnancy onset in dairy ewe-lambs of different weight, when entering the first reproductive season. Treatments were tested in different groups having low, medium and high live weight. The different outcome between protocols was evaluated by comparing the number of days from treatment to pregnancy onset.



**Materials & Methods:** On mid-July, 131 prepubertal ewe-lambs were selected from a single flock. Ovarian status was evaluated by transrectal ultrasonography to ensure the absence of cyclicity. The animals were divided in three groups according to live weight (live weight  $\pm$  SE; high weight: HW,  $39,1 \pm 0,3$ ,  $n=35$ ; medium weight: MW,  $33,8 \pm 0,2$ ,  $n=64$ ; low weight: LW,  $28,2 \pm 0,3$ ,  $n=32$ ). Within each weight group, animals were randomly allocated into two subgroups: GnRH, treated with a GnRH analogue (gonadorelin,  $40 \mu\text{g}/\text{head}$ ;  $0.8 \text{ ml}$  of Cystoreline®, Ceva Salute Animale, Italy); CTR, which was the untreated control. Both subgroups were exposed to the ram effect. GnRH groups following 1 week from the treatment administration, were evaluated again by ovarian ultrasound scanning. Ewe-lambs showing corpora lutea were injected with a PGF2 $\alpha$  analogue (cloprostenol,  $100 \mu\text{g}/\text{head}$ , PGFVeyx, Vexy-Pharma GmbH, Germany) and then placed with crayon marked rams. Ewe-lambs not showing signs of ovulations were injected with a second dose of GnRH. After 7 days, the ewe-lambs were checked again and the ones showing corpora lutea were injected with a PGF2 $\alpha$  analogue, while the remaining received a third injection of GnRH. After all ewe-lambs were exposed to fertile rams. Ewe-lambs in the CTR, after the initial ultrasound scanning, were managed as single flock with fertile rams fitted with crayon markers.

Ewes mating behaviour was checked daily for 120 days starting from rams introduction. The mated ewe-lambs, once identified, were removed from the flock. Pregnancy was diagnosed within 30 days after mating using transrectal ultrasonography. During the trial, data collection was supported by a farm management software developed by Sementusatech®. The efficacy of the protocols in anticipating pregnancy onset was determined by assessing differences between groups in the number of days (from Day 1 - treatment start to pregnancy onset) needed to reach the 25%, 50%, 75% of flock pregnancy rate.

**Results:** There was a difference in the timing of pregnancy establishment between groups ( $p < 0.005$ ). The GnRH-MW group showed the best performances in reaching the threshold pregnancy rates of 25, 50 and 75% ( $P < 0.05$ ) when compared to the other groups. Both LW groups had poorer performances ( $P < 0.05$ ). The administration of GnRH had no effect in advancing the onset of pregnancy in the HW group (GnRH-HW) when compared to (CTR-HW). Both groups showed a delay in reaching the 25% pregnancy rates compared to the GnRH-MW group, but thereafter HW groups were able to catch up.

**Conclusions:** The current study showed that the hormonal protocol can improve reproductive performances in ewe-lambs at their first reproductive season by advancing pregnancy onset and by increasing fertility rates in medium weight ewe-lambs. The ram effect alone could be used with ewe-lambs that have already reached the optimal weight at the onset of the breeding season.

**Keywords:** Puberty, male effect, GnRH, ultrasound, dairy sheep.

## SR-21

### Effect of a topical anaesthetic formulation on viral load in lambs naturally infected with orf virus

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**Objectives:** Orf is a highly contagious eruptive skin condition of sheep and goats, caused by a Parapoxvirus with a worldwide distribution. It affects mainly lambs and kids, with more serious outbreaks often associated with intensive husbandry, causing significant financial losses to livestock production. It is also a zoonotic disease, affecting mainly people via direct or indirect contact with infected animals. Vaccination remains the preferred option to control the disease. However, currently in Spain and many other countries, no orf vaccine is available. The treatment of this disorder referred to as Contagious Ecthyma and Scabby Mouth, involves standard hygiene practices and management of presumptive secondary infections.

The wound dressing formulation, Tri-Solfen® (Animal Ethics Pty Ltd, Australia) is registered for use in sheep in Australia, cattle in Australia, New Zealand and Laos, and is in the process of being registered to be used in cattle and pigs in Europe and other countries. This formulation offers numerous advantages over current therapies, particularly as it has been confirmed as providing pain relief and more rapid healing of lesions (Windsor et al, 2016). The formulation contains two local anaesthetics (lignocaine and bupivacaine), adrenalin and an antiseptic (cetramide) in a gel formulation that creates a barrier effect with a pH of  $\sim 2.7$ . This numbs the pain of lesions, reduces their infectivity, hastens healing, potentially reduces weight loss, and the antiseptic properties avoids the need for other treatments, including antibiotics.

This study explores potential antiviral roles and healing properties of Tri-Solfen® in orf naturally infected lambs through viral genome real time PCR quantification and tissue culture in ovine primary cells.

**Materials and methods:** This study was approved by the Ethical Commission of the University of Zaragoza. Fourteen one-month-old Rasa Aragonesa lambs, naturally infected with orf, were recruited from a farm where an outbreak of orf disease was occurring. The animals were selected at the early stages of the infection when lesions were initially advised and divided into two cohorts: Group A ( $n=11$ ) consisting of animals with orf lesions treated with Tri-Solfen® and Group B ( $n=3$ ), a control group without treatment.

Cotton swabs were obtained before treatment (T0) and days 1 (T1), 3 (T2) and 5 (T3) post-treatment, then submitted to direct DNA extraction and real-time PCR quantification (Exopol) or to incubation with primary tissue cultures from ovine skin fibroblasts (OSF) and T-immortalized goat embryonic fibroblasts (TIGEF). Orf virus quantification was performed

by real time PCR on DNA from cultured cells at day 0 and 5 post-treatment. Data were analyzed using the non-parametric Wilcoxon test for paired samples and by T-Student's test for unrelated samples.

**Results:** In the study carried out using quantitative PCR, no significant differences were found between day 0 pre-treatment (T0) and day 5 post-treatment (T3) ( $p=0.722$ ). However, when the viral load was assessed in primary tissue cultures of ovine skin fibroblasts (OSF) and T-immortalized goat embryonic fibroblasts (TIGEF), there was a reduction in both groups between T0 and T3 that was significant in the OSF cell cultures ( $p<0.05$ ).

**Conclusions:** These results suggest that despite the presence of the viral DNA in the orf lesions at 5 days post-treatment, this may belong to inactivated virus as the viral load obtained after cell culture of the samples of the treated animals was significantly less than that obtained from controls. These findings suggest that as treatment of orf lesions with Tri-Solfen® reduces the viral load present in lesions, such therapy may also alter the clinical progression and transmission in outbreaks of Contagious Ecthyma.

**Keywords:** Sheep, orf virus, topical anaesthetic formulation, viral load.

## SR-22

### Clinico-serological and molecular study of *peste des petits ruminants* in sheep and goats in Al Muthanna province/ Iraq

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**Objectives:** This study intends to investigate the *peste des petits ruminants* virus (PPRV) infection in sheep and goat using Clinico-serological and molecular tools in Al Muthanna province/ Iraq.

**Materials and Methods:** In October 2017 outbreak was occurred in sheep and goats in Al Muthanna province/ Iraq. There were obvious deaths both in young and adult sheep and goats during the period extended from October/ 2017 to March 2018. According to the case history, clinical symptoms, and post-mortem investigations, the PPR virus was accused. Blood samples were collected from the jugular vein of totally 75 animals of the infected flocks (50 sick and 25 healthy animals) using sterile vacutainer tubes (without anticoagulant), and the serum was extracted after centrifugation and kept at  $-20^{\circ}\text{C}$  deep freeze in the laboratory until the test is conducted. Competitive Enzyme-Linked Immunosorbent Assay (C-ELISA) was down to detect the presence of antibodies against PPRV. Mouth swabs (epithelial tissue from the lesions) from sick animals were collected, kept in a frozen box, and sent to the laboratory. RNA was extracted from all samples according to the instructions using the RNeasy\_ Mini Kit (Qiagen, Germany). The extracted RNA was kept at  $(-120^{\circ}\text{C})$  until amplification procedures. Later on, the RNA samples were used for cDNA synthesis, and specific two sets of primers were used

for the detection of PPRV fusion (F) gene and nucleocapsid (N) gene.

**Results:** Typical clinical signs of PPRV have obviously appeared on the sick animals including fever, loss of appetite, marked depression, erosive stomatitis, ocular catarrhal inflammation, profuse diarrhea. Some cases revealed fetid & blood-stained and respiratory signs that included nasal mucous discharge, different stages of bronchopneumonia and mouth breathing and erosions of the nasal cavity. The post-mortem examination of few numbers of dead animals showed congested trachea, red lung hepatization with area firm to touch, particularly in the cardiac and anterior lobes and enlargement of pulmonary and mesenteric lymph nodes accompanied with congestion of small intestinal mucosa. The serological test shows positive results in 90 % (45 out of 50) and 48 % (12 out of 25) of sick and healthy animals respectively. The results of molecular analysis revealed 10 positive samples for PPRV, and 7 F genes (PPRV/ Al Muthanna / 2017) were genetically close to the (PPR \ Kurdistan 2012), (KF478924) and (JF 274480) strain previously determined in Kurdistan/ Iraq, Turkey and Egypt respectively with homogeneity reached to 95% nucleotide sequence. Moreover, three samples were genetically identical for the N gene of %100 (PPR \ Kurdistan 2012), %99 (DQ840197), %99 ( FJ 795511), and %97 (DQ 840190) strain from Kurdistan/ Iraq, Saudi Arabia, Emirate and Israel respectively.

**Conclusions:** In conclusion, PPRV was diagnosed in small ruminants in Al Muthanna province, and the diagnosis was done according to clinical signs & post-mortem examination and confirmed by serological C-ELISA test and RT-PCR molecular technique. Moreover, the results of the molecular technique revealed the close genetic relationship between local PPRV strain and strains isolated previously from neighboring countries. Another molecular epidemiological study is recommended by the author to determine the origin of PPRV in Iraq.

**Keywords:** Peste des petits, Sheep, Molecular , Iraq.

## SR-23

### Use of thermography for the diagnosis of chronic proliferative rhinitis in sheep and for the differential diagnosis of the first case affecting the dorsal turbinate

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**Introduction:** Chronic proliferative rhinitis (CPR) is an upper respiratory tract disease that affects sheep and is associated with *Salmonella enterica* subsp. *diarizonae* serotype 61:k:1:5:(7). It may be unilateral or bilateral, and, although minor changes have been seen in dorsal turbinate, the ventral nasal turbinate is always affected, which makes it possible to observe the proliferative tissue emerging from the nostril.

In the following case it is described the use of a thermal



imaging camera in the diagnosis of an atypical case of chronic proliferative rhinitis in sheep. Contrary to what is normally observed, this time the dorsal turbinate and ethmoidal area were mainly affected.

**Materials and methods:** The Small Ruminant Clinical Service of the Veterinary Faculty of Zaragoza, Spain, (SCRUM) received, in 2018, a Lacaune ewe with severe upper respiratory tract clinical signs. The 3-year-old female presented a very low body condition, despite no loss of appetite, and extreme weakness. The ewe also showed bilateral seromucous nasal discharge, an upper airway snoring and inspiratory dyspnea. Moreover, a nasal deformation in the middle area of the nose was clearly present.

In order to obtain more information, further complementary imaging techniques such as thermography, x rays and computed tomography scanner (CT) were carried out.

**Results:** Thermography revealed an increase in the temperature of both nasal cavities, corresponding to the difficulty of the cold air passing through, and a hottest area in the middle part of the nose, which corresponded with the area of the swollen dorsal turbinate. Thereafter, CT of the cranial region enabled us to obtain a clear image of the damaged tissue, showing the increase in size of swollen dorsal turbinate and the partially affected ethmoidal area. Along with these, two lateral radiographic projections were made, one on each side of the affected area, and an increased opacity was observed inside the dorsal nose chamber. All these images were compatible with chronic proliferative rhinitis although affecting, for the first time in such a strong extent, the dorsal nasal turbinate and the ethmoidal area.

After the death of the animal, a post-mortem examination was performed. When sectioning the head sagittally, an inflammation of the ventral and dorsal nasal turbinates of both nasal cavities were observed. When the dorsal turbinate from the right side was removed, different polypoid formations could be observed.

Histopathological study of the swollen turbinates revealed a thickened nasal mucosa and an epithelium composed of several layers of disorganized epithelial cells. All the tissue presented a chronic inflammation. The lamina propria was expanded and densely infiltrated by plasma cells and a few macrophages. The intracellular presence of the bacteria in the epithelium and the lamina propria was also confirmed by immunohistochemistry of the affected tissue.

Both the microbiological (culture) and molecular (qPCR) study of the samples taken from the affected tissue of both turbinates revealed a massive presence of *Salmonella enterica* subsp. *diarizonae*, which was typed as serovar 61:k:1,5,(7).

**Discussion/conclusions (literature; acknowledgments):** Chronic proliferative rhinitis is becoming a more commonly diagnosed disease, not only in Spain, but also in other countries in Europe and America. To date, in all the descriptions made of this disease the ventral turbinate was always affected unilaterally or bilaterally and only minor changes were observed in dorsal turbinate or ethmoidal area. In the case here reported, a detailed description is made of the condition of the dorsal and partially ethmoidal turbinate, showing how this bacterium can deepen the respiratory tract, affecting more seriously internal structures.

The use of thermography, a cheap and handle diagnostic imaging technique, can be very helpful to locate the affected area, thereby facilitating clinical diagnosis of upper respiratory tract diseases of sheep, especially at the beginning of the process.

**Keywords:** Chronic, proliferative, rhinitis, thermography, dorsal.

## SR-24

### Vaccination against ovine footrot in lactating ewes has only a transient effect on milk production

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**Objectives:** A multivalent inactivated vaccine against ovine footrot (Footvax®, MSD Animal Health) is indicated for the active immunization of sheep against footrot caused by *Dichelobacter nodosus*. The need of a non-restrictive claim on lactating ewes becomes obvious to reduce the use of antibiotics, to avoid milk discard in relationship with milk antibiotic residues and to avoid footrot related discomfort.

The aim of the study was to assess the immediate impact on milk production of vaccination against footrot in lactating ewes.

**Materials & Methods:** Forty-four lactating ewes were included and randomised (milk production) in one farm with no history of footrot. Twenty-two ewes were vaccinated subcutaneously with the inactivated multivalent footrot vaccine (Footvax®, MSD Animal Health) on D0 and D28 and twenty-two received a saline solution (negative control or NaCl group) on the same days. During the period following the vaccine injections, the milk production was individually measured daily for 42 days after the first injection.

**Results:** First administration of the vaccine (D0):

Cumulative milk yield by ewe (L/Ewe) was 2.9% lower in the vaccinated group ( $38.6 \pm 4.9$  L and  $39.8 \pm 6.1$  L respectively) from 0 to 14 days after vaccination. Moreover, both groups showed similar time curve of mean milk yield during the first 2 days after product administration. After that, the average daily milk yield in the vaccinated group was slightly below the average daily milk yield in the NaCl group until 12 days after vaccination when milk production was recovered in the vaccinated group compared to the NaCl group.

Second administration of the vaccine (D28):

Time curve of mean milk yield per ewe in the vaccinated group decreased sharply the day after product administration until D31 (i.e. 3 days after vaccination) compared to the curve in the NaCl group. There was a significant decrease in milk yield of 8.8% in the vaccinated group compared to the NaCl group from D28 till D42. The milk yield in the vaccinated group



increased to the same yield of the NaCl group from D32 to D39, demonstrating once again that vaccination led only to transient milk reduction and was fully recovered 11 days after vaccination.

The second vaccination can be considered as a worst-case safety scenario which will in veterinary field practice not that often occur in lactating ewes, because the basic repeated vaccination schedule is normally given to lambs at a younger age.

**Conclusion:** Vaccination against ovine footrot (Footvax®, MSD Animal Health) in lactating ewes led to a transient and slight decrease in milk production of 2.9 %, which recovered around 11 days after the first administration. From this, it is clear that the milk loss due to vaccination is very moderate compared to potential footrot related negative consequences such as economic impact, extra antimicrobial use and extra labour.

**Keywords:** Ovine footrot, vaccination, lactation, transient effect, anti microbial reduction.

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## SR-25

### The impact of veterinarian-farmer engagement on sheep lameness: a clinical impressions study

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**Objectives:** The objective of the study was to encourage vets and farmers to engage with reducing sheep lameness in UK flocks and to increase implementation of the 'Five Point Plan' (developed by Food Animal Initiative farms in 2014 and adopted as the UK national strategy for tackling lameness in sheep).

**Materials & Methods:** Between January and June 2020 veterinary practices across the UK were approached by convenience sampling to assess interest in participation in a clinical impressions study. Veterinarians were required to carry out a visit to each enrolled farm to work through the Five Point Plan and identify measures which could be taken to reduce lameness on an individual flock basis. A veterinary assessment of lameness prevalence was undertaken and where possible, farm medicine and treatment records consulted to establish antibiotic usage. Individual flock-level data were retained by the veterinarian to maintain confidentiality. The participating farms were provided with a primary course of *Dichelobacter nodosus* vaccine (Footvax®, MSD Animal Health) to be administered to each animal in the flock. A subsequent visit was carried out by the veterinarian 6-9 months later to reassess lameness, and medicine and treatment records. An anonymised questionnaire was distributed to all participants to gain feedback on farmer perceptions of the impact of the study on lameness in their flocks.

**Results:** Twenty-seven veterinary practices were recruited. Veterinarians from these practices enrolled a total of 70 farms. Average flock size was 630 with a range from 60 to

2300 breeding ewes. In total 44,000 sheep were vaccinated. During the 6-9 months of the study, this targeted approach more than halved average flock lameness from 13% (range 3-40%) to 5% (range 1-15%). This is compared to a national reduction in lameness prevalence over 17 years from 11% (2004) to 3.2% (2021). Among the participating farmers, 33% were previously unaware of the Five Point Plan, while 54% considered there were difficulties in implementing parts of the Five Point Plan on their farm.

The majority of participating farmers (97%) said they found the project and subsequent veterinary engagement useful and 95% said they would continue to implement the Five Point Plan. Moreover, 82% of farmers stated that they would continue to vaccinate their sheep against footrot.

**Conclusion:** Significant gains can be made in a short period of time with concentrated focus between vet and farmer on lameness in sheep flocks. The Five Point Plan has been demonstrated to be a highly effective way in which to reduce lameness and this study demonstrates that farmers value veterinary engagement in this area. It highlights a simple, significant opportunity for veterinarian-farmer engagement to increase the use of the Five Point Plan and reduce lameness on sheep farms.

**Keywords:** Sheep, lameness, footrot, vaccination.

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## SR-29

### Cost-benefit analysis of ultrasonographic pregnancy diagnosis in dairy ewes in Greece: a simulation study.

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**Objectives:** Ultrasonography is widely used for pregnancy diagnosis in sheep; however, in order to be cost-effective, pre-lambing feeding strategies must be accordingly adapted. The objective of this study was to perform a cost-benefit analysis of ultrasonographic pregnancy diagnosis (UPD) in dairy ewes in Greece, based on different fertility outcomes and feeding management strategies.

**Materials and Methods:** This simulation study was based on available local milk recording schemes' data. For 100 ewes entering the milking period and after accounting for barren ones, abortion during the last 2.5 months of gestation, losses at lambing and ewe culling at the end of the lamb-rearing period due to mastitis and low milk production, a flock size of 120 ewes at the beginning of the breeding period was considered. The 2.5-month breeding period was divided in 4 successive conception sub-periods (CSP), each lasting 17 days. Fertility outcomes included 2 levels of barren ewes (5% and 10%) and 3 conception patterns in respective CSPs: a) early (E: 82.5%-17.5%-0.0%-0.0%), medium (M: 51.0%-41.0%-8.0%-0.0%) and delayed (D: 25.0%-35.0%-28.0%-12.0%). For the simula-



tion, UPD was performed twice, 45 days after the start of the breeding season and 30 days after its end; pregnancy status and number of fetuses would therefore be established for all ewes. At the first UPD visit, all ewes would be examined while at the second one, only those previously found empty or dubious (10% of positives). Each UPD visit was charged 20€ plus 0.75€ per ewe examined, resulting in total expenses of 140€, 155€ and 180€, for the 3 conception patterns, respectively. Barren ewes were removed from the simulated flocks immediately after the second UPD visit and no feed was allocated to them thereafter, nor any expense on preventive health management (vaccinations, treatments for parasites etc; 1.5€ per ewe) was considered. Pregnant ewes of all fertility outcomes were virtually allocated in either 2 or 3 feeding groups. When allocated in 2 groups, and for the 3 conception patterns, these consisted of: a) E, ewes conceiving at the first and second CSP, b) M, ewes conceiving at the first and second+third CSP and c) D, ewes conceiving at the first+second and third+fourth CSP. When allocated in 3 groups: a) E, ewes conceiving at the first CSP bearing a single lamb, those conceiving at the first CSP bearing twin lambs and those conceiving at the second CSP, b) M, ewes conceiving at the first, second and third CSP, and c) D, ewes conceiving at the first+second, third and fourth CSP. The feeding plan included a maintenance period (starting from the second UPD until 6 weeks before presumed lambing), and a 3-period step-up pre-lambing program each lasting 2 weeks as proposed by INRA-2019. Rations were formulated according to INRA-2019 recommendations using alfalfa hay, wheat straw, corn grain, wheat bran, soybean meal and a mineral/vitamin supplement. Cost of maintenance, step-1, step-2 and step-3 rations (for ewes carrying twin lambs) were 0.24, 0.29, 0.36 and 0.44€, per ewe per day, respectively. For conception pattern E, cost for single-lamb carrying ewes were 0.29, 0.33 and 0.39€, for step-1, step-2 and step-3 rations, respectively.

When combining CSPs, feeding of step-3 ration started with the earliest lambing ewes and lasted until the end of this sub-group's lambing period. All the above fertility scenarios were compared to identical ones with no UPD and no culling of barren ewes; the start of the 3-period step-up feeding program for all ewes was based on the starting date of the breeding period and the step-3 ration was fed to all ewes until they lambed. Costs were calculated accordingly. Results are reported as differences per 100 ewes between UPD and no pregnancy diagnosis scenarios.

**Results:** Net benefit of UPD for the E pattern with 2 feeding management groups was 200€ and 480€, for 5% and 10% barren ewes, respectively. Forming 3 groups would increase benefit to 240€ and 530€, respectively. For conception pattern M, net benefit was 300€/580€ and 330€/610€, respectively. For conception pattern D, net benefit was 240€/460€ and 510€/760€, respectively.

**Conclusion:** Ultrasonographic pregnancy diagnosis is cost-effective when results are used to adapt grouping and feeding management. When conception is delayed, the formation of 3 feeding groups is highly recommended.

**Keywords:** Ultrasonography, pregnancy diagnosis, sheep.

## SR-30

### Surgical treatment of abomasal impaction in goat

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Information about disorders of abomasum in goats are scarce and the aim of this report is to describe the success of abomasotomy as a treatment of abomasal impaction in a goat. An 8-month-old goat weighing 33kg was referred to the School of Veterinary Medicine and Animal Science - UNESP, Brazil with an apathy and decreased appetite four days ago. According to clinical history, the diet consisted of crushed hay *ad libitum* and 300g of ration bran. Clinical examination revealed apathy, pale mucous membranes, ruminal hypomotility, reduced faecal output, tense abdomen with distension of the ventral quadrants and loss of ruminal stratification. Increased chloride ion concentration (67 mEq/L) evidenced by rumen fluid analysis was indicative of abomasum-ruminal reflux and as well as compatible with the observed metabolic acidosis. Transabdominal ultrasonography revealed dense hyperechoic content in ventral field of the left abdomen corresponding to the location of all abomasum, thus suggest an abomasal impaction. The animal underwent exploratory laparotomy by the left flank, which revealed an empty rumen, distended and compacted abomasum occupying much of the abdominal cavity. In contrast to the treatments described, by severe distension of abomasum, we opted for an abomasotomy followed by emptying of the viscera, removing about 7 kg of the digest. In addition to constant monitoring, antimicrobial medication with ceftiofur (5mg/kg; IV once daily; six days) associated with gentamicin (6.6mg/kg; IV; once daily; six days) and analgesic with flunixin meglumine (2.2mg/kg; IV; once daily; four days) was performed postoperatively vitamin B1 (2mL; SC; once daily; five days), dexamethasone (0.2mg/kg; IV; once daily; two days), transfaunation and calcium replacement for six days. Furthermore, was performed the prokinetic to stimulate abomasal emptying like bromopride (20mg; IM; three times a day; three days), metoclopramide (0.2mg/kg; SC; three times a day; two days), and promethazine (0.5mg/kg; IM; twice daily; four days). Although the goat defecated normally on the second postoperative day, abomasal emptying remained reduced to auscultation. Thus, with clinical suspicion of posterior functional stenosis, the atropine test and radiographic examination of the thorax and abdomen were performed, and they revealed no changes. After 14 days after surgery, the goat no longer presented abomasal motility changes, being discharged with recommendations to provide good quality food and water. The abomasotomy associated with viscera emptying and supportive therapy resulted in the successful treatment of a goat affected by abomasal impaction in the present study.

**Keywords:** Abomasotomy, abomasal disorder, small ruminant.



## SR-31

### Obstructive rhinopathy associated with chronic pithomycotoxicosis in sheep

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**Objective:** Two sheep were received at the Clinical Service of Ruminants of the University of Zaragoza (SCRUM) with respiratory and dermatologic clinical signs. A complete clinical examination was performed, emphasising the respiratory tract and skin lesions to arrive at a final diagnosis.

The practitioners reported that most of the flock animals presented upper tract respiratory clinical signs.

The objective of this work is the clinical and aetiological study of a case characterised by an inspiratory dyspnea, which affected a herd kept in an extensive regime, located in the north of Spain.

**Materials and methods:** The sheep came from a dairy farm located in Anzuela, Basque Country, and were reared in an extensive production system, grazing most of the year, except the winter months.

After their arrival at the SCRUM, the protocol was as follows: clinical examination, highlighting in detail the signs or lesions presented by the animal, haematology, biochemistry, computed tomography and postmortem examination.

**Result:** The animals showed inspiratory dyspnea with a characteristic respiratory noise, had bilateral serous nasal discharge and an enlarged submandibular lymph nodes. In one of the animals, different skin lesions with alopecias and scabs in the dorsal area of the face, eyelids and ears were noticed. Blood biochemistry showed an elevation in gamma-glutamyl transferase, alanine aminotransferase, and lipase.

The computerised tomography scan revealed an increase of the soft tissue on the rostral part with variable degrees of obstruction of the meatuses of the nasal cavities in both sheep. Both nasal cavities were generally blocked with different degrees of severity. Intravenous injection of contrast did not result in the differentiation enhancement.

Necropsy detected intense liver atrophy and fibrosis associated with chronic pithomycotoxicosis in the studied sheep. It confirmed slight elevations and roughness on the alar folds and close nasal areas in all sheep. Histopathology of the nasal lesions using histochemical and immunohistochemical techniques to study connective tissue, identified moderate to severe arteriosclerosis in the small arteries investigated with fibrosis and oedema. These lesions were similar to the ones described in blood vessels of the liver in chronic pithomycotoxicosis and our cases. The results of this study suggest a direct action of the sporidesmin on this area of the nasal cavity.

**Conclusions:** The clinical and postmortem findings sug-

gest that both lesions, respiratory and dermatologic, were caused by the sporidesmin produced by the fungus *Pithomyces chartarum*. Pithomycotoxicosis (facial eczema) has been well studied as a seasonal hepatogenous photosensitisation of sheep caused by the ingestion of sporidesmin contained in the spores of the fungus *Pithomyces chartarum*. However, to the knowledge of the authors, respiratory clinical signs have not been reported to date associated with this disease.

**Keywords:** Sheep, pithomycotoxicosis, facial eczema.

## SR-32

### The Effects of Oxytocin and PGF2 $\alpha$ Injections on Semen Quality and Libido in Buck

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**Objectives:** The use of assisted reproductive techniques in goat breeding provides additional advantages for cryopreservation and artificial insemination. In some cases, certain animals constantly have a high libido and good semen quality for evaluation, freezing or insemination, while others are reluctant for collection and have low quality ejaculates with decreased volume and concentration or other inadequate spermatological characteristics. During the last decades, specific hormones (oxytocin, prostaglandins, testosterone and GnRH) were introduced to increase the sperm output, quality of male-related reproductive deficiencies and to regulate the breeding activity. The aim of the present study was to evaluate the effects of exogenous oxytocin and PGF2 $\alpha$  on seminal quality and libido sexualis in bucks.

**Materials and methods:** This study was conducted according to ethical laws and regulation of Ankara university animal experiments local ethics committee. 20 Norduz bucks (3-4 years of age) were barned at Research Farm of Ankara University, Faculty of Veterinary Medicine (40°05'53.5"N 32°37'19.6"E). The bucks were maintained under the constant nutritional regime and with water ad libitum. To investigate the role of these hormones on male fertility, semen samples from 20 Norduz bucks (3-4 years of age) were collected with an artificial vagina twice a week with five replications in breeding season. Bucks were randomly assigned to five groups, control group was administered with 2 mL of sodium chloride, 0.9% (w/v) i.m., whilst the experimental groups were administered with oxytocin 10 IU, i.v. (Group 1, n = 5), oxytocin 20 IU, i.v. (Group 2, n = 5), PGF2 $\alpha$  5 mg, i.m. (Group 3, n = 5) or PGF2 $\alpha$  10 mg, i.m. (Group 4, n = 5) 20 min before each collection. The behavioural signs of libido (leg kicking, sniffing, vocalization, flehmen reaction, mounting without thrust and mounting with ejaculation) were recorded as the total score for libido. Semen volume, total sperm motility, sperm concentration, sperm morphology, membrane integrity and pH were recorded.

**Results:** According to obtained results, there was not any



statistical difference between the dosage groups regarding motility. However, in terms of semen volume, concentration, abnormal spermatozoa rate, intact membrane rate, and libido test, results were statistically significant among the different dose groups ( $P < 0.05$ ). Average semen volume of Group two ( $1.34 \pm 0.16$  mL) was found significantly higher than other groups, whereas mean values of PGF2 $\alpha$  groups (Group-3 and Group-4) were found lower than control value ( $P < 0.05$ ). When the concentration was taken into account, Group two has statistically higher mean value than PGF2 $\alpha$  groups. For abnormal spermatozoa rate, a significant increase in Group one was observed regarding other groups. A significant decrease of mean intact membrane percentages was evident at Group four comparing to group one. A significant increase in libido evaluation scores for oxytocin groups ( $3.04 \pm 0.64$ ;  $3.2 \pm 0.5$ ) was observed. The bucks, which were administered PGF2 $\alpha$ , were reluctant for mating and showed a decrease in libido. Thus, it might have enhanced hyperthermic and psychological stress in animals. Therefore, the duration of ejaculation was longer in PGF2 $\alpha$  groups than the other groups.

**Conclusions:** In conclusion, administration of 20 IU oxytocin twenty min before the semen collection have increased both the semen volume and the concentration of spermatozoa in bucks. Administration of oxytocin has increased the libido although there were not any improvements in semen quality. In contrast to oxytocin, PGF2 $\alpha$  administration has led to a decrease of libido and has detrimental effects on semen quality. We concluded that administration of oxytocin stimulates sexual behaviour and performance in bucks.

**Keywords:** Buck, Libido, Oxytocin, PGF2 $\alpha$ , Semen quality.

attour and cecum digesta was collected from 2 representative lambs per pen. Data was analyzed using the MIXED procedure of SAS Studio (v3.81) with pen as the experimental unit, dose as fixed effect and block as random effect. Orthogonal contrasts were included to test for linear and quadratic trends, and control (dose 0) vs. HFCG (rest of the doses).

**Results:** There were no differences on final body weight or average daily gain. Cecum digesta pH, dry matter content, and ammonia concentration were not different among treatments. Butyrate and total VFAs molar concentrations increased (22 and 10%, respectively;  $P \leq 0.15$ ) in HFCG-supplemented lambs compared to controls. However, no differences in VFA concentrations were detected when normalized to dry matter content.

**Conclusions:** Overall, these results show signs of altered hindgut fermentation in response to HFCG supplementation. We hypothesize that the application period was too short to adequately detect differences in response to supplementation, as it has been previously demonstrated that relatively long treatments (.56d) are necessary to influence hindgut ecology and improve performance in beef and dairy cattle.

**Keywords:** Gluconate, hindgut, ruminants.

### SR-33

#### Effects of hydrogenated fat-embedded calcium gluconate on performance and intestinal parameters in growing lambs

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**Introduction:** Upon fermentation in the hindgut, the prebiotic gluconic acid and its salts have shown benefits on growth in monogastric species, attributed to changes in fermentation patterns and volatile fatty acid (VFA) production.

**Objectives:** The objective of the current study was to evaluate the effects of rumen-protected hydrogenated fat-embedded calcium gluconate (HFCG) on performance and intestinal parameters in a model of growing ruminants.

**Materials and methods:** A total of 300 male Rasa Aragonesa feedlot lambs were enrolled in a dose response study divided in 2 identical rounds. Within round, animals were blocked by body weight at arrival ( $20.1 \pm 2.0$  Kg) and randomly assigned to pens corresponding to 1 of 5 HFCG supplementation doses (0, 0.4, 0.75, 1.5, or 3 g/lamb/d). After 20 d of supplementation, lambs were slaughtered at a commercial ab-