PT-01

Temperatures of pharmaceutical storage areas in large animal veterinary practice vehicles in the winter

Elizabeth R. Homerosky[1], Jeff D. Ondrak[2], Meredyth L. Jones[3], Virginia R. Fajt[4].

[1]Veterinary Agri-Health Services, Ltd., Airdrie, Canada; [2]Great Plains Veterinary Educational Center, University of Nebraska, Clay Center, United States; [3]Oklahoma State University, Stillwater, United States; [4]Texas A & M University, College Station, United States.

Objective: Many manufacturers recommend that non-refrigerated products be stored at room temperature (20–25 °C). Large animal veterinary practice vehicle storage units are commonly outfitted with an adjustable heater to prevent freezing as ambient temperatures in many locations in North America drop below these temperatures in winter. The authors have previously evaluated storage units in vehicles in the summer, so the objective of this study was to measure storage area temperatures in the winter to evaluate the effectiveness of heaters and the extent to which temperatures fall outside the manufacturers' recommended range.

Materials and Methods: A convenience sample of six vehicles from an ambulatory beef cattle practice in Southern Alberta was used. Ambient temperature and temperatures in two storage areas (close to the heater and far from the heater) in each vehicle were recorded from November 1, 2018 – February 28, 2019, at 15-minute intervals using self-contained, battery operated temperature recording devices.

Results: The lowest and highest overall temperatures recorded in a storage unit were -29.7 °C and 62.6 °C, respectively. The mean temperature recorded across all six storage units was 16.8 °C near the heater and 11.6 °C far from the heater, while ambient temperature averaged 2 °C. During the 120-day data collection period, temperatures below freezing were recorded between 3-48 days for each of the six vehicles. Readings far from the heater fell within the recommended range for room temperature 11.3% of the time, whereas only 5.6% of readings near the heater fell within the recommended range.

Conclusion: Temperatures in practice vehicle storage units were outside recommended pharmaceutical storage temperatures a significant portion of the winter. Furthermore, there is a discernable variation between storage areas near and far from the heater. Research is needed to determine the extent to which these fluctuations outside the manufacturers' recommended storage temperatures impact efficacy of stored pharmaceuticals.

Keywords: Drugs, vet box, heater, frozen.

PT-02

Efficacy of an oral solution of paromomycin for the treatment of newborn dairy calves with cryptosporidiosis. Results from a comparative European multicentric field study

Damien Achard[1], Béatrice Besche-Barbazanges[1], Gaëlle Pagny[1], Jean-François Collin[1], Dejan Cvejić[2].

[1]Ceva Santé Animale, Libourne, France; [2]Klifovet AG, Munich, Germany.

Objective: Cryptosporidiosis is a frequent parasitological infection of mammals including but not limiting to humans and neonatal ruminants. In newborn calves, cryptosporidiosis has been repeatedly identified as a major contributor of neonatal diarrhoea, a dominant calfhood disease with detrimental health and economic consequences. Current treatment options for clinically affected newborn calves are limited. A recent meta-analysis work reported halofuginone and paromomycin as valuable oral treatment options while recognizing that halofuginone can present important safety issues and that data is insufficient to fully support the use of paromomycin (*Brainard et al.,* 2020, 2021). To address this lack of evidence, a study under field conditions was performed in dairy newborn calves clinically affected by cryptosporidiosis comparing a new dose regimen of an oral paromomycin solution (Gabbrovet Multi®, Ceva Santé Animale) to a reference product based on halofuginone (Halocur®, MSD).

Material and methods: A GCP compliant study was performed according to a blinded, randomized, positive controlled, multicentric design in 4 commercial farms located in Germany, Hungary, and Portugal. The farms enrolled had a recent history of cryptosporidiosis and complied with several conditions such as vaccination for BVDV and neonatal diarrhea. Each newborn calves in this study were required to meet the following criteria for enrollment: age between 3-14 days, faecal score ≥ 2, negative rapid test for *E. coli*, coronavirus, rotavirus, and positive rapid test for *C. parvum*. Any calf that presented with diarrhoea for > 24 hours or that has been previously exposed to antibiotics, parasiticides or probiotics was excluded. Animals were randomly allocated to two treatment groups. Calves in group A received 150 mg paromomycin sulfate/ kg b.w., once daily, for 5 days by oral route while calves in group B were orally administered 100 µg halofuginone/ kg b.w., once daily, for 7 days. The following clinical and parasitological parameters were monitored at fixed times during the 21 days of the study: fecal score (0-3), general health observation (0-3), hydration score (0-3), oocyst counts (number of oocysts per gram of dry faeces), bodyweight and mean daily body weight gain (MDBWG). Percentages of calves cured at day 8 and MDBWG between day 0 and day 21 were the main criteria to evaluate the efficacy in both treatment groups. Other criteria such as parasitological cure at day 3, 5, 7, 14 and 21 and time to clinical cure from day 2 to day 8 were also assessed. The statistical unit was the calf and the 5% level of significance ($p < 0.05$ for two-sided tests) was used to assess statistical differences.

Results: Three hundred thirty-four dairy calves, mostly Holstein-Friesians, with a mean age of 9.1 days and a mean body weight of 41.8 kg were enrolled in this study. 165 calves

were allocated to treatment group A, 169 in the treatment group B. At inclusion, the treatment groups were found comparable. 94.6% (156/165) of newborn calves in group A and 87.6% (148/169) in group B were considered clinically cured by day 8. Clinical cure rate in group A was found superior to those observed in group B (lower bound of the 95% CI >0). In addition, MDBWG was higher for calves in group A in comparison to calves in group B, with a difference between groups of 0.0405 kg/day (p = 0.0086, best model); that is a bodyweight advantage of 850 g at the end of the 3 weeks follow-up period. Calves in group A were found to be cured faster in comparison to calves in group B. More precisely, the probability of healing first was 56.75% in favor of group A. The qualitative assessment of oocyst counts (positive / negative) assessed at each time point showed no significant difference, except on Day 7 (76.4% and 87.0% for negative calves in group A and B respectively). Overall, seven adverse events were documented from seven animals in group B but were not found to be related to the treatment.

Conclusion: In this European field study, daily oral treatment with 150 mg/kg of paromomycin (Gabbrovet Multi®) for 5 days was found safe and highly effective to cure sick dairy calves with cryptosporidiosis and to control their oocyst burden. In addition, this new treatment regimen was found superior to the current reference treatment based on halofuginone.

Keywords: Dairy calves, cryptosporidiosis, treatment, paromomycin, field study.

PT-03

Effects of early treatment with non-steriod anti-inflammatory drugs (NSAIDs) against bovine respiratory syncytial virus (BRSV)

Sara Hägglund[1], Eve Laloy[2], Ignacio Alvarez[3], Katarina Näslund[4], Yongzhi Guo[3], Sara Rosenberg[3], Mansour Naghibi[3], Gabriella Hallbrink Ågren[3], Haleh Yazdan Panah[3], Ganna Shevchenko[5], Jonas Bergquist[5], Anna Hillström[6], Edouard Timsit[7], Jean Francois Valarcher[3].

[1]Swedish University of Agricultural Sciences, Uppsala, Sweden; [2]Laboratoire VETODIAG, Saint-Pierre-en-Auge, France; [3]Swedish University of Agricultural Sciences, Uppsala, Sweden; [4]National Veterinary Institute, Uppsala, Sweden; [5]Uppsala University, Uppsala, Sweden; [6]University Animal Hospital, Uppsala, Sweden; [7]Ceva Santé Animale, Libourne, France.

Objectives: Non-steroid anti-inflammatory drugs (NSAIDs) are increasingly used as a single and early treatment against respiratory disease caused by bovine respiratory syncytial virus (BRSV). These drugs reduce fever, but consistent reduction of pulmonary inflammation has not been demonstrated [1]. While some of the induced mechanisms are anti-inflammatory, others might augment inflammation in the lung and delay the healing process. The drawbacks include increased levels of leuokotriene B4, a chemoattractant for neutrophils, and decreased levels of prostacyclin and prostaglandin E2, which mediate a switch from leukotriene to lipoxin production. On the other hand, the production of lipoxin and resolvins, which are important for lung epithelial repair, can also be triggered by aspirin, by another pathway [2][3]. The effect of aspirin is higher on COX-1 *versus* on COX-2, whereas the opposite is valid for meloxicam. The objective of this study was to map and compare the inflammatory events following early treatment with these different drugs against BRSV in calves.

Material and methods: Fifteen 3-8-week-old dairy calves were allocated to three groups, according to age, bodyweight and BRSV-specific maternal antibodies, and were infected with BRSV by aerosol. The calves received either 0,5 mg/kg meloxicam (MET) subcutaneously on D4 post infection (PI, n=5), 20 mg/kg acetylsalicylat-DL-lysin (ASP) intravenously on D4 and D5 (n=5), or no treatment (n=5, CRT).

Clinical signs of disease were scored and peripheral capillary oxygen saturation was measured on a daily basis. Bronchoalveolar lavages (BAL), plasma and nasal swabs were repeatedly collected until post mortem examination of lungs on D7 PI. The extent of macroscopic lung lesions were quantified and histological analyses were carried out. BRSV was detected in swabs and BAL by RT-qPCR. Inflammatory cells were counted in BAL and BAL proteins were semi-quantified by liquid chromatography and tandem mass spectrometry. Eicosanoids were analysed in plasma and BAL by liquid chromatography triple quadrupole tandem mass spectrometry.

Results: The calves developed mild to moderate clinical signs of respiratory disease. All calves shed virus from D2 or D3 throughout D7. No significant difference was observed with regard to clinical signs, virus shedding or extent of lung lesions. The two calves with the most severe clinical signs and most extensive pathological lesions, had been treated with ASP and MET, respectively. Meloxicam significantly increased the proportion of neutrophils in BAL compared to aspirin (24h after treatment) and compared to no treatment (72h after treatment). Histopathological, proteomic and metabolomic data will additionally be presented at the meeting.

Conclusions: In contrast to aspirin, treatment with meloxicam increased the influx of neutrophils in the lungs of calves infected with BRSV, compared to controls 7 days after infection and 3 days after treatment. Neither aspirin, nor meloxicam had a curative effect on clinical signs or lung lesions. The effect of these drugs will be analysed by taking into consideration systemic and local eicosanoids, as well as proteins in BAL fluids and histology. The results might contribute to evidence-based practical handling of acute outbreaks of BRSV in the field, as well as to the general understanding of pulmonary inflammation, needed for development of efficient drugs.

References:

1. Francoz, D. et al. Evidence related to the use of ancillary drugs in bovine respiratory disease (anti-inflammatory and others): are they justified or not? *Vet Clin North Am Food Anim Pract* 2012, *28*, 23-38, vii-viii, doi:10.1016/j.cvfa.2011.12.003.
2. Zheng, S., et al. Lipoxin A4 promotes lung epithelial repair whilst inhibiting fibroblast proliferation. *ERJ Open Res* 2016, *2*, doi:10.1183/23120541.00079-2015.
3. Gilligan, M.M., et al. Aspirin-triggered proresolving mediators stimulate resolution in cancer. *Proc Natl*

Acad Sci U S A 2019, *116*, 6292-6297, doi:10.1073/pnas.1804000116.

Keywords: BRSV, inflammation, NSAID, Eicosanoids, BAL proteome.

PT-04

Evaluation of the clinical effects of epidural butorphanol in cattle

Tomas Trucco[1], Mazzini Ruben[2], Cristian Leiva[2], Oscar Garnero[2], Reza Seddighi[3].

[1]Veterinaria Trucco, Private Practice, Argentina; [2]Universidad Nacional del Litoral, Facultad de Ciencias Veterinarias, Argentina; [3]University of Tennessee, College of Veterinary Medicine, United States.

Introduction and Objectives: Epidural administration is one of the commonly used regional blocks in bovine anesthesia for pain control. Caudal epidural anesthesia is commonly utilized in veterinary medicine to allow diagnostic, obstetrical, and surgical intervention in the perineal region of large animals, while allowing animals to remain in the standing position. Local anesthetics are the most frequently used drugs for producing analgesia by epidural injection, but other classes such as α-$_2$ adrenoceptor agonists, ketamine, and opioids have also been widely used. It has been shown in several species that opioids and alpha-adrenergic agents produce selective caudal epidural analgesia via binding to the spinal receptors. Stimulation of these spinal receptors results in the inhibition of rostral transmission of nociceptive (pain) impulses. Thus, a potential advantage of these agents is the selective sensory blockade, without the unfavorable depression of motor or autonomic neurons. Although many opioids have been evaluated and clinically used for epidural analgesia in cattle, there are no clinical trials on the use of butorphanol (BTL) epidurally in cows. Therefore, the objectives of this study were to determine the sedative and analgesic effects of butorphanol administered epidurally (C1-C2 intercoccygeal space) in standing cattle.

Methods: Five 2 - 3 year-old Holstein heifers (mean 14.6 months ± 0.7); weighing 423 ± 41 kg were used in this study. During the experiment, cows were restrained in a chute. The skin area over the first intercoccygeal (Co1-Co2) space was identified and aseptically prepared. Epidural puncture was performed with an 18-gauge, 38 mm needle, that was directed at the right angle to the general contour of the croup. The correct needle placement in the epidural space was confirmed by hanging-drop technique and the lack of resistance during administration of the injectate. Each animal received epidural anesthesia with butorphanol at a dose of 0.02 mg/kg BW. For each animal, butorphanol was diluted in saline (0.9%) to a final volume of 5 ml. Sedation, ataxia, and analgesia were assessed before butorphanol administration and at 5 min intervals after epidural administration for 60 min, and every 15 min thereafter for 120 min. Analgesia was tested by applying a standard noxious stimulus (skin pinching using a kocher hemostat) and subjectively scored based on a 3-point scale: *1)* no response *2)* depressed response; and *3)* normal response. The onset, magnitude, and duration of caudal epidural analgesia were also determined. Sedation was subjectively evaluated by the attitude of the cow, including the response to noise, carriage of the head, and the presence of excessive salivation. Ataxia was evaluated by observing the position of the pelvic limbs, swaying and leaning against the chute, or any knuckling of the hindlimbs. Physiological variables including heart rate (HR), respiratory rate (RR), rumen contraction (RM), and rectal temperature (RT) were assessed before epidural administration (baseline) and at 15 min intervals thereafter for 120 min. Data were analyzed using descriptive statistics and presented as mean ± SEM.

Results: Caudal epidural anesthesia was produced in all heifers following the administration of BTL. The epidural injection was easy to perform and well tolerated by all animals. Caudal epidural analgesia ranged from the tail, vulva, perineum, paralumbar fossa, flank fold and last rib in all heifers. Time to the onset of analgesia was 15 ± 2 minutes and duration of epidural analgesia was 69 ± 7.2 minutes. In all cattle, mild to moderate sedation (slight lowering of the head carriage and lower lip and ptyalism) was noted for 68 ± 6 minutes. No signs of ataxia were detected. No significant differences in heart rate, respiratory rate, rumen motility and rectal temperature were observed between measurements before and after epidural administration.

Conclusion: Butorphanol (0.02 mg/kg) administered epidurally to adult cattle produced adequate cutaneous analgesia and mild sedation without affecting the cardiopulmonary and rumen motility at the doses used in this study.

Keywords: Bovine, opioids, epidural, pain, butorphanol.