



**23rd World Buiatrics Congress, Quebec City, Canada, July 11-16, 2004**  
**23e Congrès mondial de buiatrie, Québec, Canada, 11-16 juillet 2004**

501 (2556)

**PARATUBERCULOSIS IN WILD ANIMAL SPECIES AND CATTLE IN STYRIA**

Deutz A.1, Spargser J.2, Köfer J.3, Rosengarten R.2

1Styrian Animal Health Service, Zimmerplatzgasse 15, Graz, Styria, A - 8010, Austria; 2Institute of Bacteriology, Mycology and Hygiene, V, Veterinärplatz 1, Vienna, 1120, Austria; 3Department for Veterinary Administration, Styria, Zimmerplatzgasse 15, Graz, A - 8010, Austria

This report describes an increase of paratuberculosis in nine wild animal species since 2002 and the detection of the causative agent of paratuberculosis in sections of cattle in Styria, a province in the south of Austria. The analysis comprised a total of 314 wild animals and 147 cattle. The tests revealed that 63 hoofed game animals (23 red deer, 27 roe deer, 9 chamois, 3 mouflon, and 1 farmed fallow deer), four foxes, a mountain hare, a yellow-necked field mouse, a capercaillie and 49 cattle tested positive for paratuberculosis. The study proved the intrauterine transmission of *M. paratuberculosis* in red deer and chamois and other extraintestinal manifestations of this pathogen for the first time worldwide. Affected wild animals suffered from extreme weight loss and weakness and had enlarged mesenteric lymph nodes. Evidence of diarrhoea was only observed in individual cases.

Samples were taken from mesenteric lymph nodes and, in case of pathologic lesions, also from affected organs. For cultivation, samples were decontaminated and subsequently inoculated onto Harold's egg yolk and Middlebrook agar and incubated at 37°C for up to 20 weeks. Molecular detection of *M. paratuberculosis* was accomplished by PCR amplification of IS900 and ISMav2. Up to now, only *M. paratuberculosis* of the cattle type has been detected in the wild animal samples examined by IS1311 PCR restriction enzyme analysis.

22 wild animal and 3 cattle isolates have so far been subjected to molecular typing. All 25 *M. paratuberculosis* strains exhibited the PstI/BstEII RFLP profile B-C1. RAPD analysis, however, revealed five different DNA fingerprint patterns. Identical RAPD profiles were obtained for 3 cattle and 14 wild animal isolates recovered from animals from a geographically limited area. This leads to the assumption that infections of this wild population originated from a few genetically closely related strains. Distinct genomic polymorphisms were determined for the other 8 wild animal isolates using RAPD analysis.

The increase in clinical cases in wild animal species is assumed to have been caused by a strong increase in suckler cow farming, inadequate feed hygiene (such as feeding on the ground) for wild animals, the purchase of farmed deer as well as a concentration of pathogens in the environment.

502 (860)

**AN ABATTOIR STUDY OF THE MYCOBACTERIUM PARATUBERCULOSIS INFECTION IN CATTLE IN AHWAZ-IRAN**

Haji hajikolaei M.R., Ghoorbanpour M., Amir Solaimani M.

Shahid Chamran University, Faculty of Veterinary Medicine, Ahwaz, Khozestan, 61357-13793, Iran (Islamic Republic of)

John's disease which is caused by *Mycobacterium paratuberculosis* is a chronic, infectious granulomatous and incurable enteritis in ruminants. The most important clinical signs of the disease are diarrhea and emaciation.

In order to investigate the prevalence of *Mycobacterium paratuberculosis* infection among slaughtered cattle in Ahwaz abattoir, samples were taken from 250 cattle. Before slaughter, sex, body condition and age (by use of dental formula) were assessed and after that, smears were taken from ileocecal valve, rectum mucosa, ileocecal lymph node and stool. All smears were stained by Ziehl-Neelsen technique and examined by light microscope. At first, ileocecal samples were examined and other samples were examined whereas ileocecal sample was positive.

Based on examination of ileocecal sample, out of the 250 cattle, 5 (2%) were positive and clumps of *Mycobacterium paratuberculosis* were seen. With the exception of one cattle where clumps of *Mycobacterium paratuberculosis* were also observed in ileocecal lymph node, in the other positive cattle, clumps of *Mycobacterium paratuberculosis* were seen only in the ileocecal sample. In other samples such as rectum mucosa, ileocecal lymph node and stool, clumps were not seen.

Positive cattle were <2, 3, 4 and >5 years old and all of them had normal body condition and faeces, therefore they suffered from subclinical form of John's disease.

Funding: Shahid Chamran University.

503 (712)

## MILK PRODUCTION LOSS DUE TO JOHNE'S DISEASE IN FOUR NEW ZEALAND DAIRY HERDS

Heuer C.1, Lopez-Villalobos N.2, Jackson R.2, Lawton D.1

1Massey University, Private Bag, EpiCentre, IVABS, 11222, Palmerston North, Manawatu, 5301, New Zealand; 2Massey University, Private Bag, EpiCentre, 11222, Palmerston North, 5301, New Zealand

The analysis of a longitudinal study of Johne's Disease (JD) in 4 seasonal calving New Zealand dairy herds aimed to estimate the effect of JD-infection on daily milk production. Herd test information of two successive seasons (1999/00 and 2000/01) from 906 cows was evaluated by a marginal GLM model with fixed herd and repeated cow within season effects. The model included up to 4 polynomial terms for days in milk, herd, parity (1, 2, 3, 4, 5, 6+), breed (Friesian, Jersey, Ayrshire), JD (1/0) and interaction terms. All cows were tested twice a year in October (early lactation) and April (late lactation) starting in May 2000 until October 2001. An infected cow was defined as being positive at least once to either a serum antibody ELISA or to culture. A non-infected cow had at least 2 negative culture or 3 negative ELISA test results or both.

Herd infection levels ranged from 7.1% to 17.5%. A significant three-way interaction between herd, breed and JD suggested that 2 herds (one with only Friesian cows, one with all 3 breeds) revealed no difference in fat-protein-corrected milk production. The herd with the highest infection level suggested that JD-infected Friesian cows had 3 kg/d more milk than JD-uninfected cows ( $p = 0.04$ ) while milk production of Jersey cows were unaffected by JD. JD in the herd with the lowest infection level decreased milk production by 1.5 kg/d in Friesian cows ( $p = 0.03$ ) and 1.8 kg/d in Jersey cows ( $p = 0.01$ ). We concluded that JD can reduce milk production but that the effect is highly dependent on other herd factors. Other than breed and herd level infection, such effects may include culling practice (e.g. time that clinically affected JD cows are retained in the herd), herd production level, herd size and age at first exposure (i.e. level of within cow bacterial load).

Funding: Meat and Wool Innovation

504 (2000)

## EVALUATION OF CULTIVATION METHODS OF MYCOBACTERIUM AVIUM PARATUBERCULOSIS FOR THE BACTERIOLOGICAL DIAGNOSIS OF BOVINE PARATUBERCULOSIS IN RIO DE JANEIRO, BRAZIL

Lilenbaum W.1, Ristow P.2, Fonseca L.2

1Universidade Federal Fluminense, Rua Prof. Hernani Mello, 101, Niteroi, RJ, 24210-130, Brazil;

2Universidade Federal do Rio de Janeiro, Rua Prof. Hernani Mello, 101, Niteroi, RJ, 24210-130, Brazil

Paratuberculosis or Johne's Disease is a chronic enteritis caused by *Mycobacterium avium paratuberculosis* (Map), that affects ruminants causing diarrhea, emaciation and death. In Brazil, although there are important evidences of its existence, the disease is considered rare or even exotic, probably due to few studies and the absence of bacteriological isolation routine. The high serological prevalence of Paratuberculosis in bovines in Rio de Janeiro and the existence of clinically suspected animals led us to implement bacteriological diagnosis of Paratuberculosis in our laboratory. Beyond the inherent difficulty on the cultivation of Map, there is no consensus about mostly used medium HEYM's formulae and on the feces treatment protocols (decontamination and mycobacteria concentration). In order to verify the most adapted protocol to be used in our routine, we tested three HEYM's formulae: that recommended by the International Epizootics Office (OIE, 2000), the one suggested by Whipple, Callihan & Jarnagin (1991) and one with higher concentration of piruvate. Two standardized Map suspensions were inoculated on tubes prepared by each of the three formulas. The necessary time for growth was similar on all the tubes (60 days), but the number of colonies was significantly greater on the OIE formula. Four feces treatment protocols were tested with the aim of settling the most efficient: 1-Sedimentation (OIE, 2000), 2-Centrifugation (Stable, 1997), 3-Double Decontamination (Whitlock & Rosenberger, 1999) and 4-Double Centrifugation/Decontamination (Stable, 2000). Bovine feces were spiked with standardized Map suspensions, treated by the four protocols and inoculated on HEYM tubes prepared by the OIE formula with and without antibiotics. In relation to the feces treatment protocols, significant differences were observed among them. Good recovery rates (>100 col/tube) were observed on tubes treated by protocols 2 and 4, but contamination was also observed on 21.9% and 6.2% of the tubes, respectively. Growth on tubes treated by protocols 1 and 3 was reduced (<10 col/tube), but no contamination was observed. In conclusion, due to its capability of supporting growth, our isolation routine is nowadays based on the OIE formula for HEYM's medium and protocols 2 (Centrifugation) and mainly protocol 4 (Double Centrifugation / Decontamination) as the most adapted to our conditions.

Funding: CAPES, CNPq and FAPERJ

505 (3161)

## COMPARATIVE ANTIBODY RESPONSES TO 5 RECOMBINANT ANTIGENS FOR SEROLOGICAL DETECTION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN RELATED SHEDDING LEVELS

Shin S.J.1, Han H.R.2, Chang Y.F.3, Yoo H.S.1

1Seoul National University, College of Veterinary Medicine, Department of Infectious Diseases, Sillim-dong, Kwanak-Gu, Seoul, 151-742, Republic of Korea; 2Seoul National University, Shillimdong Kwanak-gu, Seoul, 151-742, Republic of Korea; 3Cornell University, College of Veterinary Medicine, Ithaca, NY, 14853, United States of America

Paratuberculosis (Johne's disease), a chronic enteritis produced by *Mycobacterium avium* subsp.

paratuberculosis (MPT), affects a large proportion of ruminants in all continents and causes important economic losses. One of the most important things to control paratuberculosis in dairy herds showed to be based on preventing the transmission of MPT from cows to calves by management measures, supported by removal of cows excreting these bacteria through the fecal route. However, there are no simple and accurate diagnostic methods for detection and herd screening of MPT shedders so far. The identification of well-characterized and specific antigens of MPT could provide the means to improve the specificity and sensitivity of serodiagnostic assay for Johne's disease. Eighty-five complex (85A, 85B and 85C), 35-kDa and superoxide dismutase (SOD), which have been considered as major antigens of MPT and could elicit immune response to MPT infection, were cloned, expressed with pET22b(+) in *E. coli* and purified by preparative continuous elution using a polyacrylamide tube gel apparatus in this study. Then, we compared the serological reactivity of the 5 recombinant antigens with respect to shedding levels of MPT (fecal culture and IS900 PCR test used as indicators) using enzyme-linked immunosorbent assay (ELISA). Initially, screening tests were performed using crude antigens such as purified protein derivative; however, the results with this antigen were indistinguishable in relation with shedding levels. Antibody response to the 35-kDa in high shedding cattle were significantly higher than those to the others ( $p < 0.01$ ). Out of 85 complex, 85B showed slightly higher response than others. Also, 35-kDa antigen was shown to have the highest sensitivity and predictive values compared to other antigens.

Finally, an ELISA based on 35-kDa was compared with a commercial ELISA kit with 200 positive and 200 negative sera to find out possibility to use for field situation and herd screening. The positive, negative predictive values and sensitivity of 35-kDa were 95.45%, 86.36% and 87.5%, respectively. These sera which showed difference in comparison with commercial ELISA kit, they also did not react with 35-kDa in Western blot. These results suggested that 35-kDa could be a useful serodiagnostic candidate in ELISA against MPT infection.

506 (3130)

#### APPLICATION OF RISK ASSESSMENT AND RISK ZONES TO DAIRY FARM BIOSECURITY PROGRAMS

Van Saun R., Wolfgang D.

Pennsylvania State University, 115 Henning Building, University Park, Pennsylvania, 16802-3500, United States of America

Biosecurity benefits to animal welfare, product quality and farm profitability are well recognized and have been known for many years. Despite obvious benefits of effective biosecurity programs, most dairy farms have few to no procedures in place to protect their herds. Producers view biosecurity as a bewildering array of infectious agents, expensive tests and time consuming animal handling schemes. Vaccine use has become the emphasis or sole form of biosecurity, focusing solely on the animal and infectious agent(s). Environmental influences are often overlooked as an important component of biosecurity. The objective of this presentation is to outline a risk assessment/risk zone approach to implementing a functional biosecurity program.

Biosecurity programs must be designed for each individual farm; one program does not fit all. Farms must determine the level of disease risk they are willing to accept. Risk level will depend upon farm products being exported (heifers, cows, milk, meat, embryos) and potential disease agents. To decrease complexity, disease prevention should be focused on common modes of transmission (fecal-oral, aerosol, blood) and not individual diseases. Thus farm design, traffic lanes and facility layout determine ease with which biosecurity programs can be implemented.

All farm areas should not be considered at equal risk relative to biosecurity concerns. Physical areas and animal groups can be divided into low (green), moderate (yellow) or high (red) risk zones depending upon their relative risk of disease spread or animal susceptibility. Manure storage, feed mixing area and sick cow pens are examples of high disease spread risk zones, whereas maternity and calf raising areas are high risk areas for disease susceptibility. Likewise, all farm activities and visitors can be assigned relative risk categories. Biosecurity programs should focus on farm activities that increase disease risk, especially within and between those identified high risk areas. Once the farm risk assessment has been completed, a farm biosecurity plan balanced to farm goals and addressing identified high-risk practices can be developed.

Veterinarians due to their understanding of disease transmission should play an important role in developing farm biosecurity programs. A focused biosecurity plan that is easier to understand and implement is more likely to be followed and therefore is more likely to be effective.

Funding: Pennsylvania Dept of Agric

507 (2751)

#### ECONOMY IN THE SUCKING COWS: A SPECIFIC SOFTWARE - EVA - FOR THE MANAGEMENT OF SUCKING HERDS OF REGION WALLONNE IN BELGIUM

Arendt J.1, de Behr V.2, Istasse L.1

1Cellule Agriculture/Agro-alimentaire, Prov Hainaut, 46 rue de Nimy, Mons, Hainaut, 7000, Belgium;

2Université de Liège, FMV, Animal Nutrition Unit, Sart Tilman Bat B43a, Liège, 4000, Belgium

The management of suckling herds started in the early sixties in Belgium. It quickly appeared as a low-income production system. In order to improve profitability, software was developed at the Nutrition Unit of the Veterinary Faculty of Liege University and at the Agriculture/Agro-Alimentaire Unit of the Hainaut province. It is an integrating system working on an annual basis and built with data usually available in commercial

farms of Region Wallonne such as animal number on the end of the financial year, live weight records and growth curves, feedstuff stocks for winter, daily rations, pasture and arable management, reproduction data, pathologies and financial statement. Technical indices were built on an animal-day basis. The animals were divided in 18 classes according to the age, the physiological status (growth, pregnancy, maintenance) and the presence either at grass or indoors. The young suckling stock at grass is, e.g., in class 1 while the pregnant cow indoor is in class 14.

In the present system, farm profitability is estimated by the calculation of the animal - day gross margins. The software allows the calculation of the total animal-day number with the corresponding variable and fixed costs, and the calculated incomes for each animal according to the number of days spent in each class. The animal-day gross margin is finally calculated.

The system was experimented in 10 commercial farms of Region Wallonne from 1998 to 2001. Large differences were observed between farms. So, in 1998 the gross margin was 0.80 €/animal/day in the most poorly managed farm, the corresponding value was 1.54 € for the best performing farm. Gross margin increased when the average daily gain of the young stock (0 up to 20 months old) was improved.

“Eva” allows comparison between farms for a particular year, between years for a particular farm and between animals groups within a farm. It is also possible to simulate management based on real conditions when, e.g., the animals number changes or when prophylactic measures are planned. “Eva” could be a management tool of interest for the breeders. From records, one can therefore assess the impact of inputs and the impact of weaning date or the date of first serving for the heifers. The effects of low animal performances could also be assessed.

Funding: Région Wallonne

508 (3253)

#### PHARMACOKINETICS OF CEFTIOFUR CRYSTALLINE FREE ACID STERILE SUSPENSION IN CATTLE AFTER INJECTION IN THE MIDDLE THIRD OF THE EAR AND IMPORTANCE OF INJECTION TECHNIQUE

Brown S.1, Lehman F.2, Robinson J.1, Lucas M.1, Robb E.1

1Pfizer Animal Health, 7000 Portage Road, Kalamazoo, MI, 49001, United States of America; 2Pfizer Animal Health, 150 East 42nd Street, New York City, NY, 10017, United States of America

Ceftiofur is a time-dependent, bactericidal antibiotic that is effective against bovine respiratory disease pathogens when concentrations remain above the minimum inhibitory concentrations (MIC) for at least 3-5 days. A newly approved product, Ceftiofur Crystalline Free Acid Sterile Suspension (CCFA-SS), is a prolonged release formulation of ceftiofur that is administered subcutaneously (SC) in the ear. The objective of this study was to generate plasma ceftiofur concentration data following administration of CCFA-SS SC in the middle third of the posterior aspect of the ear. Crossbred beef cattle (n=24; 270-400 kg) were administered a single dose of 6.6 mg ceftiofur equivalents (CE)/kg BW in the middle one-third of the posterior aspect of the ear by inserting the needle approximately 1/3 of the length of the ear from the tip and pointed toward the base of the ear. This location is parallel to the rostral branch of the middle auricular artery. The needle was fully inserted with the thumb positioned perpendicular to the needle to allow deposition in the middle third of the ear. Once the full dose was administered, the bleb was massaged away from the injection site. The consequence of an inadvertent arterial injection can be retrograde delivery of the product to the external carotid artery, which in the bovine continues as the internal carotid artery and is a major blood supply to the brain. Blood samples were collected at 0, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 168 and 240 h after treatment administration. Plasma was harvested and assayed for ceftiofur and desfuroylceftiofur-related metabolites using the validated HPLC-DCA method. The LOQ of the assay was 0.150 mg CE/mL plasma. Maximum concentrations were  $6.3 \pm 2.3$  mg/mL, observed from 4-24 hours after drug administration. The AUC<sub>0-LOQ</sub> (by trapezoidal summation) was 376 mg•h/mL and  $t_{>0.2}$  (the time plasma concentrations remained above the therapeutic level of 0.2 mg/mL) was 183 hours, or over 7 days above the therapeutic concentrations for bovine respiratory disease (BRD) pathogens. These concentrations provide therapeutic concentrations for sufficient time to allow single dose efficacy for BRD. The data from this study support the hypothesis that one dose of CCFA-SS Sterile Suspension (6.6 mg/kg BW) will provide therapeutic concentrations in plasma for at least seven days after SC injection in the ear of cattle.

Funding: Pfizer Animal Health

509 (5022)

#### EVALUATION OF THE EFFECT OF INCLUSION OF RECYCLED POULTRY BEDDING AS A PROTEIN AND MINERAL SUPPLEMENT ON GROWING CALF PERFORMANCE AND BLOOD AND LIVER MINERAL STATUS

Capucille D.J.1, Poore M.H.2, Rogers G.M.1

1North Carolina State University, College of Veterinary Medicine, Department of Population Health and Pathobiology, Raleigh, NC, United States of America; 2North Carolina State University, College of Agriculture and Life Sciences, Department of Animal Science, Raleigh, NC, United States of America

Sixty Angus-cross steers were used to compare the effects of recycled poultry bedding (RPB) versus a traditional growing diet on performance and blood and liver mineral status. Steers were individually fed balanced, growing diets for a period of 84 d. The diets were: control (CON), CON + monensin (+M), deep-

stacked RPB (DS), DS+M, shallow-stacked RPB (SS), and SS+M. Dry matter intake (DMI), average daily gain (ADG) and feed efficiency, or gain to feed ratio (G:F), were monitored throughout the trial. At the end of the trial, whole blood and serum samples were collected (n=60), and liver biopsies (n=30) were performed. Whole blood was analyzed for selenium (WBSe). Serum was analyzed for BUN and selenium (Se). Steers on CON diets had higher ADG, DMI and G:F than SS (1.53 vs. 1.19 kg/d ADG; 10.12 vs. 8.6 kg/d DMI; 0.152 vs. 0.138 G:F;  $P<0.01$ ) and higher ADG and G:F than DS (1.29 kg/d ADG and 0.134 G:F;  $P<0.05$ ). Steers on DS diets had higher DMI than SS (8.63 kg/d,  $P<0.05$ ). Inclusion of monensin in the growing diets increased G:F and decreased DMI (0.147 vs. 0.136 G:F; 9.12 vs. 9.81 kg/d DMI;  $P<0.05$ ). Steers in the CON group had higher WBSe than steers fed DS diets (159 and 146 ng/ml,  $P<0.01$ ). Monensin inclusion in the diet decreased WBSe (148 +M and 156 ng/ml,  $P<0.05$ ). Serum Se concentration in steers fed CON diets was higher than steers fed either RPB diet (73, 65 and 63, for CON, DS and SS, respectively,  $P<0.01$ ). Steers fed CON diets had higher BUN (13.4 mg/dl) concentrations at the end of the growing period than steers fed DS and SS diets (11.9,  $P<0.02$  and 12.2 mg/dl,  $P<0.06$  respectively). Liver copper concentration was higher for steers fed RPB diets than for CON diets (475 and 426 vs. 235 ppm for DS, SS and CON fed calves respectively,  $P<0.01$ ). Liver magnesium concentration in the steers fed CON diets was higher than in the steers fed SS diets (521 vs. 487 ppm,  $P<0.04$ ). While analysis of the presence of monensin on liver Mg concentration showed no difference overall ( $P>0.75$ ), there was a significant interaction by diet ( $P<0.02$ ). There was a difference in steers fed CON diets (491 ppm for CON+M vs. 551 ppm for CON-M;  $P<0.02$ ) and for steers fed SS diets (514 ppm for SS+M vs. 459 ppm for SS;  $P<0.03$ ). Results indicate that steers consuming diets with RPB perform better if the material has been processed by deep-stacking prior to consumption. Differences were noted in mineral status by diet, but were not of biological significance for short-term feeding.

510 (3497)

#### FIELD EFFICACY STUDY FOR THE CONCURRENT USE OF TWO VACCINES AGAINST MAJOR BOVINE RESPIRATORY PATHOGENS IN CALVES

Cavirani S.1, de Schwartz N.2, Valla G.3, Taddei S.1, Munoz Bielsa J.2, Bussacchini M.3, Piroddi S.4  
1Dipartimento di Salute Animale, Via del Taglio 8, Parma, 43100, Italy; 2Intervet International B.V., Wim de Horverstrat 35, Boxmeer, 5830 AA, Netherlands; 3Intervet Italy, Via Walter Tobagi 7, Peschiera Borromeo, Milano, 20068, Italy; 4DVM, c/o Sez. Mal. Infettive - Via del Taglio 8, Parma, 43100, Italy

The objective of the study was to assess the efficacy of the concurrent use of Bovipast® RSP and Bovilis® IBR in calves under field conditions. The study was carried out in Italy at one farm as a randomised, partially blinded and controlled field study. Five replications (270 animals, 90 animals per group) were included. Animals aged 2 weeks to 4 months were randomly assigned to group A (Bovipast® RSP + Bovilis® IBR), group B (Cattlemaster® 4) and group C (unvaccinated). The groups were homogeneous with respect to age, body weight and serological status at admission. Blood samples from 5 calves in each group were taken at week 0, 4, 8 and 16 post vaccination and tested for antibodies against bovine respiratory-syncytial virus (BRSV), parainfluenza 3 virus (PI3V), Mannheimia haemolytica (iron proteins and leucotoxin) and bovine herpesvirus 1 (BoHV-1). The number of animals treated due to respiratory disease, occurrence of antibiotic treatments for respiratory disease per calf, culling rate and mortality due to respiratory disease, weight gain for 112 days and serological response to vaccination were evaluated. The percentage of calves treated for respiratory disease was 17.8% for group A, 26.7% for group B and 28.9% for group C. Statistical analysis showed that the odds of being treated for respiratory disease was less for group A than group C (OR = 0.53; 95% CI 0.26 - 1.08). Meanwhile the odds of being treated in group B and group C were comparable (OR = 0.90; 95% CI 0.47 - 1.72). Mortality rates for respiratory disease were 3.3% for group A, 7.8% for group B and 6.7% for group C. Post mortem examination showed that several species of bacteria, of which M. haemolytica was the most common, played a role in respiratory tract disease at this farm. In contrast to the groups B and C, in none of the animals from group A evidence of M. haemolytica infection was found. Obtained mean antibody titres indicated BRSV and M. haemolytica as the major respiratory pathogens circulating on this farm. Bovine herpesvirus 1 and PI3V antibody titres were declining with time for each replication, indicating that these viruses were not present actively at the farm. The use of Bovipast® RSP in combination with Bovilis® IBR resulted in lower numbers of animals treated for respiratory disease and a reduced mortality rate due to pneumonia in comparison to the animals that were vaccinated with Cattlemaster® 4 or left unvaccinated.

511 (3261)

#### THERAPEUTIC EFFICACY OF TULATHROMYCIN AGAINST NATURALLY OCCURRING BOVINE RESPIRATORY DISEASE

Evans N.A.1, Skogerboe T.L.2, Mann D.D.3, Weigel D.J.2, Hassfurther R.L.3, Canning P.C.3  
1Pfizer Animal Health, Veterinary Medicine Research and Development, New York, NY, 10017, United States of America; 2Pfizer Animal Health, 7000 Portage Road, Kalamazoo, MI, 49001, United States of America; 3Pfizer Animal Health, Terre Haute, IN, 47802, United States of America

Three studies were conducted to a common protocol at sites in Greeley, CO, Tulare, CA, and Terre Haute, IN to evaluate the therapeutic efficacy of tulathromycin (Draxxin®), a novel triamilide antimicrobial, against naturally-occurring BRD. Animals were purchased at sale barns and commingled for approximately 2 days at the assembly point. After arrival, calves with respiratory disease (clinical illness score (CAS) of  $>1$ ; 1=mild

depression; 2=moderate depression; 3=severe depression and a rectal temperature  $>104^{\circ}\text{F}$ ) were selected for the study. At each location, calves were allotted to one of five treatment groups with 20 calves per group (randomized block design). Treatments were a single SC injection of saline, tilmicosin (Micotil®) 10 mg/kg or tulathromycin at 5, 2.5, or 1.25 mg/kg. Each calf was evaluated daily for 14 days post-treatment with rectal temperatures and CAS assessments made by a clinician masked to treatments. Treatment failure was a CAS  $>1$  and a rectal temperature  $>104^{\circ}\text{F}$ . Body weights were determined on days 0, 7, and 14. On day 14 post-treatment, lungs were removed and examined for gross pneumonic lesions. BRD cure rates were 4, 11, 49, 54, and 43% for the saline, tilmicosin, and tulathromycin- 5, 2.5, or 1.25 mg/kg groups, respectively. Cure rates for the tulathromycin-treated groups were significantly ( $P<0.05$ ) higher than the saline and tilmicosin groups. Differences among the three tulathromycin-treated groups were not different ( $P>0.05$ ). Mortality rates were 46, 16, and 3% for the saline, tilmicosin, and all tulathromycin groups, respectively, with significant ( $P<0.05$ ) differences between the saline and antimicrobial-treated groups. There were no differences ( $P=0.053$ ) in mortalities between tilmicosin and each tulathromycin group. Overall, rectal temperatures were significantly ( $P<0.05$ ) lower in the antimicrobial-treated groups compared to the saline group over the 14-day period, but not different ( $P>0.05$ ) among the antimicrobial groups. Percentage of pneumonic lung tissue was 33% in the saline-treated group, 18% for the tilmicosin group, and 8, 7, and 11% for the tulathromycin-5, 2.5, and 1.25 treated groups with the differences significant ( $P<0.05$ ) between tilmicosin and each tulathromycin group. Weight gain was significantly ( $P<0.05$ ) greater for the tulathromycin 5 and 2.5 groups (1.2 kg/day) compared to tilmicosin (0.6 kg/day). Tulathromycin given at a dosage of 2.5 mg/kg body weight was highly effective in the treatment of BRD.

Funding: Pfizer Animal Health

512 (1092)

#### CLINICAL EFFICACY OF MELOXICAM (METACAM®) IN THE TREATMENT OF RESPIRATORY DISEASE IN FEEDLOT CATTLE

Friton G.1, Cajal C.2, Ramirez Romero R.3, Kleemann R.1

1Boehringer Ingelheim Animal Health GmbH, Binger Strasse 173, Ingelheim/Rhein, 55216, Germany;

2Boehringer Ingelheim Vetmedica S.A. de C.V., Calle 30 No. 2614, Guadalajara, Jalisco, 44940, Mexico;

3Universidad Autónoma de Nuevo León, Av. Lázaro Cárdenas, Monterrey, Nuevo León, 64930, Mexico

Bovine respiratory disease (BRD) is the leading cause of illness and death and economically the most important disease of feedlot cattle. The objective of this blind, randomised study was to evaluate the clinical efficacy of a single meloxicam treatment (Metacam® 20 mg/ml) in combination with an antibacterial therapy in cattle infected with BRD in comparison to an established reference product.

Animals were treated with a single injection of 20 mg oxytetracycline/kg body weight by subcutaneous injection, in conjunction with either meloxicam (0.5 mg/kg, subcutaneously,  $n=100$ ), or flunixin (2.2 mg/kg, intravenously,  $n=101$ ). Meloxicam was administered once, whereas flunixin could be administered once daily for up to 3 consecutive days (re-administration, if rectal temperature  $\geq 40.0^{\circ}\text{C}$ ). Rectal temperature, respiratory rate, appetite, dyspnoea, coughing, nasal discharge and general condition were recorded on Days 0 (prior to treatment), 1, 2, 3 and 7 using a weighted numerical score. Scores were summed to generate a 'Clinical Sum Score' (CSS, range 7 to 24 points). The clinical efficacy was evaluated on Days 1, 2, 3, and 7. Individual animal body weights were measured on Days 0 and 7.

Clinical parameters and the mean CSS showed no significant differences between treatment groups with mean CSS on Days 0 and 7 of 16.18 and 10.55 in the meloxicam group and 16.41 and 10.88 in the flunixin group. However, a significantly lower mean rectal temperature was measured in the meloxicam group on Day 2 ( $p<0.01$ ). The assessment of clinical efficacy was significantly better for animals in the meloxicam group on Day 7 ( $p<0.01$ ). No significant differences in mean body weights were found between groups (Days 0 and 7: 232.3 kg and 252.6 kg in the meloxicam group and 234.1 kg and 254.7 kg in the flunixin group). Repeated administration of flunixin was performed in 45% of the animals.

A single subcutaneous dose of meloxicam was as clinically effective as up to 3 consecutive daily intravenous doses of flunixin when used as an adjunctive therapy for acute febrile respiratory disease in feedlot cattle.

Funding: Boehringer Ingelheim Animal Health GmbH

513 (3127)

#### THE EFFICACY OF TULATHROMYCIN (DRAXXIN®) IN THE PREVENTION OF NATURALLY-OCCURRING BOVINE RESPIRATORY DISEASE (BRD)

Godinho K.1, Sherington J.1, Rowan T.1, Sunderland S.2

1Pfizer Animal Health, Veterinary Medicine Research & Development, Pfizer Ltd., Sandwich, Kent, CT13 9NJ, United Kingdom of Great Britain and Northern Ireland; 2Pfizer Ltd, VMRD, IPC 896, Ramsgate Road, Sandwich, Kent, CT13 9NJ, United Kingdom of Great Britain and Northern Ireland

The efficacy of Draxxin® (tulathromycin) in the prevention of Bovine Respiratory Disease (BRD), where *Mannheimia haemolytica*, *Pasteurella multocida* and *Mycoplasma bovis* were isolated, was determined during an outbreak of respiratory disease. Three hundred and forty five animals were housed in a common airspace at the same time and when 15% ( $n=51$ ) of the animals were diagnosed with clinical BRD in this acute outbreak of disease, the remaining 85% ( $n=292$ ) of the animals were allocated randomly, in a ratio of 1:2:2, to receive either Saline at 0.025 mL/kg (T1: 66 animals), tulathromycin at 2.5 mg/kg (T2: 113 animals) or

Micotil® at 10 mg/kg (T3: 113 animals). Treatments were administered subcutaneously once on day 0. From day 1 to day 14, animals were observed once daily for signs of general health. Any animal showing signs of abnormal health was clinically examined and withdrawn for reasons related to BRD if its rectal temperature was  $\geq 40^{\circ}\text{C}$ , and if it presented with abnormal respiration and depression. The presence of BRD pathogens during the outbreak was confirmed following isolation of *M. haemolytica*, *P. multocida* and *M. bovis* from deep nasopharyngeal swabs. The percentage of animals completing the study in the tulathromycin treatment (100%) was significantly greater than in either the Saline (78.5%) or Micotil® (92.9%) animals ( $P=0.0001$  and  $P=0.0069$  respectively). On days 5 and 12 respectively, a total of 16.7% and 21.2% of the Saline-treated animals had been withdrawn for reasons related to BRD; compared with 0% on both days for the tulathromycin-treated animals and 2.7% and 7.1% of the Micotil®-treated animals. Tulathromycin-treated animals showed significantly higher weight gain during the study, compared with either Saline or Micotil®-treated animals ( $P<0.05$ ). Significantly fewer treatments for BRD were required for the animals treated with tulathromycin compared with animals treated with Saline or Micotil® ( $P<0.05$ ) in the 60 days after treatment. In conclusion, tulathromycin administered as a single injection at a dosage of 2.5 mg/kg was highly effective in the prevention of bovine respiratory disease involving *M. haemolytica*, *P. multocida* and *M. bovis*. Efficacy was superior to that of a commercial product containing Micotil® in preventing BRD in cattle, which were in contact with animals diagnosed with BRD.

514 (3213)

#### PENETRATION OF CEFTIOFUR INTO STERILE VERSUS MANNHEIMIA HAEMOLYTICA-INFECTED TISSUE CHAMBERS COMPARED TO PLASMA AND LUNG TISSUE

Johnson R.1, Washburn K.2, Clarke C.3, Anderson K.3, Lucas M.1, Robb E.1

1Pfizer Animal Health, 7000 Portage Road, Kalamazoo, MI, 49001, United States of America; 2Oklahoma State University, BVMT, Farm Road, Stillwater, OK, 74078, United States of America; 3Oklahoma State University, 264 Veterinary Medicine, Stillwater, OK, 74078, United States of America

Ceftiofur crystalline free acid (CCFA) sterile suspension is a single administration antimicrobial formulation for the treatment of bovine respiratory disease associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus* in cattle. Inferences regarding antimicrobial concentrations attained at the site of infection are usually made from plasma drug concentrations. Tissue chambers, surgically implanted in the paralumbar fossae of beef cattle, have been utilized successfully to characterize antimicrobial concentrations attained in lung tissue interstitial fluid. The study was the 2nd phase of a two-phase study conducted with 12 feedlot calves (4-8 months of age; 200-300 kg; heifers and steers). In phase I implanted tissue chambers on one side of the animal were randomly assigned to receive sterile saline while the other side was inoculated with *M. haemolytica*. Following a washout period from phase I, a 2nd injection of CCFA was administered subcutaneous in the other ear of each animal in phase II. Calves were randomly assigned to sacrifice groups (3, 5, 7, 9 days post-dosing). Blood and tissue chamber fluid from all chambers of each animal were taken predosing. Blood, tissue chamber fluid and lung tissue were collected at the time of sacrifice. Collected samples were analyzed for ceftiofur and related-metabolites and total protein levels (plasma, tissue chamber fluid). Results showed plasma concentrations of ceftiofur significantly higher than infected and sterile tissue chamber fluid and lung tissue, resp. at sacrifice days 3 ( $2.40>1.32$ ,  $0.53\ \mu\text{g/mL}$ ,  $0.60\ \mu\text{g/g}$ ) 5 ( $1.03>0.42$ ,  $0.40\ \mu\text{g/mL}$ ,  $0.31\ \mu\text{g/g}$ ), 7 ( $0.64>0.24$ ,  $0.22\ \mu\text{g/mL}$ ,  $0.21\ \mu\text{g/g}$ ) and 9 ( $0.42>0.08$ ,  $0.18\ \mu\text{g/mL}$ ,  $0.15\ \mu\text{g/g}$ ) ( $P<0.05$ ). However, in general ceftiofur levels followed a rank order of plasma>infected chamber fluid>sterile chamber fluid>lung tissue. Plasma and infected chamber fluid protein levels were not different, but were significantly higher than sterile chamber fluid levels ( $P<0.05$ ). In summary, results of phase II support 1) a long duration of therapy for CCFA administered SC in the ear at 6.6 mg CE/kg with plasma and tissue chamber fluid levels above  $0.2\ \mu\text{g/mL}$  for 9 days post-dosing and lung tissue levels above  $0.2\ \mu\text{g/g}$  for 7 days post-dosing, and 2) interstitial fluid, represented by tissue chamber fluid, may be a better indicator of target site ceftiofur and related metabolites concentrations than plasma concentrations.

Funding: Pfizer Animal Health

515 (1429)

#### EFFICACY OF A FUSOBACTERIUM NECROPHORUM-ARCANOBACTERIUM PYOGENES BACTERIN-TOXOID AS AN AID IN THE PREVENTION OF LIVER ABSCESSSES IN CATTLE

Jones G.1, Jayappa H.1, Hunsaker B.1, Sweeney D.1, Rapp-Gabrielson V.1, Wasmoen T.1, Nagaraja T.G.2, Swingle S.3, Branine M.3

1Schering-Plough Animal Health Corp., 21401 West Center Road, Elkhorn, NE, 68022, United States of America; 2Kansas State University, Department of Animal Sciences, Manhattan, KS, 66506-1600, United States of America; 3Cactus Feeders, 2209 West 7th Street, Amarillo, TX, 79106, United States of America  
Condemnation of bovine livers because of abscesses causes substantial economic loss. *Fusobacterium necrophorum* and *Arcanobacterium pyogenes* are believed to act synergistically to cause liver abscesses in cattle. A study was done in a research feedlot under field conditions to test the efficacy of a single-dose, bivalent *F. necrophorum/A. pyogenes* bacterin-toxoid to reduce liver abscess incidence and severity when given to cattle entering a feedlot. Steers ( $n = 1,510$ ) were randomized to 15 pens. The pens, in 3 blocks, were assigned to 5 treatment groups. Steers fed a non-medicated ration were vaccinated with a high-antigen dose bacterin-toxoid (A), a low-antigen dose bacterin-toxoid (B), or a placebo (control group). Steers fed a tylosin-

medicated ration were vaccinated with the bacterin-toxoid A or a placebo. Steers were followed to slaughter and liver abscesses recorded and scored by personnel blinded to the treatment groups. Livers were scored for abscesses as normal (0) to severe, as A- (1), A (2), or A+ / A++ (3). Carcass and yield grades were tabulated as desirable grades [Prime + Choice and yield grade Y1 + Y2] versus less desirable grades (Select and lower and Y3 to Y5). A significant pen effect for liver abscess incidence was not detected when the data were tested with pen as a random variable ( $p = 0.20$ , logistic regression). The non-medicated, control steers had a significantly higher incidence (48%) and mean liver score ( $1.02 \pm 1.24$ ) than steers vaccinated with bacterin-toxoid A (30%,  $p < 0.001$ , Fisher's Exact test, 2 sided, mean score,  $0.66 \pm 1.14$ ,  $p < 0.0001$ , Wilcoxon Rank Sum test) or bacterin-toxoid B (36%,  $p < 0.004$ , mean  $0.71 \pm 1.15$ ,  $p < 0.005$ ). The incidence and severity of liver abscesses in the vaccinated, tylosin-medicated steers (8%, mean score,  $0.15 \pm 0.57$ ) were not significantly less than that of the tylosin-medicated, control steers (12%,  $p = 0.26$ , mean score,  $0.23 \pm 0.71$ ,  $p < 0.18$ ). Group carcass grades did not differ. The proportion of carcasses graded Y1 and Y2 was significantly lower in the control groups (55% and 53%, non-medicated and tylosin-medicated rations) than in steers given the high-antigen dose bacterin-toxoid (64% in each ration group,  $p < 0.04$ , logistic regression). Bacterin-toxoid A significantly reduced liver abscess incidence and severity in cattle fed the non-medicated ration and improved yield grades in cattle fed both rations.

Funding: Schering-Plough Animal Health

516 (3416)

#### EFFICACY OF A LIVE VACCINE (BOVILIS® RINGVAC) AGAINST TRICHOPHYTON VERRUCOSUM IN VEAL CALVES

Kuijk H.A.1, Munoz Bielsa J.2

1Intervet Nederland, Wim de Korverstraat 35, Boxmeer, 5830 AA, Netherlands; 2Intervet International B.V., Wim de Korverstraat 35, Boxmeer, 5830 AA, Netherlands

A field trial was carried out in the Netherlands to evaluate the efficacy of a live *T. verrucosum* vaccine (Bovilis® Ringvac, Intervet International BV) to reduce *T. verrucosum* induced lesions in veal calves.

A total number of 1179 calves aged 14 to 21 days originating from four herds were included in the study (240-345 calves/herd). Calves were regularly allocated in groups of five per pen with management and housing conditions being very similar between the four farms. On each farm calves were randomly divided into 3 groups. Group one was vaccinated twice 14 days apart ( $n=403$ ), group 2 ( $n=370$ ) was vaccinated once and group three ( $n=403$ ) was left unvaccinated but was treated prophylactically with Imaverol® (Enilconazole 100 mg/ml). First vaccination in groups 1 and 2 took place one week after the arrival of the animals on the farm. Treatment in group 3 took place 3 weeks post-arrival. Calves in group 3 were treated again with Imaverol® if they showed clinical signs of *T. verrucosum* infection. Animals were checked for presence of *T. verrucosum* lesions on week 0, week 7, week 12 and week 19 post-arrival. They were slaughtered on week 25 and the hides were inspected for *T. verrucosum* lesions.

In the vaccinated groups the average lesions at slaughter were 1% in both group 1 and 2. There was no significant difference between one or two vaccinations. The intensity of *T. verrucosum* lesions in group 3 increased during the trial. Average lesion scores for four farms were 2% (week 7), 8.1% (week 14), 10.6% (week 18) and 13.8% at slaughtering. There was a variation between the four farms. Lesions at slaughter time were 21.2% (farm 1), 4.7% (farm 2), 6% (farm 3) and 27.5% (farm 4). The financial losses associated with the hide damage caused by *T. verrucosum* infection were estimated for €4 per calf with an additional treatment cost of €3.

Vaccination with one or two doses of Bovilis® Ringvac proved to be more efficacious than prophylactic and therapeutic treatment with Imaverol®. In veal calves protection is required only for a short period of time (approximately 5 months). In these conditions vaccination with one dose of Bovilis® Ringvac can be an efficacious and economical way to reduce *T. verrucosum* lesions in veal calves.

517 (5300)

#### COMPARISON OF BELGIAN BLUE X NELORE CROSS AND BRAFORD KEPT ON PASTURE CONDITION IN BRAZIL

Leroy P.L., Leroy E., Michaux Ch., Cassart R.

University of Liège, Faculty of Veterinary Medicine, Department of Animal Production, B43, Liège, B-4000, Belgium

43 Belgian Blue x Nelore cross (BBB x Nelore) (21 males and 22 females) were compared to 57 Braford (29 males and 28 females) on the AgriBahia Fazenda Lagoa do Morro (GES), Bahia State, Brazil, from 2001 to 2003. Nelore cows were inseminated with 2 Belgian Blue Bulls belonging to the Company: Belgian Blue Group. Braford animals were already kept on the same farm. Calving was normal without assistance for all cows.

During the last three months of fattening, animals got a complementation of 1% of live weight per day. The average daily gain was 938.5 g ( $926.0$  g for the BBB x Nelore,  $948.7$  for the Braford) before 300 days and lower afterward due to a strong dry period reducing the total daily gain (on average  $752.8$  g from birth to slaughter). 10 BBB x Nelore steers and 10 Braford steers were slaughtered at an average age of 25 months. Average live weight, carcass weight and killing out % were respectively 553.5 kg, 286.6 kg and 51.8% for the Braford. Corresponding values were 539.7 kg, 292.0 kg and 54.1% for the BBB x Nelore cross having, on

average, lower live weight (-13.8 kg), heavier carcasses (+5.4 kg) and a higher value of killing out% (+2.3%). The 7th right rib from each of the 20 steers, taken one day after slaughter, were dissected. Measured values of fat, meat and bone and also the weight of Longissimus Dorsi, Trapezius and Latissimus Dorsi revealed that BBB x Nelore had 2.54% less fat, 6.9% less bone and 9.44% more meat in comparison to Braford. The experiment conducted in Bahia indicated that Belgian Blue crosses were born without assistance, can survive in very dry conditions, have higher dressing out percentages than Braford with carcasses characterized by less fat, less bone and more meat. Belgian Blue is thus suggested to increase meat production in Brazil.

518 (3069)

#### EFFECTS OF SUPPLEMENTAL DIETARY VITAMIN E AND BETA-CAROTENE ON PERFORMANCE AND CONCENTRATIONS IN PLASMA AND MILK IN BEEF SUCKLER HERDS

Männer K.

Free University Berlin, Faculty of Veterinary Medicine, Institute of Animal Nutrition, Brümmerstraße 34, Berlin, Berlin, 14195, Germany

Objective of the study was to evaluate the efficiency of supplemental dietary alpha-tocopheryl acetate and beta-carotene on performance, health and vitamin E (alpha-tocopherol) and beta-carotene concentrations (analysed by HPLC) in plasma and milk of 634 suckler cows (550 kg body weight) and 593 crossbred calves during the winter (November to March) and grazing (April to October) feeding period. The different supplementation levels were either within the recommendations given by GfE (German Society of Nutritional Physiology) and NRC (Nutrition Research Council) or within a modified supplementation concept for vitamin E (daily 3000 mg per cow 3 weeks a.p. up to 5 weeks p.p.; daily 1000 mg per cow from 6 to 11 weeks p.p.; daily 500 mg per cow from 12 weeks p.p. onwards) and beta-carotene (daily 300 mg per cow from 2 weeks a.p. and during the lactation period). All cows were offered mainly grass-silage and hay (winter) or pastures (summer). The grasslands for grazing and harvest were not treated with soluble fertilizers since 12 years. The overall average daily intake of vitamin E and beta-carotene (native and stabilized supplementation) in the control group was 730 and 330 mg per cow in winter and 1600 and 1190 mg per cow in summer, respectively. The vitamin E and beta-carotene concentrations in plasma and milk of cows fed with the modified concept were significantly higher than in cows supplied with the NRC or GfE recommendations, particularly during winter feeding. The incidence of mastitis tended to be reduced by the modified concept even during the summer period with a high native supply. With regard to the low stability during storage of grass-silage and hay, data suggest that the current recommendations may not be sufficient to assure vitamin E and beta-carotene adequacy during winter feeding. The vitamin E and beta-carotene concentrations in plasma of the neonatal calves of cows fed with the modified concept were only during the winter-feeding with 3.0 and 0.95 mg/l plasma significantly higher compared to calves from cows fed within the NRC or GfE recommendations. No response of feeding cows with the modified supplementation concept was observed during rearing.

519 (3478)

#### VALIDATION OF A DIAGNOSTIC TEST FOR CHRONIC PNEUMONIA AND POLYARTHRITIS SYNDROME (CPPS) IN FEEDLOT CATTLE

McDonald L.1, Bateman K.1, Ribble C.1, Campbell J.2

1Ontario Veterinary College, Rm 2514 Stewart Building, Guelph, Ontario, N1G 2W1, Canada; 2Western College of Veterinary Medicine, Saskatoon, SK, S7N 5B4, Canada

Necropsies were performed on calves under 320 kg dying within 60 days of arrival in southwestern Ontario feedlots. The purpose of the evaluation reported here was to determine if days from treatment until death would give an accurate diagnosis of chronic pneumonia and polyarthritis syndrome (CPPS). This would then be used in a later study examining the risk factors associated with CPPS where it was not possible to access all cases for post mortem examination. Histories, which included treatment records, date and time of death, were taken on all dead cattle submitted for necropsy. Gross necropsies were conducted and deemed the gold standard test. Diagnosis for CPPS by using greater than seven days from treatment to death as a cut-point was compared to the gold standard test. Over the course of the two years of study, 48.2% of all mortalities in which a post mortem was conducted were diagnosed as due to CPPS. An exact test of homogeneity showed both years data were proportionally similar ( $p=1.00$ ) which allowed the authors to merge both years for analysis, since this meant year was not a confounding factor. The positive predictive value (PPV) of using 7 days or less as a diagnostic cut-off was 69.16% (59.5-77.7), while the negative predictive value (NPV) was 65.31% (50.36-78.33). The sensitivity and specificity of using 7 days from treatment to death as a cut-point was 81.32% (71.78-88.72) and 49.23% (36.6-61.93) respectively. The number of days found to have the maximum PPV was 16, where the resulting PPV was 74.1% (63.1-83.2) and the NPV was 58.67% (46.70-69.92). The sensitivity and specificity in this situation was found to be 65.93% (55.25-75.55) and 67.69% (54.95-78.77). Deaths due to *Haemophilus somnus* infections, calves persistently infected with bovine viral diarrhoea virus (BVDV) and those with fibrinous bronchopneumonia were those most often misclassified. While using the cut point of greater than seven days between treatment and death does not have the optimal diagnostic effect, this method of classification is a valuable tool as a simple and free surrogate measure of cause of death in the population of interest.

Funding: OCA, BCRC

520 (2726)

## DIAGNOSIS AND EPIDEMIOLOGY OF LIVER FLUKE IN BURGUNDY BEEF CATTLE

Meissonnier E.1, Courouble F.2, Abrial D.3, Barnouin J.3

1Janssen Santé Animale, Issy-Les-Moulineaux Cedex, TSA 91003, 92787, France; 2Bovine veterinary practitioner, Epinac, Burgundy, France; 3 INRA, Animal Health Department, Clermont-Ferrand, France

*Fasciola hepatica* (the liver fluke) is a very common trematode parasite in French beef cattle. It is located in the liver of infected animals and significantly affects both milk and beef production and reproductive performance. The routine coprological diagnosis method is first described for fluke diagnosis and then compared with a more sensitive method. We then describe a serological method applied to pooled blood samples with an automated LISA method (based on f2 antigen).

The serological survey was undertaken on 591 beef herds in 3 Burgundian departments (Allier, Nièvre and Saône-et-Loire): average percentages of infected herds respectively are 35, 62 and 82%. The results of both diagnosis methods are discussed and displayed on maps according to different criteria: average ELISA results or average percentages of positive results per canton. This original mapping method based on a Geographic Information System makes up an interesting mean of communication to promote the deworming programmes towards the beef cattle breeders.

521 (3043)

## SINGLE TREATMENT CURE RATE OF CEFTIOFUR CRYSTALLINE FREE ACID STERILE SUSPENSION FOR THE TREATMENT OF BOVINE RESPIRATORY DISEASE (BRD) WITH A 3 OR 7 DAY RE-TREATMENT MORATORIUM

Meyer J., Moseley W., Lehman F., Bryson W., Robb E., Lucas M.

Pfizer Animal Health, 7000 Portage Road, 0225-190-40, Kalamazoo, MI, 49001, United States of America

Ceftiofur is an injectable cephalosporin developed solely for veterinary use. Ceftiofur crystalline free acid (CCFA) sterile suspension is a recently approved ready-to-use formulation developed as a single administration product for cattle bacterial respiratory disease (BRD). This study evaluated the 28 and 56 day BRD cure rates following single administration treatment of CCFA with no subsequent re-treatment (moratorium) permitted for 3 or 7 days after initial treatment, compared to a positive control, enrofloxacin with no re-treatment permitted for 3 days. This complete block design study was conducted at seven feedlots. Crossbred beef calves (n=3819) weighing between 94.5 - 359.0 kg were used. Beginning the first day after arrival, pen riders, blinded to treatment assignments observed cattle daily. Starting day two after arrival, first-time BRD pulls that met the inclusion criteria (abnormal respiration, depression evident, and rectal temperature greater than 40.0°C) were randomly assigned to the treatment groups; CCFA (6.6 mg ceftiofur/kg body weight (BW) subcutaneously (SC) in the middle third of the posterior aspect of the ear) with a 3 or 7-day moratorium, or enrofloxacin (at 12.5 mg/kg BW SC in the neck) with a 3-day moratorium. Treated animals were placed in a hospital pen for 7 or 8 days. Daily clinical evaluations continued. Re-treatment criteria included abnormal respiration, depression evident and having met the assigned moratorium. Cattle were weighed 56 days after arrival. Data from 1736 cattle were included in the analysis and were analyzed using PROC MIXED. The 28-day single treatment cure rates (STCR) were 34.4, 51.7 and 35.1% for the 3 and 7 day CCFA and enrofloxacin groups. The 56-Day STCR were 30.9, 43.8 and 30.1% for the 3 and 7 day CCFA and enrofloxacin groups. A 7-day re-treatment moratorium with CCFA-SS resulted in a 17% (p<0.05) and 14% increase (p<0.05) in 28-Day and 56-Day STCR compared to the 3-day re-treatment moratorium for enrofloxacin. Mortality rates were 4.4, 3.3 and 5.2% for the 3 and 7 day CCFA-SS and the enrofloxacin groups (p>0.05). A single dose of CCFA is effective for the treatment of BRD when a 7-day moratorium is used. This study confirms that adequate plasma concentrations of ceftiofur metabolites are present through 7 days post-treatment and that additional treatments during this period are not required.

Funding: Pfizer Animal Health

522 (3116)

## FEED INTAKE RESPONSE OF FEEDLOT CATTLE FOLLOWING SINGLE DOSE TREATMENT OF CEFTIOFUR CRYSTALLINE FREE ACID STERILE SUSPENSION OR FLORFENICOL

Moseley W.1, Bryson W.1, Robb E.1, Boyd M.2, Engelken T.2, Bowers A.3, Lucas M.1

1Pfizer Animal Health, 7000 Portage Road, Kalamazoo, MI, 49001, United States of America; 2Mississippi State University, 4025 Wise Center Road, Mississippi State, MS, 39762, United States of America;

3Mississippi State University, 214 W. Critz St., Suite A, Wiggins, MS, 39577, United States of America

In a replicated, three-treatment crossover design with three 21-day observation periods conducted at two separate locations, daily dry matter intake (DDMI) was measured in healthy feedlot cattle receiving a single dose treatment of ceftiofur crystalline free acid (CCFA-SS, 6.6 mg/kg BW) in the middle third of the posterior ear, florfenicol (40 mg/kg BW) subcutaneous in the neck or untreated Controls. At Mississippi State University (MSU), 48 cattle (286 ± 3.7 kg) were individually fed in 4 pens with 12 Calan gates in each pen. At Pfizer Animal Health Research Farm (PAH), 192 cattle (385 ± 4.3 kg) were group fed in 24 pens each containing 8 head. Treatment sequences were randomly assigned to animals within pen at MSU and to pens at PAH. Within each 21-day period, DDMI and daily feed orts were recorded on Days 0-21. Significant (p < 0.05) sequence by treatment by day interaction for DDMI led to an analysis based on the first period only for both

MSU and PAH. At MSU, DDMI for CCFA-SS cattle was not significantly less ( $p > 0.05$ ) than Control for any of the days. Administration of florfenicol resulted in a significant decrease ( $p < 0.05$ ) in DDMI compared to Control for days 2, 4, 5, 8, and 9 and was numerically lower than Control on days 3, 6, 10, 11, 12, 14, 15, and 16. At PAH, DDMI of Control and CCFA-SS were not significantly different ( $p > 0.05$ ) for any of the days following treatment. Administration of florfenicol resulted in a significant decrease ( $p < 0.05$ ) in DDMI (15% to 40%) compared to Control for days 2 through 14, but not for days 1 and 15 through 21. Given the significant sequence by treatment by day interaction in the initial analysis with inclusion of all three periods, caution is required when interpreting comparisons of treatments within periods two and three. Treatment differences may also include period, carryover, and interaction effects. However, in agreement with the results of Period 1 analysis at both locations, there does appear to be a pronounced decreased DDMI during the first days following treatment administration during each period for florfenicol compared to Control and CCFA-SS groups. In conclusion, cattle receiving CCFA-SS continued to consume feed similar to untreated Controls whereas, cattle responded with reduced DDMI in response to florfenicol. Feed intake is an important clinical sign in the diagnosis and retreatment of BRD.  
Funding: Pfizer Animal Health

523 (3501)

## ARTHROSIS SYNDROME IN BEEF STEERS WITH CHRONIC SUBCLINICAL ACIDOSIS

Quintavalla F.1, Bonati L.1, Biasin S.2, Milani D.2, Soriolo R.2, Martini F.1, Taddei S.1, Cavirani S.1

1Dipartimento di Salute Animale, Via del Taglio 8, Parma, 43100, Italy; 2DVM, c/o Sez. Mal. Infettive, Via del Taglio 8, Parma, 43100, Italy

Charolais steers ( $n=20$ ; 520 -550 kg BW) belonging to a beef herd located in Northern Italy and fed with a concentrated diet (corn silage + protein concentrates + corn meal + beet pulp) were examined and divided into 2 groups on the basis of the clinical status: 10 clinically healthy animals (controls) and 10 ill animals affected by chronic arthrosis syndrome. The principal clinical signs included weakness, laminitis, enlargement of one or two tarsal joints and ataxia.

Ruminal fluid and blood samples were collected for biochemical analysis. Arthroscopy of pathologic joints was performed and synovial fluid was aseptically collected for microbiological investigations.

Titrimetric evaluation of rumen fluids showed an increase of glucose fermentation and a decrease of cellulose digestion time and protease population motility. The graphic pattern concerning titrimetric assay showed two pH steps: the first one located at pH 3.8 ("chemical space" lactated acid) and the second one at a pH range 4.0-5.0 ("chemical space" VFA). These aspects gave evidences for a chronic rumen acidosis, as also indicated by ruminal fluids pH values. Blood biochemical parameters (NEFA, total cholesterol, amylase, iron, blood urea nitrogen, chloro, calcium, inorganic phosphorus, alkaline phosphatase, total blood protein, albumin, creatine kinase, ASAT/GOT, total bilirubin) did not showed major alterations. Titrimetric values and blood biochemical parameters of healthy and ill animals were not statistically different. Arthroscopic surgery showed capsular thickening, severe joint effusion with chronic synovitis with a large amount of fibrin clots and diffuse cartilage degeneration. Microbiological investigation on synovial fluids did not demonstrate the presence of either bacteria or mycoplasma. NSAIDs treatment performed on animals with arthrosis led to the recovery of 7 out of 10 affected animals. This result confirmed the microbiological findings indicating the absence of bacteriological agents in the synovial fluids.

Taken together our data pointed out that arthrosic syndrome was not related to infectious agents (bacteria or mycoplasma). Moreover, a chronical ruminal acidosis may be considered a necessary but not sufficient cause at triggering chronic arthrosis syndrome involving in particular the animals with a quicker body-weight increasing. This latter condition might be the factor favouring the development of the articular pathology in presence of ruminal acidosis.

524 (3262)

TIME KILLING KINETICS AND IMPACT OF CULTURE CONDITIONS (PH, CO<sub>2</sub>, AND SERUM) ON MIC VALUES OF TULATHROMYCIN AGAINST HAEMOPHILUS SOMNUS

Reese C.P.1, Norcia L.J.2, Skogerboe T.L.1

1Pfizer Animal Health, Veterinary Medicine Research and Development, 7000 Portage Road, Kalamazoo, MI, 49001, United States of America; 2Pfizer Animal Health, Groton, CT, 06340, United States of America

Macrolide antimicrobials are generally assumed to be bacteriostatic based on a mechanism of action targeting inhibition of protein synthesis. Bactericidal activity toward some bacteria species has been reported for some members of the macrolide/azalide class. The purpose of this study was to evaluate the effects of pH on in vitro activity of tulathromycin (Draxxin®) against *Haemophilus somnus* as assessed by measuring Minimum Inhibitory Concentrations (MIC) and to assess the effects of bovine serum on tulathromycin MICs and killing kinetics against *H. somnus*. Broth microdilution MIC tests were conducted using two-fold serial dilutions of tulathromycin ranging from 16 to 0.0156 mg/ml. Tulathromycin MICs were determined in VFM adjusted to various starting pH values ranging from 6.8 to 8.0. Tulathromycin MICs at any given starting pH were lower for all strains in the presence of serum than in its absence. Killing kinetics were determined for two *H. somnus* clinical isolates. Organisms were inoculated into VFM containing various concentrations of tulathromycin without serum or supplemented with 40% bovine serum. Under standard NCCLS conditions, the MIC was 1.0 mg/ml. Killing kinetics were assessed at 0.01, 0.1, 1.0, 2.0 and 4.0 mg/ml tulathromycin (0.01X to 4X the

MIC). In the absence of serum, cell numbers and viability were reduced at a faster rate with increasing tulathromycin concentration, with 4.0 mg/ml cidal (<sup>3</sup>99.9% kill) for both strains within 24 h. Bactericidal effects in the presence of 40% bovine serum were even more dramatic. At a concentration of 1.0 mg/ml viable counts decreased at a rate identical to that seen at 4.0 mg/ml in the absence of serum, with no detectable viable cells remaining after 24 h. At 2.0 mg/ml in the presence of serum, no viable cells were detected by 6 hrs and at 4.0 mg/ml viability dropped off rapidly, with >99.9% decrease in cell counts at the 4 h time-point. Addition of 40% bovine serum to VFM apparently buffered the pH of the medium during incubation in 5% CO<sub>2</sub>. Thus, the bactericidal activity of tulathromycin toward *H. somnus* was dramatically enhanced in the presence of bovine serum. In summary, a slight variation in medium pH can have a relatively dramatic effect on the in vitro MIC values of tulathromycin for *H. somnus*. The bactericidal activity appears to be concentration-dependent, in that increasing concentrations above the MIC result in faster loss of viability, particularly noticeable in the presence of serum.

Funding: Pfizer Animal Health

525 (2546)

#### CALF HEALTH IN SWEDISH BEEF SUCKLER FARMS

Stengärde L.1, Törnquist M.2

1Swedish Animal Health Service, Svenska Djurhälsovården, Skara, Se-532 89, Sweden; 2Swedish Animal Health Service, Svenska Djurhälsovården, Kävlinge, Se-244 82, Sweden

There are few data available on health status in Swedish beef suckler herds. Some information is available through KAP - the official Swedish beef recording system. The aim of the present study was to broaden the knowledge of herd health, management, feeding and housing in Swedish beef suckler operations. The material will be used as an advisory tool and as a base for further studies in the field.

The study is descriptive, based on inquiry forms. The herds (n=250) were selected due to geographical spread, size of the herd (most with more than 20 cows), and proper documentation of the herd health and management. The herds were sent information about the study and an inclusive inquiry form. Of these, 124 were positive to participate. From July 1, 2001 to June 30, 2003, the 124 selected herds were followed through inquiry forms every third month, in total nine forms. The herd managers have been asked to answer questions about the herd, among others about calf health and mortality. Herds that have answered seven or more inquiry forms (n=92) are now being processed. Data from the inclusive inquiry form is however available as preliminary results.

The number of beef cows in Sweden is 168 000 in 13 105 herds with an average herd size of 12.9 cows. The median size of herds in the study is 45 cows (10-247 cows). Twenty-two percent are pedigree breeders, 37% keep cows to produce fattening animals and the others have multipurpose herds. Seventy-eight of the farms are part of KAP. Most farms have the calving season in the first half of the year (January-June), 70% calve indoors, 27 % both indoors and on pasture, and 3% only on pasture. Stillbirths on herd basis were an average of 2.6 calves/herd and deaths during the milk period were 0.7 calves/herd. When asked about calf mortality in the herd over the past five years, the average was 4.8%. KAP gives calf mortality up to weaning as 6.1%, half of which are stillborn. In Swedish dairy herds the average mortality due to stillbirths is 4.8% and during the first 90 days a total of 8%. The number of treated calves the first twelve months of life was 3.2%. About 40% of the farmers considered calf health to be a problem during the 2001 calving season. The most common problems were diarrhea, navel infections and pneumonia.

Further data from the survey concerning calf health are now being processed and will be presented at the World Buiatrics Congress in Quebec 2004.

Funding: Swedish Farmers' Foundation for Agricultural Research

526 (595)

#### RESPONSES OF METAPHYLACTIC TREATMENT WITH MICOTIL ON THE INCIDENCE OF BOVINE RESPIRATORY DISEASE (BRD) IN SOUTHEASTERN STOCKER CATTLE

Vann R.1, Engelken T.2, McClary D.3

1Brown Loam Branch Experiment Station, 1676 Brown Loam Rd, Raymond, MS, 39154, United States of America; 2 Mississippi State University, College of Vet Med, PO Box 6100, MS, 39759, United States of America; 3Elanco Animal Health, 2001 W Main Street, PO Box 708, Greenfield, IN, 46140, United States of America

The objective of this study was twofold: 1) to determine the effect of metaphylactic treatment with Micotil on bovine respiratory disease (BRD) morbidity and mortality compared to the non-medicated control steers; and 2) to monitor feed intake and body weight to determine if a reduction in the incidence of BRD affects the performance of calves during a 45-d pre-conditioning phase prior to grazing. Feeder calves (n=239) ranging in weight from 179 to 238 kg from southeastern US origin were assigned to a Micotil treatment (n=120) group or a non-medicated control (n=119) group. The two treatment groups were evenly distributed among twelve pre-conditioning pens (n=20 calves per pen). Upon arrival calves were weighed, body temperature recorded, vaccinated, castrated, and administered treatment therapy. Calves were revaccinated at d 10 after processing and body weight was recorded on d 28 and 45. Calves were monitored daily for signs of clinical illness and treated according to post-processing BRD therapy guidelines. Following daily treatments, steers were returned to their original pen. At the completion of the 45-d pre-conditioning period, all steers were weighed,

branded, and removed from pen assignments and allowed to graze regrass pasture for 107 d prior to shipment to the feedlot. Weight gain and mortality during the grazing period (107 d) were recorded. During the pre-conditioning period, the overall pull rate was greater ( $P < 0.05$ ) in the non-medicated control group compared to the Micotil treated group (88.1 vs. 70.0%, respectively). The Micotil treated group was slightly heavier and had greater total gain and greater average daily gains ( $P < 0.05$ ) compared to the non-medicated control group during the pre-conditioning phase. However, there were no differences between the two treatment groups in average body weight, average total gain, or average daily gain during the grazing period. Metaphylactic treatment with Micotil upon arrival decreased the overall pull rate of calves exhibiting signs of BRD compared to non-medicated controls in southeastern origin stocker calves during the pre-conditioning period.

Funding: Elanco Animal Health

527 (5051)

#### COMPARISON OF ASSAYS USED TO DETECT BRANDING-AGE BEEF CALVES PERSISTENTLY INFECTED WITH BOVINE VIRAL DIARRHEA VIRUS

Walz P.H., Davis C., Kapil S., Gnad D.P.

Kansas State University, College of Veterinary Medicine, Departments of Clinical Sciences, Manhattan, KS, 66506-5606, United States of America

Infection of pregnant cattle prior to fetal immunocompetence can lead to the birth of calves that are immunotolerant to and persistently infected (PI) with bovine viral diarrhea virus (BVDV). These cattle shed BVDV throughout their life and are the major source of virus transmission within and among beef cattle herds. Identification and elimination of PI beef calves is an important strategy for controlling the transmission of this virus, and this is ideally performed by testing branding-age beef calves prior to the start of the breeding season. The purpose of this study was to compare BVDV detection assays for identifying BVDV infection in beef calves. Four BVDV detection assays were compared, and included skin biopsy immunohistochemistry (IHC), serum microplate virus isolation (VI), whole blood VI, and a serum antigen capture ELISA. Skin biopsies (ear notches), whole blood, and serum samples were collected from all beef calves ( $n=807$ ) from 12 herds that had a history of BVDV infection as defined by positive BVDV identification within the year prior to inclusion in this study. Skin samples were tested for BVDV by IHC. Serum samples were tested by the serum microplate VI for BVDV by an immunoperoxidase monolayer assay (IPMA) using primary embryonic bovine kidney cells in a 96-well test format. Serum samples were also tested for BVDV by a commercially available antigen capture ELISA. Mononuclear cells were isolated from whole blood samples, passed once on primary embryonic bovine kidney cells and tested for BVDV by IPMA. Where possible, calves that were determined positive by any of the tests were retested to document persistent infection. Of the 12 test herds, PI calves were identified in 8 herds. From the 807 calves tested, 32 (4%) were positive by whole blood VI, 31 (3.8%) were positive by skin biopsy IHC, 20 (2.5%) were positive by ELISA, and 8 (1%) were positive by serum microplate VI. All calves that were positive by the skin biopsy IHC, the ELISA, and the microplate VI were positive by whole blood virus isolation. The 1 calf that was negative by skin biopsy IHC but positive by whole blood VI was confirmed to be a PI. These results indicate that the skin biopsy IHC and whole blood virus isolation are preferred tests for identifying branding-age beef calves persistently infected with BVDV. Testing and eliminating PI beef calves prior to the breeding season is important in BVDV control.

528 (3087)

#### ANTI-PYRETIC EFFICACY OF RESFLOR™ INJECTABLE SOLUTION IN THE TREATMENT OF BOVINE RESPIRATORY DISEASE (BRD)

Weingarten A., Lewis V., Cao J., Simmons R., Varma K.

Schering-Plough Animal Health, 1095 Morris Avenue, Location U-23-3650, Union, NJ, 07083, United States of America

SCH 529752 (RESFLOR™) is a novel formulation containing florfenicol and flunixin meglumine, and is currently in development for the treatment of Bovine Respiratory Disease (BRD) and its associated pyrexia. The objective of this study was to evaluate the field efficacy and safety of SCH 529752 (RESFLOR™) in the control of pyrexia associated with naturally occurring BRD compared to both negative and positive controls. The study was designed as a prospective, randomized, masked, multi-center pivotal clinical study. Recently transported calves, of feedlot age, showing clinical signs of BRD were enrolled into the study upon meeting the inclusion criteria for pyrexia, depression and abnormal respiratory character. Once qualified each calf was randomly assigned to receive one of three treatments as a one-time dose of 20 mL/150 kg administered subcutaneously. The three treatments included saline, Nuflo® (equivalent to 40 mg/kg florfenicol), and SCH 529752 (equivalent to 40 mg/kg florfenicol and 2.2 mg/kg flunixin). A rectal temperature (°F) was measured prior to treatment and at Hour 6 post-treatment.

Five commercial feedlots within North America participated in the conduct of this study. Collectively, all five sites enrolled and treated five hundred twenty five (525) calves. Five hundred eleven (511) qualified for the efficacy portion of the data analysis (170 in the saline group, 169 in the Nuflo® group, 172 in the SCH 529752 group).

Hour 6 Results: SCH 529752 Nuflo Saline

Mean Rectal Temperature (°F) 102.5 104.0 105.2

## Mean Decrease in Temperature

at Hour 6 from Baseline (°F) 2.79 1.23 0.27

p&lt;0.0001 (SCH 529752 vs Saline; Nuflor vs Saline; SCH 529752 vs Nuflor).

\* One-sided p-value with Dunnett's adjustment; mixed model ANOVA

In conclusion, SCH 529752 (RESFLOR™) exhibited a superior antipyretic effect by demonstrating a statistically significant decrease in rectal temperature at Hour 6 compared to both the Nuflor® (p&lt;0.0001) and saline (p&lt;0.0001) treatments.

Funding: Schering-Plough

529 (3081)

## PHARMACOKINETICS OF FLORFENICOL AFTER SUBCUTANEOUS ADMINISTRATION OF NUFLOR® INJECTABLE SOLUTION OR SCH 529752 (RESFLOR™) INJECTABLE SOLUTION TO CATTLE AT A DOSE OF 40 MG/KG

Weingarten A.1, Mushtaq M.2, Lockwood P.3, Crouch L.2, Brianceau P.3, Assaf M.2, Xu M.4, Wislocki P.2, Simmons R.1

1Schering-Plough Animal Health, 1095 Morris Avenue, Location U-23-3650, Union, NJ, 07083, United States of America; 2Schering-Plough Research Institute, 144 Route 94, Lafayette, NJ, 07848, United States of America; 3Schering-Plough Animal Health, 2458 No. Chamberlain Street, Terre Haute, Indiana, 47805, United States of America; 4PPD Development, 2244 Dabney Road, Richmond, VA, 23230, United States of America

SCH 529752 (RESFLOR™) is a novel formulation containing florfenicol and flunixin meglumine. RESFLOR™ is in development for treatment of Bovine Respiratory Disease and its associated pyrexia. The objective of this study was to evaluate the bioequivalence of florfenicol after subcutaneous administration of SCH 529752 and Nuflor® Injectable Solution 300 mg/mL (Schering-Plough Animal Health) at a dose of 40 mg/kg of florfenicol. The study was designed as a randomized, two-period, two-sequence crossover study. Twenty-eight (28) cattle (14 males and 14 females) of feedlot age were randomly assigned to two groups (I or II) of fourteen cattle (seven per sex) per group. Group crossover occurred after a washout period of 56 days.

Blood samples were collected prior to dosing (0 hours) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108 and 120 hours post-dose. Plasma prepared from the blood samples, was analyzed for florfenicol by a validated LC-MS/MS method. The pharmacokinetic parameters for each animal in each phase were calculated using WinNonlin Professional Software Version 3.2. Bioequivalence of the two formulations was assessed as per the U.S. FDA Center for Veterinary Medicine Bioequivalence Guidance (Final), 2002.

The pharmacokinetics of florfenicol are described in the following tables:

Pharmacokinetic Parameters of Florfenicol in Cattle (n=28) Receiving RESFLOR™

Cmax Tmax AUClast AUC(obs) T1/2 MRT(obs) TMIC90

Mean 4.31 5.46 132.76 156.04 53.68 59.46 39.54

SE 0.21 0.32 3.92 3.94 3.98 5.39 1.73

CV 25.24 31.30 15.64 13.38 39.19 47.97 23.13

Pharmacokinetic Parameters of Florfenicol in Cattle (n=28) Receiving NUFLOR®

Cmax Tmax AUClast AUC(obs) T1/2 MRT(obs) TMIC90

Mean 3.23 6.00 122.89 165.31 72.00 87.42 39.14

SE 0.15 0.27 3.42 5.77 5.22 6.79 1.45

CV 24.50 24.00 14.71 18.47 38.33 41.11 19.66

Units: Cmax (µg/mL); Tmax (hr); AUClast (µg\*hr/mL); AUC(obs) (µg\*hr/mL); T1/2 (hr); MRT (obs) (hr); TMIC90 (hr)

The total exposure (AUClast) and time over MIC90 (TMIC90) were the same for both formulations, however, peak plasma levels (Cmax) were higher in RESFLOR™-treated cattle. As such, RESFLOR™ would be expected to be at least as clinically efficacious as NUFLOR®.

Funding: Schering-Plough

530 (2065)

## IMPACT OF THE TEXAS BEEF PARTNERSHIP IN EXTENSION PROGRAM (BEEF PEP) ON THE PROFITABILITY OF STUDY HERDS

Wikse S.1, McGrann J.2, Falconer L.2, Herd D.3, Holland P.1, Abello F.1, Rodgers G.4, Richardson D.2

1Texas A&amp;M University, Large Animal Medicine and Surgery, College Station, Texas, 77843-4475, United States of America; 2Texas A&amp;M University, Agricultural Economics, College Station, Texas, 77843, United States of America; 3Texas A&amp;M University, Animal Science, College Station, Texas, 77843, United States of America; 4Pfizer Animal Health, 138 Rim Rock Road, Aledo, Texas, 76008, United States of America

The purpose of this study was to demonstrate that beef cow/calf herds can increase profitability by increasing production and/or reducing unit cost of production through implementation of advice from veterinarian/county extension agent teams supported by university specialists. The project was carried out for 3 years in 6 commercial beef cow/calf operations in Texas. There were 1927 cows in the 6 herds with a mean of 321 cows per herd. In January 2000, veterinarian/county extension agent teams began visiting herds to give owners advice on 25 profitable ranch management practices in 7 management categories. Management categories included: 1) nutrition, 2) pasture/haying, 3) reproduction, 4) health, 5) calf husbandry, 6) marketing and 7)

performance records/accounting. Use of each practice by the herds was scored on a 1 to 5 scale the baseline year of 1999 and compared to use at the end of 3 years. Standardized Performance Analysis was used to measure the production and economic outcomes of the project.

Mean weaning weight per exposed cow of the 6 study herds increased significantly ( $p < 0.05$ ) 26.8 kg (17%) in 2000, 49.1 kg (31%) in 2001 and 43.2 kg (27%) in 2002, compared to 1999. Mean cost per 45.5 kg of the 6 herds declined significantly ( $p < 0.05$ ) \$20.04 (-18%) in 2000, \$33.40 (-29%) in 2001 and \$22.52 (-20%) in 2002, compared to 1999. Additional profits (at constant sale price of calves and herd size) for the 6 herds were \$54,407 in 2000, \$135,695 in 2001 and \$116,089 in 2002 for a total of \$306,191. Mean increase in management score of the herds was positively correlated to increase in net income ( $p < 0.07$ ) and accounted for over 60% of increased profits.

The profitability of beef cow/calf operations can be substantially increased through a team approach that identifies opportunities for improved management and helps ranch managers implement them. Local veterinarians are the best choice to lead beef herd consultation teams because of their broad knowledge base and large amount of contact time with ranch managers.

Funding: Pfizer Animal Health

531 (5059)

#### PERFORMANCE OF A TIMED ARTIFICIAL INSEMINATION (TAI) PROGRAM FOR FIRST SERVICE FOLLOWED BY TAI COMBINED WITH NATURAL HEAT DETECTION VERSUS TAI WITH NATURAL HEAT DETECTION FOR FIRST SERVICE AND SUBSEQUENT BREEDINGS IN A COMMERCIAL DAIRY

Capel M.1, Nydam D.V.2, Saltman R.3

1Perry Veterinary Clinic, 4884 Burbank Dr, Geneso, NY, 14454, United States of America; 2Cornell University, Animal Health Diagnostic Lab, Ithaca, NY, United States of America; 3Pharmacia Animal Health, Kalamazoo, MI, United States of America

The goal of this study was to evaluate the effects of breeding dairy cattle on first service with 100% timed artificial insemination (TAI) as compared to TAI combined with natural heat detection for first service. In both groups, all breedings subsequent to the first service were accomplished through natural heat detection or TAI if found open at pregnancy exam.

A dairy milking 1,900 cows, 3 times/day was selected for study. For the previous year, the Rolling Herd Average was 24,500 lbs, average Heat Detection Rate was 67%, and average Pregnancy Rate was 20%. The breeding program involved prostaglandin (PG) injections at 30-36 days in milk (DIM) and 44-50 DIM (Pre-Synch). The voluntary wait period (VWP) was set at 46 DIM. All cows not bred by natural heat detection were given Gonadotropin Releasing Hormone (GnRH) at 57-63 DIM, PG at 64-70 DIM, GnRH at 66-72 DIM, and automatically time bred 12-16 hours later (Ov-Synch).

Cows were randomized into two treatment groups. The control group followed the herd's historical breeding program. The treatment group was given PG at 9-15 DIM and 23-29 DIM, GnRH at 36-42 DIM, PG at 43-49 DIM, GnRH at 45-51 DIM, and automatically time bred 12-16 hours later. The VWP in the treatment group was 46 DIM, and all of the cows in the treatment group were time bred between 46-52 DIM. Inclusion criteria for randomization included ambulatory cows present on the farm at 9-15 DIM. Pregnancy was evaluated by rectal palpation at 35-41 days post breeding. In both groups, cows were subsequently bred by observed estrus or if diagnosed open at pregnancy examination by TAI. The main outcome of interest was the overall average pregnancy rate for four cycles after the VWP. Pregnancy rate is defined as the average proportion of cows open and eligible for insemination that become pregnant per 21-day estrus cycle. A sample size of 500 cows per treatment group allows for detection of a meaningful difference in the Pregnancy Rate between the 2 treatment groups.

Preliminary results identified a statistically significant difference between treatment and control groups for first service pregnancy rate (31% vs. 21%). After four full cycles, average pregnancy rate and average heat detection were higher for the treatment group than the control group.

It is biologically possible to achieve excellent pregnancy rates breeding cows at 46-52 DIM through either TAI alone or TAI in combination with excellent heat detection on first service.

532 (2706)

#### EFFECT OF LEPTOSPIRA SPP SEROVAR HARDJO NATURAL INFECTION ON THE REPRODUCTIVE PERFORMANCE OF A NELORE HERD MANAGED UNDER EXTENSIVE CONDITIONS

Genovez M., Castro V., Gregory, L., Oliveira J.C., DelFava C., Pituco E., Scarcelli E., Paulin L., Cardoso M. Instituto Biologico, Av. Cons. Rodrigues Alves, 1252, São Paulo, São Paulo, 04014-002, Brazil

Bovine leptospirosis occurs worldwide, and serovar Hardjo is the most frequent one (genotype Hardjobovis or Hardjoprajtino) affecting bovines, maintenance hosts of this serovar. Hardjo infection pattern varies with herd husbandry practices and type of Hardjo involved. In many countries, serovar Hardjo is recognized as a significant cause of infertility and reproductive failure. In Brazil, Hardjo has already been isolated from cattle and is a widespread endemic infection, with unclear effect on reproduction, though. The purpose of this trial was to monitor the effect of a *Leptospira* spp serovar Hardjo natural infection on reproductive performance of a Nelore herd (*Bos indicus*), representative of the extensive management scheme from the second largest beef-producing region in the state of São Paulo, Brazil. Herd was kept in *Brachiaria decumbens* rotational grazing system, 0,30 animal/ha, with mineral salt ad libitum, and presented  $4,78 \pm 0,54$  as body condition

score mean. Differential diagnosis for brucellosis, BVD, Campylobacteriosis and Trichomoniasis were found negative. The animals were vaccinated against IBR. Pregnancy (Pr), parturition (P) and calving-interval rates (CI) were evaluated. Serum samples of all females at reproductive age were examined by Microscopic Agglutination test using 6 serovars of *Leptospira* spp (cutt off > 50) in June 1999; July 2000 and July 2001, and resulted positive to serovar Hardjo, 12,4% (33/266); 64,6% (122/189) e 71,5% (128/179), respectively. Although high seroconversion rates were observed for Hardjo, the influence on reproductive performance, in both cow ( $7,9 \pm 4,1$  years) and heifer groups ( $2,15 \pm 0,20$  years) was not significant. The Pr, P and CI from seroconverted group have not shown any difference with the negative group rates in each period examined and also with the whole period negative group. Geometric means of agglutinin titers revealed an increasing tendency when compared to the first evaluation, without correlation with the reproductive performance. Conclusion: Under Brazilian climate and herd management conditions, the interference of serovar Hardjo on reproduction is less evident. Further studies on genotypic variation of Hardjo strains in Brazilian herds are required, mainly in relation to their adaptation to bovines and interaction with the environment. Australia, New Zealand and Netherlands, serovar Hardjo is also endemic but not a significant cause of reproductive failure in cattle.

Funding: FAPESP-Fund.Amparo à Pesquisa

533 (1960)

**EVALUATION OF VULVOVAGINAL LESIONS, CICLICITY AND FERTILITY RATES OF EXTENSIVELY BRED NELORE (*BOS INDICUS*) COWS SEROREAGENT TO INFECTIOUS BOVINE RHINOTRACHEITIS IN A FARM IN THE STATE OF SÃO PAULO, BRAZIL**

Gregory L.1, Genovez M.2, Carneiro P.1, Pacheco W.2, Oliveira J.2, Del Fava C.2, Pituco E.2, de Stefano E.2, Duarte F.3, Calil F.2

1University of Sao Paulo, Av.Prof.Dr.Orlando Marques de Paiva 87, Cidade Universitária, São Paulo, São Paulo, 05508-900, Brazil; 2Instituto Biologico, Av. Conselheiro Rodrigues Alves 572, São Paulo, São Paulo, 04014002, Brazil; 3Instituto Biologico, Av.Prof.Dr.Orlando Marques de Paiva 8, Cidade Universitária, São Paulo, São Paulo, 04014002, Brazil

In order to assess the influence of bovine herpesvirus1 (HVB-1) in the fertility of cows, clinical and reproductive evaluations were performed in Nelore females in a farm in the Araçatuba region, state of São Paulo, Brazil. Cows were kept in a natural breeding system, and mating season lasted three months. During two complete reproductive cycles, these animals were submitted to gynecological examination with inspection of external genitalia, rectal palpation, ultrasound examination in the first, middle and final trimester of pregnancy, and antibody survey for HVB-1, using seroneutralization. Vulvovaginitis, cyclicity and pregnancy rates were calculated, both for animals reagent and non-reagent to HVB-1. Fischer's exact test was used at 5% of significance, in order to compare rates in different years. Rate of HVB-1 positive animals and vulvovaginitis was 83.3% (20/24), rate of animals positive to HVB-1 without vulvovaginitis, 74.5% (155/208) in the first year. In the second year, these rates were respectively, 79.6% (43/54) and 80.9% (93/115). Rate of animals seroreagent for HVB-1 and presenting normal cycles was 77.8% (21/27), and those seroreagent and in anestrus, 42.9% (3/7) in the first year. In the second year, these rates were, respectively, 80.0% (4/5) and 84.2% (16/19). Rate of animals seroreagent to HVB-1 and pregnant was 75.2% (112/149), and rate of HVB-1 positive cows that were not pregnant was 63.5% (33/52) in the first year. These rates in the second year were, respectively, 80.5% (91/113) and 84.4% (27/32). Data indicate that HVB-1 was not responsible for vulvovaginitis, and did not interfere either with normal cycles or with pregnancy rates.

Key words: Fertility, vulvovaginitis, IBR, *Bos indicus*

Funding: Fapesp

534 (5078)

**SEROPOSITIVITY FOR AGENTS CAUSING NEOSPOROSIS, BOVINE VIRAL DIARRHEA, ENZOOTIC BOVINE LEUKOSIS, AND JOHNE'S DISEASE AND THEIR RELATIONSHIP WITH INCREASED CALVING TO CONCEPTION INTERVAL IN CANADIAN DAIRY COWS**

Haddad J.P.A.1, Dohoo I.1, VanLeeuwen J.1, Tiwari A.1, Keefe G.P.1, Tremblay R.2

1Atlantic Veterinary College, 550 University Avenue, Charlottetown, Prince Edward Island, C1A 4P3, Canada; 2Boehringer Ingelheim (Canada) Ltd, Eastern Canada, Quebec, J2S8B1, Canada

Neosporosis (*Neospora caninum* - NC), Bovine Viral Diarrhea (Bovine Viral Diarrhea Virus - BVDV), Enzootic Bovine Leukosis (Bovine Leukosis Virus - BLV), and Johne's disease (*Mycobacterium avium* subsp. Paratuberculosis - MAP) are transmissible diseases that are considered to have economic impacts on dairy cattle productivity and can affect international trade of animals/products. The purpose of the present study was to investigate relationships between the sero-status for these four infectious agents and calving to conception interval (CCI).

The data were collected from 136 farms (with dairy herd improvement (DHI) records), 2802 cows and 4786 lactations, selected in 5 provinces in Canada. On each farm, blood samples were collected from up to 30 randomly selected milking cows for diagnosis of infection by NC, BLV, and MAP, and 5 unvaccinated cattle over 6 months of age for diagnosis of exposure to BVDV. The blood sampling was conducted from May to Aug. 1998, except for Saskatchewan (Jan. to Mar. of 2001). Data on milk production and reproduction from all sampled cows was obtained electronically from DHI records.

For each agent, the test used and their sensitivity and specificity were NC (Biovet ELISA, 92.9%, 98.4%), BLV (IDEXX ELISA, 98.5%, 99.9%), and MAP (IDEXX ELISA, 43.0%, 99.0%). A herd was considered positive for BVD if at least one animal with titre greater than 1:64 was present in the herd.

Three mixed logistic models, with herd included as a random effect, were built using three different outcome variables: CCI higher than 200 days (CCI200) or not, CCI higher than 250 days (CCI250) and CCI higher than 300 days (CCI300). Explanatory variables offered to the models were: serologic status for the four agents (positive/negative), lactational 24 hour peak milk production in kilograms (PMP), median of the linear score of the somatic cells count from 40 to 200 days of lactation (MSCC), province, and lactation number as a 3 level categorical variable (1st, 2nd and 3rd+).

For BVDV, BLV and MAP infection, none of the models showed significant effects. For the CCI200 model, NC seropositive cows had a 1.26 ( $p=0.057$ ) higher risk of having a CCI higher than 200 days, and this risk increased to 1.44 ( $p=0.027$ ) in the CCI250 model and to 1.59 ( $p=0.093$ ) in the CCI300 model.

The increasing risk of prolonged CCI for NC positive cows was interpreted as evidence of fetal loss or abortion since previous work had indicated no effect of NC on conception.

535 (1667)

#### EXAMINATIONS ON THE ACCURACY OF PREGNANCY TESTING WITH A PORTABLE ULTRASOUND UNIT AND THE INFLUENCE ON FERTILITY PARAMETERS IN COWS

Lejeune B., Siegwart N., Hässig M., Kähn W.

University of Zurich, Department of Farm Animals, Winterthurerstr. 260, Zürich, 8057, Switzerland

The objective of the present study was to determine how easy it is for the veterinarian to learn to perform pregnancy testing using a portable ultrasound unit. Also of interest was the accuracy of this method and its economic benefit to the farmer.

The subjects of the study were 500 cows that are regularly examined by the University of Zurich. The animals were examined using a portable ultrasound unit with a 5 MHz vector probe 4 weeks after insemination. This was followed up after the 6th week with a transrectal palpation as a control. The ultrasound method had a sensitivity of 98.3% and a specificity of 78.2%. The positive predictive value was 92.2%, the negative predictive value 94.7%. The accuracy of the method was 92.8%.

The time between the first insemination and the successful insemination and calving intervals of 13 dairy herds over 4 years were determined by means of a herdcare programme. There was a significant difference ( $p<0.05$ ) in time between the first insemination and the successful insemination and calving intervals in 1998 (before using a portable ultrasound unit) and 2001 (using a portable ultrasound unit).

Using a portable ultrasound unit for early pregnancy testing is easy to learn by a veterinarian and results in a high specificity of 98.3% and an accuracy of 92.8% even when the first few months of learning the method are included. In negative results of controls the incidence of embryonic death during early gestation has to be considered.

There was a significant difference ( $p<0.05$ ) in calving intervals from 1998 (without ultrasound) and 2001 (using an ultrasound) and also for the time between the first insemination and the successful insemination. In 1998 the calving interval was 386.7 days, in 2001 it was reduced to 361.49 days. The time between the first insemination and the successful insemination was reduced from 36.4 days in 1998 to 21.4 days in 2001. The reduction of the days open improves the economic situation for the farmer.

Key words: cow, pregnancy testing, ultrasonography, time between the first and the successful insemination, calving interval

536 (2665)

#### DETERMINATION OF PREGNANCY-ASSOCIATED GLYCOPROTEINS (BPAG) IN COW'S MILK

Metelo R.1, Silva S.1, Sulon J.2, Beckers J.F.2, Moreira da Silva F.1

1University of the Azores, Praça Alberto Sá de Oliveira, 17 2º esq., Coimbra, 3030 Coimbra, Portugal;

2University of Liege, Faculty of Veterinary Medicine, Physiology of Reproduction, B41, B-4000, Liege, Belgium

Introduction: Pregnancy diagnosis is an important part of reproductive management, particularly in the dairy industry. The pregnancy-associated glycoproteins (PAGs) are placental antigens that were initially characterized as pregnancy markers in the maternal circulation of bovine species. The use of milk to determine levels of hormones has been investigated in many studies as the milk sampling avoid stressful effects of venipuncture, does not require special experience and the milk collection is easier than the blood. Ali (1999) as well as Tainturier et al. (1996) speculated the possibility to detect PAG in cow milk samples.

Aims and Methods: The aim of the present study was to validate a new bovine pregnancy method evaluating the bPAG in milk. Blood and milk samples were collected weekly from a group of 60 Holstein-Friesian cows for one year, and plasma was separated by centrifugation. For bPAG milk RIA test, samples were homogenized by heating at 40°C for 30 min and fat was removed by centrifugation. Pure bPAG was used as a tracer and as a standard, as previously described by Zoli et al (1992). For the milk, the size of the sample was increased to 500 µl and the buffer was only used on the preparation of the tracer and antiserum solutions.

Results: Milk profiles showed a concentration of bPAG variation from 10% in values very high, very near the parturition, to 1% when the concentrations achieved basal values. The drop of bPAG concentration was faster

and more accentuated in the milk, than in the blood. A good correlation was obtained between the results obtained from the blood and the milk ( $r=89.2\%$ ;  $P<0.0001$ ).

Conclusions: This study clearly indicates that milk can be used for pregnancy diagnosis in cows, giving a very helpful tool to farmers. This can become a very important tool especially in countries, e.g. New Zealand and Portugal - Azores, where the animals spend whole year in the field, especially for milking cows. Milk can be used as a later diagnosis of gestation, a non invasive and non stressed method to follow easily a gestation.

537 (1969)

#### INTERFERENCE OF MYCOPLASMA SPP OR UREAPLASMA SPP IN OVINE SEMEN QUALITY

Mettifogo E.1, Rizzo H.1, Cardoso M.2, Buzinhani M.1, Oliveira J.3, Timenetsky J.1, Gregory L.1

1University of Sao Paulo, Av.Prof.Dr.Orlando Marques de Paiva 8, Cidade Universitária, São Paulo, São Paulo, 05508-900, Brazil; 2Instituto Biológico, Av. Conselheiro Rodrigues Alves 572, São Paulo, São Paulo, 04014002, Brazil; 3Instituto Biológico, Av.Prof.Dr.Orlando Marques de Paiva 87, Cidade Universitária, São Paulo, São Paulo, 05508-900, Brazil

The reproductive disturbances in animals with commercial importance may interfere to the economy of ovine breeding. The adherence of mycoplasmas to the ovine spermatozoa is the initial feature of this host-parasite relationship. In this view it was compared the presence of mycoplasma in ovine semen and the influence of this infection in macroscopic and microscopic aspects of this fluid. Ovine semen was obtained from 33 ovine (race Sta Inês, Poll Dorset e Texel). The animals were from three properties of a region named Piedade/SP-Brasil. The clinical samples were separated in vials for culturing and other series for microscopic and macroscopic analysis. Strains of mycoplasma and ureaplasma were inoculated respectively in SP4 and UB agar. The semen samples were observed for physical, morphological and cellular aspects. The parameters were based as mentioned by COLÉGIO BRASILEIRO DE REPRODUÇÃO ANIMAL (1998). Risk estimation, Odds Ratio, was obtained by software GrapfPad Instat, version 3. for Windows. The presence of mycoplasma in ovine semen was associated in 9.09% (3/33) to the microscopic and macroscopic alterations of this fluid. Mycoplasma spp was isolated in 36.36% (12/33) from semen samples while Ureaplasma spp was isolated in 12.12% (4/33). The mycoplasmal infection rates in studied semen samples indicate that the diagnosis of these bacteria in ovine should be a routine procedure for animal quality.

Key words - Mycoplasma, ovine, semen, infertility

Funding: Fapesp

538 (5073)

#### EFFECT OF GnRH DAY 12 AFTER ARTIFICIAL INSEMINATION ON PREGNANCY RATE IN THE COW

Szenci O.1, Perényi Zs.1, Karen A.1, Takács E.1, Sulon J.2, Beckers J.F.2

1Szent István University, Faculty of Veterinary Sciences, 1Clinic for Large Animals, Üllo, Hungary; 2University of Liege, Faculty of Veterinary Medicine, Physiology of Animal Reproduction, Liege, Belgium

Gonadotropin-releasing hormone (GnRH) has been used to increase pregnancy rate between Days 11 and 14 after artificial insemination over the past decades. The scientific rationale for this treatment is to enhance embryo survival rates by suppressing the luteolytic mechanism, which may occur if there is a failure in maternal recognition. Some studies have been able to report significant improvements of 10 to 12% in pregnancy rates while others were not. In most cases, pregnancy diagnosis were based on rectal palpations. The objective of our study was to evaluate the effect of GnRH (Gonavet-50, Veyx-Pharma GmbH, Germany) on the fertility rate of Holstein-Friesian dairy cows when administered on Day 12 after insemination.

Lactating healthy cows ( $n=103$ ) on a large Hungarian dairy farm were allocated randomly into treated ( $n=54$ ) or control ( $n=49$ ) groups. Alternatively, treated cows received 50  $\mu$ g (1 ml) GnRH-agonist intramuscularly while the control cows received a placebo.

Progesterone (P4) RIA test was used to evaluate the time of artificial insemination (Day 0) and ultrasonography, P4 and pregnancy associated glycoprotein (PAG-1) RIA tests were used to detect pregnancy and embryonic mortality (Days 21, 32 and 55). Blood samples were withdrawn from the jugular vein into heparinised vacutainer tubes on Days 0, 12, 21, 32 and 55 after service and plasma was stored at -20°C until assayed.

In each group three cows were inseminated at the wrong time ( $P4 > 1,0$  ng/ml) and late embryonic mortality occurred in three (treated group) and two cows (control group), respectively.

The mean pregnancy rates after first service were 46,3% and 49% for treated and control cows, respectively. The difference between the two groups was not significant. There was no significant difference between the treated and non-treated cows when they were inseminated <100 days or >100 days after calving. In conclusion, GnRH treatment, 12 d after service, cannot be recommended as a method of improving reproductive performance in dairy cows.

539 (3500)

#### EFFECT ON REPRODUCTIVE PARAMETERS IN DAIRY CATTLE TREATED WITH INACTIVATED BVDV VACCINE IN THE POST-PARTUM

Toni F.1, Guazzetti S.2, Valla G.3, Grigoletto L.4, Cavirani S.5

1Dipartimento di Patologia Animale, Via L. da Vinci 44, Grugliasco, Torino, 10095, Italy; 2AUSL Reggio Emilia, Viale Bagnoli 85, Castelnovo ne' Monti, Reggio Emilia, 43100, Italy; 3Intervet Italy, Via Walter Tobagi

7, Peschiera Borromeo, 20068, Italy; 4DVM, c/o Sez. Mal. Infettive, Via del Taglio 8, Parma, 43100, Italy; 5Dipartimento di Salute Animale, Via del Taglio 8, Parma, 43100, Italy

Bovine viral diarrhoea virus (BVDV) is a major pathogen in cattle and causes considerable losses. Depending on the stage of gestation at the time of the infection, the outcome may be embryonic death, abortion, stillbirth or the birth of immunotolerant calves persistently infected. During viremia (transient or persistent) BVDV actively replicate in the ovary. The ovarian infection causes functional hormonal change: reduction of the number and of the dimension of folliculi and of the activity of the corpus luteum. In general BVDV is responsible of reproductive problems in dairy cattle.

To evaluate the clinical effect of treatment with an inactivated vaccine to BVDV (Bovilis® BVD) on reproductive parameters, 390 dairy cows belonging to the same herd were included in the study. Despite a routinely treatment with a combined BoHV-1/BVDV/PI3V vaccine, the herd showed BVDV circulation as demonstrated either by viral isolation and serological evidences. In particular, 194 animals were vaccinated 30-40 days post partum and 196 animals were kept as control unvaccinated, and treated with a placebo. The animals admitted to the study had to fulfill the following criteria: normal uterine involution 30-40 days after calving, a body condition score =2.75, no signs of disease. The following reproductive parameters were recorded and assessed by statistical analysis: mean value of number of insemination/pregnancy (Student T test), mean period of calving/conception interval (time in days where 50% of the inseminated cows become pregnant) (log rank test), percentage of pregnant cows and of calving (?2 test). The percentage of pregnancy was 88% and 83% in vaccinated and in control groups respectively (no significant difference,  $p=0.16$ ). The percentage of calving was 93% and 98% in vaccinated and in control groups respectively (no significant difference,  $p=0.79$ ). The mean number of insemination/pregnancy was 2.14 and 2.44 in vaccinated and in control groups respectively (significant difference,  $p=0.028$ ). The mean period of calving/conception interval was 119 and 126 days in vaccinated and in control groups respectively (significant difference,  $p$  associated to log rank test =0.067). The data pointed out that post-partum vaccination with Bovilis® BVD elicited a significant improving either in the mean value of the number of insemination/pregnancy rate and in the mean period of calving/conception interval.

540 (3222)

#### FIELD OBSERVATION ON THE EFFECT OF TIMING OF INSEMINATION ON PREGNANCY RATE

van Werven T., Jorritsma R.

Utrecht University, Faculty Veterinary Medicine, Yalelaan 7, Utrecht, 3584 CL, Netherlands

During the last decades, the average calving interval in the Netherlands increased from 387 days (1990) to 404 days (2002). In general, an extended calving interval may be due to a prolonged interval calving to first artificial insemination (AI) and/or to a prolonged interval first AI to conception. A prolonged interval first AI to conception is caused by failure of oestrus detection before and after first AI and/or by a low conception rate. A Dutch farm with an average herd size of 50 Holstein Friesian cows and a 305 day milk production of 9943 kg (4.32% fat and 3.48% protein) had a calving interval of 423 days. The interval first AI to conception was 67 days and pregnancy rate after first AI was 35%. Cows were inseminated in the morning or the afternoon by AI technicians, applying standardised semen handling procedures. Cows were fed according to requirements and housed in a free stall with slatted floors and comfortable cubicles. Therefore, we investigated whether timing of insemination was on this farm responsible for the low pregnancy rate after first AI. Between April 2002 and September 2003 the farmer recorded time of observed heat and time of insemination. Pregnancy was diagnosed by rectal palpation. From 73 cows, a total of 153 of heat observations were recorded, resulting in 60 cases of pregnancy and 93 cases of non-pregnancy. Inseminations resulting in pregnancy were performed between 2.5 and 30 hours (mean 19.0) after observed oestrus, while inseminations resulting in non-pregnancy were performed between 5.5 and 31 hours (mean 19.5) after observed oestrus. The effect of timing of insemination on pregnancy was evaluated using survival analysis with cow as random variable. Confirmed pregnancy was defined as event and number of AI was used as time variable. Independent variables were time between observed heat and AI, its quadratic, parity, morning versus afternoon AI, and their interactions. The results showed that first calf heifers had the lowest pregnancy rates, while no effects of morning or afternoon insemination were detected. We also found significant cow effects ( $p=0.07$ ), while there were no significant effects of timing of AI on pregnancy rate. We concluded that despite the large variation in intervals between observed heat and AI, low pregnancy rates after insemination were on this farm not due to inadequate timing of insemination.

541 (2787)

#### STUDY OF THE ROLE AND ORIGIN OF ENDOTOXEMIA IN THE PATHOGENESIS OF RESPIRATORY DISTRESS SYNDROME IN THE NEWBORN CALF

Aliaoui H., Danlois F., Guyot H., Rollin F.

Université de Liège, Faculté de Médecine Vétérinaire, Service de Médecine Interne des Grands Animaux, Liège, Liège, 4000, Belgium

In Belgium, respiratory distress syndrome (RDS) in mature hypermuscled Belgian Blue (BB) calves is frequently observed. It concerns 65% of BB farms whose 36% lose one or more calves each year. Septicemia and enteritis lesions found respectively in 30% and 42% of RDS necropsied calves made us to consider the hypothesis that an endotoxemia of digestive origin is associated with RDS. The aims of the study were to test

this hypothesis by dosage of blood endotoxin in RDS and in healthy comparable calves and to investigate the origin of this endotoxemia by bacteriological culture and dosage of endotoxins in colostrum of their respective dams, and by complete necropsy of dead calves.

We examined 14 eleven-hours to six-days old BB presenting RDS as well as 9 healthy calves. Calves physical examination was supported by arterial and venous blood gases and pH analysis, as well as blood endotoxin dosage by the Limulus Amebocyte Lysate test (LAL). The colostrum endotoxin content was also tested by LAL.

In 10 out of these 14 RDS calves, mental status and appetite were severely depressed. This led us to distinguish two groups: RDS undepressed (4) and RDS depressed calves (10) which presented, beside tachypnea and hypoxemia, acidosis, hypercapnea and cyanotic mucous membranes. The endotoxemia of depressed calves was significantly higher ( $0.14 \pm 0.17$  Endotoxin Unit (EU)/ml, mean  $\pm$  SEM) comparatively to healthy ( $0.01 \pm 0.01$  EU/ml) and undepressed calves (endotoxin level under limits of detection).

Bacterial examinations on 4 necropsied calves revealed pathogenic *E. coli* in the intestines of 3 calves. Only 1 calf showed simultaneous presence of *E. coli* in both intestine and heart's blood. Concerning the bacteriological culture and endotoxin dosage in colostrum, no difference was found between the three groups of dams.

This study suggests that the colostrum is not the origin of endotoxemia. The presence of endotoxemia only in depressed RDS and not in undepressed RDS calves pleads for a secondary consequence rather than primary cause of RDS. Such endotoxemia can suppress the synthesis of surfactant by pneumocytes II and, combined with higher lung vascular permeability, induces hyaline membranes formation. However, other factors like trace elements are known to influence pulmonary maturation and thus to play a role in the pathogenesis of RDS, especially iodine and selenium deficiencies. Further study is needed to clarify the pathogenesis of RDS in calves.

Funding: Service de Médecine Interne des Grands Animaux, Faculté de Médecine Vétérinaire de Liège

542 (2872)

#### EFFECT OF STEROIDAL AND NON-STEROIDAL ANTI-INFLAMMATORY DRUGS ON INFLAMMATORY MARKERS IN CALVES WITH EXPERIMENTALLY-INDUCED BRONCHOPNEUMONIA

Bednarek D.1, Kondracki M.1, Friton G.2, Trela T.3, Niemczuk K.1

1National Veterinary Research Institute, Dept. of Cattle and Sheep Diseases, Pulawy, 24100, Poland;

2Boehringer Ingelheim Animal Health GmbH, Binger Strasse 173, Ingelheim/Rhein, 55216, Germany;

3Boehringer Ingelheim Austria GmbH, Dr. Boehringerergasse 5-11, Vienna, 1120, Austria

The study evaluated the influence of treatment with steroidal (SAIDs) and non-steroidal (NSAIDs) anti-inflammatory drugs on inflammatory markers in thirty, 6-8 weeks old, healthy calves, allocated randomly into three equal groups. Local inflammatory processes were induced by infusion of paraffin oil directly into lungs. Animals received a single intravenous treatment with either meloxicam (0.5 mg/kg body weight; Metacam; group I), flumethasone (0.05 mg/kg body weight; group II) or sterile 0.9% NaCl (10 ml per animal; group III). Body temperature, respiratory and heart rate, concentration of PGE<sub>2</sub>, PGF<sub>2a</sub>, thromboxane (TXB<sub>2</sub>), leukotriene (LTB<sub>4</sub>) and malonyldialdehyd (MDA) and pro-inflammatory cytokines i.e. tumor necrosis factor (TNF $\alpha$ ) and interferon (INF $\alpha$ ) were recorded in serum, bronchoalveolar lavage (BAL) and blood platelets (BP). Serum concentrations of iron, zinc, and copper were investigated. The routine clinical and laboratory parameters were evaluated 48 h after induction of lung inflammation (T<sub>0</sub>, before initiation of therapy) and 48 hours after initiation of therapy (T<sub>1</sub>). The application of both anti-inflammatory drugs influenced clinical parameters positively with a significant decrease in rectal temperature in group I (T<sub>0</sub> to T<sub>1</sub>:  $p < 0.01$ ) and group II (T<sub>0</sub> to T<sub>1</sub>:  $p < 0.05$ ). The heart rate and respiratory rate decreased from T<sub>0</sub> to T<sub>1</sub> in groups I and II. NSAID and SAID treatment resulted in significant decrease of PGE<sub>2</sub>, PGF<sub>2a</sub>, TXB<sub>2</sub> and MDA mean concentration in serum, BAL and BP (T<sub>0</sub> to T<sub>1</sub>:  $p < 0.001$  in group 1;  $p < 0.01$  in group 2). A significant increase of mean serum and BAL concentrations of LTB<sub>4</sub> was observed in group I ( $p < 0.05$ ). The mean serum and BAL concentrations of TNF achieved a significant increase in group III ( $p < 0.05$ ). The mean concentration of IFN in serum and BAL was significantly higher in groups I and II ( $p < 0.05$ ). Iron concentration in serum decreased in all groups, however changes were significant in group II ( $p < 0.001$ ) and group III ( $p < 0.01$ ). The content of zinc and copper stayed within the physiological range for all groups and time points investigated. The significant reduction of main inflammatory mediators PGE<sub>2</sub>, PGF<sub>2a</sub>, TXB<sub>2</sub> and MDA after meloxicam treatment in calves with induced bronchopneumonia indicates a beneficial effect on the inflammatory processes. Contrary to effects observed by flumethasone, meloxicam induced an increase of LTB<sub>4</sub> and IFN indicating it is not immunosuppressive.

Funding: Boehringer Ingelheim Animal Health GmbH

543 (2325)

#### EFFICACY OF COMBINED USE OF MELOXICAM (METACAM®) AND ANTIBIOTICS IN TREATMENT OF CALF BRONCHOPNEUMONIA

Bednarek D.1, Kondracki M.1, Trela T.2, Friton G.3, Niemczuk K.1

1 National Veterinary Research Institute, Dept. of Cattle and Sheep Diseases, Pulawy, 24100, Poland;

2Boehringer Ingelheim Austria GmbH, Dr. Boehringerergasse 5-11, Vienna, 1120, Austria; 3Boehringer

Ingelheim Animal Health GmbH, Binger Strasse 173, Ingelheim/Rhein, Germany, 55216, Germany

Bronchopneumonia in calves is a common, multi-factorial disease syndrome of significant economic importance for cattle producers. The aim of this study was to investigate the efficacy of a combination of antibiotic and non-steroidal anti-inflammatory therapy in the treatment of bronchopneumonia in calves under field conditions.

Animals with typical symptoms of bronchopneumonia received a single intramuscular dose of 10 mg long acting oxytetracycline/kg body weight either in combination with a single intravenous meloxicam treatment (0.5 mg/kg body weight; group 1; n=60) or as stand alone therapy (group 2; n=60). The body temperature, breathing, heart rate, nasal discharge and coughing were evaluated over 5 consecutive days using weighted numerical scores summed to a Clinical Illness Index Score (CIIS; range from minimum 0 to maximum 5.5 score points). Body weights were recorded over a 9 week period. Additionally, the acid-base balance and gas exchange parameters were evaluated.

Mean rectal temperature decreased significantly faster in the combined treatment group 24 hours after treatment (Day 0 and Day 1:  $41.1 \pm 0.4^\circ\text{C}$  to  $39.6 \pm 0.4^\circ\text{C}$  in group 1;  $41.5 \pm 0.5^\circ\text{C}$  to  $40.4 \pm 0.7^\circ\text{C}$  in group 2;  $p < 0.01$ ). The number of animals with fever ( $> 39.5^\circ\text{C}$ ) was significantly lower in group 1 on Days 1, 2 and 3 ( $p < 0.05$ ). Mean values of the CIIS were significantly lower in group 1 from Study Day 2 until Day 5 ( $p < 0.01$ ). Mean body weights (b.w.) and mean average weekly weight gain (AWG) achieved significant differences in favour of the combined treatment group (group 1:  $65.9 \pm 9.1$  kg b.w. and  $122.3 \pm 8.2$  kg b.w.; AWG:  $7.8 \pm 0.7$  kg; group 2:  $65.3 \pm 8.3$  kg b.w. and  $112.6 \pm 9.7$  kg b.w.; AWG:  $6.6 \pm 1.1$  kg) ( $p < 0.05$  b.w.;  $p < 0.05$  AWG). In both groups the evaluation of basic indices of the acid-base balance and gas in blood showed clear improvement over the course of the study, with a significant difference for the mean concentration of hydro carbonates ( $0.72 \pm 2.4$  mmol/l in group 1 and  $1.79 \pm 2.11$  mmol/l in group 2;  $p < 0.05$ ). The combined therapy of oxytetracycline with meloxicam achieved a significant better reduction of the severity of clinical symptoms, faster recovery, better performance and beneficial effects on functional parameters of the respiratory system when compared to a stand alone antibiotic therapy.

Funding: Boehringer Ingelheim Animal Health GmbH

544 (3407)

#### ANTIMICROBIAL RESISTANCE IN COMMENSAL RESPIRATORY PASTEURELLACEAE

Catry B., Feyen B., Opsomer G., Haesebrouck F., de Kruif A.

University Ghent, Faculty of Veterinary Medicine, Salisburylaan, 133, Ghent, BE-9000, Belgium

Most studies dealing with antimicrobial resistance in livestock are focused on the commensal enteric flora, while less attention has been paid to the commensal respiratory flora. Considering bovine enzootic pneumoniae, the presence of antimicrobial resistant Pasteurellaceae in the upper respiratory tract could cause therapy failure because of the facultative pathogenic character of these bacteria. The purpose of this study was to investigate the presence of antimicrobial resistant Pasteurellaceae within the nasopharynx of calves, with special reference to tetracycline resistance. A total of 57 apparently healthy calves aged 2 to 3 months and originating from 13 dairy herds were included in the study. After disinfection of the nostril with alcohol, a sterile swab was inserted in the ventral meatus, stored on dry ice for transport and further processed within 2 hours after sampling. Nasal swabs were plated directly onto two selective media: Columbia sheep blood agar with 16 µg/ml bacitracine and Columbia sheep blood agar with 16µg/ml bacitracine and 4µg/ml oxytetracycline. Identification of the isolates onto species level was done by fenotyping and molecularly confirmed by means of tDNA-intergenic spacer PCR. Susceptibility profiles towards common veterinary antimicrobial drugs of the identified Pasteurellaceae were performed by the Kirby Bauer disk diffusion test according to NCCLS guidelines. A total of 40 Pasteurellaceae were isolated in 34 calves out of 10 herds. Identification of the strains resulted mainly in *Pasteurella* (*P.*) *multocida* (n=35). Other bacteria were *P. canis* (1), *P. trehalosi* (1), *Mannheimia* (*M.*) *varigena* (2) and one untypable *Mannheimia* spp. None of the detected *Mannheimia* strains was identified as *M. haemolytica*. Antimicrobial resistance patterns of the strains identified as *Pasteurella multocida* (n = 35) revealed 100% susceptibility for ampicillin, amoxicillin+clavulanate, enrofloxacin, ceftiofur and the combination sulphonamides+trimethoprim. Five *P. multocida* isolates, all originating from the same herd, were resistant to tetracycline and 12 *P. multocida* strains were intermediate susceptible for tylosin. The remaining organisms were fully susceptible for the given antimicrobials with the exception of the three *Mannheimia* organisms that were intermediate resistant to tylosin.

In conclusion, overall antimicrobial resistance in commensal respiratory Pasteurellaceae from calves of dairy herds is low.

Funding: IWT-Flanders

545 (2480)

#### COMBINED ISOLATION OF CORYNEBACTERIUM PYOGENES AND FUSOBACTERIUM NECROPHORUM FROM CATTLE AFFECTED WITH PLEURISY

Haji Hajikolaei M.R., Ghoorbanpour M., Ghadiri A.R.

Shahid Chamran University, Faculty of Veterinary Medicine, Ahwaz, Khozestan, 61357-13793, Iran (Islamic Republic of)

A six old native cattle was referred to veterinary hospital of Faculty of Veterinary Medicine, Ahwaz. In the history, the onset was gradually and began 2 weeks ago with complete anorexia and marked drop in milk

yield at the time of examination. The temperature was 39.1°C, heart rate was 100/min and respiratory rate was 26/min. In the hemogram PCV, WBC, neutrophil, lymphocyte, monocyte and eosinophil were 25%, 12300/µl, 68%, 29%, 1% and 2% respectively. The animal was depressed and showed breathing distress. Dyspnea was evident particularly during inspiration and the animal stood with her elbows abducted. The respiration was shallow and movement of the thoracic was restricted. Pleuritic friction sound was heard over the thoracic wall at the left side. At the right side, the lower third space of the thoracic wall, splashing sound was heard with each cardiac cycle which extend to 9th intercostals space but respiratory sounds were not heard. The dull area was horizontal and pleural fluid was detected as anechoic by ultrasonography. There were bright echoes (gas echoes) swirling in pleural fluid. An abscess with marked wall in the lung was also revealed. In the right site pericard was normal and in the left site there was few amount of fluid around the pericard. Sample of the fluid was obtained through thoracocentesis in the 9th and 8th intercostals spaces at right site. The sample was turbid and had foulsmelling. It was cultured and corynebacterium pyogenes and fusobacterium necrophorum were isolated. Probably she had multiple lung abscesses and one of them ruptured and spread from the pulmonary parenchyma and caused secondary pleurisy.

Funding: Shahid Chamran University

546 (2526)

#### BACTERIAL PATHOGENS ASSOCIATED WITH RESPIRATORY DISEASE IN CALF REARING UNITS IN FINLAND

Pentikäinen J.1, Pohjanvirta T.1, Kuronen H.1, Rusanen H.2, Autio T.1, Härtel H.3, Aho P.4, Herva T., Pelkonen S.1

1National Veterinary and Food Research Institute, P.O. Box 92, Kuopio, 70701, Finland; 2National Veterinary and Food Research Institute, P.O. Box 45, Helsinki, 00581, Finland; 3LSO Foods Oy, Turuntie 4, Forssa, 30100, Finland; 4A-Farmers, Härkätie 4, Oulu, 90400, Finland

Respiratory disease is the major health problem in all-in-all-out calf rearing units in Finland. The calves originating from several dairy farms are brought to the rearing unit at 1-3 weeks of age. The average size of compartments in rearing units is 38 calves. A total of 20 calf rearing units with acute respiratory disease were examined to evaluate the bacterial pathogens associated with the disease. In each rearing unit lung lavage samples were taken from ten calves twice (3-4 weeks interval) resulting in 397 samples. The calves were categorised according to the clinical status into three classes (I-III): I clinical respiratory disease, II suspected respiratory disease, III normal clinical status. The samples were examined bacteriologically by standard laboratory methods. Detection of Mycoplasmas was performed using selective enrichment and plating, and further confirmation for *M. bovis*, *M. bovirhinis* and *U. diversum* by PCR. In total, bacterial pathogens and Mycoplasmas were isolated in 208 and 357 samples, respectively. Species detected were *M. bovirhinis* (50% of the samples), *P. multocida* (40%), *U. diversum* (40%), *M. dispar* (37%), *A. pyogenes*/Fusobacterium sp. (10%), *Pasteurella* sp. (9%), *Str. suis* (4%), *M. haemolytica* (3%) and *H. somnus* (0.5%). Fifty-six samples (14%) contained more than one bacterial species. None of the samples were positive for *M. bovis*. According to the clinical status, 37%, 22%, and 40% of the calves were classified into class I, II, and III, respectively, in the first sampling. In the second sampling, proportion of the calves in classes I and III (25% and 22%, respectively) was decreased and in class II increased (53%). Bacteria were detected in 60% of class I calves, whereas no specific bacteria were detected in 68% of class III calves. No association between clinical status and isolation of *M. bovirhinis*, and *M. dispar* was observed. *P. multocida* and *U. diversum* were more commonly isolated from class I calves (54% and 45%), than class III calves (21% and 27%), respectively. Similarly, *H. somnus* (n=2) was present only in class I calves and absent in class II and III calves. These results indicate that *P. multocida* and *U. diversum* are the bacterial pathogens most commonly associated with clinical respiratory disease in calf rearing units in Finland. Despite *M. bovis* has been shown to be one of the most important causative agents of bovine respiratory disease in several countries, in Finland no *M. bovis* was found in lung lavage samples.

547 (2748)

#### VIRAL RESPIRATORY INFECTIONS IN CALF REARING UNITS IN FINLAND

Rikula U.1, Rusanen H.1, Härtel H.2, Aho P.3, Herva T.4, Juvonen H.1, Huovilainen A.1, Sihvonen L.1, Pelkonen S.5

1National Veterinary and Food Research Institute, P.O. Box 45, Helsinki, FIN-00581, Finland; 2LSO Foods Oy, Turuntie 4, Forssa, FIN-30100, Finland; 3A-Farmers, P.O. Box 147, Kuopio, FIN-70101, Finland; 4A-Farmers, Härkätie 1, Oulu, FIN-90400, Finland; 5National Veterinary and Food Research Institute, P.O. Box 92, Kuopio, FIN-70701, Finland

A new fashion of rearing surplus calves of dairy farms for slaughter was introduced by abattoir companies in 1999. This rearing system aims at utilizing the full growing capacity of calves by providing them an optimal nutrition and environment. Calves are brought to all-in-all-out rearing units at age of 1-3 weeks and milk fed. Respiratory infections turned out to be the major health problem in these units although dairy herds in Finland are free of bovine herpes virus-1 and practically also of bovine viral diarrhoea virus infections. A number of agents have been associated with the infections; among these are parainfluenza-3 (PIV3), bovine corona (BCV) and bovine respiratory syncytial (BRSV) viruses. This study was set out to assess the complicity of these 3 viruses to the respiratory problems and the correlation of the symptoms with the agents.

A total of 18 calf rearing units were visited during an acute respiratory disease outbreak. Paired sera and lung

lavage samples from 10 calves per unit were taken at 3-4 week intervals. At the first visit all the animals in the same compartment were clinically examined and assigned to categories according to the body temperature, respiratory frequency, abnormal lung sounds, cough and nasal secretions. Antibody titres to the PIV3, BCV and BRSV were determined with indirect ELISA (SVANOVA, Sweden). The calves aged 1-8 weeks at the first visit and 45 %, 54% and 69% had antibodies against BRSV, BCV and PIV3, respectively, according to the cut-off-point suggested by the manufacturer. The level and dispersion of optical density values from calves healthy at both visits was taken to represent the background. A rise of over two standard deviations of the background was taken to indicate a true, and a rise over one sd a suspected infection.

According to paired serology PIV3 infection was detected in 8, BCV infection in 10 and BRSV infection in 8 units. Four units had PIV3 infection only and 3 units BCV infection only. Mixed infection with 2-3 viruses was found in 8 units. There was no evidence of these 3 viruses in 3 units and the symptoms were probably due to other infectious agents. BRSV and BCV detection with PCR from lung lavage samples agreed with the serological findings.

On average mixed virus infections caused more severe symptoms in calves than infections caused by a single virus. The importance of BCV is underlined. Indirect ELISA can be useful also in investigating calves with maternal antibodies.

Funding: MAF 4122/501/2001

548 (2532)

#### CLINICAL EFFICACY STUDY OF A 3% KETOPROFEN ORAL SOLUTION AS AN ADJUNCTIVE THERAPY TO ANTIBACTERIAL THERAPY OF BOVINE RESPIRATORY DISEASE (B.R.D.) IN FEEDER CALVES

Sabaté D.1, Homedes J.1, Almajano J.2, Oliveros J.A.2

1Veterinaria ESTEVE, Laboratorios Dr. Esteve S.A., Mare de Déu de Montserrat 221, Barcelona, 08041, Spain; 2PORTAVET S.A., Zaragoza nº 25, Binéfar, Huesca, 22500, Spain

In order to assess the clinical efficacy of a 3% Ketoprofen oral solution as adjunctive treatment to antibacterial therapy of Bovine Respiratory Disease (BRD) in feeder calves, a multicenter, controlled, randomized, blind clinical trial was conducted, in accordance to Good Clinical Practice current guidelines.

The study was performed in 350 feeder calves of different breed and sex, weighing between 81 and 248 kg and with a BRD-compatible clinical chart, selected from three different outbreaks. The animals were randomly distributed in two groups. Both groups received antibiotic treatment (two administrations of Florfenicol at 20 mg/kg, IM, q 48 h). Additionally, the animals of one group received 0.1ml/kg/d of a 3% Ketoprofen oral solution (DINALGEN® Solución Oral, Laboratorios Dr. Esteve S.A., Spain), equivalent to 3 mg/kg/d of ketoprofen, for 3 consecutive days, whereas the animals of the other group received equivalent quantities of a Placebo (excipients of the oral solution) for the same treatment period. Test substances were administered by oral route through drinking water. The experimental period of the study had a duration of 10 days, distributed as treatment administration (Days 1 to 3), clinical follow-up (Days 1 to 4) and possible relapse follow-up (Days 4 to 10). Daily clinical examinations consisted on the evaluation of the degree of disease by clinical index calculation and rectal temperature recording.

The base line values of clinical index, rectal temperature and animal weight were very homogeneous, thus allowing direct comparisons between both treatment groups. After the first day of therapy, average clinical index was significantly lower in the group treated with antibiotic + oral ketoprofen compared with the group treated with antibiotic + placebo (Day 2  $p < 0.001$ ; Day 3 and 4  $p < 0.05$ ). Likewise, mean rectal temperature was also significantly lower in the ketoprofen treated group (Days 2 and 4  $p < 0.001$ ; Day 3  $p < 0.05$ ). At the end of treatment, percentage of cured animals (defined by their clinical index) was significantly higher in the ketoprofen treated group (98% vs 86%;  $p < 0.001$ ). No relapses were seen in any group.

In conclusion, the results of this study show that oral ketoprofen, administered at 3 mg/kg/d as an oral solution through drinking water has good clinical efficacy as adjunctive treatment to antibacterial therapy of BRD in feeder calves, accelerating clinical recovery and further healing of the disease.

Funding: Laboratorios Dr. Esteve S.A.

549 (799)

#### SAFETY ISSUES OF RECOMBINATION BETWEEN GLYCOPROTEIN E DELETED MARKER VACCINE AND WILDTYPE STRAINS OF INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS

Thiry E.1, Schynts F.2, Meurens F.1, Muylkens B.1

1University of Liège, Faculty of Vet. Medicine, Virology, Bd de Colonstr 20, B43b, Liège, B-4000, Belgium; 2Centre d'Économie Rurale, Virologie Animale, rue du Carmel 1, Marloie, B-6900, Belgium

Control of infectious bovine rhinotracheitis (IBR), a disease caused by bovine herpes virus 1 (BoHV-1) is performed by intensive vaccination programs in countries which exhibit a high seroprevalence of the infection. In several European countries, glycoprotein gE negative live attenuated marker vaccines are used. They can be administered intranasally at the natural portal of entry of wild-type BoHV-1 virus. It is especially the case in young animals, where the risk of natural infection is high and where it is appropriate to overcome the interference by colostral antibodies.

It can be therefore postulated that situations of double nasal infections can be encountered in natural conditions. This can lead to the production of recombinant viruses. We have therefore examined three issues regarding the potential rise of such recombinant BoHV-1 viruses.

The rise of recombinant viruses was studied by in vitro and in vivo experiments. In cell cultures and in primary calf infections, co-infection with two distinguishable BoHV-1 led to the rise of high amount of recombinant BoHV-1, including recombinants with a gE negative phenotype. However, after reactivation from latency, no gE negative viruses, either parental or recombinant, could be reisolated from calves.

However, a situation of co-infection must be rare in natural conditions. We therefore examined the effect of superinfection on recombination. A time interval of 2 to 8 hours between two successive infections allows the establishment of a barrier, which reduces or prevents any successful superinfection needed to generate recombinant viruses.

The virulence of BoHV-1 obtained after recombination between a gE negative virus and a highly virulent wild-type strain was studied in vitro. Some of the obtained gE negative recombinants still exhibited a significant virulence by three different in vitro tests.

In conclusion, based on the weak likelihood of co-infections in natural conditions, and on the results obtained in our studies, recombination and its potential adverse consequences must be rare events. However, based on the virulence studies, a single recombinant keeping virulence and acquiring the gE negative genotype is enough to severely impair IBR control programs based on vaccination. Measures should be therefore taken to reduce the risk of recombination in the field.

Funding: Service public fédéral, FRFC

550 (3425)

#### A META-REGRESSION STUDY AIMING TO EVALUATE FREQUENTLY ANALYSED METABOLITES

Agenäs S.1, Heath F.2, Nixon R.3, Wilkinson M.2, Phillips C.4

1University of Cambridge and the Swedish Univ. of Agric. Sci., Kungsängen Research Centre, S-753 23 Uppsala, 753 23, Sweden; 2University of Cambridge, Farm Animal Epidemiology and Informatics Unit, Department of Clinical Veterinary Medicine, Cambridge, CB3 7HU, United Kingdom of Great Britain and Northern Ireland; 3University of Cambridge, 3MRC Biostatistics Unit, Institute of Public Health, University Forvie Site, Cambridge, CB2 2SR, United Kingdom of Great Britain and Northern Ireland; 4University of Queensland and Univ. of Cambridge, School of Veterinary Science, Gatton 4343, QLD, QLD, Australia

Evidence of suffering in cases of undernutrition in farm animals is currently a matter of subjective veterinary judgement, often based on assessments of the body condition of the animals. In addition to this, metabolic profiling is used. Objective measures of undernutrition are needed to assist in the determination of possible infringements of legislation by livestock farmers. The objective of the present study was to evaluate the value of frequently analysed metabolites as indicators of long-term undernutrition in cattle, by combining literature data. For this purpose a meta-regression study was performed.

The literature databases ISI Web of Science, PubMed and Vet/Beast CD 1973-1988 (CABI Silverplatter Information) were searched. The searches were performed in September 2002 and included all combinations of a set of the animal terms and a set of nutrition terms. 16082 references were identified. These were then searched for studies that matched the following criteria: 1) treatment periods at least 21 days 2) cattle at least 2 years 3) information about body condition score (BCS), body weight (BW) and/or change in BW (BWC). 4) dairy cows only post peak production or non lactating or had passed peak production. 5) reports in English or Scandinavian languages. There were only ten references that matched these criteria and included information about at least one plasma parameter. In addition to these ten references, three references that were already known to the authors, and that fulfilled the criteria, were included. The references provided sufficient information to perform meta-regressions on plasma glucose, urea, non-esterified fatty acids and beta-hydroxybutyrate with BCS, BWC and BW as true measures and the plasma metabolites as surrogates. Means and 95% credible intervals of the posterior distributions were used to summarise the results. The credible intervals for all of the overall gradients spanned zero. Therefore, it could not be shown that any of the tested metabolites is correlated with BCS, BW or BWC. However, the regressions against BWC showed a promising pattern and suggest that it may be possible to find a correlation across studies, with additional data. Furthermore, it is possible that a combination of BWC and BCS would improve the strength of the regression and give significant gradients. The data available for this study was not adequate to attempt such an analysis. It would be valuable to repeat this analysis when more data is available.

Funding: RSPCA and TetraLaval

551 (3193)

#### ABOMASAL DISPLACEMENT IN GERMAN SIMMENTAL (DEUTSCHES FLECKVIEH) CATTLE: 24 CASES IN A BAVARIAN VETERINARY PRACTICE FROM 1999 TO 2003

Berchtold J.1, Constable P.2, Prechtel J.3, Brunner B.4

1Veterinary Practice Obing, Germany, Bach 2, Bad Endorf, Bavaria, 83093, Germany; 2University of Illinois at Urbana-Champaign, Dept. of Veterinary Clinical Medicine, Urbana-Champaign, Illinois, 61801, United States of America; 3Veterinary Practice Obing, Haiming 4, Obing, Bavaria, 83119, Germany; 4Klifovet AG, Munich, Geyerspergerstr. 27, Munich, 80689, Germany

The German Simmental (Deutsches Fleckvieh, DFV) is a dual-purpose cattle breed with a population of approximately 1 million cows in Bavaria. Data on the signalment and incidence of displaced abomasum in this breed is not available. The objective of this study was therefore to characterize the epidemiology of left displaced abomasum (LDA), right displaced abomasum (RDA), and abomasal volvulus (AV) in DFV cattle

treated by a Bavarian veterinary practice.

This prospective study identified 30 cattle (24 DFV, 6 Holstein-Friesian, HF) in 22 herds with a diagnosis of DA obtained during a right flank laparotomy between October 1999 and 2003. The number of lactating and dry cows in each herd ranged from 12 to 80 ( $41 \pm 17$ ; mean  $\pm$  SD), with a total of 864 DFV and 31 HF adult cows. Seventeen herds contained only DFV cattle (647 adult cows); in these herds LDA was diagnosed in 13 adult cows and 1 late gestation first calf heifer, and RDA (n=1) or AV (n=7) was diagnosed in 6 adult cows, 1 three-month-old heifer calf, and 1 eight-month-old bull calf. The remaining 5 herds contained both DFV cattle (217 adult cows) and HF cattle (31 adult cows); in these herds LDA was diagnosed in 2 DFV and 6 HF cows. Deutsches Fleckvieh cattle with LDA (n=16) were  $5.0 \pm 1.8$  years old and adult cows had  $3.1 \pm 1.7$  calvings and were in early lactation (geometric mean days in milk=20, range 7 to 240 days). For comparison, DFV cattle with RDA or AV (n=8) were  $4.6 \pm 2.9$  years old, and adult cows had  $3.1 \pm 2.3$  calvings and were later in lactation (geometric mean days in milk=56, range 7 to 365 days). The mean annual milk production in DFV herds with at least 1 case of LDA ( $7,030 \pm 966$  kg) was numerically higher than that for DFV in Bavaria in 2001 (6,261 kg, n=763,682). Left displaced abomasum in DFV occurred throughout the year, with 4 cases in spring, 6 in summer, 3 in autumn, and 3 in winter. The relative risk of LDA in DFV cattle was markedly lower than that in HF cattle (relative risk=0.05; 95% confidence interval, 0.01-0.23). The average annual incidence of LDA over the 4-year period was 0.5% ( $[(16 \times 100) / (864 \times 4)]$ ) for DFV and 4.8% ( $[(6 \times 100) / (31 \times 4)]$ ) for HF cattle. We conclude that the epidemiology of LDA in DFV in Germany is similar to that reported for HF cattle in the United States, except that the incidence of LDA in DFV is much lower.

552 (2370)

#### REFERENCE VALUES AND SEASONAL VARIATIONS OF MILK CONSTITUENTS IN RELATION TO A PRIORI NON-DIETARY FACTORS IN BROWN SWISS COWS

Brülisauer F.1, Eicher R.2

1Federal Veterinary Office, Schwarzenburgstrasse 161, Bern, 3003, Switzerland; 2University of Bern, Bremgartenstrasse 109a Postfach 8466, Bern, 3001, Switzerland

Optimal milk yield and high health status in a dairy farm can only be achieved with adequate feeding. But how can inappropriate feeding be detected before metabolic diseases and milk yield drop occur? One possibility is the analysis of milk components. However, data about objective standards and reliable references for physiological values are limited. The goal of this study was to establish reference values of milk components and its seasonal variations in relation to chosen a priori non-nutritional factors.

The study included data from 1000 Brown Swiss farms in Switzerland over a period of two years. Dependent variables observed were milk fat, milk protein, milk fat-protein-quotient and milk urea. Factors investigated were geographic zone, average annual milk yield of the entire herd, individual daily milk yield, somatic cell count, and parity. Analyses have been done both at the individual cow level and at the herd level. To estimate the seasonal variation, these calculations have been repeated monthly over two years.

A negative relationship between daily milk yield and milk fat and milk protein could be observed. Reference values for this dilution effect were generated. In this context, it was remarkable that, in herds with high annual milk yield, cows generated higher milk fat and protein percentages over the year than cows in herds with lower annual milk yields. This points out that it is possible to achieve both a high production level and generate milk with high levels of protein and/or fat. The influence of the investigated factors on milk urea was only small, except for seasonal variations. In winter, however, more stable and higher urea levels were observed in high yielding herds than in low yielding herds. The wet summer of 1999 had a negative influence on milk protein, but on the other hand, the effect of this wet grown forage on milk components was only moderate in the following winter.

Funding: University of Bern, SBZV

553 (5045)

#### IMPACT OF TREATMENT WITH MONENSIN CONTROLLED-RELEASE CAPSULE ON ENERGY-RELATED BLOOD BIOCHEMICAL CONSTITUENTS

Cécyre D., DesCôteaux L., Brodeur M., DuTremblay D., Tremblay A.

Université de Montréal, Faculté de médecine vétérinaire, CP 5000, Saint-Hyacinthe, QC, J2S 7C6, Canada

In the context of a large post-approval randomized clinical trial on Rumensin CRC, blood samples were collected to determine the impact of treatment on energy-related blood constituents of lactating dairy cows. This study was performed on 730 cows from 38 Quebec herds. Cows were randomly assigned to receive a Rumensin CRC capsule (n=354) or a placebo capsule (n=376) between two and four weeks before the expected calving date. Two blood samples were taken for each cow: once between 2 and 4 weeks postpartum and again between 6 and 8 weeks postpartum. Blood analyses were performed on serum for the following biochemical constituents: glucose, urea, total protein, albumin, cholesterol and beta-hydroxybutyrate (BHB).

Herd means for serum BHB were calculated from the first blood samples taken from the placebo cows. Those means were ranked, and three groups of risk for ketosis were created based on quartiles. For the low-risk-for-ketosis (LRK) herds, herd BHB was the first quartile; for the medium-risk-for-ketosis (MRK) herds, between the first and third quartile; and for the high-risk-for-ketosis (HRK) herds in the fourth quartile. A linear mixed-effect model was applied to assess the effect of treatment on each biochemical constituents. Herd was

entered in all models as a random variable and the following variables were included in the models to control for their fixed effects: treatment, biochemistry number, herd risk for ketosis, cow's and herd levels of production, type of feeding, lactation number and body condition. Tests for BHB were performed on log transformation.

Serum glucose tended to be increased with treatment ( $p=0.13$ ). Interaction between herd risk for ketosis and treatment was significant ( $p=0.04$ ). Effect of treatment was more important on cows in HRK herds. Serum urea was also increased by Rumensin CRC ( $p=0.001$ ). The rise in serum urea with treatment was of greater amplitude in cows fed concentrates distributed by robot than for cows fed a component feeding program distributed manually or a total mixed ration ( $p=0.02$ ). Treatment reduced significantly BHB concentration ( $p<0.001$ ). The fall on BHB concentration with treatment was more pronounced at the first biochemistry evaluation ( $p=0.03$ ). Interaction between treatment and risk for ketosis was also significant ( $p=0.01$ ) in the later model. Cows in HRK and MRK herds had an important decrease of BHB concentration with treatment, but no effect was found for cows in LRK.

554 (891)

#### MONITORING SUBCLINICAL KETOSIS USING MILK STRIP TEST AND CONTROL CHART IN DAIRY HERDS

DesCôteaux L., Bélanger A.M., Brodeur M., Bouchard E.

Université de Montréal, Faculté de médecine vétérinaire, 3200 Sicotte, Saint-Hyacinthe, Québec, J2S 2M2, Canada

Subclinical ketosis (SCK) is a metabolic disease that has been associated with reduced milk production and increased risk of other metabolic disorders. A milk strip cow-side test (Ketotest, Elanco-Provel, Guelph, Ontario) has been shown to have a sensitivity of 91% and a specificity of 74% to detect SCK in dairy cows when using a cut-off value 100  $\mu\text{mol/L}$  of BHB. Statistical Process Control (SPC) and the use of Control Charts (CC) are tools that can be used in order to monitor health status in production medicine programs. Our objectives are to provide an overview of the SPC & CC and to present a practical application of this tool using milk Ketotest to monitor SCK in dairy herds.

The basic concept of CC is to distinguish between inherent random variation and real changes in measured performance. The CC has two axes. The horizontal axis is time and the vertical one is the base of a normal distribution of SCK prevalence. A horizontal line is drawn across the chart representing the target prevalence of SCK. Horizontal lines drawn at the target level plus or minus 3 SD of the prevalence are called the control limits. Additional lines are drawn at plus or minus 1 and 2 SD's. In our herd example, a single milk sample collection from all cows in the herd was taken once between 3 and 10 days in milk (DIM) and tested for BHB after collection using the Ketotest strips. Cows were classified as positive for SCK if the milk strip test changed color to the threshold level greater than or equal to 100  $\mu\text{mol/L}$ . Herd monthly prevalence of positive cows was plotted on the chart over time, and if the SCK prevalence was in control, then the plotted proportion was expected to fall within the control limits and lie on either side of the accepted proportion. In our example, a target monthly prevalence was set at  $0.30\pm 0.15$ . Decision rules to decide if the prevalence was in control were applied. The situation needed to be investigated if 1) 1 point was outside of the control upper limit (0.75); 2) 2 of 3 consecutive points lay between +2 and +3 SD's (0.60-0.75); 3) 4 of 5 consecutive points lay between +1 and +2 SD's (0.45-0.60); or 4) 8 consecutive points lay on the same side of the mean (over 0.30).

When the apparent prevalence of SCK is monitored over time using a CC, the information can be used as an alarm system in order to determine when to make a change.

555 (5071)

#### SELECTED ANALYTES IN BONE BIOPSY AND BLOOD OF FATTENED BULLS DURING SUBCLINICAL METABOLIC OSTEOPATHY

Doubek J.1, Bouda J.2

1University of Veterinary and Pharmaceutical Sciences, Faculty of Veterinary Medicine, Brno, 61242, Czech Republic; 2National Autonomous University of Mexico, Faculty of Veterinary Medicine, Av. Universidad 3000, CP 04510, Mexico-City, 04510, Mexico

Selected chemical analytes were determined in bone biopsies and blood of healthy fattened bulls and during subclinical metabolic osteopathy. The study was carried out on 83 bulls divided in two groups (Holstein x Czech Spotted Breed) aged 12-15 months with a body weight of 400 to 450 kg. Group I of bulls (control,  $n = 33$ ) were healthy animals proceeding from 3 herds with balanced rations and all animals in these herds were without clinical signs of rickets. Group II of bulls included animals without clinical signs of rickets ( $n = 50$ ) proceeding from 5 herds with frequent cases of clinical rickets (21% to 50%) in other animals. Ration of Group II was deficient in phosphorus. Blood samples ( $n = 83$ ) were collected from jugular vein 2-7 days before sending animals to the slaughterhouse. Samples of bone biopsies ( $n = 83$ ) were obtained from the tuber coxae of bulls at the slaughterhouse post-mortem. Selected biochemical analytes: blood acid-base values (pH,  $p\text{CO}_2$ , bicarbonate, base excess), ash weight/g bone spongy fat-free dry matter (BFFDM),  $\text{Ca}^{2+}$  and inorganic P in blood plasma and bone spongy ash were determined. Blood plasma concentrations of  $\text{Ca}^{2+}$ , inorganic P and blood acid-base values were not significant for the diagnosis of subclinical rickets. Significant difference ( $P < 0.01$ ) was found in bone biopsies regarding ash weight/g BFFDM in bulls of Group II in comparison with bulls of Group I. In bulls of Group II subclinical rickets was diagnosed as ash weight/g

BFFDM decreased. Chemical analysis of bone biopsies demonstrated the applicability on the diagnosis of subclinical metabolic osteopathies in fattened bulls.

556 (2664)

#### HYPERKETONAEMIA 3-5 WEEKS POST PARTUM IN ORGANIC AND CONVENTIONAL DAIRY HERDS Dredge K., Soveri T.

University of Helsinki, Pohjoinen pikatie 800, Saarentaus, 04920, Finland

Hyperketonaemia is a common condition of dairy cows in early lactation, defined as an increase of ketone bodies beyond normal levels in blood, milk and urine. It is caused by negative energy balance, leading to mobilisation of the fat reserves of the body and increased production of ketone bodies. Hyperketonaemia causes economical loss by decreasing milk production, and by predisposing to other diseases. The purpose of this study was to get preliminary information of the incidence of hyperketonaemia, and its association with some predisposing factors, in organic and conventional dairy herds in Finland.

From the province of South-Savo, a randomly selected sample of organic and conventional dairy farms were asked to participate. Altogether 10 organic and 13 conventional farms volunteered. Information was collected by interviewing the farmers, by making observation during two farm visits, and from the national herd health recording database. Farmers collected three milk samples, at weekly intervals, 3-5 weeks after calving, from all of the cows that calved between October 2001 and April 2002. All three samples were collected from 123 organic and 103 conventional cows. Milk acetone was detected by flow-injection-analysis-technique. Cows were divided into normal and hyperketonaemic, based on their highest acetone value, with the cut off value of 2.5 mg/100ml.

Incidence of hyperketonaemia was explored at herd level, the median being about 18% (conventional 17%, organic 22%). A great variability in the incidence was noted between individual herds (0-50% in organic, and 0-86% in conventional). Some predisposing factors were explored within the two production systems. The level and form of the lactation curve differed significantly between normal and hyperketonaemic organic cows. The difference in the mean energy corrected milk production between the ketonaemia groups in organic cows was non-significant, but a marked trend was noted, suggesting that hyperketonaemia can be more common among high producers. The incidence of hyperketonaemia was significantly lower in loose-houses. Results suggest that the variation in the incidence of hyperketonaemia is bigger between individual herds, than the production systems. Even though organic farms have some factors predisposing to hyperketonaemia, there can be some elements whose influence is preventive. However, it may be wise for organic farmers to favour moderate milk production while selecting cows for a herd.

Funding: Ministry of Agr. and Forestry

557 (3503)

#### RELATIONSHIP BETWEEN GAMMA-GLUTAMYL TRANSPEPTIDASE (GGT) SERUM ACTIVITY AND THE PRODUCTION OF LIVE EMBRYOS DURING SUPEROVULATORY TREATMENT IN DAIRY CATTLE

Dupras R.1, Chorfi Y.2, Tremblay A.2

145, Rang Saint-Edouard, Saint-Liboire, Quebec, J0H 1R0, Canada; 2University of Montreal, Faculty of Veterinary Medicine, 3200 Sicotte, Saint-Hyacinthe, Quebec, J2S 2M2, Canada

Clinic observations reveals relationship between live embryo production during superovulatory treatment and high GGT serum activity in dairy cattle exposed to mycotoxins. The aim of this study is to verify the relationship between the number of live embryos and GGT serum activity after superovulatory treatment for commercial production of embryos. The study related to 103 Holstein cows (76 lactating and 27 dried up) with an average age of  $6.6 \pm 1.5$  years and  $7.59 \pm 2.32$  respectively and subjected to a superovulatory treatment for embryos production. Uterine washing fluid allowed the counting of live and dead embryos and non-fertilized oocytes. During the embryo harvest, individual blood samples were taken to determine GGT serum activity. Results: lactating cows had an average of  $4.83 \pm 3.81$  transferable embryos among  $9.25 \pm 6.90$  of total embryos and GGT serum activity was  $29 \pm 10$  U/l. Dry cows had an average of  $4.67 \pm 4.47$  transferable embryos among  $7.74 \pm 4.92$  of total embryos and GGT serum activity was  $23 \pm 7$  U/l. There were no significant effects of age, lactational stage or milk production on the total embryo production or transferable embryos ( $p < 0.10$ ). In 25 cows giving less than 1 live embryo, GGT serum activity ( $27 \pm 8$  U/l) remained similar as in 7 cows giving more than 14 live embryos. In this group of 103 cows, GGT serum activity had no predictive value on the embryo production.

558 (5057)

#### CLINICAL RELEVANCE AND THERAPY OF FATTY LIVER IN COWS

Fürll M., Bekele H., Röchert D., Jäckel D., Delling U., Wittek Th.

Universitaet Leipzig, An den Tierkliniken 11, Leipzig, 04103, Germany

Problem: The fatty liver is the most important disturbance of liver function in dairy cows. According to literature it is not easy or impossible to cure a severe fatty liver. Therefore the fatty liver syndrome is one of the main reasons for cow loss especially after parturition. However, there are doubts about the significance of primary and secondary fatty liver.

Objective: We measured the liver fat content 1) in 35 healthy cows with different milk yield after calving, 2) in 100 slaughtered cows, 3) and the state of liver cell organelles in 10 cows 1 and 8 weeks after calving, 4) in 38

cows with abomasal displacement, 5) and therapeutic results in 40 cows with "severe liver damages". Results: 1) The liver fat content differed significantly between healthy cows with a milk yield of 4500 kg/a and 9500 kg milk/a 1 and 8 weeks after calving. 2) In the livers of 100 forced slaughtered cows the most frequent alterations were fat infiltration (66%), degeneration (16%) and re-active inflammation (29%). The highest liver fat contents were found in cows with nephritis (110 g/kg) and mastitis (92 g/kg). 3) Electron microscopically we did not find a relationship between alterations of cell organelles (mitochondrions and lysosomes) and liver fat content. However, the nucleus alterations correlated significantly with liver fat in cows with high as well as with low liver fat contents. 4) In 38 cows with abomasal displacement the liver fat content was always elevated; 37% had a simple fat infiltration, 13% a degenerative fatty liver and 29% necroses. All cows were cured independent of the liver fat content. 5) 40 cows of Leipzig Veterinary Medical Hospital with "severe liver damages" (AST > 200 U/l, GLDH > 100 U/l, Bilirubin > 50 µmol/l) were selected and studied with regard to therapeutic results and prognostic value of laboratory tests. The therapy was performed with continuous infusions of glucose (750 g/day), 0.9% NaCl, propylene glycol, antiphlogistics, furthermore in some cows antioxidants and glucocorticoids. 65% of these cows were cured, 35% of the cows had to be euthanized because of primary diseases with a poor prognosis (botulism, mastitis, muscle rupture, nephritis, peritonitis, ulcera or pulmonary thrombosis).

Conclusion: The fatty liver is the most frequent pathological liver alteration; however, the clinical significance is only moderate. Without a primary disease, a fatty liver had almost always a good prognosis. Decisive is a consequent and prognostic therapy.

559 (3435)

#### EARLY DETECTION OF METABOLIC DISEASES OF DAIRY CATTLE BY USING MILK DATA, BODY CONDITION AND METABOLIC PROFILES

Gelfert C.C., Staufenbiel R.

Klinik für Klauentiere, Königsberg 65, Berlin, 14163, Germany

Introduction: Metabolic diseases (MED) in dairy cattle reduce milk yield and fertility, and increase the economic losses of the farmer. Therefore, prevalence of MED should be low. Diagnostic methods of early detection of MED and their risk factors are needed. The following presentation describes our approach in monitoring MED in herd health management of dairy cattle.

Methods: Data of monthly milk control are used first or monitoring milk yield, and milk quality, and looking for weak points in milk production of the herd. Lactation curves are calculated each for cows of first lactation, second lactation and more than second lactation. Milk yield at beginning, time of peak and persistence of milk yield are compared with norm curves, calculated milk data of all cows in Germany for one year. Depression in milk yield at beginning of lactation is related to MED like ketosis in late dry period. Milk fat percentage is an indicator for ketosis and acidosis, but must be taken in account together with milk yield. Low protein in feed together with low energy content result in low milk protein percentage and high urea concentrations in milk. Body condition (BC) is the best parameter for energy balance in dairy cow. Body condition is calculated by measuring back fat thickness (BFT) by ultrasound. Body condition has been measured monthly to use changing of BFT in monitoring energy balance. Small changes of BC are easier detected by ultrasound than by Body-Condition-Scoring.

Metabolic profiles are the third component in monitoring MED. Serum and urine samples are taken from 10 cows each of five different stages of lactation. Early dry period, late dry period, 1st week of lactation, 3-5 week of lactation, 15-18 week of lactation. Serum samples are used for liver enzyme activities and monitoring supply of vitamins and minor elements. Urine samples are used for monitoring acid-base-equilibrium and supply of major elements.

Conclusions: Together with milk date and BFT, serum and urine profiles can detect safely MED and their risk factors.

560 (3233)

#### INFLUENCE OF STARVATION ON FERMENTATION IN BOVINE RUMEN FLUID (IN VIVO)

Hoehling A., Hoeltershinken M., Holsten N.B., Scholz H.

School of Veterinary Medicine, Clinic for Cattle, Bischofsholer Damm 15, 30173 Hannover, Lower Saxony, D-30173, Germany

Starvation in cattle is a frequent consequence of illness and further a consequence of longer transportation of the animal. As the consistent lack of energy is well documented, the impact on the fermentation in bovine rumen fluid is not known. The aim of this in-vivo-investigation was to look at the effects of starvation on the rumen fermentation. Five cattle (Deutsch-Schwarz-Bunt) were investigated for nine days. After a control period of three days, while they were fed with hay and concentrate, the animals were starved for three days. Afterwards, they were again fed with hay and concentrate. Twice a day, the influence on ruminal pH, ammonia, the concentrations of volatile fatty acids, potassium, sodium and chloride were investigated. The following effects of starvation on the rumen fluid were noted: pH: +14,9%; ammonia -74,2%; volatile fatty acids: -65,4%; potassium: +672%; sodium: +105%; chloride: -29,5%.

In summary it may be said that the fermentation in the bovine rumen is clearly decreased during starvation. Furthermore, one day after the end of the period of starvation, when hay and concentrate are fed again, the rumen of a healthy animal is able to compensate the three-day-long impact of starvation. In ill animals and

after a longer period of starvation, however, it is rather to expect a delayed recovery of the ruminal fermentation. A working fermentation, though, is important to prevent aftereffects such as ketosis. Therefore it should be considered to support the recovery of the cattle by replacing rumen fluid from a healthy animal. Further investigations on the impact of starvation on the rumen fermentation in ill cattle are carried out.

561 (5018)

#### ULTRASOUND MEASUREMENT OF RUMP FAT THICKNESS AS A MONITORING TOOL FOR POSTPARTUM METABOLIC DISEASES IN HOLSTEIN COWS

Joshi N.P.1, Herdt T.H.1, Neuder L.2

1Michigan State University, Dept. Large Animal Clinical Sciences, East Lansing, MI, 48824, United States of America; 2Green Meadows Farm, Inc, Michigan State University, East Lansing, MI, 48824, United States of America

Ultrasound measurements of Backfat thickness (BFT) and Rump-fat thickness (RFT) in live animals are extensively used in monitoring carcass quality in the beef industry. Rump fat thickness and BFT are positively correlated with sub-dermal fat mass. Changes in RFT reflect changes in energy metabolism. The objective of this study was to determine the relationship of prepartum RFT to postpartum metabolic disease risk in dairy cows.

**Materials and Methods:** Forty multiparous Holstein cows were used in this study. They were group fed a totally mixed ration ad lib. Ultrasound images of RFT were captured weekly at a point 2" cranial to the tuber ischi using a portable Sonosite-180. Blood was sampled weekly from 3 weeks before calving to 4 weeks after calving to determine plasma nonesterified fatty acid (NEFA) and beta-hydroxy butyrate (BHB) concentrations. A logistic regression model was formulated using a "step-down" technique. Dystocia, RFT, parity, days from parturition at the time of sampling, and twin or single birth were the initial independent variables. Dependent variables were total morbidity, ketosis, metritis, and displaced abomasum (DA). Variables were eliminated from the model when p values were > .25.

**Results:** Mean RFT and NEFA the week before calving were  $0.75 \pm 0.09$  cm and  $0.24 \pm 0.03$  mEq/L respectively. Mean BHB 1 week after calving was  $14.97 \pm 2.17$  mg/dl. Of the 40 cows studied, 15 developed ketosis, 5 metritis, 5 mastitis, and 1 DA. Odds ratios for the effect of RFT on total morbidity and ketosis were 3.01 ( $p=.1$ ), and 3.65 ( $p=.05$ ). This suggests that for a 1 cm increase in RFT during the week before calving there is 3 fold increase in risk of total morbidity and 3.6 fold increase in the risk of ketosis.

**Conclusions:** Monitoring RFT, 1 week before calving may be a reliable method of predicting risk of peripartum diseases, especially ketosis in dairy cows. RFT measures were strongly associated with ketosis. No association of metritis and DA could be established to RFT.

**Funding:** Michigan State University

562 (2875)

#### CENTRAL CHOLECYSTOKININ-OPIOIDERGIC SYSTEMS INTERACTION IN THE MODULATION OF RUMINANTS GASTRO-INTESTINAL MOTILITY

Kania B.F., Matczuk J., Debski B.

Agricult. Univ., Fac. Vet. Med., Department of Physiological Sciences, Nowoursynowska 159, Warsaw, 02-776, Poland

**Introduction:** Since 1992 in many publications it has been suggested that cholecystokinergic system participate in opioid effects on gastro-intestinal tract (GIT) motility and feeding behavior of monogastric animals (1,2). The purpose of this study was to evaluate  $\mu$  and  $d$  opioid receptors agonists or antagonists on GIT motility and to study cholecystokinin (CCK) receptors role in modulation of opioid action.

**Material and methods:** Non-lactating female ewes ( $n=12$ ) were surgically treated by placing of electrodes in the muscular layer in 6 different GIT locations and permanent stainless steel canula was inserted into cerebral ventricle (3). Specific  $\mu$  [D-Ala<sup>2</sup>, D-Leu<sup>5</sup>]-CENkephalin (DAGO) and  $d$  opioid receptors agonist [D-Pen<sup>2,5</sup>]-Enkephalin (DPDPE) were used at doses 5-100 ng/kg BW. Naloxonazine and naltrindol were used in doses 1-5  $\mu$ g/kg BW, as specific antagonists of  $\mu$  and  $d$  opioid receptors, respectively. Experiment was repeated but prior to opioid receptors modulators animals were given icv CCK1 receptors antagonist devazepide (L364.718) or CCK2 (L 365.260) at dose 1-2  $\mu$ g/kg BW.

**Results:** DAGO and DPDPE stimulated migrating myoelectrical complex (MMC) duodenal frequency and inhibited myoelectrical activity of the stomach and large intestine. Naloxonazine and naltrindole icv infusion given 10 min prior to DAGO and DPDPE prevented this inhibitory effect. The CCK1 antagonist treatment prevented the modulatory effects of DAGO and DPDPE on myoelectrical activity of GIT.

**Discussion:** Based on obtained results of myoelectrical activity of ewes stomach, small and large intestine it seems that  $\mu$  and  $d$  opioid receptors are involved in the central modulatory action of opioides. CCK receptors antagonist protect the modulatory opioid effects on myoelectrical activity. It is possible that opioid receptors stimulation cause CCK release from the some cerebral neurons.

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563 (3218)

#### EFFICACY OF HERB PAHARI PUDINA (MENTHA SPICATA) IN INDIGESTION OF CATTLE

Kumari S.1, Prasad B.2

1HPAU, COVAS, Dept Animal Husbandry, Govt. of Himachal Pradesh, c/o Dept. Vet Medicine, Palampur, Kangra, Himachal Pradesh, 176 062, India; 2HPAU, COVAS, Dept. Vet. Clinical Medicine, Ethics & Jurisprudence, Palampur, Kagra, Himachal Pradesh, 176 062, India

Purpose: Indigestion is very common in bovine, limiting the production and economics of the farmers. The allopathic approaches for its correction are with side effects and not economical. There appears global resurgence of interest for application of population knowledge of herbal medicines. Accordingly, pahari pudina (*Mentha spicata*), a common glabrous herb of 30-90 cm height, which grows luxuriantly in the area and known to have stimulant, carminative and antispasmodic properties in human, was considered for its efficacy in indigestion of cattle.

Methods: Fifty-nine adult crossbred cattle of both sexes, suffering from simple (16 cattle, 27.14%), alkaline (13 cattle, 25.71%) and acid (30 cattle, 47.41%) ruminal indigestion, were drenched with dry whole-plant powder of pahari pudina @ 200mg/Kg body weight for three consecutive days.

Summary: Clinical parameters comprising body temperature, respiration rate, ruminal motility, faecal consistency, rumen fluid pH, protozoal count and motility, glucose fermentation test, sedimentation activity time and blood values restored to normal within 24-72 hr of medication.

Conclusions: Based on the observations, pahari pudina (*Mentha spicata*) was found to have good therapeutic efficacy in correcting simple, alkaline and acid indigestion in cattle.

564 (940)

#### EVALUATION OF MILK UREA NITROGEN IN BULK TANKS OF DAIRY FARMS OF MINAS GERAIS STATE, BRAZIL

Machado P., Corassin C., Cassoli L., Coelho K.

Universidade de São Paulo - ESALQ, Av. Pádua Dias, 11 CP 9, Piracicaba, São Paulo, 13418-900, Brazil  
Several studies have shown the importance of the protein nutrition and high productive performance of dairy cows. As well as the deficiency, feeding excess amounts of protein in the diet may be detrimental to the herd productivity. Milk urea nitrogen concentration (MUN) has been proposed and can be used as a tool to monitor dietary protein efficiency and dietary protein energy balance in dairy cows. The purpose of the present study was to evaluate the MUN concentrations in the bulk tanks of dairy herds of Minas Gerais State - Brazil. In a period of 10 months, 3044 samples were analyzed from 410 farms (bulk tanks). The average of MUN was 8,8 mg/dL, with a standard deviation (SD) of 3,7 mg/dL and a variation coefficient (VC) of 41,9%. How literature suggested values, considered ideals for MUN, should vary between 10 and 14 mg/dL, the samples were divided in three classes: Low MUN (LM): 2-10 mg/dL (65,3%), ideal MUN (IM) 10-14 mg/dL (25,4%) and high MUN (HM) 14-25 mg/dL (9,3%). The medium values, DP and CV for each one of the classes were: 6,6 mg/dL, 1,9 mg/dL and 29,4% for LM, 11,6 mg/dL, 1,1 mg/dL and 9,7% for IM and 16,4 mg/dL, 2,13 mg/dL and 13% for HM. The largest percentage of the farms presented values of MUN lower than the considered ideals, possibly due to protein and energy deficiency in the diets.

Funding: FAPESP

565 (2610)

#### METABOLIC PROFILE ANALYSES IN UNITED KINGDOM DAIRY HERDS: 1998-2003

Macrae A., Whitaker D., Burrough E., Dowell A., Kelly J.

University of Edinburgh, EBVC, Dairy Herd Health and Productivity Service, Division of Veterinary Clinical Studies, R(D)SVS, Easter Bush, Roslin, Midlothian, EH25 9RG, United Kingdom of Great Britain and Northern Ireland

Metabolic profiles are the quickest and most accurate way of asking "what the cows think" of their diet and its management, after forage analysis and ration planning has set up the theory of the ration. They are an integral part of modern dairy herd productivity and preventative medicine programmes. Properly planned metabolic profiles can identify constraints before changes in productivity appear. The correct timing and selection of cows and the use of background information are critical to success. The Dairy Herd Health and Productivity Service has been analysing blood samples for metabolic profiles in dairy cattle for over 25 years in the UK (Kelly, J.M. and Whitaker, D.A., 2000. A multidisciplinary approach to dairy herd health and productivity management. *British Society of Animal Science Occasional Publication*, No. 26, 1, 209-222). During the period April 1998 to March 2003, blood samples were received from an average of 6968 cows annually (range 6490 to 7448 cows). 62% of early lactation cows (EL; 10-20 days post partum) and 31% of mid lactation cows (ML; over 30 days post partum) had energy problems as demonstrated by beta-hydroxybutyrate (BHB) values above 0.9 mmol/l, plasma glucose values below 3.1 mmol/l and/or non-esterified fatty acid (NEFA) values above 0.6 mmol/l. 58% of dry cows (7-10 days pre-partum) also had energy constraints, as shown by BHB values over 0.5 mmol/l, plasma glucose values below 3.2 mmol/l and/or

NEFA levels above 0.4 mmol/l. These results illustrate how widespread energy constraints are, including the late pregnancy period which should be utilised for maintenance and restoration of resources in preparation for the next lactation.

12% of EL, 6% of ML and 18% of dry cows had urea-N values below 1.8 mmol/l, indicating an inadequate intake of Effective Rumen Degradable Protein.

Low magnesium levels (below 0.8 mmol/l) were identified in 8% of EL, 4% of ML and 13% of dry cows.

Inorganic phosphate levels below 1.4 mmol/l were recorded in 3% of all cows.

All of the cows sampled had been fed rations planned and formulated according to requirements. These findings show that ration formulation should only be considered to be the theoretical first step, and that it is vital to carry out metabolic profile testing in order to check how well the diet is working in practice. These results demonstrate that the management of dairy cow nutrition does not always live up to the accuracy of its planning.

566 (2300)

#### SUBCLINICAL RUMEN ACIDOSIS (SRA) IN ITALIAN DAIRY HERDS: PRELIMINARY FIELD SURVEY

Morgante M.1, Stelletta C.1, Tacchio G.1, Lettig I.1, Berzaghi P.2

1Dipartimento di Scienze Cliniche Veterinarie, Viale dell'Università 16, Legnaro (PD), 35020, Italy;

2Dipartimento di Scienze Zootecniche, Viale dell'Università 16, Legnaro (PD), 35020, Italy

Subclinical rumen acidosis (SRA) as well known as subacute rumen acidosis (SARA) represents one of the most common disorder in the intensive dairy farms that affects ruminal fermentations, animal health, productivity and farm profitability. The aim of the present study was to verify the presence of SRA in some Italian dairy herds where the condition was suspected, and to determine the possible causes. Five commercial dairy herds suspected of SRA were investigated because of high incidence of laminitis, metritis and culling rate for various pathological conditions. Twelve cows in each herd were selected randomly among animals without clinical signs of disease, good body condition, and between 40 to 170 DIM to perform rumenocentesis, urine and blood samples. The visit to each herd was organized to obtain the rumen fluid samples 5-8 hours after feeding and in coincidence with the monthly individual control of milk (somatic cell, fat, lactose and protein) parameters. Rumen fluid and urine pH were determined immediately after sampling. Concentration of SCFA in ruminal fluid were determined on the stored samples (-80°C). Blood samples were used to determine BUN concentration. TMR and residuals at sampling time were analysed for FM, CP, NDF and Starch. The results indicated the presence of SRA in three herds (n° cows with rumen pH < 5.5 more than 33%) and a critical situation in the others (cows with rumen pH < 5.5 less than 33% and cows with rumen pH between 5.6 - 5.8 more than 33%). High concentration of SCFA can be at the base of a pathogenic rumen acidosis because there were found negative correlation between rumen pH and the levels of lactate ( $r = -0.388$ ;  $P < 0.01$ ), acetate ( $r = -0.706$ ;  $P < 0.01$ ) and propionate ( $r = 0.733$ ;  $P < 0.01$ ). It was not possible to understand the exact causes of the condition that not appear to be related with the diet formulation. Animal management seems to be an important factor in developing this condition. Finally, interesting positive correlation was found between somatic cell count and n-valerate rumen levels ( $r = 0.778$ ;  $P < 0.01$ ).

Funding: Statal funds

567 (3464)

#### DIAGNOSIS OF FATTY LIVER BY ULTRASONOGRAPHY

Nichols S., Babkine M., Desrochers A.

Université de Montréal, Faculté de médecine vétérinaire, 3200 Sicotte, Saint-Hyacinthe, Québec, J2S 7C6, Canada

Fatty liver is frequently diagnosed in overweight and high producing dairy cows. Liver biopsy is the only reliable technique use to diagnose fatty liver on live animals. The objective of this study was to evaluate ultrasound examination as a diagnostic tool in cattle affected with fatty liver. The liver of 21 Holstein cows was scanned with an Aloka 1700 ultrasound unit equipped with a 3.5 MHz sectorial probe. The number of hepatic veins and portal veins, the angle of the edge of the liver and the comparison between the echogenicity of the liver and the spleen were used to assess fatty infiltration. Scanned livers were classified as normal, mildly, moderately or severely infiltrated. A needle aspiration biopsy was then performed on each animal for histopathologic evaluation. Out of the 21 subjects, 5 were diagnosed as normal and 16 with a certain degree of infiltration by ultrasound (9 mild, 4 moderate and 3 severe). Twelve animals had the same classification based on biopsy and ultrasound evaluation. Three out of 4 were correctly diagnosed as being severely infiltrated and 5 out of 6 as being normal. Echogenicity was the most reliable criteria ( $\kappa = 0.4$ ). The number of vessels and the angle were less reliable with a  $\kappa$  equal to 0.34 and 0.25 respectively. The ultrasound evaluation encompassing all of the above mentioned criteria had a  $\kappa$  of 0.41. The sensitivity and specificity of the ultrasound evaluation to distinguish an infiltrated liver from a normal one were 100% and 83% respectively. The number of pixels on the spleen (reference value) and liver were measured. Ratio was then calculated. A linear model was use to try to establish a relationship between the ratio and the degree of fat infiltration. The results were not significant. In conclusion, we find that the ultrasound evaluation of the liver is a reliable diagnostic tool. However, it is of limited value in the field and for inexperienced users.

568 (2450)

**RISK FACTORS IN HERDS WITH LOW AND HIGH KETOSIS INCIDENCE IN NORWAY**

Østeras O.1, Nordløy H.2

1TINE Norwegian Dairies BA, Pb 58, Ås, N-1431, Norway; 2National Veterinary Institute, Trondheim, Tungasletta 2, Trondheim, N-7047, Norway

Ketosis is the second most frequent recorded disease treatment in the Norwegian Health Card System. During 1994 the incidence rate was 0.14 and in 2002 reduced to 0.059 treatments per cow-year. The aim of this study was to identify risk factors with different occurrence in herds with very low compared to very high incidence of ketosis in Norway.

Herds were grouped according to the mean incidence during 1992, 1993 and 1994. The herds have to be motivated for the project, the same herdsman and the same stall for the last three years as well as having tied stalls and no automatic feeding of concentrate.

The field study was done during 1996/97 and the herdsmen were interviewed on feeding management, harvesting and conservation methods, use of concentrate and pasture. Body condition scoring was performed every month during one year. Altogether 71 herds fulfilled the study. There were 23 herds in the low and 48 herds in the high incidence group.

Three risk factors differ between the two groups of herds. The cows in the high incidence group had an odd ratio (OR) of 3.25 having body condition score > 3.25 during the period 60 to 30 days prior to calving ( $P < 0.001$ ), they had an OR 2.65 of not having roughage on the feeding board at morning ( $P < 0.01$ ) and an OR of 1.77 of not practise two step harvesting (drying the roughage) before conservation ( $P = 0.08$ ).

The estimated population attributable fraction (PAF) for cows having ketosis showed different distributions of the risk factors within the two groups of herds. For the group with high incidence the PAF was highest for not having roughage on the feeding board at morning (25%), then high body condition score during 60 to 30 days period before calving (19%) and lack of two level harvesting (16%). For the herds with low incidence the PAF was 5%, 9% and 8%, respectively.

The conclusion is that the ranking of the risk factors are different in the two groups and this should be taken care of when given advice. The most important advice for high incidence herds is to give more access to roughage and for low incidence herds to avoid fat cows during early dry period.

Funding: Agricultural dep. of the county Nord-Trøndelag, Regional veterinary authority in Trøndelag, Geno, TINE BA, BU-funding in Nord-Trøndelag, Norwegian Research Council 569 (2939)

**COAGULATION TESTS AND SELECTED BLOOD BIOCHEMICAL ANALYTES IN DAIRY COWS WITH HEPATIC LIPIDOSIS**

Padilla S., Bouda J., Constantino F., Núñez L.

UNAM, Faculty of Veterinary Medicine, Av. Universidad 3000, Col. Copilco, Coyoacan, Mexico-City, 04510, Mexico

Hepatic lipidosis (HL) is most common in periparturient dairy cows. The majority of the proteins that act in the clotting cascade are synthesized by the liver, therefore the determination of optimised prothrombin time (PT) and partial thromboplastin time (PTT) can help to detect liver failure problems in their initial stages. The aim of this study was to determine the values and changes in conventional and optimised clotting tests, as well as those of selected biochemical analytes during HL in postpartum dairy cows. Twenty Holstein cows within 30 days postpartum from two commercial dairy farms in central Mexico were selected based upon their clinical history, physical examination, liver biopsy, flotation test of liver tissue (in two  $\text{CuSO}_4$  solutions and water with various densities) and divided into a Control group ( $n = 10$ ) without clinical signs of illness, and an Experimental group ( $n = 10$ ) presenting HL. Blood was drawn from the caudal vein. Prothrombin time and PTT were determined in total plasma (100%) and in two subsequent dilutions (50%; 25%) using isotonic NaCl solution. Selected biochemical analytes: free fatty acids (FFA), aspartate aminotransferase (AST), gamma-glutamyl transferase, total protein, albumin, urea, total bilirubin, triglycerides and creatine kinase in blood serum and haemogram were determined. To corroborate the diagnosis of HL, a histological analysis of the liver samples was carried out and confirmed the findings that had been diagnosed by flotation tests. The histological analysis for the Experimental group showed seven cows to be in Grade 2 (moderate lipidosis), the remaining three were classified as Grade 3 (severe lipidosis). The Control group presented normal results by the flotation test and in the histological analysis. Optimised clotting test times were prolonged in animals with HL and there was a PT difference at both 50% and 25% plasma dilutions between both groups ( $P = 0.004$  and  $P = 0.001$ ). Significant differences were also observed in FFA, AST and triglycerides,  $P = 0.001$ ,  $P = 0.007$  and  $P = 0.044$ , respectively. The use of optimised PT allows for the detection of alterations that cannot be appreciated using conventional PT.

Funding: University project

570 (914)

**SURGICAL TREATMENT OF LEFT ABOMASAL DISPLACEMENT - PARAMEDIAN ABOMASOPEXY - MODIFIED APPROACH**

Podpecan O.1, Zemljic B.2

1Veterinary practice, Celjska c. 3a, Zalec, 3310, Slovenia; 2Veterinary polyclinic, Ljutomerska c. 25, Ormoz, 2270, Slovenia

Twenty-one dairy cows Holstein-Friesian breed, with left abomasal displacement (LDA), from 15 different

farms were surgically treated by using paramedian abomasopexy. Abomasal wall (serosis, I. muscularis and part of submucosis) was sutured together with abdominal wall (peritoneum, internal rectus sheet, rectus muscle, external rectus sheet and fascias) in a simple continuous pattern, subcutaneous tissues separately also. Skin was closed with interrupted "U" pattern.

16 cows were treated during first 24 hours, 4 cows during 48 hours and one cow four days after manifestation of clinical signs. In average LDA was diagnosed on a day 13 post partum (min. 2nd day - max. 30th day). Animals were surgically treated inside 12 hours after diagnosis was made.

In 20 cases (95 %) surgical procedure succeeded. One animal (5%) was culled on the same day, because of abomasal ulcerations and perforation diagnosed during surgery. Four animals (19%) were culled in a year because of reproduction or production problems.

17 cows (81 %) restored normal milk production, got pregnant and started next lactation.

This modified method is quick and efficient; animals were placed in dorsal recumbence for 20 -25 minutes. Only one type of suture material (Polysorb® Duble No. 2) was used to fix abomasum and to close abdominal wall. For skin closure non-absorbable No.4 material was used. The efficiency of the method is better then classical ones and comparable to similar studies.

571 (1333)

#### ABOMASAL DISPLACEMENT ON A STALL BARN DAIRY FARM IN IRAN

Rahimi M.

Razi University, College of Veterinary Medicine, P.O Box: 1451, Kayhanshahr Ave., Kermanshah, Kermanshah, 67145, Iran (Islamic Republic of)

All cases with abomasal displacement occurred in a stall barn dairy farm (Ravansar Dairy Farm, Iran) over a 5-year period (February, 1996 to March, 2001) were investigated in this study. The herd consists of 500 black and white Friesian cows. The cows were fed corn silage (15-16 kg/cow/day), commercial concentrates for cattle (12-14 kg/cow/day) and alfalfa hay (4-5 kg/cow/day).

Over the 5-year period all suspected cases of displaced abomasums (DA) were presented to the Veterinary clinic of the farm. Physical examinations were performed and the cases of DA were confirmed at the time of surgery. Since the herd history was available, incidence rates by the age-group and calving status could be analyzed.

During five years, the farm encountered 108 cases of DA. Eighty out of 108 cases (74.1%) were diagnosed as left displaced abomasums (LDA) and 28 cases (25.9%) as right displaced abomasums (RDA). The incidence rate for LDA was significantly higher than that for RDA. The mean annual herd incidence of DA was 4.6% (3.4% LDA and 1.2% RDA).

Thirty cases (27.8% of DA cases) were diagnosed within the first 2 weeks post partum, 27 cases (25.0%) within 2-4 weeks post partum, 13 cases (12.0%) within 4-6 weeks post partum and 18 cases (16.7%) after 6 weeks post partum. Twenty cases (18.5% of DA cases) were pregnant. Higher incidence of DA was diagnosed among the cows after the fourth lactation. In pregnant cows, the occurrence of DA was higher in second trimester, but the difference was not statistically significant.

The cause of DA in cattle is multifactorial. A prerequisite for the development of DA is abomasal atony or hypotony. Abomasal atony can result from limited exercise, endotoxemia, histamine release and consumption of a diet low in crude fiber or high in concentrates.

It is thought that contributory factors to the high incidence of DA on this farm are limited exercise, the feeding of poor quality roughage, low fiber or high concentrate ration, and histamine release. Since relatively high occurrence of metritis, mastitis, arthritis, digital dermatitis and phlegmon is common in the population being studied, increased histamine that arises from tissue breakdown may be an important etiological factor.

Funding: Razi University

572 (638)

#### MONTHLY AND SEASONAL VARIATION IN MILK PLASMA MAGNESIUM CONCENTRATION IN FRIESIAN DAIRY HERDS IN URMIA

Ramin A.G., Asri-Rezaie S., Salamat J.

Urmia University, Veterinary college, Urmia, West Azarbyjan, 0091-0441, Iran (Islamic Republic of)

Monthly and seasonal variation in milk plasma magnesium (Mg) concentration between industrial and semi-industrial dairy herds was investigated in Urmia in years 2002-3. 1112 milk samples from 96 herds including 53 industrial with 615 samples and 43 semi-industrial dairy herds with 497 samples were selected. 10 mls milk was taken from each herd when they were delivered to milk factory. Milk samples prepared monthly up to 12 months. Milk fat separated by cooling and centrifugation methods. Milk plasma was separated after casein precipitated by HCl in pH 6.4. Spectrophotometer method was used to measure milk Mg concentration using Mg kit (Ziest Chimi). Mean  $\pm$  SD milk plasma Mg concentration for all samples during a year in industrial and semi-industrial dairy herds was  $5.47 \pm 1.15$  and  $5.35 \pm 1.15$  mmol/l, respectively, which was significantly different ( $P < 0.05$ ). The highest mean monthly Mg concentration in industrial and semi-industrial dairy herds was 6.39 and 6.05, and the lowest was 4.29 and 4.02 mmol/l, respectively. The range was 1.32-10.1 and 0.02-9.03 mmol/l, respectively. The minimum and maximum mean monthly milk Mg concentration in industrial and semi-industrial dairy herds were observed in September and December, respectively. The concentration decreased from March to September and from then increased gradually up to December. The monthly

comparison of the mean milk Mg concentration between industrial and semi-industrial herds showed differences only in September ( $P < 0.05$ ) and December ( $P < 0.01$ ). The lowest and highest mean seasonal milk Mg concentration in industrial (4.61, 5.79 mmol/l) and semi-industrial herds (4.53, 5.71 mmol/l) was observed in summer and winter, respectively. The seasonal comparison of the mean milk Mg concentration showed differences ( $P < 0.05$ ) between summer and other seasons. Thus, it can be concluded that milk Mg concentration in industrial and semi-industrial dairy herds (except in 2 herds) were at the recommended values for dairy herds. Secondly, milk Mg concentration in industrial was higher than in semi-industrial dairy herds. The lowest milk Mg concentration was observed in September and summer in Urmia dairy herds; that could be important to consider and prepare macro-minerals in dairy food.

Funding: Urmia University

573 (2542)

#### RELATIONSHIP BETWEEN CLINICAL AND LABORATORY PARAMETERS IN COWS WITH DISPLACED ABOMASUM AND THEIR RECOVERY AFTER RIGHT FLANK OMENTOPEXY

Rohn M.1, Tenhagen B.A.2, Hofmann W.3

1Landesamt fuer Verbraucherschutz u. Landwirtschaft, Ringstrasse 1010, Frankfurt/Oder, Brandenburg, D 15236, Germany; 2FU Berlin, Tierklinik fuer Fortpflanzung, Koenigsweg 63, Berlin, D 14163, Germany; 3FU Berlin, Klinik fuer Klautiere, Koenigsweg 65, Berlin, D 14163, Germany

The objective of this study was to analyze the relationship between clinical findings prior to right flank omentopexy and survival in cows with abomasal displacement (DA). Data from 564 animals with DA (466 left DA, 98 right DA) between 01.01.1994 and 31.12.1997 were analyzed.

Cure rates were higher for cows with mild cases of LDA (89.6%) than in moderate to severe cases (81.7%).

Cure rates were lower in RDA cows with abomasal torsion (70.3%) than in RDA cows without torsion of the abomasum (87.5%). When omasum or reticulum were involved in the volvulus, cure rate dropped to 40%.

Only 14% of RDA cows survived abomasotomy to empty the abomasum to facilitate reposition.

LDA was most frequent in the postpartum period, whereas incidence of RDA was higher later in lactation.

More cows with RDA than with LDA were pregnant. Cows affected by RDA later in lactation had a better prognosis than those affected in earlier stages.

Cows with LDA often had a longer duration of disease and concurrent diseases like endometritis, ketosis or claw diseases were diagnosed more frequently. Ketotic cows with LDA had a higher cure rate than non ketotic animals. Animals with diarrhea or no faeces in the rectum and poor appetite prior to surgery had lower cure rates. Cows with RDA more frequently had diarrhea or no faeces in the rectum, a lower rectal temperature, higher heart- and respiratory rate and decreased feed intake prior to surgery than did animals with LDA. In these animals higher heart rates, along with poor general attitude and poor appetite prior to surgery were associated with a poorer prognosis.

Cows with LDA had higher bilirubin, sodium, chloride and hemoglobin concentrations than cows with RDA.

Animals that died or were euthanized showed higher AST, bilirubin and urea concentrations as well as increased hemoglobin, erythrocyte and hematocrit values, i.e. characteristics of hemoconcentration and lower sodium concentrations. Cows with RDA had higher urea, phosphate and leukocyte values than those with LDA. Cured animals with RDA had lower magnesium, hematocrit and leukocyte values prior to surgery than animals that did not recover.

Post mortem findings of cows with LDA were mainly hepatic lesions and alterations related to metabolic disorders. In contrast, in salvaged cows with RDA, lesions of the abomasum were the most common finding. Of those animals that had a follow up period of 15 months, 51% of LDA cows and 42% of the RDA cows survived this period.

574 (3346)

#### AN EPIDEMIOLOGICAL STUDY ON SUBCLINICAL DEFICIENCY OF TRACE ELEMENTS IN BUFFALOES OF PUNJAB STATE IN INDIA

Singh S.1, Uppal S.2

1Punjab Agricultural University, Department of Clinical Veterinary Medicine, E & J, Ludhiana, Punjab, 141004, India; 2Punjab Agricultural University, Department of Veterinary Clinical Services Complex, Ludhiana, Punjab, 141004, India

Subclinical deficiency of major and trace elements in dairy animals is a widespread problem, which often limits their performance but generally go unnoticed. An assessment and correction of mineral status in dairy animals, plants and soils has been considered important criteria to increase the productivity. The present study was conducted with the objective to assess the trace element status of buffaloes.

A base line survey was conducted on blood samples of 224 buffaloes randomly selected from 84 dairy units of 33 villages of Punjab State in India. Samples of fodder being fed and soils where these fodders were cultivated were also collected. Trace element concentrations in plasma, fodders and soils were estimated by Inductively Coupled Argon Plasma Atomic Emission Spectroscopy (ICP-AES).

The mean plasma Cu level was  $11.01 \pm 0.37 \mu\text{mol/l}$  and 19.2% of the buffaloes had low plasma Cu levels ( $< 9.60 \mu\text{mol/l}$ ). The majority of the hypocupraemic buffaloes were not showing typical signs of Cu deficiency. The plasma Mo concentration was  $0.45 \pm 0.05 \text{ ppm}$  and 52.2% buffaloes had high Mo concentration (plasma levels  $> 0.30 \text{ ppm}$ ). The fodder mineral analysis revealed that 71.74% of samples had Cu levels less than the

dietary requirements (10 ppm) and 21.74% had high Mo (>3 ppm). It was observed that 66.7% maize (*Zea mays*), 100.0% bajra (*Pennisetum typhoides*) and oats (*Avena sativa*), and 54.6% barseem (*Trifolium alexandrinum*) samples had low Cu, whereas 45.5% of barseem samples had high Mo. None of the soil samples were however, deficient in Cu but about 10% samples had high Mo concentration.

The plasma Co, Zn and Fe concentrations were  $11.17 \pm 0.74$ ,  $21.35 \pm 0.71$ ,  $64.95 \pm 1.90$   $\mu\text{mol/l}$ , respectively and no animal was deficient in Co, Zn and Fe. However, 45.7% fodder samples had low Zn levels (<40 ppm), The majority of soil samples were low in Co while few were low in Zn. The study indicates that the considerable buffalo population under study had low Cu and high Mo plasma levels which could be due to low Cu and high Mo levels in fodders.

Funding: Punjab Agricultural University

575 (3401)

#### AN EXPERIMENTAL INVESTIGATION ON THE METABOLIC EFFECTS OF DIFFERENT "ACID SALTS" AND CONCLUSIONS FOR THE USE IN THE PROPHYLAXIS OF SUBCLINICAL HYPOCALCAEMIA AND MILK FEVER

Staufenbiel R.1, Froemer S.1, Loeffler L.2, Engel M.3, Gelfert C.1

1Free University Berlin, Clinic for cattle and pigs, Koenigsweg 65, Berlin, D-14163, Germany; 2Free University Berlin, Institute of Physiology, Koenigsweg 65, Berlin, D-14163, Germany; 3Institute of Animal Nutrition, Koenigsweg 65, Berlin, D-14163, Germany

Introduction: Due to the economical importance, modern herd management has to have strategical concepts for prophylaxis of milk fever and subclinical hypocalcaemia. One possible approach is the use of anionic salts. But in practice there were gained quite different experiences including good or no prophylactic effect or provocation of animal losses too. That is why we need more detailed knowledge about the metabolic effects of anionic salts in milk cow feeding.

Material and methods: In a Latin square design eleven fistulated cows received two equivalents of ten different salts or salts mixtures in eleven experimental periods. They have also been given portions of water for controlling the experiment. Each salt period lasted 14 days and was followed by a salt free period of 14 days too. In all, the experimental trial takes 11 months. Beside the ingestion monitoring of food and water, body weight and backfat thickness, ruminal fluids, blood and urine samples were taken from the animals on two days to appoint clinic chemical parameters. On the last day of salt substitution, a 24 h cross-section has been determined.

Results: Only two ( $\text{CaCl}_2$ ,  $\text{CaSO}_4$ ) of the proved salts ( $\text{CaCl}_2$ ,  $\text{MgCl}_2$ ,  $\text{NH}_4\text{Cl}$ ,  $\text{NaCl}$ ,  $\text{CaSO}_4$ ,  $\text{CaSO}_4$  (D3),  $\text{MgSO}_4$  ( $\text{NH}_4$ ) $\text{SO}_4$ ) or a mixture of it had a significant influence to lower pH and the base excess value in the blood. In contrast to blood, a significant and marked acidogenic reaction was provable in the urine for all salts including  $\text{NaCl}$ . The strongest effects had  $\text{CaCl}_2$ ,  $\text{NH}_4\text{Cl}$  and  $\text{CaSO}_4$ . The effects on the acid-base equilibrium occurred immediately with ingestion of the salts with most of the parameters and were no longer traceable after stopping the salt ingestion. With the elimination of calcium via urine, an adaptive period of about one week was proved. Neither the ruminal pH value nor the ruminal fermentation were influenced by giving the salt.

Conclusions: For the tested acid salts a significant but also different effect on the acid-base equilibrium were provable.  $\text{CaCl}_2$  had an intensive effect but weak sensory properties. An unexpected and positive effect in the potency of  $\text{CaCl}_2$  had the  $\text{CaSO}_4$  which is rather tasteless. It is taken by cows gratuitously without depressive effect on the feed intake. Using  $\text{CaSO}_4$ , an application of anion rations in a total mix-ration is possible without negative effect on the ingestion.  $\text{CaSO}_4$  can be recommended as the anionic salt of first choice. It can be used in all herds independently of herd size and feeding system.

576 (3085)

#### HEALTH STATUS AND TIME RELATIVE TO CALVING EFFECTS ON BLOOD METABOLITE CONCENTRATIONS

Van Saun R.

Pennsylvania State University, 115 Henning Building, University Park, Pennsylvania, 16802-3500, United States of America

Blood chemistry analyses are frequently used by veterinarians for disease diagnosis. Blood metabolite measures are compared to laboratory defined reference ranges, however, these reference ranges often are based on mid to late lactation cow populations and may not be appropriate for evaluating transition cows. Objectives of this study were to determine effects of time relative to calving and health status on blood metabolite concentrations.

Metabolic profiles were performed on plasma samples collected from 111 cows selected from 13 commercial dairy farms over three time periods relative to calving. These periods were defined as: Early dry (ED), >30 days precalving; Close-up Dry (CU), 3 to 21 days precalving and Fresh (FR), 3 to 30 days postcalving. Metabolic profile analyses included urea nitrogen (BUN), creatinine (Cr), glucose (Glu), total protein (TP), albumin (Alb), total bilirubin (TB), alkaline phosphatase (ALP), creatine kinase (Ck), gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST), sorbitol dehydrogenase (SDH), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), phosphorus (P), magnesium (Mg), total cholesterol (Chol), triglycerides (TG), beta-hydroxybutyrate (BHB), and nonesterified fatty acids (NEFA). Disease diagnosis and

treatment events were recorded. Blood metabolites were evaluated by a model with period, health and their interaction as main effects and herd as a covariate.

Of all cows, 58% had one or more disease events postcalving. Percent healthy calvings varied greatly between herds. Herd was significant in all metabolite models, except NEFA and Ck. Time period influenced ( $P<0.05$ ) all metabolite concentrations, except Ca, P and K. Health status influenced NEFA ( $P<0.002$ ), BHB ( $P<0.005$ ), TG ( $P<0.03$ ), GGT ( $P<0.02$ ) and AST ( $P<0.04$ ) independent of time period. An interaction between time period and health status was found for Alb ( $P<0.03$ ), BUN ( $P<0.001$ ), Glu ( $P<0.001$ ), Chol ( $P<0.02$ ), TG ( $P<0.02$ ), AST ( $P<0.0002$ ), BHB ( $P<0.005$ ) and NEFA ( $P<0.001$ ). Sick cows had lower Alb, BUN, Glu and Chol and higher AST, BHB and NEFA compared to healthy cows in the FR period. These data suggest reference ranges for diagnostic interpretation of blood metabolite concentrations should be adjusted to time periods relative to calving. Interactions between time period and health status suggest prepartum blood metabolite concentrations may provide some indication to postpartum disease risk and can be useful as a herd monitoring tool.

Funding: Pennsylvania Dept of Agri

577 (5036)

#### INFLUENCE OF MANAGEMENT FACTORS ON METHODS TO EVALUATE THE NUTRITIONAL AND METABOLIC STATUS IN DAIRY HERDS AND COMPARISON OF THESE METHODS

von Tavel L.1, Kirchhofer M.1, Doherr M.G.2, Küpfer U.1, Eicher R.1

1University of Berne, Dept. of Clinical Veterinary Medicine, Div. Herd Health, Bremgartenstr. 109a, Berne, Switzerland; 2University of Berne, Div. Clinical Research, Dept. of Clinical Veterinary Medicine, Berne, Switzerland

The most important influence factor on milk protein is the amount of energy of the ration that is available for the microorganisms in the rumen. The microorganisms need the energy for their growth, and the digested microorganisms are the most important source of protein for the dairy cow. In this study, the usefulness of milk protein to assess the energy balance of the herd was evaluated. Three different methods and their practicability in the field were investigated. Additionally, the potential influence of management factors on milk protein was analysed.

The study included 47 farms: a questionnaire was filled up for each farm and Dairy Herd Improvement (DHI) tests data from 653 cows (for a total of 3097 tests) were collected over a winter period. The three methods evaluated were: 1.) the graph of Spohr about the 'efficiency of the feeding management', 2.) the calculation of the energetic situation of the herd with a new method based on the Swiss standard calculations, and 3.) a percentage value of cows per herd with an average milk yield, but a low milk protein.

A correlation could be shown between the first and the third method, but no correlation could be found with the second method. Furthermore, milk protein and the results of the three methods of evaluation were significantly influenced by several management factors.

In order to feed a dairy herd according to its requirements, it seems that it is not enough to calculate a ration, because the farmer has to feed the calculated ration, the cow has to eat, digest and finally absorb it. The absorbed ration is the one which is relevant for the maintenance of production and health of the cow, but this ration is difficult to calculate. The DHI-tests provide direct information from the cow about her metabolic situation. The graph of Spohr seems to provide good information about the nutritional efficiency of the herd. The percentage value developed for this study to obtain a simple indicator of the energy status of the herd could give a simple overview about the energy metabolism and may reveal management problems. However, its relevance has to be verified in further studies. Several management factors influence milk protein, which have to be taken into account under field conditions.

578 (3403)

#### RELATIONSHIP BETWEEN KETOSIS AND SOCIAL RANK OF DAIRY COWS IN AN AUTOMATIC MILKING SYSTEM (AMS) VERSUS A CONVENTIONAL MILKING SYSTEM - INTERMEDIATE RESULTS

Wenzel C., Nitzschke A.

Clinic for Ruminants and Pigs, Frankfurter Strasse 110, Giessen, 35392, Germany

Introduction: In husbandry with an AMS, social rank plays a major part in the daily routine of dairy cows. All resources are freely accessible (e.g. concentrate in the AMS) and cows adapt to the system according to their social rank, e.g. low-ranking cows visit the AMS at times when the visiting frequency is low. It could be deduced that some individuals are more susceptible to ketosis and that this susceptibility depends on social rank. Therefore, a study is currently being conducted to determine whether the incidence of ketosis is related to social rank of cows kept in automated husbandry. Results from the first half year are presented.

Material and methods: Social rank is determined by direct observation and a rank index is calculated to establish an artificial linear order with high, middle and low-ranking cows. Blood samples are taken every 3 weeks over the course of one year from all cows in early lactation (= 70th day) in a herd of 60 cows milked by an AMS ( $n = 21$ ; experimental group). Samples are also taken every three weeks from 5 randomly selected cows at the same stage of lactation in a conventionally milked herd of the same size ( $n = 8$ ; control group). All cows are either Red or Black Holstein breed. Blood serum is evaluated for glucose, beta-hydroxybutyrate, urea and aspartate aminotransferase. All cows are kept in loose housing, with the compartments for the experimental and control groups separated by the feeding table. Both herds are given the same feed. The

experimental group receives concentrate in the AMS, and the control cows have access to two concentrate feeders. Mean and standard deviation are calculated for all samples of each group. Non-parametric tests are used to investigate differences between rank groups.

Results and discussion: In the AMS group, 8 cows were high, 5 were middle, and 8 were low-ranking. Distribution in the control group was more irregular (5/2/4). A significant difference ( $p < 0,05$ ) in urea between the social ranks in the control group could indicate that these results have merely descriptive value. However, a slight tendency ( $p = 0,07$ ) to significant difference was found in glucose between social ranks of AMS cows. These preliminary results suggest that social rank does not increase the risk of ketosis for individual cows. Cows seem to adapt to a system where they choose their own routine. Perhaps, instead, it should be investigated whether this is due to endogenous adaptations.

579 (2566)

#### STUDY ON THE INCIDENCE OF KETOSIS IN DAIRY COWS IN AN AUTOMATIC MILKING SYSTEM VERSUS A CONVENTIONAL MILKING SYSTEM - PRELIMINARY RESULTS

Wenzel C., Nitzschke A.

Clinic for Ruminants and Pigs, Frankfurter Strasse 110, Giessen, 35392, Germany

Introduction: It is a known fact that ketosis is influenced by the husbandry system. In automatic milking systems (AMS), cows choose their own daily routine. If intake of concentrate (which is provided in the milking stall) decreases, the risk of ketosis is high. However, data on incidences of disorders other than mastitis in AMS is rare. Therefore, a study is currently being conducted to determine whether the incidence of ketosis is influenced when cows are kept in automated husbandry. Results from the first half-year are presented.

Material and methods: Samples are taken every three weeks from all cows in early lactation (= 70th day) in a herd of 60 cows milked by an AMS (Lely Astronaut®) ( $n = 26$ ). These cows make up the experimental group. Samples are also taken every three weeks from 5 randomly selected cows at the same stage of lactation in a conventionally milked herd of 60 cows ( $n = 16$ ) (control group). All cows are either Red - or Black Holstein breed. Urine and blood samples are taken from all cows. Using test strips, the urine is tested for ketone bodies. Blood serum is evaluated for glucose, beta-hydroxybutyrate (BHBA), urea and aspartate aminotransferase (AST). If urine ketone bodies are detected, the cow is clinically examined.

All cows are kept in the loose housing, with the compartments for the experimental and control groups separated by the feeding table. Both herds are given the same feed (grass -, maize silage, hay, brewers' grains). The experimental group receives concentrate in the milking stall, and the control cows have access to two concentrate feeders.

Mean and standard deviation are calculated for all samples of each group. Non-parametric tests are used to investigate differences between both milking systems.

Results and discussion: 2% of all cases in the automatic group show a concentration of ketone bodies  $> 4$  mmol/l in the urine, while 11% in the controls show the same level. Clinical investigations revealed no primary cause. No significant differences between the two groups were found in serum concentration of glucose, BHBA, urea and AST, however.

This preliminary results didn't support the hypothesis that cows which have all basic requirements freely accessible to them and are therefore left alone have a higher risk of ketosis than those with a conventional daily routine. Until now, it appears as if all cows have been able to take in enough concentrate to cover metabolic energy needs.

580 (1042)

#### DIAGNOSIS AND TREATMENT OF ABOMASAL IMPACTION IN 80 LACTATING HOLSTEIN COWS

Wittek T.1, Constable P.2, Morin D.2

1Medizinische Tierklinik der Universität Leipzig, An den Tierkliniken 11, Leipzig, 04103, Germany; 2University of Illinois, Department of Clinical Veterinary Medicine, 1008 West Hazelwood Drive, Urbana, Illinois, 61802, United States of America

Abomasal impaction has generally been considered a rare disease in cattle, and procedures for the diagnosis and treatment of affected animals have not been well described. The main aims of this retrospective study were therefore to characterize the clinical examination findings, serum biochemical values, surgical management, and postoperative outcome in dairy cattle with abomasal impaction. The medical records of 80 lactating Holstein cows ( $>2$  years old), diagnosed with abomasal impaction at laparotomy ( $n=71$ ) or necropsy ( $n=9$ ) between 1980 and June 2003, were reviewed. All cows were reported to have decreased or no food intake; cows also had metritis ( $n=21$ ), mastitis ( $n=18$ ), abdominal pain ( $n=9$ ), abdominal adhesions ( $n=8$ ), lameness ( $n=7$ ), left displaced abomasum ( $n=6$ ), right displaced abomasum ( $n=4$ ), and pneumonia ( $n=4$ ). Impaction was confined to the pyloric antrum alone ( $n=55$ ) or to both the antrum and abomasal body ( $n=25$ ). Cows were  $4.9 \pm 1.8$  years old (mean  $\pm$  SD) and 23 days in milk (geometric mean) at diagnosis, and had a rectal temperature of  $38.9 \pm 0.9^\circ\text{C}$ , a pulse rate of  $85 \pm 23$  beats/minute, and a respiratory rate of  $39 \pm 18$  breaths/minute. Serum biochemical analysis at admission ( $n=30$ ) indicated mild hypocalcemia ( $7.5 \pm 1.1$  mg/dl), hypokalemia ( $3.7 \pm 0.7$  mEq/l), and hyperbilirubinemia ( $1.0 \pm 0.9$  mg/dl), whereas mean values for serum Cl concentration ( $96 \pm 10$  mEq/l) and total CO<sub>2</sub> ( $26.8 \pm 4.1$  mmol/l) were within normal limits. A right flank laparotomy was performed in 71 cattle, and massage of the abomasum was reasonably successful (73% cases) in breaking down the impaction; resolution sometimes required the injection of dioctylsodium

sulfosuccinate solution or 0.9% NaCl into the abomasal lumen. A pyloric omentopexy was performed after abomasal massage and the abdomen closed routinely. After surgery, most cattle received 3-4 l of mineral oil orally each day for 1-5 days. Information on postoperative survival rate was available for 69 cows; survival rate for cows with antral impaction alone (93%, 42/45) was much higher ( $P < 0.0001$ ) than that for cows with antral and body impaction (50%, 12/24). We conclude that physical examination findings and the results of serum biochemical analysis do not facilitate diagnosis of abomasal impaction in lactating Holstein cows, and that a right flank exploratory laparotomy is necessary to make a diagnosis. Abomasal impaction should be considered as a differential diagnosis for inappetence and poor milk production in lactating dairy cows. Funding: Max Kade Foundation

581 (2816)

**EFFICIENCY OF ALPHA TOCOPHEROL TRANSFER TO BOVINE MILK THROUGH ORAL OR INTRAPERITONEAL SUPPLEMENTATION**

Abdellatif H.1, Pennie K.2, Fredeen A.2

1Cairo University, Faculty of Veterinary Medicine, Giza Square, Giza, Giza, 12211, Egypt; 2Nova Scotia Agricultural College, Dept. of Animal Science, Truro, Nova Scotia, B2N 5E3, Canada

The efficiency of oral and intraperitoneal (IP) supplementation of alpha tocopherol acetate was studied to determine which route would be more effective in increasing the level of alpha tocopherol in bovine milk. Eight multiparous Holstein cows were randomly allocated into two equal groups. The first group received oral supplementation of 600 IU of alpha tocopherol acetate/h/d for 10 days. Each cow from the second group received a single intraperitoneal injection of 6000 IU of alpha tocopherol acetate. Milk samples were collected every 12 hours from the cows in each group for 10 successive days. Tocopherol extractions from milk were analyzed by HPLC with fluorescence detection. Total alpha tocopherol content per milking was determined. There was no increase in the alpha tocopherol level in milk for the oral-supplemented group. The IP group, however, had an increase from 10.1 mg alpha tocopherol to 14.5 mg/milking within the first 12 hours following supplementation. By 72 hours levels for the IP group had returned to pretreatment quantities. There were significant differences between the two treatment groups at 36 and 48 hours ( $P = 0.0685$  and  $0.0809$ ) regarding the alpha tocopherol level in milk. The IP injection proved to be the most effective method of increasing the alpha tocopherol levels in bovine milk.

582 (2609)

**RELATIONSHIPS AMONG MILK UREA NITROGEN, DIETARY NUTRIENTS AND FECAL NITROGEN LOSS ON CANADIAN DAIRY FARMS**

Arunvipas P.

Atlantic Veterinary College, UPEI, Dept. of Health Management, AVC, 550 University avenue, Charlottetown, PEI, C1A 4P3, Canada

A total of 83 dairy herds were visited three times (fall 1999, spring 2000, fall 2000) between October 1999 and January 2001 within 48 hours of a herd test for milk production. Forages were sampled and analyzed for nutrient levels, which were entered into a computerized ration evaluator (Spartan). For each stage of lactation (early, mid and late), Spartan was used to determine the following parameters: crude protein percent, net energy supplied, and total protein supplied, and protein energy ratio ( $PER = (\text{total protein supplied less total protein required}) / (\text{net energy supplied less net energy required})$ ). A handful of feces was also obtained from each of 6 cows (2 early, 2 mid, and 2 late lactation) in each herd. The feces were pooled, mixed, and analyzed for nitrogen content using a conventional wet chemistry method. Milk from the most recent (relative to each herd visit) herd test for milk production was tested for milk urea nitrogen (MUN) by an infrared method (FOSS 4000). A weighted (for milk production) average MUN for all milking cows in each stage of lactation (early, mid, and late) was calculated for the test date immediately prior to each farm visit. Because the herd fecal sample was a mixture of 2 cow fecal samples from each of these stages of lactation, an average of the weighted average MUNs was calculated to obtain a herd average MUN for each sample date. Mixed linear regression modeling was used to evaluate relationships among MUN, ration composition and fecal N, with fecal N as the dependent variable, adjusting for clustering within herds, and controlling for other possible confounders. In the final model, total protein supplied in the ration was significantly positively associated with fecal N while the net energy of lactation had a smaller positive effect. While MUN values had a positive association with fecal N, the association was not statistically significant ( $P > .05$ ). In the model, 27% and 73% of the variation in fecal N came from differences between herds and between sampling dates within herds, respectively, indicating that there is considerable seasonal variability of fecal N and nutritional management within herds. The nutrient data were only able to explain approximately 8% the variation in fecal N, suggesting that average MUN values may be of limited use as a predictor of herd fecal N levels on commercial dairy farms.

583 (2724)

**EFFECT OF ANIONIC SALTS ON BLOOD SERUM ANALYTES IN DAIRY COWS**

Avila G J.1, Bouda J.1, Doubek J.2, Rivera J.1, Nuñez O L.1, Quiroz-Rocha G.1

1University of Mexico, National Autonomous FMVZ-UNAM, Ciudad Universitaria, Mexico, DF, 04510, Mexico;

2University of Brno, Faculty of Veterinary Medicine, 612 42 Brno, Brno, 612, Czech republic

Parturient paresis (PP) and subclinical hypocalcemia (SH) are common in postpartum dairy cows. The effect of administration of anionic salts (AS) in prepartum cows on the selected serum analytes, urine pH, frequency of PP and SH was studied in the period of transition in one herd of dairy cows with high frequency of PP. The study was carried out in a farm of 800 Holstein cows with milk yield of 8800 kg/lactation in central Mexico, where the frequency of PP had been 12.4%. This study included cows from the 3rd to 5th lactation, 3 weeks prior to their expected calving date, and body condition ranging from 3.5 to 3.7. The diet of an experimental group (EG) of cows (n = 11) was supplemented with 300 g of AS (NH<sub>4</sub>Cl, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and MgSO<sub>4</sub>)/cow/day during approximately 14 days before calving. A control group (CG) (n = 16) was on the same diet, without AS. The dietary cation-anion difference was determined. Samples of blood and urine were collected from all the animals, 21 and 7 d prepartum; at the day of calving, and 3, 7, 14 d postpartum. In addition, urine pH was measured in 5 animals from the (EG) twice a week. The concentration of calcium, inorganic phosphorus, magnesium, free fatty acids, AST, urea, proteins, albumin were determined in serum. Urine pH was determined by a portable pH meter and ketone bodies were determined by dipsticks. Results were analyzed using the "t" test for repeated measures between both groups of cows. In the CG, without addition of AS, four cows were treated for PP and their postpartum results were not included in the statistical analysis. The serum Ca concentration (1.68 mmol/L) was significantly decreased (P < 0.01) only in the CG at day 1 postpartum in comparison with Ca of the EG (2.07 mmol/L). The concentration of inorganic P was decreased (P < 0.05) in the CG postpartum at day 3. The urine pH in the EG, after addition of AS, until 3 d postpartum was significantly decreased (P < 0.01; 6.47-6.70) in comparison with the CG (7.82-8.29). SH was detected in 9 of 12 cows in the CG. In the other serum selected biochemical analytes there were no significant differences between both groups. The supplementation of the AS diet with AS to cows in the 2 weeks prepartum period, was effective in the prevention of PP and SH.

584 (5054)

#### THE EFFECTS OF A NON STARCH POLYSACCHARIDASE ENZYME PREPARATION FROM THERMOMYCES LANUGINOSUS ON THE RUMINAL VOLATILE FATTY ACID PRODUCTION, ENERGY AND PROTEIN METABOLISM AND MILK YIELD OF DAIRY CATTLE

Brydl E.1, Rafai P.1, Jurkovich V.1, Könyves L.1, Tirián A.1, Tegzes L.1, Kutasi J.2, Bata Á.2, Nagy G.3, Bartyik J.4, Fülöp A.5

1Szent István University, Faculty of Veterinary Medicine, Department of Animal Hygiene, P.O. Box 2, Budapest, H-1078, Hungary; 2Bata Research and Development Ltd (LLC), Pesti úti major, Ócsa, H-2364, Hungary; 3Szent István University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, P.O. Box 2, Budapest, H-1078, Hungary; 4Enying Agricultural Share-holders Co, Kossuth L. u. 42, Enying, H-8130, Hungary; 5Szent István University, Faculty of Veterinary Medicine, Kossuth L. u. 42, Enying, H-8130, Hungary

In the present study the effects of an enzyme preparation (Rumino-Zyme) high in xylanase activity were studied on the ruminal volatile fatty acid (VFA) production, parameters of energy and protein metabolism, milk yield, feed conversion rate (FCR) and body condition score of high yielding dairy cows. The lignolytic enzyme preparation applied in the present experiment and fed to dairy cows at 34 g/day dosage increased the VFA concentration in the rumen from about 32 days after calving and onward. Increased VFA production has been followed by about 5 to 10% increase in the milk production and almost 0.1% increase in the butterfat production. Increased VFA production produced more balanced energy metabolism in the experimental cows as indicated by the lower incidence rate of hyperketonaemia, and lower aceto-acetic and Non Esterified Fatty Acid (NEFA) concentration in the blood of the experimental cows. Aspartate Amino-transferase (AST) activity was tendentially higher in the control group and the proportion of control cows that had AST activity higher than 100 U/l was also higher in the control group. Both control and experimental cows showed balanced protein and acid-base metabolism throughout the experiment. Enhanced VFA production contributed to the betterment of energy balance in the experimental cows with a resultant improvement of feed intake and feed utilisation. Due to more balanced energy metabolism post-parturient weight loss of the treatment cows was lessened.

Keywords: Dairy cattle, Thermomyces lanuginosus, Rumino-Zyme, milk production, body condition, feed conversion rate

Abbreviation key: Volatile Fatty Acids (VFAs), aceto-acetic acid, Non Esterified Fatty Acid (NEFA), aspartate amino-transferase (AST), Net Acid-Base Excretion (NABE)

585 (5024)

#### THE INTERRELATIONSHIP OF MAGNESIUM, CALCIUM AND INORGANIC PHOSPHORUS IN RUMINANTS

Dua K.1, Care A.D.2

1Punjab Agricultural University, Department of Clinical Veterinary Medicine Ethics and Jurisprudence, Ludhiana, 141012, India; 2University of Wales, Institute of Rural Studies, Aberystwyth, United Kingdom of Great Britain and Northern Ireland

In the present study the interrelationship of Mg, Ca and Pi in ruminants have been studied as a part of an assessment of their role in pathogenesis of various metabolic disorders. The experimental approach involved estimation of Ca, Pi and Mg concentrations in plasma following i.v. infusion of salts of these minerals in

sheep. In five experiments infusion of (25%) magnesium sulphate at the rate of 12 ml/hr for two hours after the initial loading dose of 15 ml resulted in 270% increase in the plasma Mg concentration. The hypermagnesaemia induced was responsible for about 20% increase in the plasma Pi concentration without having any significant effect on the plasma Ca concentration. In two experiments hyperkalemia induced by infusion of potassium chloride at the rate of 0.8 mmol/min for 90 minutes did not had any significant effect on the concentrations of the Mg, Ca and Pi in the plasma. In three experiments, 1 a cholccalciferol (3.5 mg) was given twice a day for 3 days which resulted in about 27% increase in the total plasma Ca concentration, 10.85% increase in the ionized Ca concentration and 91.3% increase in the plasma Pi concentration. In three experiments, hyperphosphatemia was induced by infusion of sodium phosphate at the dose rate of 0.75 mmol/min for 180 minutes which resulted in 17.3% decrease in the total plasma Ca concentration and 20.3% in ionized Ca concentration without having any significant effect on plasma Mg concentration. The interrelationship of Mg, Ca and Pi has significant implications in the treatment of various metabolic diseases of ruminants.

Funding: Association of Commonwealth Universities, UK.

586 (2710)

#### DIAGNOSTIC USE OF RUMINAL PH IN SAMPLES COLLECTED BY ORO-RUMINAL PROBE AND BY RUMENOCENTESIS IN DAIRY COWS

Gomez R.1, Bouda J.1, Quiroz-Rocha G.1, Doubek J.2

1National Autonomous University of Mexico, FMVZ-UNAM, Ciudad Universitaria, Mexico, DF, 04510, Mexico;

2Faculty of Veterinary Medicine, University of Brno, 612 42 Brno, Brno, 612, Czech republic

Subclinical ruminal acidosis is frequent in dairy cows. Collection of ruminal samples and determination of pH are very important for the diagnosis of ruminal disorders. The aim of this study was to compare values of pH in ruminal fluid collected via oro-ruminal probe and by rumenocentesis and evaluate their diagnostic utility. We used 26 Holstein-Friesian non-pregnant cows with mean milk production of 26 kg/day. Ruminal samples were collected via oro-ruminal probe and by rumenocentesis 5 to 7 hours after the first feeding of a total mixed ration. Ruminal fluid pH was measured using the portable pH meter. The mean value was 0.30 pH unit higher ( $p < 0.05$ ) in samples obtained by the oro-ruminal probe than for fluid collected by rumenocentesis. These results indicate a positive correlation ( $r = 0.79$ ,  $p < 0.05$ ) between ruminal pH values obtained by two different collection methods. Employment of the mouth-wedge, the appropriate oro-ruminal probe and a discharge of the first 200 mL of ruminal fluid before obtaining a sample, enable the collection of an adequate sample by a non-invasive method for the determination of ruminal pH and diagnosis of ruminal acidosis.

587 (2583)

#### INFLUENCE OF MOULDY HAY ON FERMENTATION IN BOVINE RUMEN FLUID (IN VITRO)

Hoeltershinken M.1, Hoehling A.1, Hoffman P.2, Scholz H.1

1Clinic for Cattle School of Veterinary Medicine, Bischofsholer Damm 15, Hannover, 30173, Germany;

2German Collection of Microorganisms a Cell Culture, Mascheroder Weg 1b, Braunschweig, 38124, Germany

For Cerebrocorticalnecrosis (CCN) or Polioencepalomalazia (PEM) the main reasons is not well known. In the literature are discussed mouldy feedstuffs, sulphite, sulphate or high energy feedstuffs.

Mouldy feedstuffs from grassland of Northern Germany have shown a thiaminolytic effect on the rumen fermentation. So the influence of mouldy hay (kind of moulds: *Fusarium culmorum* FC, *Fusarium poae* FP, *Fusarium graminearum* FG, *Cladosporium hebarum* CH, *Alternaria alternata* AA, *Epicoccum nigrum* EN, *Ulocladium chartarum* UC, *Mucor racemosus* MR) on the in-vitro-fermentation especially the thiamine-metabolism of bovine rumen fluid was investigated using the long term rumen simulation technique:

After a control period of nine days (normal hay), a 10 days lasting test phase followed. During this time two reaction vessels (KF) were charged with normal hay, two vessels (VFII) with mouldy hay (for example: *Fusarium culmorum*) and two vessels with another mouldy hay. During the last five days of the test period 0.3 mg thiamine/reaction vessel were added daily. In the last six days all reaction vessels were fed with normal hay.

The following effects of mouldy hay during the investigation period on rumen fluid and rumen gas, respectively, could be noted: gas production: FC -8%, FP -14%, EN -2.5%; protein fraction: FG -13.5%, CH -4.2%, AA -5.3%, UC -14.6%, MR -8%; sVFA: AA -5.7%, EN +1.5%, UC -6.3%, MR -3.1%; cellulose activity: FC -38%, FP -38%.

In contrast to older experiments (maize silage with different moulds) of our laboratory, the fermentation patterns (gas-, methane-, sVFA) decreased or increased (ammonia production) significantly less in these investigations. Additionally a thiaminolytic effect as seen in trials with moulded grass was missing.

Thus, although a clear influence of these moulds on rumen fermentation could be shown, a relation to CCN was not detected.

588 (5066)

#### METABOLIC CAUSES OF SEASONAL MORBIDITY VARIATIONS IN HIGH YIELDING DAIRY COWS RESPECTING FEEDING

Hoops M., Füll M.

Universitaet Leipzig, An den Tierkliniken 11, Leipzig, 04103, Germany

The morbidity in cows is characterised by their concentrated occurrence at certain times of the year. A relation between inadequate foodstuff and morbidity can often be established, although the feeding of high yielding dairy cows should be improving continually in quality and continuity. Therefore the following aim was derived: to monitor the metabolism, morbidity and yield in cows with a high yield systematically for one year and then to relate the values to feeding.

Experimental design: 117 SB cows (ca. 8200 kg FCM/a) were used for the investigation from March to April of the next year. Parameters of energy, protein, mineral and acid base metabolism as well as haemogram were analysed and checked 10 day a.p., 3 day p.p. and 4 week p.p. in blood (B) and urine (U).

Results: During the transit period, feeding remained mostly constant. Only the structure effective crude fibre varied more and was less than 2 kg/d in May as well as from November to January. The metabolites creatinine, protein, phosphor, Fe and leukocytes did not vary significantly. The main variations showed (numbers refer to months) acid base state (5/6, 9-12); energy metabolism (3/4; 7/8, 9-12), Urea/B (3-6, 11-12). The most frequent diseases were mastitis in May/June, endometritis with their maximum from July to December as well as claw diseases with their maximum from November to February. Metabolic changes and morbidity correlate as follows: mastitis negative with pH-value, NSBA, FFA, Bilirubin ( $p < 0.05$ ), Cholesterol, Albumin, CK, Urea, ALA-AK ( $p > 0.05$ ); endometritis positive with Urea ( $p < 0.05$ ), bilirubin ( $p > 0.05$ ), negative with temperature ( $p < 0.05$ ), NSBA, Cholesterol ( $p > 0.05$ ), ALA-AK, BHB ( $p < 0.05$ ); claw diseases positive with Albumin, CK, ALA-AK ( $p < 0.05$ ), FFA, Bilirubin, BHB, Urea ( $p > 0.05$ ), Cholesterol, pH-Value, NSBA ( $p > 0.05$ ). Mastitis was especially caused by the stressed acid base state, which followed from the lack of structure effective crude fibre. Endometritis were mainly related to energy lack. Claw diseases correlated especially with the stressed energy metabolism as well as the antioxidative state.

Conclusion: Health deviations throughout the year are mainly caused by an insufficient supply of crude fibre, which is connected to the deviations of the acid base state and the energy metabolism.

589 (1777)

#### EFFECTS OF DIFFERENT DIETARY LEVELS OF VITAMIN AND TRACE-ELEMENT SUPPLEMENTATION ON MILK YIELD AND QUALITY IN GRAZING DAIRY COWS

Laudadio V., Petrera F.

University of Bari, Str. prov. per Casamassima Km 3, Valenzano, Bari, 70010, Italy

In order to evaluate the effects of different dietary levels of vitamin and trace-element supplementation on milk yield and quality in grazing dairy cows, a trial was carried out for a period of 12 weeks (January-March).

Eighteen lactating Brown cows representing three stages of lactation: Early (5-100 days), mid (101-200 days) and late (201-300 days), were divided into two homogeneous groups of 9 animals each based on number and stage of lactation and milk yield.

In addition to pasture, dairy cows received 5 kg/day of chopped oat hay and 3-9 kg/day of concentrate mixture on the basis of lactation stage, which was different between groups for the level of supplementation of vitamins and trace-elements: 0.25% in the 1st group, 0.50% in the 2nd one.

One kg of vitamin-mineral premix contained: 8000000 IU Vitamin A; 800000 IU Vitamin D3; 12000 mg Vitamin E; 2000 mg Vitamin B1; 6 mg Vitamin B12; 100000 mg Niacin and 50000 mg Choline Cl., with 250 mg Co; 20000 mg Fe; 1000 mg I; 20000 mg Mn; 40 mg Se; 43000 mg Zn and 4000 Cu.

Individual milk yields were recorded and individual milk samples were collected fortnightly to determine milk composition (fat, protein, lactose and somatic cell counts) and to evaluate renneting properties of milk (R, K20 and A30). The dietary level of vitamin and trace-element supplementation did not affect daily milk yield, fat corrected milk yield, milk composition and milk renneting properties.

The milk from early lactation dairy cows receiving the higher level of vitamin and trace-element supplementation had a significantly higher (3.31 vs 3.15%;  $P < 0.05$ ) protein content than did the milk of cows in the same stage of lactation receiving the lower level of supplementation; whereas the lactose content was higher (5.55 vs 4.81%;  $P < 0.05$ ) in the milk from late lactation dairy cows receiving the higher level of vitamins and trace-elements in the concentrate mixture than the other group.

It was concluded that the lower level of vitamin and trace-element supplementation in the concentrate mixture (0.25%) of grazing dairy cows can be economically employed without any adverse effect on their performance.

590 (5074)

#### EFFECTS OF PARTIALLY REPLACING SOYBEAN MEAL WITH CORN GLUTEN MEAL ON MILK YIELD AND COMPOSITION IN COWS AT DIFFERENT STAGES OF LACTATION

Laudadio V., Petrera F.

University of Bari, Str. prov. per Casamassima Km 3, Valenzano, Bari, 70010, Italy

An experiment was conducted to evaluate corn gluten meal (CGM) as a partial substitute for soybean meal (SBM) in ration of Brown cows. Twenty-two Brown cows, 5 days after calving, were divided into two homogeneous groups of eleven animals each according to parity number, calving date, milk yield during the previous lactation and body weight. The cows were maintained in the trial for 10 months. Experimental period was divided in three phases: early (5-100 d), mid (101-200 d) and late (201 -300 d) lactation periods.

Cows were offered a basal diet consisting of forage (Italian ryegrass hay) and 10 kg concentrate which sufficed for the production of 20 kg milk, together with a daily allowance of 1 kg concentrate for each 2.5 kg

milk produced more. Each group was fed a different experimental concentrate: control group concentrate contained SBM, while in the other concentrate CGM replaced SBM on an isonitrogenous basis at 25% replacement.

Milk yield (kg/d) and composition from early lactation Brown cows were not affected by dietary treatment; however, the percentage and yield of milk protein were increased by about 0.10 percentage unit and by about 32 g per day per cow, and the percentage and yield of milk fat were decreased by about 0.11 percentage unit and 33 g per day per cow when SBM was partially replaced by CGM in diet. Milk yield and composition from mid and late lactation cows were not affected by dietary treatment. Feeding CGM to mid and late lactation dairy cows significantly increased ( $P < 0.05$ ) milk protein content (3.58 vs 3.35% and 3.78 vs 3.51%, respectively) and tended to increase milk protein yield (805 vs 769 g/d and 667 vs 651 g/d, respectively); instead it tended to decrease milk fat content (from 3.62 to 3.75% and from 3.82 to 4.06%, respectively) and milk fat yield (from 807 to 864 g/d and from 675 to 739 g/d, respectively) compared with feeding SBM.

During the milking period, total milk yield, total 4% FCM yield and total milk fat yield tended to be higher for cows in group SBM than those in CGM (+ 119 kg, + 239 kg and + 50kg respectively), while total milk protein yield tended to be higher (+ 29 kg) in CGM compared with SBM.

In conclusion, increasing rumen undegradable protein (RUP) in the diet by partially replacing SBM with CGM significantly improved protein content of the milk but did not have a positive effect on milk fat content or milk yield.

591 (2351)

#### EFFECT OF ORAL DRENCHING WITH SODIUM HEXAMETAPHOSPHATE ON SERUM AND URINE LEVELS OF CALCIUM, PHOSPHOROUS AND MAGNESIUM IN LACTATING DAIRY COWS

Ottesen J., Jørgensen R., Thilsing T., Enemark J.

The Royal Veterinary and Agricultural University, Dyr-laegevej 88, Frederiksberg, 1870, Denmark

This paper describes a first attempt to monitor an expected calcium binding effect of sodium hexametaphosphate (SHMP) drenching on serum concentrations and on renal excretion of calcium, phosphorus and magnesium, in a pilot study with lactating dairy cows.

Three lactating non-pregnant Jersey cows were randomly assigned in a prospective experimental factorial study as either control ( $n=1$ ) or experimental ( $n=2$ ). The study was divided into two halves, using one of the experimental cows in each half while the third cow acted as control during the entire experiment. Over a period of two days the experimental cows were drenched twice daily. Each drench consisted of a freshly made solution of 150 g SHMP in water. Such two-day procedure was carried out three times, one week apart, on each of the experimental cows. In each half of the study daily blood and urine samples were drawn from both cows involved, starting 4 days before the first SHMP-drenching and continuing until 8 days after the last drenching.

In the first days after SHMP-drenching the experimental cows experienced a non-significant decline in serum calcium down to the threshold of hypocalcaemia, followed by a significant increase in serum calcium that tended to be above pre-treatment levels. This was interpreted as a calcium homeostatic response. Parallel to the increase in serum calcium, renal calcium excretion was significantly elevated. Serum and urine phosphorus peaked shortly after SHMP-drenching, with serum phosphorus reaching hyperphosphataemic levels. Intestinal absorption of hydrolysed SHMP was suggested as an explanation to these results. Any effect of SHMP-drenching on serum Mg could not be documented. SHMP-drenching did not affect serum carbamid, milk yield or body condition of the cows. The mechanisms and interactions involved in SHMP-drenching are discussed in relation to the observed oscillations in serum and urine mineral levels. It was concluded that SHMP drenching affected blood calcium in a way similar to that observed by our research group after drenching dairy cows with the zeolite synthetic sodium aluminium silicate. However, a preventive effect of SHMP supplementation on milk fever remains to be shown.

Funding: The Royal Veterinary and Agricultural University, Denmark

592 (3361)

#### SEASONAL COBALT DETERMINATION IN SLAUGHTERED COWS' RUMEN CONTENTS AROUND TABRIZ CITY IN EAST AZARBAYJAN PROVINCE

Rezaee Saber A.P.1, Rezaie A.1, Yousefi Oskui M.A.2

1University of Tabriz, Veterinary college, Department of clinical sciences, Tabriz, 5167784177, Iran (Islamic Republic of); 2Veterinary, Iran (Islamic Republic of)

Amount of cobalt was determined in 80 samples taken from rumen contents of slaughtered cattle around Tabriz.

The samples were taken on 15th of the second month of each season (autumn, winter, spring, summer), air dried, and their Co content measured by the atomic absorption spectrophotometry. The measured values were mostly in the low normal range (normal range=4-7.2 ppb) around the year, except for 6 measurements (2 spring and 2 autumns in the Osku district, and 1 spring 1 autumn in the Vanyar district) which indicates possible Co deficiency in the area.

Funding: Islamic Azad university of Tabriz (Iran)

593 (2592)

## INFLUENCE OF ANIONIC SALTS ON RUMEN FERMENTATION PATTERN (IN VITRO)

Scholz H.1, Mueller-Oekzan E.2, Chawanit M.2, Hoehling A.1, Hoeltershinken M.1

1Clinic for Cattle School of Veterinary Medicine, Bischofsholer Damm 15, Hannover, 30173, Germany;

2School of Veterinary Medicine, Bischofsholer Damm 15, Hannover, 30173, Germany

The dietary cation-anion-balance concept (DCAB-concept) is used all over the world to prevent hypocalcemic paresis in high yielding cows. Since the advantage for the Ca metabolism is well documented, nobody looked at consequences for the ruminal capacity. Although these salts may have a serious impact on the ruminal environment, which may hold some risks for the nutrient supply of the cow during high lactation. The aim of this long term in-vitro-investigation (RUSITEC) was to look at effects of different DCAB-diets (-100, -200 and -300 meq/kg DM as different mixtures of NH<sub>4</sub>Cl + MgSO<sub>4</sub> and CaCl<sub>2</sub> + CaSO<sub>4</sub> + MgSO<sub>4</sub>, respectively) on the rumen fermentation. In six experiments lasting 27 days each, consisting of a preliminary phase (9 days), a test phase (10 days) and a regeneration phase (8 days) the influence on the pH, the concentrations of volatile fatty acids, ammonia, protein, thiamine and its derivatives, long chain fatty acids, nucleobases and the gas production as well, were investigated; additionally the distribution of vitamin B1 and its derivatives (TDP = thiaminediphosphate, TMP = thiaminemonophosphate) in different fractions of the rumen fluid (f.e. plant and protozoal fraction, bacterial fraction) was examined. The influences on ruminal fermentation patterns were not striking. In general a moderate dose dependent decrease of the ruminal activity was seen, expressed as a reduction (in comparison to controls) of the methane production (-9%), the n-butyrate production (-5%) and the i-valerate production (-13%). But there was no influence on the main volatile fatty acids or parts of the protein metabolism (protein production). Particularly the thiamine metabolism was involved. In different fractions the concentrations decreased in remarkable amounts. For example, in the plant and protozoal fraction, thiamine diminished by about -18.4%, TDP -46%, TMP -21%. It is to assume that the sulphuric parts of the diet had been the reason for this phenomenon. In diets with ammonia compounds as ammonium chloride an increase of the ammonia concentrations up to 25% of the controls has to be expected. Since these changes were mainly seen with the high concentrated DCAB diets (-300meq/kg DM) it is to conclude that the normal use of DCAB-diets (between -100 and -150 meq/kg DM) in the feeding of cattle before calving will be without risks for the rumen metabolism during the adjacent period of high lactation.

594 (1661)

## EFFECT OF FEEDING BALED GRASS SILAGE ON THE SENSORY CHARACTERISTICS AND THE VOLATILE FRACTION OF RAW MILK

Wichtel J.1, Bosset J. 2, Dohoo I.1, Stryhn H.1, Mouchili A.1

1UPEI, Atlantic Veterinary College, 550 University Avenue, Charlottetown, Prince Edward Island, C1A 4P3,

2Swiss Federal Dairy Research Station, Liebefeld, Bern, CH-3003, Switzerland

The association of feeding baled silage to lactating cows and the occurrence of feed off-flavour in raw milk established by the study conducted by Mouchili et al. (2003) prompted a more extensive investigation of not only the sensory characteristics of milk samples collected from cows before and after they were fed baled silage, but also the volatile organic compounds (flavour compounds) of these samples. A total of 27 milk samples (nine pre-silage feeding and 18 post-silage feeding) collected from 9 Holstein Friesian cows were organoleptically evaluated by two panels of five trained milk graders and further analyzed for their volatile fraction using headspace (HS) solid phase microextraction (SPME), gas chromatography-mass spectrometry (GC-MS) and flame ionization detection (GC-FID) techniques. Organoleptic assessment revealed that all the 9 pre-silage feeding samples as well as 2 of the post-silage feeding samples were milk of good quality (free of any objectionable flavour). The chromatograms from GC analyses showed that ½ hour post-silage feeding samples had higher concentrations (peak heights) of ethanol, acetone, dimethyl sulfide, 2-butanone, hexanal, heptanal and 2,3-octanedione ( $p < 0.05$ ) and lower concentrations of butane 2,2,3,3-tetramethyl, pentane 2-methyl, and pentane 2,3,4-trimethyl ( $p < 0.10$ ) than pre-silage feeding milk samples; whereas 3 hours post-silage feeding milk samples differed from pre-silage feeding samples by their high content of acetone, dimethyl sulfide, 2-butanone ( $p < 0.05$ ) and hexanal ( $p = 0.09$ ). Although multivariable logistic regression analysis using both sensory and gas chromatographic data indicates that the occurrence of feed off-flavour could be predicted by the concentration of dimethyl sulfide alone, compounds such as acetone, 2-butanone or hexanal, either singly or in combination, have been reported to be as important in the development of feed off-flavour in milk following the feeding of grass silage.

These findings may form the basis of a potential practical and more reliable diagnostic assay to identify unacceptable flavours in raw milk.

Keywords: off-flavour, volatile organic compound, gas chromatography, organoleptic

595 (1322)

## CHARACTERIZATION OF VTEC E. COLI STRAINS ISOLATED FROM CALVES IN NORTHERN JORDAN

Al-Majali A., Abu-Ashour H., El-Sukhon S.N.

Jordan University of Science &amp; Technology, P.O. Box 3030, Irbid, Irbid, 22110, Jordan

In Jordan, 105 fecal samples of one day to 6 months old calves were collected between August 2001 and August 2002. They were cultivated on SMAC and CT-SMAC media. A total of 89 (88%) isolates having the cultural characteristics of E.coli were accumulated. They were characterized by determination of their: 1) biochemical activities, 2)  $\beta$ -glucuronidase activity, 3) enterohaemolysin production, 4) serotyping, 5)

verocytotoxicity, using the ECACC, WHO cell line and 6) production of VT1 and VT2 using the RPLA and VTEC-RPLA kits.

The  $\beta$ -glucuronidase activity was detected in 15 (34%) isolates while 6 (6.7%) produced enterohaemolysin. The O26, O111 and O157 serotypes were determined in 9 (10%), 18 (20%) and 14 (16%) isolates, respectively.

Eight (8.9%) isolates revealed verocytotoxic effect which was observed in approximately 85% of cell population within 18-24h, was increased by prolonged incubation and was enhanced by treatment of their crude toxin extraction with polymyxin. All of them were sorbitol and rhamnose fermenters and variable on inositol. Of those, only 1 isolate could be typed with the above mentioned three antisera and was belonging to O111 serotype. By the RPLA and VTEC-RPLA techniques, 5 isolates were confirmed to produce only the VT1. The VT2 was not determined in any of them. It is concluded that calves represent hazardous source for spreading EHEC among Jordanians.

Funding: JUST, Irbid, Jordan

596 (2544)

#### PREVALENCE OF SHIGA-LIKE-TOXIC ESCHERICHIA COLI (STEC) IN HAEMOLYTIC AND UREMIC SYNDROME (HUS)-ASSOCIATED FARMS IN FRANCE

Arné P., Millemann Y., Oppici S., Buatier V., Colmin C., Sanaa M.

Ecole Nationale Vétérinaire d'Alfort, 7 avenue du général de Gaulle, Maisons-Alfort cedex, 94704, France  
STEC strains are major food-borne bacterial pathogens that have been implicated in diarrhoea, haemorrhagic colitis and HUS. One serotype: O157:H7, is the dominant serotype associated with disease worldwide but other serotypes (O26, O103, O111) are also frequently implicated in disease. The ability of STEC strains to cause severe troubles in humans is undoubtedly related to their capacity to secrete the Stx1 and/or Stx2 Shiga toxins. Several other virulence factors that might contribute to the pathogenicity have been described like the intimin, product of the eae gene and the enterohemolysin encoded by the ehx locus.

Cattle appear to be the main reservoir of STEC strains and contaminated ground beef is the most common source of human infection.

In the global frame of the surveillance of HUS by children (under 15 year-old) related to animals in France, and in order to provide data useful for Quantitative Risk Analysis on diseases due to STEC, a case-control study was conducted in 10 groups of cattle farms. Each group consisted in a case farm linked to a HUS and 3 control farms of the same size and located in the same geographical area. Samples of silage, water and animal faeces were collected in all the livestock and screened using a multiplex PCR for the presence of stx, eae and ehx genes simultaneously on crude extracts (Paton and Paton, 1998). All PCR reactions were negative in only 4 farms. Virulence genes were not detected in both water and silage samples but more than 50% of the faeces samples were positive for one or more genes. Notably stx1/2 genes were amplified in 95% of positive faeces extracts.

About thirty faeces-derived positive samples for (stx + eae + ehx) genes were further subjected to isolation of strains using both selective media and an O157-specific immunomagnetic separation. None of the 5 isolated strains harbouring the 3 loci belonged to the O157 serotype.

This survey confirmed that the prevalence of STEC related virulence genes is high in cattle but pathogenic strains are rarely encountered. An efficient control of STEC hazard should therefore associate the detection of genes and the identification of specific serotypes.

Paton, A. W., and J. C. Paton. 1998. J Clin Microbiol 36(2):598-602.

Funding: DGAI (Min Agriculture)

597 (3291)

#### DETERMINATION OF ANTIGENIC CHARACTERISTICS OF HYDATID FLUID IN SHEEP AND HUMAN

Hashemi Tabar G.R., Fakoran J.

Ferdowsi University of Mashhad, Iran, Mashhad, Khorasan, 91775-1793, Iran (Islamic Republic of)

Hydatidosis is one of the zoonoses, which is very important in the field of medicine, veterinary and also economy. The adult Echinococcus granulosus, which lives in the small intestine of canines is swallowed by intermediate host (human and sheep). This parasite causes cysts in tissues, which are called "hydatid cysts". Hydatid cyst contains hydatid fluid (HF) and also hydatid sand. These proteins stimulate immune system and produce antibody in host body.

This research was done on ten samples of hydatid fluid of sheep and one from human. Hydatid fluids were separated from sand hydatid by centrifugation in order to determine dominant subunits HF, similarities and differences between human and sheep. HFs were analyzed by 12.5% SDS-PAGE. Dominant bands which consist of four different bands in the ranges of 85-97, 55-65, 30-36 and 20-24 kDa showed similarities between sheep and human. To determine antigenic characteristics of bands, one sample of human HF and two samples of sheep HF were used for Dot blotting and also Western blotting. In this research sera were taken from healthy and infected sheep and human.

The results showed that color reactions were observed on the nitrocellulose paper in all of the mentioned sera in 20-24 kDa band on human (HF). No reaction on sheep samples was shown. These experiments showed different structures in humoral immunity between sheep and human.

Key words: Hydatidosis, Hydatid fluid, Sheep, Human

598 (3327)

## APPLICATION OF POLYMERASE CHAIN REACTION (PCR) AND SEROLOGICAL METHODS TO DRIED SAMPLES ON FILTER PAPERS STERILIZED BY ETYLENE OXIDE GAS

Muramatsu Y., Shin-No Y., Kirisaswa R., Ueno H., Morita C.

Rakuno Gakuen University, Dept. of Veterinary Public Health, 82-1 Bunkyo-dai-Midorimachi, Ebetsu, Hokkaido, 069-8501, Japan

The filter paper has widely been employed for blood-sampling to research many kinds of bacterial and viral diseases. This procedure is particularly useful in seroepidemiological studies. Dried sample on the filter paper is also suitable for the molecular screening of the infectious diseases and autosomal disorder by PCR. A DNA fragment specific for a targeted microorganism was amplified and detected from the dried blood sample stored at room temperature for several days extend to more than 10 years. For several years, we have used dried blood samples on filter paper for serodiagnosis of spotted fever group rickettsia and/or *Coxiella burnetii*. The purpose of this study is to assess the utility of this sample collection method both for PCR and serological study post-sterilization by ethylene oxide gas (EOG).

Five blood samples were collected from cows in our university farm. Three of them were positive for anti-*C. burnetii* antibody and the remaining two were negative neither for the antibody and the coxiella DNA. The seropositive samples were absorbed and dried on filter papers. The negative bloods were added with purified, inactivated *C. burnetii*, and then the mixtures were absorbed and dried on filter papers. These dried samples were eluted into PBS, and then the eluates were used for serological assay by indirect fluorescent antibody test (IFA), or detection of the coxiella DNA by nested-PCR. Plural pieces of filter papers were used for preparation of each dried sample. All of the dried bloods on filter papers were separated into two groups. The filter papers in the one group were sterilized by EOG prior to use for IFA or PCR. The filter papers in the other group were subjected to IFA or PCR without sterilization.

After sterilization by EOG for 2 h at 55°C, there were no differences for the antibody titers to *C. burnetii* between the two groups. Similarly, EOG-sterilization for 12 h at 37°C did not influence the results of IFA of the both groups. After sterilization by EOG for 2 h at 55°C, we could detect the coxiella DNA by the nested-PCR, however we could not detect the coxiella DNA after EOG-sterilization for 12 h at 37°C. After EOG-sterilization for 2 h at 55°C, the serological investigations and the PCR could be performed in safety. Especially, The EOG-sterilization is suitable for the serological method such as IFA. It is suggested that the EOG-sterilization is available for mass-screening of the infectious diseases in safety.

Funding: High-Tech Res. Program and RGU

599 (857)

## SEROLOGICAL SURVEY OF SHEEP INFECTION TO TOXOPLASMA GONDII IN TABRIZ SLAUGHTER HOUSE

Nematollahi A.

Tabriz University, Tabriz, East Azarbaijan, 51666, Iran (Islamic Republic of)

Toxoplasmosis is a zoonotic disease in various species of animals caused by a sporozoan parasite, *Toxoplasma gondii*, distributed throughout the world including Iran. Toxoplasmosis is a common infection of sheep and goats worldwide and it is recognised as one of major cause of infectious reproductive failure. This survey was carried out to show the prevalence of this disease in Tabriz (Iran). 486 sera sample were collected from both domestic and industrial sheep. The counter-current immunoelectrophoresis test (CCIEP) was used to determine the rate of infection. The results indicated that the rate of infection was 26.33%. There was significant differences in infection to *Toxoplasma gondii* in the age group of sheep ( $p < 0.05$ ). Also a significant difference was observed between rate of infection and sex in sheep ( $p < 0.01$ ). Results of this study indicated no significant differences between rate of infection and gestation period.

Funding: Tabriz University

600 (2822)

## OCCURRENCES OF ORTHOPOXVIRUS IN DAIRY COWS ON FARMS OF THE COUNTIES OF CAMPOS DOS GOYTACAZES AND SÃO FIDÉLIS, R.J., BRAZIL

Cardoso de Melo V.1, Santos J. A.1, Moussatche N.2, Damaso C.2

1Zoonosis Control Division and Environmental Surveillance "Dr. Arnaldo Rosa Viana", City hall of Campos dos Goytacazes, Voluntários da Pátria, 522 / 501, Campos dos Goytacazes, Rio de Janeiro, 28.030-260, Brazil;  
2Universidade Federal do Rio de Janeiro, Biophysics Institute Carlos Chagas Filho, Rio de Janeiro, Brazil.

The infection caused by orthopoxvirus (Poxviridae) in dairy cattle presents lesions in teats, lips and faces of calves in nursing phase. This illness has zoonotic characteristics, which could be transmitted to human beings, causing lesions in the hands, arms and faces of the milkers. The pustule lesions become ulcers and scabs in the teats of the cows and in the superior limbs and on the face of human beings that could cause widespread lymphadenopathy, high fever with an average course of seven days and in some cases edema in the affected limb. The illness may provoke economic losses, due to complications such as mastitis, reduction of milk production and loss of animal value, in addition to removal of the worker from the service. This work aims at pointing out the etiologic agent that causes a vesicular-pustule illness that attacked the dairy cattle of the farms of the counties of Campos dos Goytacazes and São Fidélis, RJ in the month of September 2002.

Clinical examinations of the animals were performed as well as the removal of the scabs from the affected teats. The scabs were conditioned in ice by the team from the Zoonosis Control Division and Environmental Surveillance "Dr. Arnaldo Rosa Viana". The material was sent to the Virus Molecular Biology Lab of the Biophysics Institute Carlos Chagas Filho-UFRJ for viral isolation of the scabs and observation of the cytopathic effect.

As per the molecular diagnosis, the total DNA of the infected cells has been extracted and used as mode in PCR tests for the enlargement of the gene of hemagglutinina (HA). The amplicons have been submitted to RFLP test and analysis of the restriction pattern through agarose gel. The result obtained was compatible with the profile observed for the virus "Cantagalo". These data suggest that the isolated materials from Campos dos Goytacazes are similar to the samples of the virus "Cantagalo" identified in the county of Cantagalo, RJ in 1999.

Support: CCZ, SMS, City hall of Campos dos Goytacazes, RJ, Brazil

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23rd World Buiatrics Congress, Quebec City, Canada, 2004

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