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301 (3400)

STREPTOCOCCUS BOVIS AS INDICATOR BACTERIUM FOR CATTLE

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The purpose of the study was to demonstrate *Streptococcus bovis* to be more appropriate as a Gram-positive indicator bacterium compared to *Enterococcus faecalis* and *Enterococcus faecium*. Although the latter two bacteria are used as indicator bacteria in national monitoring programmes, they are only frequently isolated in faeces of pre-ruminating calves. They are rare in adult cows. Indicator bacteria should have a high prevalence and a zoonotic character, and should be receptive to resistance genes.

Faecal samples were taken from 927 animals in three different age categories located on 10 dairy farms and 10 beef cattle farms. Isolates were identified according to Devriese et al. (1992). *Streptococcus bovis* was isolated on all 20 farms. *Enterococcus faecalis* was isolated on 10 of the 20 farms and *E. faecium* only on 3 of the 20 farms. On the dairy farms, 117 of 453 samples (25.8%) were positive for *S. bovis*. On the beef cattle farms, 167 of 474 samples (35.2%) were positive for *S. bovis*. In total, *S. bovis* was isolated from 30.6% of all samples. In calves younger than 6 months, *S. bovis* was isolated from 46 of 127 samples (36.2%), in young cattle from 6-12 months, 27 of 158 samples (17.1%) were positive for *S. bovis* and in cows older than 12 months, 211 of 642 samples (32.9%) were positive. Susceptibility testing was performed on 102 *S. bovis* isolates from 10 dairy farms by a disk agar diffusion test according to NCCLS guidelines. Only 21 isolates (20.6%) were susceptible to all antimicrobials tested, 15 (14.1%) were resistant to at least 1 antimicrobial and 66 isolates (64.4%) were intermediate resistant to at least 1 antimicrobial.

It was concluded that *S. bovis* is suitable as a Gram-positive indicator bacterium for cattle because of the higher prevalence of *S. bovis* in faeces compared to the prevalence of *Enterococcus* spp. and because of their ability to harbour resistance determinants. Concerning the zoonotic aspect of *S. bovis*, although bovine non specific serotypes were involved, the organism is recognized as a cause of endocarditis in elderly people and an uncommon cause of septicaemia and meningitis in newborn infants. Even though different subtypes of a bacterial species may affect man and animals, the ability of bacterial strains to transfer genes between each means that either population may act as a reservoir of resistance genes (Teale, 2002).

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302 (2725)

IN VITRO ACTIVITY OF GENTAMICIN AGAINST CLINICAL STRAINS OF SALMONELLA TYPHIMURIUM ISOLATED FROM CATTLE IN FRANCE

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The serovar Typhimurium of *Salmonella enterica* ssp. *enterica* is a common cause of clinical salmonellosis among cattle in France and is frequently associated with septicaemia [1]. Clinical signs and mortality can be reduced by treatment with antimicrobial agents and gentamicin, a broad-spectrum aminoglycoside antibacterial, is sometimes administered for that particular purpose.

The aim of the present work was to evaluate the in vitro activity of this antibacterial against strains of *S. Typhimurium* isolated from infected cows, it could be a treatment of choice against clinical salmonellosis. Material and methods: Fifty two strains of *S. Typhimurium* were isolated from cows with clinical salmonellosis. Minimal inhibitory concentrations (MIC) of gentamicin were determined by dilution in Mueller-Hinton agar according to NCCLS guidelines. The NCCLS veterinary breakpoint MICs were used. Additionally, kill kinetics were performed against 10 strains in Mueller-Hinton broth, with an initial inoculum of approximately 10⁷ CFU/mL. Samples were collected at 0, 1, 2, 3, 4, 6 and 24 hours and recultured on agar medium for counting after 24 h incubation. Results were expressed in the form of time-kill profiles of *S. Typhimurium* against seven gentamicin concentrations.

Results: MIC₅₀ and MIC₉₀ of gentamicin were both 1 mg/L, with MIC values ranging 0.25 mg/L to 64 mg/L. Fifty-one strains (98%) were susceptible (MIC ≤ 4 mg/L) and one strain was resistant (MIC = 64 mg/L). Time-kill curves were similar for the 10 strains tested (MIC = 1 mg/L). Gentamicin demonstrated a concentration-dependent bactericidal activity. A strong bactericidal effect was observed within the first 3 hours of exposure at concentrations equal 2xMIC. A bactericidal effect was also observed within a 4 to 6 hours of exposure at the MIC, but this concentration did not prevent a bacterial regrowth.

Discussion: *S. Typhimurium* strains isolated from clinically affected cattle often have a high level of resistance against antimicrobial drugs. Our results demonstrated the low occurrence of resistance to gentamicin and the strong concentration-dependent bactericidal activity of this antibacterial. Moreover, the value of MIC₉₀ showed its possible indication in the treatment of infections caused by this serovar. However, it is recommended to consider these results together with pharmacokinetic data in order to predict the success or failure of the treatment.

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303 (3001)

ANTIMICROBIAL SUSCEPTIBILITY OF CAMPYLOBACTER ISOLATED FROM ORGANIC AND CONVENTIONAL DAIRY FARMS IN THE UNITED STATES

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From a longitudinal study of foodborn pathogens on 128 dairy farms, this objective was to describe the patterns of susceptibility in *Campylobacter* according to herd management style. Herds were enrolled in four states by herd size and management type (conventional or organic). Environmental and fecal samples were collected on a bi-monthly basis for 10 months. Specimens were processed at a central laboratory for isolation and identification of *Campylobacter*. Antimicrobial susceptibility was determined for 2,131 isolates by automated microbroth dilution to determine minimum inhibitory concentration (MIC). A panel of 17 antimicrobials, including classes of drugs used on dairy farms and in human therapy, was used. MIC values for each isolate were read manually as the lowest concentration showing no visible growth. Using NCCLS guidelines for breakpoints of enteric pathogens, overall resistance among dairy isolates was low (~ 2% resistant) regardless of herd management for azithromycin, ceftriaxone, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, and nalidixic acid. Resistance was 9% and 8.2% to ampicillin, 33% and 29% to kanamycin, and 48.9% and 58.2% to tetracycline for conventional and organic farm isolates, respectively. Using the antimicrobial concentration that inhibits the growth of 50% and 90% of the isolates, MIC₅₀ and MIC₉₀ were also determined by management type. Preliminary results of MIC₅₀ and MIC₉₀ showed that isolates were within a single dilution by herd type with the exception of the MIC₅₀ of tetracycline which required four times the tetracycline concentration in conventional farm isolates (32 µg/ml) compared to organic farms (8 µg/ml). These results, including factors associated with the above observations, will be discussed.

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304 (3395)

DRUG RESISTANCE AND SENSITIVITY OF BACTERIA FROM REPEAT BREEDING AND ABORTIONS IN BOVINES

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Over the years infectious abortions in dairy cattle have resulted in significant economic losses. Bacterial infections are incriminating factors in majority of cases. In the present communication, a wide variety of bacteria were isolated and in order to have effective control drug resistance and sensitivity patterns of isolates from normal, repeat breeding and abortion cases were studied. Out of total of 17 isolates (Cattle 8, buffaloes 9) from normal animals, the organisms were found to be sensitive to Gentamicin, Chloramphenicol, Tetracycline, Naledixic acid, Nitrofurantoin but were resistant to Erythromycin, Pencillin, Ampicillin and Neomycin. Out of total 155 isolates from repeat breeder cattle and buffaloes maximum isolates 146 (94%) were found sensitive to Cifrofloxacin followed by Gentamicin 115 (74%) and Chloramphenicol (67%). The resistant antibiotics were Penicillin and Naledixic acid with varying degree of drug resistance. Various bacteria isolated from fetal heart blood, stomach contents, fetal organs from cattle and buffaloes were subjected to drug sensitivity pattern. Out of total 41 isolates all 5 *Brucella abortus* were sensitive to Gentamicin, Chloramphenicol and Neomycin whereas they were resistant to Furazolidone, Penicillin, Erythromycin and Cotrimaxozole. *Streptococcus* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* exhibited minimum resistance pattern. The detailed resistance/sensitivity pattern was studied in special relation to control of infectious abortions and repeat breeders.

305 (2866)

MARBOFLOXACIN SUSCEPTIBILITY OF PATHOGENIC MASTITIS BACTERIA ISOLATED FROM CATTLE IN EUROPE (1994-2002)

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Background: Marbofloxacin is a fluoroquinolone developed for individual cattle treatment, which has been marketed since 1997 in Europe and registered for mastitis infection in the UK since 2000. Vétoquinol has set up a European surveillance programme (France, Germany, Italy, Belgium, Netherlands, Spain, Ireland, and the UK) to assess marbofloxacin susceptibility against pathogenic strains.

Methods: On 1673 first intention cattle pathogenic strains collected between 1994 and 2002 from cases of mastitis (528 *E. coli*, 448 *S. aureus*, 394 *S. uberis*, 188 *S. dysgalactiae* and 115 *S. agalactiae*), marbofloxacin MIC (minimal inhibitory concentration) testing was performed in a referenced laboratory by the broth microdilution method (NCCLS, M31-A).

Results: The most frequently isolated bacterial species from bovine mastitis were *E. coli*, *S. aureus* and *S. uberis*. No significant change to marbofloxacin susceptibility has occurred during this eight year period. The most susceptible strain was *E. coli* (between 94.8 and 100%) with the lowest MIC90 (between 0.016 and 0.023 µg/ml). The second most susceptible strain was *S. aureus* (between 98.4 and 100%) with a MIC90 between 0.235 and 0.357 µg/ml. The least susceptible strains without resistance development were Streptococci. The least susceptible Streptococci was *S. agalactiae* (between 50 and 94.5%) with the highest MIC90 between 0.968 and 1.741 µg/ml.

The MIC distribution for each strain demonstrated essentially one population with only some less susceptible strains. Indeed, for *E. coli* the population was between 0.008 and 0.03 µg/ml with some strains having a MIC up to 8 µg/ml depending on the year of isolation. For *S. aureus*, the population was between 0.12 and 0.5 µg/ml and some strains had a MIC up to 4 µg/ml according to the year of isolation. The MIC distribution for all the Streptococci was the same, between 0.5 and 2 µg/ml with only one strain at 4 µg/ml.

In 2002, 1.12 % of strains isolated from bovine mastitis were resistant to marbofloxacin.

Conclusions: Marbofloxacin's contribution to the increase and spreading of resistant bacteria has been limited since its launch in 1997 and since its first registration for mastitis treatment in 2000. According to the pharmacokinetic and pharmacodynamic data as well as the clinical results, marbofloxacin has a very good activity against Enterobacteria and some Gram positive pathogenic strains from bovine mastitis.

306 (5050)

CLINICAL OBSERVATION OF A CHRONIC WASTING SYNDROME IN A DAIRY HERD WITH LAMINITIS

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This clinical observation of the ambulatory clinic of the national veterinary school of Alfort concerns a dairy herd (140 cows) with an important mortality (45 deaths in two years) and abortions (25 with different diagnosis: *Chlamydia*, *Listeria*, *Coxiella burnetii*, *Neospora*). Pyogenic infections with *Arcanobacterium pyogenes* (uterine and respiratory abscesses) were observed at the necropsy of two cows. Another observation was an acute congestion of the intestine with isolation of *Aspergillus fumigatus*.

In the herd, the clinical signs were mostly a severe laminitis in every cow (lameness, tarsitis, « praying » position, clumsy efforts to rise or lie down, resulting in cutaneous injuries and important subcutaneous abscesses). Other symptoms were rumen depletion, wasting, mastitis, ptyalism. The absence of neurological sign can rule out bovine spongiform encephalopathy.

With the examination of feeding practices, we have concluded to a suspicion of subclinical rumen acidosis leading to these locomotor disturbances and abscesses.

This case seems very similar with the « chronic wasting disease in cattle » described in 1999/2001 in Netherlands. The time between subclinical rumen acidosis and apparition of severe laminitis can explain the difficulty of the etiological diagnosis of this metabolic problem (the pH of the rumen can be normal when the first clinical signs are seen and low fat milk is not systematically seen in subclinical acidosis in a herd).

Amelioration of zootechnic and feeding practices were recommended but irreversibility of lesions (severe laminitis, abscesses...) cannot solve all the problems of the herd.

307 (2718)

THE EFFECT OF HOUSING TYPE ON LAMENESS PREVALENCE IN WISCONSIN DAIRY HERDS

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The objectives of the study were to determine the prevalence of lameness as a function of season, housing type and stall surface type among dairy cows in a small, representative sample of herds in Wisconsin.

Thirty dairy herds were visited once during the summer and once during the winter and at each visit, all lactating dairy cows, including those in the sick pen, were locomotion scored using a four point system ranging from 1 (normal sound), 2 (slight lameness), 3 (moderate lameness) to 4 (severe lameness). The

proportion of cows scoring 3 and 4 were considered 'clinically lame' for the purpose of lameness prevalence assessment.

Of the thirty herds, 15 were free stall housed and 15 were housed in stanchion and tie stall barns. 13 herds used sand as a stall surface and 17 herds used non-sand stall surfaces, which included concrete bedded with straw or shavings, rubber mats bedded with straw or shavings and rubber crumb filled mattresses bedded with sawdust or shavings.

Mean (SE) lameness prevalence was significantly lower at 21.1% (2.0) in the summer than in the winter at 23.9% (2.1) ($P < 0.05$). Lameness prevalence between seasons was constant in tie stall herds at 19.6% (4.0) in the summer and 19.7% (3.0) in the winter. However, there was a significantly higher prevalence of lameness in free stall herds in the winter at 27.8% (2.6) than in the summer at 22.8% (2.1) ($P = 0.004$).

Analysis by stall base demonstrated that free stall herds with sand stalls showed no significant seasonal increase in lameness, but free stall herds using non-sand surfaces suffered a significantly higher lameness prevalence rate in the winter at 33.7% ($P = 0.007$). The lowest lameness prevalence was found in tie stall herds using sand stall surfaces at 12.1% in the winter and 12.2% in the summer. We suggest that behavioral differences related to stall use and time spent standing and lying between cows on the two different stall surfaces may impact the prevalence of lameness.

The best quartile of herds had less than 11.4% of cows in the summer and less than 14% of cows in the winter score clinically lame. Only 0.2% of cows in these herds were locomotion score 4. Thus, the presence of several severely lame cows in a herd would be indicative of a herd lameness problem.

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308 (2722)

LAMENESS TREATMENT RATES IN 10 WISCONSIN DAIRY HERDS

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Few studies have documented lameness treatment rates in North American dairy herds. Case definition, ability to detect lame individuals and frequency of delivery of the hoof trimming program significantly impact the incidence of new and recurrent cases.

Treatment records for lame cows were kept on 10 Wisconsin dairy herds for 12 months. Six herds were free stall housed and four herds were tie stall housed. Half used sand bedded stalls and half used a rubber mat or mattress. Stall base types were equally distributed by housing type. Mean (SE) herd size was 145 (28.2) cows. Mean (SE) herd lameness prevalence, determined by averaging the results of locomotion scoring at two visits during the recording period, was 22.2% (2.8).

Using the following case definitions, 1155 lameness treatment events were analyzed. A new lameness treatment was defined as a limb-case treated within one 28 day period. A recurrent treatment occurred in the same limb, greater than 28 days after the first treatment for the same condition.

Lameness affected 39.2% (7.3) of the herd on average. Herd mean (SE) lameness treatment rate was 69.1 (15.6) limb-cases per 100 cows per year. The ratio of incidence to prevalence was 3.2:1. Mean recurrence rate was 12.2%.

Digital dermatitis was the most common lesion found, accounting for 56.8% of all treatments and most of the infectious lesions. Sole ulcer (18.4%), white line disease (10.43%) and sole hemorrhage (6.4%) were the most common claw horn lesions identified. Mean (SE) treatment rate for claw horn lesions was 38.0% (14.7) in mat and mattress stall herds and 14.1% (3.7) in sand stall herds ($P = 0.16$).

The highest rates of lameness treatment by month occurred in September and during the period January - February. The proportion of limb treatments due to claw horn lesions compared to infectious lesions peaked in September at 59.4% of all treatments. For all other months, infectious lesions exceeded 50% of all treatments.

Behavior changes combined with increased risk of subacute ruminal acidosis may be responsible for an increase in the rate of claw horn lesions observed around 2 months after a period of heat stress during a typical Wisconsin summer.

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309 (5046)

LEFT DISPLACED ABOMASUM: TO TOGGLE OR NOT TO TOGGLE, THAT IS THE QUESTION !

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An observational study was conducted at the Université de Montréal in Saint-Hyacinthe (Québec) from October 1998 to April 2000 on 215 cows diagnosed with left displaced abomasums (LDA) in order to evaluate the risk factors associated with success of the toggle pin fixation procedure (TPF, $n = 108$) and the omentopexy (OPX, $n = 80$). For each LDA case, a questionnaire was completed before proceeding with one of the fixation techniques chosen by the clinician. The cow's medical history and clinical examination were recorded. Follow-up evaluations were done between days 4-7 (phone), days 10-14 (veterinarian's visit) and 30-35 (phone). Follow-ups consisted essentially in evaluating the cow's return to normal feed intake and normal milk production according to the producer's opinion. Culling decisions were also recorded at follow-ups. Two-tales

Fisher exact test chi-squares were used to evaluate factors associated with the success of the procedures. A significance level of $p < 0.1$ was used.

The TPF method was performed by 5 clinicians and the OPX method was mostly used by one clinician. The two groups were similar according to age (2.6 lactations), number of days in milk (12 days) and BCS (3.2). At time of diagnosis, the TPF group had a higher prevalence of clinical signs compared to OPX. In fact, for the TPF group, 85% of the cows were mostly off-feed compared to 63% for the OPX group and, 50% of the TPF cows were ketotic compared to 32% of the OPX. Cows from TPF group appeared to be more likely to be culled by 30-35 days (24/108 or 22.2%) than OPX cows (11/80 or 13.7%).

The risk factors associated with the success of the TPF group were: 1) the number of trocar perforations necessary to fix the abomasum (6% (5/77), 14% (3/22) and 50% (3/6) of cows had a culling decision taken by days 4-7 respectively when 2, 3 and 4 perforations were done, $p=0.02$) and, 2) the duration of the condition before the LDA diagnosis (7% (4/56) and 24% (6/25) of the cows had a culling decision taken on them by days 4-7 if it took less than 4 days to find the problem vs between 4 and 14 days; $p=0.08$). There was no effect of the use of antibiotics for this group. The only risk factor associated with the success of the OPX was the recurrence of a LDA. In fact, when it was a LDA relapse, the cows were more likely to be culled by days 30-35 compared to the first occurrence of LDA (40% (2/5) and 7.7% (5/65) respectively; $OR=8$, $p=0.07$).

310 (1260)

UNILATERAL NEPHRECTOMY IN CATTLE

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Introduction: Renal disease is usually bilateral and medical therapy the treatment of choice. We hypothesized that unilateral nephrectomy can be performed successfully and cattle can remain productive. The purpose of this study was to determine survival and productivity in affected cattle. MandM: A search was performed and records retrieved for analysis. Results: 17 cattle were Holsteins, 4 beef cattle. Of these, 12 were Holstein, 3 Ayrshire, 1 Beefmaster, 1 Simmental; 14 were cows, 3 bulls; the mean age 3.5 years (13 were 2-9 years old, 3 <6 months, 1 unknown). Cattle had decreased appetite, poor milk production, weight loss, diarrhea, pyuria, infertility, lethargy, pollakiuria, hematuria swelling of the rear limbs, abdominal distention, and mastitis. Eight lactating dairy cows were a mean of 100 DIM. Affected cattle had a mean rectal temperature 38.8 C, mean HR 91 beats per minute, mean RR 40 breaths per minute. Rectal examination revealed an enlarged left kidney in 10 of 14 cattle, enlarged ureter in 2 of 14. Hematology revealed that affected cattle had a mild leukocytosis and neutrophil:lymphocyte reversal. Cattle with pyelonephritis had hyperfibrinogenemia.

Biochemistry analysis revealed azotemia, hyperkalemia, hyperproteinemia. Urinalysis revealed a mean spgr = 1.015, pH = 8, proteinuria, hematuria and pyuria, bacteria in 7 cattle. Culture yielded *Corynebacterium renale*, *E coli*, *Actinomyces pyogenes*, *Proteus sp.* Both kidneys were involved in 6 cattle, the left kidney in 5, and the right kidney in 5. Nephrectomy was performed via a standing paralumbar fossae (PLF) approach in 13 cattle, PLF approach under general anesthesia in 3, and paramedian incision under general anesthesia in 1. The affected kidney was removed via right PLF ($n=11$), left PLF ($n=1$), by combination right and left PLF ($n=4$), or a paramedian incision in 1. 1 cow died from severe post-operative hemorrhage, 2 cattle were euthanized because of renal failure, and 14 cattle (82%) were discharged. Follow-up was available for 6 cattle. 1 cow had 6 calves, 2 dairy cows were lactating at an average level of production, 1 dairy cow was culled because of low production, 1 calf was smaller than age matched calves, and 1 cow had low dry matter intake. Discussion: Unilateral nephrectomy is a useful treatment for renal disease in cattle where one kidney is principally involved. Affected cattle may return to productivity, but clients should be aware that performance may be sub-optimal.

311 (5063)

BEHAVIOURAL AND PHYSIOLOGICAL RESPONSES OF CALVES TO DEHORNING USING A LONG-ACTING LOCAL ANESTHETIC

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Dehorning of calves has been shown to be a noxious stimulus as demonstrated by increases in serum cortisol and behavioural changes. These changes can be attenuated in the short-term by the use of 2% lidocaine (L) to block the corneal nerve prior to dehorning. A more concentrated solution of lidocaine (5%) has been shown to produce a prolonged block of C-fibre conduction and produce long-lasting analgesia in human clinical studies.

Behavioural and physiological responses after dehorning by heat cauterization were measured in 32, 10-12 week old, female, Holstein calves. Each calf was randomly assigned to one of 4 treatments; 5% L followed by dehorning (F), 2% L followed by dehorning (T), saline followed by dehorning (S) or 5% L followed by sham dehorning (C). Physiological responses were assessed by collecting blood via a jugular catheter at -0.5, 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 9, 12, 24, 48 and 72 h. Feeding, drinking, scratching, rubbing, licking and

inactivity behaviours were observed in the standing and recumbent positions using a 10-min scan sampling method and analysed on a time period and daily basis for 72h following the procedure. Acute phase proteins and immunological components were determined at 0, 12, 24, 48 and 72h.

Plasma cortisol was not affected by treatment ($P=0.07$) or treatment by time interaction ($P=0.06$), but there was an overall significant ($P<0.001$) time effect. Calves had an increase in cortisol within the first 0.5h (9.3 ± 1.1 vs 18.8 ± 2.2 ng/ml) and again from the 4h to 6h samples (5.7 ± 1.4 vs 10.4 ± 1.3 ng/ml). Fibrinogen concentration and neutrophil:lymphocyte ratio (N:L) were not altered ($P>0.1$) by treatment. However, N:L was highest ($P>0.1$) at 12h. For the first 24h post-dehorning, behaviours of all calves were similar and frequencies of scratching, licking, grooming and head rubbing in the recumbent and standing positions were near zero. Treatment differences in recumbent activity were not significant ($P=0.09$), but there was an overall increase in inactivity for all groups over time ($P<0.001$). Eating and drinking frequency were not affected ($P>0.05$) by treatment. Thus, injection of 5% lidocaine prior to dehorning does not appear to provide any additional pain/stress relief over previously established methods.

312 (2455)

PRELIMINARY RESULTS OF PREVALENCE AND CORRELATIONS BETWEEN MAJOR REAR CLAW DISORDERS IN 500 DUTCH DAIRY HERDS

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Sound feet and legs are of paramount importance to the cow for optimal productivity, health and animal welfare (Brand et al, 1996). Since most claw disorders have a multifactorial background, monitoring of claw health is instrumental to good herd health management. With the overall aim to improve claw health in the Netherlands, a research project was initiated (Holzhauer, 2002) to study the prevalence of major rear claw disorders in dairy cattle and additionally to develop a monitoring tool on claw health for dairy farmers and advisors. After an additional training as to uniform assessment of diagnoses, 20 professional claw trimmers registered their findings on major rear claw disorders (digital dermatitis, interdigital dermatitis, interdigital phlegmon, sole ulcer, sole hemorrhage, white line process and interdigital hyperplasia) during a one-year period (May 2002-July 2003) from 10-50 dairy herds each. From the Dutch Cattle Syndicate information was provided on age, breed, last calving date and parity of each registered animal. In addition a questionnaire was filled out on herd related situations and management like type of floor, cubicle and bedding, measures of preventive claw health (foot bath, routine pedicure), management on pasturing and purchase of new animals. Descriptive statistics of the 7 claw disorders at cow and herd level are calculated. In addition the relation between claw disorders is estimated by correlation coefficients for claw, cow and herd-level. Thirdly, univariate analysis of dependent variables is performed to identify significant factors for inclusion in a multilevel model. The multilevel model (proc mixed, glimmix macro, SAS 8.1) was used to determine risk factors for sole hemorrhages. Preliminary results of 440 dairy herds indicate that of all recorded animals (21330), 31,0% had no observed rear claw disorders while more than 13,8% of animals had 3 or more disorders present. Most prevalent cow claw disorders were sole hemorrhages (38%), digital dermatitis (22%) and interdigital dermatitis (38%). These disorders were present on both rear claws in 70%, 31% and 68% respectively.

313 (2403)

FIELD ASSESSMENT OF THE EFFICACY OF TOLFENAMIC ACID IN THE TREATMENT OF LAMENESS IN DAIRY COWS

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A total of 65 lactating dairy cows presenting a lameness (related to limb trauma (excluding fractures), foot rot or a compression of the pelvic nervous filaments) were enrolled by 12 investigators in this randomised, blind, field trial. The objective of this study was to assess the efficacy of tolfenamic acid (TA) (Tolfedine®, Vétoquinol SA) versus a placebo. The cows exhibiting one or more of the following characteristics were not included: laminitis, permanent recumbency, podal affection or lameness due to infection requiring an antibiotic treatment (except for foot rot), as well as animals treated with an anti-inflammatory drug or antibiotic within the previous 15 days, at drying off.

The animals received either TA (2 mg/kg, 1 ml/20 kg) or the placebo (1 ml/20 kg) at D0 and D2 via the intramuscular route. An antibiotic treatment (Excenel®, Pharmacia) was associated in cases of foot rot. The animals were clinically monitored on D0, D2, D5, and D14 for the following parameters: rectal temperature, general condition, appetite, lameness, posture at rest, ability to stand, lying position and warmth /swelling /pain upon pressure for the affected area. The local and general tolerance was also investigated.

Due to major deviations from the protocol, 3 cows were completely withdrawn from the efficacy analysis, leaving 33 cows in the TA group and 29 in the placebo group. A statistically significant time*treatment effect was attained ($p=0.02$, general linear model) for the lameness evolution analysis with higher cure rates in the TA group than in the placebo group especially at D5 (42.4% vs. 17.9% at D5 and 63.6% vs. 50% at D14). This advantage was confirmed by the evolution of other clinical parameters with a clear statistical trend in

favour of TA (treatment effect, $p=0.06$ for the posture at rest and recumbency analysis, general linear model) and by the global evaluation of the treatment by the investigator which was statistically significant (78.8% of satisfaction with the TA treatment versus 55.1% with the placebo, $p<0.05$, Chi-square test). Tolfedine® was, furthermore, very well tolerated locally and systemically (no side-effects, painless injection, no swelling at injection site).

In this blind field study, TA improved the comfort of dairy cows suffering from lameness by reducing the pain element, and thus making it an appealing option for the treatment of lameness in dairy cattle.

314 (2568)

DEVELOPMENT OF BODY AND CLAW TRAITS AND PRESSURE DISTRIBUTION UNDER THE CLAW IN CALVES AND HEIFERS OF DIFFERENT CATTLE BREEDS

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The objective of this study was to analyse the development of body and claw traits in German Holsteins (GH), German Brown (GB) and German Red Cattle (GR). Measurements included were body weight, height and proportions of the body as well as claw measures like length of the dorsal border, the heel and the diagonal, the height of heel, the angle of the dorsal border and the hardness of the claw horn. Furthermore, the pressure distribution under the claws was analysed using an electronic measuring system. The measurements were taken in 56 calves (34 GH, 10 GB, 12 GR) which were examined every two months from the age of 2 to 17 months. Linear animal models were employed for the analyses.

The study showed that the development of the body weight and measures in GR is in due proportion to the development of the claws, so that the relation of the pressure distribution and weight load of the claws was proportionally to the body weight and size during the first 17 months of life. On the contrary, in GH the body weight in relation to the growth of the claw and its claw horn capsule increased superproportionally leading to a weight overload of the claws. The results indicate that breeds with high growth capacity and not adequate development of the digit bones and claw horn capsule may be more susceptible to claw diseases.

315 (2025)

THERMOGENESIS ON THE CORIUM OF BOVINE CLAWS TRIMMED WITH DIFFERENT TYPES OF GRINDING DISKS

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The use of motorized disk cutters for claw trimming has been associated by several authors with excessive temperature increase of the corium and subsequent thermic damage. The temperature generation on the claw depends on the duration of direct contact between the sole horn and the rotating disk, as well as on the type of surface of the disk used for trimming - fine grain, coarse grain, or steel blades. The aim of this study was to measure the possible thermogenesis on the corium of the sole in bovine claws trimmed with different types of grinding disks.

For this experimental study, 4 different disks were tested on bovine feet from the slaughterhouse: an aluminium disk with small cutting steel blades, disks with 50% and 70% amount of fitted hard metal granulate and an abrasive disk. A correct functional claw trimming to a standard sole horn thickness of 5 mm in the toe area was carried out in 10 pairs of claws, with each of these disks, resulting in a total of 40 trimmed claw pairs. In four additional groups of 10 claw pairs each, incorrect claw trimming to a sole horn thickness of 2 mm in the toe area was performed.

The claw specimens were prepared following a standard protocol; thermocouples were introduced into drill holes to the sole corium. Measurements were taken using a laptop equipped with a program of National Instruments (Lab View) and 3 thermocouples continuously during claw trimming and during the 2 following minutes in order to measure a possible protracted temperature increase caused by the insulating properties of horn.

In the correctly trimmed claws, there was a minimal temperature increase in the corium in the groups where the disks with steel blades and with hard metal granulate were used (median < 0.26 , SD: 1.48) and a moderate increase (median < 0.74 ; SD: 1.41) in the claws trimmed with the abrasive disk. Incorrect claw trimming also caused only a minimal temperature increase in the groups where the disks with steel blades and the hard metal granulate were applied (median < 1.57 ; SD: 3.61). The highest increase was documented in the group where the abrasive semiflexible disk was applied (median: 2.46; SD: 2.44).

These results demonstrate that thermogenesis caused by motorized claw trimming with the disks described is negligible if correct functional claw trimming is performed. Even if the claws are severely over trimmed, pathological thermic effects to the corium are also unlikely, except in claws where the abrasive disk was applied.

316 (2020)

NEW SURGICAL APPROACH TO THE PLANTAR FETLOCK JOINT THROUGH THE DIGITAL FLEXOR

TENDON SHEATH WALL IN CASES OF CONCURRENT SEPTIC SYNOVITIS

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This report describes 2 cases of septic tenosynovitis of the digital flexor tendon sheath of right lateral hind-digits - one a fibrinous-purulent tenosynovitis of a Simmental cow with an age of 4.7 years, the other a sero-fibrinous tenosynovitis of a 2.5 year old Charolais bull - and a concurrent serofibrinous arthritis of the adjoining fetlock joint, caused by penetrating wounds over the digital tendon sheath area, which were treated using a novel surgical approach to the affected fetlock joint.

The cow showed a grade 3 (of 4) lameness, the bull was non-weightbearing. In both patients, the digital flexor tendon sheath was initially opened through the penetrating wound. Both digital flexor tendons and the inflammatory exudates present were removed. In both cases, a small puncture through the fetlock joint capsule was detected, located just distal to the lateral proximal sesamoid bones. The tract was surgically enlarged and a second opening into the plantar fetlock joint pouch with a 2-3 cm long and 5 mm wide incision between the two lateral suspensory ligament branches was created, giving easy access to the plantar joint aspect and pouch allowing removal of fibrin clots and effective joint lavage using 5 litres of sterile saline solution. Both incisions of the fetlock joint capsule remained unsutured and they were drained using LIGASANO-soft polyurethane foam dressing to preclude premature closure. Also, the tendon sheath wound remained unsutured.

In both patients, the digital flexor tendon sheath and the fetlock joint were lavaged daily for the 3 consecutive days. The lameness improved rapidly within the following 10-14 days, the surgical wounds healed by second intention and the patients were discharged to their owners 16 and 17 days after surgery showing a grade 1/4 lameness. Twelve months later these animals were without lameness and showed a normal conformation of the digits on the operated limbs.

This new surgical approach to the infected fetlock joint proved to be easy and consequently making a second skin incision for lateral arthrotomy unnecessary. A particular benefit of the described method is the improved access to the plantar aspect of the fetlock joint, which is necessary for meticulous tract debridement and fibrin removal.

317 (3404)

CLINICAL EXPERIENCES WITH DORSAL THORACOLUMBAL EPIDURAL ANAESTHESIA FOR LAPAROTOMY IN CATTLE

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Objective: Surgical procedures from the flank as laparotomy, ruminotomy or sectio caesarea are common in cattle. The objective of this study was to examine the efficiency of the dorsal thoracolumbal epidural anaesthesia for analgesia in the right flank of cattle.

Methods: A dorsal thoracolumbal epidural anaesthesia was carried out prior to a laparotomy in the right flank of 21 Holstein Friesian cows (body weight 460 - 744 kg, age 1 - 8 years) suffering from abomasal displacement (19 LDA, 2 RDA). Xylazine (0.75 ml; 2%) diluted with procaine (2%) to a final volume of 5 - 6 ml was injected epidurally between the last thoracic and the first lumbar vertebra. The efficiency of analgesia was evaluated by scoring the defensive reaction during surgical treatments (1 = no defensive reaction; 2 = little defensive reaction; 3 = moderate defensive reaction; 4 = substantial defensive reaction; 5 = additional restraining necessary; 6 = additional local anesthesia along incision line necessary).

Results: In 3 cases the attempt to puncture the epidural space remained unsuccessful. In 18 cases the anaesthesia ensured a satisfying analgesia of the right flank for abomasal replacement and omentopexy lasting on average 48 (25 -70) minutes. The surgeries started 15 minutes post epidural injection. No side effects as spontaneous come down, damage to the spinal cord, ataxia of the pelvic limbs or respiratory dysfunction were observed.

Conclusions: The dorsal thoracolumbal epidural anaesthesia induced a satisfying analgesia of the flank without serious complications with a single injection of a low drug volume. Thereby, dorsal thoracolumbal epidural anaesthesia was shown to be as effective as conventional methods of flank anaesthesia.

318 (1191)

CRYOSURGICAL DEHORNING OF CALF

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Cryosurgery is one of the methods used for treating soft tissue lesions in which the destructive effect of cryogens like liquid nitrogen is used to induce tissue destruction. In dairy industry horn is considered as an unfavorable and sometimes harmful tissue, which has to be eliminated. Different methods have been proposed for dehorning with different advantages and disadvantages. In this study the effect of liquid nitrogen on ceasing or suppressing. Horn bud growth is evaluated.

The study was carried out on 12 calves in two age group, one and three weeks old Each group was subdivided into two subgroups for one or three freeze-thaw cycle one horn bud of each animal was

considered as treatment and the other one as control. A metal rod introduced in liquid nitrogen and after thermal equilibrium between the rod and the cryogen, the tip of the rod was put on horn bud of each animal for one minute. In three freeze-thaw cycle group the cycle was repeated 3 times the gross appearance and horn bud growth were evaluated on distinct intervals.

The result showed no significant difference between the one - cycle and control groups. There was a significant difference between the three - cycle and control group in less than one-week old calves. The average diameter and length reduction in treatment group comparing with the control was 88 and 96%, respectively. It was also concluded that the younger and calmer the animal was the better the result could be expected.

319 (1049)

THE ROLE OF LIQUORICE IN EXPERIMENTAL SKIN WOUND HEALING IN CALVES. A HISTOPATHOLOGIC STUDY

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Liquorice (Shirin Bayan), a derivative from the root of *Glycyrrhiza glabra*, from the family leguminosae, has been proposed in ancient Iranian texts for coughs, bronchitis, constipation, and especially gastric ulcers. Its effectiveness in prevention from or treating gastric ulcers has been the subject of some experimental works. Here the role of liquorice combined with sesame oil has been evaluated in experimental wound healing in calf. The study was carried out on two female ten-month-old Holstein calves in the same conditions. An area of 10×10 cm on each side of the thorax of the animals was surgically prepared and infiltrated with local anesthetic. Eight uniform skin wounds were created in tow rows by an 8 mm skin punch on each prepared area (total of 32 wounds). Each side of the animals was used to evaluate a remedy (group 1 to 4). To make the mixture, 16 grams of powdered and sieved liquorice was mixed with 36 grams of sesame oil to make a suspension. The wounds were treated for 7 days as follow: The wounds of group 1 to 3 were rinsed daily by normal saline, after which, in group 1 the mixture and in group 2 only the sesame oil was applied on wounds. In group 4, no treatment was applied. The wounds were left open. On day 8, skin biopsies were taken from the wounds and sent for histopathologic study (H & E staining).

In group one, 7 wounds (87%) showed moderate granulation tissue and mild scab formation, and complete re-epithelialization. In group 2, there were no granulation tissue, massive scab formation and minimal re-epithelialization. In groups 3 and 4, mild granulation tissue, severe scab formation and very limited re-epithelialization were observed.

It can be concluded that the mixture can be considered as an effective remedy for skin wound healing.

320 (850)

RELATIONSHIPS BETWEEN LAMENESS AND PRODUCTION AND MANAGEMENT VARIABLES IN MICHIGAN DAIRY HERDS

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The objective of this study was to identify which herd-level production and management variables were related to the prevalence of lame cows in 72 Michigan dairy herds. Data collection took place between March 1998 and January 1999. The proportion of adult cows in each herd with arched-back posture while standing and walking and/or lameness in at least one limb was calculated. Herd managers were interviewed about management practices. Herd production variables were calculated from Dairy Herd Improvement Association data. A multivariable regression model, created in a forward stepwise manner, identified frequency of addition of new bedding material, proportion of the herd hoof trimmed in the previous year, and herd mid-lactation mean milk fat concentration to milk protein concentration ratio for the year prior to lameness assessment, as significant ($P = 0.05$, model coefficient of determination = 0.25) variability factors of the herd prevalence of lameness. These results suggest that effort to reduce the prevalence of lameness should focus on bedding management and hoof trimming and that laminitis may be an important cause of lameness, since the milk composition changes associated with increased prevalence of lameness were similar to those expected with sub-acute rumen acidosis, a risk factor for laminitis.

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321 (1825)

COMPARISON OF TETRACYCLINE CONCENTRATION IN PLASMA, SYNOVIAL FLUID AND MILK FROM PAPILLOMATOUS DIGITAL DERMATITIS DAIRY CATTLE SUBMITTED TO INTRAVENOUS AND INTRAVENOUS REGIONAL SINGLE-DOSE

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The majority of lameness in cattle originates in the foot. Currently, there are only limited treatment options available and source of significant economic loss also. The antimicrobial therapy should be based in bacterial

sensitivity and pharmacokinetics features. The antimicrobial agent should have a broad antibacterial spectrum and achieve high concentrations in all tissues infected, particularly bone and synovial fluid. Intravenous regional antibiotics consist in place a rubber tourniquet around distal limb during up to 60 minutes and injects a water-soluble antibiotic into a digital vein. This method has been used to improve podal disease treatment. The purpose of this study was compare single-dose concentrations of crystalline tetracycline chloridrate, injected intravenous (IV) and intravenous regional (IVR) in bovine plasma, synovial fluid and milk from dairy cattle. Twelve milk production dairy cattle were used with various stages of digital dermatitis. Cows were randomly assigned to two groups of six animals. Group 1 will inject a single-dose of 10 mg/Kg crystalline tetracycline chloridrate IV and Group 2 with 1000 mg of crystalline tetracycline chloridrate IVR. Blood, synovial fluid and milk samples were taken from jugular vein, left tarsal joint and mammary gland respectively at 0 (control), 22 minutes, 45 minutes, 1 hour and 22 minutes, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96 and 120 hours late injections. Tetracycline concentrations in samples were determined by high-performance liquid chromatography (HPLC). Mean values tetracyclines plasma concentration in Group 1 were higher than Group 2. Synovial fluid and milk tetracycline concentration showed opposite compartment. Group 2 tetracycline concentration in synovial fluid was higher than Minimal Inhibitory Concentration (MIC) during 24 hours for the most frequent bacteria, which cause claw disease. Mean milk antibiotic concentration in Group 2 indicated that would be necessary withdraw up to 96 hours. IVRA with tetracycline was a plus technique to dairy cattle podal disease. Milk residues decreased and synovial fluid concentration increased this regional antibiotics. Funding: NOVARTIS, FUNDUNESP 012/02-DFP

322 (1159)

AETHIO-PATHOGENESIS OF DERMATITIS DIGITALIS IN CATTLE

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Digital dermatitis (DD) was considered to be multifactorial skin disease of the bovine digit, which cause big economical losses in dairy industry. Although all bacteriological causes are more or less determined as *Fusobacterium necrophorum*, *Bacteroides nodosus* and *Clostridium perfringens* are, the role of *Treponemas* was still not totally clear.

In our experiment *Treponema denticola* and *Treponema brennaborse* were determined out of clinical cases. After 96 hours maceration of the healthy skin above claw those *Treponemas* cultures had successfully reproduced all clinical signs of the disease. On the light and electron microscope *Treponemas* were detectable adhering to the surface and between the skin cells of the outer layer of epidermis. Both gross and histopathologic investigations of the hairy skin adjacent to the interdigital space are significant factors when considering its susceptibility to infections and site of lesions. Our theory is based upon hypothesis that prior trauma to the digital skin and combined interaction of different already known infectious agents are necessary to provoke all typical signs of the digital dermatitis.

323 (3137)

THE USE OF ANIMAL-BASED MEASUREMENTS TO EVALUATE TIE STALL DESIGN AND STOCKMANSHIP IN ONTARIO DAIRY CATTLE

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A cross sectional study was conducted on 317 Ontario tie-stall dairy farms to explore the prevalence of the following variables: hock and neck lesions, arched backs, outward rotation of the hind claws, cleanliness of the udder and hind limbs, teat injury and broken and docked tails. All lactating cattle on the study farms were scored for each of the variables. The above listed variables were analyzed descriptively and for correlations between tie stall length, stall width, tie rail height, chain length and the outcome variables of milk production, somatic cell count and culling rates.

Average herd size of the study farms was 56 lactating animals. On 88% of farms, hock abrasions were observed on 10% or more of the cows. The percentage of cows with hock abrasions was positively correlated with the width of the stall. ($r = 0.1665$, $p = 0.009$) Cows with an arched back while standing were positively correlated ($r = 0.1836$, $p = 0.004$) with one or both hind claws rotated outward more than 20 degrees. Dorsal neck abrasion was positively correlated with tie rail heights, ($r = 0.2858$, $p = 0.0002$) between 35-42 inches. Farms with rails higher than 42 inches had few neck abrasions. Greater than 5% of cows had broken tails on 67 (21%) of the farms.

The average herd size of the study farms approximates the average lactating herd size of Ontario dairy farms. The finding that hock abrasions increase with the stall width could be explained by the greater amount of leg movement the cows can perform while lying or rising. This in combination with a lack of sufficient bedding could cause more hock abrasions. Lameness has previously been correlated with both arched backs while standing and rotated hind claws. As lame cows display both of these signs, it is not surprising that the two variables were positively correlated. The correlation between neck abrasions and rail height may indicate that when the rail is lower than 42 inches, the dorsal aspect of the cow's neck repeatedly grazes the rail when rising or eating. Cow tails may occasionally be broken from being stepped on by a neighbouring cow, however when multiple cows have broken tails the handling techniques of the farm staff should be reviewed. A sample

of farms will be revisited in order to investigate the causal relationships of significant correlations.

324 (5008)

INTERNATIONALIZING DAIRY HEALTH SOFTWARE SYSTEMS

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Many excellent software programs exist for collecting and reporting dairy herd health data. When there is a need for a program in a different country, barriers of language and other conventions, such as the format for displaying dates, may require an entirely new version of software.

Our goal was to develop methods to bridge international barriers to gathering health data with a single software program. Software was converted to an international format so it would be easier to create "localized" versions for each country or language when needed. Accommodation for different operating systems, monetary systems, and characters in languages were made. The techniques used can be applied to other software programs. Examples of program input and/or report outputs were developed in Vetcheck Dairy Software for English, Spanish, Portuguese, French, Japanese, and Norwegian.

Funding: Medford Veterinary Clinic, Medford, Wisconsin USA

325 (2259)

A SERO-EPIDEMIOLOGICAL STUDY OF BOVINE LEPTOSPIROSIS IN KARAJ

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A serological study was conducted in order to investigate the present status of leptospirosis in cattle in Karaj region of Tehran province between July and December 2001. For this study a total of 203 serum samples were randomly collected from 60 herds. All serum samples were serologically tested by Microscopic Agglutination Test (MAT) using live antigens representing *Leptospira interrogans* serogroups: Grippityphosa, Icterohamorigia, Canicola, Hardjo, Ballum and Pomona.

The results of this study showed that 95 (46.8%) samples belonging to 39 (60%) of the herds had a positive reaction against one or more serogroups. The most prevalent *Leptospira* serogroup was canicola (59 samples) and then ballum (37 samples), grippityphosa (36 samples). The less prevalent *Leptospira* serogroup was hardjo (7 samples). None of the samples had seropositive reaction against serogroup Icterohamorigia.

As many of visited herds had at least one unvaccinated dog for guarding the herds, it is concluded that the high prevalence of Canicola serogroup can be associated with close contact between dogs and cows.

Therefore, more attention to herd's dogs is needed in order to vaccinate the dogs and reduce the close contact between these species. In order to investigate the role of dog in bovine leptospirosis in Karaj, a serological study of leptospirosis in dogs is suggested.

Funding: University of Tehran

326 (2534)

EVALUATION OF THE ANTIBODY RESPONSE INDUCED IN NAÏVE CATTLE BY AN INACTIVATED BOVINE VIRAL DIARRHEA VIRUS VACCINE

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The aim of this study was to determine the serological response as measured by the level of neutralizing and non-structural 3 (NS3) protein antibodies induced in naïve cattle by the vaccination with the inactivated vaccine Bovilis® BVD (Intervet International) which contains a type 1A cytopathic bovine viral diarrhoea virus (BVDV) strain (C86).

The trial was performed with a total number of 30 seronegative cows and heifers (27 vaccinated and 3 controls) from one dairy herd free of persistently infected animals and never vaccinated against BVDV. During the trial, no animals were introduced in the herd.

Animals were vaccinated on days 0, 30, 270 and 654 of the experiment course. Blood samples were collected from all the animals on days 0, 30, 60, 270, 300, 654, and 684. The levels of antibodies against BVDV were determined by a standard virus neutralisation test (VNT), using Oregon C24V strain on embryonic bovine trachea cells (EBTR) in micro-titre plates, and with 2 commercially available ELISA tests that only detects antibodies to NS3 protein of Pestivirus (INGEZIM BVD, Ingenasa, Spain and SERELISA BVD/BD mono blocking Ab, Synbiotics, France).

In VNT, each serum was tested four times. Antibody titres were calculated by the Spearman-Kärber method. In vaccinated group the mean logarithmic titres were calculated and their titres were expressed as the reciprocal of their antilogarithm.

No seroconversion was detected in any of control animals during the trial. Mean neutralizing antibody titres (MNAT) obtained were 4, 138, 49, 490, 81, and 390 on days 30, 60, 270, 300, 654 and 684, respectively. The third dose of Bovilis® BVD had a high booster effect on neutralizing antibody response, as one month after its administration the MNAT increased 10 times and it was almost 1.7 times higher than the MNAT that had the six seropositive animals from the same herd at the beginning of the trial.

As none of the samples from the vaccinated animals gave a positive result by any of the two commercial ELISA tests, therefore Bovilis® BVD could potentially be used as a marker vaccine.

327 (3217)

THERMOSENSITIVE VACCINE ADJUVANTS FOR CATTLE: CONTROLLED RELEASE AND EFFICACY
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Vaccines based on inactivated antigens generally contain adjuvants to improve efficacy. Adjuvants work through two main mechanisms based on depot effect and immuno-stimulant components. A galenic formulation was used to look for a fluid vaccine (easy to inject) giving low local reactions and insuring at the same time short term and long term efficacy by controlled release pattern. A thermo-thickening emulsified system was developed and assessed for physicochemical parameters and adjuvant effect in mice with a model antigen and in cattle. The specific system produces a thousand time viscosity increase when moved from low storage temperature (4°C) to physiological one (37°C). The consequences of this thermo-thickening on release kinetic of an aqueous probe were studied in vitro. Particle sizing, stability program and optical microscopy were used to test emulsions. Thermo-sensitivity was also assessed through rheology and microscopy. Different formulations were developed ranging with variable biodegradability. The influence of biodegradability on safety and efficacy already observed for traditional water in oil emulsions was confirmed. This type of adjuvants, easy to use and rendering fluid aqueous vaccines, is the answer to some field requests for easy to inject vaccines and is suitable for cattle but also more sensitive animals.

328 (2686)

BOVINE PAPILLOMAVIRUS DETECTION FROM BOVINE TEATS BY IMMUNOHISTOCHEMISTRY AND ELECTRONMICROSCOPY

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We examined the teats of slaughtered cattle to survey the prevalence of papilloma and to detect Bovine Papilloma Virus (BPV).

Out of 880 examined heads, 432 heads (49.0%) was Holstein and 448 heads (51.0%) was Korean native cattle. Out of 432 heads (Holstein), 263 heads (60.8%) had papilloma lesions on teats. But out of 448 heads (Korean native cattle), only 33 heads (7.4%) has papilloma lesions on teats. Atypical flat form papilloma: 224 heads (85.2%) in Holstein cattle and 32 heads (97.0%) in Korean native cattle.

By immunohistochemistry (IHC), BPV antigen was detected in 35 heads (22.9%) of 153 heads (Holstein) which had papilloma lesions. But in Korean native cattle, BPV antigen was detected in 1 head (3.8%) of 26. By electron-microscopy (EM), in Holstein papilloma cases, BPV particles were detected in 31 heads (39.2%) of 79.

We concluded that papilloma is a very prevalent disease in Holstein teat and the prevalence of papilloma in Holstein teat was higher than that of Korean native cattle. It is needed to develop diagnostic methods to detect BPV from clinical cases because IHC and EM are not sensitive methods to detect BPV.

329 (1728)

LABORATORY DIAGNOSIS OF BOTULISM: WHICH SAMPLE TO SUBMIT?

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Botulism can have devastating effects on an affected farm, but since only small amounts of toxin are needed to induce clinical symptoms or death, diagnostic laboratories need a highly sensitive test to detect the toxin in biological samples. The golden standard at this moment still is the mouse lethality test, but ethical and legal restrictions make this test increasingly difficult and expensive.

Therefore it is important to select samples with the highest likelihood of yielding a positive result. A small survey was done in the two laboratories in Belgium where the diagnosis of botulism in animals was done during the period of 1994 to 2001.

A total of 469 samples were selected for this survey originating from horses (76), cattle (209), birds (42) and animal feedstuffs (142). The inclusion criteria for the samples were: multiple dead or affected animals on the premises and symptoms of acute death or symptoms of dysphagia and/or paralysis. Overall only 6.1% of the samples were positive for botulinum toxin. Samples from horses and feedstuffs yielded the lowest number of positive diagnoses (resp. 1.3 and 1.4%). Cattle samples gave 5.6% positive diagnoses and 44.8% positive results were obtained from bird samples.

The samples from cattle and birds were further subdivided in the type of material that was submitted for analysis. Only 2.1% respectively 1% of the ruminal contents and serum samples of cattle were positive, whereas 21% of the liver samples were positive. In samples from birds stomach contents, liver, serum and intestinal contents gave positive results in 33%, 44%, 75% and 33% respectively.

Statistically significant conclusions are difficult to make because of the known low sensitivity of the test and the small numbers of positive analyses. A first conclusion might be that in cattle suspected of botulism,

submission of a liver sample seems to yield the highest likelihood of obtaining a positive result. Serum samples from suspected cattle seem very unlikely to be of diagnostic value compared to serum samples from birds. Since no determination of the amount of circulating toxin was done, it is unclear whether this reflects a lower ingestion of toxin in cattle or a faster passage of the toxin to nerve endings. One indication for a possible difference of toxin distribution in different species may be that, as in birds, in human medicine botulinum toxin also can be readily demonstrated in serum.

330 (2895)

A STUDY ON MYCOBACTERIUM BOVIS IN GOAT AND SHEEP AND RELATION WITH THE INFECTED COWS

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Objective: Diagnosis of tuberculosis disease in goat by isolation Mycobacterium and to differentiate its strain.

Design: observational study. Animals: The border between midcranial and midcaudal in the left side of neck of one thousand goats. Procedure: To shave 4x20 cm dimension this site of neck; Injection of the avian and mammalian tuberculin in upper and lower points respectively with interval of 10-20 cm.; To measure thickness of skin in injected points and to compare the skin before and after the test in 72 hours after injection time (comparative intradermal test); Clinical and necropsy examination in reactor and suspicious goats; Sampling of organs with visible lesion and available lymphoid glands in non visible lesion; To do Bacteriology, Polymerase Chain Reaction and Pathological tests on samples.

Results: In all goats which were studied, 7 goats responded positive and 4 suspicious. In necropsy findings, 6 goats had non visible lesion and 5 had visible lesion. Mycobacterium was not isolated in NVL but among 5 goats with VL; Mycobacterium bovis and M. tuberculosis strains were isolated and differentiated in 3 goats and 1 goat respectively. PCR test confirmed those results. Pathological consideration showed typical lesion granulomatosis.

Clinical implications: This study is the first research about mycobacterium in goat in Iran. It shows that the presence of tuberculosis in goat is expected in Iran.

Funding: Islamic Azad University of Sanandaj, Iran

331 (5064)

FREE ENDOTOXINS (ET) AND ENDOTOXIN-EFFECTORS (ALA-AK, HAPTOGLOBIN) IN FREQUENT RUMINANT DISEASES

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Objective: Disturbances of the lipid metabolism causes a reduced ET neutralisation and clearance which could result in free ET, supported by a direct flood of ET from the adipose tissue. The diseases in the early lactation (table) are partially created due to the interactions of the ET metabolism.

This study aimed at checking two problems with regards to frequent ruminant diseases: 1. Can an increase in ET or reactions of its effectors be proven in blood? 2. Are there any changes traceable already before the outbreak of each disease including the dry period that makes pathophysiological conclusions or an early diagnostic usage possible?

Material and methods: In two dairy farms total 45 healthy cows and 144 cows that developed the different diseases were analysed before and after parturition. In detail ET, antilipid A IgG-titre (ALA-AK), TEAC, haptoglobin and Fe were tested.

Results: The ET (EU/ml) concentrations (medians) were in healthy and sick cows on 3rd day after parturition: healthy cows (n=45) 0.42; sick cows (144) 0.48; endometritis 0.72; reproduction disorders 0.38; mastitis 0.58; retained placenta 0.24; milk fever 0.91; diseases of claws 0.36. The ET concentrations of all ill cows have only tendentially and not significantly increased in comparison to healthy cows. However, increased ET concentrations were observed in cows that developed Retentio secundinarum and milk fever 1 week before parturition, only in cows with milk fever, endometritis as well as mastitis on the 3rd day after parturition. In comparison to healthy cows, the ALA-AK only increased on the 14th day after parturition in ill cows. In comparison to healthy cows the haptoglobin concentrations were generally securely higher in ill cows after parturition. The Fe concentrations were significantly lower in ill cows compared to healthy cows. Haptoglobin correlated securely negative with the Fe concentrations. The TEAC concentrations fell near parturition; in Retentio secundinarum they were significantly lower 1 week before parturition in comparison to 14 days after parturition. Earlier research proved that before the diagnosis of abomasal displacement, free ET concentrations clearly increased.

Conclusions: ET have obvious clinical significance in different diseases nearly parturition. Enhanced haptoglobin concentrations show the importance of physical stress.

332 (5058)

PARTUS INDUCED ACUTE-PHASE-REACTION (APR) AND POSTPARTAL DISEASES IN COWS

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Objective: The knowledge about the diagnostic significance of Acute-Phase-Proteins (APP) in cows is incomplete. Therefore we examined the concentrations of haptoglobin (Hp) and of serum amyloid A (SAA) before (b.p.) and during the first four weeks after parturition (a.p.) in healthy and in cows with different postpartum diseases.

Experimental design: In a first study we periodically checked 250 cows (mean: 7000 kg milk a year) five days b.p. and up to four weeks a.p. from healthy cows (n=15) and cows with Mastitis phlegmonosa (n=3), Mastitis catarrhalis (n=4), Retentio secundinarum (n=12), infections of urinary tract (n=4), Pneumonia (n=4), Arthritis and Panaritium (n=6) as well as Dislocatio abomasi (n=5). In a second study we checked Hp in 40 healthy cows (leukocytes < 10G/l) in the same way.

Results: Cows which developed Mastitis phlegmonosa had the biggest increase in Hp concentrations on the 1st and 3rd day after a.p., 3.64 ± 1.90 g/l and 5.29 ± 0.80 respectively. Equally, the Hp concentrations in cows with Retentio secundinarum ($1.05 \pm 1.00/ 3.46 \pm 1.40$ g/l) as well as in cows with Mastitis catarrhalis ($1.16 \pm 2.00/ 3.49 \pm 1.30$ g/l) increased significantly on the 1st and 3rd day a.p. in comparison to healthy cows.

Additionally, cows that developed Dislocatio abomasi also had increased Hp concentrations on the 1st and 3rd day a.p. The SAA concentrations rose in cows with Mastitis and cows with Retentio secundinarum. In the second study, we found Hp concentrations < 0.5 g/l in "absolutely" healthy cows.

Conclusion: Especially Hp is a very sensitive APP in cows. Significant changes of Hp are already detectable in the subclinical state. For example Hp increased on the 1st and 3rd day a.p. in cows which later became sick with abomasal displacement and Mastitis.

333 (2728)

SEPARATION AND PARTIAL CHARACTERIZATION OF FUSOBACTERIUM NECROPHORUM DEOXYRIBONUCLEASE

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Fusobacterium necrophorum subsp. necrophorum, one of the important pathogens of purulent infections in man and animals, produces extracellular deoxyribonuclease (DNase). Little is known about the molecular size and activity of DNase. To elucidate the problems, we attempted to separate and characterize the DNase in the fusobacterial culture.

Methods: F necrophorum subsp. necrophorum VPI 2891 strain was anaerobically cultured at 37°C for 2 days in modified heart infusion broth (200 ml) supplemented with K₂HPO₄, glucose, cysteine-HCl and ascorbic acid at pH 7.4. The culture supernatant was treated with 95% ethanol precipitation and the precipitate was collected with centrifugation, dissolved in 20 mM Tris HCl buffer (pH 7.5, 5 ml), dialysed for 4-5 h and applied to DEAE-cellulose column chromatography. Each fraction (3 ml) was estimated DNase activity by referring to the method of Schermann and Blobel (1968). The fractions showing the positive reaction were examined by SDS-PAGE. Additional activities were also inspected.

Results: After treatment of DEAE-cellulose column chromatography, the fusobacterial DNase was purified about 58-fold in relative purification with 0.7% recovery. In SDS-PAGE analysis, DNase positive fraction gave a single band which corresponded to molecular size of about 115 kilo dalton. No apparent band was demonstrated in the lane which was stained with Schiff's reagent. The active preparation showed no effect on RNA.

Discussion and conclusion: The fusobacterial DNase in the culture supernatant was separated by the treatments with ethanol precipitation and DEAE-cellulose column chromatography. SDS-PAGE profile revealed only one band.

This suggests that these simple procedures seem to be practical for the separation of the DNase, though its recovery rate was not high. Whether the fusobacterial DNase operates to decompose bovine cell DNA at the bacterial infection, is still unknown. Further work is required to elucidate the interaction of the bacterial DNase and bovine cells.

334 (1904)

ABATTOIR SURVEY OF BOVINE PYELONEPHRITIS

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Pyelonephritis is a specific infection of the urinary tract of cattle, caused by some bacteria particularly Corynebacterium renale, characterized by chronic purulent inflammation in the bladder, ureters and kidneys. E.coli, Proteus sp., Klebsiella sp., Staphylococcus sp., Streptococcus sp. and Pseudomonas aeruginosa are also common causes of lower urinary tract infection and pyelonephritis in all species.

In order to investigate the prevalence rate and detection of etiological agent of pyelonephritis in Shahrekord abattoir, kidneys of 404 slaughtered cattle (207 female and 197 male) were inspected. The number, age and gender of slaughtered cattle were recorded. Tissue samples from kidneys, which had gross changes, were obtained for histologic examination. Also urine samples from bladder renal tissue samples and were collected aseptically for microbiological examination.

In the histologic examination pyelonephritis were observed in 3 cases which one occurred in a cow 7 years

old and two in bulls under 2 years old. The lesions included a marked neutrophil infiltration in the renal pelvis, urinary tubules and interstitial connective tissue with tubular epithelial cells necrosis and tubular destruction. Clumps of polymorphonuclear leukocytes were visible in casts in many collecting tubules.

In the bacteriological examination *E.coli* was isolated from urine and renal tissue in 2 cases. In another case *Staphylococcus* isolated only from renal tissue. Therefore the prevalence rate of the disease was estimated as 0.74% and *E.coli* was the main aetiological agent of pyelonephritis in this study.

Funding: University of Shahrekord

335 (2278)

MALIGNANT CATARRHAL FEVER IN BRAZIL

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Malignant catarrhal fever (MCF) is a generally fatal disease of cattle, buffalo, and many species of wild ruminants caused by a herpesvirus. The disease is characterized by low morbidity but high mortality. The clinical signs of MCF are highly variable and range from peracute to chronic with, in general, the most obvious manifestations developing in the more protracted cases. In the peracute form, either no clinical signs are detected, or depression followed by diarrhoea and dysentery may develop for 12-24 hours prior to death. In general, the onset of signs is associated with the development of a fever, increased serous lachrymation and nasal exudates, which progresses to profuse mucopurulent discharges. Animals may be inappetent and milk yields may drop. Characteristically, progressive bilateral corneal opacity develops, starting at the periphery. In some cases skin lesions appear (characterised by ulceration and exudation), which may form hardened scabs associated with necrosis of the epidermis, and are often restricted to the perineum, udder and teats.

Salivation associated with hyperaemia may be an early sign, progressing to erosions of the tongue, hard palate, gums and, characteristically, the tips of the buccal papillae. Superficial lymph nodes may be enlarged and limb joints may be swollen. Nervous signs such as hyperaesthesia, incoordination, nystagmus and head pressing may be present in the absence of other clinical signs or as part of a broader more characteristic syndrome. In this communication, we examined 173 samples of brain tissues from bovines of São Paulo state with neurological disorders and Rabies negatives. These samples were tested for presence of MCF by direct immunofluorescence to identify viral antigen. No one of 173 tested samples was positive for MCF. The results observed in São Paulo State disagree with other reports previously described in Northeast of Brazil. In Bahia state were related 2 outbreaks of MCF in two different farms, but not confirmed by laboratory diagnostic.

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336 (5038)

HERD-LEVEL SENSITIVITY, SPECIFICITY, AND PREDICTIVE VALUES OF BOVINE TUBERCULOSIS SKIN TESTS IN MICHIGAN

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In managing the bovine tuberculosis eradication program in Michigan, correct classification of infected herds is the most relevant actionable event for regulatory intervention because, with few exceptions, the entire herd is depopulated if one or more animals within a herd are diagnosed with bovine tuberculosis. A shareware program was used to estimate herd-level sensitivity (HSe), specificity (HSp) and predictive value positive (HPVP) and negative (HPVN) for the bovine tuberculosis tests as they were implemented in Michigan. Four different scenarios were simulated: 1) serial interpretation of the caudal fold and comparative cervical tuberculin tests, 2) serial interpretation of the tuberculin tests, and mycobacterial culture and polymerase chain reaction when the later two are used in parallel, 3) as for scenario 2, but specificity fixed 1.0, and 4) sensitivity was increased to 0.9 (4a) or 0.95 (4b) and Sp=1.0. Estimates of test sensitivity (Se), specificity (Sp), true prevalence within and among herds were obtained from the literature and in collaboration with regulatory veterinarians. An empirical distribution of herd sizes in the northern part of the lower peninsula in Michigan was used to simulate the variation in herd sizes in the area of Michigan where bovine tuberculosis is most likely to occur. Furthermore, scenarios 2 and 3 were used to investigate the effect of herd size on herd-level parameters. In general HSe estimates were reasonably high, ranging between 0.712 and 0.840 for the four scenarios, however, individual estimates all ranged between 0.0 and 1.0. Increasing Se only increased HSe little. Herd-level specificity estimates were lowest for the two scenarios (0.693 and 0.940, respectively) where Sp was not assumed perfect (scenario 1 and 2). HPVP estimates were very low (0.042 and 0.143, respectively) for the first two scenarios also, but increased to 1.0 when perfect specificity was assumed. The HPVN remained high for all four scenarios, ranging between 0.995 and 0.997 with very narrow confidence intervals. The size of the tested herd has a major effect on HSe, which increases as the herd size increases. Both HSp and HPVP decreased with increasing herd size. However, when Sp was assumed perfect, only minor effect was seen on HSp and HPVN. It is apparent that the tests used in identifying herds that are

infected with bovine tuberculosis work fairly well on a herd basis. However, as the herd size decreases so does the HPVN and HSe.

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337 (5039)

ESTIMATION OF SENSITIVITY AND SPECIFICITY OF BOVINE TUBERCULOSIS SKIN TESTS IN MICHIGAN WHEN A 'GOLD STANDARD' IS NOT AVAILABLE

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Four-hundred-and-ninety-four cattle from seven herds were tested with the caudal fold and comparative cervical tuberculin tests to detect infection with *Mycobacterium bovis* as part of the bovine tuberculosis eradication program in Michigan. Bayesian inference was used to estimate the sensitivity and specificity of the caudal fold tuberculin test and the comparative cervical tuberculin test in four different 'two-population-two-test' latent-class models. The two tuberculin skin tests could not be used in the same model, hence the sensitivities and specificities of the two tuberculin skin tests were evaluated against results of mycobacterial culture. Possible dependence between the caudal fold and comparative cervical tests, and mycobacterial culture was investigated using a conditionally independence model and three models allowing for dependence between sensitivities and specificities. One of the dependence models assumed perfect specificity for mycobacterial culture. There was a statistically significant negative correlation between the comparative cervical tests and mycobacterial culture, however the dependence between the caudal fold test and mycobacterial culture was not significant. Assuming conditional dependence between both skin tests and mycobacterial culture, the Bayesian estimates and posterior 95% credible intervals of the sensitivity and specificity of the caudal fold and comparative cervical tests were 0.854 (0.563-0.975) and 0.939 (0.899-0.982), and 0.758 (0.475-0.930) and 0.986 (0.962-0.999), respectively. The estimates of sensitivity and specificity obtained in this study generally agree with those reported from other studies in the United States.

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338 (1368)

SEROLOGICAL STUDY OF BOVINE VIRAL LEUKOSIS IN BORUJEN, LORDEGAN AND FARSAN

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Bovine leukemia virus (BLV) is a cancer-causing virus of cattle, which can be passed from mother cow to calf via the milk; BLV commonly infects dairy and beef cattle and is found in the marketed milk and meat of these animals. Although there are numerous causes for cancer, viruses are of major importance in this field, for example: HTLV-1 and HTLV-2. In addition, BLV is a cancer-causing virus of cattle that is related to above-mentioned viruses in the genus of deltavirus and family, retroviridae. Most infected cattle do not actually develop leukemia, but remain healthy and are not culled from the herd.

BLV infections are not limited for cattle. The virus can infect sheep and nonhuman primates experimentally and cause cancer in the sheep. In the laboratory it can infect the cells of many species including humans and other primates.

To determine the status of BLV incidence of herds in Borujen, Lordegan & Farsan (Chaharmahal & Bakhteyari Province), this investigation was carried out from September 2002 to November 2002 on 352 sera samples.

The aim of this study was to determine the BLV incidence and the effect of age, size of herds and other factors on incidence of the disease. Examination of the sera specimens by ELISA for antibodies to bovine leukemia virus revealed that the overall incidence was 8%. With respect to different cities, the incidences in Borujen, Lordegan and Farsan were respectively 3.6%, 4.3% and 20%. Obviously the highest and lowest incidences were observed in Farsan and Borujen, respectively.

There were statistical relation between some factors such as common needle and common rectal gloves use with the incidence of BLV in investigated herds ($\alpha = 0.05\%$). The lowest incidence was observed in 2-3 years old cows and the highest incidence were observed in > 5 years old cows. No statistical relation was observed between size of the herds with incidence of BLV ($\alpha = 0.05$).

Our results are in accordance with Digiacomo R.F., Henry E.T., Johnson R., Pelzer K.D., and Wentink G.H. with respect to common rectal gloves and with Pelzer K.D., Parodi A.L., Henry E.T. and Radostits O.M. with respect to common needle. We didn't find any statistical relation between size of the herds and incidence of BLV, Our results are not in accordance with Digiacomo R.F., Radostits O.M. and Blood D.C.

KEY WORDS: Bovine viral leukosis, ELISA, Incidence

Funding: Shahrekord University

339 (1317)

COMPARATIVE SEROEPIDEMIOLOGIC STUDY OF HUMAN AND BOVINE LEPTOSPIROSIS IN NEYSHABOUR (IRAN)

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A set of 300 sera from cattle and 60 sera from human was collected in Neyshabour. These sera were subjected to the microscopic agglutination test for antibodies to *Leptospira canicula*, *L.ichterohaemorrhagiae*, *L.hardjo*, *L.grypotyphosa*, *L.interrogans* serovar pomona. The serological results were confirmed by enzyme-linked-immunosorbent-assay. The results showed the highest prevalence of serogroup interrogans serovar pomona in both human and cattle. The high sero-prevalence in cattle is of public health concern because the close contact between cattle and man may provide the link between a reservoir in the environmental and susceptible human. Further informations about prevalence percent of each serogroup in human and cattle will be submitted in the presentation.

Funding: Teaching Hospital of Tehran University

340 (5072)

CLINICAL EFFICACY OF LINCOMYCIN / SPECTINOMYCIN INJECTABLE (LINCO-SPECTIN®) FOR THE TREATMENT OF EXPERIMENTALLY INDUCED MYCOPLASMA BOVIS ARTHRITIS IN CALVES

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Mycoplasma bovis associated arthritis is an increasingly important disease in young calves. A study was conducted to evaluate the efficacy of the lincomycin / spectinomycin injectable antibiotic association (Lincomycin 5 g + Spectinomycin 10 g / 100 mL)(L/S) (Pfizer, Paris, France) on experimentally induced *M. bovis* arthritis in young calves. Seventeen one week-old calves were included in the study. A live culture of *M. bovis* (Strain AFFSA Lyon # 1067, 10^8 UFC/mL) was injected (0.5 mL) in the right carpus of each calf. Twenty-four hours after inoculation, calves presenting arthritis symptoms were randomly assigned to either of two groups: Linco-Spectin (n= 10) and Control (n = 7). Calves in the Linco-Spectin group received 1 mL, L/S per 10kg per kg body weight IM). On the first day of treatment (Day 2) two injections were given 12 hours apart, This was followed by a daily L/S administration for the next 5 days (Day 3 to Day 8), starting 12h after the second L/S injection. To ensure their welfare, the calves were under strict veterinary supervision throughout the study. No treatments were administered to the calves in the Control group. Difference between right and left carpus circumference, rectal temperature and lameness intensity (ranked as 0 = no lameness; 1 = presence of lameness; 2 = marked lameness) were evaluated daily starting at the time of inoculation (Day 1) until Day 12 by blinded observers.

The difference in carpus circumferences was higher in the control group than in the Linco-Spectin group from Day 3 to Day 10 ($P < 0.001$); In the control group, out of 74 clinical evaluations for lameness, 64.9% were ranked as 2, 35.1% as 1 and 0% at 0 while in the Lincospectin group, out of 110 clinical evaluations for lameness, 21.8% were ranked as 2, 41.8% as 1 and 36.4% as 0. Lameness was both less frequent and less intense in the Linco-Spectin group ($P < 0.001$). Rectal temperatures were higher in the control group ($P < 0.05$) from Day 3 to Day 10. These results confirm the efficacy of the L/S in the treatment of *M. bovis* arthritis in non-ruminant calves.

341 (2334)

A CLINICAL STUDY OF BOVINE PESTIVIRUS DISEASE COMPLEX IN SLOVENIA

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An extensive review of clinical records kept in the archive at the Clinic for Ruminants in Ljubljana revealed the first relevant document on Bovine Virus Diarrhea Virus (BVDV) infection in Slovenia dating from the year 1962. The clinical picture and pathomorphological findings in a 6 months old Jersey heifer which was affected with incurable foul smelling profuse diarrhea resulting in death were diagnosed at that time as Mucosal Disease (MD). In our contribution about clinical findings on Bovine Pestivirus Disease Complex which were confirmed via blood analyses on BVDV antibodies and antigen were reported. The acute forms of MD, congenital defects in calf, outbreak of abortion and chronic forms of MD were established. High percentage (94%, 73%, 71% and 46%) of seropositive reactors in four herds from which our clinical cases of BVDV disease were taken, confirmed the presence of the persistent infected-viremic (PI) in cattle, which were infected via transplacental transmission of the virus during early period of gestation. All reported cases were also photo and video recorded. As it is evident from the results of our study, the BVDV has been present in herds of Slovenia cattle for a longer period of time.

342 (2348)

NATIONAL SURVEILLANCE PROGRAM FOR SALMONELLA DUBLIN IN DANISH CATTLE FARMS

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A national surveillance program for *Salmonella dublin* in Danish cattle farms was initiated in October 2002. The main purpose is to reduce the prevalence of *S. dublin* infection and thereby increase food safety. The

program is administered by the Danish Cattle Federation and controlled by the Danish Veterinary and Food Administration.

In the surveillance program all cattle farms are classified into two levels based on serological analyses (LPS-ELISA). From dairy farms bulk tank milk are sampled 4 times a year and classification is done from antibody measurements of 4 consecutive samples. In cattle farms without milk production (beef herds) the 3 blood samples from animals above 8 months of age submitted for the ongoing bovine virus diarrhea (BVD) eradication program are used for classification. All results are recorded in the Central Cattle Database and the results of calculation are stored in the Central Husbandry Register. Farms in Level 1 are considered most likely free of *S. dublin* and farms in Level 2 are considered most likely infected. Farms with a positive bacteriological diagnosis of *S. dublin* are classified into Level 3. Classification is also determined by trade in live animals. If a farmer buys animals from a farm with a poorer level than his own this poorer level is also given to the buyer's farm.

After the first year of the program 75.3% of the dairy farms are in Level 1, 24.5% (1797 herds) are in Level 2 and 0.2% are in Level 3. There are big variations in the prevalence of Level 2 farms among the geographic regions (range: 2.2%- 58.4%). In beef herds 74.8% (15,655 herds) are most likely free of *S. dublin* and 17.6% are still unclear. The latter is mainly herds without trade of live animals.

Except for farms with clinical outbreaks, there are no official restrictions put onto farms, irrespective of which level they belong. The farmers have received the program with great attention and there is a demand for control strategies in Level 2 and 3 herds.

The structure of the surveillance program will be presented together with a discussion of the consequences for the farmers.

Funding: Danish Cattle Federation

343 (1736)

THE PREVALENT PATTERN OF FIELD ISOLATES OF BOVINE VIRAL DIARRHEA VIRUS IN THE WORLD BASED ON GENOTYPING

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Bovine viral diarrhea virus (BVDV) induces various clinical manifestations in cattle. Based on the diversity of the viral gene, BVDV have been classified by genotype and subgenotype. The relationship between genotype or subgenotype and clinical manifestations is not well known. The development of BVDV infection has been reported everywhere in the world, however, regional differences in biological properties of BVDV are unclear. The clarification of these problems would be useful for the elucidation of the pathogenicity of BVDV. In the present study, genotypes and subgenotypes of BVDV field isolates from Japan, Germany and the United States of America (USA) were identified, and the prevalent pattern of BVDV in individual countries was estimated genetically. Subgenotypes were determined based on phylogenetic analyses of nucleotide sequences of the E2 coding gene of BVDV. Forty-five, 61 and 56 BVDV strains were isolated from naturally infected cattle in Japan, Germany and USA, respectively, between 1980 and 2003. In each country, two major subgenotypes were recognized as follows: BVDV1b (detection rate was 46.7%) and 1a (37.8%) in Japan, 1b (49.2%) and 1d (39.3%) in Germany, and 1b (46.4%) and BVDV2 (42.9%) in USA. BVDV1a, which has been considered a prototype of BVDV, was not prevalent in Germany (1.6%) or USA (10.7%). In USA, BVDV2 was the major prevalent genotype, but only a few isolates were classified as BVDV2 in Japan (2.2%) and Germany (3.3%). The most prevalent subgenotype 1b in each country constructed individual small clusters in the subgenotype 1b branch in the phylogenetic tree. No difference was recognized in clinical manifestations among BVDV-infected cattle in each country. Diarrhea, respiratory disorders and poor growth were most frequently recognized. In conclusion, although there was no relationship between genotypes or subgenotypes of BVDV and clinical manifestations, a peculiar prevalent pattern in subgenotypes was identified among the three countries.

344 (3196)

BOVINE PAPILLOMAVIRUS TYPE 2 INFECTION ON DIFFERENT CATEGORIES OF CATTLE WITH ENDEMIC ENZOOTIC HAEMATURIA

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The enzootic haematuria (EH) is a chronic disease that occurs in older cattle, since 2 years old, causing the death for persistent anemia, progressive emaciation and cachexia. In the EH aetiology are involved the chronic intoxication by the ingestion of bracken fern (*Pteridium aquilinum*) toxins and the bovine papillomavirus type 2 (BPV-2) infection. This assay had for objective to identify the presence of BPV-2 in the different animal categories of cattle with EH endemic and deriving from a farm with the pastures infected by bracken fern. There are used blood samples of 98 bovines stratified in three animal categories composed by: i) 41 cows; ii) 32 heifers; iii) 25 calves. In the cows, six animals presented clinical signs of EH. The Semi-Nested-PCR technique was used for the BPV-2 L1 gene detection in white blood cells. BPV-2 was detected in 46% (45/98) of blood samples. In the cows, 61% (25/41) of the samples presented the BPV-2, including the six samples of animals with EH clinical signs. In the heifers and calves, the BPV-2 was detected in 48% (14/32) and 24% (6/25) of the samples, respectively. These results present statistics significance ($p < 0.01$)

among the bovine categories and showed that in the categories where the animals are susceptible for the EH clinical signs (cows and heifers) the BPV-2 infection rate are significantly higher. The BPV-2 detection in all of the cows with EH clinical signs indicate your closely relationship with this disease. The results obtained in this assay suggest a low level transmission by vertical route what coincides with our results where the positives rates increased in agreement with the age group of the animal samples studied.

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345 (2684)

SUBPOPULATION OF LYMPHOCYTES IN HOLSTEIN-FRIESIAN CATTLE INFECTED WITH ENZOOTIC BOVINE LEUKOSIS

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Introduction: Enzoitic bovine leucosis (EBL) is a chronic disease caused by bovine leukemia virus (BLV), retroviridae (1). The characteristic feature of this disease is proliferation of lymphocytes in circulating blood or lymphoid tissues (2). Because EBL concern lymphocytes, immunological disorder or alteration in the lymphocyte subpopulation is suggested (3).

In this study, we investigated the changes of the lymphocyte subpopulation in the circulating blood of Korean native cattle infected with bovine leukemia virus.

Materials and Methods: We have used BLV infected and noninfected Korean native cattle reared in Chongnam Province for this study. Bloods are collected to the vacutainer containing EDTA. Using flow cytometry (Becton Dickinson, FACScan), we have tested the lymphocytes, and used cellquest software for analysis of FACS results. We used primary antibodies purchased from VMRD and secondary antibodies purchased from Serotec and Southern Biotech.

Results and Discussion: The average number of total lymphocytes of BLV infected and noninfected cattle is 15,000 and 9,300, respectively. The number of B cells and CD11b is increased in BLV positive cattle. The population of CD2, CD4, CD6 and CD8 is decreased in BLV positive cattle, but CD5 positive cells in BLV positive cattle is slightly increased. In dual staining, B cells and CD5 dual positive cells are prominently increased in BLV positive cattle.

Finally, B lymphocytes are increased and T lymphocytes are decreased in BLV infected cattle. But CD5 which is T cells marker is increased in BLV positive cattle.

346 (3354)

EPIDEMIOLOGICAL AND CLINICAL CHARACTERIZATION OF THE HIGH MOUNTAIN DISEASE IN CATTLE IN COLOMBIA

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High mountain disease is a clinical entity that affects bovine that are either transported or reside at high altitude. The bovine species is very prone to suffer from this disease probably due to its cardiopulmonary anatomical conformation but mainly to its great pulmonary vascular reactivity to low atmospheric O₂ pressure. This disease is commonly observed in cattle at 2000 m. Fifty percent of the milk in Colombia is produced by dairy cattle that reside above 2000 m., which places these cattle at a great risk of developing the disease. Studies in Mexico and USA have shown prevalences between 0.45 to 0.65%. The main objectives of this study were to determine the prevalence of this disease in the "Cundi-boyacence plateau" in Colombia as well as to characterize clinically the disease using the cases presented to the large Animal Clinic at the College of veterinary medicine, Universidad Nacional de Colombia.

A survey was conducted in 39 dairy farms during 6 month but information was on a complete year. The interviews were done by one of the researchers and each farm was visited three times during the study. The information was to characterize the farm regarding size, management, feeding, health programs and cases that presented in a year. All the records of the cattle admitted to the large animal clinic in the last seven years were reviewed and the ones with signs of heart failure were selected. Only cases confirmed to have high mountain disease by necropsy and histology were included in the study. Statistical analysis included a Chi-2, Fisher exact test, Odds ratio and binary logistic regression ($p < 0.05$). 33.3% of the farms had at least a case of a clinical picture compatible with high mountain disease. The prevalence in the farms that reported the disease ranged between 0.47-5.46%. At the animal level the prevalence was 0.5%. The risk factors determined by the logistic regression model ($p < 0.05$) were the extension and the topography of the farm. The clinical signs observed were pectoral oedema, jugular distension, depression, submandibular oedema, poor body condition and weakness. AST and GGT were increased, stress leukogram. Arterial PO₂ was normal. Necropsy showed transudate in all body cavities, cardiomegaly, chronic passive congestion and histologically the diagnostic lesion was hypertrophy of the median layer of the pulmonary artery.

347 (822)

PREVALENCE OF CARDIAC MURMURS IN CLINICALLY HEALTHY CATTLE

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Congenital and acquired cardiac diseases are not uncommon in cattle. Cardiac defects and valvular endocarditis have been reported in different breeds of cattle. However, no study has been done on the prevalence of cardiac murmurs in this species of animal. Therefore this study was undertaken to find out about heart sounds abnormalities in apparently healthy cattle population. 200 cows were selected randomly from different dairy farms of Shiraz area. These cows were chosen on the base of criteria: 1- Holstein breed, 2- either pregnant or in milking stage and 3- in the range of 2 to 10 years (divided in three age groups; less than 3, 3 to 6 and more than 6 years old). All cows were examined by two examiners using both the ordinary stethoscope and meditron sensor stethoscope. Finally the cows with cardiac murmurs were auscultated by both examiners simultaneously using sensor stethoscope.

In general 15 cows had heart sound abnormalities; 10 diastolic, 13 systolic and 2 had both systolic and diastolic murmurs.

The diastolic murmurs were equally divided between aortic and pulmonary valves and all were either early or mid diastolic. The systolic murmurs were mainly tricuspid origin and two of them were holosystolic. None of cattle with murmurs, but one, showed signs of cardiac diseases. The one with holosystolic murmur had jugular distention and pulsation. The history showed that the cow was suffering from a chronic mastitis. 15 cows were more than 6 years old and the remainders were 3 to 6 years and none of the cow less than 3 years had murmur. This preliminary study showed that cardiac murmurs are not common in cattle as compared to small animals and horses.

Funding: Shiraz Vet. School

348 (2982)

DISEASES WITH NEUROLOGICAL SIGNS OF CATTLE IN CENTRAL BRAZIL

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The death of bovine caused by diseases that present neurological signs can incur serious financial losses. The knowledge of the profile and incidence of neurological diseases in Central Brazil is very important for control and prevention. All the bovine at the Veterinary Hospital of the University of Brasilia that showed neurological signs were used. The bovine were submitted to a clinical exam, with samples collected for laboratory exams in accordance with clinical suspicion. Nervous tissues from animals that died were collected, examined macroscopically and, later fastened in formalin, processed and analyzed by optic microscopy. Part of the samples were sent to be diagnosed for rabies. In the animals suspected of botulism, liver samples were collected, along with ruminal and intestinal content. These samples were frozen and sent to be tested for botulinic toxin. Samples were submitted to test BHV 5 and cultured. 59 animals were examined with neurological symptoms and 20 different diseases were diagnosed. 18 cases of rabies (30,5%), five of polioencephalomalacia (8,5%), five of blackleg (8,5%), four of HVB-5 (6,8%), four of botulism (6,8%), two of urea intoxication (3,4%), two of bony demineralization with decubitus (3,4%), two of medullar compression (3,4%), two of intoxication for *Palicourea macgravii* (3,4%), two of lead intoxication (3,4%), two of purulent meningoencephalitis (3,4%), one of hepatic steatosis (1,7%), one of facial paralysis (1,7%), one of bacterial encephalitis (1,7%), one of medullar lesion (1,7%), one of leucosis with medullar lesion (1,7%), one of cerebral babesiosis (1,7%), one of downer cow (1,7%), one of cerebral eimeriosis (1,7%) and one of cerebellar hypoplasia (1,7%). The study demonstrated the importance of researching and/or instituting specific prophylactics programs for the diseases in this geographic region.

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349 (2976)

CEREBROSPINAL FLUID ANALYSIS IN CATTLE WITH RABIES

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Analysis of the Cerebrospinal fluid (CSF) is described as a good auxiliary method for the diagnoses of the diseases of the central nervous system. The result of the analysis in association with the report and the clinical signs almost always supplies enough elements to establish a diagnosis. Three samples of CSF were collected from seven bovine with rabies, treated at the Veterinary Clinic of Brasilia University. The samples were analyzed in a maximum time-frame of two hours after collection. The collection was accomplished at the atlanto-occipital site using needles with length of varied size in accordance with the weight of the animal. The samples were checked for: transparency, color, density, glucose content, protein content, differentiation and count of nucleated cells and erythrocytes count. In the physical exams the seven samples showed no color or turbidity. Two animals presented increase of erythrocytes and pleocytosis in the CSF. Three samples had normal proportion of lymphocytes. In four cases the percentage of monocytes was increased and in one case an increase in neutrophils was found. Only one of the samples showed an increase in protein content. Three

animals showed concentrations of glucose in CSF above 105 mg/dl. Although this is considered a high level in relation to this particular component of the CSF, the three animals showed normal levels in terms of glycemia. One animal presented decrease in glucose with 28.7 mg/dl. In that animal, this is the only value out of normal levels. The CSF exam presented values to differentiate the rabies from bacterial encephalitis because in that condition the fluid presents changes in both aspect and color, as well as a considerable increase in the proportion of neutrophils. In spite of being a complimentary exam in the differential diagnosis, in the cases of polioencephalomalacia, BHV-5 and rabies, the analysis of CSF has little value. In those diseases the alterations of the fluid are similar and little variation from the normal values exists. The CSF exam in animals with rabies presented reduced value in the diagnosis.

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350 (5002)

IMMUNOGLOBULIN VARIATION PROFILE IN HEALTHY HOLSTEIN CALVES; RESPIRATORY TRACT ON THE FIRST MONTH OF LIFE

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The present paper aimed at determining the immunoglobulin variation profile for lavages from the bronchoalveolar and tracheobronchial regions of the respiratory tract for healthy neonate calves. Twenty Holstein neonate and healthy male calves were studied, all of them having been fed with colostrum. The animals were assigned into two groups, with ten animals each, respectively, with a view to assess the two lavage collection methods, the nasotracheal intubation for bronchoalveolar samples and the tracheocentesis for the tracheobronchial ones. In both groups, collection was carried out at a seven-day interval, from the first days of the animal's life up to approximately one-month. The immunoglobulins (IgG, IgM and IgA) present in the lavages at different occasions were qualitatively and quantitatively studied. The methods assessed showed to be both viable, low cost and allowing repeatability, despite the fact that the nasotracheal intubation technique brought more discomfort to the animal at collection and the tracheocentesis gave rise to doubts as regards the respiratory tract lavage region, in addition to a small lavage fluid recovery. In microbiological terms, the tracheocentesis showed the lowest lavage contamination rate. As regards immunological elements, it was noted that the healthy calves fed with colostrum up to one month of life showed greater IgG content as compared to the IgA one, in both tracheobronchial and bronchoalveolar regions. Also, the respiratory tract sites impacted on the immunological and protein elements, the tracheobronchial region showing greater total protein, albumin and immunoglobulin contents. Furthermore, the time has also impacted the respiratory tract lavage element variation, with a tendency to increase these elements in BAL and reduce them at TBL during the calves' first weeks of life.

The immunoglobulins found within the animals' respiratory tracts were originated by serum-selective transfer and not by active local production. Upon analysis of percentages obtained for the three immunoglobulins under study, it was noted that these were influenced by the collection site, and not by the calves' lifetime, the average amounts found to be: BAL: 93.5% IgG, 5.2% IgM and 1.2% IgA, and TBL: 84.0% IgG, 13.6% IgM and 2.35% IgA. Higher IgM and IgA and lower IgG proportions were found in the tracheobronchial region as compared to the bronchoalveolar one.

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351 (1643)

INVESTIGATION OF NURSING STRATEGIES TO IMPROVE ADAPTATION TO EXTRA-UTERINE LIFE IN BELGIAN WHITE AND BLUE CALVES AND HOLSTEIN CALVES DURING THE FIRST 24 HOURS AFTER BIRTH

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The aim of this study was to evaluate the effect of 6 different nursing strategies immediately after birth on respiratory and metabolic adaptation to extra-uterine life in healthy newborn calves. Forty-two Belgian White and Blue (BWB) calves delivered at term by an elective caesarean section and thirty-seven Holstein calves delivered at term by vaginal way were assigned, respectively, into 3 and 4 groups according to the strategy imposed immediately after umbilical cord rupture. Calves were examined at birth, 5, 30 and 60 minutes, 6 and 24 hours after birth by the following tests: physical examination, arterial blood gas analysis, arterial and venous blood acid-base balance analysis, and an evaluation of passive immune transfer. In beef breed (BWB), an association of nursing procedures immediately after delivery influenced respiratory and metabolic adaptation to extra-uterine life in calves delivered at term by an elective caesarean section. Suspension by the hind legs for less than 110 seconds immediately after umbilical cord rupture followed by sternal recumbency had a favourable functional impact during the first day of life on postnatal pulmonary gas exchange and on postnatal correction of mixed acidosis present at birth, contributing to passive immune transfer. The application of this nursing combination in deliveries at term by an elective caesarean in healthy BWB calves should be recommended to compensate for the lack of obstetrical «stress» observed in birth by vaginal way without any complication. In dairy breed (Holstein), body positioning immediately after delivery

slightly influenced physiological adaptation to extra-uterine life in calves delivered at term by vaginal way. Suspension by the hind legs for less than 90 seconds immediately after umbilical cord rupture is the nursing procedure producing the most, but light, favourable effect during the first 24 hours of life on postnatal respiratory function and on postnatal correction of mixed acidosis due to birth. However, this impact is lower than the one observed previously in suspension in beef breed. As the functional impact of the suspension is weak in Holstein calves, a simpler nursing strategy, i.e. placing the calf in sternal recumbency, should be recommended in dairy breeding farms looking for an elementary nursing technique in deliveries by vaginal way.

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352 (1908)

PREVALENCE OF ROTAVIRUS AND CORONAVIRUS IN NEONATAL CALF DIARRHOEA IN AZERBAIJAN PROVINCE OF IRAN

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Neonatal calf diarrhoea is one of the most common health problems in calf, and causes extensive economic losses from morbidity and mortality. This study includes a total of 500 cases (under 2 months old) of calf diarrhoea in 25 farms during one-year period (2001). The objective of this study was to determine the prevalence of rotavirus and coronavirus in calf diarrhoea in Azerbaijan province, Iran. Fecal samples were taken from every calf with clinical diarrhoea. All samples were submitted for virological examination. Electron microscopic examination of fecal material showed that rotavirus was found in 170 (34%) specimens, and coronavirus was found in 75 (15%) samples. The maximum prevalence of rotavirus is found in about 4-weeks old calves and the minimum prevalence is in the first week of age. For the coronavirus, the maximum prevalence is in found in 2-weeks old calves and the minimum prevalence is in the first week of age. However, for both rotavirus and coronavirus, the maximum prevalence is in winter and the minimum prevalence is in summer.

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353 (2598)

TRACE ELEMENT DEFICIENCIES IN THE PATHOGENESIS OF RESPIRATORY DISTRESS SYNDROME IN THE MATURE NEWBORN CALF

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In Belgium, respiratory distress syndrome (RDS) is one of the leading causes of neonatal death in the mature hypermuscled Belgian Blue calf (BB) but also occurs in other cattle breeds. Major clinical signs (tachypnea, tachycardia and sometimes depression) develop in the first hours after birth and are due to insufficiency of functional surfactant. Knowing that trace elements deficiencies can slow pulmonary maturation, the aim of this study was to investigate trace elements status in 10 RDS affected BB herds in comparison with 6 reference herds without any evidence of RDS.

In each herd, blood was sampled from 10 pregnant or freshly calved healthy cows. In each blood sample, the plasmatic zinc (Zn) and copper (Cu) contents and erythrocytic glutathion peroxydase activity (GSH-pxe) were measured and considered normal when above 15 $\mu\text{mol/L}$, 14 $\mu\text{mol/L}$ and 250 IU/gHb, respectively. A herd was deficient in one element if at least 30 % of sampled animals were out of normal range for this element. Milk was also sampled and pooled from 10 other cows or, when possible, bulk milk was taken. Milk iodine (I) content was considered normal when above 80 $\mu\text{g/L}$. Results were compared between groups by Chi-square test.

All RDS affected herds had low Zn and Cu concentrations and low GSH-pxe activity. Eight out of 10 had low I in milk. In the non-RDS affected herds, only 1 herd was deficient in I, Zn, Cu and had low GSH-pxe activity, 2 herds were deficient in Zn and Cu and 1 herd was deficient in Cu. RDS affected herds were significantly more often deficient in I and GSH-pxe than non-affected herds.

It seems that the trace elements selenium (Se), Zn and Cu play an essential role in the development of RDS. The same applies for I, although not deficient in all herds. The reason for this might be that milk samples were taken during lactation, when cows' nutrition was different and, in any case, supplemented in I. In mature babies, the same RDS is observed but its etiology is still unclear, although I deficiency in the mother is suspected to play a major role in pathogenesis. Furthermore, it has been demonstrated that a Se-dependent deiodinase is responsible for transformation of thyroxine (T4) into tri-iodothyronine (T3), which is essential for effective surfactant production.

In conclusion, results suggest an association between RDS in mature newborn calves and trace elements deficiencies, especially Se and I, that can be responsible for primary surfactant insufficiency.

354 (1735)

IMPORTANCE OF ARBOVIRUS INFECTION IN CONGENITAL DEFECTS OF CALVES

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The outbreaks of calves with congenital defects caused by arbovirus have been repeated in Kagoshima, Japan since 1972. In the recent 4-year survey, we tried to clarify the importance of arbovirus infection in the whole defective calves collected. In 2000-2003, 375 calves with congenital defects were collected. They were epidemiologically, clinically, pathologically and virologically investigated and classified systemically. Calves with debility (36 cases) and retarded growth (129 cases) were removed from the analysis due to the lack of visible congenital defects. In the remaining 210 calves, central nervous system defects (41%) and musculo-skeletal system defects (mostly arthrogyrosis) (37%) predominated and most of them were caused from arbovirus infection. Other defects included cardiovascular system (5%), urogenital system (4%), ocular system (3%), digestive system (2%) and others. In winter and early spring (December-April), the peak occurrence of defective calves was found every year. Virologically, they were confirmed to be caused by Akabane virus for 1999-2000, Chuzan virus for 2001-2002 and Aino virus for 2002-2003. However, the phenotype of those defects was somewhat different from the classic type found in each of the three viruses. It may suggest variation of these viruses. Though two-third of their dams were inoculated with a mixed vaccine (Akabane, Chuzan and Aino virus), many defective calves were born. It is concluded from this research that the arbovirus plays an important role in the occurrence of defective calves and further research is needed to investigate the viral variation and to develop an effective preventive method.

355 (3195)

COLOSTRUM QUALITY AND QUANTITY IN BELGIAN BLUE COWS AND THE SUBSEQUENT MATERNAL IMMUNITY

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Colostrum quality and quantity in cows are important factors for establishing maternal immunity in order to safeguard the newborn calf. Data on Belgian blue (BB) cows regarding this subject are scarce. In this study colostrum volume of 11 BB cows and 11 BB heifers was determined immediately after parturition. Colostrum quality of first colostrum was determined by a radial immunodiffusion assay measurement for immunoglobulin G (IgG) content for 9 BB cows and 5 BB heifers. Furthermore, blood samples of 7 BB calves receiving only BB colostrum and of 6 BB calves receiving only Holstein Friesian (HF) colostrum were taken 36 hours after birth and the serum IgG content was determined by a radial immunodiffusion assay. All these data were acquired on the same farm.

Three BB heifers and 2 BB cows (5/22 = 23%) produced no colostrum at all, while one heifer was too aggressive to milk (1/22 = 5%). The colostrum volume of the animals that did produce colostrum ranged from 0.60 to 4.50 l, with a mean production of 2.30 + 1.31 l. Volume differed significantly ($P < 0.05$) between BB heifers (1.46 + 0.72 l) and BB cows (2.96 + 1.32 l). Belgian Blue colostrum of 14 animals contained between 49.50 and 240 g IgG/l, with a mean content of 111.11 + 51.13 g IgG/l. The difference in IgG content between heifers (81.70 + 24.10 g/l) and cows (127.44 + 55.83 g/l) was not statistically significant.

Serum IgG content of 7 BB calves (50.71 + 18.42 g IgG/l) receiving only BB colostrum was significantly higher than serum IgG content of 6 BB calves (21.37 + 2.49 g IgG/l) receiving only HF colostrum ($P < 0.05$). Although the administered colostrum volume did not significantly differ (3.03 + 1.41 l versus 4.22 + 0.56 l for BB and HF colostrum respectively) colostrum IgG content significantly differed (112.71 + 32.95 g IgG/l versus 55.67 + 22.79 g IgG/l for BB and HF colostrum respectively) and was responsible for the higher serum IgG content in the calves receiving only BB colostrum ($P < 0.05$).

In conclusion we can state that, although colostrum volume is quite low in BB heifers and cows, IgG content of this BB colostrum is sufficient to acquire good maternal immunity in the calves suckling this colostrum.

Immunoglobulin G content of HF colostrum is significantly lower, probably due to a dilution effect, and this must be taken into account whenever HF colostrum is fed to BB calves.

356 (1938)

ANTIMICROBIAL SUSCEPTIBILITY OF ESCHERICHIA COLI STRAINS ISOLATED FROM NEONATAL DIARRHEIC CALVES

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Colibacillosis is a prevalent pathology in neonatal calves. Regular epidemiological surveillance of antimicrobial susceptibility of Enterotoxigenic *E. coli* (ETEC) is needed in order to base the choice of antibiotic therapy on information on susceptibility trends.

Minimum Inhibitory Concentrations (MIC) of 13 antibiotics or associations for 98 *E. coli* strains were determined by the reference agar dilution method following the guidelines of the "Comité de l'Antibiogramme de la Société Française de Microbiologie". All isolates had been collected in 2003-03. They originated from seven different geographical areas in France. The strains were isolated from the feces of epidemiologically unrelated diarrheic calves. Fimbrial and afimbrial adhesins were characterized by agglutination tests. CS31A, F5, F41, F17, and F4 were present in 32%, 21%, 19%, 15%, and 2% of the isolates, respectively; 29% of the strains were not typable.

Overall, only 3.1% of the strains were susceptible to all antimicrobial compounds tested. Of the tested strains, 6.1% were susceptible to tetracycline, 27.6% to chloramphenicol, 64.3% to trimethoprim/sulfamethoxazole, and 33.7%, 35.7% and 78.6% to kanamycin, neomycin and gentamicin, respectively. A low frequency of quinolone susceptible isolates was observed in this study, with 56.1% and 72.4% of the tested strains susceptible to nalidixic acid and enrofloxacin, respectively. 6.1% of the strains were susceptible to amoxicillin, 15.3% to amoxicillin/clavulanic acid, whereas the cephalosporins retained a good activity, with 83.7% and 99% of the strains susceptible to cephalexin and cefquinome (MIC \leq 2 mg/L according to Intervet), respectively. A heterogeneous distribution was clearly observed for the colistin MIC which allowed susceptible (MIC = 0.12-0.25 mg/L) and bacteriologically resistant (MIC = 4-16 mg/L) populations to be distinguished. The studied strains have developed resistance: three strains were solely susceptible to cefquinome. Monitoring of antimicrobial resistance is a priority. This is the first report in France of ETEC with elevated colistin MIC. The effect of these observations on therapeutical outcome remains to be appraised.

357 (3301)

COLOSTRUM MANAGEMENT IN CALVES: EFFECTS OF DRENCHING VERSUS BOTTLE FEEDING

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The objective was to examine whether the administration of colostrum by drenching represents a safe method and results in satisfying levels of immunoglobulins in the serum of newborn calves compared to bottle-fed calves. Newborn HF-calves (N = 46; 43.7 \pm 6.1 kg birth weight; mean \pm SD) were used. 21 calves received 1 h post natum (p.n.) 2 L of fresh colostrum from the dam via a nipple bottle (group I). In 15 calves, 4 L colostrum from the dam were drenched 1 h p.n. (group II). Thereafter, all calves were fed milk replacer exclusively. Immunoglobulin concentration was analysed in a subsample of the colostrum after centrifugation (Sandwich-ELISA). Venous blood samples were taken prior to colostrum administration and 12, 24, 48, 72, 96, 120, 144, 168 and 336 h p.n.; total protein (TP) was analysed from plasma (Cobas Mira®). Serum immunoglobulin concentration was determined in the sample taken 24 h p.n.. To characterize the kinetics of immunoglobulin absorption, a catheter was introduced into the jugular vein of 5 calves (2 L colostrum 1 h p.n., nipple bottle; group III) and 5 calves (4 L colostrum 1 h p.n., drencher; group IV); 17 blood samples were taken within 72 h p.n. and analysed for immunoglobulins and TP. Especially calves with a low birth weight appeared depressed for 12-24 h after being drenched; adaptation to further feeding with a nipple bucket was more difficult compared to calves of group II. Concentration of immunoglobulins in colostrum serum was 53.7 \pm 14.8 g/L. Calves TP (prior to colostrum supply: 43.7 \pm 3.6 g/L) rose within 24 h p.n. by 11.1 \pm 4.6 g/L (group I) and significantly higher in drenched calves (group II; 16.5 \pm 7.3 g/L). Serum concentration of total immunoglobulins 24 h p.n. was higher in drenched calves (group II: 25.2 g/L; 18.6/36.6; median, 25/75 percentiles) compared to bottle-fed calves (group I: 14.1 g/L; 11.7/19.1). Maximal immunoglobulin concentrations were found 12 h p.n. in group III and IV. A slightly delayed increase of serum immunoglobulin concentrations (ca. 3 h) was observed in group IV compared to group III; again the drenched calves reached significantly higher immunoglobulin compared to the bottle-fed calves. It is concluded that the proper drenching of 4 L of colostrum was safe and useful to achieve an adequate transfer of immunoglobulins; the transfer of colostrum into the rumen seems to be without biological significance.

358 (3459)

ABILITY OF THE ABOMASAL LUMINAL PH-TIME RELATIONSHIP TO PREDICT THE ABOMASAL EMPTYING RATE IN HOLSTEIN BULL CALVES

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Suckling causes a rapid increase in abomasal luminal pH in neonatal calves. After suckling, the luminal pH at any given time is determined by the volume, pH, and buffering capacity of the ingested meal, the rate and extent of abomasal secretion, and the rate of abomasal emptying. We hypothesized that the time required for luminal pH to return to the preprandial value was correlated with the rate of abomasal emptying in calves. The aim of this study was therefore to determine whether the abomasal luminal pH-time relationship could be used as an index of abomasal emptying rate in calves suckling solutions of different pH and buffering capacity, and in calves with different acid secretion rates induced by atropine administration.

Five Holstein bull calves (<30 days old), with a cannula in the abomasal body, were fed 2 l of milk replacer, 2 l of milk replacer and parenteral atropine (0.01 mg/kg IV then 0.02 mg/kg SC every 30 minutes), or 2 l of an isoosmotic (150 mM) solution of NaCl, NaHCO₃, or Na-acetate. Technetium-pentetate (10 mCi) was added to the test solution immediately before suckling, and abomasal luminal pH was measured continuously using a flexible glass pH electrode placed through the abomasal cannula. The mean preprandial pH (1.45 \pm 0.41, mean \pm SD) was determined for 15 minutes before suckling, and the pH return time was defined as the time required for the post suckling luminal pH to return to 0.50 units above the mean preprandial pH value.

Scintigraphy was used as the gold-standard measure of abomasal emptying rate, and the power exponential formula of Siegel et al. (1988) was used to calculate the scintigraphic half emptying time.

The mean maximum luminal pH value occurred immediately after suckling and was 4.11 for NaCl, 5.42 for

milk replacer, 5.52 for milk replacer and atropine, 6.09 for Na-acetate, and 7.44 for NaHCO₃. Scintigraphic half emptying time ranged from 29 to 201 minutes, and pH return time ranged from 92 to 644 minutes. There was a good correlation ($r = +0.78$; $P = 0.0039$) between pH return time and scintigraphic half emptying time; $\text{pH return time} = 3.04(0.62) \times \{\text{scintigraphic half emptying time}\} + 58(90)$, with the standard errors in parentheses. We concluded that the pH return time provided a useful research method for estimating abomasal emptying rate in calves suckling solutions of different pH and buffering capacity, and in calves with different acid secretion rates.

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359 (3341)

RISK FACTORS FOR MORBIDITY AND MORTALITY IN CALVES DURING THE FIRST FOUR MONTHS OF LIFE IN SELECTED DAIRY HERDS IN THE HIGH ALTITUDE TROPIC IN COLOMBIA

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Several studies worldwide have shown that morbidity and mortality are limiting factors in the cattle industry. In Colombia, it has been established that mortality of calves represents along with mastitis and reproductive problems the main limiting factors in the dairy industry.

In order to determine the effect of management practices and season on morbidity and mortality and to identify the main diseases that affect the calves up to the first four months of life, a prospective cohort study was designed. The experimental unit was the calf nested in the 28 farms that accepted to participate in the study. The herds were located on the Colombian Andes at mean altitude of 2640 meters above sea level. Interviews to the farm owner or manager with a direct observation were done to determine the management practices. 541 female calves were followed up to fourth month of life. Each farm was visited during a whole year.

The epidemiological associations among management factors, season, immunoglobulin (Ig) levels between 24-72 hours of life and morbidity and mortality were estimated using Odds Ratio (OR) and logistic regression ($P < 0.05$).

Morbidity and mortality rates were 58% and 12% respectively. The main clinical diseases were diarrhea (44.9%), stomatitis (9.2%), omphalitis (9.2%), tympani (7.3%) and other entities (29.4). Mortality causes were tympani (21%), diarrhea (17%), stomatitis (13%), septicemia (13%), omphalitis (8%), pneumonia (6%) and other entities (22%).

The management factors that were identified as risk factor for morbidity in general were inadequate Ig absorption (OR 1.5), water source: river (OR:1.5), calf housing (OR:1.5), calf raising >9 months (OR:1.3), Milk replacer use (OR:1.3), give colostrum with bottle nipple (OR:1.3), Holstein breed (OR:2.2), average milk production >20 liters (OR:1.2). No significant risk factors were determined for mortality.

As protective factors were raising period (5 months) (OR:4), Veterinary assistance (OR:0.5), more than 5 days with the dam (OR:0.5), raising on a paddock (OR:0.6). The stepwise logistic regression for morbidity included: Holstein breed (OR: 2.7), milk replacer use (OR:2.4), water source: river (OR: 2), Ig adequate absorption (OR: 0.46). These results indicate these are the main factors associated with morbidity and mortality.

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360 (1918)

EVALUATION OF INFLUENCE OF TWO METHODS OF ARTIFICIALLY FEEDING COLOSTRUM ON SERUM GAMMAGLOBULIN CONCENTRATIONS OF NEONATAL CALVES

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Objective: To determine the influence of the presence of the dam at the time of colostrums intake on serum gammaglobulin concentration of neonatal calves, from birth to 4 weeks of age.

Animals: Forty newborn calves divided to two groups (20 per group), determination of serum gammaglobulin concentration from day 2 to 28 after birth.

Procedure: twenty newborn calves were immediately removed from their dams after birth and housed in single pen in the calf rearing unit (group 1). Calves were offered almost 6% of their body weights or 2.5 liters colostrum from the dam at the age of 1-2 h from a nipple pail. The other 20 newborn calves kept with their dams in calving pen for the first 3 h and during this time fed colostrum from nipple pail (group 2). Calves were then separated from their dams and housed in single pen in calf rearing unit. Estimation of total protein followed by gammaglobulin concentration was carried out by electrophoresis.

Statistical analysis: ANOVA test followed by Fischer's PLSD test.

Results: Mean serum total protein levels of calves in group 2 were greater than group 1 on days 2, 14 and 28 after birth but the difference were not significant. Mean serum gammaglobulin concentration in-group 2 was greater than calves' in-group 1 on days 2, 14 and 28. The difference at day 2 was significant ($P < 0.05$), but at days 14 and 28 the difference were not statistically significant.

Clinical implications: From the results of this study it seems that presence of dams in the first hours of life, and only at the time of first colostrum feeding (the most important part of received colostrum), can increase serum gammaglobulin concentration of calves. Regarding the difference between mean serum gammaglobulin

concentrations in two groups (calves received colostrum nearby their dams and calves received colostrum without their dams) were continued to one month after birth, it seems that the presence of dam during the first colostrums intake can increase calves resistance against diseases and also can decrease calves mortalities on the first month of life.

Funding: University of Tehran, Faculty of Veterinary Medicine

361 (2155)

COMPLEX VERTEBRAL MALFORMATION IN HOLSTEIN CATTLE

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Complex vertebral malformation (CVM), a lethal disorder, is newly recognized familial syndrome in Holstein cattle. Little is known about the prevalence of the disorder in dairy herds and the economic loss associated with CVM. Further diagnostic and genealogical information is necessary to understand and control this genetic disorder in the Holstein breed.

Purpose: Female stillborn Holstein calves with shortened cervical and thoracic regions, protrusion of the tongue, and bilateral symmetric flexural contraction of the anterior limbs were submitted to the laboratory for postmortem diagnostic examination. The aim of this study was to document the cases of CVM confirmed in stillborn calves, and to monitor the carrier rates of CVM in cows from 5 local dairy herds by using a newly developed DNA-PCR-RF test which employing restriction enzymes for the detection of CVM.

Results: Multiple hemivertebrae, fused and misshaped vertebrae, synostosis and scoliosis of cervical, thoracic and lumbar vertebral column were found in the affected calves by radiographic and computed tomographic (CT) analysis. Multiple morphologic abnormalities including fusion, malformation, and displacement, were found in the ribs and sternum. Cardiac abnormalities, including atrial septal defect were observed in some cases. In DNA-PCR test, a new DNA-PCR method that is based on the detection of DNA fragments of PCR amplified products digested by restriction enzymes was developed, and was proven to be specific and reproducible. The affected calves were diagnosed as having CVM based on the DNA-PCR results and the characteristic findings. DNA collected from their dams were heterozygous carrier of CVM. In a preliminary survey, CVM-carrier rates in 220 dairy cows from 5 herds ranged from 5-13%.

Conclusion: It was clear that the gene encoding CVM is widely prevalent in Holstein dairy herds. Economic losses due to early fetal death associated with CVM appear to be problematic, but the frequency of CVM-related embryonic death and abortion in early gestation remain to be clarified in the Holstein dairy herds. A program for the eradication of the CVM gene in Holstein cattle has been enacted in Japan.

362 (668)

EFFECTS OF NEONATAL CALF ORAL REHYDRATION THERAPY SOLUTIONS ON MILK CLOTTING TIME

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Oral rehydration therapy solutions (ORT) are formulated to correct or prevent dehydration and metabolic acidosis. Feeding simultaneously small volumes of whole cow's milk and isotonic metabolizable-bases ORT to diarrheic calves has been highly recommended. The purpose of the present study was to compare the in vitro effects on whole cow's milk clotting times of 50 ORT for the treatment of calf scours commercially available. In vitro milk clotting times were determined at 37°C using rennin. All milk clotting determinations were performed in triplicate. The rennin was diluted with water to a concentration of 12.5 g/L. The rennin solution was added to the test solution, and clotting time was determined by examining it through a glass beaker for the appearance of flakes within a 10-minute period. The solutions tested were homogenized cow's milk and the ORT reconstituted in milk according to the dilution's directions recommended by the manufacturer. The pH was measured in triplicate samples prior to addition of rennin, immediately after the milk had clotted, or at the end of the 10 min-period in unclotted milk samples. To assess objectively the size of the curd formation, all samples were poured through a series of four molecular sieves. ORT had usually between 40 and 100 mEq/L of alkalinizing ability. Popular bases found included bicarbonate and citrate. Some products contained mainly metabolizable bases such as acetate and/or propionate. Few ORT had almost no alkalinizing potential. In non metabolizable-bases ORT, no milk clot formation was found. After mixing into milk, these solutions were generally more alkaline. However, one of these non metabolizable-bases ORT contained high amounts of citric acid. This respective ORT was found more acidic when mixed into milk and no curd formation was found. ORT containing mainly metabolizable bases, such as acetate and/or propionate, did not interfere with curd formation. Phosphate was included as acid phosphate salts in some ORT. The presence of phosphate salts in these respective ORT was associated with decreased pH of the mixed solutions and enhanced clotting. The particular combination of citric acid and phosphate salts was found significantly more acidic when mixed into milk. Finally, high dextrose concentrations were associated with no clot formation in comparison with isotonic ORT. The addition of swelling substances was quickly associated with gelling effect which could be mistaken with milk clotting formation.

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363 (3131)

DEVELOPMENT OF A SEPTICEMIA MODEL IN NEWBORN CALVES TO EVALUATE VETERINARY MEDICINE EFFICACY

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Septicemia remains a prevalent condition in newborn calves. Few experimental models are described in the literature that reproduce septicemia in calves. In this study, 16 new-born calves deprived of colostrum, were inoculated per-os with a O78:K- strain of Escherichia coli. After inoculations, calves were monitored every 6 hours for clinical signs of septicemia. Five clinical signs were recorded and noted from 0 to 3. A Total Evaluation Grade (TEG) was attributed to each calf at each clinical evaluation and was defined as the sum of each grade attributed to each one of the 5 clinical signs. For all 16 calves, TEG was = 14 at the time of inoculation. Septicemia was defined as TEG = 11; Symptoms of septicemia were observed in 14 out 16 calves within 36 hours of inoculation. At clinical evaluation when TEG was detected = 11, 8 randomly chosen calves received an intravenous antibiotic administration (ceftiofur, 3 mg/kg). Seven treated calves survived: TEG = 14 within 24 hours of treatment. One treated animal died within 24 hours of drug administration and all (n=6) non treated animals died. Oral administration of a O78:K- strain of E. coli is a suitable model to induce septicemia in colostrum restricted newborn calves. This model could serve as the basis to test medicines efficacy in calves.

364 (1262)

THE EFFECTS OF PROLONGED ADMINISTRATION OF FROZEN BOVINE COLOSTRUM ON PERFORMANCE AND DIARRHEA IN CALVES UNDER ONE MONTH OF AGE

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Diarrhea in calves under 30 days of age is one of the most common diseases, which the large animal clinician encounters in practice. The aim of this study was to present a useful and convenient method for reduction of calf diarrhea, the significant cause of economic loss in cattle herds.

20 newborn Holstein calves, which had been born without dystocia, were allotted randomly into control and treatment groups. Calves weighing 31-40 kg at birth were offered 2 kg of colostrum immediately after birth and the same amount 4-6 hours later. Calf starter and water were available ad libitum from day 14. All calves were fed two times a day. In group 1, 10 calves were fed once daily 0.8-1 kg thawed colostrum (stored by freezing) diluted with 1-1.2 kg milk. In group 2, control treatment, 10 calves were fed only 2-2.2 kg warm milk in each meal everyday. Calves were weighed at 0, 10, 20 and 30 days of age.

Incidence and severity of diarrhea were recorded based on landmarks such as consistency of feces, skin fold to determine the degree of dehydration, consciousness and ability to stand (for metabolic acidosis). Little differences in average daily weight gain were recorded. Weight gain for 0-30 days was similar for both treatments. The calves given colostrum daily were less affected by scours. Not only incidence but also severity of diarrhea in group 1 was significantly less than in group 2.

Saif (1985) reported that daily consumption of colostrum may provide an increase in local passive immunity of intestine but that weight gain was not affected by this method. Our results are in accordance with Foley (1979), Saif (1991) and Ikemori (1997).

KEY WORDS: Diarrhea, Frozen colostrum, calves

Funding: Shahrekord University

365 (2746)

COLONIZATION, SAFETY AND FECAL RECOVERY OF A POTENTIAL PROBIOTIC STRAIN OF LACTOBACILLUS PLANTARUM IN CALVES

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Despite widespread commercial availability, there is little scientific evidence supporting the use of probiotics in calves. The lack of demonstration of efficacy is due in part to the lack of studies assessing colonization properties of selected strains in the target animal species. A bovine-origin strain of Lactobacillus plantarum B80 (LPB80) was recently identified by the authors as possessing in vitro properties that may render it useful for the prevention of Escherichia coli-associated gastrointestinal disorders in calves. This preliminary in vivo study was designed to evaluate the colonization ability, safety and effects of LPB80 on fecal coliforms and lactic acid bacteria (LAB) following oral administration to calves. 12 male 1-2-day old Holstein calves were randomly divided into three groups and fed either placebo (Group A, n=3), 10^7 - 10^8 colony forming units (CFU) of LPB80 (Group B, n=5), or 10^{10} - 10^{11} CFU of LPB80 (Group C, n=4) once daily for 5 days. Calves were housed in individual pens and monitored for 15 days. Daily physical examinations were performed. Fecal water content and quantification of LAB, coliforms and LPB80 in feces were evaluated blindly. Intestinal samples were collected at the conclusion of the study. Partial sequencing and alignment of 16S rRNA gene were used to confirm identification of LPB80 in fecal samples. No adverse clinical signs were observed at the end of the experiment in any of the calves that received LPB80. Administration of LPB80 resulted in no

statistical differences for variables studied among groups except for total fecal LAB numbers; surprising with a significantly lower number of LAB in Group C ($P=0.01$). Interestingly, LPB80 was recovered from samples of both treated and placebo animals. Alignment of the 16S rRNA gene sequences showed 100% similarity when comparing the strains recovered from feces and the offered strain. Results suggest that oral LPB80 is non-pathogenic, inhabits the gastrointestinal tract of healthy calves and might colonize the gastrointestinal tract following oral administration. Studies on the effect of oral administration of LPB80 on the incidence of *E. coli* F5 diarrhea in calves, and carriage of *E. coli* O157 are warranted.
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366 (1499)

HEMATOLOGICAL AND BIOCHEMICAL FINDINGS IN CALF DIARRHEA: DETERMINATION OF PROGNOSTIC VALUES

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The objective of this study was to evaluate the clinical and laboratory findings in diarrheic calves and to identify the prognostic values of these findings. Twenty-one calves aged one to fifteen days, were referred to the authors' clinic. All were Holstein calves. The principal reason for referral was diarrhea. The complete case history was recorded. Each calf was examined thoroughly. Jugular blood samples (10 ml) were collected at the beginning and before treatment. Two ml of jugular blood were collected into EDTA contained tubes for hematological examination. A complete blood count was made and a differential leukocyte count was done microscopically. The plasma concentrations of total protein and fibrinogen were measured with a refractometer. Serum was separated by centrifugation, frozen and stored at -20°C until analysis. Serum glucose, urea nitrogen, albumin, and creatinine were measured using an automatic biochemical analyzer. Serum sodium and potassium were measured with a flame photometry and chloride by titration method. Jugular blood samples from 15 apparently normal non-diarrheic calves of similar ages were analyzed in the same way to provide control data. Statistical tests used were unpaired Student's t test, Mann-Whitney and Chi-square tests, with significance being accepted at the $P<0.05$ level. The calves were treated with isotonic electrolyte fluids. The amount of fluid therapy was estimated on the basis of degree of dehydration. Gentamycin administered at a rate of 4.4 mg/kg every 12 h. Five calves died, despite of the treatment. The diarrheic calves had significantly higher serum concentrations of total protein, urea nitrogen, creatinine and potassium; and significantly lower levels of serum glucose and chloride than the control calves. The hematological feature of diarrheic calves was significantly different from normal ones. The diarrheic calves had significantly higher level of PCV and fibrinogen and elevated numbers of WBC, mature and band neutrophils and monocytes than healthy calves. Also, the concentrations of urea nitrogen, creatinine and potassium were significantly higher in died calves than survived ones. It was concluded that concentration of serum urea nitrogen, creatinine and potassium may be used as prognostic value in diarrheic calves.

Funding: Ferdowsi University of Mashhad, Mashhad, Iran

367 (3108)

RISK FACTORS FOR CRYPTOSPORIDIUM PARVUM INFECTION IN ONTARIO DAIRY CALVES

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Cryptosporidium parvum is a zoonotic protozoal parasite that infects several livestock species including cattle. In calves, infection with *C. parvum* is often associated with diarrhea, accompanied by the shedding of infective oocysts in the feces.

From June to October 2003, fecal and blood samples were taken from 609 calves 0 to 30 days old on 11 dairy farms affected by neonatal diarrhea in southwestern Ontario. Fecal samples were collected weekly and examined for *C. parvum* oocysts by a standardized sucrose flotation method, and in a subset of calves were also screened for other enteropathogens. Blood samples were drawn in the first week of life and were used to quantify total serum protein and globulin levels as well as, in a subset of calves, to test for bovine viral diarrhea virus. For each calf, producers completed a questionnaire on hypothesized risk factors for *Cryptosporidium* infection, and basic clinical data were also collected. Statistical analysis was performed using Stata8® (Stata Corporation).

Preliminary analysis showed that calves on all 11 farms were infected, with over 50% of calves in the study shedding *C. parvum* oocysts in the first month of life. Most calves shed oocysts between 7 to 16 days of age, and this age group of calves also shed the maximum number of oocysts. Most of the variance in infection risk occurred between farms, with a high correlation of shedding between calves within farms.

Of the hypothesized risk factors investigated in this study, a longer time of contact with the dam was associated with an increased risk of infection, while the feeding of pooled colostrum appeared to be beneficial

to the calf.

The results of this study indicate that *C. parvum* infection is common among dairy calves in southwestern Ontario, and that infection risk for this parasite varies more from farm to farm than within individual farms. This would suggest that most of the factors related to the risk of *C. parvum* infection in dairy calves are common to all calves within a farm, and supports future farm-level studies. The relationship found between time with the mother and *C. parvum* infection may implicate periparturient cows as a source of infection for their calves, while the apparent advantage of feeding pooled colostrum may support the role of maternal antibody in protecting against *C. parvum* infection in neonatal calves. Both of these findings require further investigation. Funding: DFC, OMAF, NSERC

368 (765)

HEPATIC TRACE MINERAL CONCENTRATIONS IN THE BOVINE FETUS AND NEONATE

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Understanding nutrition's role in animal health has prompted a need for accurate assessment and interpretation of mineral status relative to disease potential. Adult animal tissue mineral concentrations and their interpretation have been well defined. Diagnostic criteria for fetal and neonatal hepatic mineral concentrations are not well established. The objective of this study was to generate preliminary data for assessing bovine fetal and neonatal hepatic mineral concentrations.

Liver samples were collected from 106 bovine fetuses and 64 neonates submitted to a diagnostic laboratory. Inductively coupled plasma atomic emission spectroscopy (ICP/AES) was used to assay 22 minerals in all samples. Mineral concentrations were determined on a wet weight and converted to a dry weight basis. Liver dry matter (DM) content was determined by drying an aliquot sample in a convection oven. Fetal gestational age was estimated from crown-rump length. Reported cause of death or necropsy findings was recorded. Mean (range) fetal and neonatal age was 8.4 months (3-9.5 months) and 14.2 days (0.5 hr-90 days), respectively. Mean fetal (0.22), neonatal (0.24) and adult (0.32) liver DM ratios were all different ($P < .0001$). Fetal and neonatal liver DM ratio increased ($P < .0001$) with age. These data suggest differing liver DM content may confound diagnostic interpretation of mineral concentrations, when expressed on a wet weight basis. Fetal or neonatal wet weight-based criteria may not be appropriate given changing liver DM with age. Most fetal mineral DM concentrations exceeded current adult concentrations. Neonatal concentrations were somewhere between these values. These data are in agreement with previous studies suggesting greater mineral concentrations in fetal liver DM, compared to adults. On a DM basis, liver Cu, Mg, P, Zn and K concentrations increased with fetal age, whereas Ca, Co, Se and Na decreased and Fe and Mn were not different. In neonatal samples Se, Cu, Zn, Na and K concentrations declined, Ca, Fe, Mg and P concentrations did not change, and Co and Mn increased with age. These data support the concept that the liver plays an important role in perinatal mineral metabolism. Diagnostic interpretation of fetal and neonatal liver mineral concentrations may be improved if evaluations are based on age and hepatic DM content. Preliminary diagnostic criteria for fetal and neonatal hepatic mineral concentrations may be based on the upper 75% values for each mineral.

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369 (3443)

CYTOLOGY OF TRACHEOBRONCHIAL (TBL) AND BRONCHOALVEOLAR LAVAGES (BAL) IN HOLSTEIN CALVES WITHOUT RESPIRATORY DISTRESS ON FIRST MONTH OF LIVE

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Cytology of tracheobronchial (TBL) and bronchoalveolar lavages (BAL) in Holstein calves without respiratory distress on first month of life.

The aims of the present study were to establish relative physiological values and to follow the dynamic of variation of different cell types presented on tracheobronchial and bronchoalveolar of neonate calves lungs. For this purpose 20 male Holstein calves without respiratory distress were selected and distributed in two experimental groups: G1 - TBL collect by tracheocentesis method (n=10); G2 - BAL collect by nasotracheal tube (n=10). The collects were carried out with a 7-day interval since the first week of life until 30 d of neonate calves. For both collecting methods animals were restrained on left lateral recumbency and 40 ml of sterile saline were injected aspirating 0.5% up to 20% and 60% of the solution, respectively for the tracheocentesis (TBL) and nasotracheal intubation (BAL). The slides for cytologic evaluation were made from an aliquot of 200µl of pulmonary wash through the cytocentrifugation, followed by Rosenfeld staining method. Alveolar macrophages and neutrophils showed a negative correlation ($r = -0,90$) and were highly significant with the method of collection and the pulmonary region studied. The percentage of alveolar macrophages on G1 showed a progressive decrease, significant on fourth week (62.15%, 34.30%, 34.50%, 20.15%), differing this behavior from those obtained on G2, where medians percentage were stable (75.50%, 63.5%, 75.65%, 70.55%). On an opposite way, neutrophils percentage on G1 showed crescent values and significant higher

(15.30%, 59.40%, 63.40%, 75.80%) than those observed on G2 (12.40%, 3.65%, 19.30%, 11.00%). Giant macrophages and ciliar epithelial cells were characterized by absence of significant variations of their percentages on the comparing of the two methods of collecting utilized, the regions submitted to washes and the four weeks of the study. The general values of medians of lymphocytes on G1 (1,90%) and on G2 (1,90%) showed a little participation on cytology obtained for TBL and BAL, similarly behavior of eosinophils that were practically inexistent on both groups and weeks of life. The collect realized through nasotracheal tube showed to be less invasive when compared to that realized through the tracheocentesis method.

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370 (3254)

DETERMINATION OF CEFTIOFUR AND DESFUROYLCEFTIOFUR-RELATED RESIDUES IN MILK OF LACTATING DAIRY COWS RECEIVING SUBCUTANEOUS INJECTIONS OF CEFTIOFUR CRYSTALLINE FREE ACID STERILE SUSPENSION IN THE MID-EAR AT A NOMINAL DOSE OF 6.6 MG/KG BODY WEIGHT

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The objective of this study was to measure the concentration of parent ceftiofur and desfuroylceftiofur-related residue in milk from lactating cows administered CCFA-SS formulation (200 mg/mL) in the middle third of the posterior aspect of the ear at a nominal dose rate of 6.6 mg/kg. Twelve mid-lactation dairy cows were administered CCFA (as the 200 mg/mL oil-based sterile suspension) into the middle third of the posterior aspect of the ear, given as two 12 mL injections, one injection into each ear. The estimated mean dose rate, based on girth tape BW estimates, was 7.64 mg/kg, or 115% of the target. Milk was collected through approximately twelve milkings post-treatment. Milk samples were tested for antibiotic residue by the Parallax, Charm II, Charm MRL, Charm SL and SNAP assays. Milk samples were also analyzed by two HPLC methods; the HPLC-DCA method, which measures all desfuroylceftiofur-related residue including parent ceftiofur, and the HPLC-CEF method which measures parent ceftiofur specifically. Detectable levels of >0.015 mg/mL (LOD of the method) were found in all samples of the 2nd milking through the 6th milking by the HPLC-DCA method. However, only three samples were above the LOQ of the method (0.050 mg/mL), with concentrations of 0.073 mg/mL and 0.066 mg/mL, respectively, for the 2nd and 3rd post-administration samples from one cow and 0.051 mg/mL for the 3rd milking for one other cow. The estimated mean concentration for the 2nd through 6th milkings post-treatment ranged from 0.020 mg/mL to 0.036 mg/mL, and all other collections were <0.015 mg/mL. The results from the HPLC-CEF assay indicated that very little, if any, parent ceftiofur translocated across the udder membrane-blood barrier to be found in the milk.

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371 (2496)

HEALTH, REPRODUCTION, AND PRODUCTION IN DAIRY COWS WITH INCREASING MILK PRODUCTION

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In advancement of cattle breeding, an important role belongs to complete programs of preventive diagnosing and health control guided by veterinary surgeon. From 1999 to 2003, in one East-Slovakian farm, we performed regular preventive diagnosis of dairy cows' diseases. The farm has in average 365 dairy cows of low-land black spotted breed with 75-92 % of Holstein-Friesian proportion. Feeding system was organized in phases according to lactation stages. The farm has an increasing tendency in milk production: in 1999 - 4200 L, 2000 - 5100 L, 2001 - 5800 L, and in 2002 - 7079 L per cow. Samples for metabolic profile test were collected from three groups of dairy cows: dried cows, cows after parturition, and lactating cows. Haematological profile was analysed by cell analyser Serono Plus. Concentrations of Ca, Mg, Na, K, Cu, Fe, Zn, and Se were estimated by atomic absorption spectrophotometry; other biochemical indices including inorganic phosphorus by analyser Alize. In 1999-2000, increased occurrence of puerperal disorders in poor reproduction indices and culling rate up to 40 %. Other findings included increased numbers of somatic cells (NSC) in pooled milk samples (600-700 000) and locomotion disorders (pododermatitis, Rusterholz's ulcer). In 2001-2002, there was some improvement in the aforementioned indices, which was shown in evaluation in January 2003 when culling rate decreased to 26 % and NSC ranged around 350 000. Following results of analyses, corrective measures were suggested to eliminate biochemical dysbalancies of the animals; however, some problems persisted throughout the observation period. In all the groups of cows, we found leukocytosis, hypercholesterolaemia, low triglyceride levels, and increased concentrations of nonesterified fatty acids, marked hypocupraemia, sideropenia, hypozincaemia, and hyposelenioaemia. Examinations of rumen fluid showed increased acetate and decreased propionate concentrations. Evaluation of biochemical indices requires sound knowledge of factors influencing their concentrations or activities; e.g. advanced pregnancy, parturition, high milk production, composition of diet, as well as some pathological conditions affecting absorption and utilisation of nutrients. Neglecting these factors may lead to misinterpretation. The aforementioned data indicate necessity of continuous examinations of metabolic status of dairy cows to adopt preventive measures guarantying optimal production health of the animals.

372 (5043)

PHOTOPERIOD MANIPULATION PRE-PARTUM TO INCREASE MILK YIELD IN COMMERCIAL DAIRY HERD IN THE SUBSEQUENT LACTATION

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In commercial Holstein dairy herd, 47 dairy pregnant heifers and 73 dairy cows (total 120) paired by parity were divided, randomly 60 days pre-partum (dry period) to two photoperiod treatments: natural day photoperiod (ND), heifers: n=24, cows: n=38, 12-14h light and short day photoperiod (SD), heifers: n=23, cows n=35, 16 h dark 8 h light. After parturition all the animals were moved to the milkers barn 16 h light 8 h dark. Temperature and relative humidity data were monitored in the barns constantly during the treatment period April to November, and no differences were observed in these measures between the treatments. Parameters that were evaluated: calf weight, stillborn rate, daily milk yield 305 DIM, milk composition, post parturient diseases (milk fever, uterine prolapse, ketosis, retained placenta, metritis, displaced abomasum) were monitored. Fertility parameters were monitored as well open days, odds to conceive from 1st service and wait period.

First lactation SD cows yielded 3.1 kg milk per day ($P=0.016$) in 305 days more than the ND cows. No difference in milk yield between treatments was found in 2nd and above lactation. No differences between treatments in milk composition were observed, as well as in post parturient diseases or fertility.

Prolactin levels (RIA) measured at 12h intervals pre- and post-partum in 19 cows (SD n=10; ND n=9). Peak levels of prolactin at parturition tended to be higher ($P = 0.08$) in ND than in SD cows in both primiparous and multiparous cows. In multiparous cows there were no differences between SD and ND cows in prolactin levels pre- or post-partum. On the other hand in primiparous cows, levels of prolactin were higher ($P < 0.05$) in ND than in SD cows both pre- and post-partum. The significant response in prolactin levels of primiparous cows to the short day regimen pre-partum may explain their positive response in milk yield, in contrast to the multiparous cows. The response in prolactin levels pre- and post-partum to pre-partum SD regimen may serve as a predictor to future response in milk yield. The different response of primiparous and multiparous cows to the SD treatment in our conditions should be further investigated.

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373 (2154)

EFFECT OF GRAZING SYSTEM ON FETAL DEVELOPMENT IN NELLORE CATTLE

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Intensive grazing systems for beef females, based on abundant availability of high quality forages and supplementary concentrates, may affect fetal development. The objective of this study was to determine the effect of grazing system on length of gestation, fetal development, and characteristics of the calf at birth. Twenty-four pregnant (bred to Nellore bulls) Nellore females were allocated into two groups. The control group (G1) grazed *Brachiaria decumbens* (signal grass) in a traditional (extensive) grazing system and the second group (G2) was managed on *Panicum maximum* cv. Tanzania 1 (Tanzania grass) in an intensive grazing system. Fetal development was evaluated by ultrasonography on Days 31, 45, 59, 94, 122, 220, and 255 of gestation. The diameter of the amniotic and allantoic cavities, crown-rump length, circumference and diameter of the head and ocular orbit were determined. At birth, calves were weighed and height, length, thoracic circumference, and ocular orbit and bi-parietal diameters were measured. There were no differences ($P > .05$) in fetal development. The G1 cows had a longer gestation period (4.5 d; $P < 0.05$) and their calves had greater ($P < 0.05$) weight, height, length, and thoracic circumference at birth. In conclusion, Nellore females raised under intensive pasture management conditions had significantly shorter gestation and smaller calves at birth than those raised under extensive pasture management conditions. Therefore, adoption of new management practices (e.g. intensive pasture management), should take into consideration animal behavior and productivity.

Key words: Fetal growth; Genotype; Grazing system; Cattle; Nellore; Ultrasound

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374 (3386)

THE USE OF HCG IN EMBRYO TRANSFER

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The use of HCG to increase pregnancy rate was confirmed by Dr. W.W. Thatcher - University of Florida (Novel systems for reproductive Management of Lactating Dairy Cows and Strategies to improve embryo survival - experiment Sept. 1999) when he noted that administration of HCG increased the plasma progesterone when administered 5 days after AI. In accordance with this affirmation, we tried to improve the pregnancy rate in Holstein recipients by administration of HCG on the implantation day of an embryo frozen-

thawed.

The HCG (human chorionic gonadotrophin) has different uses: stimulate the follicular development, induce the ovulation, maintain the state of corpus luteum and increase the secretion of progesterone by the corpus luteum.

We took two hundreds (200) Holstein heifers and cows divided randomly in three (3) groups; all of them were injected with HCG or placebo (sterile water) on the implantation day (6 or 7 days after oestrus). Randomly, half of each group received HCG and the other half received placebo. All these recipients were noted as good or bad recipients (bad means repeat breeder). All the embryos were ethylene glycol frozen embryos.

The Group 1 received 1700 I.U. HCG (Chorulon- Intervet), Group 2 received 2500 I.U., and the Group 3 received 3300 I.U. injected I.M.. This experiment took place on summer 2002 (particularly hot for the Quebec area).

Respectively, the results were for animals receiving HCG or placebo: Group 1 (75% vs 60%), Group 2 (52% vs 71%), Group 3 (64% vs 60%). For the animals classified as repeat breeders, the results were 50% vs 0%. These results showed a light improvement of pregnancy rate in normal recipients (64% vs 60%) with the use of HCG. In the group of repeat breeders, although the group was very small, the result showed a marked increase for the recipients who received HCG, (50% vs 0%) and it is probably due to an accessory CL and an increase of progesterone to avoid or reduce the embryo loss.

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375 (2134)

NEOSPORA-ASSOCIATED ABORTION AND FIELD EXPERIENCE WITH A COMMERCIAL VACCINE IN A DAIRY HERD

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In cattle, the major mode of infection with Neospora is vertical. Calves infected in utero are commonly born alive with or without clinical signs. However, when Neospora infection results in abortion, it usually occurs during months 5 or 6 of gestation. Cows seropositive for Neospora are considered to be at significantly greater risk of abortion than non-infected cows. It is unclear precisely what determines whether or not a Neospora-infected cow aborts or carries her calf to term. It is speculated that the outcome of pregnancy is determined by the immune responses of both the fetus and dam.

Primiparous heifers are more likely to experience Neospora-associated abortion than are multiparous cows.

This suggests that development of immunity to the organism may abrogate some of the tendency for abortion in subsequent gestations. As additional evidence for the development of protective immunity, it has been shown that exposure of cows six weeks prior to breeding reduced vertical transmission.

There is not a proven strategy for control of vertical transmission of Neospora. In this study, we have field tested a commercial Neospora vaccine in a dairy herd with a historically high incidence of Neospora-associated abortion. The herd consists of 250 head of registered Holsteins. As each animal was diagnosed pregnant by rectal palpation at 40-70 days gestation, individual cows were assigned to either control or vaccinated groups. The vaccine was administered according to manufacturer's recommendation; two injections four weeks apart during the first trimester of gestation. Prior to vaccination, blood was drawn to determine Neospora status by ELISA. A second blood sample was obtained 30 days later and a second vaccine dose administered to animals in the vaccine group. A third blood sample was obtained 30 days after the second sampling date. All cows were followed until the gestation ended either by delivery of a calf or abortion. Aborted fetuses and stillborn calves were examined grossly and microscopically to determine cause of abortion or death. Blood was obtained presuckling on calves delivered alive to determine if fetal antibodies were present against Neospora. Preliminary results indicate the vaccine reduces the incidence of Neospora-associated abortion but may not offer protection against vertical transmission of the parasite.

Funding: Intervet

376 (1788)

EFFECT OF SHORT-TERM VARIATION OF DIETARY INTAKE ON LIVE WEIGHT GAIN, HORMONAL AND METABOLIC STATUS AND IN VITRO EMBRYO PRODUCTION IN SUPEROVULATED DAIRY HEIFERS

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This study investigated the relationships between growth rate, hormonal and metabolic status and in vitro embryo production of heifers submitted to short-term variation of energy intake and collected by Ovum Pick-Up (OPU).

Sixteen Prim'Holstein heifers (14 ± 1 months old, 340 ± 25 kg) were fed with a diet composed of hay, soybean, barley, minerals and vitamins and were collected by OPU every two weeks after superovulation treatment. Cumulus-oocyte complexes were in vitro matured and fertilized, and embryos in vitro cultured for 7 days.

Heifers received for 6 weeks (Period 1 = OPU 1 to 4) a diet aiming a 1000 g/day live weight gain (LWG). After

OPU 4, they were allocated to 2 diets (1000 or 600 g/day) for 8 weeks (Period 2 = OPU 5 to 8). Blood was sampled once a week to measure glucose, insulin, IGF1, non esterified fatty acids (NEFA), β -hydroxybutyrate (β OH) and urea plasma concentrations, and at the time of OPU for estradiol assay. Effects of period, group of growth rate and their interaction were analysed using the Mixed procedure of SAS (female effect as random) and lsmeans were subsequently compared with Scheffe's test.

Mean LWG observed during period 1 was 950 ± 80 g/d (lsmean \pm SEM, $n=16$). In period 2, it was 730 ± 70 g/d ($n=8$) and 1300 ± 70 g/d ($n=8$) for restricted and overfed group respectively. A significant period effect was observed on oocyte and embryo production variables, metabolic parameters and estradiol concentrations. Moreover, significant differences between periods were found only in the "700 g/d" group with more blastocysts and grade 1 embryos associated to more estradiol at OPU and less glucose and BHB in period 2. In the "1300 g/d" group, more follicles < 8 mm 2 days before FSH treatment, higher insulin and IGF-1 and lower NEFA in period 2 were observed. For follicles aspirated at OPU, collected oocytes, day 7 total embryos, no difference was observed between periods within groups.

These data suggest that following an overfeeding period, dietary intake restriction may increase blastocysts production and embryo quality. They illustrate the role of glucose, insulin and IGF-1 as mediators of nutritional effects on reproduction in growing animals, and show that nutritional requirements aiming to optimize follicular growth and embryonic development may be different.

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377 (3411)

USE OF REFRIGERATED SEMEN: A FIELD EXPERIENCE REPORT

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To fulfill the high demand of top proven sires, OGER has developed a fresh semen technology for many years. Since 1988, semen of 3 to 7 bulls is distributed to technicians in the fields and used refrigerated. Semen is delivered three days a week (Monday, Wednesday and Friday) from the beginning of November till the end of March. According to each bull's intrinsic fertility, doses contain between 3 and 5 millions total sperm. Thanks to this technique, AI units production is multiplied by an average of 2.82 folds when compared to frozen sperm. Semen is extended in a Tris/egg-yolk extender containing 6.4% glycerol (Optidyl TM, Biovet, France). It is diluted at room temperature, refrigerated at 4 C and then processed in .25 ml straws. AI units are stored in computerized cool-boxes and delivered by cars to four AI cooperatives. They are then dispatched to technicians in thermos flasks containing ice-cubes to maintain the temperature at $4^\circ\text{C} \pm 2^\circ\text{C}$. The duration of use is three days (day 0 to day 2).

A control in cool-boxes proved their efficacy to maintain the right temperature. While big variations were recorded in the technicians' flasks [0°C to 10°C]. Over years, the average utilization rate of fresh semen is 87% but varies according to bulls (70% to 98%).

Fertility results show significant differences among bulls. Some bulls display better fertility with fresh semen than with frozen especially on D0. Others don't stand refrigeration. Preliminary tests of semen incubation are recommended. Each year, non-return rates with fresh or with frozen semen show a drop of 2 to 3 points in favour of frozen units. AI at D0 are more fertile than frozen semen of the same bull. The average loss of fertility over years reached 9 to 11 points of non-return rates for AI made at D0 or at D2. These results depend on sires: extreme individual differences vary from 5 to 19 points over this three days period.

Fresh semen is efficient to increase top bulls semen availability. It requires a rigorous control of the storage temperature, a quick delivery network and a high density of females to inseminate. Despite a slight loss of fertility when compared to frozen semen, it allows an optimal distribution of the genetic merit of top bulls among breeders. The achievement of specific extenders and the improvement of cooling systems will be of great help to optimize fertility results.

378 (1164)

A STUDY ON THE SERUM LEVEL OF VITAMIN A AND BETA-CAROTEN IN NATIVE COWS WITH RETAINED PLACENTA IN AHWAZ (IRAN)

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The retained placenta is a complication of puerperal period and is associated with different factors such as deficiency of vitamin A and beta-carotene in serum.

Therefore, this study was conducted to evaluate the serum level of vitamin A and beta-carotene in retained placenta cows in Ahwaz city (central of Khozestan State). Samples were collected from jugular vein of 11 primiparous and 19 multiparous retained placenta cows and from normal cows (25 primiparous and 35 multiparous) without retention of fetal membranes, during 12 months. The differences between serum vitamin A and beta-carotene values for affected and control cows were evaluated statistically with case-control matching by a 2-tailed paired t test. Results showed that mean (\pm SEM) value of blood serum vitamin A (mg/dl) of case and control groups was 40.77 ± 0.5 and 43.98 ± 0.23 , respectively; the difference was not significant ($P>0.05$). Blood vitamin A level of primiparous retained placenta ($n=11$) and primiparous normal cows ($n=25$) was 43.06 ± 1.4 and 45.71 ± 0.58 , respectively; the difference was not significant ($P>0.05$). For multiparous

cows, those values were 39.45 ± 0.79 (n=19) and 42.74 ± 0.39 (n=35), respectively; the difference was not significant ($P>0.05$). Mean (\pm SEM) value of blood serum beta-carotene (mg/dl) in case group (n=30) and control group (n=60) was 67.56 ± 1.1 and 124.98 ± 1.01 , respectively; thus blood serum beta-carotene level of cases was significantly lower than control group ($P<0.05$). The mean value of serum beta-carotene in 11 primiparous of cases (70.35 ± 4.82) and 25 control primiparous (139.39 ± 2.64) was significantly different ($P<0.05$). The mean beta-carotene level of 19 multiparous cases (65.92 ± 2.71) was significantly lower ($P<0.05$) than that of 35 multiparous control (114.68 ± 1.57).

The conclusion of this study shows that deficiency of beta-carotene affected cows is prevalent and should be considered in the prevention and control of the disease.

379 (1417)

CAROFERTIN®: EFFECT OF INJECTABLE β -CAROTIN ON THE REPRODUCTIVE PERFORMANCE IN DAIRY COWS

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The aim of this study was to investigate the effect of a supplementation with β -carotin administered systemically (Carofertin®) on the reproductive performance in dairy cows. 100 cows received three intramuscular injections of 20 ml Carofertin® (=200 mg / injection) 3 to 4 weeks ante partum, 1 to 2 weeks post partum and 6 to 8 weeks post partum. Control cows (n=101) were treated with 20 ml 0.9 % NaCl-solution. Blood samples were taken before the first and the second β -carotin injection and at the time of first insemination. Serum concentration of β -carotin was determined by high pressure liquid chromatography (HPLC). For all animals, diseases, treatments, inseminations, cullings and culling reasons were recorded, and a clinical examination was performed during the puerperal period (4 to 6 weeks post partum). Furthermore, fertility parameters and cost per pregnancy were calculated. From a subgroup of cows, blood samples were taken twice weekly starting around Day 40 post partum, and serum concentrations of progesterone were measured by radioimmunoassay (RIA) throughout the luteal phase. The trial period lasted until the day of drying off or until a cow was culled.

In the study, the mean serum concentrations of β -carotin at the three sampling points did not differ between groups ($P>0.05$). The clinical examination during the puerperal phase revealed that cyclicity had started statistically significantly more often in control cows than in the cows receiving Carofertin®. With regard to the other parameters, no significant group differences were found. Cost per pregnancy were higher in the Carofertin® group (241.78 €) than in the control group (171.40 €). The main reason for the higher cost were the cost of Carofertin® (8.71 € per injection). In both groups, replacement cost for cows culled due to infertility and cost of days open beyond 85 days post partum had the highest economical impact on cost per pregnancy.

These results suggest that the use of Carofertin® in dairy cows for therapy or prophylaxis of fertility disorders under the given experimental conditions might not be recommendable.

380 (1513)

EFFECT OF EXCESSIVE FLUORIDE ENVIRONMENT ON PRODUCTIVITY AND REPRODUCTIVE PERFORMANCE IN DAIRY COWS

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The objective of this study was to evaluate the effect of excessive fluoride environment on productivity and reproductive performance in dairy cows. A total of 1400 cows from 3 herds were enrolled in this investigation. Examination on dairy cows fluoride, selenium and copper contents in external environment, such as drinking water, feedstuff and soil, and internal environment, such as serum or blood, hair and urine, have been conducted. It was confirmed that the dairy cows have excessive fluoride and low selenium/copper. While dairy cows were fed diets daily supplemented with selenium 0.25 mg/kg and copper 3.2 mg/kg for 3 months, the selenium and copper deficiency disappeared, but high fluoride symptoms still existed when compared with healthy cows. The uterine recovery time, the time of first estrous and interval period of high fluoride cows were 38.7 ± 4.0 day (n=15), 76 ± 25 day (n=15) and 146.5 ± 30.1 day (n=15), respectively, they were prolonged 6.6 day, 6 day and 38.5 day, respectively. Postpartum progesterone contents at day 5, 15 and 30 were 0.70 ± 0.07 ng/ml (n=7), 1.06 ± 0.11 ng/ml (n=7) and 1.44 ± 0.33 ng/ml (n=8), respectively. They were significantly lower than in the healthy cows (1.18 ± 0.05 ng/ml (n=6), 1.69 ± 0.12 ng/ml (n=5) and 2.31 ± 0.48 ng/ml (n=6)) at each corresponding time. Meanwhile, the morbidity of retained placenta and mastitis in excessive fluoride cows were higher 16.7% and 13.3% than in healthy cows, the average daily milk yield per cow was decreased by 0.8 kg. It was concluded that excessive fluoride affects the dairy cow's production and reproductive performance.

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381 (697)

EFFICACY OF A VACCINE AGAINST NEOSPORA CANINUM RELATED ABORTIONS

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A clinical trial was carried out to evaluate the efficacy of a new vaccine (Neoguard®, Intervet International BV) against abortion caused by *Neospora caninum* (NEO) in cattle. Five dairy herds (281 to 716 cows) were included. A total of 2,240 cows at 25-45 days of pregnancy were randomly allocated either to vaccination with Neoguard® or to injection with 0.9% NaCl solution (placebo). Treatments were repeated after 3-4 weeks but not later than at 3 mo of gestation. Serum samples were examined for antibodies against *Neospora caninum* (IFAT) and BVDV (ELISA) before first vaccination, at 4 weeks post booster and 4 and 8 weeks after any abortion. All information was triple blinded for farmers, veterinary practitioners and laboratory personnel. Vaccination was associated with a noticeable site reaction found in only 4 NEO cows at second vaccination. It was concluded that Neoguard® is safe and well tolerated. In response to vaccination, the IFAT seroprevalence (at titres <1/100) increased from 12 to 88% (independent of age) while the controls showed an increase from 11 to 23%, apparently due to natural challenge or recrudescence of *Neospora caninum* infection.

Abortion rates and vaccine effect were markedly different on the 5 trial farms: the vaccine was efficacious in two of five farms ($p < 0.01$; efficacy 30% and 54%, respectively), that is 30% or 54% of all cases in non-vaccinated cows could have been prevented by vaccination. Efficacy was small and non-significant in one other farm with a low abortion rate (3.1%; efficacy 26%) while no efficacy was observed in 2 farms. It was concluded that vaccine efficacy depended on specific farm conditions, e.g. overall abortion rates, level of natural challenge, presence of other infectious agents able to cause abortion, and possibly other stress factors related to the recrudescence of *Neospora caninum* infection.

Among 111 aborting cows, 38 were found non-pregnant at the time of expected calving. The assumption that abortions in these cows had occurred in early gestation and the finding that there were more pre-vaccination seropositive than seronegative cows in this group gave rise to the hypothesis that neosporosis may trigger abortions already during the first trimester of gestation during which the cows were presumably not yet fully protected by the vaccine. Exclusion of such cows rendered the overall vaccine effect highly significant (efficacy 59%; $p=0.007$).

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382 (1527)

DETRIMENTAL EFFECTS OF CHANGING DAIRY CATTLE OPERATION ON REPRODUCTIVE AND PRODUCTIVE INDICES

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Introduction: In a new system of dairy cattle operation, lactating cows were kept in free-stalls and calves and heifers were transferred to another farm to be raised and to receive artificial insemination and returned to the target farm repeatedly [1]. The feasibility of the new system was questionable enough to motivate us to perform a study on reproductive and productive indices.

Materials and methods: The study was performed for one year, in a small Holstein dairy herd in Tehran Vicinity. The system consists of a conventional open shading system composed of free-stalls covered with straw bedding for 79 lactating cows, and a Total Mixing Ration (TMR) feeding system based on National Research Council (NRC). Indices such as average daily milk per cow, daily milk sale, services per conception, days open, conception rate, and calving interval were calculated and compared with the target figures; some reproductive and productive diseases, including anoestrus, abortion, retained placenta, endometritis, and ovarian cysts were also monitored. Data were analyzed by Chi-square and Student t-test.

Results: Despite the increased number of cows, the average daily milk per cow slightly decreased (28 versus 25 liters, $P>0.05$); however, the average daily milk sale increased from 2112 to 2977 liters due to the number of purchased cows. Quarter infection rate for subclinical mastitis was 13.5% at the beginning of the study; however, this increased to the 38.5% at the end of the year ($P<0.01$). *Streptococcus agalactiae* was the main pathogen in the herd. Nineteen cows were culled due to Infectious Bovine Rhinotracheitis (IBR), abortion and death during the study. Services per conception, days open, and calving interval increased, but conception rate decreased in comparison with those of the target indices: 3.17, 131 days, 405.5 days, and 32% versus 1.8, 85-115 days, 365-395 days, and 45-55%, respectively. Anoestrus, abortion, retained placenta, endometritis, and ovarian cysts also increased from 43.5%, 0.5%, 23%, 19%, and 6.4% after beginning of the study to 67%, 2.9%, 56%, 42%, and 4.7%, respectively.

In conclusion, the new methods and novel approaches to operation of dairy cattle may not be applicable to all environments and all countries.

[1] Hoard's Dairyman, September 25, 2001.

Funding: University of Tehran

383 (1353)

CROSSBREEDING AT THE CROSSROADS - IS THERE A FUTURE FOR THE CROSSBRED HOLSTEIN FRIESIAN X JERSEY COW IN AUSTRALIA?

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The introduction of North American Holstein genetics into many countries of the world has broadened the genetic base of local populations resulting in substantial genetic improvement and significant production gains. In Australia this has led to a growing proportion of Holstein Friesian cattle (83%) at the expense of the Jersey (12%) and the Friesian x Jersey (5%), based on Australian 2001/2002 herd test figures.

The consequence of genetically improving the modern dairy cow through the importation of Holstein genetics has been the emergence of a breed that is heavier, leaner and has a higher milk yield potential than previous dairy breeds. However, it has been shown to have lower fertility, more health problems and a lower survival rate. One way proposed to overcome declining reproductive performance and a lowered survival rate is the strategic use of a rotational crossbreeding program in mixed breed herds.

Although the New Zealand literature records many references to the substantial and highly profitable benefits of crossbreeding in that country, there are minimal comparative statistics and findings on how crossbreds behave under Australian conditions. In fact, New Zealand has extensively exploited the advantages of crossbreeding and hybrid vigor to improve the fertility and performance of the national herd and is now progeny testing crossbred bulls.

An extensive assessment of crossbreeding in Australia has therefore been undertaken in an attempt to address a lack of comparative data in the Australian literature. This will provide some direction on whether crossbreeding is sustainable and therefore profitable for Australian seasonal calving dairy farmers.

An observational study in 17 Victorian seasonal calving herds containing a significant proportion of crossbreds plus the interrogation of herd records from the Australian Dairy Herd Information System (ADHIS) database and the Dairy Australia funded InCalf study will provide data on the comparative performance within herds of crossbred Holstein Friesian x Jersey and straightbred Holstein Friesian cows. The study will specifically look at breed differences in reproductive performance, somatic cell count, production, incidences of disease including mastitis and lameness and survival rate under the pasture based seasonal calving conditions of the Victorian dairy industry.

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384 (3431)

IS IRISH DAIRY HERD FERTILITY DECLINING?

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The objective of this study was to characterise the temporal trends in herd fertility and to investigate associated risk factors. Data were retrieved from national recording organisations and research institute databases. Throughout the 1960s and 1970s herd fertility was high with calving rates to first service of between 60 and 69%. In the 1980s research herd data first showed a significant decline in conception rate to first service between 1980 (67%) and 1988 (59%). Subsequent studies in commercial herds in the 1990s confirmed this trend where calving rate to first service declined significantly by 0.7 to 0.9% per year and calving rate to all services by 0.5% per year. In this time (1991-1998) mean lactation number decreased (4.32 to 3.45) and the proportion of bred cows not re-calving increased (7.3 to 13.5%). Calving interval increased by 0.9 days per year (1993:389 v. 1999:395 days) and reappearance rate between 1st and 2nd lactation decreased by 1% per year (1993:80.4% v. 1999:73.2%). The most recent study showed a significant decline in calving rate to first service of 0.96% per year (55 to 44%) and an increase in inseminations/conception (1.54 to 1.75) between 1990 (55%) and 2001 (44%). The physiological mechanisms underlying this decline may include increased prevalence of atypical luteal profiles post partum, reduced oestrous intensity and increased early embryonic mortality. Late embryonic mortality (day 28-84) is currently, on average, 7%. During this period of declining fertility major changes have occurred in Irish dairy farming; introgression of Holstein genes, selection entirely on milk production, increased herd size and increased use of do-it-yourself artificial insemination. The most significant risk factors associated with this decline have been strain substitution and single trait selection for milk production, mediated through body condition loss from calving to first service and actual body condition at first service. Currently 48% of cows in Irish dairy herds conceive to first service and 14% of cows are not pregnant at the end of the industry-average 15-week breeding season. However, wide inter-herd variation exists. In conclusion, Irish dairy herd fertility has been declining since the 1980s. This phenotypic trend is attributed to both genetic and environmental factors. Currently Irish dairy herd fertility falls short of the targets set for seasonal compact calving.

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385 (2934)

GESTATION RATE OF DAIRY HEIFERS FED CALCIUM SALTS OF PALM OIL OR WHOLE FLAXSEED FOLLOWING TRANSFER OF EMBRYOS COLLECTED FROM DAIRY COWS FED CALCIUM SALTS OF PALM OIL OR WHOLE FLAXSEED

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Seventy-five Holstein heifers were used in a 2 x 2 factorial arrangement of treatments to determine the importance of fat sources in the diet of donor and recipient animals on the success of pregnancy after embryo transfer measured on day 50 by ultrasonography. The four treatments were embryos collected from dairy cows fed either calcium salts of palm oil, Megalac® (MEG) or whole unprocessed flaxseed (FLA) that were transferred into heifers fed either MEG or FLA. Both FLA and MEG total mixed diets fed to heifers were equal in protein (15.3%), ether extract (5.98%), and net energy of gain (1.11 Mcal/kg) contents. Similarly, diets FLA and MEG fed to dairy cows were equal in protein (18.0%), ether extract (5.1%), and net energy of lactation (1.76 Mcal/kg) contents. Diets were fed for 8 wk to heifers before to induce heat synchronisation (2 injections of 2 ml of Estrumate 12 d apart, 500 µg cloprostenol, Sherring-Plough, Pointe-Claire, QC, Canada,). Embryos were collected from 30 cows (n = 15 per diet) following a synchronisation and superovulation program using a non-surgical procedure performed 6 d after insemination of cows. All embryos were frozen before being transferred 7 d after standing heat of heifers. There was no interaction (P > 0.05) between diet fed to cows and diet fed to heifers. The diet fed to heifers had no effect (P > 0.05) on pregnancy rate, which averaged 42.5%. The diet fed to cows had a significant effect (P < 0.05) on pregnancy rate; embryos collected from cows fed FLA resulted in greater pregnancy rate (58.8%) than embryos collected from cows fed MEG (29.3%). Therefore, the type of dietary fatty acids would be more important for donor animals than the type of fatty acids present in the diet of recipient heifers. These data suggest that feeding a source of omega 3 fatty acids such as flaxseed would improve the quality of frozen embryo as shown by the improvement in pregnancy rate following embryo transfer.

Funding: Governments

386 (2290)

STUDY OF THE PRESENCE OF NEOSPORA CANINUM IN THE SEMEN OF SEROPOSITIVE BULLS FROM ARTIFICIAL INSEMINATION CENTERS IN BRAZIL - PRELIMINARY RESULTS

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Neosporosis has been described as an important cause of abortion in bovines. Seroepidemiological surveys performed in Brazil show a high incidence of Neospora caninum in dairy and beef herds. The agent has been isolated from aborted fetuses and brain of calves presenting nervous symptoms. No reports were found on the role of the bull in the transmission of the parasite, although Toxoplasma gondii has already been isolated from caprine semen. The pathogeny and the biological cycle of this protozoon is similar to that of N. caninum. This study was performed in order to assess if N. caninum may be transmitted by semen of seropositive bulls from Artificial Insemination Centers (AI). In September 2002, serum samples of 153 bulls were examined by ELISA test, in order to evaluate the frequency of N. caninum occurrence in the AI Center and the animals continued to be monitored every six months. From 15% seroreagent bulls (23/153), an aliquot equal to 0.5 mL of fresh semen was sent to the laboratory, for the Neospora caninum study using PCR. DNA extraction was performed using Eppendorf® commercial extraction kit, and primers of the region Np-6 and Np-21 from Neospora caninum, which amplify 337 pb. Up to now, 49 semen batches from seroreagent animals have been analyzed, and none of them was positive. These are preliminary results, and the project will evaluate, in two years, all batches of semen, from seropositive bulls, that will be sold, in order to obtain a significant number of samples per animal, and enable a conclusion on whether the elimination of this agent occurs in semen.

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387 (2930)

FOLLICULAR DEVELOPMENT AND EMBRYO QUALITY OF DAIRY COWS FED CALCIUM SALTS OF PALM OIL OR WHOLE FLAXSEED

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Thirty lactating Holstein cows were used in a randomized design to study the effects of feeding two fat sources on follicle size and embryo quality. On d 21 post-calving, cows were assigned to a total mixed diet containing either calcium salts of palm oil, Megalac® (MEG) or whole unprocessed flaxseed (FLA). Both total mixed diets were equal in protein (18%), ether extract (5.1%), and net energy of lactation (1.76 Mcal/kg) contents. After 80 d of feeding the two treatments, a controlled internal drug release device (CIDR, 1.9 g progesterone, InterAg, Hamilton, New Zealand) was inserted into the vagina followed 24 h later by an injection of 3 mg of estradiol 17β (1.5 mg/ml, Denis Giroux Pharmacy, Saint-Hyacinthe, QC, Canada). Four days after the estradiol injection, cows were superovulated. The protocol used decreasing doses of follicle stimulating hormone, FSH (20 mg/ml, Follitropin V®, Bioniche, Belleville, ON, Canada) given twice a day (0700 and 1900h) for 4 days (d 5, 11, 12, and 13 following insertion of the CIDR) with injection of prostaglandin F2a (Estrumate, 500 µg cloprostenol, Sherring-Plough, Pointe-Claire, QC, Canada) at the time of the last two injections of FSH. The CIDR was removed at the last FSH injection and cows were

inseminated 12, 24, and 36 h later. Non-surgical collection of embryos was performed 6 d after insemination. All embryos were evaluated and the total number of embryos collected and their classification were recorded. In parallel, ovaries of five cows per treatment were observed using real-time ultrasound twice a week from initiation of feeding the two diets until the start of the synchronization/superovulation protocol. The number and size of follicles were noted. Number of small (< 5 mm), medium (5 to 10 mm), large (> 10 mm), and total follicles averaged 1.70, 1.44, 0.29, and 3.43, respectively, and they were not different ($P > 0.05$) between treatments. Diet had no effect ($P > 0.05$) on the number of total, transferable, viable, grad I and II, degenerated, and unfeconded structures, which averaged, respectively, 6.11, 2.90, 2.85, 1.25, and 2.29. These data suggest that feeding a source of omega 3 fatty acids such as flaxseed has no effect on embryo quality of and follicle size.

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388 (3199)

ECONOMICS OF REPRODUCTIVE MANAGEMENT IN DAIRY COWS USING SYNCHRONIZATION OF ESTRUS AND/OR OVULATION

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In the light of decreasing conception rates and unsatisfying estrus detection and AI submission rates, numerous protocols to synchronize estrus and/or ovulation have been developed to improve reproductive and economic efficiency in dairy herds. The objective of this study was to analyze the economic benefit from synchronization protocols with special reference to the routine use of prostaglandin F2a and Ovsynch. Data from 7 field trials using these protocols were included in the study.

The main cost factors considered for the economic evaluation were days open € 2.5 per day after 85 DIM, culling € 500, AI costs € 15, treatment costs (GnRH € 6, PG Euro 3, treatment € 1.5) and costs for examination of cows (€ 4 per palpation). To account for the effect of price assumptions on the economic efficiency of the protocol, values were varied over a wide range in a sensitivity analysis. Assumptions were identical for all protocols regardless of the trial site and the publication year.

A total of 15 study groups were included in the analysis. 6 groups were bred on observed estrus, 3 were Ovsynch groups, 2 used PG after rectal palpation and 4 were targeted breeding protocols with AI on observed estrus. There were considerable differences between the seven trials indicating herd and time-specific effects on the efficacy of the protocols.

Overall, costs for replacement of cows (44.8%) and additional days open beyond 85 DIM (25.3%) were the major cost factors. AI and veterinary costs (examination, drugs and treatment) accounted for 14.1 and 15.8 % of the total costs, respectively.

On average, protocols for synchronization reduced costs compared to the 6 groups using only AI on observed estrus in all scenarios. Among the synchronization protocols, use of PGF2a after rectal palpation seemed most effective. Veterinary and AI costs were highest in Ovsynch protocols. However, compared to AI on observed estrus they reduced costs for days open and culling. Compared to PGF2a protocols, Ovsynch did not reduce costs for days open and culling considerably. However, in the only trial comparing Ovsynch and a PGF2a protocol directly, both cost items were reduced substantially in the Ovsynch group.

Results of this study underline the benefit from synchronization protocols for the reproductive and economic efficiency of dairy farms. However, direct comparison of protocols on several farms is necessary to evaluate the benefits associated with a specific protocol.

389 (2636)

INVESTIGATION OF EARLY LOSS OF PREGNANCY AND THE ROLE OF NEOSPORA CANINUM AMONG HEIFERS ON ONE NEW ZEALAND DAIRY FARM

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Previous trial work has shown that some Neospora caninum (Nc) infected cows are suffering early embryonic loss and early abortion (first and early second trimester). A trial is to be conducted during the 2003-4 season to follow the pregnancies of a group of 120 dairy heifers on a farm that has a history of Nc abortion since 1997. During the first year of the abortion outbreak 9% of the herd at risk aborted including 19% of heifers. In recent years, seropositive cows have experienced up to 18% abortions while abortions in seronegative cows have been sporadic, not exceeding 1%. Since the initial outbreak, abortions have been noticeably more frequent among heifers. Regular blood sampling of the entire herd including replacements has been undertaken for the past 6 years to study the epidemiology of Nc infection and to monitor serostatus of the animals.

The heifers in this study will be ultrasound scanned for pregnancy from 8 weeks after the Planned Start of Mating and then at 4 week intervals. Serum and whole blood samples will be collected at the time of each pregnancy test and held for further testing. When a loss of pregnancy has been established, the serum samples will be analysed for IFAT titre and whole blood for the presence of Nc DNA using a Polymerase Chain Reaction (PCR) technique that has been developed at Massey University. The aim is to establish the time of infection or recrudescence of infection and antibody response and relate it to the timing of pregnancy loss. Bovine Viral Diarrhoea Virus (BVDV) sero-status before and after abortion will also be analysed. Any

aborted foetal material that is retrieved will also be examined using histopathology and PCR. Samples from matched non-aborting heifers will be similarly analysed and the results will be presented at the conference. Funding: NZ Dairy Industry Global Fund

390 (3499)

IMMUNE RESPONSES TO BOVINE HERPESVIRUS 1 INDUCED IN CATTLE BY TRADITIONAL AND DELETED LIVE VACCINES

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Five groups of infectious bovine rhinotracheitis (IBR)-free heifers (5 animals each) were treated with a traditional live vaccine (TLV - Bovilis IBR, Intervet): group A (priming intranasally, booster intranasally); group B (priming intranasally, booster intramuscularly); group C (priming intravaginally, booster intravaginally); group D (priming intravaginally, booster intramuscularly); group E (priming intramuscularly, booster intramuscularly). Other 5 groups (F, G, H, I, J) were vaccinated with a deleted live vaccine (DLV - Bovilis IBR marker, Intervet) in accordance with the same protocol.

Immune responses were evaluated before priming (T0), before and 15 days after booster (T1 and T2). For humoral response, antibodies against glycoproteins gE and gB of bovine herpesvirus 1 (BoHV-1) were detected by specific enzyme immuno-assays (EIA) and neutralizing antibodies by a seroneutralization assay (SN). Cell-mediated immunity (CMI) to BoHV-1 was investigated by an in vitro antigen-specific interferon gamma (IFN- γ) release assay.

At the T1 sampling, the animals belonging to A, B, D, E (TLV) and G, H, I, J (DLV) groups showed neutralizing antibody response. Moreover, 2 animals of the group C (TLV) and 1 of the group F (DLV) did not show antibody response at SN. Serological patterns obtained by gB-EIA resulted the same as those observed by SN. However, 2 out of the 3 SN-negative animals resulted gB positive. For groups treated with TLV, 11 animals out of 25 (44%) resulted gE positive. Eighteen animals (72%) treated with TLV and 14 (56%) treated with DLV showed CMI response.

At the T2 sampling, all the animals treated with either TLV and DLV seroconverted. For TLV, the highest neutralization response was found either in animals primed intranasally or intravaginally and boosted intramuscularly (groups B and D). For DLV, the highest neutralization response was obtained in animals primed intramuscularly and boosted intramuscularly (group J). All the animals treated with either TLV and DLV resulted gB positive. Regarding gE antibody response, 1 TLV-treated animal resulted seronegative even after the booster. All the DLV-treated animals remained gE negative. Twenty-one animals (84%) treated with TLV and 22 (88%) treated with DLV showed CMI response.

In conclusion, data pointed out the suitability of both vaccines to elicit humoral and cell-mediated responses in cattle following administration by different routes.

391 (3139)

THE VALUE OF BOVINE DIARRHOEA (BVD) AND LEPTOSPIRA SP. TITRE IN QUARTERLY MILK SAMPLES WITH CONSEQUENTIAL VACCINATION

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Bovine Viral Diarrhoea (BVD) and Leptospirosis are both serious causes of disease and economic loss in the dairy herd. BVD causes loss by clinical sign and disease, which can often be very mild and include abortion, infertility, diarrhoea, mucosal disease and immunosuppression. Leptospirosis causes loss by clinical signs and disease including infertility, abortion, and milk drop. Often clinical signs are mild and go unnoticed. The zoonotic implications of Leptospira add to the seriousness of its presence within a herd and it's welfare issue. Both diseases can be controlled by vaccination but due to the current recession within the UK dairy sector the cost of vaccination and investigation for the disease is not seen as good value for money. Investigations are only undertaken in many cases when severe clinical outbreaks causing serious economic loss and high welfare implication occur. Losses are often subclinical and therefore hard to identify.

This paper examines the value of testing herds via bulk milk testing for the presence of BVD and Leptospira titres. Test result information indicating infection was fed back to farmers. Farmers made aware of their herd infection rates then made a decision to vaccinate or not.

Testing was carried out by SAC Veterinary Services. Their test interpretation was used. Bulk milk samples were collected from participating farms and analysed on a quarterly basis.

One hundred and eighteen farms participated in the study. Analysis of the data shows that for Leptospira 33% of non-vaccinated herds started vaccination. When herds with negative bulk milk titre are taken out of the total, 42% of inconclusive or high bulk milk sample herds started vaccination of which 92% started on the strength of high titre results.

Analysis of the data showed that 23% of non-BVD vaccinated herds started vaccination. When herds with low BVD titre are taken out of the total 26% of herds started vaccination of which 61% started on the strength of high bulk milk titre and 39% started due to clinical signs.

Results indicated that bulk milk titre evaluation for BVD and Leptospira was a successful aid to start herd

vaccination to control the disease.

392 (2757)

VACCINATION IN ADULT BULLS WITH BRUCELLA ABORTUS RB51 AND CHALLENGED WITH BRUCELLA ABORTUS 2308

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The objective of the present work was to determine shedding and genital effect of vaccination in bulls with *Brucella abortus* strain RB51 (SRB51) and challenged with *Brucella abortus* strain 2308 (S2308). Fourteen adult bulls were selected from brucellosis-free herd. Bulls were previously seronegative for *Brucella*-specific antibodies on buffered plate agglutination, 2-mercaptoethanol (2ME) and standard tube agglutination (SAT) tests. Bulls were assigned in three groups: GI (n=6) bulls were subcutaneously vaccinated with SRB51 (Schering-Plough), GII (n=6) were unvaccinated but challenged bulls, GIII (n=2) were control animals. At 90 postvaccination (PV) days bulls from GI and GII were challenged intraconjunctivally with 5×10^7 cfu of S2308. Clinical genital examination, blood and seminal fluids samples were fortnightly made for clinical status, serology and culture, respectively, until 180 postchallenged (PC) days when all bulls were slaughtered. At the necropsy, entire genital tracts were collected for detailed necropsy examination, histopathology and bacterial culture. At 30 PV days, SRB51 was isolated from the semen of one bull (GI). No clinical and genital alterations were seen in any bulls at PV time. All bulls vaccinated with SRB51 did not seroconverted at PV time. Bulls from GI and GII had positive titles to SAT and 2-ME after PC time. Five of the six vaccinated bulls (GI) were 2-ME positive at 30 PC days, but at 75 PC days 3/6 remained positive. Bulls from GII were seropositive to 2-ME from 30 to 75 PC days. Bulls from GIII were always seronegative in all sampling. At 45 PC days, S2308 was isolated from the semen of one bull of GI. No relevant grossly finding was observed on post mortem examination in any bulls. *Brucella* S2308 was isolated from the scrotal lymph node in one bull of GI. Histopathologically, 2 bulls on GI showed genital inflammation (orchitis, epididymitis, ampullitis, seminal vesiculitis). Bulls from GII showed only minor genital inflammation, GIII control bulls had not histological lesions. Under conditions of this study, vaccination with SRB51 of bulls produced seminal shedding and caution should be taken before it is used in adult bulls.

393 (2782)

EFFICACY OF BOVILIS® QARAT IN CONTROLLED CHALLENGE STUDY

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The C86 BVDV strain has been shown to elicit an immune response in vaccinated animals protecting against BVDV transplacental transmission. Combination of that strain with an IBRV, PI-3V and BRSV strains in an inactivated vaccine adjuvanted with aluminium salts (Bovilis® Qarat) would facilitate herd vaccination management. Quality of short-term (4-18 weeks) and long-term (24-38 weeks) protection afforded by Bovilis® Qarat was experimentally assessed using three matched groups of six naive calves each. The basic vaccination regime of two intramuscular inoculations one month apart was staggered between the long-term and the short-term groups by about five months so that the two groups along with six unvaccinated (control) calves could be challenged at the same time. Intranasal (IN) and/or intratracheal (IT) sequential challenges at 3-8 weeks intervals were performed in the following order: IBRV (IN), PI3V (IN plus IT), 12 pestivirus isolates pool (IN) and four BRSV isolates pool (IN plus IT).

The IBRV challenge produced febrile rhinotracheitis in control calves but both the severity and the duration of reaction were significantly reduced in calves in both vaccinated groups. The latter also shed considerably less virus for a significantly shorter period than the control calves. In line with studies previously reported by other groups, PI3V challenge, pestiviruses pool challenge, and BRSV pool challenge did not induce severe respiratory disease. However, after PI3V and pestiviruses challenges, calves in both vaccinated groups shed significantly less virus in nasal mucus and for a much shorter period than controls. Also noteworthy was the observation that none of the vaccinated calves yielded pestivirus positive serum whereas all six control calves were positive for 5-8 days after IN challenge, confirming results obtained previously in challenge studies carried out in animals vaccinated with the C86 strain. Hence the vaccine will also prevent abortions due to BVDV. Although the IN plus IT BRSV challenge was asymptomatic, all six control calves shed BRSV in nasal mucus for 3-8 days and four of them also from lung washings during two weeks of monitoring after challenge when however there was no BRSV isolated from these sites in vaccinated calves.

These results allow for the conclusion that Bovilis® Qarat would efficiently protect cattle against constituent viruses in the vaccine including also transplacental infections due to IBRV virus and BVDV.

400 (1933)

DETECTION OF OSTERTAGIA OSTERTAGI ANTIBODIES USING AN INDIRECT-ELISA ON MILK SAMPLES AND ITS RELATION WITH MILK PRODUCTION PARAMETERS IN TWO DIFFERENT PRODUCTION SYSTEMS

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The main objective of the present study was to examine the associations between *Ostertagia ostertagi* indirect-ELISA measures in milk samples and farm-management practices known to be associated with gastrointestinal (GI) parasites. The associations were evaluated in two different production systems with different levels of exposure to GI parasitism to assess the potential of the ELISA technique as a monitoring tool for parasite burden in dairy cattle. Individual samples from 10 animals and the corresponding milk herd samples were collected from 133 herds in Girona and 123 herds in Menorca. Samples were tested using an indirect crude-antigen *O. ostertagi* ELISA. There was a high and significant relationship between the average optical determinations (OD) of individual animals and the OD measured in the milk tank ($r_2 = 0.77$, $P < 0.001$). In Menorca, dairy herds were smaller, with lower numbers of lactating cows, significantly lower milk daily production, more land available for pasture (ha/herd), and their cows had greater access to pasture than herds in Girona. The individual milk OD values were higher ($P < 0.001$) in Menorca herds compared to Girona herds. Bulk tank OD values were also higher in Menorca herds ($P < 0.05$). In general, herds that provided access to pasture to their animals at any time of their life tended ($P = 0.14$) to increase OD values in milk compared to those that did not provide access to pasture. Parasite control against GI nematodes in both Girona and Menorca farms was poor, but when adequate parasite control treatments against GI nematodes were applied, lower OD values were observed. Herds that treated the cows during the dry period had significantly ($P = 0.05$) lower ODs than those that did not treat their animals at that time, and the herds that treated their animals during the raising period as heifers or during lactation had lower OD values than those that did not, although differences were not statistically significant ($P > 0.10$). Overall a negative correlation between OD values and milk production was observed ($r_2 = -0.27$, $P < 0.001$). Herds that dewormed the lactating animals had higher average milk production than those that did not ($P < 0.05$) and this difference was not affected by location. As conclusion, the ELISA technique may be a useful tool for monitoring parasite burden in dairy cattle and could be especially useful to evaluate the effect of strategically epidemiological-based anthelmintic treatments.

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