NATIONAL RESEARCH COUNCIL REPORT: "DIAGNOSIS AND CONTROL OF JOHNE’S DISEASE": IMPLICATIONS FOR INTERNATIONAL PROGRESS IN CONTROL OF PARATUBERCULOSIS AND CROHN’S DISEASE

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In May 2003, a group of veterinary and medical scientists, convened by the National Research Council, National Academy of Sciences in the U.S.A., reported on a two year review of national and international research and control efforts for paratuberculosis (Johne's disease), as well as the possible association of Mycobacterium avium spp. paratuberculosis (Map) with Crohn's disease in humans. Central to the report was a series of recommendations to guide national policy and to focus research priorities. These recommendations focussed on disease control, education and training, research, diagnostics and immunology, Map genome studies, vaccine development, and human/animal health issues.

The report provides guidelines for implementation of state-federal control programs for cattle and recommends adaptation and adoption of some innovative sampling and sample-pooling strategies developed in other countries. Four areas of research were identified that could clarify the relationship between Map and Crohn's disease. Specific recommendations were made in the areas of environmental contamination with Map, paratuberculosis control in other domestic and captive ruminant species, and food safety issues related to paratuberculosis in animals.

Since the publication of this report, substantial progress has been made, particularly in the areas of development of a comprehensive paratuberculosis control and education program for the U.S.A. and in research studies to evaluate the role of Map in Crohn's disease.

The National Research Council report on Johne's (paratuberculosis) focuses on the situation in the U.S.A., but preparation of the report entailed a comprehensive review of the international literature and conclusions of this report may have broad application in other countries concerned with paratuberculosis.

ANTIMICROBIAL DRUG USE AND RESISTANCE IN FREE-STALL DAIRY HERDS IN ONTARIO, CANADA

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Quantitative information on antimicrobial use in Canadian dairy herds is fundamental to understanding antimicrobial resistance (AMR) patterns and improving antimicrobial use in the industry. An understanding of AMR patterns of enteric bacteria carried by dairy cattle is necessary in describing the potential public health impact of the dissemination of resistant foodborne bacteria from dairy farms. This presentation will describe the antimicrobial usage patterns of dairy veterinarians and free-stall dairy producers in Ontario, Canada, and the AMR patterns of E. coli and Salmonella isolated from cows and calves in 25 study herds.

A questionnaire assessing aspects of antimicrobial use was mailed to all producers with free-stall dairy barns and all livestock veterinarians in the province of Ontario. Additionally, 25 free-stall herds where enrolled in a longitudinal study in which producers tracked individual and herd level drug use for a one year period using a paper-based treatment record and a garbage can auditing (GCA) system. Fecal Salmonella and E. coli isolates from cull cows and cow-calf pairs in study herds were tested for antimicrobial resistance to 16 antimicrobials using the Sensititre System and the National Antimicrobial Resistance Monitoring System 2002 plate configuration.

The questionnaire response rates were 51% for producers and 47% for veterinarians. Both groups ranked the reasons for antimicrobial use in lactating dairy cattle: 1-mastitis, 2-uterine/vaginal infection, 3-lameness, 4-respiratory infections. Ceftiofur, penicillin, trimethoprim-sulfadoxine and oxytetracycline were the most frequently veterinarian dispensed injectable antimicrobials, while a novobiocin-streptomycin-penicillinG-polymixinB combination product and cephaloridine were the most frequently dispensed intramammary antimicrobials. These finding are supported by data from study herd records and GCA.

All 26 Salmonella isolates from cow-calf pairs were susceptible to all 16 antimicrobials. Of the 1215 E. coli isolates from 243 cow-calf fecal samples, 471 (39%) were resistant to at least 1 antimicrobial. Isolates were most commonly resistant to tetracycline, sulphonamide and streptomycin and there was no resistance to ceftiofur, ceftriaxone or ciprofloxacin. Calf isolates had a higher frequency of resistance (p<0.0001) and higher
levels of multi-drug resistance compared to cows, but there was no significant difference between heifer and bull calf isolates.
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103 (1201)
IMPACT OF AGRICULTURAL DEVELOPMENT AND BOVIDAE BREEDING ON THE ENVIRONMENT IN THE ORIENTAL BRAZILIAN AMAZONIA
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During the last forty years the Brazilian Amazon has been subjected to massive colonization which has considerably modified its environment. In an attempt to avoid the threat of internationalization, as well as an agrarian reform in the South and North-East of Brazil, the government, through the construction of numerous roads which opened it up to the other states, together with a politics of credits and fiscal incentives, was at the basis of this process. Success was rapid and entailed the arrival of important investors as well as small farmers. This social difference was reflected throughout the agrarian systems, in particular as the government neglected the small farmers because of the low efficiency of the family holdings, event though these were largely subsidized. The pressure exerted by the major landowners in obtaining more territory led to a monopolization of the rural areas which were already well developed. The small farmers were forced to migrate to the poor areas of the cities or to the pioneering fronts that are difficult to develop. Today, this phenomenon is the cause of numerous damaging effects and of violent conflicts in the Brazilian Amazon. It has also largely contributed to the erratic clearing of the forest. Another important factor in environmental degradation in the Brazilian Amazon is the large expansion of animal breeding that followed the process of colonization. This expansion was characterized by the setting up of cultivated pastures, detrimental to the forest, but with a high economical efficiency compared to the native pastures and a high sale value, thus entailing ground speculation. However, unless special care is taken, this efficiency may drop rapidly due to the process of pasture degradation. Nevertheless, the low price of forested land encourages the farmers to adapt a short-term exploitation approach, even if they have to buy new land when the original is degraded. A restoration of the social unbalance among the farmers, an intensification of the utilisation of the pastures with a long term vision and an economic development of the forest should contribute to minimizing the impact exerted on the Brazilian Amazon.
Funding: INSTITUT VETERINAIRE TROPICAL

104 (1323)
PREVALENCE OF MULTIDRUG-RESISTANT (MDR) SALMONELLA IN FRENCH BOVINE DAIRY HERDS
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Salmonella is in France the first cause of food-borne outbreaks. This bacterial genus is also often resistant to several antibiotics simultaneously, with for instance the DT104 phage type (Arcangioli et al, 2000). This latter Salmonella subtype is responsible for 65% of clinical cases of bovine salmonellosis in France (Martel et al, 2002).
In the global frame of Quantitative Risk Analysis (QRA) on foodborne diseases due to MDR Salmonella, a 3 year-study was conducted in order to appreciate: (i) the prevalence of these bacteria in bovine dairy herds situated in Western France, with and without clinical expression of salmonellosis and (ii) the contribution of antibiotic use in these herds to the emergence and persistence of MDR Salmonella strains in bovine dairy herds. These data are essential for risk assessment of human infection by MDR Salmonella of bovine origin.
With the help of an extended partnership, 494 dairy herds were included and manure or dung sampling was performed for Salmonella research during the two first years of the study. Individual fecal samples from dairy cows but also bulk milk, water, and environmental swabs were collected in each contaminated (Salmonella positive) herd. All strains isolated during the study were serotyped and tested for their antimicrobial susceptibility.
No difference could be evidenced between the three regions investigated as well as between both years of sampling. The herd prevalence of Salmonella and MDR Salmonella contamination observed was respectively 8.1% (IC95%: 4.5% - 13.3%) and 1.9% (IC95%: 0.5% - 5.4%). Serotypes encountered included Montevideo (26%) as well as Typhimurium strains (14%). Among MDR isolates, we notably found one Derby and one Typhimurium strains.
The intra herd prevalence estimated by a Bayesian approach was variable between the contaminated herds, ranging from 0% to 75% of the present cows. The major associated risk factor evidenced for MDR Salmonella contamination was a history of clinical salmonellosis during the fore-year. Also, MDR strains were isolated more often at the end of the sampling period (end of winter - beginning of spring).
Considering MDR Salmonella farms as cases and non-MDR Salmonella farms as controls, a study is now
conducted in order to identify and analyze factors, such as antibiotic use, supposed to contribute to the emergence and persistence of MDR-Salmonella isolates in bovine dairy herds.

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105 (1016)
SPATIAL ATTRIBUTES OF DAIRY HERDS SHEDDING SALMONELLA SPP. INTO BULK TANK MILK IN A CALIFORNIA DAIRY SHED, USA
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Introduction: Salmonella infections in dairy cattle are an animal and public health concern. Salmonella are commonly isolated from market dairy cattle at slaughter, shed in milk, and can be isolated in cattle feces without signs of disease. The environment may be a reservoir of Salmonella. Little is know about movement of Salmonella between farms. To examine movement of Salmonella on an ecosystem level requires use of tools that incorporate spatial, temporal, and biologic data. The purpose of this study was to identify spatial attributes of dairies within a region that would describe and provide insight into Salmonella ecology.

Materials and Methods: A geographic information system was developed that included dairy locations, waterways, crops, land use, and bacteriologic results from water samples and bulk tank milk in a three-county area in California. Bulk tank milk samples were tested every three months over two years. The samples came directly from processor samples used for quality milk programs. Dairy density was assessed for buffers ranging from 1.6-8 km from each dairy location and from each salmonella-positive dairy. Disease rates were produced using variable spatial filters. Tests for statistical significance used Monte Carlo simulations.

Results: Over 500 dairies were identified and mapped in the region. The most common Salmonella serotypes isolated from bulk tank milk were S. Montevideo and S. Dublin. Herds with >6 other dairies located within 3.22 km were 2.2 times more likely to be Salmonella-positive compared to herds with <3 dairies located within 3.22 km. There was a linear association between herds shedding the same Salmonella serotype >2 times and density of dairies in a 3.22 km buffer (p=0.04). Dairies with > 6 other dairies in a 3.22 km radius were 2 times more likely to have S Montevideo isolated from bulk tank milk compared to dairies with <3 other dairies within 3.22 km (p=0.04). Isolation of S. Dublin at any sampling period was not associated with dairy density within 3.22 km (p=0.19).

Using spatial filtering, areas of significantly high Salmonella prevalence were found to vary across time. S. Montevideo appeared to cluster in two geographic locations.

Conclusions: Dairy density appears to be a risk factor for Salmonella identification in bulk tank milk. A cattle-adapted Salmonella serotype had less tendency to cluster, whereas non-host adapted serotypes did display clustering in space and time.

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106 (2510)
ANIMAL HEALTH ON IRISH ORGANIC DAIRY FARMS
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Organic farming currently accounts for 2 to 3% of agricultural production and retail food markets in the European Union. Organic livestock management differs from conventional farming in various aspects including nutritional management, housing and bedding, and disease management. The effects of these differences had not previously been investigated in Irish organic dairy systems. Previously published literature pertaining to other countries suggested little overall change in animal disease incidence following conversion to organic dairy production, but potential problems in the area of dairy cow mastitis. A prospective animal health-monitoring program was established on an institutional organic dairy farm and its adjacent institutional conventional farm for two years. A similar animal health-monitoring program was established on five commercial organic dairy farms throughout Ireland, for one year. The mean organic herd size was 43 cows with a mean yield of 4300 liters per cow per year. In all, 250 organic cow-years were included in the study. All animal health events were recorded on the study farms throughout the study period. Data collected included individual disease incidents, group interventions, and reproductive events. This information was supplemented by laboratory investigations.

on the institutional organic farm. There was high incidence of clinical mastitis in study cows (50 cases per 100 cows per year). Herds with loose-straw-based winter housing system had a significantly higher incidence of mastitis than those with cubicle-based winter housing (74 and 27 cases per 100 cows per year respectively). Other adult cow diseases such as lameness, ketosis, milk fever, and hypomagnesaemic grass tetany occurred at rates lower than recorded previously in published studies pertaining to conventional agriculture. Low incidences of calfhood diseases, such as diarrhea and respiratory disease complex, were also reported. Non-conventional therapy was used in 60% of all animal disease events on study farms. Homeopathy was the most frequently used non-conventional therapeutic modality. Reproductive efficiency of organic study cows (mean calving to service interval 82 days; mean calving rate to first service of 62%; and mean calving to conception interval of 89 days) appeared to be better than previously published conventional cow data. This
study establishes base-line data on animal health in Irish organic dairy systems.

Funding: Teagasc

REGULATION OF ANIMAL TRADE - A TOOL TO REDUCE INFECTIOUS DISEASES IN CATTLE HERDS?

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Funding: Intramural-University of Wisconsin, 2015 Linden Dr, Madison, WI, 53706, United States of America

REGULATION OF ANIMAL TRADE - A TOOL TO REDUCE INFECTIOUS DISEASES IN CATTLE HERDS?

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TRANSMISSION OF FMD IN GROUPS OF VACCINATED AND NON-VACCINATED CALVES

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Twenty-six Holstein Friesian calves were included in an experimental study to measure transmission of FMD virus in free roaming calves. Three groups of four calves were vaccinated with DOE OManisa vaccine two weeks before challenge, three groups of four calves remained unvaccinated. In each group, two calves were challenged (day 0) with 1500 CIDs (cow infectious dose 50%) of the first cattle passage of the FMD field isolate O/NET2001. An intranasal method of inoculation was used. The other two calves in each group were contact-exposed to the inoculated calves. Two calves were only vaccinated and served as vaccine control group. This experiment was repeated once.

The first seven days after challenge mouth-swabs and heparinised blood samples were taken daily. In the consecutive week the mouth swab collection continued on a daily basis. Serum blood samples were obtained on day 0, 4, 7, 11, 14, 21 and 28 after challenge. At the end of the experiment (day 29, 30 and 31) probang samples were collected. Virus from the mouth swabs, probang and heparinised blood samples was titrated on secondary ovine kidney cells. The sera were tested in the virus neutralisation assay. Transmission was quantified by estimating the reproduction ratio R using the occurring contact infections.

Results: All inoculated calves were assumed to be infectious; contact-exposed calves were classified infectious when they, either showed clinical signs, or tested positive in virus isolation from blood or mouth swab samples. In the vaccinated groups only one contact-exposed calve was classified infectious. In the non-vaccinated contact-exposed 9 out of 12 calves became infectious. Antibody titres were found in non-vaccinated, infectious calves, but no booster effect on the titre was measured in the vaccinated calves. This resulted in a Maximum Likelihood Estimate for the R in the vaccinated groups Rv = 0.17 (0.01; 1.13); the MLE for R in the non-vaccinated groups is Rc = 3.30 (1.23; 28.88). When testing H0: Rc <= Rv, p = 0.002.
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It is a well known fact that infectious cattle diseases, either severe or not, are most effectively spread by direct contact from animal to animal, in or between herds. Thus, in large cattle herds and dense animal populations (ie auctions and exhibitions), at common pasture and in transport vehicles, there is an increased risk for spread of infectious agents by more or less direct contact between the animals.

Norwegian farmers are purchasing about 40 000 cattle per year, 2/3 of which are young stock and calves. Most of the cattle (83%) are sold and transported inside the “county” borders, but the last 17 % of the animals are sold to other regions and are sometimes also transported over long distances. Over time, it has been possible to follow the spread of an infectious disease, ie BVDV (Bovine Viral Diarrhea Virus) from one herd to another in distant parts of the country after purchase and transportation of PI (Parainfluenza)-animals to uninfected herds.

The Norwegian dairy and beef industry, therefore, from January 1st, is introducing a volunteer “quality assurance system” in cattle trade to prevent such spread of infectious diseases between cattle herds. The country is divided into four trade regions with compulsory veterinary certification of individual animals that are sold out of regions, combined with increased focus at the herd health situation in the herd of origin. There will also be a possibility for interested farmers to join a cattle herd health certification system, offered by the cattle industry. Such a system will include regular veterinary control of herd health status combined with specific demands of the hygienic standard and precautions as to reduce the risk of introducing infectious agents into the herd.

PRE-SLAUGHTER MONITORING OF E. COLI O157 AND OTHER STEC IN COMMERCIAL FEEDLOTS

PRE-SLAUGHTER MONITORING OF E. COLI O157 AND OTHER STEC IN COMMERCIAL FEEDLOTS

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Shiga-toxin producing E. coli (STEC) have become a worldwide public health concern. In North America, serotype O157:H7 is a particularly significant issue for human health and the beef industry. Since cattle feces are often implicated as the source of STEC in human infections, there is considerable interest in identifying and controlling these bacteria in cattle. Although there appears to be a role for pre-harvest control, there are currently no validated surveillance methods for STEC in live feedlot cattle. Field-based validating strategies are a necessary component of on-farm control programs. The development of methods for rapid and valid assessment of the STEC status of commercial feedlot cattle has been hindered by diagnostic test limitations.

Through a series of laboratory and field studies we are evaluating diagnostic methods for detection of STEC following OIE (Office International des Epizooties) guidelines for methods of test validation. Concurrently, we are establishing prevalence and risk factors for E. coli O157:H7 and other STEC in Alberta feedlot cattle immediately prior to slaughter. During the summer of 2003, we found that the prevalence of O157:H7 was higher than was previously reported. The fecal prevalence was 100% at the pen-level and 43% for individual cattle (range 13-77% within pen). We found that a pen-level diagnostic method (rapid on-premise evaluation devices (ROPEs) + rapid culture/ELISA) could detect O157:H7 in pens within 24 hours of slaughter. We will determine the diagnostic sensitivity and specificity of this method following a trial that is currently underway.

Previously, we found evidence of other serotypes that cause human illness, such as O111 and O26, can be detected relatively frequently in feces from pens of feedlot cattle (20% and 80% pen-prevalence; respectively). E. coli O157:H7 and other STEC are found in feces of feedlot cattle with a potentially high, but often extremely variable frequency. Preliminary results indicate that commercial feedlots may be able to use pen-monitoring strategies to assess the STEC status of pre-slaughter cattle. A valid testing strategy could identify STEC status of pens pre-slaughter, focus costly interventions on high prevalence pens, identify risk factors associated with high prevalence, and validate pre-harvest interventions in a HACCP-based approach.

IDENTIFICATION OF BOVINE GASTROINTESTINAL LACTIC ACID ORGANISMS WITH IN VITRO INHIBITORY ACTIVITY ON ESCHERICHIA COLI F5 AND O157

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At present, there is a lack of scientific evidence supporting the use of probiotics to treat or prevent diarrhea in calves. In order to identify strains with beneficial in vitro properties, 104 bovine-derived lactic acid bacteria (LAB) isolates from 54 healthy animals were assessed for resistance to low pH (2 and 4) and bile salts (0.3 and 0.15%), aerotolerance, and antimicrobial activity against Escherichia coli F5 and O157. Fourteen and
42% of isolates grew well (=80% growth compared to controls) at pH 4 and 0.3% bile respectively, whereas all isolates grew poorly at pH 2. Interestingly, eight isolates enhanced the growth of E. coli, while seven were strongly inhibitory (=85% of inhibition compared to control). This inhibition was associated with low pH of the supernatants \((r^2=0.804; p<0.001)\), and thought to have resulted from the production of organic acids. Overall survival rates and inhibitory activities were used to rank the isolates. The best isolates in all tests were identified as Lactobacillus plantarum by 16s rRNA gene analysis. Results suggested that the procedures developed in this screening study were effective to select calf LAB tolerant to low pH and bile salts with inhibitory activity on E. coli. To the authors' knowledge this is the first calf study where aerotolerance was included as a criterion to select intestinal probiotics and where bovine-derived Lactobacillus plantarum strains were identified as potential gastrointestinal probiotics for calves. Further colonization and safety studies are required for these isolates.

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112 (2887)
CAUSES FOR SPACE TIME CLUSTERING OF SWISS BOVINE SPONGIFORM ENCEPHALOPATHY (BSE) CASES
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Until November 9, 2003, a total of 449 cases of bovine spongiform encephalopathy (BSE) have been detected. Of these, 206 were born after the ban on feeding mammalian meat-and-bone meal (MBM) to ruminants became effective (BAB-cases). A cluster (CL) analysis was started to find explanations for the observed spatial variation. Exact co-ordinates of farms of putative exposure for BSE cases and of all farms and feed mills in Switzerland and results from the surveillance for MBM in cattle feed produced there, were acquired.

Testing for clustering was carried out using the SaTScan-statistic and a Bayesian approach. Odds ratios (OR) and correlations were calculated with NCSS.

Two statistically significant CL were identified for the BAB cases, whereas no clustering was detected for cases borne before the feed ban. The CL appeared in distinct time periods, indicating different time intervals of exposure. Only one CL of BAB cases came up significant in space-time analysis, whereas there was a strong time CL and some significant space-time CL for cases borne before the feed ban. CL resulting from a analysis with locations of feed mills were almost identical.

The CL regions showed high cattle and pig density and also a high proportion of mixed farms. Farms with a BSE case had an OR of 1.7 to have pigs compared to farms without. The OR to have pigs was 2.0 for farms with a BAB case compared to farms without. If BAB cases inside the cluster regions were compared to cases outside, the OR to have pigs was 3.8 for farms inside the CL.

The Bayesian approach calculated the posterior Relative Risk (pRR) for BAB cases in a region. Regions with a high pRR resembled well the regions identified as CL. The pRR was tested for correlation with farm animal data. These results indicate a high pRR in areas with a high density of farm animals, but that other factors are necessary to explain the differences in pRR in high density areas.

The facts that purely spatial CL were only shown for BAB cases in regions with high pig density and a high proportion of mixed farms, as well as the finding of feed mills producing contaminated cattle feed in these CL, support the hypothesis that contamination of cattle feed with feed for other species (containing MBM) may have caused these BAB cases. The occurrence of the CL in different time periods provided evidence for the hypothesis that clustering may be linked to the source of feed used on the farm of putative exposure.

113 (5019)
HEALTH AND PRODUCTION EFFECTS ASSOCIATED WITH SALMONELLA SHEDDING IN DAIRY CATTLE
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Salmonella enterica is associated with disease in dairy cattle but it also commonly found in dairy environments and in clinically healthy animals. What is unclear is whether asymptomatic infections affect production and health of these animals. A cohort of 564 animals from a single dairy was enrolled in a prospective study to monitor salmonella shedding dynamics and shedding status on health and production. Animals were enrolled into the study over a 3-month period. All animals, estimated to be 60 days before calving, were enrolled and sampled on a two-week schedule up to 60-days post calving. At each sampling time a fecal sample was obtained along with body condition and hygiene score. Health and production records were collected daily by farm personnel and recorded in an on-farm computer system. Fecal samples were cultured for Salmonella using a two-step selection/enrichment and plated on selective media. All suspect isolates were biochemically confirmed and serogrouped and serotyped. One hundred and ninety three of the 564 study animals were detected as shedding salmonella. The dominant serotypes recovered were S. Reading and Typhimurium. Salmonella shedding was very low during the pre-parturient period and peaked immediately post-partum. Shredding remained high during the early milking period. Cows that were detected
as shedding were 2 times more likely to be removed from the herd as non-shedders. Animals detected with Salmonella were 3 times more likely to be treated for disease. Salmonella status had no effect on mortality or milk production.

Funding: USDA, CSREES, NRI, Epidemiologic approaches to food safety

114 (2631) FEEDING DIRECT-FED MICROBIALS TO REDUCE THE PREVALENCE OF ESCHERICHIA COLI O157:H7 IN FEEDLOT CATTLE

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A clinical trial was conducted during the summers of 2002 and 2003 to test the effectiveness of feeding Lactobacillus acidophilus direct-fed microbial (DFM) to prevent feedlot cattle from shedding Escherichia coli O157:H7 in the feces. Steers (n= 448) were assigned to either of four vaccination treatments (2 hd/trt) within pen. Treatments included: 1) no vaccination; 2) vaccinated once at re-implant (d-42); 3) vaccinated upon arrival (d-0) and again at re-implant (d-42); and 4) vaccinated on arrival (d-0), at d-21, and again at re-implant (d-42). An additional 128 steers were assigned to 12 pens within the same feedyard to serve as unvaccinated external controls. Each steer was sampled every three weeks of the feeding period for 1 pre-treatment period (d-0), 2 interim periods (d-21, d-42), and 4 test-period samplings (d-63, d-84, d-105, d-126). From May 8 to September 26 2003, 4260 fecal samples were collected for culture from 608 calves. The data were analyzed by logistic regression (GENMOD of SAS) accounting for repeated measures, year, pen and block. Odds ratios were converted relative risk and efficacy was 1-relative risk. Prevalence differed between 2002 and 2003 (p=0.002). In 2002 the average probability for a DFM-treated steer to shed E. coli O157:H7 over the test periods was 13% compared to 21% among untreated cattle. In 2003 the average probability of shedding was 21% among DFM-treated steers compared to 28% for controls. Over the two years DFM treated cattle were 35% less likely to shed E. coli O157:H7 than cattle in untreated pens (p=0.002). Feeding the DFM product did not affect (P>0.10) overall ADG, DMI, or ADG: DMI. We concluded that feeding this DFM effectively reduced the proportion of feedlot cattle shedding E. coli O157:H7 in feces.

Funding: Nebraska Beef Council, USDA NRICGP, Nutrition Physiology Inc

115 (2628) VACCINATION TO REDUCE THE PREVALENCE OF ESCHERICHIA COLI O157:H7 IN FEEDLOT CATTLE

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A clinical trial was conducted to test the effectiveness of vaccinating feedlot cattle against Type III secretory proteins of enterohemorrhagic Escherichia coli on the proportion of feedlot steers shedding E. coli O157:H7 in feces. Medium-weight steers (N=480) were assigned randomly to 60 pens (8 hd/pen) and to one of four vaccination treatments (2 hd/trt) within pen. Treatments included: 1) no vaccination; 2) vaccinated once at re-implant (d-42); 3) vaccinated upon arrival (d-0) and again at re-implant (d-42); and 4) vaccinated on arrival (d-0), at d-21, and again at re-implant (d-42). An additional 128 steers were assigned to 12 pens within the same feedyard to serve as unvaccinated external controls. Each steer was sampled every three weeks of the feeding period for 1 pre-treatment period (d-0), 2 interim periods (d-21, d-42), and 4 test-period samplings (d-63, d-84, d-105, d-126). From May 8 to September 26 2003, 4260 fecal samples were collected for culture from 608 calves. The data were analyzed by logistic regression (GENMOD of SAS) accounting for repeated measures, and pen. During the post-treatment period the probability of vaccinated cattle shedding O157:H7 was 11% compared to 29% of unvaccinated cattle. Over the post-treatment period cattle receiving vaccine were 59% less likely to shed O157:H7 compared to external control cattle (p=0.0008). Vaccine efficacy of receiving 1, 2, or 3 doses of vaccine was 52, 58, and 68% respectively, compared with cattle in pens not receiving vaccine. Unvaccinated cattle in treated pens were 50% less likely to shed O157 than cattle in pens not receiving vaccine. We concluded that vaccination effectively reduced the proportion of feedlot cattle shedding O157:H7 in feces, the effect was dose-responsive, and vaccination within a pen also protected unvaccinated pen-mates (herd-immunity).

Funding: Nebraska Beef Council, USDA NRICGP, Bioniche Life Sciences

116 (1823) PREVALENCE OF GIARDIA IN CATTLE ON PRINCE EDWARD ISLAND

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The prevalence of Giardia in dairy and beef calves has been well documented. Giardia intestinalis is one of the most common intestinal pathogens of human beings and animals globally. However, few studies have
been conducted on the prevalence of Giardia in adult cattle. In this study fecal samples were collected from adult cows (n=195) and calves <6 months of age (n=179) on 11 farms on Prince Edward Island (Canada). Giardia cysts were isolated from 20g of feces using sucrose flotation and centrifugation, then stained with a Giardia specific FITC-labelled mAb and enumerated under the fluorescent microscope. Additional samples were collected from one herd and examined for Giardia over a three-month period. The overall prevalence of Giardia in the cows was 38%, with the prevalence on individual farms ranging from 21 to 52%. The overall prevalence of Giardia in the calves was 49%, with the prevalence on individual farms ranging from 0 to 82%. Mean number of Giardia cysts in the herd that was sampled every two weeks fluctuated over time, ranging from 20 to 60% with a cumulative prevalence in the herd of 65%. The mean number of Giardia cysts excreted by this herd also fluctuated over time, ranging from 15 to 47 cysts per gram of faeces. Currently, Giardia isolates from these cattle are undergoing DNA sequence analysis to determine their zoonotic potential. At this time, 6 Giardia isolates correspond to the Assemblage A genotype, which is also known to infect humans, and 2 isolates correspond to the livestock specific Assemblage E genotype.

The results of this study demonstrate that Giardia was not only highly prevalent in young calves but also in adult cattle. Despite a low number of cysts excreted per gram of feces, adult cattle may still act as a significant reservoir for Giardia due to the large amount of feces they produce. Additionally, the presence of the human infective Assemblage A genotype in cattle indicates that giardiasis in cattle may be an important source of infections in human beings, particularly farmers and veterinarians.

Funding: AVC, DVM/GRADUATE SCHOLARSHIP

118 (3436)
UTERO-OVARIAN STATUS PRIOR TO FIRST SERVICE - 2. EFFECTIVENESS OF COMMONLY USED VETERINARY THERAPEUTICS ON PREGNANCY RATE


Ultrasound reproductive tract scores (URTS), (7,797) were performed on 5,734 Holstein-Friesian cows in 61 spring-calving herds over 2 years (1999 and 2000) prior to first service. Data were restricted to URTS records carried out prior to the start of the breeding season, to herds where detailed records regarding veterinary therapeutics were obtainable, and to records where the treatments used were deemed appropriate. Thus, 6,477 URTS records were available for analysis. Twenty two per cent of cows received at least one fertility treatment between calving and the end of breeding. Statistical analysis to investigate if differences in pregnancy rate to first service (PREG1) were evident between cows that received fertility treatments and those that did not, within URTS score, was carried out using the PROC GENMOD procedure in SAS. Adjustement was made for herd, year, parity and calving to pre-service scan interval. Veterinary therapeutics, with URTS 1 (normal uterus, cycling) (predominantly a prostaglandin regime) and URTS 6 (normal uterus,
anovulatory) (predominantly progesterone supplementation), resulted in a lower PREG1 (P<0.05) compared to cows receiving no fertility treatment. Intervention with URTS 2 (mild endometritis, cycling) and URTS 4 (mild endometritis, anovulatory) had no significant effect on PREG1. Cows with URTS 3 (moderate endometritis, cycling) and URTS 5 (pyometra), however, did respond positively (P<0.05) to the fertility regimes used (predominantly washout +/- prostaglandin regime and an intensive hormonal regime including a washout, respectively). This study is the largest to report on the use of fertility treatments as used by veterinarians and farmers at commercial farm level. The results of this analysis indicate, that no more than 8% of cows justify veterinary therapeutics (produce a favourable response). These include cows with moderate to severe endometritis.

Funding: AIB, HUKI, AI Co-ps, Dairy Levy

119 (5015)

EVOLUTION AND REPEATABILITY OF THE REPRODUCTIVE PERFORMANCE ASSESSED IN HOLSTEIN DAIRY HERDS IN SOUTHWEST OF FRANCE BETWEEN 1999 AND 2002

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As in many countries, since 20 years, the reproductive performances are regularly decreasing in dairy cattle. Technical and economic consequences are important for the breeders but also for the AI cooperatives. This survey aimed firstly to determine the evolution of fertility and fecundity between 1999 and 2002 in the Holstein breed in the South-West of France. Secondly, the repeatability of the reproductive performances of each herd was assessed on several years in order to classify the herds according their reproductive profile among years and to test the relationship with some herds characteristics.

Data used originated from 2367 Holstein dairy herds in the area of MIDATEST breeding cooperatives union from 1999 to 2002. For each herd-year unit, fecundity was assessed by rate of calving-to-1rst service interval higher than 90 d (CSI90) and rate of calving to conception interval higher than 110 days (CCI110). Fertility was estimated by 1rst service conception rate (CR1) and the percentage of cows requiring 3 Alis or more to be fertilized (3AI). Those parameters were chosen because of their high coefficients of variation (CSI90=36.2%, CCI110=31.0%, CR1=15.1% and 3AI=42.3%). For each parameter, mobile means (MM) were calculated by campaign in order to classify herds in 4 following classes: high fertility (HFEC, CR1 > MM and 3AI < MM) or low fertility (LFER; CR1 < MM and 3AI > MM), high fecundity (HFEC; CSI90 and CCI100 < MM) or low fecundity (LFEC; CSI90 and CCI100 > MM).

CR1 was declining during the 4 years from 57.8% to 54.6% together with an increasing rate of cows which were served 3 times or more (16.0 to 18.6%). Rates of CSI90 and CCI110 increased respectively from 29.9% and 37.3% to 32.9% and 45.0% between 1999 and 2002. The percentages of herds defined as HFER, LFER, HFEC and LFEC averaged 37.9, 35.1, 36.4 and 32.7% respectively. Globally, 33 % of herds remained in the same class between two consecutive years and they represented only 10 % of herds over 4 years (HFEC=9.9%, LFEC=8.5%, HFER=11.5%, LFER=8.6%). LFEC and LFER herds were characterized by a higher level of milk production, a greater size and a period of calving more frequently observed between January and August in comparison to the average population. On the contrary, HFEC herds were characterized by a calving period between September and December and by high fertility results. Levels of milk production were lower in HFER herds than in the average population, but did not differ in HFEC herds. This study highlighted that the repeatability of herd reproductive performances over successive years may be used to better characterize the profiles of herds and could be a part of local intervention schemes to improve reproductive performance after AI.

120 (2509)

TRANSVAGINAL FOLLICULAR PUNCTURE AND ANIMAL WELL-BEING IN COW

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Oocyte collection for in vitro embryo production using the Ovum Pick-Up (OPU) technique requires transvaginal follicular puncture. OPU has been criticized by animal welfare groups because the method requires penetration of the vaginal wall, peritoneum and ovarian stroma with a needle. The aim of this study was to objectively evaluate the stress and ovarian lesions associated with trans-vaginal puncture.

Ten primiparous Holstein cows from the same herd were used. Trans-vaginal puncture was performed twice a week during 8 weeks (OPU group; 16 sessions) on five cows. The other five cows (control group) were manipulated in the same way as the OPU cows (transrectal palpation and insertion of an intra-vaginal device) except that trans-vaginal punctures were not performed. Both groups were manipulated under epidural anesthesia. Acute stress was measured by measuring: blood cortisol over the three hours following manipulation and milk production. Repeated acute stress was evaluated by comparing adrenal sensitivity (ACTH test) and white blood cell count before and after the 16 manipulation sessions. An additional four cows were ovariectomised at regular intervals between 0 and 30 d after OPU and the ovaries were examined.
These data show that although a large proportion of clones develop LOS in late gestation, they are actually compared to controls (4.7 ± 1.2 mm vs. 7.2 ± 1.7 mm at D50 and 5.7 ± 1.5 mm vs. 9.3 ± 4.3 mm at D64, p < 0.05) for OD or VW at any stage. Placentome width but not length was significantly smaller in clones (p < 0.05). Finally, clones alive at birth were also significantly smaller than controls at D50. There was no difference for OD or VW at any stage. Placentome width but not length was significantly smaller in clones at all stages (CRL: 31.5 ± 5.6 mm vs. 36.0 ± 4.6 mm at D50, p < 0.01; 48.4 ± 0.8 % in controls). There was no difference for MCV or Pl at any stage. Lymphocytes counts were significantly lower in clones than controls at D50 and D64 (2.4 ± 0.3 to 3.6 ± 0.4 × 10⁶/µL, p < 0.05, in clones and controls respectively). There was no difference for monocyte counts and Glu at any stage, nor for Cr and U at D1. Fib was significantly higher in clones at D4 and D35. The same trend was observed for Ht (27.7 ± 0.9 to 36.7 ± 1.0 % from D1 to D63 in clones vs mean Ht of 38.4 ± 0.8 % in controls). There was no difference for MCV or Pl at any stage. Lymphocytes counts were significantly smaller in clones at D21 and D49 and were within the lower range for calves. Neutrophil counts were significantly higher in clones at D1: 11 844 ± 890/µL vs. 8672 ± 1148/µL, p < 0.05, in clones and controls respectively. There was no difference for monocyte counts and Glu at any stage, nor for Cr and U at D1. Fib was significantly higher in clones at D4 and D35.

Five clones died after the end of the study (age >3 months) and there was no difference for all parameters measured between clones that finally died and clones that did not.

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121 (5013)
HAEMATOLOGICAL AND BIOCHEMICAL MEASUREMENTS OF CLONED CALVES DURING THE TWO FIRST MONTHS OF LIFE
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Somatic cloning by nuclear transfer has been associated with several abnormalities during pregnancy. In our laboratory, a mean of 30 % of cloned calves die mostly during the first month. It is not known, however, whether the remaining surviving animals that appear clinically normal are completely healthy. In order to determine their health status, blood samples were obtained from 23 clones and 12 control calves at day 1, 4, 11, 21, 35, 49 and 63 from birth (D0). Haemoglobin concentrations (Hb), haematocrit (Ht), mean corpuscular volume (MCV), leucocyte populations (lymphocytes, monocytes and neutrophils), platelets (Pl), glucose prior to feeding (Glu) and fibrinogen (Fib) were measured. Creatinin (Cr) and urea (U) concentrations were measured only at D1. Results are presented as mean ± standard error. Data were analysed using ANOVA for repeated measurements and Student t test as appropriate with SAS software.

All measured parameters were within normal limits for all calves. Hb and Ht, however, were significantly lower in clones compared to controls, at all stages for Hb and until D35 for Ht. Hb in clones increased between D1 and D63 from 8.3 ± 0.3 to 10.6 ± 0.3 g/dL whereas Hb in controls remained stable (mean: 11.4 ± 0.2 g/dL). The same trend was observed for Ht (27.7 ± 0.9 to 36.7 ± 1.0 % from D1 to D63 in clones vs mean Ht of 38.4 ± 0.8 % in controls). There was no difference for MCV or Pl at any stage. Lymphocytes counts were significantly smaller in clones at D21 and D49 and were within the lower range for calves. Neutrophil counts were significantly higher in clones at D1: 11 844 ± 890/µL vs. 8672 ± 1148/µL, p < 0.05, in clones and controls respectively. There was no difference for monocyte counts and Glu at any stage, nor for Cr and U at D1. Fib was significantly higher in clones at D4 and D35.

Five clones died after the end of the study (age >3 months) and there was no difference for all parameters measured between clones that finally died and clones that did not.

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122 (5023)
ULTRASOUND MEASUREMENTS OF BOVINE SOMATIC CLONES AT D50 AND D64 OF PREGNANCY
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The technique of cloning is associated with important gestational losses. In the bovine species, most losses occur between D35 and D70 of gestation, and deficient placental development has been described. Late foetal losses are associated with excessive foetal growth (Large Offspring Syndrome or LOS). The objective of this work was to compare foetal development at D50 and D64 for bovine clones and control pregnancies, to see if these could be predictive of a further development of LOS.

Thirty clone and 36 control (32 AI and 4 IVF) singleton Holstein pregnancies were used for ultrasound (US) with a 5 MHz rectal probe. Crown rump length (CRL), head length (HL), orbital diameter (OD), vesicle width (VW) and length and width of the placentome closest to the foetus were measured at each stage. Data were analysed using variance analysis with SAS software.

Seventy per cent of clone recipients aborted (one half before D90). There was no difference for foetal measurements between IVF and AI and the two groups were pooled as a single control group. CRL and HL were significantly smaller in clones at all stages (CRL: 31.5 ± 5.6 mm vs. 36.0 ± 4.6 mm at D50, p < 0.01; 48.4 ± 11.9 mm vs. 63.3 ± 4.9 mm at D64, p < 0.05, in clones and controls respectively). Moreover, clones that died before D90 were significantly smaller at D64 than clones that died after D90 or went to term (about 20 mm less). Finally, clones alive at birth were also significantly smaller than controls at D50. There was no difference for OD or VW at any stage. Placentome width but not length was significantly smaller in clones compared to controls (4.7 ± 1.2 mm vs. 7.2 ± 1.7 mm at D50 and 5.7 ± 1.5 mm vs. 9.3 ± 4.3 mm at D64, p < 0.01, in clones and controls respectively). These data show that although a large proportion of clones develop LOS in late gestation, they are actually...
smaller than controls in early pregnancy. This is probably due to delayed or abnormal early placental development. US cannot be used to predict further development of LOS and other means like maternal plasma Pregnancy Associated Glycoproteins like PSP60 concentrations may be used (Heyman et al., 2002). Funding: ENVA (Ecole Nationale Vétérinaire d’Alfort)

123 (3203)
INTRAUTERINE APPLICATION OF ENZYMES FOR THE TREATMENT OF CHRONIC ENDOMETRITIS IN DAIRY CATTLE
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Chronic endometritis in dairy cattle is associated with decreased reproductive performance and economic losses to the farmer. The treatment is based on the use of PGF2a and its analogues, or the intrauterine application of antibiotics or disinfectants. The efficacy of PGF2a and antibiotics is well documented. However, due to the risk of residues and resistances, the use of hormones and antibiotics in food producing animals is under critical public discussion.
The use of proteolytic enzymes is established in the non-antibiotic treatment of mastitis in dairy cattle. The objective of this study was to evaluate the use of enzymes in the treatment of chronic endometritis. In a field trial, cows with vaginal discharge diagnosed 21-27 days post partum (dpp) were randomly assigned to two treatment groups. Endometritis was classified into three categories, depending on the type of vaginal discharge: clear mucus with flakes of pus (E 1), mucopurulent discharge (E 2), and purulent discharge (E 3). Cows in Group A (n=192) received an intrauterine infusion containing the enzymes trypsin, chymotrypsin, and papain. Cows in Group B (n=228) were treated with 0.5 mg of the PGF2a-analogue cloprostenol. All cows were re-examined 35 to 41 dpp. In Group A, cows were re-treated with enzymes if signs of endometritis were found, while in Group B all cows received a second injection of cloprostenol regardless of clinical findings. After a voluntary wait period of 72 days cows were bred following an observed estrus.
Clinical cure rate after the first treatment was 59.4% and 68.1% in groups A and B, respectively (p>0.05). Days to first service, conception rates, percentages of cows pregnant and days open were not significantly different between the groups. In both groups, clinical cure rate and reproductive performance were better for cows with E1 or E2 than with E3, respectively.
From the results of this study, it may be concluded that the local use of proteolytic enzymes is a promising approach for the treatment of chronic endometritis in dairy cows. However, PGF2a is still the treatment of choice for chronic endometritis.

124 (2868)
COMPARISON OF TWO BREEDING PROTOCOLS TO EVALUATE THEIR EFFECTS ON SOME REPRODUCTIVE INDICES INCLUDING CONCENTRATIONS OF ESTRADIOL AND PROGESTERONE IN DAIRY CATTLE.
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Ovsynch-CIDR with timed AI and repeated injections of PGF2a 14-days apart were studied to see the effects on some reproductive indices including serum and milk estradiol and progesterone concentrations in two stages of early pregnancy.

Materials and Methods: Multiparous cows in the study had two or more parturitions without a history of dystocia, retained placenta, uterine infections and clinical mastitis and were assigned into two groups: Group 1 - nPG, n=27 and Group 2 - OC, n=25. Group 1 - nPG received two injections of PG (Estrumate® NL. 0.5mg, IM) on 35 and 45d post partum and inseminated at the detection of estrus after the second PG. Group 2 - OC cows received 5ml GnRH IM on day 1 (Gonadorelin®, Abureihan Inc. Iran) and a CIDR insert (CIDR®, InterAg Inc. NZ). On day 7, PG was injected & CIDR was removed. Another dose of GnRH was injected on day 9 and the cows were inseminated 18h later (66h after PG). Blood and milk samples were taken at the beginning of treatments, d5 and d21 after insemination. Concentrations of estradiol and progesterone were assayed in the serum samples. Milk progesterone in the last 2 samplings was measured in 20 cows of each group. All hormones were assayed using ELISA kits (DRG Instruments Inc., Germany).

Results: Conception rates to first AI were low in both groups (14.28% for nPG and 23.8% for OC, P>0.05). Conception rates to second and third AI were greater in OC as compared to nPG (71.42% and 85.71% vs 23.8% and 61.9% for OC and nPG, respectively, P<0.05). Number of services/conception and overall conception rates were 2.23 and 55.71% for OC and 3.13 and 40.3% for nPG, respectively (P<0.05). Days open and predicted calving intervals were 106 and 387 days for OC and 138 and 418 for nPG, respectively (P<0.05). Mean serum estradiol concentrations on day 5 were lower in OC than nPG. Milk fat progesterone concentrations was higher in d5 and d21 in OC compared to nPG (P<0.05).

Conclusions: According to the results of this study, Ovsynch-CIDR with timed AI increased conception rates to second and third AI and progesterone levels in d5 and d21 after first AI and decreased days open, calving interval, service/conception as compared to repeated injections of PG.
Keywords: Ovsynch-CIDR, PGF2a, Progesterone, Estradiol, Dairy Cattle
Funding: Government of Iran

125 (2308)
A PROPHYLACTIC PROGRAM USING HOMEOPATHIC REMEDIES FOR IMPROVEMENT OF REPRODUCTIVE PERFORMANCE IN AN ORGANIC DAIRY FARM
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The animal husbandry in organic agriculture in Germany is required to use complementary veterinary medicine for the general health prevention and the cure of clinical diseases. On the other hand there is still a lack of sound scientific studies proving the efficiency of herd health programs including homeopathic remedies. In a German dairy herd with 200 cows, a prophylactic program using homeopathic complex remedies was conducted. The aims of the organo- and functiotropical remedies were to stabilize liver metabolism, to regulate parturition, to enhance uterine involution and to promote the return to cyclicity after calving, respectively. A randomized, double blind, placebo controlled study was conducted. The subcutaneous application of 10 ml of the complex remedies started at drying off, and was repeated at calving, 7 and 14 days post partum (p.p.), respectively. Before each treatment, all cows were examined clinically and blood samples were collected for determination of following parameters: AST, Bilirubin, Urea, Ca, P, Mg. Examination and sampling were continued in weekly intervals until day 35 p.p. Animals not inseminated until day 60 p.p. were examined and sampled again. The effect of the treatment was measured by clinical findings of uterus and ovaries, blood and milk parameters, disease incidence, reproductive performance (days to first service, days open, conception rates), and development of body condition. The onset of cyclicitty p.p. was slightly earlier in the treatment group (p>0.05). When the average daily milk yield was considered in the model statistically significant differences of the onset of cyclicity between treatment and control group were obtained (p<0.05). Despite the earlier onset of ovarian activity the treatment group had more days to first service. Both treatment groups were similar in terms of conception rate or days open. The treatment group with increased AST, Bilirubin or Urea at drying off showed lower values after calving compared to the control group. Animals with a body condition score > 3.5 at drying off showed less cases of liver damage after calving in the treatment group (p<0.05).
The homeopathic treatment described here seemed to be able to relieve cows at risk in the peripartal period.

126 (804)
THE EFFECT OF SMALL DOSES OF NALOXONE ON THE PREVALENCE OF OVARIAN FOLLICULAR CYST IN DAIRY COWS
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In previous work it was observed that the administration of low doses of naloxone increased libido and fertility in the ewe and buck. And advanced the first oestrus after weaning in the sow. Therefore it was considered of interest to study the effect of small doses of naloxone on one of the most common ovarian dysfunctions in dairy cows, ovarian follicular cysts. For this purpose 60 cows were chosen at random from different dairy farms, and allocated at random in groups of 20, to three different treatments. Group one was treated with 200 mcg of a GnRH analogue (Gonadorelin, Cystorelin, SANOFI, México). Group 2 was treated with six im injections of 5 mg naloxone HCl, at twelve hour intervals. Group 3 was treated as group 2 with saline injections.
It was observed that in cows of group 1, when inseminated in the following estrus after treatment, 13 ovulated and conceived (60 %). In cows of group 2, when inseminated following estrus after treatment, 18 ovulated and conceived (90 %). And cows of group 3, treated with saline solution, when inseminated on the following oestrus after treatment 4 ovulated (10 %) and 2 conceived (5 %). LH preovulatory surge was advanced in cows treated with naloxone, and in cows suffering of follicular cysts LH surge was not detected. It was concluded that endogenous opioids are important modulators of oestrus behavior and ovulation in the cow.
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127 (5082)
THE EFFECT OF SUBCLINICAL ENDOMETRITIS AND ROUTINE TREATMENT WITH PROSTAGLANDIN F2 ALPHA ON REPRODUCTIVE PERFORMANCE OF DAIRY COWS
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In a triply-masked, random prospective study 529 cows in 6 central New York dairy herds were sampled at 3, 5 and 7 weeks postpartum, when uterine samples were obtained for endometrial cytology for diagnosis of subclinical endometritis (SE) and each cow was given an injection of sterile saline or prostaglandin F2a (Lutalyse, Pharmacia). Uterine samples were obtained by infusion of 20 ml sterile saline, mild agitation, and aspiration. The aspirate was centrifuged, stained and examined microscopically. Endometritis was diagnosed...
PREVENTION OF RETAINED PLACENTA BY INJECTION OF COLLAGENASE INTO UTERINE ARTERY OF COWS DELIVERED BY CAESAREAN SECTION

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In cows, administration of collagenase (200 000 U) via umbilical arteries during a caesarean section, successfully released placenta within 36 hours without inducing any adverse side effects (Eiler and Hopkins, 1993; J. A. V. M. A. 203, 436-443; Eiler et al., 1997, Theriogenol. 48, 1147-1152).

In this study, we have tested the efficiency of much lower doses of collagenase (20 000 U) using another route of administration: the enzyme was injected into the uterine artery.

This experiment was performed in 30 cows (15 collagenase-treated cows and 15 control cows). Multiparous cows of different breeds and showing no evidence of diseases were used. All cows tested negative for brucellosis, chlamydiosis and Query fever (serological controls). Only cows subjected to surgery for the first time were included. The genital tract of each cow was examined using transrectal palpation and ultrasonography. Cows were confirmed to be pregnant with a single fetus. Animals with the presence or suspicion of uterine or fetal disease were excluded. The caesarean section was performed at the same gestational stage in control and treated cows (276 ± 2 days versus 276 ± 3 days respectively). The age of the cows was similar in the two groups (6.7 ± 0.5 years vs 6.8 ± 0.8 years respectively). Sixteen to 20 hours before surgery, retained placenta was induced in all cows by an intramusculaire injection of dexamethasone (30 mg). Only one method for the caesarean section was used: standing with an incision through the left flank (Stocker and Waelchli, 1993, Vet. Rec. 132, 507-508). After replacing the closed uterus into the abdominal cavity, the cranial branch of the uterine artery was located. The characteristic vibration (thrill) helped identify the uterine artery. The artery was catheterized with a 25 G needle connected to a syringe with a catheter and 50 mL of collagenase (Sigma, C-9891) solution (20 000 U in phosphate buffer pH 7.2 with CaCl2 0.5 mM) were infused.

The average membranes retention time was 39.7 hours and 114.0 hours for experimental and control cows respectively (P < 0.001). The treated cows showed no clinical sign of abnormality over a ten-day-post-treatment observation period. Collagenase injection is simple and can be completed in two minutes. We conclude that uterine intra-arterial injection of low dose of collagenase is safe and potentially effective in preventing retained placenta during caesarean section in cow.
THE EFFECT OF STRAIN OF HOLSTEIN-FRIESIAN COW AND FEEDING SYSTEM ON POSTPARTUM OVARIAN FUNCTION, ANIMAL PRODUCTION AND PREGNANCY TO FIRST SERVICE

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Three strains of Holstein-Friesian (HF): high production North American (HP), high durability North American (HD) and New Zealand (NZ) cows were assigned, within strain, to one of three grass-based feeding systems: 1) the Moorepark (control) system (MP), 2) a high concentrate system (HC), 3) a high stocking rate system (HS). Ovarian function was assessed using milk progesterone profiling. Milk samples were collected from 117 cows in each of two successive years, with 81 animals being common to both years. Milk samples were collected thrice weekly on Monday, Wednesday and Friday during morning milking. Sampling began day 5 post-calving and continued to day 25 post first AI. Animals that received reproductive hormonal treatment (48) were removed from the database prior to analysis. Nether strain of Holstein-Friesian or grass-based feeding system had a significant effect on post-partum interval to commencement of luteal activity (CLA). The mean interval to CLA was 32.7 days (s.e. 1.18). The values ranged from 7 to 101 days, with 59 and 83 percent of cows ovulating by day 32 and 53, respectively. The HD (60%) and NZ (63%) strains had significantly (P<0.05) higher pregnancy to first service than the HP strain (44%), while feeding system was not significant.

Retrospective analysis categorised all cows into four quartiles based on interval to CLA (<21 days, 21 to 27 days, 28 to 45 days and >45 days). Cows with longest CLA interval (>45 days) calved earlier (P<0.01). Cows with the earliest CLA had longer oestrous cycle (P<0.05) and higher nadir protein content (P<0.05). Cows that did not conceive to first service (n=79) were also retrospectively compared to those that conceived to first service (n=107). Cows that conceived to first service had fewer luteal phases (P<0.001) and of longer duration (P<0.05). Additionally, cows that conceived to first service reached nadir body condition score at an earlier stage of lactation (P<0.05). Finally cows that displayed atypical progesterone profiles (n=71) were retrospectively compared to those that had normal progesterone profiles (n=115). Cows displaying atypical progesterone profiles had greater body condition loss between calving and first service (P< 0.05), had longer calving to conception intervals (P<0.05) and had later observed first heats. Milk production and liveweight appeared to have no effect on progesterone profiles postpartum.

Funding: Industry

130 (3432) 

EFFECT OF LUMPY SKIN DISEASE VIRUS ON SEMEN QUALITY IN EXPERIMENTALLY INFECTED SUSCEPTIBLE BULLS

Irons P., Gerber D.
EFFECT OF SOCIAL DOMINANCE ON THE EXPRESSION OF ESTRUS IN ANGUS COWS

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This study evaluated the effect of social dominance on the expression of the first two postpartum estrus in

UPDATE ABOUT OESTRUS BEHAVIOUR IN HIGH YIELDING DAIRY COWS

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In France, dairy cows are mainly inseminated after spontaneous oestrus detection which seems to be more difficult than earlier, especially during winter time. Our purpose was to realize an extended description of oestrus behaviour with quantification of frequency of behaviours for high yielding dairy cows housed in free-stalls. Factors of variation of duration and intensity of oestrus were examined: time to first ovulation, regularity of ovarian activity, milk production and composition, metabolites and net energy (NE) balance. The survey began at calving to 80 days postpartum or at first service if previous. In the barn, 6 high-sensitive video cameras were installed to visualise each cow at any moment of the nycthemeral. Ovarian activity was attested by milk progesterone profiles. Oestrus detection was codified and realized always by 2 trained persons. In a first methodology step, we described and codified all the behaviours of 15 cows during follicular and luteal phases at second or third postpartum ovulations. The second step has concerned the 64 cows of the trial. Only 44 cows have ovulated during the first service period i.e. between 50 and 80 days postpartum. Silent oestrus (ovulation not accompanied by any behavioural change) has concerned 14% of cows. Even though standing to be mounted remained the most specific behaviour, only 59% of cows have presented this behaviour. The others cows have presented behavioural changes, especially a higher frequency of proceptive interactions as sniffing or licking the genital area (P<0.001, change point test) without standing to be mounted. Standing to be mounted was slightly more expressed during night (P<0.05) then day. Active interactions were expressed similarly at any time. Delayed first ovulation after 50 days and prolonged luteal phase had an effect on oestrus behaviour and detection, especially on false positive cows (P<0.01). Milk efficiency (Milk yield/NE intake) of the 6 first weeks postpartum was correlated less expressive oestrus behaviour (P<0.05). NE balance of the 3 first weeks postpartum was related to oestrus duration (P<0.05). In conclusion, this trial confirms that spontaneous oestrus behaviour is less expressive in high yielding dairy cows than in others. Detection can be improved by combining heat detectors and observations of proceptive secondary signs. Chronology between oestrus and service might need to be re-evaluated.

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EFFECT OF SOCIAL DOMINANCE ON THE EXPRESSION OF ESTRUS IN ANGUS COWS

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This study evaluated the effect of social dominance on the expression of the first two postpartum estrus in

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Lumpy Skin Disease Virus (LSDV) is a Capripoxvirus which causes considerable economic losses due to emaciation, damage to hides, infertility, mastitis, loss of milk production and mortalities. The pathogenesis of infertility in bulls has not been studied. The objective of this study was to investigate the seminal characteristics in bulls with experimentally induced disease.

Six unvaccinated seronegative bulls 11 to 19 months of age were housed in vector-free housing. Two were Holsteins and four were Dexters or Dexter crosses. The animals were artificially infected by intravenous injection of a virulent field isolate strain V248/93. Animals were then observed for clinical signs of LSD. Semen was collected and evaluated weekly until 70d post-infection, and was continued fortnightly in the two animals still secreting virus in semen until 159d. Semen was tested for LSDV by PCR until three consecutive samples were negative. Macroscopic semen parameters were judged visually, motility was assessed by microscopic estimation and morphology was evaluated using phase contrast microscopy.

Two animals developed severe LSD, two developed milder signs and in two infection was inapparent besides fever for 2 and 4d. One of the severely affected animals was azoospermic on days 28-70 and the other was severely oligospermic on days 35-70. Total motility declined in all animals from day 8 (n=5) or day 14 (n=1) and returned to preinfection levels at days 21, 42, 70 (n=2), 97 and 132. Percentage normal sperm declined in all but one of the mildly affected animals, beginning at 14d (n=3) or 21d (n=2) and returning to preinfection levels at day 42, 70, and 132 (n=2). Morphology had not recovered in one animal at the time of slaughter at 70d. Semen from the two severely affected animals was positive on PCR until 132d and 159d, despite their semen quality having recovered by 132d.

The duration of the poor semen quality in the two severely affected bulls exceeds what can be explained by the febrile response alone. We therefore consider it likely that LSDV can affect semen quality by disturbance of thermoregulation in the testes due to scrotal skin thickening or by direct effects on testicular tissue. The exact mechanism warrants further investigation. Recovery of good quality semen in these animals was remarkable considering the severity and duration of the dysspermatogenesis and indicates that clinicians should not be hasty in setting a hopeless prognosis in similar cases.

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132 (1422)
Angus cows. Ninety postpartum Angus cows were allocated by parity and body frame into 3 groups of 30 each. Dominance values per cows were calculated upon the base of agonistic interactions which were daily recorded during feeding hours along the 90 d of the study. Using the obtained dominance values, a social order with dominants (D), intermediates (I), high-subordinates (HS) and low subordinates (LS) was arranged. Information on expression of estrus was obtained by fitting HeatWatch® devices on all cows. No influence (P>0.05) of social order was found on either duration of estrus or total mounts received. However, the interaction period by social order showed that overall, LS cows received more mounts across estrus (2.6 ± 0.3) than D (1.8 ± 0.3; P< 0.07), I (1.4 ± 0.2; P< 0.001) and HS cows (1.6 ± 0.2; P<0.004). The D and I cows expressed estrus more intensively (P<0.01) during the first 3 h following estrus onset compared to HS and LS cows. Nevertheless, from 3 to 6 (P<0.001) and 6 to 9 h (P<0.02) after estrus onset, LS cows expressed estrus more intensively than D and I cows. Similarly, although with poor intensity and lacking statistical significance, LS cows received mounts during 9 h more than cows from the other social orders. It was concluded that social dominance may be considered a factor influencing the expression of estrus in beef cows.

134 (3365)
THE EFFECT OF THE INCREASED FAT MOBILISATION ON UTERUS INVOLVEMENT AND OVARIAN CYCLE IN DAIRY COWS
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An experiment was conducted to study whether the increased fat mobilisation around calving would be accompanied by an impaireduterus involution and delayed onset of cyclic ovarian activity. 28 clinically healthy multiparous Holstein Friesian cows were selected randomly from groups of dry cows, 1-10 days prior to expected calving. Blood samples were taken on every 10th days until 60th day of lactation to determine the NEFA, AST, glucose and acetooacetic acid concentration. From 20th d postpartum blood samples were taken every 3 days for progesterone profile test. Uterus and ovaries were examined at 5th, 20th and 40th days postpartum by rectal palpation and 30th and 60th days postpartum by ultrasound. We described the uterus size by scores 1-4 and scored the body condition (1-5 system) as well. The cows were divided into two groups according to their blood NEFA concentration exceed the physiological threshold (0.200 mmol/l) more than one time (which means longer than 10 days) during the examined period (Group NEFA, n=12) or not (Group N, n=16). There was significantly (p<0.05) higher average plasma NEFA concentration during the 1st month of lactation in the NEFA group. Averaged blood acetooacetic acid concentration (0.080 vs. 0.350 mmol/l, p<0.05) and the incidence of hyperketaonemia (2.5 ± 0.63, p<0.05) was higher in group NEFA at day 10 postpartum compared to Group N. The prepartum body condition score was higher (p<0.05) in NEFA group (3.75 ± 3.38). On day 30 and 60 the presence of CL on ovaries was lower in NEFA group (8.3% ± 18.75% and 33.3% vs. 75% respectively). The average uterus score examined on day 30 and 60 was higher in NEFA group (2.83 ± 2.19 and 2.08 vs. 1.78), respectively. Lower percentage of NEFA group cows exhibited first progesterone wave until 35 days of lactation compared to N group. We concluded that increased fat mobilization existing more than 10 days has negative effect on uterus involution, and resumption of cyclic ovarian activity.

135 (5017)
PHARMACOKINETICS OF INJECTED VITAMIN E IN PERIPARTUM DAIRY COWS
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Supplementation of peripartum dairy cattle with parenteral vitamin E has been reported to decrease the incidence of retained placenta and metritis. Our objective was to compare the effect of one subcutaneous (SC) or intramuscular (IM) injection of vitamin E on plasma and neutrophil a-tocopherol concentrations. Cows and heifers in 2 research herds (n=46) were enrolled. Cattle were fed a total mixed ration based on alfalfa and corn silage including 750 IU/d of supplemental vitamin E. Ten days before expected calving animals were randomly assigned to receive one injection of 3000 IU of RRR- a-tocopheryl acetate IM or SC or an IM saline placebo. Blood samples were collected immediately before treatment and then three times per week for three weeks. Blood (50ml) was collected from the coccygeal vein into tubes with EDTA and chilled. Plasma was harvested and cholesterol and a-tocopherol concentrations were determined. Cholesterol concentrations were used as a surrogate measure of availability of lipoproteins for a-tocopherol transport. In a subset of animals (n=15) neutrophils were isolated, re-suspended in saline with 1% pyrogallic acid and frozen. Both plasma and neutrophil a-tocopherol concentrations were measured with standard a HPLC technique. The effect of treatment was analyzed with multivariable linear regression accounting for repeated measures (Proc Mixed in SAS). Covariates included parity, body condition score at enrolment, and occurrence of retained placenta. Overall, IM (n=14) and SC (n=17) groups had higher (P < 0.01) plasma a-tocopherol concentration than control (n=15) cows (2.51, 2.77, and 1.66 mg/ml, respectively), but IM and SC were not different (P=0.29). Similarly, plasma a-tocopherol:cholesterol mass ratio was higher (P < 0.01) in IM and SC than in controls (4.03 vs. 4.26, and 2.75 vs. 10-3, respectively), but there was no significant difference among the treated groups (P=0.5). Plasma a-tocopherol:cholesterol mass ratio peaked on day 2 after injection for both IM and SC, and was not different among these groups. IM treated cows maintained higher (P<0.05) plasma a-
tocopherol:cholesterol mass ratio than the control animals for 7 days, while SC animals were higher for 14 days after the injection. Treated animals sustained higher plasma a-tocopherol from 10 days before calving to 1 day after calving with no difference between the treatment groups. There were no significant treatment effects on neutrophil a-tocopherol concentrations, but these were numerically higher in IM and SC cows than in controls (0.20, 0.14, and 0.1 µg/10^6 neutrophils, respectively), and followed a similar pattern to the changes in plasma concentrations. These results suggest that vitamin E should be injected within 1 week of expected challenge, and that SC administration is as effective as IM.

136 (2490)
ULTRASOUND EVALUATION OF THE REPRODUCTIVE TRACT AS A TOOL FOR SELECTION OF EMBRYO RECIPIENTS IN BOVINE
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Embryo transfer is used to enhance genetic improvement by amplifying reproductive rates of valuable females and because of the relatively low reproductive rate and long generation interval, it is especially useful in cattle. The success of embryo transfer depends on factors associated with the embryo, the recipients and their management. Statistical model shown that recipient factors have a better correlation with the potential to carry a pregnancy to term after transfer of an embryo than embryonic factors (MacMillan, 1998). The purpose of the present study was to provide basic information on the potential value of ultrasound examination of various reproductive tract components to select recipients which have the best chance to become pregnant. Embryo recipients were Holstein heifers and first calf heifers. At the scheduled time of embryo transfer (D7), based on heat detection and blood discharge, a rectal palpation was performed to evaluate the presence, the dimension and the consistency of the corpus luteum in addition to detect genital tract anomalies. If the recipient was determined suitable based on history and rectal palpation then an ultrasonic examination of the ovaries and the uterine horns was performed. On the uterine horns, presence of fluid in the uterine lumen and edema in the endometrium was assessed separately and scored in a 0-3 scale (0=none; 1-3=increasing amount). Finally, blood samples were collected for estradiol 17ß and progesterone concentration. All transferred embryo were frozen and classified as 4-1 embryos (very good to excellent morula -I.E.T.S. criteria, 1989). They were frozen in ethylene glycol (1.5M) and loaded in 0.25 cc straws. At the time of transfer, they were thawed in air for 10 seconds and in a water bath at 30ºC for 30 seconds directly transferred to recipients. Pregnancy was determined by rectal palpation and/or ultrasonographic exam between 40 and 50 days following the transfer. A total of 25 embryos were transferred and the overall pregnancy rate was 58%. Of the present preliminary study, none of the measured factors did affect significantly the pregnancy rate. However, all recipients (n=2) with a grade 3 edema of the endometrium were not pregnant. The preliminary observations made in the present trial need to be investigated on a larger scale before conclusion could be draw on the usefulness of ultrasound in the selection process of competent recipients in bovine embryo transfer Lefebvre.

137 (2573)
CHANGES IN THE CONCENTRATIONS OF UREA, GLUCOSE AND BETA-HYDROXYBUTYRATE IN THE FOLLICULAR FLUID OF THE DOMINANT FOLLICLE IN HIGH-YIELDING DAIRY COWS EARLY POST PARTUM
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The intrafollicular environment, in which the preovulatory oocyte grows and matures, may be one of the major factors determining subsequent fertility. The aim of this study was to examine to what extent altered concentrations of urea, glucose and β-hydroxybutyrate (b-OHB) are reflected in the follicular fluid (FF) of the dominant follicle. Those metabolites experience remarkable homeorhetic fluctuations during the postpartum period of high-yielding dairy cows due to negative energy balance and a high-protein diet. Nine times a blood sample was taken from each animal (nine high-yielding dairy cows) between 7 days before and 46 days after parturition. At the same sampling interval, but only starting from day 14, 6 FF samples of the dominant follicle (> 8mm) were collected by means of transvaginal follicle aspiration. Serum and FF samples were analysed using commercial clinical and photometric chemistry assays for glucose, b-OHB and urea. Data were analysed using a repeated measurement design in a linear mixed effects model with cow as random factor. Glucose concentrations transiently decreased in the first two weeks post partum. Urea concentrations doubled around parturition and were relatively stable for the rest of the experimental period. Serum b-OHB concentrations gradually increased after parturition and peaked at day 33. Throughout the study, changes of glucose, urea and b-OHB concentrations in the serum were highly reflected by changes in the FF (r = 0.92; r = 0.98 and r = 0.81 respectively, P < 0.001).

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grow and mature in a dynamic biochemical environment. It indicates that the typical metabolic changes of glucose, urea and b-OHB in the serum of high yielding dairy cows shortly post partum, is reflected in the FF and, therefore, may affect the quality of both the oocyte and the granulosa cells.

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138 (2390)
**A COMPARISON OF LIQUID VERSUS FROZEN SEMEN ON CONCEPTION RATES IN DAIRY CATTLE**

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Declining fertility and reproductive inefficiency are major challenges facing the dairy industry. Several studies have looked at the use of liquid (fresh) versus frozen semen and found that comparable fertility rates are achieved when fresh semen is used at much lower sperm doses. However, no one to date has assessed the use of liquid semen at higher sperm doses as a technique to improve fertility in dairy cows. The objective of this study was to compare conception rates in dairy cows randomly allocated to receive liquid or frozen thawed semen from the same ejaculate, at a similar total sperm dose.

The study was conducted on 75 Holstein dairy farms in south-western Ontario which were enrolled in milk recording, had all of their cattle inseminated by professional inseminators (Gencor Inc.), and had veterinary reproductive herd health visits at least once monthly. Herds were enrolled on a contract basis for twenty consecutive cow inseminations, with each cow randomly assigned to be inseminated with either liquid, or frozen thawed semen. Cows were pregnancy checked by a veterinarian at routine reproductive herd health visits between 27 and 45 days post-insemination.

Based on 2,178 breedings with known outcomes, raw conception rates for cows bred with fresh and frozen thawed semen were 35%. Using a generalized linear mixed model to control for herd, bull, lactation number, DIM and current milk yield, the outcome pregnant or open was evaluated for 1,426 breedings with complete data. While parity, stage of lactation and bull did affect pregnancy, the use of fresh or frozen thawed semen did not have a significant effect on the overall outcome.

Based on the analysis of these data we conclude that the use of fresh semen at a total sperm dose similar to that of frozen thawed semen does not increase the probability to conception and does not offer an advantage over the use of frozen thawed semen.

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139 (3345)
**A RETROSPECTIVE STUDY ON THE USE OF OVSYNCH AND ITS EFFECTS ON THE FERTILITY OF DAIRY COWS WITH OR WITHOUT OVARIAN CYSTS IN QUEBEC**

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A retrospective study using paired groups was done on 12,584 cows at first AI (artificial insemination) and 6276 cows at second AI. The study was performed between June 2000 and June 2002; the cows were from 668 dairy herds in the province of Quebec. These herds use the Ovsynch program selectively. The primary objective of this study was to evaluate the effect of the use of a synchronization programme on fertility at first and second AI. The second objective was to verify the effectiveness of the Ovsynch programme on ovarian cysts diagnosed within two weeks preceding an insemination.

The Ovsynch treatment uses gonadotropin releasing-hormone (GnRH) and prostaglandins (PGF2a) according to the following schedule: day 0, one IM injection of GnRH; day 7, one IM injection of PGF2a ; day 9 (p.m.), a second IM injection of GnRH ; day 10 (a.m.), AI without previous detection of oestrus.

The mean conception rate (CR) at first AI was 39.3% and on second AI it was 40.1%. The CR of animals who underwent the Ovsynch treatment was 37.4% (n=6,129) compared to 40.6% (n=5,054) for the control group (p=0.001). On second AI, the respective rates were 40.3% (n=3,065) for treated animals compared to 40.7% (n=2,715) for the untreated group (p=0.36). These results demonstrate that a selective use of the Ovsynch programme in normal production conditions results in a CR slightly lower or similar to that obtained in animals inseminated following oestrus detection. The Ovsynch programme was revealed to be a practical and efficient reproductive tool in the herds under study.

The average rate of cows with ovarian cysts was 5.5% before first AI and 4.9% before second AI. More than 60% of animals with ovarian cysts were treated with the Ovsynch programme. The crude CR at first AI for cystic animals treated with Ovsynch was 40.2% (n=567) versus 38% (n=153) for the control group. At second AI, respective CR were 42.6% (n=279) versus 40.3% (n=57) for the treated and control groups. For cystic animals, CR was similar for Ovsynch treated animals and controls and did not differ statistically from non cystic animals.

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140 (2918)
**ESTRUS SYNCHRONIZATION IN BEEF COWS: COMPARISON BETWEEN OVSYNCH AND PRID + PGF2a + ECG**

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The aim of this study was to compare 2 protocols of estrus synchronization in suckled beef cows over a 2 years period. The population studied consisted of 172 Charolais and 168 Limousin cows in 12 and 14 beef herds, respectively. In each herd, cows were allotted according to parity, body condition score and calving difficulty. Group 1 (n = 174) received a progesterone releasing intravaginal device (PRID) for 8 days with a capsule containing 10 mg estradiol benzoate, a 25 mg IM injection of dinoprost at Day -4 and 500 IU eCG at PRID removal (Day -2). Cows were inseminated on Day 0, 56 h after PRID removal. Group 2 (n = 166) received 0vsynch: GnRH (Day -10, 100 mg IM), PGF2a (Day -3, 25 mg IM), GnRH (Day -1, 100 mg IM), AI 16 to 24 h after the last GnRH (Day 0). Plasma progesterone concentrations were measured to determine cyclicity prior to treatment (Days 20 and 10), to confirm the occurrence of synchronization (Days 0 and 10) and to determine the early pregnancy rate (Day 24). Pregnancy diagnosis was performed by ultrasonography between Days 35 and 45. The effects of variation factors on synchronization and pregnancy rates were studied using logistic mixed models. There was no significant difference between groups 1 and 2 respectively for cyclicity rate before treatment (80.5 % vs 80.1 %) and for pregnancy rates on Day 24 (62.1 % vs 54.8 %, P = 0.09) and Days 35-45 (53.8 % vs 46.3 %, P = 0.16). Synchronization rate was higher (P < 0.01) in Group 1 (90.8 %) than in Group 2 (77.1 %) and was affected by cyclicity prior to treatment in Group 2 but not in Group 1. (Group 2: 45.5 % in anestrous cows vs 85.0 % in cyclic cows; Group 1: 88.2 % in anestrous cows vs 91.4 % in cyclic cows, P interaction = 0.05). Pregnancy rates at 24 days were influenced by the year of study (52.4 % vs 68.8 %, OR = 2.12, P < 0.01) and by cyclicality before treatment (anestrous cows 46.3% vs cyclic cows 61.5%, OR = 1.86, P<0.05). Pregnancy rates at 35-45 days were influenced by the year of study (44.2 % vs 59.8 %, OR = 1.92, P < 0.01). Among synchronized cows, the plasma progesterone concentrations were higher in Group 1 than in Group 2 at Day 10, at Day 24 in pregnant cows at Day 24 and at Day 24 in pregnant cows at Days 35-45. In conclusion, although pregnancy rates were similar for the 2 treatments, in suckled beef cows Ovsynch induced a lower rate of synchronization than PRID + PGF2a, particularly in anestrous cows.

141 (2864)
EFFICACY OF A KILLED WHOLE NEOSPORA CANINUM TACHYZOITE PREPARATION AGAINST NEOSPORA-INDUCED ABORTION OF COSTA RICAN DAIRY COWS UNDER FIELD CONDITIONS.
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The efficacy of a commercial Neospora-vaccine based on whole killed tachyzoites (Bovilis® Neoguard, Intervet International), was assessed by a standard field trial, based on a cohort design. A total number of 914 were used. The animals were over 2.5 months in pregnancy and belonged to 25 Costa Rican specialised dairy herds, with an incidence of abortions between 10 and 23% (global rate of 15.2%) the 2 years previous to the study. The within herd Neospora caninum sero-prevalence of the selected farms ranged between 25 and 70%. For each cow vaccinated (454), a cow of the same herd, breed and age category, was selected as control (460). The period of administration of treatments extended from June to November of 2000. The treatments were administered in two, 5 ml doses 1 month apart, the first dose given between day 75 and 90 of gestation. The overall incidence of abortion among all treated cows was of 16.1% (147/914). The treatment specific incidence was 11.23% (51/454) and 20.87% (96/460) for the vaccinated and the placebo group, respectively. The prevented fraction by vaccination amounted to 0.46, and the risk ratio for the vaccinated group was 0.54. The Cox hazard ratio, was 1.99 (95% CI: 1.41, 2.79), meaning that the force of abortion is reduced twice in the vaccinated group. As abortion in cattle is caused by a variety of different infectious and non-infectious factors, the expected reduction in the incidence of Neospora caninum induced abortion should be higher. Therefore the results of this study, the first one following this type of design, shows that the killed whole Neospora caninum tachyzoite preparation had a reasonable efficacy to prevent Neospora caninum induced-abortion in Costa Rican dairy cattle.

Keywords: Neospora caninum; Vaccine, Efficacy; Field trial; Dairy cattle; Costa Rica.

142 (3190)
EFFECTS OF BODY CONDITION SCORE (BCS) AT CALVING AND LEVEL OF GRAIN FEEDING DURING EARLY LACTATION ON PLASMA CONCENTRATIONS OF INSULIN-LIKE GROWTH FACTOR-I (IGF-I), SELECTED BLOOD METABOLITES AND RESUMPTION OF OVARIAN CYCLICITY IN PASTURE-FED DAIRY COWS
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A study was conducted with 72 multiparous Holstein-Friesian cows to evaluate the effects of BCS at calving or level of grain supplementation during early lactation on plasma concentrations of IGF-I and blood
metabolites including non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB) and urea. Plasma concentrations of IGF-I were also compared in cycling and anoestrous cows and relationships between these concentrations and resumption of ovarian cyclicity investigated. Cows were managed over a period of 5 months to calve in BCS of 4, 5 or 6 (scale of 1 to 8). They grazed pasture and were supplemented in early lactation with 1 or 6 kg of cereal grain concentrate per day. Blood samples were taken from the coccygeal vessels of cows within 12 h of calving, and at 1, 5 and 10 weeks post-calving. Plasma concentrations of IGF-I were measured using a validated ELISA. Plasma NEFA, BHB and urea concentrations were measured using an auto-analyser. Data were analysed at calving and week 10 post-calving by ANOVA and Independent-samples t-test. The results reported are Least square means ± SE (BCS or supplement effect) or Mean ± SEM (IGF-I in cycling and anoestrous cows). The BCS at calving did not affect (P = 0.958) plasma IGF-I concentrations at calving. Plasma NEFA (1.23 ± 0.11 vs 0.84 ± 0.11 mmol/l; P = 0.022) and BHB concentrations (0.61 ± 0.04 vs 0.38 ± 0.04 mmol/l; P<0.001) were higher in cows with BCS 6 than BCS 4 at calving. The concentrations of IGF-I and metabolites were not affected (P>0.05) by BCS at week 10 post-calving. Higher (6 kg) level of grain feeding increased plasma IGF-I concentrations (87.8 ± 4.7 vs 72.4 ± 4.7 mmol/l; P = 0.022) and decreased urea (4.11 ± 0.21 vs 5.16 ± 0.20 mmol/l; P = 0.001) than the lower (1 kg) level at week 10. The data was re-organised into groups that re-commenced cycling spontaneously or were anoestrous before the start of the seasonal artificial insemination program irrespective of their BCS at calving. Cycling cows had higher plasma IGF-I concentrations than anoestrous cows at calving (56.5 ± 3.8 vs 39.8 ± 4.1 ng/ml; P = 0.009) and at week 10 (87.3 ± 4.2 vs 64.5 ± 4.3 ng/ml; P = 0.001), and shorter intervals from calving-to-1st ovulation (39.8 ± 2.0 vs 61.4 ± 4.2 days; P < 0.001). Plasma IGF-I concentrations were associated with the resumption of ovarian cyclicity.

Funding: Melbourne University

143 (3307)

USE OF INTRA-VAGINAL PROGESTIN PESSARIES ON REPRODUCTIVE EFFICIENCY IN POST PARTUM BEEF COWS

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The objectives in this study were to determine whether treatment with progestin (250 mg of Medroxy Acetate Progesterone - MAP) via an intra-vaginal pessary would induce estrus and conception in post partum beef cows, at 60 days postpartum (+/- 15 days). Two experiments were conducted. In Experiment 1, suckled beef cows (n = 90) that were anestrous with a body score condition ranging from 2 to 3 (scale from 1 to 5), received one of the following treatments: group 1) an intra-vaginal pessarie with MAP for 7 days plus an i.m. injection of 5 mg of estradiol benzoate (EB), group 2) an intra-vaginal pessarie without MAP for 7 days (a sham device - control). The overall pregnancy rate (TP) was 64.7%. Group 1 had a higher pregnancy rate (P<0.002) than group 2 (77.3% vs. 53.8%). In the second experiment, it was evaluated the effect of the sequential use of progestins during the postpartum period. We used intra-vaginal MAP in 79 pluriparous post partum beef cows (70 days post partum). Animals were allotted in 3 groups. Group 1: received one of the following treatments: group 1) an intra-vaginal pessarie with MAP for 7 days plus an i.m. injection of 5 mg of estradiol benzoate (EB), group 2) an intra-vaginal pessarie without MAP for 7 days (a sham device - control). The overall pregnancy rate (TP) was 64.7%. Group 1 had a higher pregnancy rate (P=0.002) than group 2 (77.3% vs. 53.8%). In the second experiment, it was evaluated the effect of the sequential use of progestins during the postpartum period. We used intra-vaginal MAP in 79 pluriparous post partum beef cows (70 days post partum). Animals were allotted in 3 groups. Group 1: received one of the following treatments: group 1) an intra-vaginal pessarie with MAP for 7 days plus an i.m. injection of 5 mg of estradiol benzoate (EB), group 2) an intra-vaginal pessarie without MAP for 7 days (a sham device - control). The overall pregnancy rate (TP) was 64.7%. Group 1 had a higher pregnancy rate (P<0.002) than group 2 (77.3% vs. 53.8%). In the second experiment, it was evaluated the effect of the sequential use of progestins during the postpartum period. We used intra-vaginal MAP in 79 pluriparous post partum beef cows (70 days post partum). Animals were allotted in 3 groups. Group 1: received no MAP (control); group 2: received one MAP for seven days; group three: received two MAP for seven days followed by with seven days interval between treatments. Group 2 and 3 received EB at insertion of the pessaries. After withdrawal of the pessaries, the cows were exposed to bulls in a proportion of 1 to 20 and were managed and under the same conditions during 90 days. The pregnancy diagnosis was done, by ultrasound scanning, 25 days after removing the bulls (115 days after treatment). Pregnancy rate was higher (P=0.10) in group 3 (83.3%) than the group 1 - control (53.3%). Pregnancy rate in group 2 (64.5%) was not different than group 1 or 3. The average stages of pregnancy of group 3 at ultrasound examination (65.1 days) were higher (P=0.03) than group 1 (38.1 days) and group 2 (39.6 days). Open cows were not included in the analysis. Based on these experiments it may be concluded that treatment with progestins may improve fertility in post partum beef cows and the sequential use of progesterone may increases pregnancy rate and anticipate the post partum conception.

144 (5014)

INFLUENCE OF HERD MANAGEMENT ON ARTIFICIAL INSEMINATION RESULTS DURING PASTURE PERIOD

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The relationship between herd management and artificial insemination (AI) results was quantified using the data issued from 965 AI made in 48 herds (Normande and Holstein breeds) affiliated to the AI centre AGIRE in Normandy. The frequencies and incidences of the different situations following AI were estimated using milk progesterone concentration measured the day of AI, 21 days later and with PSPB (Pregnancy specific Protein B) measurements 40-45 and 65-80 days later aiming to confirm pregnancy (Pinto et al., 2000). Likelihood of different herd management effects and of the breed were estimated using a multiple mixed logistic regression
model. Adjusted odds ratios (OR) were calculated to assess the probability of pregnancy. Incidence results (results over the population which is able to be concerned) after AI averaged 41.9 % for pregnancy 70 days after AI, 37.8 % for early embryonic death or non-fertilization (EED), 29.3 % for late embryonic death (LED) and 4 % for cows inseminated out of the ovulation period (P4 > 2ng/ml; P4+).

Incidence of EED and LED was lower in Normande than in Holstein cows (33.1 vs 41.1%, OR=0.68, P<0.02 and 20.7 vs 35.1%, OR=0.45, P<0.01 respectively). A high milk production at AI was related to increased LED in comparison to lower milk levels (40.9 vs 25.2, OR=0.48, P<0.01). Milk levels at AI tended to be higher too in EED cows, but this was not significant. Duration of the dry period and time from heat detection to AI and breed were the main factors associated with EED. Incidence of EED was decreased when the dry period lasted 55 to 75 days compared to shorter periods (32.4 vs 50.5%, OR=2.83, P<0.01, n=361) and when AIs were performed 6 to 20 hours after the first signs of detected oestrus in comparison to intervals shorter than 6 hours (34.5 vs 63.6%, OR=3.43, P<0.01, n=460).

The proportion of P4+ cows was greater than 7% in 23% of herds (11/48). Incidence of P4+ at AI was increased when cows showed "low behavioural signs of oestrus" in comparison to "normal" estrous behaviours (7.1 vs 2.8%, OR=0.33, P<0.02, n=673). On the contrary, the risk of P4+ was lower in females presenting a high difference between fat and protein milk contents than in females with a difference smaller than 3 g/kg (3.6 vs 6.8%, OR=3.86, P<0.01).

These results suggests that, under these pasture conditions, fertility may be improved with some herd management related to duration of the dry period, time of breeding according to milk production, longer intervals between oestrus and AI and specific attention to animals with "low signs of estrus".

145 (1739)
EFFECT OF INTRARUTERINE TREATMENT WITH CEPHAPIRIN ON THE REPRODUCTIVE PERFORMANCE OF SEASONALLY CALVING DAIRY COWS AT RISK OF ENDOMETRITIS FOLLOWING PERIPARTURIENT DISEASE
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Objective: To determine the effect of intraterine antibiotic treatment of cows "at risk" of developing endometritis on their reproductive performance.

Procedure: Cows (n=1325) from seasonal calving dairy herds (n=17) were enrolled in the study if they had calved >6 days and had at least one of the following conditions that placed them "at risk" of endometritis: retained foetal membranes, dystocia, a dead calf at or <24 hours of calving, hypocalcaemia, twins, calving induction or observed to have a vulval discharge >7 days after calving. They were blocked by calving date, age and "at risk" condition and randomly assigned to be treated with an intraterine infusion of 0.5 g cephalolin, or left as untreated controls prior to examination 28 to 37 days prior to mating start date (MSD) within each herd. All cows were body condition scored and had their cervical os discharge scored (VV score) on a scale of 0 (clear) to 3 (purulent). Uterine tone, size and position and ovary size and structures were assessed by transrectal palpation. Treatment immediately followed examination.

Results: Overall there was no significant treatment effect on reproductive performance. However when cows included on the basis of calving induction only were removed (remaining cows termed modified at risk (MAR) group (n=945)) the effect of treatment varied with the calving to treatment interval (CTI) and VV score. For the subset of MAR cows with CTI<28 days (n=229), treatment improved their first service conception rate (Odds ratio (OR) 3.1; p<0.01), 3 week (OR 2.8; p<0.01) and 6 week (OR 2.1; p<0.05) in calf rates (ICR) and reduced the mean MSD to conception interval (MSDCI) (84+/-6 vs 64+/-5 days; p<0.05). The improvement was greater for VV positive (VV+) than VV negative (VV-) cows. For the subset of MAR VV+ cows with CTI<42 days (n=160), treatment improved 3 week (OR 2.8; p<0.05) and 6 week (OR 2.4; p<0.05) ICR and reduced mean MSDCI (74+/-4 vs 62+/-4 days; p<0.05). Treated MAR, VV- cows with CTI>48 days (n=230) had a reduced 6 week ICR (OR 0.56; p<0.05).

Conclusion: Intraterine treatment of MAR cows with 0.5 g cephalolin improved their reproductive performance if they were treated soon after calving. A greater treatment effect in VV+ than VV- cows suggests that VV scoring is a useful tool for the diagnosis of endometritis. Treatment had a detrimental effect on VV- cows. Both early and late treatment of MAR cows with 0.5 g cephalolin improved their reproductive performance if they were treated soon after calving. A greater treatment effect in VV+ than VV- cows suggests that VV scoring is a useful tool for the diagnosis of endometritis. Treatment had a detrimental effect on VV- cows.

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146 (2276)
THE USE OF VAGINOSCOPIC (VISUAL) EXAMINATION OF POST-PARTUM DAIRY COWS FOR THE DIAGNOSIS OF ENDOMETRITIS AND ITS ASSOCIATION WITH REDUCED REPRODUCTIVE PERFORMANCE
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Objective: To use reproductive performance parameters to investigate the use of speculum examination of the cervical os discharge (visual vaginal (VV) examination) for the diagnosis of endometritis in dairy cows.

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Procedure: Cows (n=1325) from seasonal calving dairy herds (n=17) were enrolled in this study if they had been calved >6 days and had at least one of the following conditions that placed them "at risk" of endometritis: retained foetal membranes (RFM), dystocia, a dead calf at or <24 hours of calving, hypocalcaemia, multiple birth, calving induction or observed to have a vulvar discharge (VD) >7 days after calving. As part of a study to assess the effect of intrauterine antibiotic therapy, they were blocked by calving date, age and "at risk" condition and randomly assigned to be treated with an intrauterine infusion of 0.5 g cephepirin, or left as untreated controls prior to examination and treatment 28 to 37 days before mating start date (MSD) within each herd. All cows were body condition scored (BCS) and had their cervical os discharge scored (VV score) on a scale of 0 (clear) to 3 (purulent). Uterine tone, size and position and ovary size and structures were assessed by rectal palpation.

Results: Accounting for herd, age, treatment, BCS, and calving to treatment interval (CTI), cows with a positive VV (VV+) score (1 to 3) were less likely to conceive to their first service (Odds ratio (OR) 0.62; p<0.01), had lower 3 week (OR 0.56; p<0.01), six week (OR 0.51; p<0.01) and 21 week (OR 0.48; p<0.01) in calf rates and had a hazard ratio of 0.68 (p<0.01) for pregnancy. Mean MSD to conception interval was longer in VV+ cows in both control (88+/-6 Vs 60+/-3 days; p<0.05) and treatment (74+/-5 Vs 56+/-3 days; p<0.05) groups. Accounting for CTI, herd and the following factors, a VV+ score was associated with a low BCS, primiparous cows, pelvic uterus, RFM, VD, dystocia, dead calf and twins.

Conclusion: A VV+ score was associated with reduced reproductive performance and was more common in primiparous and low BCS cows. A VV+ score was less common in cows with an abdominal uterus. The sensitivity of the VV exam may be lower in these cows as the abdominal uterus may not expel as much pus due to poor uterine tone or gravity effects. Of "at risk" cows, those with RFM, VD, dystocia dead calf or twins were more likely to have a VV+ score. Further research is required to determine the role of VV examination in cows not "at risk" of endometritis.

Funding: Intervet Australia Pty Ltd

147 (3366)
A NOVEL BVDV VACCINE IMPROVES FERTILITY IN CATTLE CHALLENGED WITH NONCYTOPATHIC BVDV TYPE 1 UNDER CONTROLLED LABORATORY CONDITIONS
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Bovine viral diarrhoea virus [BVDV] is an important cattle pathogen with a global distribution. Two genotypes of BVDV are recognised, types 1 and 2, both of which can cause severe, if rarely fatal, infection and devastating economic losses. The pathogenesis of BVDV-related disease is complex and most commonly includes the intestinal mucosa, the immune system and the reproductive system. The clinical impact of BVDV infections depends upon the virulence and biotype of the strain involved. As a result of the tropism of the virus for the reproductive system it is not surprising that BVDV is thought to affect fertility in cattle.

In this study a novel inactivated BVDV vaccine was administered subcutaneously to breeding age heifers shortly before synchronised insemination. A similar number of breeding age heifers were given saline as a control. All animals were negative for BVDV serum antibody and virus before enrolment. Both the vaccine and saline were given by the subcutaneous route and comprised two administrations 21 days apart. A group of non-pregnant breeding age heifers was challenged intranasally four and seven days after synchronised artificial insemination with two heterologous noncytopathic strains of BVDV type 1. When the study terminated on approximately the 75th day of pregnancy, 21 of 22 [95%] heifers were pregnant with live calves in the vaccinated group, and only 9 of 22 [41%] in the control group [P= 0.0002]. This represents a 132% increase in fertility over this period of pregnancy. All calves were negative for BVDV by virus isolation.

In conclusion, a novel inactivated BVDV vaccine was shown to be highly efficacious in reducing infertility due to challenge with heterologous noncytopathic BVDV type 1 strains under controlled laboratory conditions.

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148 (949)
DELAYED RESUMPTION OF OVARIAN CYCLICITY POSTPARTUM IN COWS: EFFECTS ON REPRODUCTIVE PERFORMANCE AND POTENTIAL RISK FACTORS
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The fertility of lactating dairy cows has been declining worldwide. The cause is yet to be described. The objectives of the study were to investigate the incidence of the delayed resumption of ovarian cyclicity postpartum, and its effects on subsequent reproductive performance, and to identify potential risk factors. The study was conducted in a commercial dairy farm in Hiroshima, Japan. Milk samples were collected from...
149 (1202)
PREVENTION OF ABORTIONS DUE TO NEOspora canINum IN COWS
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Neospora is a cause of abortions in dairy and beef cows in France. Decoquinate is a non-antibiotic synthetic molecule, active on certain protozoa: coccidia, cryptosporidia, toxoplasma has been revealed to be effective in vitro on the tachizoits of Neospora Caninum. It has been shown to significantly reduce the effect of experimentally induced toxoplasmosis in pregnant ewes. It is approved as anticoccidial for use in cattle, sheep and goats in several countries.

In 1999-2000, on two dairy farms (Prim’Holstein breed) of western France, where the prevalence of neosporosis is 75% and parasite transmission is both vertical and horizontal, a control group of 28 cattle was made up, and a treated group was composed of 19 cows which orally received 2 mg/kg/day decoquinate for one month from drying off, and of 24 heifers which received the same dose during two one-month periods, separated by a 15-day period, as from 5.5 months of gestation.

In vertical transmission, the treatment did not prevent mother-to-calf transmission of the parasite, but in horizontal transmission the number of abortions was diminished (0/15 versus 2/4 in the controls).

In 2000-2001, decoquinate was distributed at the rate of 2mg/kg/day as from the 45th day of gestation until the 8th month to 32 dairy heifers, 45 others being used as controls. In vertical transmission, this treatment reduced mother-to-calf transmission of the parasite (28% negative versus 5%) and abortions (21% versus 38%).

In horizontal transmission, 59% of the calves were negative in the treated group versus 35% in the control group, and 6% of the cows aborted versus 17%.

In Italy, on a suckling farm (Limousin breed), 90 cows or heifers orally received 1 mg/kg/day decoquinate for 2 months as from the 4th month of gestation, leading to a 6.7% abortion rate versus 27.7% in the 44 controls.

150 (2849)
NORMALITY AND VARIABILITY IN IN VITRO EMBRYO PRODUCTION PERFORMANCE IN CLONED CATTLE DERIVED FROM EMBRYONIC CLONING
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Genetically identical animals are valuable models for research. As natural monozygotic twins calves are rare, the technique of nuclear transfer provides the possibility of generating sets of animals with the same nuclear genome (“genetically identical”). Departing from the logical assumption that clones are identical, we designed this study to answer the following questions: Do clones have normal ability to produce embryos in vitro? Are they identical, i.e. at least are oocyte and embryo production less variable than the ones of controls? Ovum Pick-Up (OPU) and in vitro fertilization were performed in four sets of cloned heifers (embryonic clones, n = 10, two sets of triplets and two sets of twins). For each group of cloned heifers, one group of non-genetically related animals of same age and breed were used as control (n = 13, three groups with three and one group with four animals). In 304 OPU sessions, a total of 1798 oocytes were recovered.

The mean number of oocytes recovered per female per OPU (± SD) was similar between cloned and control animals (5.7 ± 2.9 vs. 6.1 ± 4.5 respectively). After in vitro maturation, fertilization (with semen from the same
bull tested for a high in vitro fertility) and culture, the blastocyst formation rate for cloned animals was 35.0 % ± 29.2 % and was not significantly different from the one of controls (29.4 % ± 30.9 %).

When variances of oocyte production were compared between cloned and control animals, two sets of cloned animals were statistically more homogenous than their respective controls (7.1 vs. 23.9 and 7.3 vs. 26.7 respectively P < 0.001). The coefficient of variation (CV) for oocytes recovered (49.9 % and 73.0 %, overall for cloned and control groups respectively) and blastocyst rate (91.8 % and 105.2 %, overall for cloned and control groups respectively) were, in all comparisons, lower in groups of cloned animals than in controls. Our study demonstrates that clones animals (embryonic) have normal in vitro embryo production performances. Nevertheless, a non-negligible rate of variability between animals within each set of clones was observed, which confirms that cloned cattle are not the exact phenotypic copy of each other. Results should be interpreted with caution owing to the limited number of cloned animals in spite of the large number of oocytes analyzed. Further work has to be conducted on cloned cows obtained through somatic cell nuclear transfer.

151 (2504)
IN-VITRO EMBRYO PRODUCTION IS AFFECTED BY THE OOCYTE DONOR COW
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In mouse, the influence of the genetic origin of oocytes on embryo development has been well described, but this maternal effect is not demonstrated in cattle. The aim of this study was to examine whether such a maternal effect over blastocyst production could be evidenced in the bovine. Six Holstein cows with diverse genetic backgrounds (different fathers, mothers and grand-parents) were selected as oocyte donors. They were raised in the same farm and had similar physiological status (primiparous, non pregnant, dry cows with synchronised estrus). Oocyte collection by OPU was performed for 14, 12 and 6 weeks (periods A, B and C). The collected oocytes were in vitro matured (one microdrop per cow) for in vitro fertilization (IVF). IVF was done with the sperm from 3 bulls with high in vitro fertility (one bull for each period). Oocyte number, cleavage and blastocyst rate (day 8) were recorded.

A total of 1707 oocytes were recovered with a mean of 4.84 ± 0.31 oocytes/cow/OPU. For all 3 periods, the numbers of oocytes recovered were 225cd, 445a, 165d, 289bc, 178d and 375ab* for each experimental animal (cows C1 to C6 respectively). The mean cleavage rate and blastocyst rate were 79.2 ± 3.0 % and 28.7 ± 3 %, respectively (363 blastocysts in total). Mean period blastocyst rate (all cows) differed significantly (35.8a ± 5.8%, 19.7b ± 4.9 % and 31.1a ± 7.8 % for period A, B and C respectively) due to the paternal effect on embryo production. However multiple step-down range tests for blastocyst rate for each period show that the best (C5) and worse (C4) blastocyst producers were always the same animals despite the use of different semens. Blastocyst rates for cow 4 and cow 5 during period A / Bull 1 were 15.1 a % and 58.3 b %, for period B / Bull 2, 8.7 a % and 39.5 b % and for period C / Bull 3 C4 10.0 a % and C5 60.4 b %. This conserved ranking supports the hypothesis of the existence of a maternal influence on in vitro embryo production in cattle. Since no correlation appeared between the number of oocytes collected and the blastocyst rate, female selection on the number of blastocysts produced per OPU session should involve both criteria.

*: p < 0.05

152 (3214)
WHEN TO START OVSYNCH IN DAIRY COWS? REPRODUCTIVE AND ECONOMIC CONSIDERATIONS
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Ovsynch is a protocol to synchronize ovulation in lactating dairy cows and allow for timed AI (TAI). Field studies have shown, that conception rates to TAI increase as lactation progresses. The objective of this study was to analyze, if postponing AI further into lactation could improve the reproductive and economic efficiency of Ovsynch. A total of 290 low producing (lower 25% of the herd) and 281 high producing cows (upper 25% of the herd) of a commercial dairy herd in Brandenburg, Germany, were included in the study. Cows were assigned by their ear tag numbers to receive early TAI (53 to 59 days in milk (DIM) in low producers, 73 to 81 DIM in high producers) or late TAI (73 to 81 DIM in low producers, 94 to 102 DIM in high producers). Synchronization was achieved by 2 treatments with a GnRH analogue on days -10 and -1 and a treatment with a PGF2a analogue on day -3. AI was performed 16 to 20 hours after the second GnRH treatment. Cows returning to estrus after TAI were bred on observed estrus. Cows diagnosed not pregnant 42 to 48 days after AI were re-synchronized on day -3. AI was performed 16 to 20 hours after the second GnRH treatment. Cows returning to estrus after TAI were bred on observed estrus. Cows diagnosed not pregnant 42 to 48 days after AI were re-synchronized using the same protocol.

Conception rates were compared using Chi-square test. Conception rate to TAI was higher in the late AI groups than in the early AI groups (34.5% vs. 14.4% (p<0.05) in low producers; 41.4% vs. 28.2% (p<0.05) in high producers). Conception rate to TAI between 73 and 81 DIM did not differ significantly between low and high producing cows (34.5% vs. 28.2%). The proportion of cows pregnant by 200 DIM was numerically higher in low than in high producing cows (77.4 vs. 70.7%, p<0.1) but did not differ between cows receiving early or
late TAI (74.3 vs. 74.2%).

Economic analysis using price assumptions for the cost factors prolonged days open, culling of cows, hormonal treatment and examination of cows, revealed that in low producing cows costs per pregnant cow were lower in the late than in the early TAI group (€ 195.09 vs. 225.45). In high producing cows postponing TAI to 94 to 102 DIM was not economically beneficial compared to TAI at 73 to 81 DIM (€ 244.52 vs. 232.02).

Results of this study indicate that regarding economic aspects, for this herd there was an optimum period for starting Ovsynch between 63 and 71 DIM, resulting in TAI 10 days later. However, before this finding can be used as a general recommendation, the results should be confirmed in further studies in different herds.

153 (2618)
NORMAL ENDOMETRIAL CYTOLOGY IN POSTPARTUM COWS
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Reproductive performance of dairy cows following postpartum period (PP) depended on uterine health status which is related with previous occurrence of uterine diseases such as endometritis. Over the years, examination methods as rectal palpation, vaginoscopy, uterine biopsy and bacteriology, has been used with known limitations for the diagnosis of endometritis. Small amount or absence of uterine discharge makes the diagnosis of this condition very difficult with routine diagnostic tests. However, diagnostic methods like endometrial cytology (EC) and ultrasonography has recently shown promises (Kasmanickam et al., 2002).

Five (5) primiparous heifers and three pluriparous cows from the University herd were examined four times at 7 days apart, between d20 and d48 after normal calving. The cows were submitted to the following procedures: recording of any perineal discharge, ultrasonography exam of the uterus and ovaries using a 7.5 MHz rectal linear probe (Aloka, ISM) without manipulation of the uterus. Uterine body diameter and intrauterine fluid volume as well as the echotexture of both fluid and endometrium were assessed. Similar measures were done on both uterine horns. Palpation per rectum of position, size and consistency of the uterus and the cervix were recorded. Vaginoscopy were performed to assess the presence and type of discharge in the vagina and cervix. Samples for EC were collected using a cytobrush (Fisher Scientific Ltd) as described by Kasmanickam et al., 2002 and stained with modified Wright stain (Diff Quick, Jorgensen Lab.).

On the smears, endometrial and neutrophil cells were the two predominant types of cells with variable patterns of distribution. The entire slide was assessed for the number of neutrophils per field (400X) and the ratio of neutrophil per endometrial cells which were recorded for 10 fields. The distribution of the endometrial cells was either in sheets or detached and spread out. The neutrophils were unevenly distributed (clumps in certain areas) and never observed in sheets of endometrial cells. Analysis (linear model, SAS) of the data shown that neither parity, ovarian status and the visit did affect the number of neutrophils present on the smear. However, there is a tendency for a decrease in the number of neutrophils as the number of week postpartum increases (p=0.09). More studies are needed to further standardize the EC before its use in practice.

154 (2767)
EFFECT OF NON ESTERIFIED FATTY ACIDS ON GRANULOSA CELL PROLIFERATION
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Negative energy balance (NEB) may affect ovarian function in dairy cows early post partum through increased concentrations of non-esterified fatty acids (NEFA), which are known to be cytotoxic for several cell types.

Since the mono-unsaturated oleic acid (C 18:1) together with the saturated ones palmitic (C16:0) and stearic acid (C18:0) are the most abundant in serum during NEB, the effect of these fatty acids on granulosa cell proliferation was investigated. Bovine granulosa cells were harvested through repeated aspiration of follicular fluid from large follicles (>8mm) on slaughterhouse ovaries. After washing (800xg), cell viability was assessed by trypan-blue exclusion and cells (75000) were cultured for 48h under serum free conditions, with 1 ng/ml FSH and 10 ng/ml insulin. Cells were treated with 0, 150, 300, or 500 µM of the individual fatty acid or 450 µM of a 1:1:1 combination of all three fatty acids. At the end of culture, granulosa cell numbers per well were determined spectrophotometrically (492nm) with the CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega Benelux, Leiden, The Netherlands). As the number of cells is linearly related to the absorbance measured, a standard curve was constructed by incubating known numbers of cells with the MTS solution under the same culture conditions. The number of viable cells remaining in each well at the end of culture was estimated from the resulting regression equation. Both PA and SA had a significant inhibitory effect on granulosa cell proliferation at the three concentrations tested (P<0.01). This effect was not dose-dependent for PA (P>0.05) since all three concentrations reduced cell numbers evenly (52.9 to 60% reduction). Stearic acid on the other hand had a more severe negative effect on cell proliferation at 300 µM and 500 µM than at 150 µM (P<0.01; 69.7% and 71.1% reduction versus 33.5%). Oleic acid only inhibited cell proliferation significantly (P<0.01) at the highest concentration of 500µM (66.5% reduction). The combination treatment also reduced cell numbers significantly (P<0.01) compared to controls (34.7% reduction). We conclude that in vitro NEFA reduce cell proliferation and/or survival of bovine granulosa cells. This indicates that elevated NEFA concentrations early post partum in dairy cows may affect ovarian cells and hence ovarian functioning.
155 (942)
PERFORMANCE OF HOLSTEIN DAIRY COWS HAVING EITHER A SINGLE OR TWIN PREGNANCY
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Twin calving cows are often associated with multiple health problems and poor productive and reproductive performance. With twining rates increasing in many dairy herds, different strategies have been attempted to control associated health problems with twin pregnancies without consistent success. The objective of this study was to identify potential metabolic or performance parameters that may provide insight to properly managing twin pregnancies.

Mature Holstein cows (n=44) were used to address prepartum dietary protein supplementation on performance. Twin pregnancy (TP) occurred in 6 cows and these data were compared to the 38 singleton pregnant cows (SP). All cows were monitored from dry off through 15 weeks of lactation. Dry matter intake (DMI) and milk yield was observed daily. Body weight (BW), blood metabolic parameters and milk composition were determined 2x or 3x per week. Body condition score (BCS) was evaluated weekly. Other animal parameters such as calf and placenta weights, disease incidence and reproductive performance were recorded.

Cows with a TP had lower calf birth weight (36.8 vs 43.3 kg), heavier wet placental weight (7.5 vs 5.8 kg) and fewer days pregnant (275 vs 280 d) compared to SP cows. Combined twin calf birth weights averaged 174% of SP cows. Mean DMI was lower for TP cows prepartum and declined earlier in gestation compared to SP cows. Postpartum DMI was not different. Mean BCS was lower pre- and postpartum for TP cows. Twin pregnant cows had lower BW and greater BCS loss postpartum. Milk production and composition were not different by pregnancy status. Twin pregnant cows tended to experience more periparturient disease episodes (4 vs 2.4) compared to SP cows. Prevalence of dystocia, metritis, and mastitis was greater in TP cows. Days to first ovulation (24.9 vs 37.8 d) and total services (1.5 vs 2.5) were greater for TP cows and other reproductive intervals tended to be longer in TP cows. Lower insulin and greater beta-hydroxybutyrate concentrations prepartum were observed in TP cows compared to SP cows. Nonesterified fatty acid concentration was greater in late pregnancy for TP compared to SP cows. Much of the observed health and production problems associated with TP may result from inadequate nutrition to meet fetal needs resulting in severe maternal nutrient depletion. Feeding a prepartum diet higher in energy and protein density may help to address the lower DMI of TP cows and minimize associated health problems.

156 (3362)
EVALUATION OF THE EFFICACY OF PROSTAGLANDIN TREATMENT IN DAIRY COWS
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A retrospective meta analysis was performed on data from a Dutch computerised Herd Health Management Program. In the period from 1988 to 2003 a total of 3786 cows from 53 herds belonging to the Ambulatory Clinic of the Veterinary School were treated with prostaglandin (PGF2a). Reason for treatment was based on lactation stage more than 60 days after calving and unobserved heat in the presence of a palpable functional corpus luteum. Functionality of the corpus luteum was not proved by blood hormone concentration. Cows were inseminated based on expressed heat or 48 - 72 hours after treatment. Pregnancy was diagnosed by rectal palpation. Results showed that second and third parity cows were most treated (22.3% and 20.2% respectively). Within 21 days after PGF2a injection, 68.7% of the cows were inseminated, and within 8 days only 60.4%. Highest response was shown on day 3 and day 4 after treatment, influenced partly by blind insemination. On those two days 40% of all treated animals were inseminated. A small second peak was shown at 24 to 27 days after treatment. Within 21 days after PGF2a injection, 26.5% of all treated animals became pregnant, and within 8 days, 23.5%. Pregnancy rate per day varied during the first 8 days between 32% (day 8) and 43% (day 6). There was also an effect of days in milk. Cows that were more than 130 days in milk showed higher pregnancy rates. As expected, history also played a role. Cows that were inseminated more than 2 times before treatment showed lower pregnancy rates. Chance to become pregnant decreased from 39% when cows were inseminated twice before treatment to 14% when treatment followed the fifth insemination. This large study under field conditions shows the enormous variation in response between animals. It may be concluded that the overall effect of PGF2a-use in normal cyclic cows with unobserved heat is poor, only 26.5% pregnant cows due to the direct effect (within 21 days) of treatment. This can be due to the experience of the veterinarian (poor palpation skills), to the farmer (poor detection) or to the cow (poor expression). In making a treatment decision number of inseminations before treatment and number of days in milk of the patient should be taken into account. A cost benefit analysis of PGF2a (7.5 Euro per treatment in the Netherlands) shows that an extra farm visit for palpating the problem cows could be more beneficial.

157 (5009)
RETROSPECTIVE ANALYSIS OF THE ASSOCIATION BETWEEN SUBCLINICAL KETOSIS AND CONCEPTION FAILURE IN ONTARIO DAIRY HERDS

Proceedings of the WBC Congress, Québec, Canada, 2004
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Retrospective analysis was used to investigate the association between subclinical ketosis (SCK) and risk of conception at first service.  
Data from 1010 animals housed at 25 farms was collected as part of a large clinical field trial extending between 1995-1996. Reproductive data was collected using on-farm data sheets, veterinary records and on-farm computer records. Conception to first service, number of services, and days from calving to conception were calculated from these records. Milk production and health information was recorded for the first three Dairy Herd Improvement (DHI) tests. Serum beta hydroxybutyrate (BHBA) determined in the first, second, third, sixth and ninth weeks of lactation was used to monitor energy balance. A cutpoint of 1400umol/L BHBA was used to define SCK. Exclusion of animal records occurred if the animal was not serviced or the first service outcome was not known. After exclusion 806 records remained for analysis. A total of 667 animals were confirmed pregnant. The first service conception risk was 34.6% (279). Risk of SCK ranged from 19.1% to 4.5% in the second and ninth week respectively. Two by two tables were generated to calculate the relative risk of SCK on conception to first service. Relative risk (RR) of conception failure to first service was significant for cows with SCK in week 2 (RR 1.12; 1.05, 1.19) p<0.001. The mean BHBA value for week 1 and 2, using the same cutpoint to define SCK, was used to calculate a RR for animals with known outcome to first service. The RR of conception failure increased to 1.8. When the RR at different mean BHBA cutpoints (week 1 and 2) was evaluated a significant increased risk of failure to conceive was identified at 1100 umol/L BHBA (RR 1.4). This effect increased in magnitude from 1100 umol/L BHBA to 1600 BHBA (RR 2.0) consistent with a dose response relationship. Animals that lost 1.25 BCS units were 1.1 times less likely to conceive at first service than animals that lost less than 1 body condition score. From this data control and prevention of SCK immediately post-partum has the potential to influence reproductive performance of Ontario dairy herds.

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158 (1654)
THE EFFECTS OF SYNCHRONIZATION METHOD AND POSTPARTUM ENERGY INTAKE ON REPRODUCTIVE PERFORMANCE FOLLOWING TIMED ARTIFICIAL INSEMINATION IN BEEF COWS  
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The objectives of this study were to compare the effectiveness of two estrus synchronization methods for use in timed artificial insemination (TAI) programs in silage-fed beef cows and to examine interactions between synchronization method and postpartum energy intake. It was hypothesized that a modified CIDR program, which utilizes progesterone and estradiol benzoate, would enhance reproductive efficiency in underfed cows when compared to an Ovsynch program. Hereford-cross cows (53 and 64 head for years 1 and 2, respectively) were assigned to one of six treatments: three levels of silage [Low (L), Medium (M) and High (H)] and 2 methods of estrus synchronization (Ovsynch or CIDR), stratified by parity and predicted calving date. Cows were fed grass/clover silage, with dry matter intakes of 1.4%, 1.7% and 2.0% of body weight for the L, M and H groups, respectively. Ovsynch treatment consisted of 100 µg GnRH IM on day 0, 25 mg prostaglandin F2a (PGF) IM on day 7 and 100 µg GnRH IM on day 9, with TAI 16-18 h later. CIDR treatment consisted of intravaginal insertion of a CIDR® implant (1.9 g progesterone) with 1 mg estradiol benzoate (EB) IM and 100 mg progesterone IM on day 0, 25 mg PGF IM and removal of the implant on day 7, followed by 1 mg EB IM 24 h later, and TAI 28-30 h after the final EB injection. Percent pregnant following TAI tended to be greater for CIDR cows (69%) than for Ovsynch cows (53%) (P=0.07). The probability of pregnancy depended on diet (P=0.05) and was subject to a diet x year interaction (P<0.05). Cows with higher postpartum energy intakes had the highest conception rates in year 1 but not in year 2. In year 1, conception rates were 59%, 75% and 89% for the L, M and H groups, respectively. In year 2, they were 55%, 50% and 47%, respectively. The difference between years may have been due to lower average body condition scores (BCS) for the second year of the study. There was no clear interaction between diet and synchrony method. Nonetheless, it appeared that the advantage gained from CIDR versus Ovsynch was mainly in the L group in year 1 and all three groups in year 2. These data indicate that both the Ovsynch and CIDR methods are capable of achieving high conception rates following a single TAI in silage-fed postpartum beef cattle over a range of postpartum energy intakes. The CIDR method tended to produce better results than the Ovsynch method in underfed cows.

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159 (1774)
EFFECTS OF INTERVAL FROM CALVING TO TREATMENT AND RESUMPTION OF OVARIAN CYCLICITY ON CONCEPTION RATE IN DAIRY COWS AFTER OVSYNCH
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The objectives of the present study were to show the effect of intervals from calving to initiation of ovulation synchronization protocol (OVSYNCH) on conception rate and then to know whether status of resumption of ovarian cyclicity postpartum at the time of OVSYNCH treatment influences the conception rate in Holstein-Friesian dairy cows. In the first experiment, a total of 1,457 cows were inseminated artificially after OVSYNCH during a period ranging between 41 and 442 days postpartum. The conception rates after OVSYNCH were compared among the groups with different intervals from calving to treatment. In the second experiment, 123 cows were inseminated after OVSYNCH 44 to 149 days after parturition. Blood samples were collected twice, 7 days before and the day of OVSYNCH, for plasma progesterone assays to know whether cows were cycling or not before OVSYNCH. The conception rate was compared between cows cycling and those not cycling. In the third experiment, 39 cows were monitored for resumption of ovarian cyclicity postpartum by measuring progesterone concentrations in plasma collected weekly between 2 and 9 weeks postpartum. All the cows were, then, inseminated after OVSYNCH. The conception rate was compared between different groups of cows divided by days to resumption of ovarian cyclicity postpartum. Conception rate after OVSYNCH was lowest, 47.8%, 40 to 60 days postpartum, which increased gradually towards 101 to 120 days postpartum, 55.8%. The 123 cows were divided into 4 groups with different patterns of change in plasma progesterone before OVSYNCH; Low-Low (n=20), Low-High (n=28), High-Low (n=33) and High-High (n=42). The conception rate in 20 acyclic cows with Low-Low pattern showed a lower conception rate than the other 103 cyclic cows (30.0% vs 52.4%, P<0.10). Among the cyclic cows, cows showing High-High pattern showed a lower conception rate than those with Low-High and High-Low patterns. Of the 39 cows monitored for resumption of ovarian cyclicity, 11 cows resumed the cyclicity within 34 days postpartum and showed a significantly higher conception rate than those with delayed resumption beyond 56 days postpartum (72.7% vs 27.3%, P<0.05). In conclusion, days postpartum and status of resumption of ovarian cyclicity at the initiation of OVSYNCH significantly influences the conception rate in dairy cows. It is imperative to implement OVSYNCH in cows after resumption of ovarian cyclicity postpartum for improving the conception rate.

160 (1609)
EXPRESSON OF ESTRUS SIGNS DURING PRE-SERVICE AND SERVICE PERIODS POSTPARTUM IN HIGH-PRODUCING DAIRY COWS AND A POSSIBLE ROLE OF STRESS IN DEPRESSION OF ESTRUS
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Weakened estrus signs and difficulty in estrus detection are two major causes of declined reproductive performance in high-producing dairy cows of today. A series of experiments were carried out to show some characteristics of expression of estrus signs in high-producing Holstein Friesian cows during postpartum pre-service period as well as service period and to show a possibility that stress causes depression of estrus signs. Experiment I: Occurrence of standing estrus during postpartum pre-service period. Postpartum ovarian cyclicity was monitored by milk progesterone profiles until 70 days after calving in 27 cows. Cows were checked for estrus signs by visual observation at an interval of 4 hours with the aid of Kamar Heat Mount Detector and tail paint. Percentage of cows showing standing estrus at the first, second, third and fourth ovulations were 8, 26, 50 and 67 %, respectively. Experiment II: Intensity and duration of estrus signs after VWP. Thirty cows, which were due to be served, were observed visually for estrus at a 4 hours interval. The heat mount detector and tail paint were also used. Primary as well as secondary estrus signs were recorded. A cumulative number of cows detected in estrus were 56. Of the 56 cows, 64.3% showed standing estrus, while 35.7% expressed secondary estrus signs only. Mean (±S.D.) duration of standing estrus was 6.6 ± 6.3h. Experiment III: Response of adrenocortical progesterone to ACTH in ovariectomized lactating cows. Four ovariectomized Holstein Friesian cows, seven to nine months in milk, were injected intramuscularly with 25 IU ACTH to show the response of adrenal progesterone. All the 4 cows showed an increase in plasma progesterone after ACTH challenge. The peak plasma progesterone concentration exceeded 1.0 ng/ml in 3 of the 4 cows. The mean (± S.D.) of peak progesterone concentrations in the 3 cows after the first ACTH challenge was 3.67 ± 0.66 ng/ml, which was well comparable with the concentrations obtained in the luteal phase before the ovariecotomy. Results of the three experiments indicate that a substantial number of cows do not show standing estrus even at the third or fourth ovulations postpartum and intensity of estrus as well as duration of standing estrus have been reduced in high-producing cows. The capability of the adrenal cortex to secrete sufficient amount of progesterone to suppress estrus may suggest a possible role of stress in weakening of estrus signs in cows.

161 (3502)
METABOLIC BALANCE AND EMBRYO PRODUCTION DURING SUPEROVULATORY TREATMENT IN DAIRY CATTLE
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Superovulatory response in dairy cows is very variable and causes many problems for embryo transfer in cattle. The objective of this study is to verify the relationship between the number of live embryos and the metabolic state of cows after superovulatory treatment. Forty-nine Holstein cows (41 lactating and 8 dried up) with an average age of 6.6 ± 1.5 years were subjected to a superovulatory treatment for commercial production of embryos. Uterine washing fluid allowed the counting of total, live and dead embryos as well as non-fertilized oocytes. During the embryo harvest, individual blood samples were taken from each cow for analysis of 22 biochemical parameters. Feed samples were collected daily for two weeks before embryo collection and were analyzed for mycotoxins (vomitoxin, zearalenone and T2 toxin). The 49 cows gave an average of 9.45 ± 5.60 total embryos and oocytes of which 5.27 ± 4.20 embryos were transplantable, 0.37 ± 0.80 were dead and 3.82 ± 3.78 were non-fertilized oocytes. The production of live embryos was classified according to 4 levels of performance: 0-1 (N = 11); 2-4 (n=13); 5-9 (n=14) and more than 9 (n=11). Analysis of variance showed that serum concentrations of magnesium (p<0.07), calcium (p<0.05) and potassium (p<0.05) were significantly higher in the group of cows producing more than 9 transferable embryos. There were no significant effects of age, lactational stage or milk production on the transferable embryos (p<0.10). The metabolism of energy (glucose, cholesterol and hydroxybutyrate) and proteins (urea, albumin), hydric balance (creatinine and sodium), hepatic function (albumin, bilirubin, AST, GGT), immune system (globulins), trace mineral (Cu, Zn, Se) and beta-carotene had no significant effect on transferable embryos (p<0.10). Mycotoxin amount in feed varied from 0 and 175 ppm and it had no significant effect on embryo production. In conclusion this study in which all cows were in their natural farm environment, showed a link between metabolic factors responsible for maintaining high serum concentrations of calcium, magnesium and potassium and transferable embryos after superovulatory treatment. Transferable embryo production was not significantly affected in cows exposed to mycotoxins even at the highest detectable level (175 ppm).

162 (3024)
VENTRAL LAPAROSCOPIC ABOMASOPEXY ON ADULT CATTLE
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Left displaced abomasum (LDA) is a frequent surgical condition in large animal practice. Although efforts have been made to prevent the disease, the large animal veterinarian has to treat surgically abomasal displacement. Laparoscopic abomasopexy provides minimal invasion of the abdomen with an expected rapid return to previous production with minimal complications. A two step lapararoscopic approach has been described with good results. Our objective was to describe a one step ventral laparoscopic approach for correction and prevention of LDA in cattle. Laparoscopic abomasopexy was performed on ten healthy Holstein cows. Normal position of the abomasum and absence of adhesions were assessed by ultrasound examination of the cranial ventral abdomen. Surgery was performed under sedation (xylazine 0.1mg/kg I.V.), and local anesthesia with the animal on dorsal recumbency. After surgical preparation, an 8 mm full thickness abdominal wall incision was made on the umbilicus. A 8 mm trocar was inserted through this incision and the abdomen was insufflated. A 8 mm 0o 420 mm long rigid laparoscope was inserted through the 8 mm trocar sleeve. After laparoscopic examination of the cranial abdomen, a second 10 mm trocar was inserted 3 cm caudal to the xyphoid process and 3 cm to the right of the ventral midline. A 10 mm grasping forceps was used to grab the abomasum at middistance of its large curvature, 3 cm lateral to the greater omentum attachment. The abomasum was pulled against the ventral abdominal wall. A portal instrument was placed cranial and lateral (right) to the laparoscope. Abomasopexy was performed by suturing the abomasum to the right cranial ventral abdominal wall with 4 abdominal simple interrupted stitches (polydioxanone USP 2). The portal incisions were closed in a routine manner. For the first week, each cow was examined once a day. Three months after the surgery, abomasal adhesions were assessed by performing a second laparoscopic surgery. Complications were not observed during the experiment. The rumen was not penetrated in any of the animals. Twenty minutes was the mean surgical time. Three months after the surgery, adhesions were observed on all ten cows. Length of those adhesions was equivalent to the original fixation. In some cows, hollow spaces between stitches were observed. This laparoscopic technique was safe, easy and allows a efficient fixation of the abomasum. Therefore, it can be used to treat surgically LDA.
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163 (1616)
LAPAROSCOPIC OVARIECTOMY IN STANDING COWS
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Numerous techniques for ovarietomy in cattle have been described and are performed transvaginally or transabdominally via the flank or linea alba. The method of choice in horses is laparoscopic ovarietomy via a unilateral or bilateral flank approach in the standing animal. It is minimally invasive and allows direct visualization of the ovaries. A similar method would be desirable in cattle with ovarian disorders or for
reproductive research. Thus, the goal of our study was to establish a technique for laparoscopic ovariectomy in standing cattle.

Eight Swiss Braunvieh cows aged 3 to 9 years and without gynecological abnormalities were used. Feed was withheld for 24 to 48 hours prior to surgery. A bilateral flank approach was used in 2 cows and a left flank approach in 6. Various portal sites were evaluated to determine the optimal approach for instruments and laparoscope.

Withholding feed for a minimum of 36 hours allowed good visualization of the uterus and ovaries. Cows were sedated with xylazine, and the portal sites in the left flank were infiltrated with 2% lidocaine. The optimal site for the laparoscopic portal was at the ventral angle of the paralumbar fossa, approximately 10 cm ventral to a horizontal line drawn through the tuber coxae and at the transition from the middle to the caudal third of the flank. After trocharization, the abdomen was insufflated with carbon dioxide to an intrabdominal pressure of up to 10 mm Hg. Two instrument portals were made under laparoscopic guidance 20 and 30 cm ventral to the tuber coxae. Grasping forceps and claw forceps were used to pull each uterine horn cranially until the ovary could be held with the claw forceps. An injection cannula was used to inject 2% lidocaine subserosally into the mesovarium and mesosalpinx. Using bipolar cauterization, the mesovarium was transected. The proper ovarian ligament was cut with serrated curved scissors, and the ovarian pedicle was examined for hemorrhage. The ovary was placed in a collection bag and removed from the abdomen by enlarging slightly one of the instrument portals. The second ovary was removed in the same way. The portals were sutured, and antibiotics were administered to all cows for five days. There were no abnormalities in the general behavior and condition during a 30-day observation period after surgery. The procedure initially took 150 minutes to perform but as the operator’s experience increased, this time decreased to 120 minutes.

164 (5084 )
SURGICAL TREATMENT FOR FRACTURES IN CALVES
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For the past 20 years we have been using in our Large Animal Practice, the external fixation technique described previously. Different material can be used, depending on the case. We can assert that with this procedure the following goals can be achieved:

- good quality and fast bone healing
- quick functional recovery
- affordable cost for the owner

This procedure can be used on the metacarpus and metatarsus, the tibia and the mandibula. The pins can be set in the simplest construction or in the most complex one, depending on the practitioner's creativity.

Unfortunately we have not yet found an adequate surgical procedure to repair fractures of the humerus and the femur in the calf.

In this presentation, I will discuss anatomical specificities of the calves' bones, I will review the main steps of callus formation in young bovines, and I will develop the most important aspects of this procedure (asepsis, anaesthesia, clinical examination, radiographic examination, prognosis, setting of pins, biomechanical qualities of the construction, post-operative follow up, removal of pins). Therefore we will review a few clinical cases:

- diaphyseal fracture of the metatarsus/carpus
- fracture of the metacarpus due to assisted calving
- fracture of the tibia : blind approach or through stab incision
- fracture of the mandibula
- other fractures

I will describe the different surgical supplies (JAM, FESSA, APEF). I will give examples of possible complications, osteomyelitis and osteoarthritis, although rarely observed. I will conclude this short presentation with the results achieved in a study of 100 cases. External fixation is the best surgical procedure to repair fractures of the distal limb in calves but in many cases adequate healing can be obtained by using a cast, for a much lower cost. A good large animal orthopedic surgeon must take care of achieving the best result at an affordable fee for the owner.

165 (2007)
THE RUMEN OF CALVES - ENDOSCOPICAL VIEW OF ANATOMICAL STRUCTURES
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Introduction: Endoscopy, the inspection of body cavities by means of optical devices, has enjoyed growing
importance in veterinary medicine in recent years. Today it represents an essential method of examination, aiding diagnosis and prognosis also in cattle medicine. As mentioned in various reports endoscopy is also used for scientific works and is helpful for education.

The aim of our study was to demonstrate the inside of the rumen of calves by endoscopic examination. This work was performed within the framework of a study concerning development of rumen acidosis in calves.

Material and Method: The rumen of 6 Holstein calves (age between 1 and 2 months, male) was examined by passing the fiberscope through the oral cavity and oesophagus and through a permanent fistula, placed in the left paralumbar fossa, respectively. Depending on the access, ruminoscopy was performed with a flexible endoscope of different length and diameter. The endoscopic visible anatomical structures of the rumen were described. Additionally the impression of the left kidney and the spleen were examined by percutaneous sonographic examination. Further on the act of drinking, feeding by nipple drinker and installing of a stomach tube were documented.

Results: Following anatomical structures could be viewed by passing the flexible endoscope through the permanent fistula: dorsal and ventral sac of rumen with the caudal and cranial pillar of rumen; the impression of spleen and left kidney, the ruminoreticular opening and the reticular groove. The mucous membrane of all these structures was evaluated. During drinking an immense enlargement of the abomasums could be visualized from the inside of the rumen. The sonographic examination of the left kidney and spleen determined the endoscopic findings. Passing the fiberscope through the oral cavity just the dorsal and little parts of the ventral sac of rumen could be visualised.

Summary and conclusions: Endoscopic examinations represent non invasive or minimally invasive examination methods suitable for application in cattle. It allows for direct visualisation and thus real representation of visible surfaces. Basic knowledge of anatomy and of the normal endoscopic appearance is a necessity for successful use of endoscopy.

In summary, the diverse possibilities endoscopy offers in cattle represent an important advance in large animal medicine in regard to diagnosis, prognosis, therapy as well as education.

166 (5056)
THE USE OF INTRA-OPERATIVE ULTRASOUND IN EVALUATION OF DEEP ABDOMINAL STRUCTURES IN CATTLE
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Transcutaneous and transrectal sonographic examination have been standard procedures in large animal practice for many years. While these techniques have been developed and refined to optimize their use, detailed image acquisition from structures lying deep within the abdominal cavity remains difficult and sometimes impossible to attain.

Direct application of an ultrasound probe to the organ in question without an offset, facilitates rapid examination in all three planes (dimensions) and provides optimal image quality. The option of the colour doppler modality further facilitates detailed images and diagnostic procedures. Direct application of an ultrasound probe can be achieved by laparoscopic or direct intra-operative methods and provides detailed information with respect to small, defined areas in contrast to the transcutaneous method which provides images representing larger organ dimensions.

Intra-operative ultrasonography (IOUS) has been performed in diagnostic and therapeutic procedures for liver, kidney, pancreas, spleen, abomasum, reticulum, rumen and small intestine. Results of these studies demonstrate an improvement in imaging for all organs. Images presented demonstrate the following ultrasound characteristics of IOUS obtained from standing, mature cattle (results of kidney and liver have previously been presented, WBC Hannover, 2002).

Pancreas: examination through a right flank approach, high quality images were obtained without difficulty from all animals examined. Good quality transcutaneous images were difficult to obtain from fat cows and in those cases when intestinal structures were located between the abdominal wall and the pancreas.

Spleen, Rumen and Reticulum: the spleen was readily examined both intra-operatively and transcutaneously. Intra-operative examination of the rumen and reticulum were facilitated by using the spleen as an “off-set pad” to better visualize their wall features.

Abomasum: High quality images are readily obtained from the abomasum using a left flank approach without the necessity of “off-set” techniques. The addition of large volumes of physiological saline to the abdominal cavity further improves the diagnostic image quality.

Comparison of IOUS techniques with examination of post-mortem specimens in a water bath environment confirms the accuracy and reliability of the intra-operative methodology.

While these techniques are applicable to virtually all cows, we are currently recommending them for individual valuable animals which require further workup to improve diagnostic, therapeutic and prognostic information. For such animals these procedures are informative, readily performed and may help to reduce extensive, time consuming laboratory investigations.

167 (1444)
CLINICAL USE OF MODIFIED DORSOLUMBAR EPIDURAL ANESTHESIA IN CATTLE
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STRATEGIES TO MINIMIZE PAIN RESPONSE FOLLOWING DEHORNING IN DAIRY CALVES

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Three experiments were designed to assess ketoprofen administration in Holstein dairy calves prior to dehorning. In experiment A, heifer and bull calves between 2 days and two weeks of age were dehorned with a butane dehorner. Calves were randomly allocated to receive a lidocaine cornual nerve block and either an intramuscular injection of saline (placebo) or an intramuscular injection of ketoprofen (treatment). In experiment B, heifer calves between 4 and 8 weeks of age were randomly assigned to the same placebo and treatment allocations as in experiment A, but were dehorned with an electric Rhinehart dehorning device. In experiment C, heifer calves between 2 days and 2 weeks of age were randomly allocated to receive either a ketoprofen intramuscular injection only or a lidocaine cornual nerve block only. All injections and nerve blocks were administered at least 10 minutes prior to dehorning.

Calf behavior was video-recorded between 0-2, 3-5 and 6-8 hrs post-dehorning. The videotape observer was blinded to treatment allocation. Scan sampling methodology was used to record the frequency of ear flicks, head shakes and head rubs. The frequency of lying, standing, feeding and self-grooming were also recorded. Statistical analysis was conducted with non-parametric Mann Whitney tests and analysis of variance where appropriate.

Experiment A results indicate that a difference in cortisol concentrations from the time of dehorning until 3 hours later was significantly lower (P < 0.05) in the ketoprofen-treated group. All behavioral responses in this experiment were infrequent and there were no behavioral differences noted between treatment groups. Interim analysis from experiment B indicates a reduction in the frequency of ear flicks (placebo 48.4 ± 8.3 vs treatment 27.4 ± 8.3, P < 0.05) during the 6 hours following dehorning. Behavioural responses in the older calves dehorned with a larger dehorning device were considerably more frequent than that observed in younger calves. Data analysis for experiments B and C are in progress.

Our work to date suggests that practitioners should encourage dairymen to dehorn calves at a young age (2 days to 2 weeks) to minimize the behavioral response to dehorning. Additional treatment with ketoprofen at the time of dehorning may be beneficial in alleviating pain response following dehorning.

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169 (3212)


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Acute abdominal pain is common in calves. Although the gastrointestinal tract is frequently involved, other systems may be affected. A thorough physical examination is essential to determine the cause and to decide if the animal will be treated medically or surgically. The aim of this retrospective study was to describe the conditions causing abdominal pain in calves, to find relevant parameters indicating if surgery is required or not, and finally to establish a prognosis based on clinical laboratory findings.

Medical records from cattle under 6 months old admitted to the Centre Hospitalier Universitaire Vétérinaire of the Faculté de Montréal between 1992 and 2002 were reviewed. Cases selection was based on the final diagnosis made by the attending clinician. This diagnosis had to be related to a known cause of acute abdomen in calves. Animals with a diagnosis of enteritis only were excluded from the study.

Results from physical examination, chemistry profile, complete blood count, and blood gas analysis were recorded. Follow-up information was obtained by phone conversation with the owner. Short term prognosis was established based on discharge or not from the hospital. Long term prognosis was based on actual production state of the animal.

The gastrointestinal tract was involved in 95% of the cases. Small intestine conditions were at the origin of the colics in 43% of the animals. Atresia coli was the most common small intestine pathology. The abomasum was involved in 31%, the rumen in 16% and the cæcum in 7% of the animals. Two calves were presented for simultaneous abdominal pain and severe respiratory distress consisted with diaphragmatic hernias. Two animals had abdominal pain of unknown origin and one suffered of a primary diffuse peritonitis. Urinary tract was involved in 6 calves (4.5%).

Dehydration was frequently observed upon arrival but was not found to be a good prognosis indicator. Absence of feces was determining in the decision of going in surgery. None of laboratory parameters was significant as a prognosis indicator. There was a tendency for toxic neutrophils to be associated with a poor outcome.

The digestive tract is involved in most of colics in calves requiring surgical intervention except for condition affecting the rumen. Sixty-three % of the treated calves were discharged from the hospital. Atresia coli was the most common pathology in calves less than 8 days with a prognosis of 49% if the animal recovered from the surgery.

170 (1255)
COMPARISON OF TWO TECHNIQUES FOR LAPAROSCOPIC ABOMASOPEXY FOR CORRECTION OF LEFT DISPLACED ABOMASUM IN DAIRY COWS.
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Laparoscopic abomasopexy (LA) has been described to reduce the incidence of complications associated with traditional laparotomy and the blind toggle corrective techniques for left displaced abomasum in cattle. This surgical technique involved left paralumbar fossa laparoscopy in standing cattle for placement of the abomasopexy suture followed by right paramedian laparoscopy for suture retrieval. We hypothesized that LA could be performed successfully in cattle through a right paramedian laparoscopy alone. The purpose of this study was to compare the “two-step” procedure to a “one-step” procedure for LA. The two-step LA consisted of aseptic preparation of the left paralumbar fossa. Two laparoscopic portals were created. The laparoscope was inserted in one portal, used to identify the abomasum, and direct insertion of the trocar and cannula into the lumen along the greater curvature. A steel toggle pin was inserted via the cannula into the lumen and the abomasum deflated. The incisions were closed with a simple interrupted suture. Then the cow was placed in dorsal recumbency. After aseptic preparation of the ventral abdomen, two additional portals were made in the right paramedian area. The abomasum and suture material were visualized and a grasping forceps was used to retrieve the suture. The excess suture material was withdrawn up to a preset marker on the suture, positioning the abomasum adjacent to the body wall and the suture tied. The incisions were closed with a simple interrupted suture, and the cow returned to a standing posture. The one-step procedure was done similarly to the ventral approach of the two-step method. The laparoscope was used to guide the trocar and cannula into the abomasal lumen. The toggle suture was passed through the cannula into the lumen and the excess suture material kept exteriorized. The abomasum was deflated and the excess suture material was withdrawn. Six cows had LA (3 using the two-step, 3 using the one-step). The two-step procedure required 45 minutes surgical time compared with 20 minutes for the one-step procedure. No post-operative surgical complications were observed. The one-step technique was a simpler and more efficient technique of LA.

171 (3433)
POST SURGICAL CONVALESCENCE AFTER LAPAROSCOPIC ABOMASOPEXY OR RIGHT FLANK LAPAROTOMY AND OMENTOPEXY IN DAIRY COWS WITH ABOMASAL DISPLACEMENT
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In German HF dairy cows with left abomasal displacement post surgical convalescence and short term survival rate after laparoscopic abomasopexy (LSAP; N = 35, according to JANOWITZ) or laparotomy and right flank omentopexy (OPX; N = 35, according to DIRKSEN) were compared. Cows (mean body weight 564 kg; mean age 4.8 years; mean time post partum 18 days) were randomly assigned to either LSAP or OPX. Over a period of 6 days post surgery on daily basis cows were clinically examined and blood samples and
DEHORNING WITH ANALGESIA ON THE LEVELS OF CORTISOL AFTER HOT-IRON, CHEMICAL OR SCOOP DEHORNING

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The purpose of the study was to evaluate the effect of local anaesthesia and local anaesthesia combined with analgesia, on cortisol levels after hot-iron, chemical (NaOH) or scoop dehorning of calves. Animals were assigned to three groups (G):

G1 (hot iron): 4 calves only dehorned (C1); 3 dehorned with local anaesthesia (A1); 4 local anaesthesia + analgesia (AA1).
G2 (NaOH): 3 only dehorned (C2); 3 calves dehorned with local anaesthesia (A2); 3 local anaesthesia + analgesia (AA2).
G3 (scoop): 5 only dehorned (C3); 5 calves dehorned with local anaesthesia (A3); 5 local anaesthesia + analgesia (AA3)

Local anaesthesia (5 ml Lidocaine 2%) was injected in the supra-orbital fossa and the analgesic (5 ml Flunixin meglumine) was administered intravenously 5 minutes prior to dehorming. Blood cortisol was measured by radioimmunoassay before dehorming (-5 minutes) and 1,3,6 and 24 hours after dehorming.

Results: The A1 and AA1 sub-groups showed very small rise in mean cortisol levels compared with baseline.C1 had a slightly larger increase. There was an increase in cortisol levels in the A2 sub-group 1 hour after dehorming but it was smaller and less persistent than in C2. Animals of the AA2 sub-group didn’t show any increase in cortisol levels compared to baseline values. The sub-group A3 showed a comparable increase to C3 but later in time (3 to 6 hours in A3 versus 1 hour in C3). The increased levels of blood cortisol in sub-group A3 persisted for 24 hours(similar to C3).The sub-group AA3 also showed a noticeable increase during the 24 hours but less significant than in the other sub-groups. In group 3 the differences between C3
and the other two sub-groups were statistically significant.

Conclusions: The cortisol levels were much higher in all animals of G 3. Although the anaesthesia and analgesia seem to offer some protection it is not complete or long lasting. Anaesthesia in this group only delays the effect and so may be considered useless. In G1 and G2, the animals with only local anaesthesia showed evidence of pain 3 to 6 hours after dehorning which seems acceptable as this is the duration of the effect of the drug. In all groups only the use of local anaesthesia combined with analgesia prevented increases in cortisol levels. In terms of welfare the study seems to indicate that scoop dehorning is unacceptable and that local anaesthesia only delays the pain caused by dehorning. The use of local anaesthesia and analgesia seems advisable

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174 (2024)
RISK FACTORS FOR CLINICAL MASTITIS AND FOR NEW INTRAMAMMARY INFECTION IN DAIRY HEIFERS AROUND CALVING
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The objective of the study was to identify the risk factors for clinical mastitis and for new subclinical intramammary infection in dairy heifers around calving. During a year, 855 heifers from 57 French dairy herds were included. Only clinical mastitis occurring from 3 weeks before to 4 weeks after calving were considered. New intramammary infections were determined based on bacterial examination of 2 consecutive quarter milk samples, implemented 2 days before and 15 days after calving. A multivariable logistic regression was performed. 16 and 8 risk factors were identified for clinical mastitis and for new intramammary infection respectively. They were related to cow individual traits (rear quarters, winter calving, housing with mature cows before calving, udder dirtiness, dystocia and stillbirth, udder oedema, high milk production level, low concentrate and low milk urea in early lactation...) and to herd context and management (high incidence rate of clinical mastitis, small sized lying area, frequency of straw distribution, overmilking, ...). A sizeable reduction of clinical and subclinical mastitis problems on primiparous cows at calving can be expected from a control of heifer specific risk factors and from a herd control plan against mastitis.

175 (3299)
DEVELOPMENT OF AN EPIDEMIOLOGIC MODEL OF SUBCLINICAL MASTITIS TRANSMISSION FOR ESTIMATION OF THE IMPACT OF MASTITIS TREATMENT STRATEGIES
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The objective of this research is to develop a deterministic state-transition model of intramammary infections (IMI) to describe mastitis transmission dynamics in populations of lactating dairy cattle. We present a set of differential equations that build upon previous epidemiologic models (Lam et al. 1996 J Dairy Sci 79:62, Allore et al. 1999 Prev Vet Med 39:279, Zadoks et al. 2002 Epidemiol Infect 129:397) by including four states: uninfected susceptible (S), acute subclinically infected (IA), chronic subclinically infected (IC), and recovered susceptible (RS). Variables in the model include proportion of individuals within each state, and initial values for these variables are estimated from peer-reviewed literature and field observations. Fixed parameter estimates in the model include values for proportions of individuals entering and exiting the lactating population in each state, spontaneous recovery rates from each of the infected states, and cure rates due to antimicrobial treatment of infected individuals. Fixed parameter values are estimated using data obtained from ongoing field studies and from peer-reviewed publications. The model is fitted to the data using the computer software package Berkeley Madonna (Version 8.0.1, Macey & Oster, University of California, Berkeley, CA, 2000). Steady state conditions for the proportion of individuals in infected and uninfected states, and pathogen transmission rate parameters are estimated from the model. The model is used to explore the effect of varying the cure rate associated with antimicrobial treatment on output values describing the transmission rate for contagious mastitis pathogens, and the prevalence of infected and uninfected individuals. The model predicts that reductions in duration of subclinical infections caused by contagious pathogens results in a decrease in the prevalence and incidence of new acute IMI among susceptible individuals. Thus the model demonstrates indirect effects of mastitis control interventions that result from changes in the intensity of pathogen transmission, and our work highlights the potential importance of mathematical models in evaluating, comparing, and optimizing mastitis control strategies. In addition, limitations in understanding of mastitis epidemiology are highlighted by the development of these models. Further research to validate the model using data obtained from observational field trials is recommended.

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176 (5049)
USE OF VETERINARY DRUGS IN ORGANIC AND CONVENTIONAL DAIRY HERDS IN DENMARK WITH EMPHASIS ON MASTITIS TREATMENT
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The use of veterinary drugs in Denmark in conventional and organic dairy herds and herds converting to organic farming was studied in a longitudinal study. Twenty conventional herds, 18 organic herds converted before 1995 and 19 herds converting to organic farming in 1999 or 2000 were included in the study. The average number of antibiotic treatment for a mastitis case was 3.0 treatments (95% confidence interval: 2.8-3.2 treatments) in the conventional herds with access to follow-up treatments by the farmers and 1.5 (1.3-1.7) treatments in the organic herds and conventional herds without access to follow-up treatments by the farmer. In herds converting to organic farming the change to fewer days of treatment was seen immediately after conversion. Consequently, the length of mastitis treatments in Danish dairy herds seems to be strongly related to the cost of the follow-up treatments.

The most commonly used antibiotics were narrow-spectrum penicillins and broad-spectrum penicillins for systemic treatment. From 1998 to 2000 the most commonly used preparations for intramammary treatment were ampicillin-cloxacin and streptomycin-penicillin combinations. However, a marked change to intramammary preparations with cephalosporins was seen from 2000 to 2002 in all herd groups. The choice of antimicrobials seems to be related to the withdrawal time of the intramammary preparations, especially in the organic herds where the organic standards requires a withdrawal time three times longer compared to conventional herds. Alternative medicine was only used on a regular basis in five organic herds. No statistically significant differences in udder health indicators were seen between the organic and the conventional herds.

177 (2993)
CHRONIC MASTITIS-AFFECTED COWS DISPLAY LOWER LIPOXIN LEVELS THAN ACUTE MASTITIS-AFFECTED COWS
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Persistent accumulation of inflammatory cells in the udder, with neutrophil being the predominant cell type, is a characteristic feature of chronic mastitis in dairy cows. Leukotriene (LT) B4 is a potent chemotactic agent, known to induce recruitment and accumulation of neutrophils in the bovine mammary gland. The LTB4-stimulated neutrophil functional responses are closely opposed by lipoxin (LX) A4, which represent a unique class of lipid mediators with potent anti-inflammatory actions, like inhibition of neutrophil recruitment by attenuating their chemotaxis, adhesion, and transmigration across vascular and endothelial cells. We thus hypothesized that the chronic inflammation of the udder could be associated with an unfavourable ratio between these two eicosanoids and that the persistence of neutrophil accumulation could be due to an increase in LTB4 synthesis and/or an impaired LXA4 production. In an attempt to verify this hypothesis, we first measured LXA4, LTB4, and their ratio in the milk of healthy and acute and chronic mastitis-affected quarters. Next, we studied the relationships between these variables and the degree of udder inflammation as assessed by somatic cell count (SCC) measurement. The LTB4 concentration was low in healthy quarters, drastically increased in acute mastitis, and reached intermediate levels in chronic mastitis-affected quarters. However, whereas LXA4 concentration highly increased in acute mastitis, healthy and chronic quarters had similarly low values. The LXA4:LTB4 ratio was thus significantly lower in chronic mastitis-affected cows. The LTB4 concentrations measured in chronic quarters highly correlated to SCC and to milk neutrophil and macrophage numbers. A weaker correlation was observed between LXA4 and these variables. For both eicosanoids, the highest correlation was observed with the number of neutrophils. These results show the existence of an LXA4:LTB4 imbalance in chronic mastitis-affected cows because of low LXA4 concentrations. This imbalance could be implicated in the pathogenesis of the chronic bovine mastitis and might explain, at least in part, the persistent accumulation of neutrophils in milk from chronic mastitis-affected cows. Further studies are needed to determine whether administration of LX or stable analogs could have therapeutic interests in readjustment of the inflammatory imbalance occurring in the chronic inflammation of the bovine mammary gland.

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178 (5020)
THE PREVENTION OF NEW INTRAMAMMARY INFECTIONS DURING THE DRY PERIOD WHEN USING AN INTERNAL TEAT SEALANT IN CONJUNCTION WITH A DRY COW ANTIBIOTIC.
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The objective of this study was to assess the efficacy of an internal teat sealant to incrementally improve the preventative properties of dry cow antibiotic in commercial dairy herds in North America. New intramammary
Infections (IMI) during the dry period, clinical mastitis during the first 100 days in milk, and somatic cell count (SCC) in both late lactation and fresh cows were evaluated in cows recruited to one of two treatment groups at dry off. The treatments were either a commercially approved dry cow intramammary infusion (Quartermaster®; Pfizer Animal Health) in all four quarters of each cow alone or an internal teat sealant (Orbeseal®, Pfizer Animal Health) in addition to the dry cow infusion in all four quarters of each cow. A total of 608 cows with four functional quarters in 3 commercial herds in Wisconsin, initially milking between 265 and 1300 cows, were enrolled. At dry off, quarter samples were taken for bacteriology and cows were randomly assigned to receive either treatment. Quarter samples were taken within 3 days of calving for bacteriology. SCC was evaluated using routine monthly DHIA testing procedures. Clinical mastitis was recorded through 100 DIM. The outcomes were analyzed across herds and within herds using mixed model procedures, with the across herd analysis using treatment by herd (2 degrees of freedom) as the error term for testing treatment effects. The mean new IMI rate for all herds was lower for the Orbeseal® group at 8% than for the control group at 16.7% (P=0.08). The relative IMI rate (and control rate) was 59% (30%) and 62% (20%) in two herds (P <0.01 (n=291) and P<0.01 (n=741) respectively) whereas the third herd relative reduction was 25% (9%) (P=0.12, n=941), suggesting either a treatment by level of challenge or a treatment by herd effect was present. Clinical mastitis rates through 100 DIM were lower in the Orbeseal® treated cows at 23.4% compared to antibiotic alone at 29.9% (P=0.19). There was again marked variation between the herds with relative reductions of 39%, 26% and 11% (P = 0.38, 0.09, and 0.50). SCC did not differ between treatment groups at dry off but there was a 25% reduction in SCC at calving in the Orbeseal® group (P = 0.19). The three contributing herds all had reductions in SCC of 58%, 17% and 8% (P <0.01, 0.22, 0.68).

Factors related to the relative importance of non-lactating period new infections in dairy herds with respect to clinical mastitis will influence the overall benefits achieved through the use of Orbeseal®. However, in the present study, downward trends in new IMI, SCC at calving and clinical mastitis were observed overall, with significant reductions observed in some herds following the addition of Orbeseal® to the dry cow antibiotic. Funding: Pfizer Animal Health

179 (2818)
IMPACT OF EARLY LACTATION SOMATIC CELL COUNT IN DAIRY HEIFERS ON UDDER HEALTH AND PRODUCTION DURING THE FIRST LACTATION
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Every dairy farmer trusts that his recently calved heifers will significantly contribute to his income by producing high quantities of quality milk. All too often, however, it is realized that this goal is not reached as many heifers calve with intramammary infections (IMI) resulting in an increased number of somatic cells in the milk. This may negatively affect both udder health, either by a permanent elevation of somatic cell counts (SCC) or by an increased sensitivity to new infections, and production during the subsequent lactation. Multilevel linear and logistic regression techniques were used to assess the predictive ability of early lactation SCC [SCCel, measured between 5 and 14 days in milk (DIM)], on monthly test-day SCC and production in the continuing lactation. Data collected during 2000 and 2001 through the Belgian dairy herd improvement program were used for this purpose.

On the average, SCCel decreased progressively from 178,500 cells/ml at 5 DIM towards 74,800 cells/ml at 14 DIM, resulting in an overall geometric mean value of 104,000 cells/ml (n=14,766 heifers). Following the animals until 365 DIM, resulted in 117,496 test-day SCC from 14,243 heifers. The geometric mean value was 74,900 cells/ml, progressively increasing from 55,200 cells between 15 and 45 DIM towards 120,400 cells/ml between 345 and 365 DIM. The average production was 22.7 kg milk per day, progressively decreasing from 23.5 kg between 15 and 45 DIM towards 21.0 kg between 345 and 365 DIM.

The mixed model analysis showed that a high SCCel was significantly associated with an elevated test-day SCC and elevated odds of test-day SCCs exceeding 200,000 cells/ml during the subsequent first lactation. In addition, an elevated SCCel led to a significantly lower milk production during all subsequent measurements. These results highlight the importance of reducing the prevalence of IMI prepartum rather than on cure of existing IMI peripartum. This is because the discussed negative effect of an elevated SCCel was shown to be still present (and significant), although to a lesser extent, in heifers of which the second test-day SCC was lower than 50,000 cells/ml. Our interpretation is that heifers with an early IMI, on the average, remain more sensitive to subsequent subclinical infections.
Introduction: In bovine medicine the teat is an important application field of sonography. At the II. Medical Clinic for Ruminants and Swine (University of Veterinary Medicine, Vienna, Austria), 2-D sonography of the bovine teat in cows is done routinely in patients with disorder of milk flow. By using adequate sonographic equipment it is possible to identify the teat canal, the rosette of Fürstenberg, the teat and gland cistern. Till now 3-D sonography is not frequently used in veterinary medicine. The aim of this study was to demonstrate the utility of a quite easy and user-friendly method of bovine 3-D sonography and to demonstrate sonographically physiological and pathological findings.

Material and method: The teats of clinically healthy cows and of cows with milkflow disorder were examined sonographically (2-D and 3-D). The 2-D sonography of the bovine teats was carried out with the Technos-Esaote machine (Pro-Series LOGIQ 200) with a frequency of 8.5 MHz using a linear array transducer. To visualise the gland cistern the probe was applied directly to the organ. To examine the teat cistern and teat canal the teat was dipped into a water-filled cup. The probe was then coupled to the cupwall. Using 3-D-sonography the same way of scanning was performed. The acquired 2-D data were downloaded to a PC-workstation that digitised each picture to produce a 3-D block of digitised information.

Results: It was possible to achieve a good perspective view of the organ. In all cows the results of 2-D sonography of the teat confirmed the experiences described in various studies. With 3-D sonography it was possible to obtain a section through the whole teat. The volume data were imaged in a multiplanar display that showed the three perpendicular planes through the volume simultaneously. Also in the cows with milkflow disorder sonographic (2-D and 3-D) localisation and demarcation of the spatial extension of teat stenoses was possible.

Conclusion and clinical relevance: In this pilot study 3-D sonography of the mammary gland was performed for the first time. The described method of 3-D sonography offers the chance to produce interesting perspective images of the anatomy of the teat. The examining sonographer can obtain views and oblique planes not possible with the conventional methods. In summary, the authors conclude that 3-D sonography is a new method with promising applications in a variety of fields of veterinary research and practice.

181 (2502)

USE OF ULTRASONOGRAPHY FOR EVALUATION OF BOVINE TEAT LESION

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Some research recommended that ultrasonography might be an alternative to common visual methods as it is non invasive and it is possible to evaluate some morphological structures such as glandular parenchyma, the gland cistern, the teat cistern, the teat canal and their potential alterations or lesions.

1487 teats from 376 lactating cows were included in this study and followed during the entire lactation. 56 teat lesions from 7 herds were scanned using B-mode portable ultrasound equipment (Agro Scann Ecm Angouleme, France), with a 5 - 7,5 MHz linear probe, a 7” screen and a 4 hours continuous operation. The lesions were estimated in affected teats using clinical classifications: 1) teat congenital malformations; 2) teat abnormal connective proliferation; 3) teat metaplasia; 4) teat productive inflammation. Ultrasonography determinations were performed in milking parlors, in cubicles or in captures. Examinations were performed by only one operator and needed, on average, 3 minutes each. Linear probe was directly applied to teats using a gel moisture, silicon spacer or silicon glass water filled. Images were recorded on a pocket PC, then transferred in a data set. Lesions found were: 1) Teat canal stenosis (10); 2) Total and/or partial teat canal mucosal extroversion (10); 3) Teat cistern stenosis (2); 4) Teat canal aplasia (12); 5) Teat canal metaplasia (2); 6) Teat cistern wall polyps (13); 7) Teat cistern calculus or haematoma (7).

Using linear probes of 5 or 5-7,5 MHz., those are probes normally used in veterinary gynecology, practitioners don't have to support additional economic investment, but indeed, they could decrease amortization time of this equipment.

The diagnosis is very precise, reliable and accurate, allowing to establish an anatomic-pathological diagnosis of the lesion, and allow to prognosticate possible surgical approach to treat the lesion. Considering obtained results, we think to assert that teat ultrasonography, must be considered like a routinely used diagnostic instrument and not only like an extraordinary used aid.

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182 (5030)

EFFECTIVENESS OF AN INTERNAL TEAT SEALANT IN THE PREVENTION OF NEW INTRAMAMMARY INFECTIONS DURING THE DRY AND EARLY LACTATION PERIODS IN DAIRY COWS WHEN USED WITH AN INTRAMAMMARY ANTIBIOTIC

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Despite the success of blanket dry-cow antibiotic therapy, many new intramammary infections (IMI) still occur during the dry period due to factors such as delays in keratin plug formation, poor sensitivity of some invading pathogens to antibiotic preparations, and decreases in antibiotic concentrations near the end of the dry period. The objectives of this study were to describe the effect of treating quarters with an internal teat sealant...
in addition to an antibiotic (treated) on the risk for developing a new IMI between dry-off and calving, the risk for a clinical mastitis event between dry-off and 60 days in milk (DIM), and somatic cell count (SCC) measures after calving, as compared to quarters treated with antibiotic alone at dry-off (control).

The study enrolled 437 cows from two large dairy herds in western Wisconsin, with four functional quarters and no evidence of clinical mastitis at dry-off. On the day of dry-off all four quarters were sampled for bacteriological culture and SCC measures. After the final milking all four quarters were routinely infused with a commercially available long-acting antibiotic (Orbenin-DC®; Cloxacillin (benzathine), 500 mg)(Schering-Plough Corp., Kenilworth, NJ). Two contra-lateral quarters were then randomly assigned to be infused with an internal teat sealant (OrbeSealä, Pfizer Animal Health, Groton, CT). The teat sealant was stripped out at first milking after calving and all quarters re-sampled at both 1-3 DIM and 6-8 DIM for bacteriological culture and SCC analysis.

The treatment group infused with an internal teat sealant plus antibiotic had significantly fewer quarters that acquired a new IMI between dry-off and 1-3 DIM (treat = 20.2%, control = 25.4%), and significantly fewer quarters affected by clinical mastitis between dry-off and 60 DIM (treat = 5.9%, control = 8.0%) (P < 0.05).

Multivariable analysis showed that treated quarters were 30 % less likely to develop a new IMI between dry-off and 1-3 DIM and 33% less likely to experience a clinical mastitis event between dry-off and 60 DIM. Finally, treatment was associated with a significant reduction in SCC after calving. Mean SCC linear scores for control vs. treated quarters were 5.4 vs. 5.1 at 1-3 DIM, and 3.1 vs. 2.8 at 6-8 DIM (P < 0.05).

Use of an internal teat sealant as an adjunct to antibiotic therapy at dry-off offers the dairy industry a new management tool to prevent new intramammary infections during the dry period.

Funding: Pfizer Animal Health

183 (2412)
FIELD EVALUATION OF THE EFFICACY OF MARBOFLOXACIN IN THE TREATMENT OF ACUTE MASTITIS DUE TO GRAM-NEGATIVE BACTERIA IN THE DAIRY COW
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Acute E. coli mastitis is a major concern in lactating cows. Treatment is an emergency situation as the resulting endotoxic shock is life threatening. However, the use of antibiotics via the parenteral route is controversial. The aim of this study was to evaluate the efficacy of a broad-spectrum antibiotic, marbofloxacin, in the treatment of acute coliform mastitis through intramuscular injections.

In this international, randomised field trial, two groups of lactating cows presenting with acute clinical coliform mastitis in one or several quarters were compared. The first group (n=33) received a daily intramuscular injection of the control product (amoxicillin-clavulanic acid, Synulox® suspension, Pfizer AH) for 3 days at the dosage of 5 ml/100 kg (i.e. 7 mg of amoxicillin and 1.75 mg of clavulanic acid) whilst the second group (n=29) received a daily intramuscular injection of the test product (marbofloxacin, Marbocyl® 10%, Vétoguinal SA) for 3 days at the dosage of 1 ml/50 kg (i.e. 2 mg/kg). The associated treatments allowed were a calcium solution, a hypertonic glucose solution and an intra-mammary infusion based on cloxacillin (Orbenin®, Q.A., Pfizer AH), which is only effective on Gram-positive bacteria. The two groups had homogenous baseline data. The following parameters were evaluated on D0, D1, D2, D3, D7 and D14: rectal temperature, general condition, appetite, milk production, milk appearance and modification of the quarter. Milk samples were taken for bacteriological analyses at D0, D7, D14 and in cases of failure. The efficacy results were significantly better in the marbofloxacin group for the main criteria, i.e. the bacteriological cure rate (91.7% versus 62.5% respectively) and the overall cure rate (clinical + bacteriological cure) (61.5% versus 28.1% respectively) (p<0.05, Fisher's exact test). This was confirmed by the evolution of the clinical parameters which improved faster in the marbofloxacin treated animals (p=0.004 for the appetite and p=0.007 for the general condition). The tolerance at the injection site was also significantly better with marbofloxacin. As a conclusion, this study demonstrated the efficacy and safety of marbofloxacin in the treatment of acute clinical coliform mastitis. It also confirmed the effectiveness of using parenteral antibiotics in the treatment of acute E. coli mastitis.

184 (1326)
EFFECTIVENESS OF CONTROL PLANS FOR HIGH-SCC-LEVELS BASED ON THE IDENTIFICATION OF DEFECTIVE CONTROL POINTS IN DAIRY HERDS
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High somatic cell counts in bulk-tank milk generate economic losses for dairy producers and control plans are therefore implemented or modified. Effectiveness of such plans was not so frequently assessed using objective criteria. Therefore, the study aimed at (1) describing the evolution of somatic cell counts (SCC) before and after implementation of these kind of plans on objective criteria (prevalence, incidence and persistency) and at (2) relating this evolution with the observance of the recommended corrective actions.

A sample of 187 problem-farms was considered in Burgundy (central area of France). On-farm intervention was conducted by an extensionist together with a veterinarian, applying a systematic procedure in order to identify possible defective control points and contained also a series of recommendations for improving bulk-tank milk somatic cell count (BTSCC). From the period [3 months before the diagnosis phase] to the period [18 to 24 months after the diagnosis phase], median SCC decreased from 390,000 to 300,000 cells/ml.
TREATMENT OF SUBCLINICAL MASTITIS IN LACTATING COWS WITH PIRLIMYCIN HYDROCHLORIDE: FACTORS AFFECTING BACTERIOLOGICAL CURE AND SCC REDUCTION

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This study investigated the associations of bacteriological cure (Cure) and post-treatment quarter somatic cell count (SCC) with treatment duration, pre-treatment SCC and bacteriology results, and cow-side characteristics.

For the purpose of this paper data from two treatment groups in each of two multilocation studies were selected. These two studies were conducted in Member States of the European Union (EU) to evaluate the efficacy of daily intramammary infusions with 50 mg pirlimycin hydrochloride for the treatment of subclinical mastitis. Data from Study 1 allowed for comparison of a group of cows which received pirlimycin for 2 days (Pirli 2X) to a group which received no treatment (Untreated) whereas Study 2 provided data for comparison of Pirli 2X with pirlimycin for 8 days (Pirli 8X). Quarters from cows with a high monthly composite milk SCC were tested for positive bacteriology and an SCC > 300,000. If infected, the cow was enrolled and randomly allocated to treatment. Enrolled cows were monitored for clinical mastitis and other disease for 30 d after treatment initiation. At three and four weeks after treatment initiation, milk samples were taken from each enrolled quarter to determine the SCC and conduct a bacteriological culture. Bacteriological culture results were interpreted such that quarters where a same bacterial species was cultured before treatment and in at least one of the two post-treatment samples were considered a failure. The analysis of SCC used a mixed linear model (SAS proc mixed) while the analysis of bacteriological cure used a mixed logistic model (SAS glimmix macro).

Cure rate was significantly lower for higher parity, higher number of colonies in the pre-treatment culture, and it was significantly higher with longer treatment duration and for streptococci when compared to Staph. aureus. Cure rate of coagulase-negative staphylococci did not increase with longer treatment. Post-treatment SCC remained significantly higher with increasing parity, in rear quarters when compared to front quarters, and with shorter duration of treatment. The interaction between quarter (rear versus front) and parity was also significantly associated with post-treatment SCC, because in 2nd and 3rd parity animals post-treatment SCC was more reduced in front quarters than in rear quarters.

In conclusion, risk factors for Cure and reduced post-treatment SCC can be of use to help predict success rate for treatment of subclinical mastitis during lactation.
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A dry period internal teat sealer containing bismuth subnitrate has recently become commercially available in many countries. This paper describes the effect of the sealer on somatic cell counts (SCC) in the next lactation compared to cows that received antibiotic dry cow therapy (DCT).

Cows on 16 dairy farms in southwest England were selected for inclusion in a prospective DCT study. Animals randomly received either an internal teat sealer (OrbeSeal, Pfizer Animal Health) or an antibiotic DCT containing 250mg cephalonium (Cepravin Dry Cow, Schering-Plough Ltd, UK). Enrolment criteria specified were all routine cow level SCC = 200,000 cells/ml and no cases of clinical mastitis during the preceding lactation. All cows were individually sampled for cow level SCC analysis on a regular basis before and after the study dry period.

Data from 401 enrolled animals (198 in the teat sealer group and 203 in the antibiotic DCT group) were available for analysis. There was no significant difference between the treatment groups in last recorded yield, dry period length, or SCC in the last, second to last and third to last months before drying off. Animals that received the teat sealer were significantly older than those that received antibiotic DCT.

The geometric mean SCC of the first recording after calving was significantly higher in the group that received teat sealer compared to the group that received antibiotic (39,600 cells/ml cf. 30,400 cells/ml, P < 0.05) using both univariate and multivariate analysis. However, the second (32,300 vs 30,200) and third (32,600 vs 30,000) recording after calving and the geometric mean of the first three recordings were not significantly different between the groups.

These results indicate that animals that receive an internal sealer have a small (~9,000 cells/ml) but significantly higher SCC at the first recording after calving compared to animals that receive antibiotic DCT. The difference was not apparent from the second recording after calving onwards.

Previous work has demonstrated that the dry period cure rate for Corynebacterium bovis is significantly higher in animals that received antibiotic DCT compared to the teat sealer (99.5% cf 67.0%). At calving the number of quarters infected with C. bovis was higher in the teat sealer group. Infection with C bovis is associated with a minor elevation in quarter SCC and is a possible explanation for the small SCC differences seen between the groups in this study.

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188 (3076)

ACUTE PHASE PROTEINS AND THEIR POTENTIAL AS MARKERS OF MASTITIS IN DAIRY COWS

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Rapid and accurate diagnosis of mastitis is crucial, as it improves treatment effect, reduces discarding of milk, and minimizes recovery time - thereby reducing production losses and increasing animal welfare. There is thus continuously a need for improvement of mastitis detection through identification of new objective and reliable markers of mastitis. The acute phase proteins (APPs) are thus potentially useful markers of mastitis. The diagnostic potential of APPs such as haptoglobin (Hp) and serum amyloid A (SAA) rely on their low levels in healthy cows, their rapid and exponential elevation in acute phase states, and their rapidly decreasing concentrations with resolution of disease.

To further characterize SAA and Hp responses during mastitis, Hp and SAA concentrations were determined in serum and milk samples obtained from cows with experimentally induced E.coli mastitis, and from field cases of mastitis caused by different mastitis pathogens, cows with extramammary inflammatory conditions and healthy cows. Isoforms of SAA were determined by isoelectric focusing.

Cows with mastitis had elevated APP concentrations in both serum and milk, whereas cows with extramammary inflammatory conditions had increased levels of APPs in serum only. APP levels in healthy controls were low or below detection limit. Cows with severe mastitis seemed to have higher serum and milk APP concentrations than cows with moderate or mild mastitis. In cows with experimentally induced E.coli mastitis milk SAA levels increased within 6 to 12 hours of inoculation and returned quickly towards baseline levels after bacterial clearance. Isoelectric focusing showed that a particular isoform of SAA, which differed from known serum isoforms, was present in mastitic milk.

In conclusion, SAA and Hp possess several characteristics that make them suitable as indicators of mastitis: 1) milk levels in healthy cows are negligible, 2) milk levels are unaffected by extramammary inflammation 3) milk and serum levels seem to reflect severity of disease and they may therefore serve as indicators of prognosis and expected production loss, 4) SAA is synthesised within few hours of the udder being infected and is thus an early markers of mastitis, and 5) SAA milk and serum levels closely parallel disease activity, and may thus be used as indicators of treatment efficiency.

Moreover, identification of an udder-specific isoform of SAA further strengthens the diagnostic potential of this APP.

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THE RELATIONSHIP BETWEEN THE PREVALENCE OF SUBCLINICAL KETOSIS AND SUBCLINICAL MASTITIS IN EARLY LACTATION AND RETURN OVER FEED IN ONTARIO DAIRY HERDS

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Subclinical disease is one of the most prevalent and costly conditions in the dairy industry. Furthermore, subclinical mastitis and ketosis have been identified as important diseases to the individual dairy animal. However, to date there are few studies that quantify the effect of subclinical mastitis and ketosis prevalence on herd profitability.

The objectives of this research were to examine the relationship between profitability as measured by Ontario Dairy Herd Improvement (DHI) Return over Feed index (ROF), and subclinical ketosis and mastitis. The California Mastitis Test (CMT) and the KetoTest® Beta-hydroxybutrate (BHBA) milk test were used weekly for the determination of the prevalence of subclinical mastitis and subclinical ketosis in early postpartum cows (1-14 days).

Producers were identified through the DHI Return Over Feed and Management Club groups. The ROF was calculated from the difference between milk revenue and feed cost each month. Feed cost was determined from herd level dry matter intakes at each operation multiplied by fixed market prices for each feed ingredient. Revenue was calculated based on the Dairy Farmers of Ontario multiple component pricing formula for milk. There were 157 producers that submitted ROF information for the period January 1st, 2002 and January 31st, 2003. These producers represented nearly 50% (157/356) of all the ROF participants for the same time period. Of the original 157 producers, 71 initially participated in the postpartum monitoring of cows. Only producers with adequate compliance and participation for the submission of ROF and postpartum monitoring information were included in the study. A final group of 48 herds (2600 cows) were identified as having complete information for analysis.

For the determination of the prevalence of subclinical ketosis and subclinical mastitis as determined by the KetoTest® and CMT a cutoff of greater than or equal to 100 umol/l milk BHBA and greater than zero were chosen respectively. The median prevalence was determined to be 62.8% and 28.0% for subclinical ketosis and subclinical mastitis respectively. Using linear regression modeling (n=48; r²=0.088) it was determined that there was no significant association (p>0.05) of subclinical mastitis prevalence and ROF (intercept=$14.05/cow/day). However, there was a significant reduction (p<0.05, -$0.02/cow/day) in the ROF index with each percent increase in prevalence of subclinical ketosis.

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HOMEOEPATHIC REMEDIES FOR THE TREATMENT OF CLINICAL MASTITIS - A CONTROLLED, BLIND, RANDOMISED CLINICAL STUDY

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The efficacy of a pre-set homoeopathic treatment regime for acute clinical mastitis in dairy cows was established in a positive-controlled, blind, randomised clinical study involving 80 cases in 67 Holstein-Friesian cows. Cows suffering from mastitis with systemic involvement were excluded. Treatment consisted of nasal spray-application of belladonna-bryonia-urtica urens combination remedy (BBU30c), followed by phytolacca 10M at three consecutive milkings. Intra-muscular ampicillin at 4.95 grams once daily for three days was used as reference treatment.

Almost half (39 of 80) of all infections were caused by Streptococcus uberis. Environmental pathogens were responsible for 90% of all cases with isolates in pure culture (54 of 60). No pathogens were isolated in 13 cases. All those isolated from control group samples showed in-vitro sensitivity to ampicillin.

Clinical cure was defined as the absence of any milk abnormalities and a normal quarter. Bacteriological cure was defined as the absence of any growth of the pathogen isolated from the pre-treatment sample. Five cases were lost to the study. After 14 milkings, there was no significant difference in clinical cure (18 of 38 cows (47.4%) vs. 25 of 37 cows (67.6%), p>0.07), but bacteriological cure was significantly lower for the homoeopathy group (18 of 36 cows (50%) vs. 27 of 36 cows (75%), p<0.03; new intra-mammary infections excluded). The odds ratios for clinical cure and bacteriological cure with homoeopathic treatment were 0.43 (95% CI of 0.17 to 1.1) and 0.33 (95% CI of 0.12 to 0.9), respectively. The homoeopathic treatment regime used was significantly less successful than antibiosis in treating Str. uberis infections, but for other pathogens cure rates were similar. Cure rates were also similar at one and two week follow-up examinations. No deterioration was evident in cows treated with homoeopathy based on clinical examination, including udder score.

The number of cows with an Individual Cow Somatic Cell Count above 200,000 cells/ml at the earliest available herd test recording was the same (14 of 31 vs. 15 of 32 cows in treatment vs. control group; p>0.8), as was the individual cow linear score (mean 3.84 vs. 4.16, p>0.59). Four quarters had a recurrent clinical episode in the homoeopathy group vs. seven in the control group.

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Efficacy of an Internal Teat Sealant, OrbeSeal*, in the Prevention of New Intramammary Infections During the Dry Period

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The primary objective of this study was to evaluate the efficacy of an internal teat sealant (TS) infused at dry off to prevent new intramammary infections (IMI) during the dry period, as compared to routine intramammary dry cow antibiotic therapy (DCT). Secondary objectives included examining differences in cure rates of infections that were present before the dry period, as well as clinical cases of mastitis that occurred in the first sixty days of the following lactation, between quarters which were infused with the TS and those infused with the DCT.

Cows (883), from 16 commercial dairy herds in 3 provinces across Canada were utilized for this study. Quarter milk samples were collected aseptically at three points in time: two weeks prior to the dry off date (S1), the day of dry off (S2), and between 1-8 days in milk (S3). The S1 was performed to identify cows with quarters harboring an existing IMI to allow time for randomization to occur on the day of dry off. Cows deemed free of IMI at the S1 culture were placed in Study Part A and were randomized to receive either the internal teat sealant, OrbeSeal* or DryClox® (Ayerst Laboratories, Montreal, Canada) in ipsilateral quarters. Cows with one or more quarters found to be culture positive at S1 were placed in Study Part B and were randomized to receive OrbeSeal* in ipsilateral quarters following infusion of all quarters with DryClox®. All treatments were given after the final milking.

Preliminary results show that quarters treated with OrbeSeal* from cows in Study Part A had a significantly lower percentage of IMI after calving (tx= 10.0%, control= 14.0%). Quarters treated with a combination of OrbeSeal* and DryClox® from cows in Study Part B had a lower percentage of IMI after calving (tx= 20.8%, control= 25.0%). Quarters treated with OrbeSeal* (whether alone or in combination with DryClox®) had significantly fewer clinical mastitis cases up to 60 DIM (tx= 26.7%, control= 73.3%). Multivariate logistic regression analysis, controlling for important confounding and correlated variables, will be performed to evaluate cow and herd level factors that may be associated with the risk of developing IMI during the dry period.

Funding: Pfizer Canada Inc., NSERC

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 Mastitis Prevalence and Incidence in Organic Dairy Farms in Lower Saxony

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Existing investigations on the udder health situation and the array of pathogens encountered in organic dairy farms suggest that udder health in these enterprises is subjected to typical risks, e.g. limited usage of antibiotics and disinfectants along with specific nutritional problems (energy density of the ration, protein deficiency) in combination with the genetically-determined high yielding potential. The papers indicate that particularly the dissemination of cow-associated bacteria is to be expected.

During a dynamic cohort study performed in 12 randomly selected organic farms of Lower Saxony between April 2002 and April 2003, precise data on mastitis frequency and the pathogens involved was collected in the first place. With this base, the typical risks responsible for mastitis development in organic dairy farms were determined. Data from conventionally-managed dairy herds in the same area were used for external reference. Quarter foremilk samples were used, and sampling pattern switched from initially three weekly sessions to consecutive monthly samplings. Initial udder health status of newly acquired animals was established using the cyto-bacteriological results from double samples. Cyto-bacteriological analysis was performed according to DVG/NMC recommendations. Our study showed that no significant differences in mastitis prevalence and incidence between ecological and conventional herds in Lower Saxony existed.

Variation in results however was marked. The percentage of cow-associated pathogens and the mastitis caused by them did not differ significantly between the two enterprise types.

Funding: Government of Lower Saxony

Testing for Staph. Aureus in Low SCC Herds

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In herds with a high Bulk Milk Somatic Cell Count (BMSCC) Staphylococcus aureus can be the cause of chronic udder health problems. Since it is better to prevent than to cure problems, in herds with low BMSCC...
also attention should be given to S. aureus. Two factors are of major influence on the spread of S. aureus in a herd: 1) the number of infected quarters (being the main source of infection) 2) the average number of quarters newly infected by one infected quarter (transmission). Transmission of intramammary infections can be influenced by management. The number of infected quarters at any given point in time can be influenced by treating or culling cows. Before decisions can be made on whether or not to treat a cow, diagnosis of infected quarters is necessary. Udder health monitoring is mainly done with the help of individual SCC. Additionally, samples can be taken for bacteriological culturing (BC). Although for reliable diagnosis, consecutive samples should be taken for BC, usually only single samples are available. Aim of this study is to compare the results of these single samples with those of consecutive samples ('gold standard'), to quantify reliability of single samples for BC.

Single quarter foremilk samples were collected every 5 weeks from the quarters of all lactating cows during a 20-month study in 7 low BMSCC herds. A quarter was considered infected when >500 cfu/ml S. aureus were cultured from 2 of 3 consecutive samples or when >100 cfu/ml were cultured from 3 consecutive samples ('gold standard'). The BC of cows with an individual SCC >200.000 were evaluated. A single quarter sample was considered positive if >100 cfu/ml of S. aureus were found.

Comparing the results of single samples of high SCC cows, with those of consecutive samples, revealed a relatively high sensitivity of 0.92 and specificity of 0.88. The predictive value of a negative test was 0.99. With the generally low prevalence of S. aureus in low BMSCC herds, positive predictive value was low; in these cows in these herds PV+ was 0.42. When interpreting the results it should be realised that the test data evaluated are not fully independent, which has to be corrected for.

The general conclusion is that in low BMSCC herds single samples performed well in detecting uninfected quarters. However, due to low prevalence, it is advisable to take a second sample for BC before decisions are made on culling or treatment of cows identified with S. aureus in a single sample.

194 (1624)
MILK PROTEOLYTIC ACTIVITY AND BOVINE MAMMARY TISSUE DAMAGE DURING ENDOTOXIN-INDUCED MASTITIS
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The protease activity in the mammary gland is recognized as one of the major pathophysiological processes of mammary tissue damage during mastitis. However, the underlying mechanism is not fully understood. The objectives of this study were to investigate: 1) the kinetics of protease activity in milk during udder inflammation and 2) the relation between milk proteolytic activity and their respective mammary tissue damage. Sixteen healthy Holstein dairy cows were used for induction of mastitis with lipopolysaccharides (LPS). Proteolytic activity of mastitic milk and mammary tissue were assessed using, respectively, the zymography and in situ zymography assays before and after LPS challenge. In addition, impact of some protease inhibitors on milk proteolytic activity was investigated. Mastitic milk produced several proteolytic bands on the zymogram, most of which differed from those produced by plasmin. Peak proteolytic activity and mammary tissue damage occurred at 6 hours post challenge. Mastitic milk proteases hydrolyzed, in decreasing order of susceptibility, gelatin, casein, collagen, haemoglobin and mammary gland membrane proteins. This confirms that mastitic milk proteases function as broad spectrum proteolytic enzymes. The hydrolytic activity of mastitic milk was significantly inhibited by protease inhibitors. Unlike with normal milk, co-culturing of slice of normal mammary tissue with “mastitic milk” revealed substantial mammary tissue damage on histopathological examination; this pattern was also confirmed with in situ zymography of mastitic mammary tissue. The zymogram pattern of mastitic milk was similar to that of blood and milk PMN. This strongly suggests that proteases in mastitic milk mainly originate from milk PMN. The acceleration of proteolytic activity in milk might explain the intensity of mammary tissue damage during mastitis.
Funding: Novalait Inc.

195 (2953)
PRELIMINARY RESULTS ON THE EFFECTS OF MELOXICAM (METACAM®) ON HYPERSENSITIVITY IN DAIRY COWS WITH CLINICAL MASTITIS
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Recognition, alleviation and control of pain and stress are central to ensuring good welfare in food producing animals. Over 100 dairy cows with clinical mastitis (mild or moderate) were studied to assess pain associated with clinical mastitis. Mastitis therapy was given according to routine veterinary practice, with intramammary antibiotic drugs. A preparation without corticosteroid was selected, cefquinome (Cephaugard LC Intramammary, Intervet UK Limited, Milton Keynes), this was infused every 12 hours for three treatments for each case of mastitis. Cows with clinical mastitis were allocated randomly to one of 3 groups: Group 1:
antibiotics only; Group 2: antibiotics and one dose of meloxicam (Metacam®, Boehringer Ingelheim GmbH); Group 3: antibiotics and three doses of meloxicam on day of diagnosis, day 0, and on days 3 and 6. Healthy animals were recruited as controls.

All cows were examined clinically on 6-8 occasions over a 45 day period. Response thresholds to mechanical stimuli were measured on each hind limb. General linear model in Mintab Statistical Software (Minitab Inc.) and multi-level modelling in MLwiN were used to consider the time effect and treatment effect.

Treatment had a significant effect on threshold responses, with cows that received antibiotics alone (Group 1) showing greater differences compared to cows that received either one (Group 2) or three (Group 3) doses of meloxicam (P<0.001). There was no significant difference in cows that received one, compared to three doses of meloxicam. There was a significant effect of time on threshold responses for cows with mild cases of mastitis only on day 45 compared to the day of diagnosis (P=0.05); however, there was a significant effect of time on threshold responses in moderate cases between the day of diagnosis and all subsequent examination time points (P<0.05).

In conclusion, mechanical hyperalgesia is present in animals with mastitis of both mild and moderate severity, confirming previous studies, and treatment with one, or three, doses of meloxicam was shown to restore normal threshold responses to mechanical stimulus. These results indicate that meloxicam may be beneficial in analgesic therapy of clinical mastitis in dairy cows.

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196 (2956) ASSESSMENT OF PAIN IN DAIRY COWS WITH CLINICAL MASTITIS
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Masstis is a major disease problem for the dairy industry, causing significant economic losses and adverse cow welfare. Inflammation induces alterations in normal pain information processing, which may have serious consequences for the animal and which may be measured as hyperalgesia: an exaggerated response to noxious stimuli. This study was undertaken to assess the use of a range of clinical and laboratory parameters in assessing pain in dairy cows with mild and moderate clinical mastitis.

Dairy cows were examined clinically and milk samples were collected for bacteriological culture and quality analyses, on the day of diagnosis. The distance between the hocks was measured as a proxy indicator of altered cow stance. Response thresholds to mechanical stimuli were measured on each hind limb using a modification of the method described by Nolan and others (1987). Kruskal-Wallis and one-way ANOVA tests were used to compare parameters from mild and moderate cases of mastitis and normal cows.

Overall, 117 lactating cows with clinical mastitis (n=61 mild; n=56 moderate) and 45 normal cows were studied. The bacteriological results showed that Escherichia coli was isolated from 28%, and Streptococcus uberis from 39% of moderate cases; while in mild cases, E. coli and S. uberis, accounted for 16% and 18% of cases, respectively. The hock-hock distance and mechanical threshold difference were lower in normal cows than in cows with mastitis (both mild and moderate cases) (p<0.001). The heart rates, respiratory rates and rectal temperatures of cows with moderate mastitis were higher (p<0.001) than cows with mild mastitis, and normal animals. The individual quarter somatic cell count (IQSCC) and protein content of the milk of normal animals were lower compared to cows with mastitis (both mild and moderate cases; p<0.001) and the lactose content of milk was higher in normal animals compared to cases with mastitis (both mild and moderate; p<0.001). The results suggest that cows with mild and moderate mastitis exhibit mechanical hyperalgesia, indicating altered pain processing as a consequence of the inflammatory disease. These results indicate that techniques can be used to monitor pain indirectly in cattle with clinical mastitis. Furthermore, the response to analgesic treatments such as the non-steroidal anti-inflammatory drugs, which have known anti-hyperalgescic properties, can be assessed quantitatively.

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197 (2514) MILK AMYLOID A IN THE DIAGNOSIS OF BOVINE SUBCLINICAL MASTITIS
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Acute phase proteins (APPs) are a group of metabolites whose circulating levels increase during the systemic acute phase response which occurs following local inflammation. Raised levels of APPs such as amyloid A, haptoglobin, or fibrinogen, may be used as general indicators of inflammation. Raised levels of amyloid A have previously been shown in milk from quarters with clinical mastitis. The object of the current study was to examine the diagnostic utility of milk amyloid A (MAA) in the detection of bovine mammary quarters with subclinical mastitis. A total of 707 mammary quarters from 180 Holstein-Frisian dairy cows were studied. Full clinical examination was performed on each cow before obtaining milk samples from each mammary quarter,
SUBCLINICAL AND CLINICAL MASTITIS IN HEIFERS FOLLOWING THE USE OF A TEAT SEALANT PRE-CALVING

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This study investigated the prevalence of bacterial intramammary infection (IMI) pre-calving and the effect of treatment with a bismuth subnitrate teat-canal sealant (Teatseal, Pfizer, Auckland, New Zealand) on post-calving IMI prevalence and incidence of clinical mastitis in the first 2 weeks post-calving.

All glands from heifers (n=255) in 5 seasonally calving, pasture-fed dairy herds were randomly assigned to one of 4 treatment groups (control, mammary gland secretion (sample), infusion of Teatseal, or sample and Teatseal). Heifers in each herd were enrolled on one-day, on average 35 days (SD=15) pre-calving. Duplicate milk samples were collected from each gland within 3 days of calving for bacterial culture. Herd-owners collected duplicate milk samples from glands they defined as having clinical mastitis before treatment for bacterial culture. Relative risks (RRmh) adjusted for the confounding effect of herd were calculated for the outcome variables.

IMI was detected in 16.8% of glands pre-calving. There was no difference in prevalence among herds (p=0.65). Bacteria isolated were coagulase-negative Staphylococcus (76.9%), Streptococcus uberis (SU; 14.1%), Staphylococcus aureus (5.1%) and Corynebacterium spp. (3.8%). The prevalence of IMI within 3 days of calving was 11.5% (range across herds 3.0-15.2) of glands. Clinical mastitis within 2 weeks of calving was diagnosed in 5.8% (1.3-9.5) of glands. There was a significant difference in post-calving IMI prevalence (p=0.01) and incidence of clinical mastitis (p=0.001) among herds.

Pre-calving IMI increased the risk of IMI post-calving (RRmh=3.9 (95%CI 2.4-6.4) p=0.000) and the risk of clinical mastitis in that gland (RRmh=3.8 (95%CI 2.0-7.2) p=0.000). Sampling the glands pre-calving had no effect on post-calving IMI (p=0.67) or clinical mastitis (p=0.71). Teatseal did not reduce the prevalence of IMI post-calving (RRmh=0.73 (95%CI 0.52-1.0) p=0.08) or the incidence of clinical mastitis (RRmh=0.69 (95%CI 0.42-1.1) p=0.13). However, the prevalence of SU IMI post-calving (RRmh=0.52 (95%CI 0.28-0.98) p=0.04) and the incidence of SU clinical mastitis (RRmh=0.32 (95%CI 0.14-0.74) p=0.005) were lower in the Teatseal compared to control glands.

It is concluded that IMI pre-calving is positively associated with prevalence of IMI post-calving and incidence of clinical mastitis in heifers. Infusion of Teatseal pre-calving reduced the prevalence of SU IMI post-calving and incidence of SU clinical mastitis.

Funding: Eltham District Vet Services

DRY COW TREATMENT OF HEIFERS EIGHT WEEKS BEFORE CALVING

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Heifer mastitis is a serious and an increasing problem in The Netherlands. About 23% of first calf heifers have a significantly elevated Cow Somatic Cell Count (>150,000 cells/ml) at the first milk recording post-calving. In heifers about 40% of all clinical mastitis in the first lactation is seen in the first month post-calving. Culling rate for mastitis in first calf heifers is 5%. Coagulase negative staphylococci (CNS) are the most common milk sample isolates post-calving but Staphylococcus aureus is also frequently found.

Management measures are important in reducing the prevalence of mastitis in heifers; nevertheless, on some farms the incidence remains high. Good results following treatment of heifers, with dry cow treatment
products, 8 weeks before the expected calving date are described. A trial was conducted on 17 Dutch dairy farms. Eight weeks before the expected calving date 400 heifers were alternatively treated, with 600 mg cloxacillin (Orbenin Extra Dry Cow®, Pfizer) or were left untreated. Milk samples were taken at calving and again 10 days later. During the first lactation all clinical mastitis cases were sampled before treatment. Cow somatic cell counts, production and fertility data were recorded as were the course of calving, disease events and, if applicable, the reason for culling. Treatment of heifers 8 weeks before calving was easily accomplished with good restraint. Immediately post-calving 55% of all quarter-samples were negative in the treated group as against 42% in the untreated controls. In positive samples CNS were the most frequent isolate (30% in the treated and 39% in the untreated group). At 10 days post-calving and without any treatment the percentage of negative quarters had increased in both groups but was still highest in the treated group. The Cow Somatic Cell Count during the first 100 days of lactation was higher in the untreated group. The 305 days production was around 200 liters per cow higher in the treated group. There were no differences in the number of clinical mastitis cases, the culling rate and the fertility data between groups.

Treatment of heifers, 8 weeks before calving, is economically justified on farms with heifer-mastitis problems where control by management measures alone is insufficient.

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200 (1111)
ECONOMICS OF MASTITIS CONTROL: PROFITABILITY OF STRATEGIES TARGETING SUBCLINICALLY INFECTED COWS FOR LACTATIONAL TREATMENT OR CULLING
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Control strategies of subclinical udder infections rely mainly on culling and dry-cow treatment, but their effectiveness can be enhanced by antibiotic treatment of infected lactating cows. The objective of this study was to assess the economic worth of control strategies including lactational treatment and culling of subclinically infected cows, using a simulation model. The ECOMAM/ECOMAST model was developed as dynamic (daily time stepping), mechanistic (individual representation of cows) and stochastic (for occurrence and consequences of infections). Four different types of infections were considered to represent the simultaneous presence of different pathogens in a herd. For each cow, the risk of occurrence of a new infection was modeled according to prevalence of already existing udder infections in the herd, season, age, stage of lactation, production level, and individual cow susceptibility. Consequences of udder infection on milk somatic cell count, milk production and survival were modeled. Nine control strategies were compared. They combined possibly lactational treatment of subclinically infected young cows, strict culling rules applied to cows persistently infected, and improved prevention. A 3-year simulation horizon was considered and 100 replications were run. An annual discount rate of 5% was applied before summing the 3 annual margins of a replication. The compared plans were ranked according to this cumulated gross margin.

The highest improvement of gross margin varied from 2.2 to 6.1 € per 1000 liters of quota per year. Some plans were not profitable. For current French situations, the strategies including lactation treatment of young cows (together with improved prevention) were among the most profitable ones when the udder-health situation of the herd resulted in penalties higher than 1.5% of the price of a liter of milk. Strict culling rules resulted in a rapid improvement of bulk milk somatic cell count but were often not profitable because of non-fulfillment of the quota. The model allowed investigating mechanisms influencing the profitability of control plans (including sensitivity analysis on treatment cost and cure rate). To generalize the results of comparisons, economic implications of country-specific penalties for somatic cell counts and quota system should be considered.

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