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201 (5101)

MASTITIS RESEARCH FOR MAXIMUM INDUSTRY BENEFIT: CANADIAN BOVINE MASTITIS RESEARCH NETWORK

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Bovine mastitis has been researched for decades, but is still the costliest disease of dairy cattle. Researchers and the dairy industry in Canada are forming a mastitis research network to address this problem. The Network is a partnership between scientists and members of the industry, and provides a framework for concerted research collaboration and proactive knowledge and technology transfer. Thirty-four scientists from eight institutions conduct a concerted and collaborative research program under the themes of mastitis prevention, elimination and monitoring.

There is a core research platform comprising a national cohort of dairy farms, a mastitis pathogen strain-bank, a network of diagnostic laboratories, and a biosecurity level 2 facility. By planning and executing research cooperatively, resources are utilized efficiently, trainees benefit from multi-disciplinary experiences, duplication of research is prevented, and relevance to the dairy producers is maintained. The Network promotes a balance of applied and theoretical research including epidemiological strategies for mastitis control, novel technologies for enhancing host resistance, and alternatives to traditional antimicrobial therapy. A key to the Network's success is the successful transfer of knowledge and technology to dairy producers. For knowledge transfer, the Network will disseminate existing and new mastitis control information into existing channels that are normally accessed by dairymen in Canada. The Network will collaborate with knowledge transfer professionals and the National Mastitis Council to articulate mastitis management standards for Canadian dairy farmers and promote implementation of those standards through field based knowledge transfer activities. The Network will provide a framework for dialogue and monitoring of progress and impediments to implementation of control practices.

The Network offers opportunities for international scientific collaboration, the multidisciplinary training of highly qualified personnel, and provides an advanced educational curriculum for domestic and international students.

202 (3207)

COMPARISON OF ANTIMICROBIAL SUSCEPTIBILITY PATTERNS FOR BOVINE STAPHYLOCCUS AUREUS BEFORE AND AFTER DRY COW THERAPY

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Hypothesis: There would be little change in the antimicrobial resistance (AMR) profiles for *S. aureus* isolates before and after antimicrobial treatment (AMT).

Materials and Methods: ~ 300 different isolates of *S. aureus* from 75 dairy herds in Canada were derived from a study of the efficacy of tilmicosin and risk factors for biological cure where cows were randomly assigned to dry cow therapy with either cloxacillin (500mg) or tilmicosin (1500mg)¹. After calving, each cow was resampled to determine AMT success. In AMT failures, *S. aureus* the bacteria were again isolated, purified, subcultured and frozen. Antimicrobial susceptibility profiles were run for isolates obtained from both before dry off and post-calving samples and MIC values were compared for multiple antimicrobials. Data were analysed using X²square statistical methods for categorical data (significance: p=0.05).

Results: For cows receiving the tilmicosin AMT, resistance patterns for antimicrobials of the macrolide-lincosamide-streptogramin (MLS) group were observed to be significantly different than pre-dryoff samples. Significant increases in the proportion of resistant isolates after the dry period were also observed for MLS antimicrobials. No significant differences were observed in either MIC patterns or proportion resistant for *S. aureus* isolates from cows receiving the cloxacillin AMT.

Discussion: MLS AMT is widely used against staphylococci. The primary mechanism of AMR in staphylococci is ribosomal methylation² and cross resistance among MLS members has been described. Macrolides

(e.g. erythromycin) can be AMR inducers especially after low dose exposure. Here *S. aureus* isolates from the tilmicosin group, a macrolide, had changes in MIC values and in the proportion of resistant isolates after the dryoff period while those exposed to cloxacillin had no change, implying that treatment with tilmicosin influenced the transfer or expression of genetic material coding for MLS resistance. Molecular typing results will be presented to elucidate which resistance genes are present in our isolates whether resistance is constitutive or inducible.

1. Dingwell, RM et al.. (2003). Efficacy of intramammary tilmicosin and risk factors for cure of *Staphylococcus aureus* infection in the dry period. *J Dairy Sci.*86:159-68.
2. LeClercq, R. and P. Courvalin. (1991). Bacterial resistance to macrolide, lincosamide, and streptogramin antibiotics by target modification. *Antimicrob. Agents Chemother.*35:1267-1272.

203 (2698)

VACCINATION OF LACTATING COWS IMPROVES THE EFFICACY OF ANTI-STAPHYLOCOCCUS INTRAMAMMARY THERAPY

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Staphylococcus sp. is the most frequently isolated microorganism from cases of subclinical mastitis. The treatment of this type of mastitis is seldom recommended in lactating cows, because of its cost and low efficacy. We tested the hypothesis that immunizing cows with an anti-*Staphylococcus* sp. vaccine would improve the effectiveness of antibiotic treatment. Twenty-seven cows with subclinical mastitis caused by *Staphylococcus* sp. as determined by the California Mastitis Test (CMT) and bacteriological culture were used. These cows had a total of 56 infected quarters, and were split in two groups. Each group had approximately the same number of infected quarters. One of the groups was previously vaccinated with two doses, two weeks apart, of a commercial anti-staphylococcal vaccine (MASTAPH, IRFA Química e Biotecnologia Industrial Ltda.). One week after the second vaccination the cows received one daily application for three days of an intramammary formulation of an antibiotic, selected by sensitivity test. The other group received only the intramammary treatment. Milk samples were collected for culture and testing with CMT at the day of the first application of antibiotic and at weekly intervals for three weeks. The vaccinated cows showed a higher CMT score. The percentage of quarters identified as infected that were eliminating *Staphylococcus* sp. in vaccinated and unvaccinated cows was, respectively, 40% and 76.9% in the first week, 26.6% and 65.4% in the second week, and 26.6% and 42.3% in the third week. These differences were significant in the first ($p=0.0072$) and second ($p=0.0066$) weeks, but not in the third week ($p=0.2650$). The increase in the SCC of the vaccinated cows can be attributed to an increased immune response in these animals. The enhancement of the efficacy of the treatment in vaccinated cows may make the treatment of subclinical mastitis in heavily infected herds feasible, especially in animals in the beginning of the lactation. Funding: UDESC

204 (1269)

MEASURING PREMILKING STIMULATION EFFECTS WITH MILK FLOW CURVES

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The Lactocorder® (LC) is a tool that can quantify the actual milk flow curves for individual cows thereby providing instantaneous visual evidence with regard to the quality of the udder preparation during milking. The objective of the trial was to evaluate individual cow milk flow graphs and determine cow response time to changes in milking procedures. A normal udder preparation with a lag-time between stripping and attachment of the cluster of at least 90 seconds was compared to an udder preparation with a short time (<60 sec) between stripping and attachment. Prep procedures consisted of pre dipping, fore stripping, and drying teats with an individual towel. 24 Holstein dairy cows from the Cornell University research barn were used in the trial. Cows were housed in tie stalls and were milked twice daily. The LC was used to collect milk flow data for 7 milkings. Treatment was assigned to all cows for that given milking and was as follows; 2 milkings- normal; 2 milkings- short prep lag; 2 milkings- normal; and 1 milking- short prep lag. Data that was collected from the milk flow curve included; peak milk flow, presence or absence of bimodality, time from start of milking to a milk flow of 0.5 kg/minute (t_{500}), duration of increase in milk flow from t_{500} to plateau phase, presence or absence of air influx (liner slip), total milk (kg), days in milk and lactation number. Data was downloaded from the LC® generated tables, and statistical analysis was performed in SAS. Within animal differences between the two treatment procedures were evaluated. The results indicated that cows experiencing a short prep lag time took longer to reach a flow rate of 0.5kg/min ($P=0.09$). As well, with short prep, cows took 0.5 minutes longer to reach peak milk flow ($P<0.001$). There were no differences in peak milk flow between the two groups. There was a significant difference in the percentage of bimodal milk flow patterns between the prep procedures. Short udder prep lead to 66% bimodal graphs versus 51% with normal prep. In this experiment all cows were actually prepped rather well. It is expected that with a less complete preparation (i.e. skipping the stripping component) an even larger difference between procedures would have been expected. In terms of milk flow dynamics, our research has shown that changes in milking procedures may be seen immediately in the quality of the milk flow curve.

205 (2601)

SURVIVAL ANALYSIS TO ASSESS THE CONTRIBUTION OF VACCINATION AGAINST BRSV TO THE CONTROL OF RESPIRATORY DISORDERS IN NON-WEANED CHAROLAIS CALVES

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Difficulties to manage respiratory disorders in non-weaned calves have become a major complaint of farmers operating calving-to-finishing cattle units in western France. Effectiveness of vaccination in diminishing the incidence of the clinical disorders is often challenged. At the farm level, farms implementing vaccination against the BRSV (Bovine Respiratory Syncytial Virus) were shown having higher incidence rates of the disorder. This may result from decisions to vaccinate in farms where respiratory disorders occur. Therefore, a new approach was implemented at the calf level and under field conditions to study the relationship between vaccination and consecutive occurrence of respiratory disorders. This approach took into account the time-relationship between 0, 1 or 2 injections of commercial vaccines, age of calf at injections and occurrence of respiratory disorders.

Data were provided by a survey involving 156 Charolais herds in western France from September 2000 to March 2001. Data collected were: (1) demographic data; (2) dates of vaccine injections; (3) dates of occurrence of respiratory disorders and related treatments; and (4) housing characteristics and group of housed animals structure. Independent variables were designed from these data as time-dependent or not and offered to a Weibull model. Software used was the Survival Kit vers. 3.12 (Ducrocq and Sölkner, 1998). Contrary to common belief, a proper vaccination protocol (1 or 2 injections in calves aged more than 45 days, before any occurrence of cases in the yard) was associated with a significant reduction in the incidence of clinical disorders, despite the young age of the calves (and possible presence of maternal antibodies). Vaccination implemented once a case of respiratory disorder had already occurred in the yard was unable to reduce this incidence. Results also suggest that BRSV was playing a significant role in etiology of the disorders under concern, in our study population, which was consistent with preliminary data from another survey aiming at identifying the pathogens implicated.

206 (3398)

CATTLE DNA IMMUNISATION AGAINST BVDV-I AND BVDV-II

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DNA immunisation is a promising vaccinal approach because of safety, ease of production, handling of the material and possible polarisation of the immune response. In order to induce cross-protection against the major genotypes of BVDV we selected the major surface glycoprotein E2 because it is the major target of the neutralizing antibodies and the well-conserved nucleocapsid protein C because it is a target for the cellular response. The coding sequences for these two antigens from field isolates of BVDV-Ib and BVDV-II were cloned into the pcDNA3 mammalian expression vector and optimised for expression. The two constructs were efficiently expressed in COS cells and recognised differentially by bovine specific antisera.

Three routes of administration of the DNA were selected: intramuscular, intradermal and intranasal. Three groups of five cattle received a pool of the three DNA constructs by each of the three vaccination routes. A fourth group was kept as a control. All cattle were challenged with thrombocytopenic type II BVDV strain 890. The results of the virological and immunological follow-up will be presented.

207 (876)

A CYTOLYSIN BASED VACCINE FOR PROTECTION OF CATTLE AGAINST INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

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The cytolysin of *Moraxella bovis* is a leukocidic, hemolytic, pore forming toxin that initiates corneal ulceration. Because of the pathogenic role of this protein in infectious bovine keratoconjunctivitis (IBK), we hypothesized that it may have immunogenic potential. Consequently, we developed a method for concentration and for partial purification of the cytolysin, and then examined the efficacy of Quil A adjuvanted purified toxin as a vaccine against IBK.

Cytolysin was concentrated from sterile broth culture supernatants using a tangential flow filter with 100 kDa nominal molecular weight limit. The cytolysin, which has a molecular mass of 98.8 kDa was retained by the large pore size filter because it was aggregated with bacterial lipopolysaccharide. Diafiltration of the retained cytolysin increased the specific activity. A further increase in specific activity and purity was obtained by chromatographing the diafiltered retentates on a gel with 4×10^7 exclusion limit. The cytolysin was eluted in the void volume, in intimate association with endotoxin. The diafiltered cytolysin was adjuvanted with Quil A, and 2 ml doses were given to each of 35 mixed breed beef calves on May 19, and again on June 17. Other calves (N=34) were given 2 ml of saline solution, and another group of calves (n=35) were given 2 ml of a Quil A adjuvanted diafiltered retentate of sterile media. Eyes of calves were examined and were photographed weekly until September 9. Cytolysin vaccinated calves had fewer corneal ulcers on observation weeks 8, 10,

11, and 12. The cumulative proportion of calves with corneal ulcers was lower in vaccinates beginning on week 10. The corneal surface areas of the cytolyisin vaccinated calves were less than those of the other groups. Cytolyisin based, Quil A adjuvanted vaccine induced protective immunity in calves.

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208 (3225)

USE OF A MANNHEIMIA HAEMOLYTICA INTRATRACHEAL CALF CHALLENGE MODEL TO EVALUATE CEFTIOFUR CRYSTALLINE FREE ACID COMPARED TO TILMICOSIN FOR DURATION OF PROTECTION
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Ceftiofur is an injectable cephalosporin developed for veterinary use only. Ceftiofur crystalline free acid sterile suspension (CCFA) is a new single dose formulation with a long duration of effectiveness. An enhanced model was used to evaluate the duration of protection against pasteurellosis when a single dose of CCFA was administered at 6.6 mg ceftiofur/kg SC in the posterior aspect of the ear at various times before challenge, compared to tilmicosin (10 mg/kg, SC, single dose). At arrival (day -4), Holstein calves (n=144; 76.7 + 9.2 kg) were randomly assigned to challenge 3, 5, 7 or 9 days after treatment. Daily evaluations began on day -3. On day 0, calves within challenge group were randomly assigned to negative controls (n=6), CCFA (n=15) or tilmicosin (n=15) and treated. Enhanced model handling was performed on the day before challenge. The first challenge (isolate D9707257) contained 108-109 Mannheimia haemolytica organisms (pH 4.5). The second challenge 4 h later contained 109-1011 M. haemolytica organisms. Plasma ceftiofur and serum haptoglobin concentrations were determined in randomly selected calves. All surviving calves were euthanized for lung lesion scoring 9 days post-challenge. Variables included mortality, clinical scores, rectal temperature, and lung lesion scores 9 days post-challenge. Data were analyzed using PROC GLM. Mortality was 0, 3.3 and 33% for CCFA, tilmicosin and negative controls. Rectal temperatures for CCFA calves were lower than negative controls through 7 days (p<0.05) and were lower than tilmicosin calves through 3 days (p<0.05). Lung lesion scores 7 and 9 days were 1.46 and 3.94% for CCFA calves and 4.88 and 13.7% for tilmicosin calves (p<0.05). Ceftiofur plasma concentrations were 0.39 ug/mL 7 days post-treatment. A single SC administration of CCFA at 6.6 mg CE/kg SC in the posterior aspect of the ear provided superior protection against BRD compared to tilmicosin when a severe M. haemolytica challenge was administered up to 9 days after treatment administration.

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209 (2132)

FETAL INFECTION FOLLOWING BOVINE VIRAL DIARRHEA VIRUS (BVDV) TYPE 2 OR TYPE 1B CHALLENGE OF MODIFIED LIVE VIRUS TYPE 1A BVDV VACCINATED OR NON-VACCINATED PREGNANT ANIMALS

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Two trials were conducted to determine if vaccination with Modified Live Virus (MLV) Type 1a Bovine Viral Diarrhea Virus (BVDV) vaccine could prevent fetal infection following non-cytopathic BVDV challenge of the pregnant dam. The BVDV challenge in Trial 1 was a type 2 isolate; in Trial 2, challenge was with a type 1b isolate.

In both trials, BVDV susceptible females were randomly divided into vaccinates and controls. The vaccinates were given a subcutaneous injection with a combination Bovine Rhinotracheitis - Virus Diarrhea (Singer strain), Parainfluenza-3, Respiratory Syncytial Virus Vaccine (MLV) and a Leptospira canicola, L grippotyphosa, L hardjo, L icterohaemorrhagiae and L pomona Bacterin. The controls received phosphate buffered saline. Breeding began 30 days post vaccination. Intranasal challenge occurred between 75 to 96 days of gestation. The type 2 challenge included 25 vaccinates and 10 controls; the type 1b challenge included 25 vaccinates and 8 controls.

Between 148 and 174 days of gestation, the fetuses were removed by C-section. Fetuses were evaluated for BVDV infection by virus isolation (VI) of pooled fetal tissues, immunohistochemical staining (IHC) for BVDV antigens and BVDV-capture ELISA.

Results: Trial 1: All fetuses from the 25 vaccinated animals were negative for BVDV by VI, IHC and BVDV-capture ELISA; whereas BVDV was detected in the fetal tissues from the 10 control animals by all methods (p<0.0001). The genotype of the BVDV from each positive fetus was confirmed as type 2.

Trial 2: Twenty-four fetuses from vaccinated cows and six from controls were available for examination.

Twenty-three fetuses from the vaccinated animals were negative for BVDV by VI, IHC and BVDV-capture ELISA. One fetus from a vaccinated cow was positive for BVDV by VI and IHC (no skin sample available for BVDV-capture ELISA testing). BVDV was detected in the fetal tissues from the 6 fetuses removed from the control animals by VI, IHC and ELISA (p<0.0001). The genotype of the BVDV isolated from each of the six control fetuses was confirmed as type 1.

These results suggest that vaccination with MLV type 1a BVDV vaccine can significantly reduce fetal infection following challenge of the pregnant cow with type 2 or 1b BVDV.

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300 (3297)

DETECTION OF ANTIMICROBIAL RESIDUES USED IN DRY COW THERAPY AT THE POSTPARTUM MILK OF DAIRY COWS

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The purpose of this study was to evaluate in the postpartum (PP) of dairy cows, the occurrence of antimicrobial residues) used in dry cow therapy. Seventy-four animals were randomly assigned in 3 groups. The animals of each group received one of three different treatments (two b-lactams and one association form). Strip cup and CMT either at the drying off and postpartum were carried out. Milk samples for microbiological examination were collected in both periods. In the postpartum, milk samples to test for were collected. Samples were screened for by DelvotestOSP. The data had been analyzed using Software Instat GPAHPAD. The occurrence of antimicrobial residues in milk of mammary quarters treated at drying off in farms A, B and C were 18%, 22.2% and 26.5% respectively. The occurrence was higher when the dry period was less than 60 days and when postpartum sampling interval was less than 70 days. The results showed that in cows the presence of antimicrobial residues predominantly occurred in all the mammary quarters (58.5%) and in at least three of them (21.9%), therefore increasing the possibility of contamination of the bulk tank milk. It was not detected any statistical difference among the groups in respect to the used treatments, production in the drying, either in relation to the milk production, or presence of inflammatory process or mammary infection. The dry period considered as ideal for providing the mammary gland time to involution and to get prepared for colostrogenesis and new lactation (60 days), did not constitute guarantee for absence in milk in the postpartum, when, 19.4% of the mammary quarter milk samples taken from cows with a dry period interval of 60 to 70 days, treated at drying off, had presented detectable antimicrobials residues in milk. Only the information of the manufacturer on the withholding period showed not to be enough to assure product quality, the animals treated at drying off should be tested in the postpartum. Cost:benefit analysis performed showed that the use of the antimicrobial residues screening test when compared with milk discarding was economically more profitable.

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