

# Tuesday, July 14, 2009

## POSTER PRESENTATIONS

### Animal Health: Mastitis and Associated Microbiology

**T1 Natural autoantibodies in milk and their role in the development of mastitis in dairy cows.** A. T. M. Van Kneysel\*, G. De Vries Reilingh, A. Lammers, B. Kemp, and H. K. Parmentier, *Adaptation Physiology Group, Wageningen Institute of Animal Sciences, Wageningen University, Wageningen, the Netherlands.*

Natural antibodies (NABs) are antigen-binding immunoglobulins present in non-immunized individuals. They can be considered as humoral part of the innate immune system. Two types of NAB are distinguished. *Overt* NAB (which plasma concentrations rise with age) are readily detected towards exogenous antigens. *Cryptic* NAB bind autoantigens in short temporal intervals. They are suggested to clear intracellular proteins upon their leakage from necrotic cells and regulate inflammatory processes. In cows, overt NAB concentrations in plasma and milk were related to parity, energy balance and diet composition. The objective of the current study was to detect cryptic NAB in cow milk, and relate them to somatic cell count (SCC) and the incidence of mastitis. Milk samples were collected weekly from cows (n=96) from calving till week 9 postpartum (pp) and analyzed for fat, protein, SCC, and NAB to auto-proteins (myosin, thyroglobulin, transferrin). NAB titers are expressed as the  $^2\log$  values of the highest dilution giving a positive reaction. Data are expressed as MEANS  $\pm$  SEM. Repeated observations were analyzed in a mixed model. Cows produced 40.3 ( $\pm 0.3$ ) kg of milk with 3.20 ( $\pm 0.01$ ) % protein, 4.00 ( $\pm 0.02$ ) % fat, and a SCC of 150 ( $\pm 16$ )  $\times 10^3$ /ml. NAB binding myosin (5.66  $\pm$  0.06), thyroglobulin (4.85  $\pm$  0.06), and transferrin (5.76  $\pm$  0.07) were found in milk. Week pp affected ( $P \leq 0.05$ ) NAB titers binding thyroglobulin and transferrin. Clinical mastitis incidence (9%) tended to be related to NAB binding myosin ( $P=0.06$ ) and transferrin ( $P=0.08$ ), while NAB binding thyroglobulin tended to be related to SCC ( $P=0.09$ ). This study demonstrates the presence of cryptic NABs in cow milk, and shows trends for a relation between enhanced cryptic NAB concentrations and mammary inflammatory processes, as indicated by SCC and mastitis. Future studies should confirm these trends and shed light on the exact role of cryptic NAB in cow milk.

**Key Words:** somatic cell count, natural antibodies, early lactation

**T2 Psoriasin expression in bovine udder is induced by *E. coli* infection.** P. Regenhard\*<sup>1</sup>, W. Petzl<sup>2</sup>, H. Zerbe<sup>2</sup>, and H. Sauerwein<sup>1</sup>, <sup>1</sup>*Institute of Animal Science, Bonn, NRW, Germany*, <sup>2</sup>*Clinic for Ruminants, Munich, Bavaria, Germany*.

Human psoriasin (S100A7) has originally been described as a member of the family of S100 calcium-binding proteins and is overexpressed in patients suffering from psoriasis. The bovine homolog was first identified as a cow-derived respiratory allergen. Human psoriasin as well as the bovine homolog exhibit antibacterial activity especially against *Escherichia coli*. During *E. coli*-mastitis, the host defense status is a cardinal factor influencing systemic disease severity and outcome of the disease. Because of its antibacterial properties psoriasin might be an evolutionary ancient component of innate immunity in the udder. *Escherichia coli*-mastitis is a common problem in dairy cattle, and bovine psoriasin was found to exhibit antimicrobial activity against *E.*

*coli*. We therefore examined by immunoblotting whether and in which localisation the bovine protein is expressed in the mammary gland, and whether this expression is inducible by infection with *E. coli*. To obtain an antiserum, rabbits were immunised with recombinant bovine psoriasin. Six German Holstein cows in their first lactation (26 to 30 months of age, 3 to 5 months in milk) were used; 4 of them were intramammarily infected with 500 CFU of an *E. coli* strain isolated from udder secretions of a cow with clinical mastitis, 2 cows served as control animals and received no treatment. All challenged cows developed clinical mastitis after 12 h. After 24 h, the cows were slaughtered and samples were collected from 3 different localisations of the quarters. Psoriasin expression was limited to the teat cistern of the *E. coli*-infected cows, but was absent in the teat cistern of the non-infected cows and in the parenchyma of both groups, whereas the expression on udder skin was demonstrated in both infected and non-infected cows. Psoriasin thus appears to be a part of the local host defense mechanisms in the udder and seems to be inducible by infection with *E. coli*.

**Key Words:** antimicrobial peptides, mastitis, psoriasin

**T3 Innate immune responses in dairy cows and study of a promising candidate: Osteopontin.** K. Alain<sup>1,3</sup>, N. A. Karrow<sup>3</sup>, C. Thibault<sup>1</sup>, M. Lessard<sup>1</sup>, and N. Bissonnette\*<sup>1,3</sup>, <sup>1</sup>*Dairy and Swine Research and Development Center, Agriculture and Agri-Food Canada, Sherbrooke, Québec, Canada*, <sup>2</sup>*Université de Sherbrooke, Sherbrooke, Québec, Canada*, <sup>3</sup>*University of Guelph, Guelph, Ontario, Canada*.

Mastitis is the most important disease in dairy cows and the main cause of economic losses for producers. Search for genes involved in innate immune response and their genetic variants is highly appropriate to identify markers associated with resistance to mastitis. Genetic selection of bulls using those markers associated with innate immunity will result in cows with greater resistance to mastitis, thereby reducing antibiotic use. Following the discovery of a key gene involved in immune response, we evaluated the genetic potential of the osteopontin gene as a candidate for the selection of animals with greater natural resistance to mammary gland infection. The use of the subtractive hybridization technique on cDNA libraries of milk cells from cows infected with *Escherichia coli* revealed an abundant transcript expressed early during infection, namely osteopontin. The search for DNA polymorphisms (SNP) for this *SPP1* gene was conducted by comparing bulls with extreme estimated breeding value (EBV) for the somatic cell count (SCC), an indicator of mammary gland health. Amplification of genomic regions including the promoter and the seven exons was performed and used to identify four SNPs: *SPP1c. 1301G>A*, *SPP1c. 1251C>T*, *SPP1c. 430G>A*, and *SPP1c. \*41A>C*. A population consisting of 578 Holstein bulls divided into 26 families (10–60 offsprings per bull-sire family) was genotyped. The study presents the EBVs for the SCC health trait in relation to the different SNP and their associated haplotypes in Canadian Holstein bovines. Results show that *1301G>A* had an impact on the SCC value ( $p < 0.001$ ). The SNP *\*41A>C* affected the SCC ( $p < 0.01$ ), as demonstrated by allelic substitution ( $p < 0.05$ ). Haplotype analysis did not provide any statistically valid results, however, owing

to a low proportion of certain alleles in the population under study. In addition, certain SNP and their locations (promoter and 3'-untranslated regions) potentiate the genetic impact (EBV) of this candidate gene for the SCC trait. Because the SCC is directly related to mastitis, these DNA polymorphisms could affect mastitis resistance.

**Key Words:** osteopontin, DNA polymorphisms, innate immunity

**T4 Expression of Toll like receptor 4 on bovine neutrophils is not dependent on transcriptional activation.** M. Worku\*, A. Morris, H. Mukhtar, and N. Mikiashvili, *North Carolina A&T State University, Greensboro.*

Endotoxin released from the cell wall of Gram negative bacteria is associated with the pathogenesis of mastitis caused by *Escherichia coli* (*E. coli*). The objective of this study was to evaluate the effect of smooth (S) and rough (R) forms of lipopolysaccharide (LPS) on the expression of Toll-like receptor 4 (TLR4) in bovine blood neutrophils. Isolated neutrophils ( $10^7$  cells/mL) from three Holstein Friesian cows were treated with *E. coli* LPS serotype O111:B4 smooth (S) or rough (Rd) forms. Reverse transcriptase PCR was done using primers specific for TLR-4. Flow cytometry was used to assess cell surface expression of TLR-4. Analysis of variance was performed to determine the differences between the groups. No mRNA was detected for TLR-4. Exposure to LPS induced increased cell surface expression of TLR-4. The percentage of total neutrophils binding the monoclonal antibody to TLR-4 was significantly increased on treatment with both S and R forms of LPS ( $P < 0.05$ ) compared to the PBS control. Mean binding increased from  $13.57 \pm 1.51$  in PBS to  $24.42 \pm 2.61$  in Rd treated and  $24.41 \pm 4.57$  in O111:B4 treated neutrophils. No significant difference was observed in the level of TLR-4 expression between treatments with S and R forms of LPS or the PBS-treated control ( $P > 0.05$ ). Enhanced TLR-4 activity from exposure to LPS in bovine neutrophils was not dependent on transcriptional activation as evidenced by lack of mRNA induction. Increased cell surface TLR4 may be the result of recruitment of TLR4 from an intracellular pool to the bovine neutrophil cell membrane in response to LPS exposure. This mechanism has implications for the immediacy of the innate immune response to LPS from Gram negative organisms.

**Key Words:** neutrophil, TLR4, mastitis

**T5 Comparison of in vivo and in vitro mammary cell expression of selected inflammatory genes in response to  $\alpha$ -linolenic acid.** P. Rezamand\*, B. P. Hatch, K. Parnell, K. M. Hunt, J. E. Williams, W. Price, and M. A. McGuire, *University of Idaho, Moscow.*

Specific fatty acids (FA) such as  $\alpha$ -linolenic acid (ALA: 18:3  $\omega$ 3) affect immunity. One such effect is modulation of inflammatory responses. The objective of this study was to compare in vivo and in vitro effects of ALA on bovine mammary cell expression of selected inflammatory genes. In an in vivo experiment, canola meal (18:1  $\omega$ 9) was replaced with camelina (*camelina sativa*) meal (24.4% ALA) at 0, 3, 6, and 9% (DM basis) to provide rations with incremental concentrations of ALA. Primiparous Holstein cows in mid-lactation ( $n=18$ ) were randomly assigned to a treatment sequence in a  $4 \times 4$  Latin square design. Periods lasted 16 d with milk samples collected during the final 2 d of each period. Milk cells (MC) were harvested for RNA extraction, from which cDNA was synthesized. Quantitative PCR analysis demonstrated that expression of pro-inflammatory TNF- $\alpha$  in MC was linearly reduced

(up to 40%) as dietary ALA increased ( $P < 0.01$ ). No significant differences were detected among treatments in gene expression of IL-1 $\beta$ , intra-cellular adhesion molecule (ICAM)-1 or IL-6 in MC. In vitro, bovine mammary epithelial cells (Mac-T) were grown in DMEM containing 10% FBS. Cells were then sub-cultured in a medium void of FBS, to which incremental concentrations (up to 90  $\mu$ M) of ALA: BSA (FA-free) solution were added and cells were collected at specific time-points over 48 h. Although expression of IL-1 $\beta$  and ICAM-1 (6 h) mRNA were increased, that of IL-6 was reduced (time  $\times$  dose  $P=0.001$ ) by ALA. Whereas mRNA expression of IL-8 was reduced by 50% when treated with ALA up to 20  $\mu$ M, treatment with 90  $\mu$ M ALA up-regulated IL-8 mRNA expression. More notably, mRNA expression of TNF- $\alpha$  was reduced by 55% when cells were exposed to 60  $\mu$ M of ALA (48 h;  $P < 0.01$ ), similar to MC obtained from cows fed rations containing increased levels of ALA. Overall, ALA regulated expression of some of the major pro-inflammatory markers in MC and Mac-T cells but responses were not identical.

**Key Words:** dairy, inflammatory marker,  $\alpha$ -linolenic acid

**T6 Development of a multiplex-PCR detection assay for simultaneous identification of the major pathogens causing mastitis in dairy milk.** B. Cressier\*<sup>1,2</sup>, C. Thibault<sup>1</sup>, and N. Bissonnette<sup>1,2</sup>, <sup>1</sup>*Dairy and Swine Research and Development Center, Agriculture and Agri-Food Canada, Sherbrooke, Québec, Canada,* <sup>2</sup>*Université de Sherbrooke, Sherbrooke, Québec, Canada.*

Mastitis remains the major disease affecting the dairy industry worldwide. Microbiological methods remain the "gold standard" for mastitis pathogen detection in Canada. Introducing a molecular detection system would reduce the delays to identify the causative pathogen and allow proceeding with the adequate veterinary treatment. A molecular approach would speed up the detection process and must show unambiguous results with an improved sensitivity. To detect specie-specific genes, one predilection technique relies on multiplex-PCR to amplify multiple loci each localized to a specific pathogen's genomic DNA. In this study, we challenged this method for detecting in milk the major mastitis causing bacteria in a single PCR reaction: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Mycoplasma bovis*, *Streptococcus uberis*, *Str. agalactiae*, and *Str. dysgalactiae*. Specific DNA primers have been designed for each species and tested for optimal sensitivity and specificity. Because each specific primer is labelled with a different fluorescent dye, the analysis of multiple fragment length by capillary electrophoresis is a suitable system for detecting simultaneous multiple pathogen loci with improved sensitivity provided by the fluorescent signal. To challenge the molecular detection assay, we optimized an extraction assay for isolation of bacterial genomic DNA from milk. The extraction assay proposed is convenient for all detected species and greatly reduces the presence of PCR inhibitors inherent to milk. The sample is clarified using a chelator, milk pellet is washed, and a chelating resin is introduced before performing the boiling DNA extraction. This method provides a simple, efficient and inexpensive detection assay, which can be easily amenable into a high-throughput scheme using 96- or 384-well plaques. This development opens up possibilities for the functional integration of both extraction and detection of mastitis pathogens in milk of dairy herd using a high-throughput automated screening system.

**Key Words:** mastitis, pathogen detection, multiplex-PCR

**T7 Microbiology results of milk samples from California dairies received between 1999 and 2008.** D. F. Resende\*, K. Glenn, J. S. Cullor, and R. G. S. Bruno, *University of California-Davis, Tulare.*

The objective of this study was to determine prevalence and temporal distribution of bovine mastitis pathogens isolated from milk samples received in the Milk Quality Laboratory at the Veterinary Medicine Teaching and Research Center – UC Davis between 1999 and 2008. Records evaluated on this study included individual cow milk samples obtained from clinical and subclinical mastitis cases and milk samples collected during mastitis screening programs. Samples were cultured according to the National Mastitis Council guidelines and results were classified as negative or positive for bacterial growth and then evaluated for specific pathogens. Contaminated samples were defined based on the presence of more than two environmental pathogens identified in the same plate. Presence of contagious microorganisms was considered positive for determined pathogen disregarding the numbers of other environmental pathogens isolated in the same plate. Data were evaluated based on annual and seasonal trends of major mastitis pathogens. A total of 370,748 milk samples were received between January of 1999 and December of 2008, with the majority of the samples being delivered on the winter season (n=98,815). Multiple mastitis pathogens were isolated from 64.2% of the samples (n=237,968), and among these positive samples Environmental *Staphylococcus* spp were the most prevalent (44.8%) followed by Coliforms (33.5%), Environmental *Streptococcus* spp (14.8%), *Bacillus* spp (10.4%), Coagulase-negative *Staphylococcus* (5.6%), *Staphylococcus aureus* (5.6%) and *Streptococcus agalactiae* (0.1%). Contaminated samples accounted for 8.8% of positives samples being more prevalent during winter seasons (P<0.05). Environmental *Streptococcus* was the only species consistently more prevalent in the winter compared to other seasons in all years and *S. agalactiae* was the least prevalent ranging from 0.01% to 0.28%. There was no annual or seasonal trend for isolation of contagious microorganisms. In conclusion, environmental were more prevalent than contagious mastitis pathogens in all years of this study and milk samples submitted during the winter had higher risk to be contaminated.

**Key Words:** dairy cows, mastitis, milk samples

**T8 *Prototheca* mastitis outbreak investigation in lactating Jersey cows.** A. G. Kenyon\*, D. F. Resende, K. Glenn, R. Moeller, and R. G. S. Bruno, *University of California - Davis, Tulare.*

A mastitis outbreak was investigated in a 2700 Jersey cow dairy in California from March through September 2008. The only change reported in the dairy was the replacement of rubber with silicon based milk liners in the milk equipment (March 2008) and adjustment of parlor setting for the new liners. Mastitis was defined as abnormal milk with or without udder or systemic changes. Epidemiological investigation started in July 2008 when milk samples from all mastitis cases and monthly string sample were submitted for microbiological analysis at the Milk Quality Laboratory, VMTRC – UC Davis. Plates were incubated at 37° C and evaluated at 24 and 48h after incubation. Isolated pathogens were identified according to laboratory guidelines and *Prototheca zoopfi* was identified by gram stain and then by the API 20C system. Four animals with a record of chronic mastitis and lack of response to any mastitis therapy had milk samples collected and their mammary glands submitted for histopathology. String milk sample were collected once monthly to monitor mastitis pathogen in the herd. Examination of the parlor under the new settings identified pulsators overheating and some of them melting. Baseline data (BL) of the herd were retrieved retrospectively from the months prior to the mastitis

outbreak. Epidemiological investigation revealed a point source type of outbreak with recurrent infection. Mastitis incidence rate increased from 1% (BL) to 7% (July 2008) after the new parlor settings and incidence of repeated cases of mastitis also increased from 0.7% (BL) to 2.6% after March 2008. Milk sample collected from the 4 chronic cases had a pure growth of *Prototheca zoopfi*. Histopathology of the udders identified a chronic mastitis with numerous fungal organisms present in alveoli, intralobular ducts and the interstitial connective tissue. The presence of *Prototheca* in the string samples were observed in 75% of pens during the peak of the outbreak and has been decreased to 12% after recommended changes. The recommendations consisted of selective culling of affected animals, replacement of silicon to rubber based liners, and readjusting the parlor setting to the new liners.

**Key Words:** Jersey cows, mastitis, *Prototheca zoopfi*

**T9 Comparison of 16S rRNA gene sequence analysis with aerobic milk culture for the identification of potential bacterial etiologies of bovine clinical mastitis.** J. R. Wenz\*, T. E. Besser, L. K. Fox, and Y. Zhang, *Washington State University, Pullman.*

A preliminary study was performed to evaluate the potential use of 16S rRNA gene sequence analysis (16S) to better understand the ecology of bacteria in the milk of the bovine mammary gland with clinical mastitis (CM). Aerobic bacterial culture of milk is the standard method to identify the etiology of CM. Bacteriologically negative (BN) aerobic milk cultures have been reported as high as 40%. Furthermore, numerous CM cases fail to respond to therapy as would be predicted by milk culture results. Anaerobic bacteria have been identified in cases of CM but have been generally ignored. Taken together this suggests that pathogens not identified by standard aerobic milk culture may be involved in the pathogenesis of CM in dairy cattle. Milk from 17 cows with CM in a single quarter and known milk culture results were chosen for this study. Milk (2 ml) was centrifuged, DNA was extracted from the pellet and the V3 region of the small ribosomal subunit was amplified using PCR primers broadly complementary to eubacterial conserved sequences, and cloned. Five to 10 clones from each milk sample were sequenced. Two to 7 different bacterial clones were identified in the majority of samples. The 16S analysis was concordant with the milk culture result for 8/12 (67%) samples, though most 16S results included bacteria other than those found on milk culture, some of which were anaerobes. Predominant bacteria observed in 5 BN milk samples included *Pseudomonas* spp., *Bacteroides* spp., *S. aureus* and *S. dysgalactiae*. This preliminary work needs to be furthered by a study including control milk samples from contralateral, unaffected quarters as well as anaerobic and mycoplasma cultures for better interpretation of 16S results. Nonetheless, these results suggest a more complex bacterial ecology in cases of bovine CM than is indicated by aerobic milk culture. Furthermore, CM cases with a BN milk culture result may be caused by a heterogeneous population of bacteria, including Gram negative and positive organisms and anaerobes.

**Key Words:** clinical mastitis, dairy cattle, 16S rRNA

**T10 Effect of year period on mastitis prevalence and routine procedures characteristics of milking in Culiacán, Sinaloa.** M. Valdez\*, M. A. Luque, L. Almeida, J. Rodríguez, F. T. Olivas, and D. C. Ochoa, *Investigación y Transferencia de Tecnología para Rumiantes, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Sinaloa, Culiacán, Sinaloa, México.*

The objective of present new work was to know the effect of year period on mastitis prevalence and to characterize the routine procedures of milking in dairy herds of Culiacan, Sinaloa, Mexico. Total cows analyzed in the year were 976 with 3,904 quarters, in 8 representative herds randomly selected. The work was realized of September 2007 to June 2008, taking like representative year periods different environmental conditions, September month (it warms up and humid, 28.95 °C t and 78% RH); January (fresh, 19.05 °C t, 70% RH); and June (it warms up and dry, 29.65 °C, 60% RH). The health of each quarter was determined with the California Mastitis Test (CMT). The routine practices of milking in each herd were characterized taking 10 minutes from video with hidden camera, udder preparation, teats disinfection before and after milking, milking unit positioning, and over milking occurrence was analyzed. Traces result level was considered not affected, the proportion of negative quarters to CMT was 67.88% without differences ( $P>0.05$ ) between year periods, corresponding 64.68, 69.26 and 69.69% to September, January and June, respectively. The proportion of positive quarters to CMT was 28.42% without differences ( $P>0.05$ ) between year periods, corresponding 31.74, 26.89 and 26.63% to September, January and June, respectively. The fibrous quarters proportion was 3.70%. Udder preparation is not adapted one, teats disinfection before milking is not realized, in 50% herds use teat sealant, milking unit positioning is not advisable one, and the over milking is frequent. One concludes that the mastitis prevalence is elevated and is not affected by the year period, because the routine procedures of milking are not adapted.

**Key Words:** mastitis, prevalence, milking

**T11 Effects of *Mangifera indica* peel extracts on *Staphylococcus aureus* mammary infections.** S. Stella and D. Tedesco\*, *University of Milan, VSA Dep., Milan, Italy.*

In our previous trial, lower *Staphylococcus aureus* mammary infections was evidenced in milk obtained from dairy cow treated with *Mangifera indica* peel (personal communication). To verify this important effect, we performed an in vitro trial to evaluate the activity of *Mangifera indica* peel water and ethanol extracts on *Staphylococcus aureus* by agar gel dilution method. One g of each tested extract (water and ethanol) was solubilised in distilled water (10 mL) and sterilized by filtration. One mL aliquots of each solution were inoculated into fluid trypticase soy agar (100 mL) at 45°C. Then the inoculated media were plated into Petri dishes. *Staphylococcus aureus* suspensions ( $10^2$  and  $10^3$  CFU/mL) were spread on the surface of the media and the plates were incubated aerobically at 37°C for 48 hours. After the incubation period, the number and the size of colonies in the extract-containing and extract-free plates were compared. *S. aureus* colonies were observed by optical microscope Olympus BX41 (12.5X); images were acquired by Image ProPlus software (Media Cybernetics Inc.). The diameter of at least 30 colonies for each medium was measured. The test also included plates containing only the culture medium and the culture medium plus ethanol, in order to obtain a control of the solvent antimicrobial effect. *S. aureus* strains were considered susceptible in case of complete growth inhibition (bactericidal effect) or when colonies were smaller than colonies in control plates (bacteriostatic effect). *S. aureus* strains were considered resistant when the number and size of colonies didn't change compared to the control plates. Our results showed an efficient bacteriostatic action due to all *Mangifera indica* extracts tested: sizes of the tested colonies were smaller than in control (Table 1). The extract effects are following reported according to their efficacy: - *Mangifera indica* ethanol extract 1 mg/mL - *Mangifera indica* water extract (0.5 mg/mL) + ethanol extract (0.5 mg/mL) - *Mangifera indica* water extract

1 mg/mL. In conclusion treatment with this natural wastes extracts can reduce mammary infection.

**Table 1. Bacteriostatic effect of *Mangifera indica* peel extracts on *S. aureus***

Blank (TSA)	Water extract	Ethanol extract	Mixed W/Et extract
2109,5 +/- 228,1	1549,2 +/- 189,2	1188,5 +/- 54,9	1477,0 +/-54,6
<i>S. aureus</i> colony size (µm, mean +/- st. dev.)			

**Key Words:** *Staphylococcus aureus*, *Mangifera indica* peel extracts, mammary infections

**T12 Effects of OmniGen-AF on mammary mucosal responses to an *Escherichia coli* challenge.** Y.-Q. Wang\*, A. Rowson, N. E. Forsberg, and S. B. Puntorney, *OmniGen Research, Corvallis, OR.*

Previous studies (Wang et al., 2007, 2009) have shown that feeding OmniGen-AF to animals increases blood-borne markers of immunity. The goal of this study was to examine effects of feeding OmniGen-AF on the mammary mucosal response during a pathogen challenge. A murine model of bovine mastitis was used. Twenty-four timed pregnant CD1 mice were assigned to three treatments: 1) negative control, 2) positive control and 3) OmniGen-AF-fed. Negative control animals were fed a control diet. At d10 of lactation, they were anesthetized and both L4 and R4 mammary glands infused with sterile PBS. Positive control animals were fed the same diet but infused with 50 colony forming units (CFU) of a bovine clinical isolate of *Escherichia coli*. The final group of animals was fed OmniGen-AF (0.5% w/w) for 14 d prior to the infusion of 50 CFU of *E. coli* into the L4 and R4 mammary glands. Infection was allowed to progress for 24 h after which animals were euthanized (ca. d10 of lactation) and whole mammary samples were recovered and RNA isolated using Trizol reagent. Concentrations of mRNAs encoding myeloperoxidase (MPO), major histocompatibility complex 2 class II (MHC), macrophage inflammatory protein (MIP) and beta-actin were assessed using either Sybr green or TaqMan-based quantitative PCR assays. Beta-actin was used as a housekeeping gene to standardize mRNA concentrations. Mammary concentrations of MPO and MHC mRNAs were elevated significantly ( $P<0.05$ ) in OmniGen-AF-fed animals. MIP mRNA was unaffected by treatment ( $P>0.05$ ). Enhanced mammary MPO mRNA implies that neutrophil infiltration into infected tissue was increased. An increase in mammary MHC mRNA implies that antigen presentation was enhanced by provision of OmniGen-AF in the diet. Wada et al (2008) reported that OmniGen-AF reduced incidence of mastitis in dairy cattle. Possible mechanisms may include enhanced neutrophil infiltration into infected mammary tissue and enhanced antigen presentation in mammary antigen presenting cells.

**Key Words:** OmniGen-AF, mastitis, mucosal immunity

**T13 Decision-making for early postpartum subclinical mastitis.** V. E. Cabrera\*, J. Pantoja, P. Ruegg, and G. Shook, *University of Wisconsin, Madison.*

A decision tree model was developed to study the economic outcomes of testing and treating early postpartum cows for subclinical mastitis. The model evaluates sequential decisions that determine economic outcomes based on a 305-d lactation. Logistic regression models were used to predict positive and negative results of 2 diagnostic tests: quarter

somatic cell count (SCC) or California Mastitis Test (CMT). The tests were used to detect intramammary infection (IMI) for different DIM (2 to 8), parity statuses (heifer or cow), and a defined SCC threshold. Producer decisions for each cow included (1) test or no test, (2) if test is pursued, what type of test (CMT or SCC), and (3) a final decision: cull, segregate, administer antibiotics, or take no action. Each intermediate or final node of the model was associated with an economic outcome that the decision tree used to find the economically optimal pathway. The cost of subclinical mastitis was assessed as the aggregation of five factors: (1) milk loss, (2) milk premium loss, (3) premature culling, (4) clinical flare-ups, and (5) transmission to herd mates. These costs were a function of the lactation curve, milk price, defined SCC threshold, live-stock prices, and a defined prevalence of contagious mastitis pathogens. Preliminary results indicate, in general, the selection of CMT and no action for negative cows. Seems that the administration of antibiotics could be a feasible option for positive cows, especially when a cow is in first parity (increased rate of cure), milk from a treated cow is used for heifer feeding, and the prevalence of contagious pathogens is high. The cost of mastitis under an optimal policy would vary between \$142 to \$225 per cow per 305-d lactation, and depend strongly on mastitis prevalence, SCC threshold, milk price, milk production level of cow, and parity.

**Key Words:** decision tree, mastitis cost, mastitis economic impact

**T14 Effects of CpG ODN adjuvant on the immune responses elicited by a quadrivalent mastitis vaccine in dairy cows.** S.-C. Lee<sup>1</sup> and J.-W. Lee\*<sup>2</sup>, <sup>1</sup>Graduate Institute of Animal Vaccine Technology, National Pingtung University of Science and Technology, Neipu, Pingtung, Taiwan, <sup>2</sup>Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Neipu, Pingtung, Taiwan.

*Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) remain to be common pathogens inducing bovine mastitis worldwide. Prevention of mastitis by using vaccines has not been very successful. Accumulated lines of evidence indicated that the efficacy of a vaccine can be enhanced by using bacterial DNA, or synthetic CpG oligodeoxynucleotides (ODNs), as the adjuvant. In the present study, a quadrivalent mastitis vaccine, containing formalin inactivated three strains of *S. aureus* (T5, T8, and smith compact) and *E. coli* J5, was formulated with or without a sequence of CpG ODNs that has been shown to be immunostimulatory to bovine cells. Eighteen healthy dairy cows were randomly assigned to three groups and received (1) the control (Freund's incomplete adjuvant, FIA, alone, n=6), (2) Vaccine + FIA (n=6), and (3) Vaccine + FIA + CpG (n=6). Serum antibodies specific to the four strains of bacteria and the expression of cytokines, including interferon-gamma (IFN- $\gamma$ ) and IL-4, in peripheral blood mononuclear cells (PBMC) in response to killed bacteria were analyzed by real-time PCR. In comparison with the control, titers of serum antibody specific to the three *S. aureus* strains were significantly ( $p < 0.05$ ) increased.

Addition of CpG ODNs into the vaccine did not enhance the production of antibodies. However, PBMC from cows immunized with CpG ODNs as the adjuvant had a significantly increased expression of IFN- $\gamma$  (11 v.s. 4 folds) and decreased expression of IL-4 (2 v.s. 10 folds) at the transcriptional level. Results indicated that inclusion of CpG ODNs as the adjuvant in an inactivated mastitis vaccine can enhance Th1 type immune responses, which might be beneficial to the elimination of bacteria by phagocytes.

**Key Words:** vaccine, CpG, mastitis

**T15 Intramammary glucocorticoid treatment during LPS-induced mastitis.** O. Wellnitz, M. Saudenowa, and R. M. Bruckmaier\*, *University of Bern, Vetsuisse Faculty, Veterinary Physiology, Bern, Switzerland.*

Therapeutically used glucocorticoids have dose-dependent effects on the immune system. Glucocorticoids such as prednisolone (Pred) are traditionally added to antibiotic intramammary injectors aiming to support the cure of the inflamed mammary gland. The goal of the study was to evaluate the effects of Pred at the dosage commonly used in intramammary injectors on the immune system of the mammary gland and to evaluate the influence of Pred on the mammary immune response to *E. coli* lipopolysaccharide (LPS) stimulation. Five healthy lactating dairy cows with quarter somatic cell counts (SCC) below  $120 \times 10^3$  cells/mL were intramammarily infused with either Pred (10 mg), LPS (100  $\mu$ g), Pred+LPS, or saline solution (9 g/l) in one out of four quarters, respectively. Udders were completely emptied by machine milking every 12 h. SCC of each quarter, tumor necrosis factor alpha (TNF) in milk, and lactate dehydrogenase (LDH) in milk were measured at 0, 3, 6, 9, 12, 24, and 36 h. mRNA expression of TNF, interleukin (IL)-1beta, IL-8, IL-10, and lactoferrin (LF) were measured in milk cells at 0, 12, 24, and 36 h using qRT-PCR. Differences between treatments were considered significant if  $P < 0.05$ . SCC increased in LPS stimulated quarters independent of Pred within 6 h until the end of the experiment. TNF milk concentrations increased immediately after LPS stimulation independent of Pred lasting until the 12 h milking. Milk LDH was elevated at the 9 h sample in the LPS quarters and at the 12 to 36 h samples in the LPS+Pred quarters. SCC, TNF, and LDH remained unchanged in the control quarters and in the Pred treated quarters. mRNA expression of TNF, IL-1beta, IL-8, IL-10, and LF increased in LPS treated quarters independent of the presence of Pred. No changes in mRNA expression of these factors in milk cells were observed in controls and Pred treated quarters. In conclusion, stimulation of udder quarters with LPS had a pronounced effect on the mammary immune response. The investigated parameters responded to LPS as typically expected. Based on the measured parameters no immune modulating effects of Pred were observed in healthy udder quarters despite a slightly delayed LDH response.

**Key Words:** prednisolone, mammary immunity, cow

## Breeding and Genetics: Dairy Cattle Breeding II and Rabbit Breeding

**T16 Ketosis – Manageable by breeding strategies?** F. Rehbock<sup>1</sup>, G. Freyer<sup>2</sup>, F. Klug<sup>3</sup>, and N. Vukasinovic\*<sup>4</sup>, <sup>1</sup>Landesforschungsanstalt für Landwirtschaft und Fischerei M-V, Institut für Tierproduktion, Dummerstorf, Germany, <sup>2</sup>FBN, Unit Genetics and Biometry, Dummerstorf,

Germany, <sup>3</sup>Alexandrastr. 4, Graal-Müritz, Germany, <sup>4</sup>Newsham Choice Genetics, STL Research Center, Chesterfield, MO.

Improving health and fertility is an economic prerequisite for increasing longevity and life performance in dairy cows. According to the literature, ketosis has been reported to be the cause for 9 to 26% reduction