

parlor milking. Furthermore, milk yield is increased in these cows from the increased milking frequency. In contrast, unexperienced cows need intensive adaptation to the AMS.

**Key Words:** Automatic milking, Milk yield, Dairy cow

**M50 Use of digital pictures to study udder morphology in dairy sheep.** M. Rovai<sup>1</sup>, D. L. Thomas<sup>1</sup>, Y. M. Berger<sup>1</sup>, and G. Caja<sup>2</sup>, <sup>1</sup>University of Wisconsin-Madison, <sup>2</sup>Universitat Autònoma de Barcelona, Bellaterra, Spain.

Ewe udder shape and size are related to milk yield and milking time, and culling for undesirable udder traits can improve the efficiency of machine milking. Measurements from digital pictures of ewe udders may provide an easy and accurate method for measuring ewe udders. Udder traits were measured on 120 dairy ewes and from digital pictures of their udders taken at the time of the in vivo measurements. Measurements were taken at wk 5, 11, and 17 of lactation 4 hr before the pm milking. Ewes were milked 2X/d. Udder height, udder width, teat length, teat angle, and cistern height were measured in vivo using a ruler and protractor. Udders also were assigned scores from the 9-point scoring system developed by De la Fuente et al. (1995) for teat size, teat angle, udder height, and udder shape. Following in vivo scores and measurements, digital pictures of the rear udder of each ewe were taken. While taking each picture, a ruler was held parallel to the ground in the same vertical plane as the back of the udder and a few cm below the bottom of the udder to serve as a calibration device for measurements on the digital pictures. Likewise a plumb bob was suspended vertically in back and in the middle of the udder while taking each picture to give a true vertical line as a reference for measuring teat angle. Measurements from the digital pictures were obtained using the public domain software, Image Tool from Texas University, available on the Internet. All digital measurements were significantly ( $P < 0.0001$ ) correlated with those measured in vivo. Correlations were 0.73 for udder height, 0.67 for udder width, 0.47 for teat length, 0.88 for teat angle, 0.68 for teat size score, 0.79 for teat angle score, 0.88 for udder height score, and 0.89 for udder shape score. Advantages of the digital picture method over in vivo measurements are that pictures can be taken faster than the in vivo measurements at the farm, they can be analyzed at your convenience, and they provide a permanent record for future use.

**Key Words:** Digital pictures, Dairy sheep, Udder traits

**M51 Udder traits of dairy ewes on U.S. commercial farms and their effects on milk yield.** M. Rovai<sup>1</sup>, D. L. Thomas<sup>1</sup>, Y. M. Berger<sup>1</sup>, and G. Caja<sup>2</sup>, <sup>1</sup>Univ. of Wisconsin-Madison, <sup>2</sup>Univ. Autònoma de Barcelona, Spain.

Rapid scoring systems have been developed in Europe to categorize udder shapes of dairy ewes, and these scores are related to milk yield and milking time. These scoring systems were evaluated in U.S. ewes of dairy-meat crosses. Ewes were scored by one classifier 3 hr before the pm milking using a linear udder scoring system (De la Fuente et al., 1995) based on a 9-point scale per trait: udder depth, teat angle, and udder shape. Udders also were scored for typology (Gallego et al., 1983): 1 = horizontal teats, 2 = teats at 45 degrees, 3 = vertical teats, and 4 = misshaped udder. Ewes were from two commercial dairy sheep farms (A, n = 177; B, n = 166) and one university farm (C, n = 120). Ewes were milked 2X/d. Flocks differed in breed composition: A: East

Friesian (EF) crosses (10 to 50% EF); B: EF crosses (10 to 75% EF); and C: EF (50 or 75% EF), 50% Lacaune (LC), and 25% EF-50% LC (EF-LC) crosses. Remainder of breeding was of non-dairy domestic breeds. Effects of parity and stage of lactation also were considered. Percentage of EF breeding had no effect on milk production or udder trait scores in farms A and B. In farm C, LC ewes had the most ( $P < 0.01$ ) horizontal teats, and EF-LC ewes had the highest ( $P < 0.0001$ ) milk yield. Milk production ( $P < 0.05$ ) and udder depth score ( $P < 0.001$ ) increased significantly as parity number increased in all farms. Udder depth score and milk production decreased ( $P < 0.05$ ) through lactation in farms A and B. Udder depth scores did not change with stage of lactation in farm C. Ewes in later lactation tended to have more horizontal teats and faulty (type 4) udders than ewes in earlier lactation. Udders of type 2 were more frequent than other types in all flocks. Farm C also had a high percentage of ewes with udders of type 1 which may be related to LC ewes. Within all genotypes and farms, positive correlations were observed between udder depth scores and milk yield (0.20 to 0.46;  $P < 0.05$ ). High correlations were observed between udder shape and teat angle scores (0.80 to 0.93;  $P < 0.0001$ ).

**Key Words:** Dairy sheep, Udder traits, Linear scores

**M52 Udder traits of U.S. dairy ewes and their effects on milking time and milk yield.** M. Rovai<sup>1</sup>, D. L. Thomas<sup>1</sup>, Y. M. Berger<sup>1</sup>, and G. Caja<sup>2</sup>, <sup>1</sup>Univ. of Wisconsin-Madison, <sup>2</sup>Univ. Autònoma de Barcelona, Spain.

Udder shape and size is related to milk yield and milking time in specialized dairy sheep breeds in Europe. This study determined if similar relationships exist among U.S. dairy-meat cross ewes. Ewes (n=120) of 4 breed groups (50% East Friesian, EF; 75% East Friesian, EE; 50% Lacaune; LC; and 25% East Friesian-50% Lacaune crosses, EF-LC; remainder breeding of each group was domestic non-dairy breeds) were utilized. Ewes were milked 2X/d. Measurement of udder size (depth, height, width, and circumference), teat size (length and width), teat angle, and cistern height was done 6 hr after the am milking by one technician at wk 5, 11, and 17 of lactation. Cistern area by ultrasonography and kinetics of milk emission (lag time, volume the 1st minute, total volume, and milking time) also were measured. Cisternal scans were obtained by a portable ultrasound scanner with 3.5 MHz sectorial transducer. Milk yield was highest ( $P < 0.0001$ ) in EF-LC ewes, increased ( $P < 0.001$ ) with age, and decreased ( $P < 0.0001$ ) through lactation. LC ewes had the shortest ( $P < 0.01$ ) teats (3cm) and highest ( $P < 0.0001$ ) teat insertion (59). Cistern height and udder size were larger ( $P < 0.05$ ) in LC and EF-LC ewes than in the other two breed groups. EE and EF-LC dairy ewes had greater ( $P < 0.001$ ) cistern area (30 and 32 cm<sup>2</sup>) than ewes of the other two breeds. Udder and teat size increased ( $P < 0.001$ ) with parity. Udder size decreased ( $P < 0.0001$ ) through lactation while teat angle and cistern height increased ( $P < 0.0001$ ). Cistern area decreased through lactation ( $P < 0.0001$ ) and increased ( $P < 0.0001$ ) with parity. Total milking time was greatest ( $P < 0.05$ ) in EF-LC ewes, increased ( $P < 0.05$ ) with parity, and decreased ( $P < 0.05$ ) during lactation. Udder traits correlated with daily milk yield ( $r = 0.21$  to  $0.50$ ;  $P < 0.01$ ) and milking kinetics ( $r = 0.15$  to  $0.38$ ;  $P < 0.05$ ). Cisternal area correlated with daily milk yield ( $r = 0.63$ ;  $P < 0.0001$ ), milk volume during the 1st minute ( $r = 0.34$ ;  $P < 0.0001$ ), measures of teat size ( $r = 0.18$  to  $0.25$ ;  $P < 0.01$ ), and udder height ( $r = 0.20$ ;  $P < 0.01$ ).

**Key Words:** Dairy ewes, Udder traits, Milking kinetics

## Animal Health

**M53 Binding of IgM to non-apoptotic bovine blood neutrophils.** S. N. Knight\*, M. Worku, and P. L. Matterson, NC Agricultural & Technical State University, Greensboro, NC.

Receptors for IgM have been identified on bovine neutrophils. The objective of this study was to evaluate the association of IgM binding with apoptosis of bovine blood neutrophils. A modified assay to detect apoptosis by comparing the effect of actinomycin-D (160 $\mu$ M), sodium butyrate (160 $\mu$ M), E.Coli lipopolysaccharide (LPS) (10ng/ml) treatments versus untreated isolated neutrophils in the presence or absence of purified bovine IgM was used. Whole blood was collected from healthy, lactating Holstein cows (N=4) in 15 ml vacutainer blood collection tubes pretreated with 250 IU of heparin sodium. The blood was pooled, diluted with 1X PBS, separated by gentle centrifugation and RBC were

lysed with 0.83% ammonium chloride several times until a white pellet and clear supernatant was obtained. Viable, isolated PMN were verified by microscopic observation and counting, using Trypan Blue exclusion for viability (98.0%) and Wright stain differentials. Treated and control PMN were spotted onto poly-L-Lysine, subbed slides. After drying, slides were then assayed for the apoptosis using Promega's Apoptosis Detection Kit which is based on the TUNEL method of labeling fragmented DNA of apoptotic cells with Fluorescein. The percentage of cells incorporating green fluorescence was evaluated microscopically. Neutrophil isolation, Actinomycin D and Dexamethasone induced apoptosis. Bacterial endotoxin, Sodium butyrate and IgM binding showed the least amount of apoptosis. Treatment with IgM had no effect on apoptosis

due to treatment. The Fc receptor for IgM may serve as a marker of non-apoptotic neutrophils.

**Key Words:** Neutrophils, Bovine, Apoptosis

**M54 Dissociation of glucocorticoid and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) responses to repeated endotoxin (LPS) challenges: effects of individual versus group penning.** S. Kahl\* and T.H. Elsasser, *USDA, Agricultural Research Service, Beltsville, MD.*

The development of effective intervention strategies to limit overproduction of proinflammatory cytokines during immune challenge depends on an accurate assessment of how animal-to-animal variability influences the interpretation of data and subsequent conclusions. Our objective was to determine the effect of two consecutive LPS challenges (LPS1 and LPS2, 5 d apart; 0.2  $\mu$ g/kg BW, i.v., *E.coli* 055:B5,) on plasma TNF- $\alpha$  and cortisol (C) responses in heifers kept in individual or group pens before and during the challenge. Forty two heifers (309  $\pm$  4 kg) were fed a forage-concentrate diet (15% CP) to appetite and assigned to individual (IND, n = 32) or group (GRP, n = 10) pens. For LPS challenges and each blood collection, GRP heifers were moved from a group pen to a semicircular holding pen leading to the squeeze chute. The total moving distance was less than 50 m. In IND heifers LPS challenges and blood collection were performed in each animal's assigned individual pens, loosely haltered, without animal transfer. Indwelling Teflon™ jugular catheters were implanted one d prior to challenge. For each challenge, blood samples were obtained at 0, 1, and 2 h relative to LPS injection. The primary response to LPS challenge was measured as area under the time  $\times$  concentration curve (AUC, ng/ml  $\times$  h). Overall mean plasma TNF- $\alpha$  and C responses were lower after LPS2 than LPS1 (respectively, 2.64 vs 4.57,  $P < 0.01$  and 48.2 vs 54.8,  $P < 0.05$ ). However, TNF- $\alpha$  responses were greater in IND than GRP heifers both after LPS1 (5.20 vs 3.92,  $P < 0.05$ ) and LPS2 (3.93 vs 1.35,  $P < 0.01$ ). There were no differences in C responses between IND and GRP heifers in both LPS1 and LPS2 (52.3 vs 50.8,  $P > 0.05$ ), although initial plasma C concentrations at 0 h of LPS2 were higher in GRP than IND heifers (10.8 vs 3.1 ng/ml,  $P < 0.01$ ). Results indicate that handling and management of heifers prior to and during acute phase response (APR) to LPS challenge affect the magnitude of proinflammatory cytokine release as modeled with TNF- $\alpha$ . The data also suggest an animal management-related dissociation between glucocorticoid and TNF- $\alpha$  response during APR that compromises interpretation of the degree to which LPS tolerance develops.

**Key Words:** Cortisol, Endotoxin, Tumor necrosis factor- $\alpha$

**M55 Effects of age at transport on health and development of neonatal dairy calves.** T. A. Johnson\*<sup>1</sup> and S. D. Eicher<sup>2</sup>, <sup>1</sup>*Purdue University, West Lafayette, IN*, <sup>2</sup>*USDA-ARS, West Lafayette, IN.*

Stress associated with transportation at an early age can have immunological effects as well as effects on performance. The purpose of this study was to evaluate the effects of age at transport on immune development. Holstein calves (n=47) were randomly assigned to treatments by day of transport; 2-3 d (A), 4-5 d (B), or 6-8 d (C) within a completely randomized design. Colostrum was administered to each calf within 24 h of birth followed by two equal feedings a day of all milk replacer (4 L/d) and offered ad libitum grain-based dry feed. Calves were transported (6h) and then placed in outdoor individual hutches for the remainder of the study. The mean plasma protein was 6.38 dl ( $\pm$  0.70) at 2 d-of-age. Blood samples, obtained by jugular venipuncture, were collected pre- and post-transport then on d 7, 14, 21, 28, 35, and 42. Mixed model procedures of SAS were used to analyze the data as a repeated measure. Hematocrit percents of peripheral blood were different over wk ( $P < .01$ ). Both granulocyte counts and white blood cell counts increased ( $P < .05$ ) during the first 2 wks, but remained constant throughout the study. At wk1, groups A and B had more cells positive for CD18 than group C ( $P < .05$ ). Group A had greater phagocytosis in wk 1 through wk 3 and at wk 6 compared to group C ( $P < .01$ ). Both chemiluminescence and phagocytosis increased over time ( $P < .01$ ), but were not different among treatments. Plasma fibrinogen was different among treatments ( $P < .01$ ), but no time effect or interaction was detected. Group A had greater fibrinogen concentration than group C on d 0 ( $P < .05$ ) and at wk 2 ( $P < .01$ ). Group A also had a greater fibrinogen concentration than group B on d 0 ( $P < .01$ ) and group B tended to

have greater concentrations than group C at wk 5 ( $P < .10$ ). This study indicated that age at transport primarily impacts the health of a dairy calf during the first few weeks of age, however differences after that are minimal.

**Key Words:** Dairy calves, Transport, Stress

**M56 Carbadox does not alter immune cell phenotypes in mesenteric lymph nodes of pigs challenged with *Salmonella enterica* serotype Typhimurium.** K. A. Skjolaas, T. E. Burkey, M. R. Barker, S. S. Dritz, and J. E. Minton\*, *Kansas State University.*

Carbadox is widely used in nursery pig diets for growth promotion, but the mechanism of action of this class of antibiotics has not been thoroughly elucidated. One action of carbadox could be to change pathogen load in the lower gut and thereby alter populations of immune cells in mesenteric lymph nodes of pigs. Weaned pigs were housed in an environmentally controlled nursery and fed diets containing no added antibiotic (n = 8) or carbadox at 55 ppm (n = 8). No other antimicrobials were included in the diets. Pigs were fed their respective treatment diets for 14 d, then all pigs were challenged orally with 10<sup>6</sup> CFU *Salmonella enterica* serotype Typhimurium. Samples of mesenteric lymph nodes draining the terminal jejunum and ileum were obtained from all animals at sacrifice 14 d after bacterial challenge (after 28 d on experimental diets). Lymph nodes were disrupted mechanically to obtain single cell suspensions. Cells were prepared for flow cytometric analysis with primary antibodies to cell surface antigens, followed by FITC-labeled secondary antibodies. Primary antibodies recognizing CD4, CD8, B cell, and granulocyte/monocyte surface antigens were used. The proportion of lymphocyte-gated cells positive for CD4 (29.0  $\pm$  2.7 and 31.6  $\pm$  2.7), CD8 (24.0  $\pm$  1.4 and 24.5  $\pm$  1.4), and B cell (64.9  $\pm$  5.3 and 56.7  $\pm$  5.3) markers did not differ between pigs fed carbadox and control diets, respectively. Similarly, proportions of gated cells labeled with the granulocyte/monocyte marker did not differ between carbadox (85.4  $\pm$  4.5) and control (86.3  $\pm$  4.5) treatments. The results suggest that dietary carbadox, at levels commonly used for growth promotion in swine nursery diets, does not affect major immune cell populations in mesenteric lymph nodes following challenge with an enteric pathogen.

**Key Words:** Carbadox, Pig, Immune cells

**M57 Effects of conjugated linoleic acid (CLA) and trans-C<sub>18:1</sub> fatty acids (TFA) on production variables and immune indices following castration in beef cattle.** L. H. Baumgard\*<sup>1</sup>, C. E. Moore<sup>1</sup>, C. R. Baily<sup>1</sup>, M. BenAbdallah<sup>1</sup>, P. S. Cuneo<sup>1</sup>, S. Dial<sup>1</sup>, D. Luchini<sup>2</sup>, and G. C. Duff<sup>1</sup>, <sup>1</sup>*The University of Arizona, Tucson*, <sup>2</sup>*BioProducts Inc., Fairlawn OH.*

Feeding CLA alleviates the growth-suppressing effects (cachexia) caused by an activated immune system in rodent models. Larger animals often experience cachectic symptoms (decreased feed efficiency and reduced ADG) immediately (7-14 d) post-castration. Growing male beef cattle (n=30, 359 60 kg BW) were fed isoenergetic diets (steam-flaked sorghum based) supplemented (top dressed) with rumen protected (RP) palm oil (559 g/d; EnerGII® [EII]; control), RP TFA (594 g/d) or RP CLA (609 g/d) from #7 to 28d relative to castration. Each treatment provided 475 g lipid/d and RP TFA consisted of 17.2% *trans*6-8, 8.7% *trans*-9, 8.8% *trans*-10, 5.8% *trans*-11 and 7.3% *trans*-12 C<sub>18:1</sub> and the RP CLA contained 6.5% *cis*-9, *trans*-11, 5.4% *c/t*-8, *c/t*-10, 8.25% *c/t*-11, *c/t*-13 and 7.9% *trans*-10, *cis*-12 CLA. All bull calves were weighed on d #7, 0, 3, 6, 9, 12, 15, 21, 28 and blood collected on d #7, 0, 3, 6 and 9 relative to castration. On d 0 testicles were banded with latex tubing and scrotums surgically removed on d 3 (post-banding). During wk 1 and 2 post-castration all animals had reduced DMI (7.8%), lost BW (ADG, -0.69 kg/d) and reduced feed efficiency (G:F, -0.15) and there was no treatment by wk interaction on these measurements. Overall (d-7 to 28), CLA supplementation decreased DMI ( $P = 0.04$ ; 7.6, 7.4 and 6.1 kg/d for EII, TFA and CLA, respectively) and did not effect G:F (0.013) or ADG (0.17 kg/d). Compared to d 0, body temperature on d 3 and 6 were elevated ( $P < 0.05$ ) by 0.54 and 0.19C. Castration had little effect on total white blood count, monocytes and eosinophils. but neutrophils were reduced ( $P < 0.001$ ) 23%, lymphocytes increased 10%, basophils increased 266% and the neutrophil:lymphocyte ratio decreased 29% on d 9 (post-castration). Treatment had no effect on aforementioned immune variables. Unique fatty acids evaluated in this trial were ineffective at

preventing the negative side effects on production immediately following castration.

**Key Words:** CLA, Castration, Beef cattle

**M58 Suppression of Th1-like BoCD4<sup>+</sup> T lymphocyte proliferative response by BoCD8<sup>+</sup> T lymphocytes stimulated with staphylococcal enterotoxin C is induced by type II cytokines.** Y. H. Park\*<sup>1</sup>, W. A. Ferens<sup>2</sup>, W. C. Davis<sup>3</sup>, J. S. Ahn<sup>4</sup>, N. H. Kwon<sup>1</sup>, and G. A. Bohach<sup>2</sup>, <sup>1</sup>Seoul National University, Seoul, Korea, <sup>2</sup>University of Idaho, Moscow, USA, <sup>3</sup>Washington State University, Pullman, USA, <sup>4</sup>National Veterinary Research and Quarantine Services, Anyang, Korea.

Staphylococcal isolates from bovine mastitis often produce superantigen (SAg) exotoxins. We previously demonstrated that the SAg staphylococcal enterotoxin C (SEC) leads to an inversion of the CD4:CD8 T cell ratio and generation of an atypical CD8<sup>+</sup> T-cell subpopulation. In the present study, we examined T cell proliferation and apoptosis profiles of subpopulations of bovine peripheral blood mononuclear cells (PBMC) in cultures stimulated with SEC. DNA synthesis in cultures stimulated with SEC was low during the first four days and increased greatly on day 5. In contrast, DNA synthesis in concanavalin A (ConA) stimulated cultures increased continuously from day 1 through day 5. SEC stimulated cultures showed exceed apoptosis of CD4<sup>+</sup> T cells at early stage and predominant proliferation of CD8<sup>+</sup> T cells at late stage. Type II cytokines were predominantly transcribed at late stage of culture. While transcription of type I cytokines reached peak, but low level compared with ConA stimulated PBMC. Our results suggest that SEC promotes *Staphylococcus aureus* survival by induction of a specific subset CD8<sup>+</sup> T cells and suppression of CD4<sup>+</sup> T cells may be via type II cytokines in CD8<sup>+</sup> T cells.

**Key Words:** Staphylococcal enterotoxin C, Bovine T cells, Cytokine mRNA

**M59 Increased levels of LPS-binding protein (LBP) in bovine blood and milk following bacterial lipopolysaccharide challenge.** D. Bannerman\*<sup>1</sup>, M. Paape<sup>1</sup>, W. Hare<sup>1</sup>, and E. J. Sohn<sup>2</sup>, <sup>1</sup>USDA-ARS, Beltsville, MD, <sup>2</sup>University of Maryland, College Park, MD.

Approximately 40% of the clinical cases of mastitis that occur annually are caused by Gram-negative bacteria. The most common Gram-negative pathogens implicated in mastitis are *Escherichia coli*, *Klebsiella pneumoniae*, and various species of *Enterobacter*. A common denominator to all of these bacteria is the presence of endotoxin or lipopolysaccharide (LPS), which is found in the outer membrane of all Gram-negative bacteria. LPS is a highly pro-inflammatory molecule that is shed from the bacterial surface during bacterial replication or death. The bovine mammary gland is highly sensitive to LPS, and LPS has been implicated, in part, in the pathogenesis of Gram-negative mastitis. Recognition of LPS is a key event in the innate immune response to Gram-negative infection and is mediated by the accessory molecules CD14 and LPS-binding protein (LBP). Previous studies have demonstrated an increase in soluble CD14 in milk following intramammary challenge with LPS. The objective of the current study was to determine whether LBP levels increased in the blood and mammary gland following LPS challenge. The left and right quarters of five mid-lactating Holstein cows were challenged with either saline or LPS (100 ug), respectively, and milk and blood samples collected. Basal levels of plasma and milk LBP were 38 and 6 ug/ml, respectively. Plasma LBP levels increased as early as 8 h post-LPS challenge and reached maximal levels of 138 ug/ml by 24 h. Analysis of whey samples derived from LPS-treated quarters revealed an increase in milk LBP by 12 h. Similar to plasma, maximal levels of milk LBP (34 ug/ml) were detected 24 h following the initial LPS challenge. These data suggest a possible role for LBP in mediating mammary gland response to LPS.

**Key Words:** Mastitis, Endotoxin, LPS-binding protein

**M60 Establishment of a bovine cell-culture system to study the genomic response of mammary epithelial cells to infection with *Staphylococcus aureus*.** O. Wellnitz\* and D. E. Kerr, University of Vermont, Burlington, VT.

A cell-culture system was developed to study changes in gene expression during mammary epithelial cell infection. Primary cell cultures from three healthy Holstein cows were prepared, passaged twice, and frozen in liquid nitrogen until infection experiments. Cryopreserved cells were thawed, grown in plastic culture flasks, then split once into 6-well plates. After 24 h cells were infected with  $2 \times 10^8$  cfu/well of *Staphylococcus aureus*. Sterile, mock-infected plates were included as controls. Three hours post-infection the extracellular bacteria were removed by changing the medium and addition of gentamicin (100µg/ml). Cells were grown for another 21 h after which medium and total RNA were harvested. Lactoferrin concentrations in conditioned medium, measured by ELISA, were  $1.2 \pm 0.1 \mu\text{g/ml}$  and  $2.3 \pm 0.4 \mu\text{g/ml}$  in sterile or infected cells, respectively ( $P=0.07$ ). Lactoferrin mRNA expression, as detected by northern blot analysis, was  $1.8 \pm 0.3$  fold higher ( $P<0.05$ ) in infected cells compared to sterile cells. Tumor necrosis factor alpha (TNFα) mRNA expression was detected by quantitative RT-PCR using SYBR Green. The TNFα response to the infection protocol was variable, being numerically increased 43, 22 and 2 fold as compared to the mock-infected cells ( $P>0.05$ ). The analysis of lactoferrin and TNFα were chosen to detect an infection response, because bovine lactoferrin concentrations in milk often increase during mastitis and the cytokine TNFα is known to play an important role in inflammatory processes. The increase of lactoferrin expression in cells after infection establishes a direct connection between infection and epithelial cell lactoferrin production and reflects the increased milk lactoferrin concentrations seen in mastitis. The biological replication from simultaneous analysis of cells from different animals is an advantage over experiments with an immortalized cell line. The current model provides substantial quantities of RNA (> 100µg/plate) that will be useful for techniques such as microarray analysis.

**Key Words:** Mastitis, Cell culture, Gene expression

**M61 Results of bovine mastitis target pathogen susceptibility monitoring program for 2001.** C. J. Lindeman\*, E. S. Portis, and S. A. Salmon, Pharmacia Animal Health.

A bovine mastitis pathogen susceptibility monitoring program was initiated by Pharmacia Animal Health (PAH) in 2001 to monitor *in vitro* activity of ceftiofur, pirlimycin, a lincomycin/neomycin combination, and a penicillin/novobiocin combination. Minimum inhibitory concentrations (MICs) were determined for 354 bacterial strains isolated from bovine mastitis cases at 6 veterinary diagnostic laboratories in the US and Canada. Isolates were forwarded to PAH for MIC determinations using a commercially available broth microdilution system that conforms to National Committee for Clinical Standards (NCCLS) guidelines. MIC<sub>90</sub> values were calculated from data obtained for the bacterial strains that were received. Ceftiofur was the only compound tested which exhibited consistent activity against both gram-negative and gram-positive pathogens tested, with the exception of the enterococci. Penicillin/novobiocin showed excellent activity against the gram-positive organisms and was highly active when tested against the enterococci. Lincomycin/neomycin showed excellent activity against *S. agalactiae*, *S. aureus* and other *Staphylococcus* spp. Pirlimycin was also active against the staphylococci and streptococci. Minimum Inhibitory Concentrations (MIC<sub>90</sub>) for Antimicrobial Agents Against Organisms Isolated from Cases of Bovine Mastitis

Organism (No. tested)	Ceftiofur	Lincomycin/ Neomycin	Penicillin/ Novobiocin	Pirlimycin
<i>Streptococcus agalactiae</i> (20)	≤0.06	≤0.06	≤0.06	0.12
<i>Streptococcus dysgalactiae</i> (32)	≤0.06	0.12	≤0.06	≤0.06
<i>Streptococcus uberis</i> (17)	0.12	64.0	0.12	8.0
<i>Streptococcus</i> spp. (25) <sup>a</sup>	≤0.06	8.0	0.12	1.0
<i>Enterococcus</i> spp. (15)	>64.0	16.0	1.0	16.0
<i>Staphylococcus aureus</i> (68)	1.0	0.5	≤0.06	0.25
<i>Staphylococcus</i> spp. (88)	1.0	.25	≤0.06	0.5
<i>Escherichia coli</i> (63)	0.5	>64.0	32.0	>64.0
<i>Klebsiella</i> spp. (19)	0.5	2.0	32.0	>64.0
Other Gram-negative bacilli (7) <sup>c</sup>	** <sup>b</sup>	**	**	**

<sup>a</sup>Includes *Streptococcus* spp. (23), *Lactococcus* spp. (2) <sup>b</sup>MIC<sub>90</sub> not calculated for organisms with less than 10 isolates <sup>c</sup>Includes *Pasteurella* spp. (2), *Citrobacter* sp. (1), *Enterobacter* sp.(1), *Serratia* sp. (1), *Acinetobacter* sp.(1), *Pseudomonas* sp. (1)

**Key Words:** Mastitis pathogen, Minimum inhibitory concentrations, Susceptibility

**M62 Comparison of Petrifilm<sup>TM</sup> with standard and augmented culture techniques for the isolation of pathogens on milk samples.** B. O. Silva, D. Z. Caraviello\*, and P. L. Ruegg, *University of Wisconsin - Madison.*

The objective of this study was to compare the 3M<sup>TM</sup> Petrifilm<sup>TM</sup> with different methods of isolation of udder pathogens from milk samples. Composite (n = 29) and quarter (n = 362) milk samples were collected. Staph express Petrifilm<sup>TM</sup> and Enterobacteriaceae Petrifilm<sup>TM</sup> were compared with standard and augmented culture techniques for isolation of *Staphylococcus aureus* and coliforms respectively. Standard culture technique consisted of streaking 0.01 ml of milk on sheep blood agar and processing samples using NMC procedures. Augmented culture techniques consisted of samples processed using centrifugation (5 ml centrifuged at 2000 x G for 15 min) or incubation (18 h at 37°C) and plated similar to the standard method. *S. aureus* was isolated from 5.4%, 6.1%, 6.9% and 7.7% of samples for standard, centrifuged, incubated and Petrifilm<sup>TM</sup>, respectively. Coliforms were isolated from 10.5%, 9.4%, 17.4% and 15.7% for standard, centrifuged, incubated and Petrifilm<sup>TM</sup>, respectively. Overall, 8.2% of the samples were positive for *S. aureus* and 20.2% of the samples were positive for coliforms. Test characteristics were determined using the number of isolates positive by any method as the gold standard for comparisons. The sensitivity for *S. aureus* was 65.6%, 75.0%, 84.4% and 87.5% for standard, centrifuged, incubated and Petrifilm<sup>TM</sup>, respectively. The sensitivity for coliforms was 52.0%, 46.6%, 86.3% and 78.1% for standard, centrifuged, incubated and Petrifilm<sup>TM</sup>, respectively. Two presumed *S. aureus* isolates from Petrifilm<sup>TM</sup> were determined to be coagulase negative *Staphylococcus* species resulting in a relative specificity of 99.4% for the 3M<sup>TM</sup> Staph Express. The specificity of the 3M<sup>TM</sup> Enterobacteriaceae Count Plate was 100%. According to McNemar's test for paired data, both Petrifilm<sup>TM</sup> tests were significantly different (P < 0.05) from standard technique. Results show a great potential for the use of Petrifilm<sup>TM</sup>, an easy and rapid method, in a herd evaluation program when *S. aureus* and coliforms are the pathogens of interest.

**Key Words:** Mastitis, Petrifilm, Microbiology

**M63 Effect of intramammary infection at calving caused by environmental pathogens on lactation performance, mastitis incidence, and somatic cell counts in lactating Holstein cows.** S. O. Juchem\*<sup>1</sup>, L. G. Corbellini<sup>2</sup>, K. N. Galvao<sup>1</sup>, J.E.P. Santos<sup>1</sup>, and M. Villaseñor<sup>1</sup>, <sup>1</sup>*Veterinary Medicine Teaching and Research Center, University of California - Davis,* <sup>2</sup>*Universidade Federal do Rio Grande do Sul - Brazil.*

An aseptic milk sample composited from all 4 quarters was collected in the first 3 d after calving from 1290 Holstein cows not displaying signs of clinical mastitis. Samples were cultured in blood agar and BHI medium for microbiological analyses at the Milk Quality Laboratory (VMTRC, Tulare). Results were grouped into 5 treatments: no growth (NG), coliforms (COL), coagulase negative *Staphylococcus* spp. (SS), non-agalactiae *Streptococcus* spp. (STC), and a mixed culture of SS and STC (MX). Data from monthly production and clinical mastitis (CM) cases were collected for the first 300 d in milk (DIM). Diagnosis of CM was performed at every milking by the herd personnel. Continuous and binomial data were analyzed using, respectively, the MIXED and the LOGISTIC procedures, and number of mastitis cases per cow by the GENMOD procedure of SAS (2001). Interval from calving to first CM case was analyzed by the Survival Analysis procedure of MINITAB (2000). Results are shown according to the following order: NG, COL, SS, STC and MX. Yields (kg/d) of milk (37.3 vs 34.3; P<0.05) and 3.5% fat-corrected milk (37.6 vs 34.2; P<0.05) were lower for STC than NG, but they did not differ among the other groups. Fat and true protein in milk was similar for all 5 groups (P>0.15) and they averaged 3.6 and 3.2%, respectively. Similar to yields of milk, fat and true protein production was higher for NG than STC cows (1.32 vs 1.2 and 1.19 vs 1.09 kg/d; P<0.05). Linear SCC scores (1.8 vs 2.4 vs 2.5 vs 3.3 vs 2.7; P<0.01) were higher for STC, but not different from MX. Cows in NG had lower incidence of CM during lactation (11.4 vs 31.3 vs 21.7 vs 43.2 vs 28.6%; P<0.01). The mean number of CM cases per cows during lactation was lower for NG than the other groups (0.04 vs 0.6 vs 0.26 vs 1.54 vs 0.55; P<0.01). The interval from calving to the first CM case was affected by bacterial isolate at calving (254 vs 134 vs 248 vs 201 vs 102 d; P<0.01). Intramammary infection with no signs of clinical mastitis in the first 3 d postpartum affects lactation performance, increases linear SCC and occurrence of CM.

**Key Words:** Mastitis, Milk culture, Dairy cows

**M64 Safety and compatibility of Orbeseal<sup>®</sup> during the dry period and early lactation when used in conjunction with commercially-available intramammary dry cow therapies.** R. Hassfurther\*<sup>1</sup>, D. Earley<sup>2</sup>, and N. A. Evans<sup>2</sup>, <sup>1</sup>*Pfizer Veterinary Medicine, Terre Haute, IN USA,* <sup>2</sup>*Pfizer Animal Health Group, New York, NY USA.*

Orbeseal<sup>®</sup> is an internal teat sealant designed for use at dry-off. It is an inert viscous paste consisting of bismuth subnitrate in a paraffin base and is aseptically administered at dry off. The sealant mimics the natural keratin plug and provides immediate closure of the teat canal thus helping to prevent intramammary infections during the entire dry period. It is likely the US dairy industry will use Orbeseal<sup>®</sup> in combination with intramammary dry cow antibiotics. The current study examined the safety and compatibility of Orbeseal<sup>®</sup> when administered concurrently with any 1 of 4 commercial dry cow antibiotics. Thirty cows were treated at dry off with Orbeseal<sup>®</sup> or a dry cow antibiotic in conjunction with Orbeseal<sup>®</sup> in each quarter. Systemic observations and gland assessments were made throughout the dry period and post-calving in order to evaluate product compatibility. Bismuth subnitrate is radiopaque, and radiographs of teats were taken throughout the dry cow period and at calving to evaluate the presence of the plug. At calving, foremilk was stripped and physical presence of Orbeseal<sup>®</sup> was determined. Milk production was measured for 20 days post-calving and composite milk samples were collected to investigate bismuth levels. Orbeseal<sup>®</sup> did not compromise gland health and was physically compatible with licensed dry cow antibiotic therapies. The radiographs and the physical presence of the seal at calving support evidence that Orbeseal<sup>®</sup> remains intact in the teat cistern until physically removed post calving. Treatment with Orbeseal<sup>®</sup> had no effects on early lactation milk production and no deleterious impact on antibiotic residues in milk. Collectively, these data demonstrate that Orbeseal<sup>®</sup> is a safe and reliable dry cow product when used alone or in conjunction with licensed dry cow antibiotics.

**Key Words:** Orbeseal<sup>®</sup>, Dry Cow, Teat Sealant

**M65 Test-day milk loss associated with elevated test-day somatic cell score.** R. H. Miller\*, H. D. Norman, G. R. Wiggins, and J. R. Wright, *Animal Improvement Programs Laboratory, Agricultural Research Service, USDA, Beltsville, MD.*

To determine usefulness of current and previous test-day somatic cell score (SCS) in predicting test-day milk yield, test-day records from Holstein first and second calvings between 1995 and 2002 were examined. Initial selection required that cows have at least the first four test days with recorded milk yield and SCS for both parities 1 and 2. Least-squares analyses were conducted for milk yield on test days 2 through 10 within herd and cow. The model included regressions on both current test-day SCS and mean SCS of all previous test days with separate estimates by parity; effects for parity and calving year also were included as well as a regression on days in milk on test day 1. Error degrees of freedom ranged from 143,748 to 214,526. Highest SCS was most often on test day 1 (20%) followed by test day 10 (14%). Ranges of regression

coefficients (kilograms of milk per unit of SCS) are in the table. Effect of current SCS on test-day milk yield was much greater for parity 2 than for parity 1 but only slightly greater than effect of mean of previous test-day SCS on milk yield for parity 1. Milk loss from elevated SCS likely results both from mammary status on test day and from direct and residual influences of elevated SCS earlier in lactation. Mastitis in early lactation appears to have a carryover effect on milk yield for the remainder of the lactation.

Parity	Previous test-day Maximum effect		SCS mean Minimum effect		Current test-day Maximum effect		SCS Minimum effect	
	Coeffi- cient	Test day	Coeffi- cient	Test day	Coeffi- cient	Test day	Coeffi- cient	Test day
1	-0.346	9	-0.142	2	-0.401	10	-0.216	3
2	-0.366	4	0.021	10	-1.209	10	-0.489	4

**Key Words:** Somatic cell score, Test-day milk, Mastitis

## Breeding & Genetics

**M66 Optimising genetic gain in a small population.** A. Karlsen\*, T. Steine, and E. Sehested, *GENO Breeding and A.I. Association.*

Small farms characterize dairy production in Norway. The average herd size in 2002 was 15.3 cows. Although the herd size is increasing, the number of cows has been slowly decreasing over the last years. The Norwegian Dairy Cattle (NRF) population is currently about 300,000 cows. Phenotypic data on health, fertility traits, production (yield and beef), calving information and management has been reported to the Norwegian Dairy Herd Recording System (NDHRS) since 1978, and recordings are compulsory for members. In 2002 96% of the cows were part of the NDHRS. These cows represent the breeding population of NRF. Dams are considered as sire mothers if their total merit index, milk production and pedigree meet the requirements. Every year about 400 bull calves are purchased based on their pedigree information at approximately 3-4 months of age. In the period between 5-12 months of age, they are evaluated for growth rate, conformation and semen quality. The 120 best young bulls are then selected for progeny testing. Several functional traits (fertility, mastitis resistance, other diseases, calving ease and stillbirths) are included in the total merit index. To perform progeny testing for these traits, breeding values are based on 250-300 daughters. After progeny testing the best 10-12 sires are selected as elite sires. Selection is based on total merit index and also on number of sire lines represented and number of close relatives in use. To prevent inbreeding, a restriction is put on the use of sires. The optimum distribution between use of young bulls for progeny testing and elite sires are 40:60 in the NRF population. A computer program is distributed to all farmers that optimise use of young bulls and elite sires in the herd, and suggest the optimum mating combinations. Farmers can download the program freely, or get a breeding plan from the dairy advisor that runs this program on routine basis for the farmers. It is assumed that approximately 90% of the farmers use the breeding plan. Through this breeding scheme a small population is turned into a large breeding population.

**Key Words:** Genetic gain, Small population, Norwegian dairy cattle

**M67 Identification of quantitative trait loci affecting birth and weaning weights in pigs.** J. W. Holl\*<sup>1</sup>, J. P. Cassady<sup>2</sup>, and R. K. Johnson<sup>1</sup>, <sup>1</sup>University of Nebraska, Lincoln, NE, <sup>2</sup>North Carolina State University, Raleigh, NC.

A whole-genome scan was used to identify chromosomal regions and estimate quantitative trait loci (QTL) that affect individual pig birth weight (BWT) and weaning weight (WWT). A three-generation resource population was developed by crossing a randomly selected control line with high-indexing pigs from a line selected for an increased index of ovulation rate and embryonic survival. Phenotypic data were collected on F<sub>2</sub> females, born in three replicates, for BWT (n = 428) and WWT (n = 405). Grandparent, F<sub>1</sub>, and F<sub>2</sub> animals were genotyped for 151 microsatellite markers. Calculations of logarithms of odds (LOD) scores were by least squares. The full model included fixed effects of replicate, sire-dam combination as a polygenic effect, and coefficients for additive and dominance effects as fixed regression coefficients. The reduced model included fixed effects of replicate and sire-dam combination. Genome-wide critical  $\alpha = 0.01$ ,  $\alpha = 0.05$ , and  $\alpha = 0.10$  levels

were established using a permutation approach. There was evidence (P < 0.10) for QTL affecting BWT on SSC8 between markers OPN and SO178 and on SSC12 between markers SO143 and SX957, with additive effects of the allele inherited from the control line of  $-0.020 \pm 0.017$  kg and  $-0.059 \pm 0.019$  kg and dominance effects of  $0.085 \pm 0.031$  kg and  $-0.073 \pm 0.037$  kg, respectively. No QTL were detected for WWT. Knowledge of QTL for BWT should be considered to maintain neonatal survival in selection programs that may indirectly have an adverse affect on BWT.

**Key Words:** Pigs, Quantitative trait loci, Weight

**M68 Detecting quantitative trait loci for twinning and production traits in Holstein dairy cattle.** J. Cruickshank\*<sup>1</sup>, M. R. Dentine<sup>1</sup>, P. J. Berger<sup>2</sup>, and B. W. Kirkpatrick<sup>1</sup>, <sup>1</sup>University of Wisconsin-Madison, Madison, Wisconsin, <sup>2</sup>Iowa State University, Ames, Iowa.

Twinning in dairy cattle has been associated with many negative health and reproductive events that cause economic loss to the producer. Reports have suggested that twinning rates are increasing and that there may be a positive relationship between milk production and twinning frequency. Quantitative trait loci (QTL) for twinning rate on bovine chromosomes 5, 7, 19, and 23 have been previously identified in other populations. The objectives of this study were to detect and confirm the existence and effects of these QTL and to look for QTL for milk yield, fat and protein yield and percent, somatic cell score (SCS), and productive life in those same chromosomal regions. Half-sib families of 25 North American Holstein sires with high twinning rate PTA comprised the population under investigation. This project utilized sire predicted transmitting ability (PTA) values for the production traits from USDA. Twinning rate PTA values were estimated from calving data. DNA extracted from semen samples was analyzed using 45 microsatellite markers across the four chromosomes. Marker heterozygosity of the patriarchs averaged 56%. Evidence of twinning QTL was found in multiple families on chromosomes 5 and 23 at a chromosome-wise p < 0.05. Similarly, evidence of QTL was found on chromosome 7 for milk; chromosomes 5, 7, and 19 for fat yield and fat percent; chromosome 7 for protein yield; chromosome 5 for protein percent; and chromosomes 5, 19, and 23 for SCS. Most of these families are related within three generations and will be combined into larger, multi-generation families for further analysis. For twinning QTL replicated in Holsteins, chromosomal positions will be more narrowly defined by haplotype analysis. Frequencies of haplotypes associated with twinning will be estimated in elite Holstein cow populations.

**Key Words:** QTL, Twinning, Cattle

**M69 Development of three repeat microsatellite loci in Korean cattle.** S. Chen, H. Chung\*, D. Yoon, I. Cheong, and S. Lee, *National Livestock Research Institute, Suwon, Korea.*

Microsatellite, which contained tri repeats, have been isolated in Korean cattle (Hanwoo). The pooled Korean cattle genomic DNA, which was digested with Sau3AI and separated onto agarose gels, was recovered