696 Effect of a micronutrient supplement on the functional capacity of neutrophils harvested from the blood of dairy cows during the periparturient period. X. S. Revelo*, A. L. Kenny, N. M. Barkley, and M. R. Waldron, *University of Missouri, Columbia.*

The objective of this study was to investigate the effect of a micronutrient supplement on the functional capacity of neutrophils (PMNL) isolated from the blood of dairy cows during the periparturient period. Cows received 56 g/day of either OmniGen-AF (n = 8) or sham control (soybean hulls; n = 12) mixed into total-mixed rations from d 46 ± 1 before calving until d 31 after parturition. PMNL were collected from cows on d 49 ± 2, 28 ± 1, 19 ± 1, and 9 ± 1 prepartum and 1, 7, 14, and 30 postpartum to determine their luminol-dependent generation of reactive oxygen species (ROS), formation of extracellular traps (NETs), chemotaxis toward interleukin-8 (IL-8) and antimicrobial capacity against Staphylococcus aureus. There was no effect of dietary OmniGen-AF on any of the parameters used to assess PMNL function ($P \geq 0.20$). In contrast, ROS production and NET release by phorbol 12-myristate 13-acetate (PMA)-activated PMNL changed between days relative to parturition (time effect, $P \leq 0.01$). ROS production increased by 40% between 49 and 19 d prepartum ($P \leq 0.05$) and then declined 63% to reach a lowest level on d 1 postpartum ($P \leq 0.05$). ROS generation by PMA-activated PMNL did not differ and persisted low on d 1, 7 and 14 ($P \geq 0.20$), however, it recovered to a level similar to that found prepartum by d 30 postpartum. NET release by PMA-stimulated PMNL collected from all cows was highest on d 49 prepartum but decreased 26% and 40% by d 28 and 19 prepartum, respectively ($P \leq 0.05$). The expression of NETs by PMNL remained low until d 14 relative to parturition, but slightly increased on d 30 postpartum (25% higher compared with d 19 prepartum, $P \leq 0.05$). There was no effect of day relative to parturition on PMNL chemotaxis toward IL-8 or killing ability against S. aureus ($P \geq 0.05$). These results suggest that impairment of ROS production and release of NETs contribute to the altered PMNL function in periparturient dairy cows.

Key words: neutrophil, immunosuppression, micronutrient supplement

697 Multiple *Mycoplasma* spp. detected in bulk tank milk samples using real-time PCR and conventional culture, and agreement between test methods. D. J. Wilson*[1], A. Justice-Allen[2], J. D. Trujillo[3], and G. Goodell[4], *[1]Utah State University, Logan, [2]Arizona Game and Fish Department, Phoenix, [3]Iowa State University, Ames, [4]The Dairy Authority, Greeley, CO.*

A PCR method and standard culture were both used to test for Mycoplasma spp. in bulk tank milk samples, and results were compared. Bulk tank milk samples (n = 165) from 16 dairy farms that had been found mycoplasma-positive were tested as were 15 samples from a farm previously negative. All samples were cultured on modified Hayflick medium and incubated at 37°C in 10% CO_2. DNA extraction, PCR reaction using SYBR green, and dissociation curve analysis to determine the melt curve and Tm were also performed in triplicate. For selected samples, and samples with Tm different than the M. bovis positive control, bp length was determined by capillary electrophoresis, and DNA sequence of amplicons was compared to known sequences using BLAST to detect multiple *Mycoplasma* spp. A true mycoplasma-positive sample was defined as having at least 1 positive test, whether culture or at least 1 SYBR PCR; specificity was 100% by definition. Test sensitivity was calculated as the number of positive cultures or PCR tests divided by the number of tests performed on true positive samples. The confidence interval for sensitivity of the 2 tests was calculated and evaluated for overlap, and test method agreement was also assessed with a kappa statistic. Results are in the table. Ninety samples were mycoplasma-negative and 90 were positive. Forty-eight samples were positive on all 4 tests (1 culture, 3 PCR). Culture sensitivity = 62/90 = 68.9%; PCR sensitivity (3 tests/sample) = 207/270 = 76.7%; Agreement = 141/180 = 78.3% ($\kappa = 0.54$, moderate agreement); 95% CI overlap indicated comparable sensitivity. Of 52 speciated samples there were 39 *M. bovis* (75%) isolates, 13 *M. alkalescens* (25%), 4 *M. arginini* (8%), 2 *M. gateae* (4%), 1 *M. bovigenitalium* (2%). All 7 double-positive samples included *M. bovis*. The *M. bovigenitalium* was found in 1 tank sample from the herd previously mycoplasma-negative. Both test methods demonstrated useful and comparable sensitivity for detection of mycoplasma in bulk milk.

Table 1. Comparison of mycoplasma culture and PCR results on bulk tank milk

	PCR positive	PCR negative	Total
Culture positive	51	11	62
Culture negative	28	90	118
Total	79	101	180

Key words: mastitis, mycoplasma, PCR

698 Multiple tests based estimates of *Mycobacterium avium* ssp. *paratuberculosis* prevalence in domestic ruminant population suspected for Johne's disease. S. V. Singh*[1], P. K. Singh[1], A.V. Singh[1], B. Singh[1], A. Kumar[1], A. Srivastav[2], S. Gupta[1], H. Singh[1], A. Mittal[1], S. Yadav[2], and J. S. Sohal[1], *[1]Central Institute for Research on Goats, Mathura, Uttar Pradesh, India, [2]College of Veterinary Sciences, Mathura, Uttar Pradesh, India.*

Mycobacterium avium subspecies *paratuberculosis* (MAP), the cause of Johne's disease (JD) is a major animal pathogen worldwide and is endemic in the animal population wherever investigated. Despite low per animal productivity, JD received low priority outside developed countries. JD is endemic in the domestic ruminant population of the country. Planning major initiative on the control knowledge of epidemiology of MAP is essential. Due to lack of "true estimates" on the prevalence of MAP, disease is not a priority for the control in the domestic livestock in the country. In 2010, 3007 samples were submitted to Animal Health Division (Central Institute of Research on Goats, Mathura) for screening against MAP. Samples originated from cattle, buffaloes, sheep and goats suspected for JD from 9 states (Uttar Pradesh, Tamil Nadu, Himanchal Pradesh, Gujarat, Assam, Kerala, Madhya Pradesh, Rajasthan and Punjab). Samples (3007) were screened by microscopy (716 feces), IS900 PCR (183 feces and 510 blood) and "indigenous ELISA kit" (1598 serum). Using microscopy, fecal PCR, blood PCR and indigenous ELISA kit, 51.0, 27.3, 19.6 and 68.4% samples were positive, respectively. With respect to the 4 tests, study showed moderate to high levels of prevalence in the suspected livestock population. Study revealed value of microscopy and indigenous ELISA kit as screening tests, whereas IS900 PCR (blood and feces) was at best confirmatory. Higher presence of MAP in the test samples showed the need for wider study to estimate sero and molecular prevalence of MAP in domestic ruminants population of the country.

Key words: paratuberculosis, epidemiology

699 Evaluation of a BVD milk ELISA test detecting anti-p80 antibody and comparison with ear notch testing for PI cattle. D. J. Wilson*[1], K. A. Rood[1], and G. Goodell[2], [1]*Utah State University, Logan,* [2]*The Dairy Authority, Greeley, CO.*

A milk ELISA test for antibody (Ab) against Bovine Viral Diarrhea (BVD) was studied and compared with standard ear notch testing for Persistently Infected (PI) cows. Milk metered samples were tested from a dairy herd with past diagnoses of BVD abortions and PI cows. BVD MLV vaccine was given to calves 3 mo and 4 mo old, to all cows at dryoff and 15-21 DIM post calving. 247 and 258 cows were tested 1 mo apart using a competitive ELISA for milk antibody (Ab) binding to p80 BVD protein. Results are reported as % binding by a second test kit Ab; higher second Ab binding means the milk had less anti-BVD p80 Ab. Interpretation: 90-100%, little Ab - PI or vaccine failure if consistent; 60-89%, moderately low Ab; 30-59%, moderate Ab; 10-29%, high Ab; 0-9%, very high anti-BVD Ab. Four samples from each cow were handled differently: fresh milk, fresh with preservative pill, frozen 7 days, room temp 7 days with preservative. Ear notches were sampled concurrently from all cows for BVD antigen (Ag) testing. No PI cows were found from ear notch Ag tests of 345 cows. Milk handling method was significant; fresh milk mean 49% second Ab binding was higher than other methods, 7 days preserved was especially lower at 42% ($P \leq 0.01$, ANOVA, Tukey's). All further results here are from fresh milk. Binding ranged from 3%-98%, quartiles 29%, 47%, 62% 1st mo, 35%, 56%, 71% 2nd mo. 15 cows had 90-98% binding on one test, but 14 were milking each mo and were below 90% on the other test; 60% mean binding the other mo. For cows >90%, DIM was 41-188, 305ME mean 12,935 kg, daily milk mean 44 kg. No PI or vaccine failures (consistently >90%) were found by milk ELISA. DIM significantly affected Ab binding: 1-9 DIM, 16%*; 10-30 DIM, 34%*; 31-60 DIM, 46%; 61-150 DIM, 60%*; 151-300 DIM, 47%; 301-360 DIM, 40%; >360 DIM, 46%. * = $P \leq 0.025$, ANOVA, Tukey's. Lactation number did not affect binding. The milk ELISA agreed with ear notch testing in finding no PI cows. Anti-BVD Ab was high in early lactation and then decreased. The ELISA warrants further study.

Key words: BVD, antibody, ELISA

700 Biophotonic imaging as a method to evaluate efficacy of intramammary antibiotics against *Staphylococcus aureus* in vitro. J. Curbelo*, J. Brett, C. Steadman, H. L. Sanchez, T. Rowlison, K. S. Seo, P. L. Ryan, and S. T. Willard, *Mississippi State University, Mississippi State.*

In this study we evaluated whether biophotonic imaging (BI) could be adapted to evaluate antimicrobial efficacies of intramammary antibiotic preparations (IAP) against bioluminescent *Staphylococcus aureus* (S. aureus-Xen8.1) in bovine milk in vitro. Whole bovine milk (500 mL; n = 4) were inoculated with S. aureus-Xen8.1 and treated with commercial IAP (cephapirin sodium (CEP), hetacillin potassium (HET) and/or pirlimycin hydrochloride (PIR)), and a control (CON) containing no antibiotics. Aliquots were collected from each inoculated sample (100µL; n = 5) over a 9 h period, placed in a 96-well plate, imaged and plated to correlate the number of cfu with photonic emissions (PE). An ANOVA and Fisher LSD test were performed to determine significant differences in PE and cfu between treatments. Photonic emissions from S. aureus-Xen8.1 increased in CON, HET and CEP treatments in a parallel manner with no differences in PE and cfu ($P > 0.05$) from 0 to 2 h post antibiotic addition. PE in the PIR treatment decreased during the same period ($P < 0.05$), but remained stable for the remaining 7 h. However cfu did not correspond with these results, remaining unchanged during the first 4 h. Differences in PE between HET and CEP were first observed at 2 h post antibiotic addition (243.3 ± 4.5 and 236.9 ± 1.4, respectively; $P < 0.0001$), which corresponded to the differences first observed in cfu between these 2 treatments after 2 h ($3.4 \times 10^7 \pm 4.4 \times 10^6$ and $2.2 \times 10^7 \pm 1.1 \times 10^6$, respectively; $P < 0.0001$). In summary, the discrepancies found between PE and cfu in the PIR treatment could be attributable to its mode of action. Pirlimycin is a bacteriostatic antibiotic and interferes with protein synthesis, therefore suppressing the production of light at the ribosomal level. In contrast, CEP and HET are bactericides and target cell wall synthesis. Therefore, the degree of impairment caused by these 2 antibiotics against S. aureus-Xen8.1 corresponded to the changes in PE detected, which allows for the quantification in real time of bacterial progress during in vitro and/or in vivo antimicrobial studies.

Key words: biophotonic, *Staphylococcus aureus*, mastitis

701 Experimental induction of *Streptococcus uberis* mastitis in bred dairy heifers: A challenge model. K. A. Jackson*, D. J. Hurley, F. M. Kautz, L. O. Ely, and S. C. Nickerson, *University of Georgia, Athens.*

A challenge model for experimentally inducing *Streptococcus uberis* mastitis in 7 bred dairy heifers from a university herd was developed. Heifers were randomly assigned 2 contralateral quarters to receive an infusion of the *S. uberis* challenge strain NIRD-0140J. To challenge the heifers, a bacterial suspension was created to achieve a concentration of 1000–2000 cfu/mL in 1 mL of physiological saline. For a successful challenge, 3 of 4 consecutive microbiological cultures had to be positive for *S. uberis* based on the API 20 Strep miniaturized identification kit. Once infection was confirmed, the challenged quarter was treated with SPECTRAMAST DC. Preliminary analysis showed that 6 of the 7 heifers (85%) were challenged successfully with the dose used. The average concentration used to challenge the 7 heifers was 1,080 cfu/mL, which fell within the goal range of 1000–2000 cfu/mL. Data showed that before challenge, SCC averaged 11.5×10^6/ml across control and challenge quarters. At 24 h post challenge, SCC increased to 23.8×10^6/ml in challenged quarters and remained elevated. In contrast, unchallenged quarters resulted in no change in SCC and the concentration remained at or below 10.1×10^6/mL. Differential leukocyte data showed that before challenge, macrophages predominated in secretions followed by lymphocytes and neutrophils. By 24 h after challenge, there was a marked spike in neutrophils, which lasted for the duration of the trial. Results suggest that the challenge model developed was successful in routinely establishing experimental S. uberis mastitis in dairy heifers, which was controlled (100% cure) by the administration of nonlactating cow therapy.

Key words: experimental challenge, mastitis, *Streptococcus uberis*

702 Effects of OmniGen-AF on enhancing immunity in dairy heifers vaccinated with a *Staphylococcus aureus* bacterin. V. J. Eubanks*[1], N. E. Forsberg[2], Y. Q. Wang[2], K. Zanzalari[3], J. Chapman[3], D. J. Hurley[1], F. M. Kautz[1], L. O. Ely[1], and S. C. Nickerson[1], [1]*University of Georgia, Athens,* [2]*Oregon State University, Corvallis,* [3]*Prince Agri Products Inc., Quincy, IL.*

The purpose of this study was to evaluate the effect of a commercial feed additive on amplifying heifers' immune response to *Staphylococcus aureus* vaccination, with the goal of calving heifers free of infection with low somatic cell counts (SCC) to meet the proposed

legal limit of 400,000/mL. Overall animal health was monitored by measuring blood immune parameters, body growth, health incidents, and prevalence of mastitis. Bacterial culture of teat canal swabs and mammary secretions to determine presence of mastitis revealed that 40% and 55.1%, respectively, were infected with *S. aureus* and other *Staphylococcus* and *Streptococcus* spp. along with elevated SCC $\geq 1 \times 10^6$/ml. S. aureus was found to be the most prevalent pathogen in both the teat canal swabs (11.4%) and mammary secretions (20.5%). Heifers fed the commercial feed additive did not show a significant advantage or disadvantage in growth based on weight and height measurements. However, L-selectin and IL-8 receptor mRNA analyses on blood leukocytes showed that post-treatment L-selectin (3.6) and Il-8 (2.38) receptor mRNA levels were higher for heifers fed the commercial feed additive than control heifers (1.53 and 1.19, respectively), suggesting greater antibacterial leukocyte activity and elevated immune status in treated animals. S. aureus antibody titers across both treatment groups before vaccination averaged 1:400. By 1 mo post-vaccination, titers increased 2–10 fold, and the titers of several heifers continued to increase through the 3rd mo post-vaccination; however, no differences have been detected between treatments.

Key words: mammary immunity, mastitis, *Staphylococcus aureus*

703 Genetic parameters of adaptive immune response traits in Canadian Holsteins and implications for health. K. Thompson-Crispi*[1], A. Sewalem[2,3], F. Miglior[2,3], and B. Mallard[1], *[1]Dept. Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada, [2]Guelph Food Research Center, Agriculture and Agri-Food Canada, Guelph, Ontario, Canada, [3]Canadian Dairy Network, Guelph, Ontario, Canada.*

Infectious diseases contribute to substantial economic loss in the dairy industry with human and animal health implications. The immune system is a tightly genetically regulated system that largely controls response to infectious disease. Including estimated breeding values (EBV) of immune response (IR) traits in a selection index has the potential to improve inherent animal health. Previously, cows classified as High Immune Responders in dairy herds in Ontario and the US were found to have improved response to vaccine, increased milk and colostrum quality and decreased incidence of diseases like mastitis, metritis, ketosis and retained placenta. The objectives of this study were to evaluate antibody-mediated (AMIR) and cell-mediated IR (CMIR) traits in Holstein cattle on a national scale and estimate genetic parameters of these traits. In collaboration with the Canadian Bovine Mastitis Network, 690 Holsteins from 58 herds across Canada were immunized to measure delayed-type hypersensitivity as an indicator of CMIR and serum antibody (AMIR) to putative type 1 and type 2 test antigens. The statistical procedure included the fixed effects of parity and stage of lactation and the random effects of herd-technician, animal and residual. A linear animal model was used to estimate genetic parameters and breeding values using a DMU software package. Heritability of CMIR was 0.18 and for AMIR between 0.14 – 0.41 depending on time and antibody. The genetic correlations between CMIR and AMIR were negative and ranged from −0.13 to −0.45. EBV were used to classify cows as High (H), Average or Low (L) for IR. Health and production records are available for correlation with these diverse immune response profiles. Preliminary results show no difference in 305 d production between IR categories, indicating selection for IR would not impact production. Selective genotyping of H and L immune responders using the Bovine SNP50 BeadChip is underway. Identifying H and L immune responders and determining genetic profiles of these phenotypes may make it feasible to include IR in breeding indices to improve health of dairy cows.

Key words: immune response, genetic parameters, dairy cattle

704 The relationship between measured optical density of uterine lavage samples and clinical endometritis. V. S. Machado*, M. L. S. Bicalho, and R. C. Bicalho, *Cornell University, Ithaca, NY.*

The objective of this study was to evaluate a novel clinical endometritis diagnosis technique using optical density of uterine lavage samples. Clinical endometritis is the inflammation of the endometrium diagnosed by the detection of purulent or mucupurulent uterine exudates after 21 d postpartum, not accompanied by systemic signs. In the present study, the diagnosis of clinical endometritis was evaluated using low-volume uterine lavage technique at 35 ± 3 DIM, examining the presence of purulent or mucupurulent content only in the uterine secretion. The study enrolled 554 cows from a dairy farm located near Ithaca NY from May 4th 2010 until January 17th 2011. After they were cultured for *A. pyogenes*, the uterine lavage samples were frozen in −80°C until they were processed for the assessment of optical density (OD), as follows: hypertonic saline solution (10%) was added to the uterine lavage in a 1:1 proportion, followed by incubation at room temperature for 30 min and centrifugation for 30 min at 3,000 x g at 4°C. The supernatant was collected and an aliquot of 0.2 mL was added to microplate wells. The OD200, OD352, OD620, OD790, OD860 and OD960 were assessed. The prevalence of clinical endometritis in the present study was 16.8% (93 cows). The ROC analysis of the accuracy of ODs in the detection of clinical endometritis was done for several OD wavelengths. The OD620 (optical density at 620 nm) presented the highest area under the curve in the ROC analyzes and was selected for further analysis. The ROC analyzes indicated an optimal cut-off point of 0.049 (OD620); sensitivity was 88.17% and sensitivity was 70.93%. The positive predictive value and negative predictive value for the test were 37.96% and 96.75%, respectively. The mean OD620 for endometritis negative cows was 0.07 (±0.005), which was significantly lower than for cows diagnosed with endometritis 0.24 (±0.03). The OD620 mean for *A. pyogenes* culture negative cows was significantly lower (0.9 ± 0.007) than for *A. pyogenes* culture positive cows (0.17 ± 0.03).

Key words: endometritis, uterine health, dairy cows

705 Survey of individual cow records to identify factors associated with lameness in dairy cattle in New Zealand. C. M. Lira-Diaz[1], J. K. Margerison*[1], and N. Lopez-Villalobos[2], *[1]Massey University, IFNHH, Palmerston North, New Zealand, [2]Massey University, IVABS, Palmerston North, New Zealand.*

This study aimed to assess the main factors associated with the occurrence of lameness of dairy cattle, using 1969 individual cow records from 3 dairy farms in the North Island of New Zealand and monitored cows for locomotion score (LS) using a 5 point scale (1 not lame, 3 tender footed, 4 lame, 5 very lame (not weight bearing on a limb/ limbs)) on a daily basis for a 12 mo period. A total of 9.34% of cows were diagnosed as lame at least once during the year (LS > 3) and assessment of individual claws was recorded using the international foot map (IFM) (0 = inter-digital, 1+2 = white line, 3 = axial lateral sole, 4+5 = sole area, 6 = heel, 10 = inter-digital heel, 11 = other cause of lameness). The incidence of lameness was significantly higher in hind claws 3.73 times more frequently (78.88% of cases) than fore claws (21.12% of cases), with no significant difference (*P*

< 0.8498) between right (Rt) and left (Lt) claws (Lt: 49.40%, O.R.1, C.I.95%; Rt: 50.60%; O.R.1.024, 95% C.I.). Lateral claws had the highest incidence of lameness (55.38%, O.R. 1.24, C.I 95%; $P < 0.05$) from medial claws. Results indicate a significantly ($P < 0.001$) higher percentage of LS 4 (59.20%, O.R. 7.05, C.I.95%), followed by LS 3 (32.40%, O.R.1.83, C.I. 95%), while LS 5 was lowest (8.40%, O.R. 1.00, C.I.95%). Highest incidence related to areas of the sole (IFM 4+5: 23.11%) followed by abaxial (IFM 3: 22.71%) and while line (WL) (IFM 1+2: 16.33%) areas, followed by inter-digital (IFM 0+10: 15.54%) and heel (IMF 6: 10.76%), while in 11.55% of cases the exact area affected was not identified. Claw examination showed the type of lameness most frequently diagnosed was WL disease (26.29%), followed by sole penetration (23.11%), inter-digital necrosis (16.33%), sole ulcer (11.16%), medial claw overgrowth (4.78%), thick hock (3.19%) and 15.14% was other causes. In conclusion, the main claws affected were the hind (88.45% of cases) and the main types of lameness were WL disease and foreign body penetration of the sole.

Key words: lameness, dairy cattle

706 Claw horn disease and claw horn anatomy: A meta-analysis in UK and New Zealand first-lactation dairy cattle. L. A. Lethbridge and J. K. Margersion*, *IFNHH Massey University, Palmerston North, New Zealand.*

In dairy cattle lameness is one of the main economic and welfare issues and claw horn related issues are the main pathogenesis. There is considerable amounts of data exist in many countries, there have been few similarly detailed studies assessing claw horn disease (CHD) and anatomical characteristics in the first lactation completed in New Zealand (NZ). This research used meta analysis to compare studies of CHD in the UK and NZ by the same research methods over a 3 year period. Days postpartum (dpp) significantly affected the number, percentage and total lesion score for both sole and white line, and peak hemorrhaging in NZ occurred at 110 dpp and had declined by 160, a similar pattern where peak lesions were seen at 100 dpp and levels had reduced by 150 dpp (UK). The median locomotion score of 1 with a peak score of 2 occurred between 71 and 98 dpp (NZ), which was a little sooner than the 110 to 120 dpp found in many other studies. A similar pattern for claw lesion score (CLS) to peak approx 110 to 120 d occurs irrespective of location. However the UK based research had far lower CLS (0.3) for both sole and white line lesions than in NZ. Overall, there were no significant relationships found between claw horn growth and wear rates, CLS dpp and PR (growth = Average growth = 5.98 - 0.386 Sole PR + 0.0324 d + 0.0033 CLS Sole R-Sq(adj) = 15.4% $P = 0.402$ and average wear = 9.52 - 0.458 Sole PR + 0.0173 d - 0.0112 CLS Sole R-Sq(adj) = 3.4% $P = 0.600$, and net change Net = - 11.2 + 0.244 Sole PR + 0.0396 d + 0.0303 CLS Sole R-Sq(adj) = 12.6% $P = 0.573$). Monthly growth rates were generally higher in the NZ compared with the UK, while claw horn wear rates were higher in NZ compared with the UK at 100 dpp. NZ heifers maintained a shallower claw angle, shorter dorsal border and higher heel height than UK heifer resulting in a smaller more compact foot and smaller wearing surface. The dorsal borders and heel depth in UK heifers were longer and shallower than those in NZ heifers, but UK foot angle was steeper than NZ.

Key words: claw horn disease, dairy cattle