

## Animal Health: Beef cattle health, lameness and mastitis

**504 Evaluating the metagenome of nasal samples from cattle with bovine respiratory disease complex (BRDC).** Tara G. McDanel<sup>\*</sup>, Larry A. Kuehn, and John W. Keele, *US Meat Animal Research Center, Clay Center, NE.*

Bovine respiratory disease complex (BRDC) is a multi-factor disease, and disease incidence may be associated with an animal's commensal microbiota (metagenome). Therefore, evaluation of the animal's resident microbiota in the upper nasal cavity may help us to understand the effect of the metagenome on incidence of BRDC in cattle. Nasal swabs from approximately 600 calves were collected at various time points including preconditioning, weaning, and when the animal enters the feedlot and is diagnosed with BRDC. Samples from healthy cohorts were also collected for each time point evaluated in the feedlot to compare metagenome profiles of healthy and sick animals. Samples from animals diagnosed with BRDC in the feedlot were pooled in groups of 10 based on when the animal was diagnosed with BRDC (one week, 3 weeks, or 4 weeks after weaning). Samples from these same animals were also evaluated at the time points previous to entering the feedlot (preconditioning and weaning) to evaluate changes in the metagenome across time. Additionally, samples from the preconditioning and weaning time points were pooled in groups of 10 based on location origin of the animals before entering the feedlot, as calves came from 4 pasture locations before being weaned and comingled in the feedlot. To evaluate and compare the metagenome of each pooled sample, the variable region (approximately 1,500 bp) along the 16S ribosomal RNA gene was amplified by PCR to include v1-v8. These amplified products were then sequenced using next-generation sequencing (Pacific Biosciences RSII instrument) and sequence reads were analyzed by WebMGA and GreenGenes to identify subfamilies for the bacterial populations present. Overall, *Mannheimia haemolytica* (34–87%) was the predominant bacterial subfamily present in all pools evaluated at the feedlot, compared with preconditioning and weaning time points. Additionally, metagenome profiles differed across animal location origin before entering the feedlot. These results demonstrate a change in the metagenome of the nasal cavity across different time points of production and confirm the likely role of *Mannheimia haemolytica* in BRDC. USDA is an equal opportunity provider and employer.

**Key Words:** bovine respiratory disease complex, metagenome, 16S sequence

**505 Acute and chronic stress models differentially affect the inflammatory and antibody titer responses to respiratory vaccination in naïve beef steers.** Nathan D. May<sup>\*1</sup>, Jeff A. Carroll<sup>2</sup>, Nicole C. Burdick Sanchez<sup>2</sup>, Shelby L. Roberts<sup>1</sup>, Heather D. Hughes<sup>1</sup>, Paul R. Broadway<sup>2</sup>, Kate P. Sharon<sup>3</sup>, Michael A. Ballou<sup>3</sup>, and John T. Richeson<sup>1</sup>, <sup>1</sup>West Texas A&M University, Department of Agricultural Sciences, Canyon, TX, <sup>2</sup>USDA-ARS, Livestock Issues Research Unit, Lubbock, TX, <sup>3</sup>Texas Tech University, Department of Food and Animal Sciences, Lubbock, TX.

The objective of this research was to determine the effect of an acute vs. chronic stress model on serum antibody titer and acute phase responses. Seronegative beef steers ( $n = 32$ ;  $209 \pm 8$  kg) were stratified by BW and assigned randomly to 1 of 3 treatments (1) Chronic stress (CHR), 0.5 mg/kg BW dexamethasone (DEX) administered i.v. at 1000h on d -3 to 0; (2) Acute stress (ACU), 0.5 mg/kg BW DEX administered i.v. at 1000h on d 0 only; or (3) Control (CON), no DEX. On d -4, steers

were fitted with jugular catheters and placed into individual stanchions in an environmentally controlled facility. At 1200h on d 0, steers were administered a modified-live virus respiratory vaccine containing isolates of infectious bovine rhinotracheitis virus (IBRV), bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV) and parainfluenza-3 virus (PI3V). On d 4, cattle were transported (177 km) to an isolated facility and housed in a single pen. Serum was harvested from d 0, 7, 14, 21, 28, 35, 42, and 56 and subsequently used to determine IBRV-, BVDV-, BRSV-, and PI3V-specific antibody titers. Additionally, serum from d -2, 0, 1, 3, 7, and 14 was used to quantify haptoglobin (Hp) and ceruloplasmin (Cp) concentrations. There was a  $\text{trt} \times \text{d}$  interaction ( $P < 0.01$ ) such that CHR steers had a greater ( $P \leq 0.07$ ) BVDV antibody titer from d 14 to 28; whereas, CHR was greater ( $P = 0.06$ ) than ACU on d 56. Moreover, IBRV antibody titers were increased beginning on d 14 for CHR and d 28 for ACU, and remained elevated through d 56 compared with CON ( $P \leq 0.03$ ). Stress treatment altered Hp such that CON exhibited a greater ( $P < 0.01$ ) Hp concentration than CHR but was not different from ACU ( $P = 0.16$ ). On d 3, Cp was greatest for CON, intermediate for ACU, and least for CHR ( $\text{trt} \times \text{d}$ ;  $P \leq 0.01$ ). Results suggest that immunosuppressive conditions in CHR and ACU may have allowed enhanced viral replication from the vaccine, resulting in a greater antibody titer response. Data further indicate that DEX administration blunted the acute phase response and these alterations were particularly evident in the CHR stress model.

**Key Words:** cattle, stress, vaccination

**506 Effect of injectable trace mineral administration on health, performance and vaccine response of newly received beef cattle.** Shelby L. Roberts<sup>\*1</sup>, Nathan D. May<sup>1</sup>, Casey L. Brauer<sup>2</sup>, Wes W. Gentry<sup>2</sup>, Caleb P. Weiss<sup>2</sup>, Jenny S. Jennings<sup>2</sup>, and John T. Richeson<sup>1</sup>, <sup>1</sup>Department of Agricultural Sciences, West Texas A&M University, Canyon, TX, <sup>2</sup>Texas A&M AgriLife Research, Amarillo, TX.

Previous research has established that trace minerals are necessary for optimal animal health and performance. The objective of this study was to evaluate the effect of an injectable trace mineral supplement containing copper, zinc, selenium, and manganese (Multimin 90) on vaccine response, growth performance and morbidity of beef calves upon entry into a feedlot. A total of 128 crossbred bull ( $n = 40$ ) and steer ( $n = 88$ ) calves were utilized. Cattle were stratified by initial BW ( $276 \pm 3$  kg) and gender, then assigned randomly to treatment pens ( $n = 8/\text{treatment}$ ). Treatment protocols were (1) negative control (CON), or (2) Multimin 90 (MM) administered at 1 mL/45.5 kg BW subcutaneously on d 0. Cattle were also administered a pentavalent modified-live respiratory vaccine, anthelmintic, and metaphylaxis with tilmicosin phosphate on d 0. Individual BW data and blood were collected on d 0, 14, 28, and 42. Harvested serum was used to determine bovine viral diarrhea virus (BVDV) type 1a antibody titer as a proxy for vaccine response. Health was monitored daily by trained personnel blinded to treatment pen assignment. Calves were pulled when assigned a clinical illness score of  $\geq 2$ , and considered morbid and administered antimicrobial treatment if rectal temperature was  $\geq 39.7^\circ\text{C}$  or if lung auscultation score was  $\geq 3$  on a 1 to 5 scale. Overall DMI was not different ( $P = 0.82$ ) between CON and MM. Also, no difference in overall ADG ( $P = 0.21$ ) was detected between CON (1.36 kg/d) and MM (1.25 kg/d) steers. The overall morbidity rate observed for this study was low (14%). There was no statistical difference ( $P = 0.71$ ) in morbidity between treatments,

which averaged 15.6 and 12.5%, for CON and MM, respectively. There was a treatment x d effect ( $P = 0.09$ ) for BVDV-specific antibody titer. On d 14, the MM group had a greater ( $P = 0.02$ ) BVDV antibody titer than the CON group. This data suggests that while administration of an injectable trace mineral did not improve performance or morbidity rate when disease incidence was low, the BVDV-specific antibody response to a respiratory vaccine developed faster for Multimin 90 treated animals.

**Key Words:** cattle, trace mineral, vaccine

**507 Effect of different combination viral-bacterial respiratory vaccines on serum leukotoxin antibody, acute phase response, and performance in beef heifer calves.** Heather D. Hughes<sup>\*1</sup>, Sjoert Zuidhof<sup>2</sup>, Shelby L. Roberts<sup>1</sup>, Joelle L. Pillen<sup>1</sup>, Garrett D. Bigham<sup>1</sup>, and John T. Richeson<sup>1</sup>, <sup>1</sup>Department of Agricultural Sciences, West Texas A&M University, Canyon, TX, <sup>2</sup>Boehringer Ingelheim Vetmedica, St. Joseph, MO.

Vaccination of newly received cattle against respiratory pathogens is a common practice in the stocker or feedlot setting; however, this practice may induce an acute inflammatory or febrile response that could affect clinical presentation or transiently reduce performance. Our objective was to determine if different combination viral-bacterial respiratory vaccines affect the leukotoxin (LKT) antibody concentration, acute phase response, rectal temperature (RT), or gain performance. A total of 30 clinically healthy beef heifer calves (BW = 222.3 ± 27.1 kg) were stratified by pre-trial serum antibody against *Mannheimia haemolytica* (MH) whole cell wall, then assigned randomly to 1 of 3 vaccine treatment regimens consisting of (1) Pyramid 5 + Presponse SQ (P5PS; n = 10), (2) Bovi-shield Gold One Shot (BGOS; n = 10), or (3) unvaccinated control (CON; n = 10). Heifers were housed in a single pen, and blood, BW and RT were collected on d 0, 4, 7, 14, 28, 42 and 56. A treatment x d interaction was observed for MH-specific LKT ( $P < 0.001$ ). Cattle administered either of the vaccines had greater LKT antibody concentrations than CON on d 14 and 28 ( $P \leq 0.04$ ), whereas BGOS was greater than CON on d 7 ( $P = 0.03$ ) but did not differ from P5PS ( $P = 0.49$ ). No differences were detected for RT ( $P = 0.85$ ) or ADG ( $P \geq 0.33$ ), which averaged 1.17, 1.11 and 1.23 kg/d for P5PS, BGOS, and CON, respectively from d 0 to 56. Respiratory vaccination affected serum haptoglobin (Hp) concentration; P5PS exhibited greater Hp concentrations than CON ( $P = 0.01$ ) but was not different from BGOS ( $P = 0.53$ ). No difference in serum ceruloplasmin was observed ( $P = 0.88$ ). Results indicate that either vaccine produced a greater LKT antibody and Hp response compared with CON, but RT and performance were not affected. Respiratory vaccines may have slight inflammatory effects when administered to clinically healthy cattle, yet further research is warranted to elucidate vaccine-induced inflammation in highly stressed cattle.

**Key Words:** acute phase response, cattle, vaccine

**508 Probiotic supplementation improves performance, neutrophil function, and antibody responses of post-weaned Holstein heifers during the commingling phase.** Caleigh E. Payne<sup>\*</sup>, Luis G. D. Mendonça, Lucas D. S. Rocha, Sophia C. Trombetta, Suzy Q. Fowler, Juan C. Gordienko, Sonia J. Moisés, and Lindsey E. Hulbert, Kansas State University, Manhattan, KS.

The risk of disease increases for post-weaned calves when they are transitioned from individual housing to group housing (commingling). Therefore, this study was conducted to determine if probiotic supplementation of mannan-oligosaccharide (MOS) and b-glucan (BG) would

assist calves in the transition from individual hutches to groups of 3. Feed intake, body weight gain, in vivo adaptive immune responses, and ex vivo neutrophil responses were measured from 60, weaned Holstein heifer calves (age 52 ± 4.0 SD d; 83 ± 14.7 SD kg). One week before commingling (-1 wk), calves were randomly assigned to either a daily bolus dose of oral prebiotics (3 g; 10% MOS, 18% BG) dissolved in 15 mL of molasses or control (15 mL molasses only) for 7 weeks. Daily DMI was collected and calves were weighed weekly. Whole blood was collected via jugular venipuncture on wk -1, 1, 2, and 6 relative to commingling. In addition, all calves were administered an innocuous protein injection, ovalbumin (OVA; subQ; 0.5 mg/mL), at commingling and 4 weeks after commingling. All blood samples were measured via flow-cytometry for peripheral neutrophil phagocytosis (PG) and oxidative burst (OB) responses to heat-killed *E. coli* (8739). Plasma OVA-specific IgG and IgA were measured at 2 weeks after each OVA injection. Two weeks after commingling, prebiotic-treated calves had neutrophils with greater OB intensity than control calves ( $P = 0.005$ ). Prebiotic-calves also had greater primary IgA ( $P < 0.01$ ) and IgG ( $P = 0.04$ ) responses to OVA than control calves, as well as greater secondary IgA response ( $P < 0.01$ ) than control calves. One week after commingling, prebiotic-calves had greater ADG (1.07 vs. 0.90 ± 0.059 kg/d;  $P = 0.04$ ) and as well as lower F:G (2.61 vs. 3.60 ± 0.204;  $P = 0.001$ ) than control calves. Probiotic supplements enhanced innate and adaptive immune measures and performance during the commingling phase. This supplementation may help reduce the risk of disease and improve vaccination response during commingling in post-weaned dairy heifers.

**Key Words:** probiotic, immunology, bovine

**509 Shotgun metagenomic analysis of bovine digital dermatitis.** Martin Zinicola<sup>\*</sup>, Hazel Higgins, Svetlana Lima, Vinicius Machado, Charles Guard, and Rodrigo Bicalho, Cornell University, Ithaca, NY.

Metagenomic methods amplifying 16S ribosomal RNA genes have been used to describe the microbial diversity of healthy skin (HS) and lesion stages of bovine digital dermatitis (DD) and to detect critical pathogens involved with disease pathogenesis. In this study, we characterized the microbiome and for the first time, the composition of functional genes of HS, active (ADD) and inactive (IDD) lesion stages using a whole-genome shotgun approach. A total of 16 biopsy samples (HS, n = 8; ADD, n = 4; IDD, n = 4) were collected from Holstein dairy cows housed in one dairy farm. DNA was extracted and the microbiome was determined by shotgun techniques using Illumina MiSeq platform. Metagenomic sequences were annotated using MG-RAST pipeline. Six phyla were identified as the most abundant. *Chordata*, *Firmicutes* and *Actinobacteria* were the predominant phyla in the microbiome of HS, while *Spirochetes*, *Bacteroidetes* and *Proteobacteria* were highly abundant in ADD and IDD. *T. denticola-like*, *T. vincentii-like* and *T. phagedenis-like* constituted the most abundant species in ADD and IDD. Recruitment plots comparing sequences from HS, ADD and IDD samples to the genomes of specific *Treponema* spp., supported the presence of *T. denticola* and *T. vincentii* in ADD and IDD. Comparison of the functional composition of HS to ADD and IDD identified a significant difference ( $P < 0.05$ ) in genes associated with motility/chemotaxis and iron acquisition/metabolism. We also provide evidence that the microbiome of ADD and IDD compared with that of HS had significantly higher ( $P < 0.05$ ) abundance of genes associated with resistance to copper and zinc, which are commonly used in footbaths to prevent and control DD. In conclusion, the results from this study provide new insights into the HS, ADD and IDD microbiomes, improve our understanding of the disease pathogenesis and generate unprecedented knowledge regarding

the functional genetic composition of the DD microbiome. Additionally, an increase in the abundance of copper and zinc resistance genes has been reported, suggesting that further research is necessary to optimize the foot bathing technique for the control and treatment of DD.

**Key Words:** bovine digital dermatitis, dairy cow, metagenomic

### 510 Comparison of milking and lying behavior between lame and sound cows on dairy farms with automated milking systems.

Meagan T. M. King<sup>\*1</sup>, Ed A. Pajor<sup>2</sup>, Stephen J. Leblanc<sup>3</sup>, and Trevor J. DeVries<sup>1</sup>, <sup>1</sup>Department of Animal and Poultry Science, University of Guelph, Guelph, ON, Canada, <sup>2</sup>Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada, <sup>3</sup>Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.

To develop better ways to use behavioral data to detect lame cows, comparisons of milking visit behavior, milk yield, and lying behavior were made between lame and sound cows on automated milking system (AMS) farms. Data were collected for 30 cows, over a 6-d period, from each of 26 AMS farms in Eastern Ontario, Canada. Cows were gait scored using a 5-point numerical rating system (1 = sound to 5 = lame). Cows with gait scores < 3 were classified as sound (n = 527) while those with gait scores ≥ 3 were classified as lame (n = 245). Milking visit behavior was extracted from the AMS computer at each farm, while lying behavior was measured continuously using electronic data loggers. Body condition and hygiene were also scored, and were compiled with other cow-level variables such as parity and DIM. Data were summarized across the 6-d observation period/cow and analyzed in multivariable general linear mixed models. When controlling for DIM, milking frequency (#/d; mean ± SE) was lower ( $P < 0.001$ ) for lame cows ( $2.8 \pm 0.1$ ) compared with sound cows ( $3.1 \pm 0.1$ ). Milk yield was lower ( $P = 0.05$ ) for lame cows ( $32.0 \pm 0.9$  kg/d) compared with sound cows ( $33.4 \pm 0.8$  kg/d), while accounting for parity, DIM, and body condition. Compared with sound cows, lame cows had fewer AMS refusals/d (1.0 vs. 1.8; SE = 0.2;  $P < 0.001$ ). Lameness status did not affect the frequency of AMS failures/d ( $0.1 \pm 0.01$ ). Overall, lame cows made fewer visits to the AMS than sound cows (4.0 vs. 5.0 visits/d; SE = 0.3;  $P < 0.001$ ). When accounting for parity and DIM, lying time was greater ( $P = 0.002$ ) for lame cows ( $728.2 \pm 13.7$  min/d) compared with sound cows ( $693.8 \pm 12.0$  min/d). These results demonstrate the differences in behavior and resulting productivity between lame and sound cows, indicating the potential use of behavioral indicators to identify lame cows on AMS farms.

**Key Words:** automatic milking, dairy cow behavior, lameness

### 511 Comparing the prevalence of hoof lesions in dairy cattle classified as high, average or low antibody and cell-mediated immune responders.

Shannon L. Cartwright<sup>\*1</sup>, Kathleen Thompson-Crispi<sup>1,2</sup>, Marlene Paibomesai<sup>1</sup>, Filippo Miglior<sup>2,3</sup>, and Bonnie Mallard<sup>1,2</sup>, <sup>1</sup>Department of Pathobiology, University of Guelph, Guelph, ON, Canada, <sup>2</sup>Center of Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada, <sup>3</sup>Canadian Dairy Network, Guelph, ON, Canada.

Several studies have shown cattle classified as high immune responders have lower incidence of disease, however the occurrence of hoof lesions has yet to be evaluated in dairy cattle classified for immune response. Therefore the objective of this study was to compare the prevalence of hoof lesions in dairy cattle classified as high, average or low antibody (AMIR) and cell-mediated immune responders (CMIR).

Cattle (n = 190) from a commercial dairy farm in Ontario were evaluated for immune response (IR) using a patented protocol that captures both AMIR and CMIR. They were classified as high, average and low responders based on Estimated Breeding Values for AMIR and CMIR. Hoof health data was collected by the farm's hoof trimmer using Hoof Supervisor software. Only the first trim date for each animal was included in this data set, and multiple lesions per cow were considered. All lesions were analyzed both as individual lesion types and grouped into infectious (digital dermatitis) and horn (sole hemorrhage, sole ulcer, toe ulcer, interdigital hyperplasia and white line). The trimmer scored each lesion for severity as: 1 = least, 2 = middle, 3 = most. Data was analyzed using a SAS mixed model, which included the effects of parity and IR category (high, average and low). Data is presented as number of cases per cows within IR category, and significance is reported at  $P < 0.05$  with trends at  $P < 0.10$ . Results showed that high antibody responders had significantly less digital dermatitis (20%) compared with average (36%) and similarly significantly less severe digital dermatitis (3%) compared with low (16%). Conversely, high antibody responders (50%) had significantly more non-infectious horn lesions compared with average (25%) and low (21%). High cell-mediated responders had a trend toward less interdigital hyperplasia (0%) compared with average (8%). Therefore, not only do cows classified as high immune responders have lower disease incidence, but this study suggests they also have lower prevalence of infectious hoof lesions, a critical problem facing dairy producers.

**Key Words:** hoof health, immune response, dairy cattle

### 512 Calf macrophages exhibit a robust response to LPS which is not affected three weeks after an early life challenge with LPS in vivo.

Filiz T. Korkmaz<sup>\*</sup>, Aimee L. Benjamin, and David E. Kerr, University of Vermont, Burlington, VT.

Genetic and epigenetic factors may contribute to animal-to-animal variation in innate immune response to infection with epigenetic effects being imposed by differences in early life environment. To evaluate lasting effects of a severe early-life infection on innate immune response capability we exposed 6 pairs of Holstein bull calves, at 8d of age, to either a 0.5 µg/kg intravenous dose of LPS or saline. Three weeks later we established cultures of monocyte-derived-macrophages (MDMs) from 100 mL blood samples by culturing monocytes for 8 d. The cells were then challenged for 24h with 100 ng/mL of LPS. The LPS treated calves demonstrated clinical signs of endotoxin challenge and markedly elevated ( $P < 0.05$ ) plasma levels of TNF-α ( $3.2 \pm 1.0$  ng/mL) and IL-6 ( $14.4 \pm 2.8$  ng/mL) at 2h post-challenge. Although there was no significant difference ( $P > 0.05$ ) between cells from LPS or saline treated calves, the MDMs produced considerable quantities of IL6 and TNF-α in response to LPS, which averaged  $2.3 \pm 0.4$  ng/mL and  $190 \pm 81$  pg/mL, respectively. Likewise, there was a large LPS-mediated induction of IL8 and IL1-β ( $88.1 \pm 37.6$  and  $1954.9 \pm 1166.5$  fold change, respectively). Readily detectable expression of TLR4 and CD14 was measured with minor induction due to LPS. No differences ( $P > 0.05$ ) were measured between groups in LPS-induced gene expression. Relatively large inter-animal variation was found in most parameters with a significant correlation between expression of IL1-β and IL8 ( $R^2 = 0.68$ ,  $P < 0.01$ ) and TLR4 and CD14 ( $R^2 = 0.57$ ,  $P < 0.01$ ). Dermal fibroblasts were also isolated from 10 calves to determine how their response to LPS compared with that of the MDMs. Fibroblast TLR4 expression was less than MDM TLR4 expression and this was reflected in lower LPS-induced induction of IL8 and IL-6 gene expression. In conclusion, LPS response in MDMs and fibroblasts is variable between animals, lower in fibroblasts, and is not affected in MDMs 3-wks after a single

exposure to LPS at 8d of age. Future studies are needed to determine genetic and epigenetic components which may be influenced by early life environment.

**Key Words:** innate immunity, epigenetic

**513 Dysbiosis of the fecal microbiota in cattle infected with *Mycobacterium avium* ssp. *paratuberculosis*.** Marie-Eve Fecteau, Raymond Sweeney, Sanjay Kumar, Nagaraju Indugu, Bonnie Vecchiarelli, Bhima Bhukya, and Dipti Pitta\*, *Department of Clinical Studies, School of Veterinary Medicine, New Bolton Center, University of Pennsylvania, Kennett Square, PA.*

Johne's disease (JD) is a chronic gastrointestinal infection of cattle caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP). In the US, more than 68% of dairy herds are infected with MAP, leading to considerable financial losses for producers. We hypothesized that cattle naturally infected with MAP have a reduction in gastrointestinal microbial biodiversity and gastrointestinal dysbiosis may play a role in the pathogenesis of JD. In the present study, fecal samples from 20 naturally infected (positive group); 25 JD-negative herd mates (exposed group); and 25 JD-negative cows from a MAP-free herd (negative group) were obtained from New Bolton Center, JD repository. All fecal samples were processed for genomic DNA and amplified for the V1-V2 regions of the 16S rDNA gene. Sequencing was performed on a 454 Roche Platform and sequences were analyzed using QIIME. Approximately 252,380 reads were analyzed from 70 bacterial communities with an average of 2,843 reads per sample. Approximately 34,606 operational taxonomic units (OTUs) were produced by clustering at 97% sequence similarity. Weighted and unweighted Unifrac distances by principal coordinate analysis showed a substantial difference ( $P < 0.001$ ; Permanova test) in the bacterial community composition of the positive group versus the exposed and negative groups. Taxonomic assignment of the OTUs identified a total of 18 bacterial phyla across all samples. Across negative and exposed groups, *Bacteroidetes* and *Firmicutes* constituted more than 95% of the total bacterial population. However, in the positive group, lineages of *Actinobacteria* and *Proteobacteria* increased and those of *Bacteroidetes* and *Firmicutes* decreased ( $P < 0.001$ ). *Actinobacteria* was highly abundant (30% of the total bacteria) in the positive group compared with <0.1% in exposed and negative groups. Therefore, it is apparent that bacterial communities in exposed and negative groups were homologous whereas significant variation in positive samples was observed. Bacterial diversity in positive group was compromised compared with negative and exposed groups.

**Key Words:** dysbiosis, fecal, Johne's disease

**514 Use of a novel adjuvant to enhance the protective effect of a commercial vaccine against *Staphylococcus aureus* mastitis in dairy heifers.** Charles Hall, Stephen Nickerson\*, David Hurley, Lane Ely, and Felicia Kautz, *University of Georgia, Athens, GA.*

Use of a novel adjuvant (Immunoboost) to enhance antibody titers in response to a commercial vaccine (Lysigin) against *Staphylococcus aureus* mastitis in dairy heifers was evaluated in a 2-phase trial. Heifers 5 to 12 mo of age were used in both study phases and blood samples were collected weekly for processing (ELISA) to determine anti-*S. aureus* antibody levels; baseline titers before each phase did not exceed 1:1600. In phase 1, hyper-immunization with Lysigin to enhance the level and duration of titers did not result in titers that consistently exceeded conventional immunization. In phase 2, anti-*S. aureus* titers

in heifers immunized with Lysigin + 2 mL Immunoboost tended to be elevated ( $P = 0.10$ ) over those of heifers immunized conventionally with Lysigin alone by d 7 after initial immunization, a trend that continued through d 14. By d 21, titers in the Immunoboost group were elevated ( $P = 0.05$ ) over conventional vaccinates and remained significantly elevated through d 35, returning to baseline by d 42. After receiving booster injections on d 42, the Immunoboost group experienced an increase ( $P = 0.05$ ) in titers over conventional vaccinates on d 49 of the trial, and titers remained significantly elevated through d 63. Titers in the Immunoboost group remained elevated over conventional vaccinates through d 84 of the trial, but the difference was not significant, and titers in both groups were approaching baseline values. Findings suggest that Immunoboost is capable of enhancing the antistaphylococcal titer response to the commercial vaccine Lysigin, albeit in the short term. These studies indicate that continued study of using immunization to control *S. aureus* mastitis in dairy heifers is justified.

**Key Words:** antibodies, heifer, mastitis

**515 The efficacy of PlyC endolysin as an alternative therapy for *Streptococcus uberis* mastitis in vitro.** Sara Linden<sup>1</sup>, Parimala Sharma<sup>1,2</sup>, Kasey M. Moyes\*<sup>2</sup>, and Daniel C. Nelson<sup>1,3</sup>, <sup>1</sup>*University of Maryland, College Park, MD*, <sup>2</sup>*Institute for Bioscience and Biotechnology Research, Rockville, MD*, <sup>3</sup>*Department of Veterinary Medicine, College Park, MD.*

The bacteriophage endolysin, PlyC, displays antimicrobial activity against select streptococcal species and may be a promising new therapy for *S. uberis*-associated mastitis. This study investigated the antimicrobial activity of PlyC against a broad spectrum of *S. uberis* isolates, established an in vitro dose response, measured ex vivo binding and antimicrobial efficacy in *S. uberis* infected milk, and assessed safety and response to antibodies. For antimicrobial activity, 2 µg/mL of PlyC was incubated with 7 different strains of *S. uberis* isolated from cows with mastitis as well as ATCC 27958 and BAA-854 (strain 0140J). For dose-response studies, PlyC (0–16 µg/mL) were incubated with 27958. The linear proportion of the initial lytic velocity was measured using a spectrophotometric turbidity reduction assay for both antimicrobial activity and dose-response assays. To demonstrate streptococcal-specific binding in raw cow's milk, 20 µg of AlexaFluor-555-labeled PlyC was incubated with milk containing BAA-854 and binding was visualized via fluorescent microscopy. Finally, polyclonal antibodies against PlyC (pre-immune sera and hyper immune sera [titer ranging from 1:10–1:10,000]) were mixed with 32 µg/mL of PlyC and activity was measured using the spectrophotometric turbidity reduction assay. All data were statistically analyzed using the Student's *t*-test. Our results showed that PlyC possessed potent lytic activity ( $P < 0.01$ ) against all strains of *S. uberis* tested. The dose-response profile demonstrated lytic activity ( $P < 0.01$ ) at concentrations ranging from 1 to 16 µg/mL. Microscopy results indicated that PlyC specifically labeled cell walls of BAA-854 in raw milk ( $P < 0.01$ ). In addition, PlyC retains full lytic activity ( $P < 0.01$ ) against 27958 in the presence of high titer anti-PlyC antibodies, suggesting that even if antibodies are present, they are non-neutralizing. Taken together, the results indicated PlyC has the potential to be used as a novel therapeutic against *S. uberis*-associated bovine mastitis via its specificity against several strains of *S. uberis*, the components in milk do not interfere with activity, and it is not inhibited in the presence of antibodies.

**Key Words:** mastitis, PlyC, treatment