P121 Genetic relationship of *Escherichia coli* isolated from the reproductive and gastrointestinal tracts of dairy cows pre- and postpartum. K. L. Jones*[1], F. Cunha[1], S. J. Jeon[2], K. C. Jeong[1], Y. Yang[3], and K. N. Galvão[1], [1]*University of Florida, Gainesville, FL,* [2]*Long Island University, Brookville, NY,* [3]*Sun Yat-sen University, Guangzhou, China.*

The objective was to investigate the source of bacterial colonization of the uterus by exploring the genetic relationship among *E. coli* strains isolated from the recto-anal junction (RAJ) and the reproductive tract (RT) of dairy cows pre- and postpartum. Cows (n = 34) had a swab sample collected from the vulva, vagina, and RAJ every 3 d starting 6 d before expected calving until 9 d postpartum. A blood sample was collected at all time points. A swab sample was collected from the uterus at the same time points postpartum. All samples were cultured aerobically on CHROMagar *E. coli* selective medium for 24 h at 37°C. Isolates from cows with growth from the vulva and/or vagina in addition to the uterus (n = 8) were used for whole-genome sequencing (WGS). All RAJ samples grew and none of the blood samples grew. A total of 44 isolates were selected for WGS, which was performed using an Illumina MiSeq. PATRIC was used to annotate each genome. The Harvest Suite was used for core genome alignment, SNP identification and phylogenetic tree rendering. Clades with no branching were evaluated for SNPs. Strains were considered clonal isolates if ≤4 SNPs difference between strains. Clonal strains were isolated from individual cows from the RAJ and vulva (1 cow; 1 SNP), the RAJ, vulva and vagina (1 cow; 0 SNP), the vulva and uterus (1 cow; 0 SNP), the vagina and uterus (2 cows; 0 SNP between strains in each cow), and the vulva, vagina, and uterus (1 cow; 0 SNP) postpartum. Clonal strains were also isolated from different cows from the vulva prepartum, and vulva and vagina postpartum (0 SNP between strains), the RAJ prepartum, and vagina and uterus postpartum (max 1 SNPs between strains), the RAJ, vulva and vagina postpartum (max 1 SNPs between strains), and the uterus, vulva and vagina postpartum (max 2 SNP between strains). Finding clonal *E. coli* strains in the RAJ from the same cow or different cows in the vulva, vagina, and uterus postpartum indicates that the GI is a source of *E. coli* that can colonize the RT, and that *E. coli* can be transferred among cows.

Key Words: *Escherichia coli*, genetic characterization, uterus

P122 Genes associated with immune function are downregulated in blood-derived neutrophils from periparturient cows. E. Asiamah*[1], K. Ekwemalor[2], S. Adjei-Fremah[2], B. Osei[3], and M. Work[2], [1]*University of Arkansas, Pine Bluff, Pine Bluff, AR,* [2]*North Carolina Agricultural and Technical State University, Greensboro NC,* [3]*Oklahoma Medical Research Facility, Oklahoma City, OK.*

The periparturient period (3 weeks pre-calving and 3 weeks postcalving) is a challenging period for dairy cows in large part due to the dysfunction of their immune system. The compromise in the immune system puts the dairy cow at a higher risk of infections and other diseases. The impairment of neutrophil function plays a crucial role in the immunosuppression of the periparturient dairy cow. Research is emerging to better understand the molecular mechanisms underlying the impaired immune function in dairy cows during the periparturient period. This study aimed at evaluating the global gene expression of blood-derived neutrophils from periparturient cows. Blood was collected from Holstein Friesian periparturient cows (n = 3) at −14 d relative to expected calving date and 7 d relative to the actual calving date. Neutrophils were isolated based on procedures described by Abdelmegeid et al. (2017; *Journal of Dairy Science* 100:3155–3165). Isolated neutrophils were subsequently used for transcriptional profiling using the Agilent bovine (v2) 4 × 44 K array. Data normalization and statistical analysis were performed using GeneSpring GX software version 13.0. The analysis was carried out using a *t*-test unpaired statistical method with Benjamini-Hochberg FDR method. Fold changes in gene expression calculated were filtered at a cut-off of ≥ 2 ($P < 0.05$). The results showed that 249 genes were differentially expressed (FC ≥ 2, $P < 0.05$.). Eighty-seven genes were downregulated and among the top 20 downregulated genes were genes essential to neutrophil response and immunity. These included *PGLYRP1*, which is involved in pathogen recognition, and *SERPINB4*, which is a protein that inhibits neutrophil-derived proteinases to protect tissue damage at inflammatory sites. Additionally, genes associated with cellular adhesion and migration (*ADRM1* and *THY1*) were also significantly downregulated. Concurrently, the pathway analysis also revealed that the TLR, inflammation response, oxidative stress, and MAPK signaling pathways are affected in bovine neutrophils during the periparturient period ($P < 0.05$). This work sheds some light on the altered gene expression in neutrophils during the periparturient period and the knowledge generated will ultimately be used for the development of novel management strategies to combat immunosuppression and disease susceptibility during this stage in the dairy cow.

Key Words: periparturient, neutrophils, dairy

P123 Effect of Holstein milk yield genotype on ex vivo innate immune response to lipopolysaccharide and lipoteichoic acid during the periparturient period. A. A. Brink*[1], W. J. Weber[1], J. D. Lippolis[2], J. B. Cole[3], S. M. Godden[4], and B. A. Crooker[1], [1]*Department of Animal Science, University of Minnesota, St. Paul, MN,* [2]*USDA-ARS National Animal Disease Center, Ames, IA,* [3]*USDA-ARS Animal Genomics and Improvement Laboratory, Beltsville, MD,* [4]*Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN.*

Our objectives were to determine effects of milk yield genotype on innate immune response to ex vivo lipopolysaccharide (LPS) and lipoteichoic acid (LTA) stimulation. Unselected (UH, stable milk yield from 1964, n = 10) and contemporary (CH, n = 11) Holsteins that differ by more than 4,500 kg milk/305 d were fed the same diet *ad lib* and milked twice daily. Cows were blocked (1/genotype) by parity and expected calving date. Heparinized blood was collected at −14, 7, 28, and 49 DIM, mixed with a low or high dose of LPS (10 and 100 μg LPS/mL blood) or LTA (0.01 and 1.0 μg LTA/mL blood), and incubated for 4 h at 37°C. Plasma concentrations (pg/mL) of IL-6 and IL-1β were quantified by ELISA (Invitrogen, Carlsbad, CA), log-transformed and analyzed by repeated measures using PROC MIXED (SAS) with DIM as the repeated effect. Means differed when $P < 0.05$. Milk yield was less in UH than CH (29.0 vs. 45.3 kg/d FCM). The response of IL-6 and IL-1β to LPS and LTA were greater ($P < 0.01$) with the high dose of antigen. The IL-6 response to LPS was greatest ($P < 0.01$) at d7 and did not differ among other days while the IL-1β response was greatest ($P < 0.01$) at d-14 and decreased postpartum (2.7, 3.2, 2.8, 2.6 ± 0.14 for IL-6 and 3.8, 3.6, 3.3, 3.3 ± 0.08 for IL-1β on d-14, 7, 28, and 49, respectively). There was a genotype by dose interaction ($P < 0.01$) for IL-1β response to LPS as the low dose was greater in UH than CH (3.2 vs. 2.9 ± 0.10) but did not differ for the high dose (4.0 vs. 3.9 ± 0.10). The IL-6 response to LTA was greatest ($P < 0.01$) at d7 and did not differ among other days

while the IL-1β response was greatest ($P < 0.01$) at d-14 and decreased postpartum (2.6, 3.0, 2.8, 2.5 ± 0.14 for IL-6 and 3.5, 3.2, 3.1, 3.0 ± 0.07 for IL-1β on d-14, 7, 28, and 49, respectively). There was a trend ($P = 0.07$) for IL-6 response to LTA to be greater in UH than CH cows. The UH cows had a greater ($P < 0.02$) IL-1β response to LTA than the CH cows (3.4 vs. 3.1 ± 0.08). Cytokine profiles demonstrate postpartum alterations in the innate immune response and a more sensitive IL-1β response to both antigens by the UH cows.

Key Words: milk yield genotype, LPS, LTA

P124 Whole-blood transcriptomic signature after 17 and 35 days of feeding OmniGen AF to prepartum Holstein cows. M. Garcia*, J. Chapman, and B. Humphrey, *Phibro Animal Health Corporation, Teaneck, NJ.*

OmniGen AF (OG; Phibro Animal Health, Teaneck, NJ) is a feed additive with demonstrated benefit on improving the immunocompetence of stressed dairy cows. This study aimed to identify an early whole-blood transcriptomic signature, focused on immunity, during the first 35 d of feeding OG to prepartum Holstein cows. Sixteen cows (60 d before expected calving date) were randomly assigned to 1 of 2 treatments at dry-off: CTL (no supplementation) or OG (56 g OG/cow/d). Cows were bled at d 17 and 35 after OG feeding began. Isolated whole-blood RNA samples with RNA integrity number >7 (n = 5 per treatment) were submitted for RNA sequencing (Novogene Co., Ltd.). Sequencing libraries were generated using NEBNext Ultra™ (NEB, Illumina, USA). Processed reads were mapped to the bovine genome using HISAT2. Differentially expressed genes (DEG, $P < 0.05$) were identified using DESeq2 R package. Gene ontology (GO) analysis of DEG was performed with clusterProfiler R package, and enriched GO terms with corrected $P < 0.05$ were deemed significantly different. Cows fed OG had 1,130 and 1,574 DEG at d 17 and d 35, respectively. At d 17, 18 DEG, 17 upregulated, were part of 4 enriched GO terms. Three GO terms were ancestor terms of neutrophil chemotaxis (neutrophil migration, granulocyte chemotaxis, and granulocyte migration). At d 35, 325 DEG, 264 upregulated, were part of 46 enriched GO terms. Two GO terms enriched at d 17 were also enriched at d 35. Noticeable, 10 of the 18 DEG enriching GO terms at d 17 were also part of enriched GO terms at d 35. Furthermore, the other 44 enriched GO terms at d 35 were mostly biological processes involved in innate (e.g., inflammatory response, phagocytosis) and adaptive (e.g., regulation of T-helper 1 type immune response, positive regulation of IFNγ production) immunity. A unique set of genes were identified that are similarly regulated at d 17 and d 35 of OG feeding. These findings confirm that biomarkers of immunity, regulated by OmniGen AF, can be detected as early as 17 d, and are further enhanced after 35 d of initial day of feeding.

Key Words: OmniGen, dairy cow, immunity

P125 Effect of in vivo heat stress on respiration rate, rectal temperature, and blood mononuclear cell function of dairy cows ranked for immune response. S. Cartwright*[1], J. Schmied[1], M. McKechnie[1], A. Livernois[1,2], and B. Mallard[1,2], [1]*Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada,* [2]*Centre of Genetics of Livestock Improvement, University of Guelph, Guelph, ON, Canada.*

Heat stress (HS) causes disease in dairy cattle. High (H) immune response (IR) dairy cattle have reduced disease, however variations in response to HS have not been evaluated. Therefore, the objective was to evaluate the effect of in vivo HS on respiration rate (RR), rectal temperature (RT), and blood mononuclear cell (BMC) function related to heat shock protein 70 concentration (HSP70 conc) and cell proliferation (CP) in IR phenotyped cattle. Holstein cattle (n = 24), ranked for IR [8 H, 8 average (A), 8 low (L)], based on estimated breeding values, were evaluated. Cattle were HS at the same time on 2 subsequent days (HS1 = 1 HS, HS2 = 2 HS), in the tie-stall wing of the barn, by increasing temperature to 29°C for 4 h. Blood samples were taken pre and post HS1 and HS2. Manual RR and RT and barn temperature (temp) and humidity (hum) were taken pre-HC and every 30 min during HS. Temperature-humidity index (THI) was calculated for each measurement using temp and hum. From blood samples, BMC were obtained to assess HSP70 conc and CP. Repeated-measures models, run in R, evaluated differences in RR and RT and included effects of IR phenotype, THI, parity, pregnancy status and production. General linear models, run in R, evaluated differences in HSP70 conc and CP with similar models. Results showed HIR (THI 76–81 LSM from 43.2 to 55.7 breaths per min (bpm)) had lower RR at higher THI compared with AIR (THI 76–81 LSM from 53.2 to 66.1 bpm) and LIR (THI 76–81 LSM from 50.4 to 73.4 bpm). Differences in RR were observed at THI of 76 (H vs A $P = 0.0247$, H vs L $P = 0.0202$), 77 (H vs A $P = 0.0419$, H vs L $P = 0.0453$) and 81 (H vs A $P = 0.0247$, H vs L $P = 0.008$). No differences in RT was observed. Results also showed HIR had greater HSP70 conc after HS1 (H LSM HS = 12.1 ng/mL SEM = 0.5, A LSM HS1 = 9.2 ng/mL SEM = 0.8, L LSM HS1 = 7.5 ng/mL SEM = 0.5) compared with A ($P = 0.0247$) and L ($P = 0.001$) and greater CP after HS1 (H LSM = 3.36 SEM = 0.65, L LSM = 0.99 SEM = 0.3, $P = 0.0279$) and HS2 (H LSM = 2.83 SEM = 0.62, L LSM = 0.59 SEM = 0.1, $P = 0.0425$) compared with L. Therefore, results may indicate HIR are more thermotolerant compared with A and L.

Key Words: heat stress, immune response, blood mononuclear cells

P126 Persistent, transient or delayed hypocalcemia in Jersey cows: Associations with metabolites and milk production. A. M. Fillus*[1], C. C. Baccili[2], V. Gomes[2], R. B. Navarro[3], and R. Almeida[1], [1]*Universidade Federal do Paraná, Curitiba, Paraná, Brazil,* [2]*Universidade de São Paulo, São Paulo, São Paulo, Brazil,* [3]*Capal Cooperativa Agroindustrial, Arapoti, Paraná, Brazil.*

The aim of this study was to determine the types of subclinical hypocalcemia (SCH) and relate it to milk yield (10, 30, and 60 d postpartum) and blood metabolites. Thirty-one primiparous and 85 multiparous Jersey cows were housed in a compost barn with a robotic milking system. Blood samples were collected at 1, 2, 3, and 4 d postpartum to characterize the level of plasma total Ca and diagnose cows with SCH. Primiparous cows were categorized in 4 early postpartum Ca status groups: NC (normocalcemic; > 2.15 mmo/L at 1 and 2 DIM), tSCH (transient; ≤ 2.15 mmo/L at 1 DIM and >2.15 mmol/L at 2 DIM), dSCH (delayed; > 2.15 mmol/L at 1 DIM and ≤2.15 mmol/L at 2 DIM), and pSCH (persistent; ≤ 2.15 mmol/L at 1 and 2 DIM). The plasma Ca thresholds for multiparous were the same described above, but the second blood collection was at 4 DIM. Statistical analyzes were performed by SAS procedures (v.9.4) using the GLM for single measures and MIXED for repeated measures over time. We found 52.6% NC cows (n = 61), 26.7% tSCH (n = 31), 12.1% dSCH (n = 14), and 8.6% pSCH (n = 10). Average milk yield in the first 10 d was greater ($P = 0.04$) for tSCH than pSCH cows (23.24 vs. 18.96 kg/d, respectively), while NC and dSCH cows showed intermediate yields (21.66 and 20.54 kg/d, respectively). No differences on average milk yield in the first 30 and 60 d were found ($P > 0.10$) among groups. Haptoglobin concentrations were higher ($P < 0.05$) for pSCH and dSCH cows than for tSCH ones; 24.5 ± 3.3 and 22.8 ± 2.7 vs. 14.9 ± 2.1 mg/dL, respectively. NEFA concentrations were higher ($P < 0.05$)