Animal Health 3: Mastitis

W1 Hoof-impact and slide measurements for common Ontario dairy farm floorings. J. E. French*, J. J. Thomason, J. C. Wright, and V. R. Osborne, 1Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada, 2Department of Biomedical Sciences, University of Guelph, Guelph, ON, Canada.

In the context of preventing lameness and injury from slipping, our objective was to characterize hoof impact and slide on 6 flooring types (FT) commonly used in Ontario dairy farms: DC: diamond-grooved concrete, EC: sanded epoxy-covered concrete, GR: grooved rubber mat, HR: high-profile rubber mat, LR: low-profile rubber mat, and TG: turf grass (Kentucky bluegrass/fescue mix). Surface hardness (SH) was measured on each FT using a Clegg drop hammer. Five pre-trained lactating Holstein cows were each walked over all 6 surfaces sequentially in a randomized order. Walking speeds were determined from 200-fps videos in MATLAB. A 3-axis accelerometer that was epoxied to the lateral claw of each hindfoot captured continuous horizontal (ah) and vertical (av) accelerations at 2500 Hz during each trial. All ah and av waveforms were inspected on-screen to identify irregularities that indicated hoof slipping. Impact events (from contact to the hoof being still) were isolated for 3—6 stances/trial, and peak decelerations (ahMax, avMax) were determined in MATLAB. Each ah impact event was double integrated backward, from still to moving, to estimate normal hoof slide. Ranking ahMax and slide values gave separate indicators of relative grip/slip among surfaces. The influences on ahMax and avMax of FT, SH, cow, speed and claw (and all significant higher-order factors) were assessed by ANOVA in SAS 9.4. After verifying data normality, SH positively affected avMax (P < 0.05), but not ahMax (P = 0.75), which are expected results. ahMax was affected by FT (P < 0.01), strongly implicating differences in surface friction (not measured here). The ranking of surfaces (from low to high grip) on ahMax was: LR, EC, HR, GC, DC, TG, and on hoof slide was: EC, HR, GR, LC, TG, with only LR moving substantially, indicating that the variables measure similar aspects of surface properties. Unexpected hoof slips were only seen for 3 stances, 2 on turf and 1 on HR. The hoof-surface interaction can be assessed in subtle detail, and such data may be of use in preventing injury by slipping.

Key Words: dairy cattle, hoof-surface interaction

W2 Detecting the bacterial variation of recycled manure solids for use as bedding in freestalls. H. Wu*, N. Zheng, and J. Wang, Laboratory of Quality and Safety Risk Assessment for Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.

Recycled manure solids (RMS) was separated from cow feces as bedding. However, the influence of bedding microflora on milk bacteria remains unclear. To investigate the variation of bacteria in samples of RMS process and compare the contribution to microbe in mastitis/healthy bedding to milk microbe. Three consecutive days in 2019, the samples of rectum feces from healthy/mastitis dairy cows were collected. All feces were mixed and then subjected to fecal mixing pool, extrusion, store, sun-cure according to the RMS process. Samples were collected for each step. Meanwhile, the Mastitis/healthy cowshed bedding and mastitis/tank milk were collected in the farm of Hebei Province. All samples were used to investigate the presence of pathogenic bacteria of mastitis by qPCR mastitis screening kit. And the bacterial diversity of those sample was determined by the variable region of v3-v4 in the flora determined by MiSeq. The Qiime2 software, SPSS, Galaxy and R software were used to analyze the Miseq data. All pathogenic bacteria of mastitis were not found in all samples except the milk samples of mastitis. The microflora similarity of faces and bedding was low in the mastitis/healthy shreds. Moreover, Lefse results showed that bacterial proportion of feces from healthy dairy cows was significant different with those from mastitis cows. F16 (1.8 ± 1.3%), Erysipelototrichaceae (1.4 ± 1.4%) and Coriobacteriaceae (0.2 ± 0.2%) had a higher proportion in the feces of healthy dairy cows, while only Streptococcaceae (0.5 ± 0.1%) had higher proportion of mastitis feces (P < 0.05). The enrichment of microorganisms in rectal feces decreased during the RMS processed steps. Compared with rectal feces, there were a higher proportion of Xanthomadaceae (9.2 ± 2.2%), Sphingobacteriaceae (2.6 ± 1.4%), Saprospiraceae (4.4 ± 2.4%), Phyllobacteriaceae (6.7 ± 2.4%), Flavonirviraceae (1.0 ± 0.6%), Flavobacteriaceae (9.1 ± 4.9%) and Altermomonasaceae (3.7 ± 2.1%) in the bedding samples. The predominant bacteria in milk was not abundant in feces or bedding materials. In general, RMS for use as bedding may not increase the incidence of mastitis in dairy cows.

Key Words: recycled manure solids, microflora

W3 Identifying factors associated with lameness and its impact on productivity in automated milking herds. R. D. Matson*, M. T. M. King*, T. F. Duffield, E. Santschi, K. Orsel, E. A. Pajor, G. B. Penny, T. Mutsuwagwa, and T. J. DeVries, 1Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, 2Department of Population Medicine, University of Guelph, Guelph, ON, Canada, 3Lactanet, Sainte-Anne-de-Bellevue, QC, Canada, 4Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada, 5Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada.

Voluntary milking is critical for success in automated milking systems (AMS); impaired gait (lameness) may negatively affect the ability and desire for cows to milk voluntarily. The objective of this study was to assess the effect that lameness has on the productivity of AMS herds and identify factors associated with lameness. From April to September 2019, 76 robot herds were visited, and data on barn design and farm management practices were collected. Data from AMS units were collected, along with milk recording data for the 6 mo period before farm visits. Farms averaged 99 ± 73 lactating cows, 2.3 ± 1.4 robot units/farm, 43.9 ± 9.0 cows/robot, 36.7 ± 4.7 kg/d of milk, a milking frequency of 3.0 ± 0.4x/d, and a herd-average SCC of 198.3 ± 88.1 (x1,000) cells/mL. Thirty percent (minimum of 30 cows/farm) were scored for body condition (BCS 1 = underconditioned to 5 = over conditioned) and gait (1 = sound to 5 = lame); with clinically lame ≥ 3: 28.6 ± 11.7%; and severely lame ≥ 4: 3.0 ± 3.2%). Univariable models were used to screen independent variables (as fixed effects) in mixed-effect linear regression models and variables with P < 0.25 were offered to multivariable models. Clinical lameness was 10.2 percentage points (p.p.) less prevalent on farms with sand bedding (P < 0.01) and tended to be 2.8 p.p. less for each additional time stalls were raked/d (P = 0.07) and 5.7 p.p. less for farms that built new barns vs. retrofitting existing barns (P = 0.07). Herd average milk yield/cow decreased with greater prevalence of clinical (−0.1kg/d for 1 p.p. increase; P = 0.01) and severe lameness (−0.8kg/d with doubling of prevalence from 3 to 6%; P < 0.01). Milk yield/robot decreased with a greater prevalence of clinical lameness (−7.1kg/d for 1 p.p. increase; P = 0.01). Lesser milking frequency was associated with a greater proportion of over-conditioned cows (P = 0.04). SCC was associated with a greater proportion of clinically lame (P < 0.01) and under-conditioned cows (P = 0.05). Overall, this study demonstrates that productivity and milk quality in AMS herds are optimized by maintaining mobility and body condition of cows.

Key Words: automated milking system, lameness, herd management

W4 Screening stable flies and house flies as potential vectors of digital dermatitis in dairy cattle. R. Thibodeaux*, J. Brady, S. Swiger, and B. Jones, 1Tarleton State University, Stephenville, TX, 2Texas A&M Agrilife Research, Stephenville, TX.

Key Words: digital dermatitis, cattle, vector, screening
Digital dermatitis is a bacterial infection that causes lesions above the heel bulbs on cattle hooves. *Treponema* bacteria are present on the lesions of an infected hoof. Transmission is suspected through mechanical exposure in dirty bedding areas, but insects as potential vectors have not been explored. Flies can lead to other diseases like mastitis. Thus, the vast number of flies on a dairy may contribute to the spreading of digital dermatitis. Therefore, the objective of this research was to determine if flies captured from a dairy farm known to have digital dermatitis carry *Treponema* bacteria on their legs and heads. Autoclaved mesh sweep nets were used to collect the stable flies (*Stomoxys calcitrans*) and house flies (*Musca domestica*) for transfer to a plastic crate with a fabric opening until transport to the lab. The heads and legs of the fly were cut off with a sterile scalpel to use in the DNA extraction. Flies (n = 417) had DNA extracted using a Qiagen Blood and Tissue Kit. DNA from one tube was extracted from samples was measured by using a Qubit 2.0 Fluorometer kit (Life Technologies, Carlsbad, CA). A plasmid with *Treponema phagedenis* gene sequence was used as a qPCR positive control because this species was most prevalent in digital dermatitis infections. The negative control used was molecular biology grade water. The plasmid was serial diluted 1:10,000 before use as a qPCR positive control. *Treponema* bacteria were not detected on any of the flies we tested. The DNA of treponemes may not have been detected due to the small sample size compared with the overall population, or that other species of flies need to be analyzed to broaden the possibility of detecting the DNA. Further analysis should be conducted to confirm if other flies may carry *Treponema* bacteria, and to explore other potential vectors of *Treponema* bacteria.

**Key Words:** digital dermatitis, *Treponema*, qPCR

**W6**

Transcriptional alterations due to sole ulcer revealed via RNA-seq analysis of corium tissue in lactating dairy cows. F. Rosa¹, N. A. Carpinelli², J. T. R. Carvalho², K. Mjoun³, and J. S. Osorio¹, ¹Dairy and Food Science Department, South Dakota State University, Brookings, SD, ²Animal Science Department, Universidade Federal de Lavras, Brazil, ³Alltech Inc, Brookings, SD.

Ulceration of the sole can damage the corium and affect the keratinization of the hoof tissue. An RNA-seq analysis was performed in corium tissue biopsies to provide a comprehensive transcriptome profile of the hoof. Multiporous Holstein dairy cows were selected based on their condition as clinically healthy (HSU, n = 7), or diagnosed with sole ulcers (SU, n = 7). Lameness was assessed based on a locomotion score (1–5 scale). Hoof hardness was assessed using a durometer (Shore A0–100HA). Locomotion scores were 1 ± 0 and 2.5 ± 0.5 (mean ± SD) for HSU and SU, respectively. Hoof hardness was 79.8 ± 6.1 and 69.9 ± 4.4 for HSU and SU, respectively. Hoof biopsies were performed according to Osorio et al., 2012; *J Dairy Sci, 95*:6388. All corium samples were immediately flash-frozen in liquid nitrogen. The RNA integrity number for all samples was 8.0 ± 1.0. The RNA samples were sequenced (NGS; Illumina, NovaSeq S4) at the University of Minnesota Genomics Center. Processed reads were aligned to the bovine genome using HISAT2. Differential transcript analysis was performed using a quasi-likelihood test in R-package (EdgeR) and False Discovery Rate < 0.05 correction was applied. Among the 500 differentially expressed genes identified, SU upregulated the expression of genes related to the Toll-like receptor (TLR) pathway including TLR2, 5, and 6 (P < 0.01) in comparison to HSU. Genes mediators of pro-inflammatory response *IL1A*, *IL1B*, *IL1RA*, and *IL18RAP* (P ≤ 0.03) were upregulated in the SU in comparison to HSU. Keratin-associated proteins (KRTAP) including KRTAP17–1, KRTAP15–1, KRTAP19–3, and KRTAP27–1 were downregulated (P < 0.002) in SU than HSU. The greater P = 0.005) expression of COL6A2 in hoof tissue of SU cows than HSU can be associated with extracellular matrix structural function impacted by sole ulcer. Our preliminary data further confirms that ulcerations can develop inflammatory-like conditions while decreasing keratinization of the hoof. Further analysis of this RNA-seq data can provide novel molecular targets for nutritional interventions to reduce sole ulcer incidence and prevalence in dairy herds.

**Key Words:** dairy cow, hoof, transcriptome

**W7**


A data-driven, selective dry-cow therapy algorithm has been used often in trials by the authors and has been implemented by US dairy producers to target prudent use of antimicrobials. This case report retrospectively evaluated the recurrence, characteristics, and culture results of cows from a NY dairy that were identified as “low risk” by the algorithm during the 2nd year of using the program. All cows eligible for dry-off had the opportunity to be enrolled from April 2018 to Oct 2018 and again from May 2019 to Aug 2019. “Low risk (LR)” cows (cows that likely will not benefit from antibiotics) were identified as having all of the following: < 200k cells/mL at last test dry-off, an average somatic cell count < 200k cells/mL on the last 3 test days, no signs of mastitis at dry-off, and no more than 1 clinical mastitis event in the current lactation. Quarter-level milk samples were aseptically retrieved at dry-off during both periods and were submitted for diagnostic aerobic culture and MALDI-TOF follow-up, epithelial permeability and keratinization. Enrichment pathway analyses will be performed to better understand these findings and to further identify molecular targets for nutritional or management interventions.
Dry-cow antibiotic therapy is used to both cure existing intramammary infections (IMI) at drying off and to prevent new IMI from occurring during the dry period. IMI increases the risk of clinical mastitis (CM) in the subsequent lactation, therefore, dry-off antibiotics are commonly used during this period to decrease the impact of IMI or CM on milk quality and quantity. The objective of this research was to determine the relative efficacy of dry-off antibiotics labeled for use to cure IMI in dairy cattle through a systematic review and network meta-analysis approach. A systematic search of relevant databases (Medline, CAB Abstracts, Science Citation Index, Conference Proceedings Citation Index – Science, and Agricola), conference proceedings, and FDA summaries was conducted to identify relevant articles. Eligible studies were controlled trials with natural disease exposure evaluating dry-off antibiotic treatment for cure of existing IMI, in comparison to a different antibiotic treatment, a non-antibiotic treatment, or no treatment. From 3749 unique citations identified by the search, there were 149 eligible trials with 236 treatment arms remaining for data extraction, which was completed independently in duplicate. Data from these studies will be synthesized using a network meta-analysis to assess the efficacy of multiple dry-cow antibiotic options through the use of both direct and indirect evidence. If products are equivalent in efficacy, one with a lesser level of importance for human health can be chosen for use by producers. This research will aid veterinarians and dairy producers in making evidence-based decisions concerning antibiotic use and will support the judicious use of antibiotics in dairy cattle.

**Key Words**: bovine mastitis, *Staphylococcus aureus*

**W9** Characterization of serine-aspartate rich (Sdr) proteins in *Staphylococcus aureus* isolates from bovine intramammary infections in Canada. A. Dubé-Duquette*1, É. Demontier1, J.-F. Lucier1, S. Rodrigue1, J.-P. Roy2, C. Ster1, and F. Malouin1, 1Département de Biologie, Faculté des Sciences, Université de Sherbrooke, Sherbrooke, QC, Canada, 2Département de Sciences Cliniques, Faculté de Médecine Vétérinaire, Université de Montréal, St-Hyacinthe, QC, Canada, 3Agriculture and Agri-Food Canada, Sherbrooke Research and Development Centre, Sherbrooke, QC, Canada.

Many *Staphylococcus aureus* surface proteins represent key elements in the pathogenic process leading to bovine intramammary infection (IMI) and mastitis. We characterized the diversity of serine-aspartate repeat (Sdr) surface proteins found in *S. aureus* strains collected from quarter milk samples in Canada. Strains were selected to represent the main *spa*-types found in Canadian dairy cattle (t259, t267, t359, t605, t13401, t2245). Two groups (t605 and t13401) were strong biofilm producers and *spa* type t2445 was composed of weak biofilm producer strains. The *spa* type t529 was the most prevalent and associated with the presence of the superantigen gene *seg*. Whole-genome sequencing data from the mastitis-associated *S. aureus* strains (36 strains in total) was used to analyze the distribution of 3 specific *sdr* genes (*sdrC, sdrD* and *sdrE*) in the different *spa* groups. *SdrC* was present in all studied strains except one from the t13401 group. Interestingly, strains from t605 and t529 had the same mutation leading to an early stop codon in *sdrC*. Also, there was a deletion of 330 bases in the *sdrC* gene of t2445 strains (the low-biofilm producers), occurring in a repeated region of the protein rather than in the functional region. *SdrE* was found in all *spa* groups except for t13401. Surprisingly, an allelic variant of *sdrE* was noticed in t529 isolates. This variant, named bone sialoprotein-binding protein, is characterized by a modified functional domain of the protein and was previously linked to strains leading to bone infections in humans. *SdrD* was present in 3 *spa* types (t267, t2445 and t359) and its sequence was very similar in all those strains. These results revealed that most *S. aureus* strains from quarter milk samples carry *sdrC* and *sdrE* and that important sequence differences exist between some *spa* groups. However, *sdrD* seems less common. Considering that *S. aureus* can cause variable infection patterns in dairy cattle, understanding the diversity of virulence factors should lead to a better identification of problematic strains and lead to more precise herd management decisions.

**Key Words**: bovine mastitis, *Staphylococcus aureus*