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229 Fecal microbiome profiles of pre-weaned Jersey and Holstein calves with gastrointestinal disease. G. S. Slanzon*1, L. M. Parrish, S. C. Trombetta, W. M. Sischo, and C. S. McConnel, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA.

Gastrointestinal (GI) disease is the most common illness in pre-weaned dairy calves. The fecal microbiome composition is associated with health status, but changes in the microbiome across different levels of GI disease and different breeds remain unclear. Our objective was to associate symptoms of GI disease and breed differences with the fecal microbiome. Fecal samples were collected from 282 calves: 194 Holstein (H) and 88 Jersey (J). Health status was evaluated daily. Calves with a fecal score ≤ 2 and no clinical illness were classified as healthy. Calves with a fecal score ≥ 3 were diagnosed with GI disease and classified as bright-sick (BS) or depressed-sick (DS) according to their behavior. A total of 45 samples from healthy calves (n = 38 H, n = 7 J), 11 samples from BS calves (n = 5 H, n = 6 J), and 15 samples from DS calves (n = 6 H, n = 9 J) were randomly selected to represent different breeds, ages (4–15 d of age) and disease severity. The V3-V4 region of 16S rRNA gene was sequenced and an ASV table was used to compare taxonomic profiles. Differences were identified by LEfSe (P < 0.05; LDA score > 2). Firmicutes was identified as the most dominant phylum in calves with GI disease, and Actinobacteria in healthy calves. Healthy calves showed abundant Bifidobacteriaceae, Bacteroidaceae and Enterobacteriaceae. BS calves showed abundant Listeriaceae, Clostridiaceae and Lachnospiraceae. DS calves showed abundant Lactobacillaceae, Streptococcaceae and Enterobacteriaceae. Specific breed differences included a greater abundance of Bacteroides fragilis (LDA = 4.44) in healthy Jersey calves, and Bifidobacterium longum (LDA = 5.25) in healthy Holstein calves. Lactobacillus reuteri_vaginalis (LDA = 5.02), was abundant in DS Jersey calves, and Escherichia_Shiella coli (LDA = 5.01) was abundant in DS Holstein calves. Notably, the abundance of the family Lactobacillaceae in diseased calves raises questions regarding its role in sustaining a favorable microbial balance, given that certain lactic acid bacteria have been shown to reduce the incidence of diarrhea in calves and provide protective effects against opportunistic pathogens at the intestinal level.

Key Words: microbiome, calf, diarrhea

230 Implementation of an antimicrobial-use algorithm for treatment of diarrheic calves: Impact on antimicrobial treatment and mortality rates. D. Gomez1, L. Arroyo1, D. Renaud2*, and J. S. Weese3, 1Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, 2Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, 3Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.

Calf diarrhea is the main cause of disease and usage of antimicrobial agents in calves <30 d of age on dairy farms. Approaches are needed to reduce, refine and standardize the use of antimicrobial (AB) therapy in diarrheic calves. The objective of this study was to evaluate the impact of implementing an antimicrobial-use algorithm on AB treatment and mortality rates of diarrheic calves. The study consisted of 2 periods: (a) Data analyses of AB treatment and mortality rates of dairy calves from 10 farms (F1-F10) before the implementation of the algorithm (before period, BP; 1 year) and (b) data analyses of AB treatment and mortality rates after implementation of the algorithm (after period, AP; 1 year). The algorithm directed AB therapy based on the presence of fever (≥39.5°C) and hypoxemia in calves suffering from diarrhea. Treatment records of 2049 and 2251 calves were available in the BP and AP. Overall, the incidence of diarrhea was similar between periods (BP; 77% (1,573/2,049) vs. AP; 75% (1,698/2,251); P = 0.316). Implementation of the algorithm resulted in a marked reduction in antimicrobial treatment rates. In the BP, 85% (1303/1573) of the diarrheic calves were treated with antimicrobials, while in the AP, 18% (310/1698) were treated (P < 0.001). Reduction of the antimicrobial treatment rates of diarrheic calves was observed in 7 farms in the AP (P < 0.01), but differences were not observed in 3 farms (P > 0.05). In general, there were no differences in the overall mortality (BP: 4% (75/2,049) vs. AP: 3% (72/2,251); P = 0.449) or the mortality attributable to diarrhea between periods (BP: 1% (22/1,573) vs. AP: 0.6% (12/1,592); P = 0.73). The use of user-friendly antimicrobial-use algorithms can reduce significantly the use of antimicrobials with no identifiable negative impact on calf health.

Key Words: calf diarrhea, antimicrobial use, mortality rates

231 Antimicrobial use and decision making with respect to treatment of diarrhea in Canadian dairy calves. T. Uyama*1, D. Kelton1, S. LeBlanc1, D. Léger2, S. Dufour3, J. Roy1, H. Barkema4, E. de Jong1, K. McCubbin1, M. Fonseca1, L. Heider1, and D. Renaud1, 1Department of Population Medicine, University of Guelph, Guelph, ON, Canada, 2Centre for Food-borne, Environmental & Zoonotic Infectious Diseases, Public Health Agency of Canada, Guelph, ON, Canada, 3Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, QC, Canada, 4Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada, 5Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI, Canada.

Antimicrobial resistance in livestock is a growing concern due to possible transmission to humans so it is important to understand antimicrobial use in farm animals. Dairy calves may receive antimicrobials for the treatment of diarrhea, but it is unclear under what circumstances antimicrobials are used. The objective of this study is to investigate antimicrobial use and case-specific information used in treating diarrhea in Canadian dairy calves. A total of 105 dairy farmers (Ontario: 31; Alberta: 28; British Columbia: 26; Nova Scotia: 20) were selected purposively and completed a questionnaire in person about calf health. First, farmers were asked, “Do you use antimicrobials to treat calf diarrhea?” Second, only those who used antimicrobials to treat diarrhea were asked, “What case-specific information do you use to select a diarrhea case for antimicrobial treatment?” Respondents were instructed to select all that apply from a list of 4 symptoms (fecal consistency, fever, attitude, level of dehydration) or otherwise specified. The average herd size was 162 milking cows (range 36–560). Among 105 farmers, 72% used antimicrobials to treat diarrhea. Among those who used antimicrobials for diarrhea, 78% used “fetal consistency,” 61% used “fever,” 55% used “attitude,” 53% used “level of dehydration,” and 28% used 11 other characteristics as indicators to treat diarrhea with antimicrobials. The most common answer was selecting all 4 symptoms given, which was selected by 20% of those who used antimicrobials to treat diarrhea. Among 59 farmers who used fetal consistency as an indicator to treat diarrhea with antimicrobials, only 15% solely used this criterion. Among 91 farmers who were asked whether they had a written treatment protocol for calf diarrhea, 35% reported that they have the protocol and 94% of them were discussed with veterinarians. Treatment decisions could be improved for those who solely depend on fetal consistency as an indicator to treat diarrhea with antimicrobials, as well as developing protocols with a veterinarian, to encourage prudent use of antimicrobials in dairy calves.

Key Words: dairy calf, treatment protocol, case-specific information
Diarrhea continues to be the primary cause of morbidity and mortality in preweaned dairy calves. This disease results in economic losses and long-term effects on health and productivity of surviving animals. Hence, monitoring systems that can be correlated with fecal scores in preweaned calves can allow dairy farmers to implement effective response protocols to such disease. Therefore, the objective of this study was to use 3-D accelerations to derive behavior activity (e.g., standing or lying time) and associate these with fecal scores in neonatal dairy calves. Forty-two healthy newborn dairy calves were used from birth until 21 d of age in a retrospective analysis. Calves were housed in individual hutches, had free access to starter and water, and were fed 2.8 L of milk replacer 2 × /d during wk 1 to 3. Calves were fitted with an accelerometer (Onset; Pocasset, MA) mounted in the rear left leg set to record every 60-s throughout the experiment. Fecal score (FS), respiratory score, rectal temperature, and starter intake were recorded daily, while BW and withers height were measured weekly. Overall fecal score (FS) of all calves reached its highest points during wk 2, with a maximum value on 10 d (FS = 2.1 ± 0.1). Based on average individual FS, calves were classified as low FS (LFS; FS = 1.47 ± 0.1; n = 21) and high FS (HFS; FS = 2.14 ± 0.1; n = 21). Data were analyzed using PROC MIXED of SAS with group, time, and their interaction as fixed effects and calf within group as random effect. Data were analyzed using PROC MIXED of SAS with group, time, and their interaction as fixed effects and calf within group as random effect. As expected, FS was greater ($P < 0.01$) in the HFS group during the first 21 d of life. LFS calves showed greater ($P < 0.01$) starter intake (0.23 vs 0.10 kg/d), BW (50.0 vs 44.1 kg), and withers height (82.0 vs 78.8 cm), while lower respiratory score ($P = 0.02$) and rectal temperature ($P < 0.01$) than HFS calves. The HFS calves tended ($P = 0.11$) to have greater lying time than LFS. Although standing bouts were unaffected ($P = 0.64$) by FS, lying bouts tended to be greater ($P = 0.06$) in HFS calves than LFS. Differences in standing and lying behavior indicate that these parameters could be used to monitor diarrhea in neonatal dairy calves.

**Key Words:** calf scour, accelerometer, diarrhea

### Table 1 (Abstr. 233). *Bacillus licheniformis* and *B. subtilis* enzymatic activity profile

<table>
<thead>
<tr>
<th>Species</th>
<th>Protease</th>
<th>Cellulase</th>
<th>Amylase</th>
<th>Xylanase</th>
<th>Galactomannanase</th>
<th>Galactanase</th>
<th>X-gal</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. licheniformis</em></td>
<td>—</td>
<td>xx</td>
<td>(x)</td>
<td>(x)</td>
<td>x</td>
<td>(x)</td>
<td>(x)</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>x</td>
<td>x</td>
<td>xx</td>
<td>xx</td>
<td>xx</td>
<td>(x)</td>
<td>—</td>
</tr>
</tbody>
</table>

*Where (x), x, and xx represent increasing zones of clearance/more enzyme activity; — = none.*

233 **In vitro evaluation of Bacillus licheniformis and Bacillus subtilis enzyme activity, Clostridium perfringens Type A inhibition, and biofilm formation.** A. Segura*, L. Drehmer1,2, A. J. Triminio1, N. A. Carpinelli1, and J. S. Osorio1, 1South Dakota State University, Brookings, SD, 2Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil, 3Escuela Agrícola Panamericana El Zamorano, El Zamorano, Francisco Morazan, Honduras. The use of Bacillus as direct-fed microbials (DFM) has been investigated due to their ability to produce antimicrobials and synthesize enzymes. In ruminants, Bacillus supplementation has been reported to improve performance and limit diseases. The aim of this study was to investigate (1) the enzymatic activity profile of 2 Bacillus strains (*B. licheniformis* [BL], DSM 5749, and *B. subtilis* [BS], DSM 5750), (2) their inhibitory capacity toward *Clostridium perfringens* Type A (CPa, DSM 756), and (3) their biofilm synthesis capacity. Enzyme activity was measured qualitatively with insoluble chromogenic substrates and on milk agar for protease activity. CPa inhibition was evaluated qualitatively by using an agar diffusion assay. In addition, the Ankom RF Gas Production System was used to measure gas production of CPa with or without BL or BS incubated in a Brain Heart Infusion (BHI) broth (37°C, 24h, in triplicate). Biofilm formation was assessed by crystal violet staining after overnight incubation in BHI broth at 37°C. Statistical analysis was performed using the one-way ANOVA with the Dunnett’s post-hoc test (GraphPad Prism). Table 1 reports the enzyme activities and shows that *Bacillus* differed in their capacity to produce enzymes. BL and BS were able to generate similar inhibition zones against CPa. The quantitative method also revealed that BL and BS significantly reduced CPa gas production (20%, $P < 0.001$, and 12%, $P < 0.01$, respectively). Finally, BL appeared to be a strong biofilm producer ($OD_{595nm} > 2$) while BS weakly produced biofilm ($OD_{595nm} < 1$). The results show that the enzyme activity, and the ability to produce antimicrobials and biofilm appear to be specific features for each *Bacillus*. In vivo studies need to be conducted to test if BL and BS could enhance ruminant performance and health.

**Key Words:** Bacillus, direct-fed microbial (DFM), ruminant