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Dysbiosis of the fecal microbiota in feedlot cattle with hemorrhagic diarrhea

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ABSTRACT

The bovine gastrointestinal microbiota is a complex polymicrobial ecosystem that plays an important role in maintaining mucosal health. The role of mucosal microbial populations in the pathogenesis of gastrointestinal diseases has been well established in other species. However, limited information is available about changes in the fecal microbiota that occur under disease conditions, such as hemorrhagic diarrhea in feedlot cattle. The objectives of this study were to characterize the differences in fecal microbiota composition, diversity and functional gene profile between feedlot calves with, and without, hemorrhagic diarrhea. Deep fecal swabs were collected from calves with hemorrhagic diarrhea (n = 5) and from pen matched healthy calves (n = 5). Genomic DNA was extracted, and V1-V3 hypervariable region of 16S rRNA gene was amplified and sequenced using the Illumina MiSeq sequencing. When compared to healthy calves, feedlot cattle with hemorrhagic diarrhea showed significant increases in the relative abundance of *Clostridium*, *Blautia* and *Escherichia,* and significant decreases in the relative abundance of *Flavobacterium, Oscillospira, Desulfonauticus, Ruminococcus, Thermodesulfovibrio* and *Butyricimonas.* Linear discriminant analysis effect size (LEfSe) also revealed significant differences in bacterial taxa between healthy calves and hemorrhagic diarrhea calves. This apparent dysbiosis in fecal microbiota was associated with significant differences in the predictive functional metagenome profiles of these microbial communities. In summary, our results revealed a bacterial dysbiosis in fecal samples of calves with hemorrhagic diarrhea, with the diseased calves exhibiting less diversity and fewer observed species compared to healthy controls. Additional studies are warranted in a larger cohort of animals to help elucidate the trajectory of change in fecal microbial communities, and their predictive functional capacity, in calves with other gastrointestinal diseases.

1. Introduction

The bovine gastrointestinal microbiota is comprised of a diverse and complex microbial population that coexists in a coordinated, complex mucosal ecosystem, and contributes to host health and immunological development [1]. Certain components of this microbiota are always present in large, and approximately constant, numbers in the healthy gut [2]. These symbionts act as a defense barrier against colonization by transient enteropathogens, support the host in digestion and energy harvest from the diet, and provide stimulatory signals to the immune system [3]. While the composition and functional roles of the bovine fecal microbiota have been significant research topics for decades, the majority of previous studies were restricted by their dependence on traditional culture-dependent methods. These techniques provide only a relatively narrow picture of these complex microbial populations [4]. Recent advances in next generation sequencing platforms, and their accompanying bioinformatics analytics, have revealed the extent of diversity of microbial communities in the gastrointestinal environments of many species, including cattle, and have demonstrated the complex interplay between the host and microbial ecosystems [5].

Recently, changes in the composition of fecal microbiota has been associated with various gastrointestinal diseases; for example, certain community structures appear to be protective against pathogens that can cause diarrhea [5]. Disturbances in gastrointestinal health are often multifactorial, and can cause economic losses in cattle through diminished fertility, milk production and/or weight gain [6]. Poor gastrointestinal health is also associated with impaired digestive and absorptive processes, disruptions of the resident microbiota [3] and a higher incidence of morbidity and mortality, particularly in young animals [7]. Potential causes of hemorrhagic diarrhea in calves have not yet been determined, but growing evidence indicates that it is due to a combination of factors that include high levels of grain feeding, parasitic (coccidiosis) and bacterial infections (*Clostridium perfringens and Escherichia coli*) [4,6,8]. While several investigators have employed

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next generation sequencing to describe the changes in the fecal microbiota associated with enteric disease in calves [5,9], there are no studies that have evaluated alterations in the fecal microbiota in feeder cattle with hemorrhagic diarrhea. Therefore, the objective of this study was to characterize the differences in fecal microbial community composition, diversity, and predictive functional metagenomic profile, using 16S rRNA sequencing, between cattle with, and without, hemorrhagic diarrhea. We hypothesized that feedlot cattle with hemorrhagic diarrhea would have a reduction in fecal microbial richness and diversity when compared to the pen matched healthy controls.

2. Materials and methods

2.1. Animal populations and sample collection

Samples for this study were collected as part of a clinical investigation from a large (> 70,000 head one-time capacity) cattle feeding facility located in the west central United States that had a multi-year, seasonal history of hemorrhagic diarrhea late in the feeding period. A total of 10 feedlot cattle (5 calves which exhibited profuse bloody diarrhea of less than 24 h duration and 5 pen matched control calves) that were more than 100 days into the feeding period, and were over 550 kg of body weight, were enrolled in this study. Management practices in this enterprise were typical of those used in large cattle feeding operations across the Unites States. Briefly, cattle were housed in open, packed soil pens, with concrete feed bunks on one side and a free access water source. Cattle were fed a total mixed ration multiple times per day that was matched to stage of feeding, and all cattle had a steroidal implant placed at the beginning, and again in the middle, of the feeding period. Each pen was comprised of multiple sources of cattle that were comingled at the beginning of the feeding period. All cattle received a similar preventative health program at arrival that included anthelmintics, a commercial 5-way viral respiratory vaccine, and a 7-way Clostridia bacterin.

Cattle were observed daily, by trained feed yard staff, for common signs of disease. Upon observation of hemorrhagic diarrhea in an individual, each calf was moved to a hospital area for individual examination (case animals). At the same time, a calf without clinical signs was removed from the pen to the hospital area for examination (control animals). In the hospital area, a complete physical examination was performed by trained hospital personnel. Across all individuals, the only abnormal findings in the case animals was the presence of profuse bloody diarrhea. Following examination, a single, deep fecal swab was collected using a 15 cm long culture swab (Pur-Wraps®, Puritan Medical Products, Gulford, Maine, U.S.A.). The tips of the swabs were then broken off into sterile 2-mL cryo-tubes, transported on dry ice to the laboratory, and stored at −80 °C until analysis.

The use of all animals for this study was approved by University of Illinois Institutional Animal Care and Use Committee.

2.2. Genomic DNA extraction

Total genomic DNA was extracted from each fecal swab using the power® Fecal DNA isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions [10]. For each sample, the total DNA concentration and integrity was quantified using a Nanodrop™ spectrophotometer (NanoDrop Technologies, Rockland, DE, USA) at wavelengths of 260 and 280 nm, and agarose gel electrophoresis (Bio-Rad Laboratories, Inc, Hercules, CA, USA). The extracted nucleic acids (NAs) were immediately stored at −20 °C pending further analysis.

2.3. 16S rRNA gene amplification and Illumina sequencing

The extracted DNA was subject to Fluidigm Access Array Amplification (Fluidigm Corporation, South San Francisco, CA, USA) as previously described [11]. Briefly, the primer sequences F28-2-for (GAGTTTGATCNTGGCTCAG) and R519-2-rev (GTNTTACNGCGGCKGCTG) were used to amplify the V1-V3 hypervariable region of the 16s rRNA gene. PCR reactions were performed on a Fluidigm Biomark HD™ PCR machine (Fluidigm Corporation, South San Francisco, CA, USA) using the default Access Array cycling program without imaging. PCR products were quantified on a Qubit fluorometer and the quality of the amplicons regions was assessed using a Fragment Analyzer (Advanced Analytics, Ames, IA, USA) to confirm amplicon regions and sizes. PCR products were then sized on a 2% agarose E-gel™ (Life technologies, Grand Island, NY, USA) and extracted from the isolated gel slice with QIAquick Gel extraction kit (Qiagen, Valencia, CA, USA). Cleaned, size-selected product was run on an Agilent Bioanalyzer™ to confirm appropriate profile, and determine the average size. The final pooled Fluidigm libraries were transferred to the DNA Services lab at the W. M. Keck Center for Comparative and Functional Genomics, at the University of Illinois at Urbana-Champaign, for Illumina sequencing. The Illumina MiSeq platform (Illumina, San Diego, CA, USA) was used to sequence the V1- V3 region of the 16S rRNA gene according to the Illumina instructions.

2.4. Bioinformatics and statistical analysis

The raw sequence data were preprocessed and analyzed using the open-source software package, Quantitative Insights into Microbial Ecology (QIIME®) version 1.9 (http://qiime.org/) [12]. Sequences were filtered for quality using the default parameters of the split_libraries.py command [13]. Chimeric sequences were detected and removed using UCHIME® [14]. The remaining sequences were clustered into operational taxonomic units (OTUs) using open reference OTU selection protocol (97% identity cutoff) with the UCLUST algorithm [15], and assigned a taxonomic identity, using an open reference protocol against the Greengenes® database, to the genus level [16]. The QIIME® software was then used to calculate alpha diversity indices (an estimate of bacterial community richness in a sample) using the Chao1, observed species, and Shannon diversity index. Due to unequal sequencing depth between the different treatment groups, and to standardize our analysis, all samples were randomly rarefied to 713 sequences per sample using QIIME software.

The OTU relative abundance values were analyzed using the linear discriminant analysis (LDA) effect size (LEfSe) algorithm to identify OTUs that display significant differences between the two experimental groups [17]. Finally, the OTUs data were submitted to PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) pipeline to predict the alterations in the fecal microbiota function using KEGG (Kyoto Encyclopedia of Genes and Genomes) databases [18].

Statistical analyses were performed in using JMP 13 software (SAS Institute Inc., North Carolina, USA). The least-square mean of bacterial diversity indices, relative abundance, and functional profile of the fecal microbiota between the healthy calves and calves with hemorrhagic diarrhea, were compared using non-parametric Wilcoxon/Kruskal-Wallis Tests fitted in JMP 13 (SAS Institute Inc.). Beta diversity was estimated using Principal component analysis (PCA), using the two experimental groups as variables, and the prevalence of most abundant bacterial genera as covariates. P values of < 0.05 were considered significant for all analyses.

Fastq data obtained in the current study were uploaded to the sequence read archive (SRA) on National Center for Biotechnology Information (NCBI) website to make the files available for a public database with bio-project accession number PRJNA326197.

3. Results

3.1. Overall sequence analysis

The sequencing analysis of the V1-V3 hypervariable regions of the bacterial 16S rRNA resulted in a total of 25,407 sequences in all fecal

swabs. The number of sequences per sample ranged from 1130 to 7131 (mean 3175.87, SD 1746.75) and comprised 1355 OTUs (97% identity cutoff) across all samples.

3.2. Dynamics of change in the fecal microbiota between calves with hemorrhagic diarrhea and healthy calves

At the phylum level, the dominant bacterial phyla across all samples were *Firmicutes* (48.69%), *Bacteroidetes* (27.42%) and *Proteobacteria* (12.36%). In calves with hemorrhagic diarrhea, we observed a predominance of *Firmicutes* (60.21% versus 37.17% in healthy calves: $P = .031$) and *Actinobacteria* (4.21% versus 2.33% in healthy calves: $P = .032$). While in the healthy calves, we observed a predominance of *Spirochaetes* (5.32% versus 0.58% in calves with hemorrhagic diarrhea: $P = .047$), *Nitrospirae* (2.76% versus 0.36% in calves with hemorrhagic diarrhea: $P = .001$) and *Synergistetes* (0.83% versus 0.27% in calves with hemorrhagic diarrhea: $P = .045$) (Fig. 1 and Fig. S1).

At the family level, the most abundant bacterial families in the fecal microbiota across all samples were *Clostridiaceae* (22.27%), *Bacteroidaceae* (13.51%), *Lachnospiraceae* (13.38%), *Ruminococcaceae* (6.53%), *Flavobacteriaceae* (5.65%), *Prevotellaceae* (4.63%) and *Spirochaetaceaea* (3.29%). In calves with hemorrhagic diarrhea, we observed a predominance of *Clostridiaceae* (32.81% versus 11.73% in healthy calves: $P = .029$), *Lachnospiraceae* (16.86% versus 9.91% in healthy calves: $P = .014$), and *Corynebacteriaceae* (1.42% versus 0.13% in healthy calves: $P = .041$). While in the healthy calves, we observed a predominance of *Ruminococcaceae* (8.97% versus 4.08% in calves with hemorrhagic diarrhea: $P = .041$), *Flavobacteriaceae* (8.51% versus 2.79% in calves with hemorrhagic diarrhea: $P = .032$), *Desulfohalobiaceae* (5.03% versus 1.41% in calves with hemorrhagic diarrhea: $P = .003$), *Thermodesulfovibrionaceae* (3.17% versus 0.41% in calves with hemorrhagic diarrhea: $P = .001$), *Odoribacteraceae* (2.54% versus 0.88% in calves with hemorrhagic diarrhea: $P = .016$) and *Veillonellaceae* (2.14% versus 0.66% in calves with hemorrhagic diarrhea: $P = .017$) (Fig. 2 and Fig. S2).

At the genus level, the most abundant bacterial genera in the fecal microbiota across all samples were *Clostridium* (19.31%), followed by *Bacteroides* (14.04%), *Blautia* (8.86%), *Flavobacterium* (5.09%), *Prevotella* (4.91%), *Treponema* (3.42%), and *Oscillospira* (3.71%). In calves with hemorrhagic diarrhea, we observed a predominance of *Clostridium* (29.68% versus 8.94% in healthy calves: $P = .012$), *Blautia* (12.43% versus 5.29% in healthy calves: $P = .031$), and *Escherichia* (1.94% versus 0.06% in healthy calves: $P = .047$). While in the healthy calves, we observed a predominance of *Flavobacterium* (7.77% versus 2.41% in calves with hemorrhagic diarrhea: $P = .032$), *Oscillospira* (6.28% versus 1.12% in calves with hemorrhagic diarrhea: $P = .047$), *Desulfonauticus* (5.13% versus 1.31% in calves with hemorrhagic diarrhea: $P = .004$), *Ruminococcus* (2.89% versus 1.46% in calves with hemorrhagic diarrhea: $P = .024$), *Thermodesulfovibrio* (3.31% versus 0.43% in calves with hemorrhagic diarrhea: $P = .001$) and *Butyricimonas* (2.53% versus 0.43% in calves with hemorrhagic diarrhea: $P = .017$) (Fig. 3 and Fig. S3).

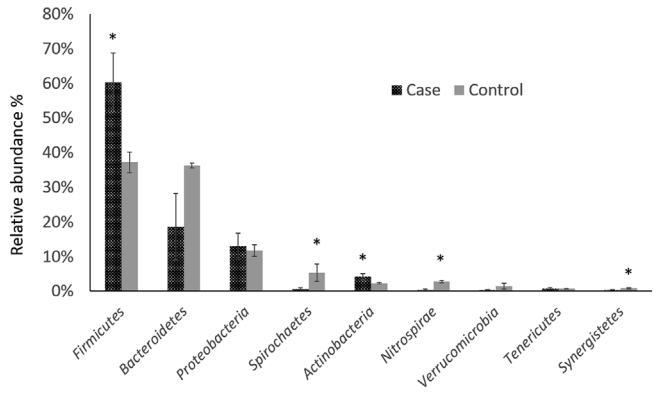

Fig. 1. Bar graphs illustrating the relative abundance of top bacterial phyla that averaged more than 1% of the relative abundance observed in the fecal swabs from healthy calves and calves with hemorrhagic diarrhea. Error bars represent standard errors. The asterisks indicate significant differences between healthy control calves and calves with hemorrhagic diarrhea. *$p \leq .05$.

To further evaluate the potential change in fecal microbiota associated with hemorrhagic diarrhea, the differences in microbial relative abundance between healthy calves and calves with hemorrhagic diarrhea, were compared using the LEfSe algorithm (Fig. 4). The OTUs with the highest LDA (LDA log score threshold ≥ 2) from each group are depicted in (Fig. 4). Using LEfSe, 42 OTUs and 11 OTUs were identified as enriched taxa in healthy calves and calves with hemorrhagic diarrhea respectively.

3.3. Fecal microbiota predictive functional metagenomic profile

The most abundant functional features across all samples were the Membrane Transport; Transporters (6.91%), Membrane Transport; ABC transporters (3.42%), DNA repair and recombination proteins (2.56%), Ribosome Translation (2.11%) and Peptidases (2.06%). The relative abundance of the 30 most abundant functional profiles are shown in (Fig. 5). The relative abundance of some functional profiles tended to be significantly different between samples from diseased calves compared to the healthy control calves (Translation; Ribosome; $P = .043$; Pyrimidine metabolism: $P = .036$; Signal Transduction; Two-component system: $P = .039$; Arginine and Proline metabolism: $P = .037$; Replication and Repair; Chromosome: $P = .044$; Aminoacyl-tRNA biosynthesis: $P = .041$; Carbohydrate Metabolism; Pyruvate metabolism: $P = .016$ and Alanine, aspartate and glutamate metabolism: $P = .024$) (Fig. 5).

3.4. Overall variation in the fecal microbial community structure and diversity

The alpha-diversity of the fecal microbiota was measured using several metrics, namely Chao1, observed species, and Shannon diversity index. The overall average Chao1 of all samples was 232.28 ± 37.54. While, the average number of observed species of all samples was 124.3 ± 14.85. The average value for the Shannon diversity of all samples was 4.95.07 ± 0.37.

Comparisons of the bacterial diversity metrics of the fecal microbiota (Chao1, observed species and Shannon diversity index), revealed a statistically significant difference, in each bacterial diversity indices, between the healthy calves and calves with hemorrhagic diarrhea P value $< .05$ (Table 1 and Fig. 6).

PCA was used to evaluate the fecal microbial community β diversity (an estimate of microbial community diversity within a group). Using the most abundant bacterial genera, PCA revealed that the healthy calves was compositionally distinct from the calves with hemorrhagic diarrhea (Fig. 7).

Finally, a Venn diagram was generated to describe the unique, and shared, OTUs between the two groups. Using OTU counts, the data set within the two groups were represented by a total of 1355 OTUs. The OTU distribution showed that there were 746 and 339 unique OTUs identified in healthy control and hemorrhagic diarrheic calves respectively. Furthermore, a total of 270 OTUs, representing the core microbiota, were shared between the two groups (Fig. 8).

4. Discussion

The bovine gastrointestinal tract is a complex and diverse ecosystem, where many interactions between host, microbiota, and the surrounding environment, occur simultaneously [2]. Maintenance of a healthy gut, and the prevention of infectious diseases, are critically dependent on intestinal homeostasis and proper immune competence

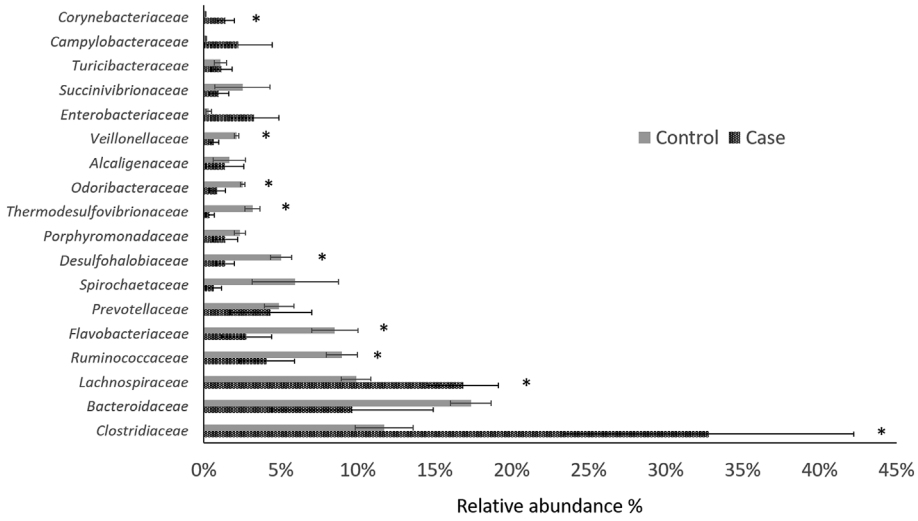

Fig. 2. Bar graphs illustrating the relative abundance of top bacterial families that averaged more than 1% of the relative abundance observed in the fecal swabs from healthy calves and calves with hemorrhagic diarrhea. Error bars represent standard errors. The asterisks indicate significant differences between healthy control calves and calves with hemorrhagic diarrhea. *$p \leq .05$.

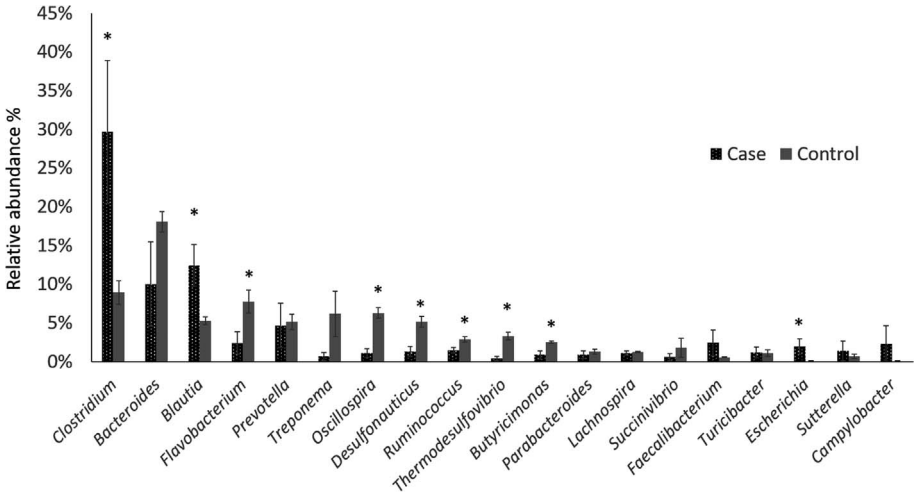

Fig. 3. Bar graphs illustrating the relative abundance of top bacterial genera that averaged more than 1% of the relative abundance observed in the fecal swabs from healthy calves and calves with hemorrhagic diarrhea. Error bars represent standard errors. The asterisks indicate significant differences between healthy control calves and calves with hemorrhagic diarrhea. *$p \leq .05$.

[19]. The gastrointestinal microbiota at equilibrium is thought to benefit the host by modulation of the mucosal and systemic immune system, and by providing resistance against pathogen colonization. There is a growing understanding that an imbalanced microbiota can interfere with host health through local bacterial dysbiosis and immunological dysregulation [5]. Understanding the intrinsic and extrinsic factors that impact the gastrointestinal microbiota is important in determining whether management strategies could be used to foster a balanced gut microbial ecosystem, particularly during the more hazardous phases of the production cycle [2]. The general composition of healthy gastrointestinal bacterial communities have been described in many species, including cattle [1–3,9]. However, limited information is available about the changes in fecal microbiota that may arise during specific diseases, such as hemorrhagic diarrhea in calves. This study compared the composition, diversity, and predicted functional profiles of the fecal microbiota between healthy feedlot calves and calves with hemorrhagic diarrhea.

Our results demonstrated significant differences in microbial community composition, and predicted functional profiles, between the healthy calves and calves with hemorrhagic diarrhea. The most abundant bacterial phyla seen in both hemorrhagic diarrhea and healthy calves were *Firmicutes,* and *Bacteroidetes, w*hile the predominant bacterial genera were *Clostridium, Bacteroides*, *Blautia*, *Flavobacterium* and *Prevotella.* This pattern is very similar to that described in previous studies that have characterized the fecal microbiota of cattle [2,20,21]. The significant differences ($p < .05$) in bacterial phyla, families and genera between the healthy and diseased calves is strong evidence that a change in microbial structure is associated with the occurrence of hemorrhagic diarrhea in feedlot cattle. In fact, similar changes at different taxonomic levels have also been reported in feline diarrhea [3], acute hemorrhagic diarrhea in dogs [1], and colitis in horses [22]. It is important to understand that our data does not provide definitive evidence that the microbial community changes had a primary or causal role in the clinical signs. It is possible that the hemorrhagic diarrhea was caused by an independent pathophysiological process, and that the observed microbial changes were a secondary outcome to the perturbations in the gastrointestinal environment arising from the causal pathology.

The two main bacterial taxa present in greater proportions in the calves with hemorrhagic diarrhea were *Clostridium* and *Escherichia* spp. *Clostridium* spp. are ubiquitous in farm environments and the bovine gastrointestinal tract [23] and can have either positive or negative influences on the host [24]. While many *Clostridium* species are pathogenic (e.g. *C. perfringens*) [25], others are without specific toxins or virulence factors, and provide host benefits through improved digestion of complex organic matter and enhancement of local or systemic immunity [26,27]. *Escherichia* spp. are also commonly found in the farm environment and are considered indigenous to the calf gut microbiota [28,29]. In our feedlot population, the increased proportions of *Escherichia* spp. in calves with hemorrhagic diarrhea is similar to that observed in feline diarrhea [3]. *Escherichia* spp. can be pathogenic, and include many taxa associated with neonatal diarrhea in calves [30].

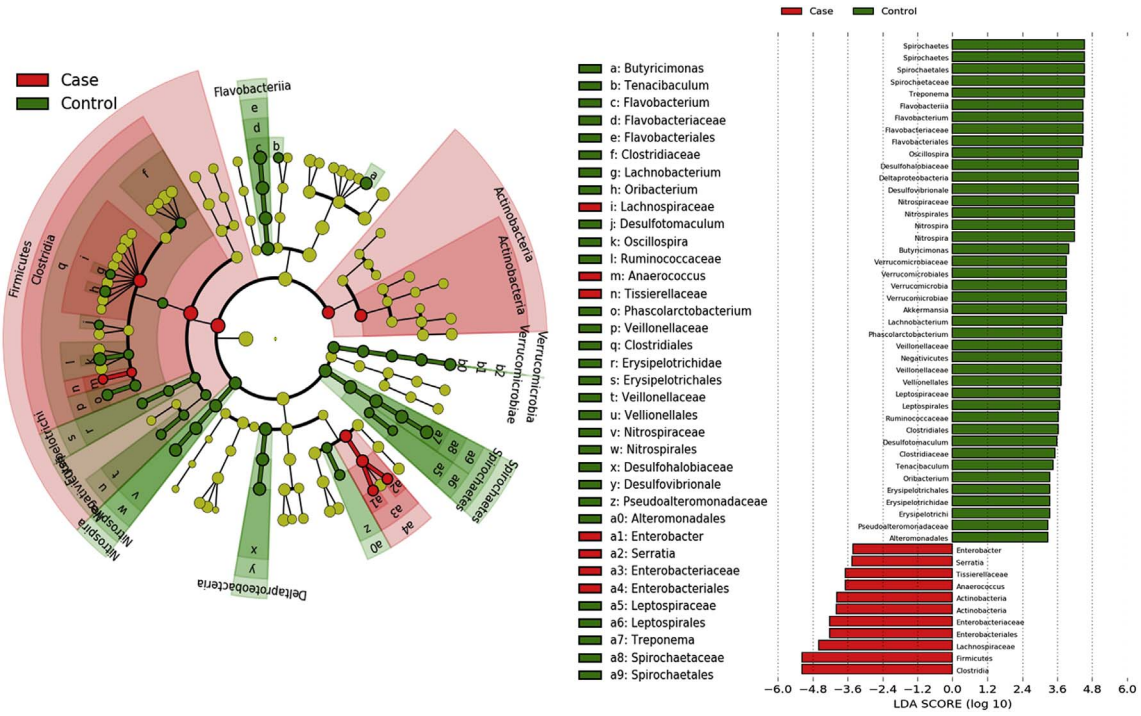

Fig. 4. LEfSe comparison of the fecal microbiota of healthy calves and calves with hemorrhagic diarrhea, depicting operational taxonomic units with absolute Linear Discriminant Analysis LDA score log10 ≥ 2.0. Red: cases; green: healthy. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Further studies evaluating the role of *Clostridia* and *Escherichia,* either as markers of gastrointestinal dysbiosis, or as contributory enteropathogens to hemorrhagic diarrhea, are warranted. Of additional interest in this study was the higher proportion of member of *Ruminococcaceae* and *Butyricimonas* in the healthy calves. Member of these taxa are widely associated with gut health, through generation of short-chain fatty acid which is important for reduction colonization of opportunistic pathogens in the intestines [31]. The abundance of *Blautia* in calves with hemorrhagic diarrhea also deserves special attention and further investigation, as the role of this genus in cattle is currently unknown.

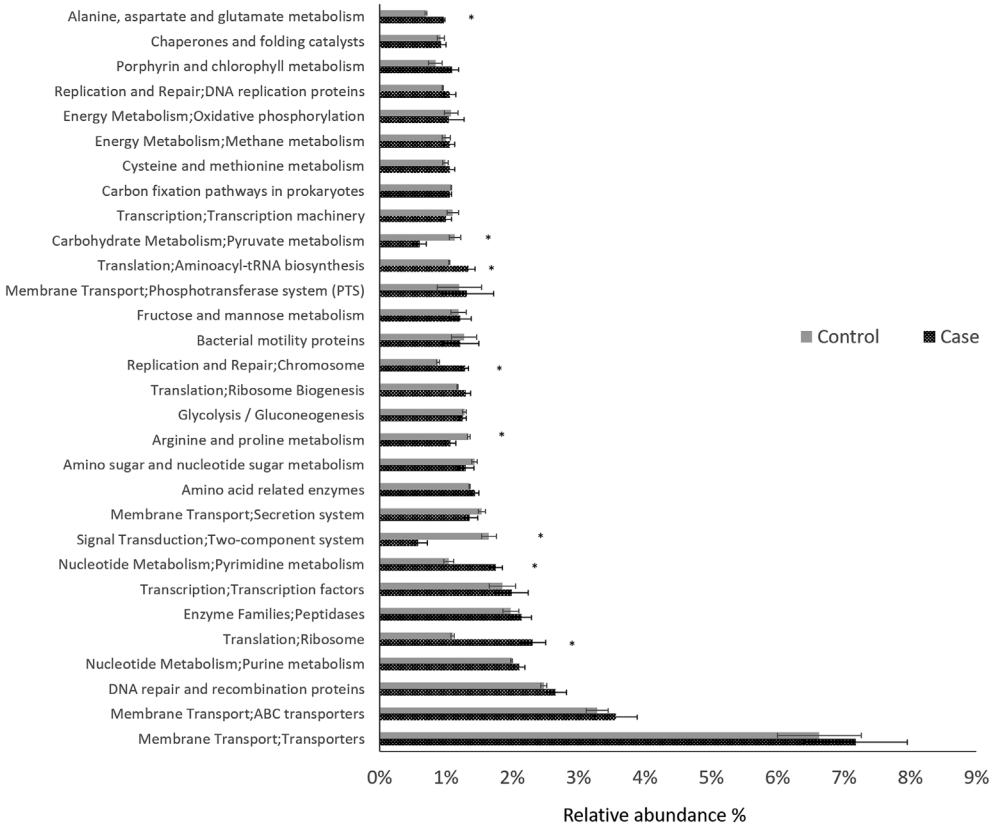

Fig. 5. Relative abundance of the 30 most abundant functional profile features in the fecal swabs from healthy control calves and calves with hemorrhagic diarrhea. Error bars represent standard errors. The asterisks indicate significant differences between healthy calves and calves with hemorrhagic diarrhea. *$p \leq .05$.

Table 1
Bacterial diversity indices (Chao1 index, Observed species and Shannon diversity index) measures for fecal microbiota between the healthy calves and calves with hemorrhagic diarrhea. The data are presented as the mean ± standard error. There was a statistically significant difference in different bacterial diversity indices between the healthy calves and calves with hemorrhagic diarrhea. *P* value < .05.

Calves group	Chao1 index	Observed species	Shannon index
Healthy control	326.23 ± 39.73	196 ± 57.54	6.02 ± 0.04
Hemorrhagic diarrhea	138.55 ± 19.32	89.6 ± 9.23	3.89 ± 0.23
P value	0.012	0.008	0.009

In this study, we used 16S rRNA gene profiles to predict the metagenomic functional profiles, based on analysis through the PICRUSt pipeline [18], and identified several functional features that were significantly different between healthy calves and calves with hemorrhagic diarrhea (Fig. 4). While these are only statistical inferences, based on the known functional profiles of the most abundant taxa in our study, similar changes have been described in cats with diarrhea [3]. Further investigations into the functional implications of the observed microbial community changes, either by direct measuring of metabolites or by gene transcript analysis, will be an important next step for better understanding the pathophysiology of hemorrhagic disease in feedlot cattle.

Bacterial species richness and diversity are also thought to be important measures of community health of the gut microbiota [32]. The study results revealed significant decreases in bacterial diversity indices (Chao1, observed species and Shannon diversity index) between the healthy calves and calves with hemorrhagic diarrhea (Table 1). PCA also revealed differences in microbial community composition between the two groups. In combination, these results indicate that there are marked differences in the overall microbiota structure between healthy and diseased calves, and supports the notion that there is significant bacterial dysbiosis in the gastrointestinal tract of the affected animals, with less diversity and fewer observed species compared to healthy controls. These results are in accordance with previous observations in dogs with diarrhea [1]. Once again, these results cannot be used to attribute causality but, since microbial species richness and diversity are important factors in intestinal homeostasis [33], it is clear that a disrupted gastrointestinal ecosystem is strongly associated with the presence of hemorrhagic diarrhea.

While the results of this study were compelling, the experimental limitations of our study should be considered. Firstly, this study was performed on a relatively small number of calves, though equivalent to other published studies [9]. It follows that data from a larger population is required before definitive conclusions can be made regarding the specific microbial community changes that contribute to hemorrhagic diarrhea outbreaks in feedlot populations. Secondly, assessment of the impact of various contributory management practices such as diet transition, commingling, vaccination and housing on the fecal microbiota was beyond the scope of this study. Despite these experimental drawbacks, the results of this study provide preliminary insight into an area of investigation that could be of great relevance to bovine gastrointestinal health. However, investigation into the causes, and clinical significance, of the trajectory of change in fecal microbial populations are indicated before preventative or corrective strategies against hemorrhagic diarrhea can be recommended.

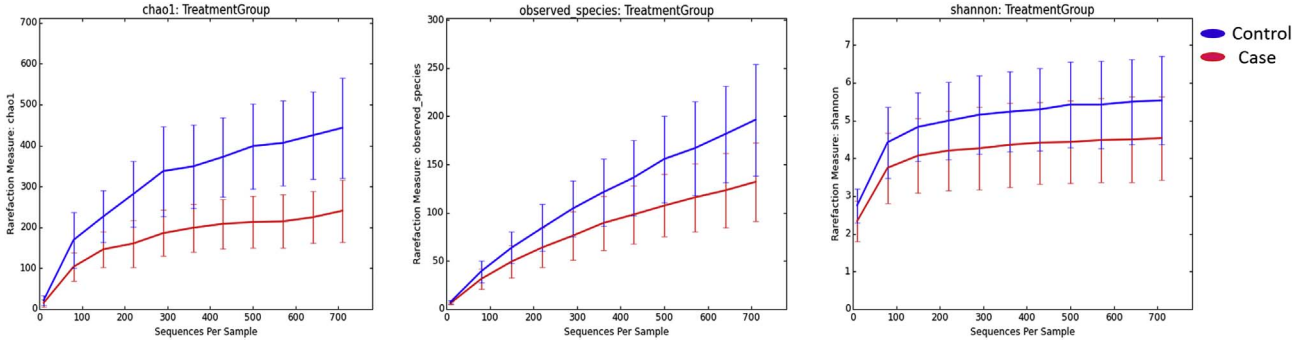

Fig. 6. Rarefaction curves of 16S rRNA gene sequences obtained from fecal samples from healthy calves and calves with hemorrhagic diarrhea. The graphical lines represent the mean and error bars represent standard deviations. The analysis was performed on a randomly selected subset of 713 sequences per sample.

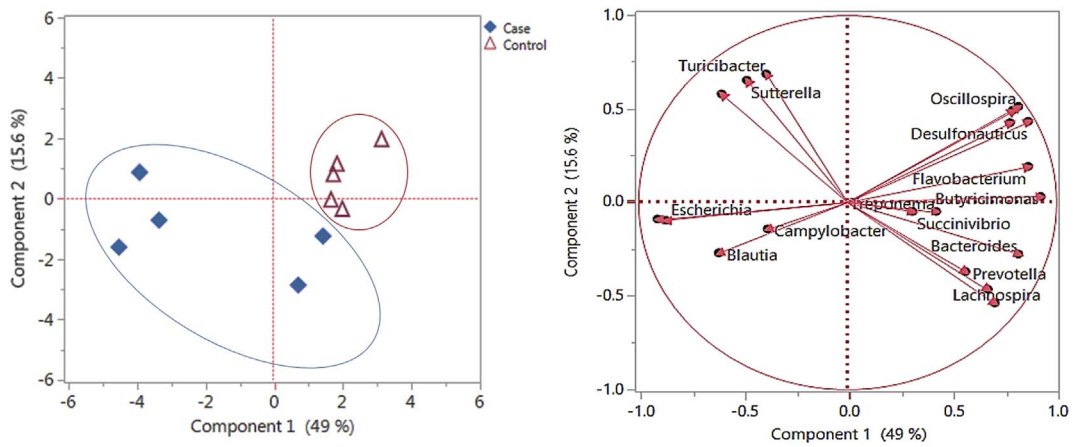

Fig. 7. PCA plot depicting the community membership of the fecal microbiota of calves taken from healthy calves and calves with hemorrhagic diarrhea. Component loading plot with each vector corresponding to one genus being proportional to its component loading. PCA was performed using JMP 13 based on the prevalence of the most abundant bacterial genera as covariates and the two calves group as variables. The percentage of variation explained is indicated on the axes.

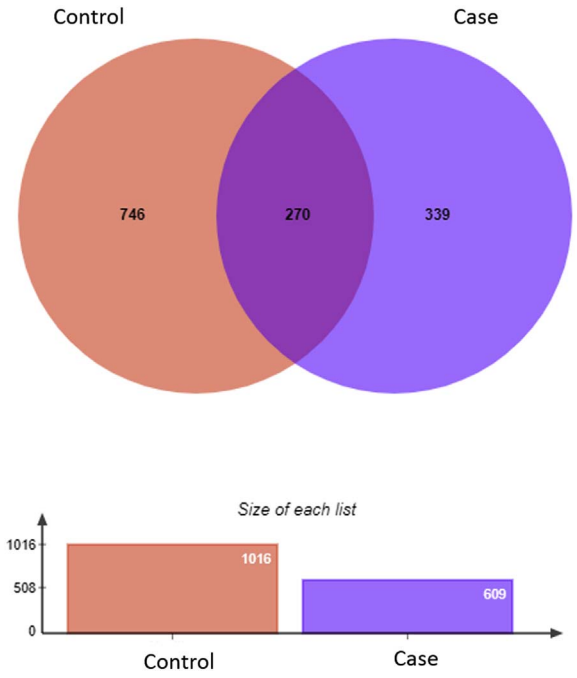

Fig. 8. Venn diagram depicting the common and unique OTUs among the two groups (healthy and hemorrhagic diarrhea calves). There were 746 unique OTUs identified in healthy control cattle, 339 OTUs unique in the hemorrhagic diarrhea calves, and 270 OTUs were represented the core microbiota between the two groups.

5. Conclusion

This is the first study to describe the differences in fecal microbial community composition, diversity and predicted functional profile between feedlot calves with and without hemorrhagic diarrhea. In this cohort of cattle, we observed differences in the fecal microbiota composition and predicted functional metagenome profiles between healthy calves and calves with hemorrhagic diarrhea. Results of this study indicate the presence of bacterial dysbiosis in the gastrointestinal tracts of calves with hemorrhagic diarrhea, with affecting animal samples exhibiting less diversity, and fewer observed species, compared to healthy controls. Future studies, in larger populations, are required to evaluate the role and clinical significance of dysbiosis of resident microbiota in health and susceptibility to the development of gastrointestinal diseases in feedlot cattle.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.micpath.2017.12.059.

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