Effects of preconditioning on the nasopharyngeal microbiota of beef calves grazing winter wheat

Josiah M. Brooks,† Jennifer J. Randall,‡ and Glenn C. Duff||

†Department of Animal and Range Sciences, New Mexico State University, Las Cruces, New Mexico 88003, USA ‡Department of Entomology, Plant Pathology, and Weed Science, New Mexico State University, Las Cruces, New Mexico 88003, USA ||Clayton Livestock Research Center, New Mexico State University, Clayton, New Mexico 88145, USA

INTRODUCTION

Bovine respiratory disease (BRD) remains the most problematic disease affecting the beef cattle industry because of losses in calf performance due to morbidity and mortality rates and increased production and treatment costs (Duff and Galyean, 2007; Timsit et al., 2016). Despite well-funded research, numerous collaborations, and changes made in vaccination and other health protocols to reduce the prevalence of BRD, incidences of BRD continue to rise (Peel, 2020). There are several approaches researchers have used to study the prevalence of BRD. First, preconditioning is a beef calf management strategy that generally includes a vaccination program, a minimum of a 45-d weaning period, dehorning and castration, adaptation to feed bunks and water troughs, and individual identification. Preconditioned (PRECON) calves have been shown to display improvements in health and performance in the feedlot compared to nonpreconditioned (NONPRE) calves (Step et al., 2008) although there appears to be limited studies evaluating the potential effects of PRECON in stocker calves on winter wheat pasture. Second, recent advancements in genomic technology have provided researchers with an alternative avenue to study BRD by using culture-independent techniques (e.g., next-generation sequencing) to evaluate the effects of BRD on the bovine nasopharyngeal (NP) microbiota (Timsit et al., 2020). Additionally, studies have shown that Mannheimia haemolytica serotype A1 is commensal bacterium in the NP that becomes pathogenic following viral infection or stress (Rice et al., 2007; Griffin et al., 2010) and thus may alter the NP microbiota in calves after market entry. To the best of our knowledge, however, no studies have been conducted in these topics for beef cattle upon arrival on winter wheat pasture. Therefore, the objective of the current study was to investigate the effects of preconditioning on the NP microbial community composition of beef steers received on winter wheat pasture. Our hypothesis was that PRECON steers would have a greater diversity in NP microbial composition with a reduction in BRD associated genera (i.e., Mannheimia and Pasteurella) compared to NONPRE and commingled (COMM) steers.

MATERIALS AND METHODS

All animal procedures, facilities, and personnel were approved by the New Mexico State University (NMSU) Institutional Animal Care and Use Committee (IACUC).
Animals, Health Protocols, and Treatments

Mixed breed steers (n = 145) were utilized in a completely randomized design for a 112-d trial to compare the effects of preconditioning on the nasopharyngeal microbial community. In November 2018, steers were purchased from a local auction barn in Dalhart, Texas, as preconditioned (PRECON; n = 70) or nonpreconditioned (NONPRE; n = 75). Previous health history was not known for either group; however, PRECON calves were reported as weaned and receiving vaccinations for respiratory viral pathogens (e.g., infectious bovine rhinotracheitis [IBR], parainfluenza, [PI3], bovine viral diarrhea [BVD], and bovine respiratory syncytial virus [BRSV]). No information on management was reported for NONPRE steers.

Steers were transported separately by truck approximately 80 km to the NMSU Clayton Livestock Research Center in Clayton, New Mexico. Upon arrival, steers were offloaded into separate pens with ad libitum access to grass hay and water. Approximately 8 h later on day 0, steers received subcutaneous injections (neck) of IBR-BVD-IP3-BRSV-Mannheimia haemolytica-Pasteurella multocida (Vista Once SQ, Merck, Madison, NJ) and clostridial (Covexin 8, Merck, Madison, NJ) vaccines, an injectable (oral) dewormer (Dectomax, Zoetis, Florham Park, NJ), and a growth promotion implant (under the skin of the front middle third left ear) (Synovex C, Zoetis, Florham Park, NJ) and were weighed, branded, and tagged with an ear tag on the right ear denoting animal identification (number of tag) and treatment group (color of tag). No steer was identified as unhealthy on day 0 or 2. Two 15.24 cm Puritan sterile polyester-tipped nasopharyngeal swabs (NS) were inserted approximately 23 to 26 cm in both the right and left nostrils of each steer and were swabbed in a clockwise and counterclockwise direction with simultaneous rotation; care was taken to ensure saturation of the swab with no external contamination. For day 0, NS were collected at the end of processing. Both swabs were immediately stored in a snap cap 5-mL polystyrene tube and were stored in liquid nitrogen. A second set of NS were collected on day 2 using the same procedure. All swabs were transported to a −80° freezer for later analyses. Following processing on day 0, steers were randomly allocated to 1 of 3 treatment groups: PRECON (n = 50), NONPRE (n = 50), or COMM (n = 20 PRECON and n = 25 NONPRE) and were released onto a 120-acre winter wheat pasture divided into three paddocks for each of the treatments with access to a common water source. Animal caretakers were blinded to treatment groups. Steer health was monitored throughout the study and were retroactively classified a health status as healthy (H) or unhealthy (U) based on their clinical symptoms, treatment, or mortality due to BRD using the D.A.R.T. system as described by Step et al. (2008). Only one steer was pulled and treated for gaseous bloat and was removed from study.

DNA Extraction, Sample Quality, and PCR

All NP swabs were transported on dry-ice to the National Animal Disease Center in Ames, Iowa, and were briefly used for cell culturing on bovine blood agar to identify any M. haemolytica and Pasteurella multocida colonies. For more detailed analyses, all samples were transported on dry-ice and processed at a quarantine laboratory at NMSU. Extractions of deoxyribonucleic acid (DNA) from NS for each animal and by day was completed using the DNA PowerLyzer PowerSoil Kit from Qiagen (Hilden, Germany) following the manufacturer’s instructions; sterile, blank cotton swabs were used during each extraction set as a control for potential bacterial contamination from extraction kits. Quantification and quality of NS DNA samples were evaluated using a NanoDrop One (Thermo Scientific). One set of swabs were unaccounted for in each day 0 and 2 sets, resulting in n = 144 DNA samples for each day. PCR was used in all DNA samples to detect the presence of M. haemolytica serotype A1.

Sequencing and Bioinformatic Analysis

A subset of DNA samples (n = 120) were sent to the University of Minnesota Genomics Center (Minneapolis, Minnesota) for next-generation sequencing. Selection of DNA samples were influenced by type of comparisons but were randomly chosen. Comparisons were used to evaluate the NP microbiota composition and included health status comparisons (H vs. U; HSC), presence of M. haemolytica serotype A1 in steers across days comparison (MAC), and treatment group comparisons (PRECON, NONPRE, and COMM; TGC). For HSC, n = 16 DNA samples were randomly selected on days 0 and 2 from steers classified as H or U with a total of n = 64 samples. For MAC, all steers positive for A1 on day 0 (n = 6) and day 2 (n = 8) were chosen. For the TGC, n = 7 DNA samples were
randomly selected from each treatment group on day 0 with the same steers and number of samples selected again on day 2. No sample was present in more than one comparison but the effect of steer health status in MAC and TGC was not considered. The V4 hypervariable region of the 16s rRNA gene was amplified and sequenced using Illumina MiSeq platform, and data were returned as fastq files. Total NP microbial composition was determined using the Qiagen CLC Microbial Genomics Module (Hilden, Germany) by each comparison separately (HSC, MAC, or TGC) using the same parameters following the manufacturer’s instructions. Reads for each sample were paired, trimmed, and examined for sufficient high coverage. Only $n = 10$ samples from the HSC did not pass the coverage cutoff and were excluded from further analyses. Alpha diversity (Shannon’s entropy) and beta diversity (Bray–Curtis dissimilarity matrix) were analyzed for within and between sample comparisons with PERMANOVA. A $P$-value $\leq 0.05$ was considered statistically significant. Operational taxonomic units (OTU) tables were generated using the SILVA 138 (99%) 16s rRNA annotated database as a reference. Detection at the genus level could be made but species level could not be accomplished with the current methods employed.

Figure 1. The bacterial taxa (genera) abundances of randomly selected steers ($n = 27$) on day 0 classified as healthy (H; $n = 13$) or unhealthy (U; $n = 14$) at the end of the study based on clinical symptoms, treatment, or mortality due to BRD. Microbial community composition was determined using the V4 hypervariable region of the 16s rRNA gene sequences analyzed with the Illumina platform. H steers did not display clinical symptoms of, receive treatment for, or suffer mortality due to BRD, whereas U steers met at least one of the criteria.

Figure 2. The bacterial taxa (genera) abundances of randomly selected steers ($n = 27$) on day 2 classified as healthy (H; $n = 13$) or unhealthy (U; $n = 14$) at the end of the study based on clinical symptoms, treatment, or mortality due to BRD. Microbial community composition was determined using the V4 hypervariable region of the 16s rRNA gene sequences analyzed with the Illumina platform. H steers did not display clinical symptoms of, receive treatment for, or suffer mortality due to BRD, whereas U steers met at least one of the criteria.
RESULTS AND DISCUSSION

For HSC, day 0 H steers had a lower abundance of *Mannheimia* (33%) with a greater abundance of *Pasteurella* (8.0%) compared to U steers (34% and 0.91%, respectively; Figure 1). On day 2, abundances of *Mannheimia* and *Pasteurella* in H steers (43% and 16%, respectively) were greater than U steers (21% and 2.7%, respectively; Figure 2). Holman et al. (2015) reported differences in NP microbiota composition of healthy cattle and cattle affected with BRD in the feedlot. In the current study, the NP microbial community of H and U steers was different on day 0 ($P < 0.05$) and on day 2 ($P < 0.01$) using PERMANOVA, although similar proportions of *Mannheimia* were observed in each group. For MAC, the abundance of *Mannheimia* on day 0 (36%) increased on day 2 (45%) with *Pasteurella* decreasing from day 0 (14%) to day 2 (1.4%; Figure 3). No difference was observed in abundance between days 0 and 2 ($P > 0.05$). Previous research shows that virulence factors of *M. haemolytica* serotype A1 enable the bacterium to become the predominant serotype

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**Figure 3.** The bacterial taxa (genera) abundances of steers positive for *M. haemolytica* serotype A1 on day 0 ($n = 6$) and on day 2 ($n = 8$). Microbial community composition was determined using the V4 hypervariable region of the 16s rRNA gene sequences analyzed with the Illumina platform. Steer health status was not considered in this analysis.

**Figure 4.** The bacterial taxa (genera) abundances of steers by treatment group and by day using the V4 hypervariable region of the 16s rRNA gene sequences analyzed with the Illumina platform. Steers ($n = 7$) were randomly selected from each treatment group (PRECON, NONPRE, and COMM) on day 0 ($n = 21$) with the same number of DNA samples and steers chosen on day 2 ($n = 21$). Steer health status was not considered in this analysis.
Following disruption of the more commensal NP bacteria, likely leading to BRD (Rice et al., 2007; Griffin, 2010). Although individual M. haemolytica serotypes and their proportions could not be distinguished in the OTUs using the methods employed in this study, it appears that M. haemolytica serotype A1 may have contributed to the dramatic shift in Mannheimia across days 0 and 2 for the calves positive for this serotype in this comparison. For TGC, comparisons were made within and across days by treatment group (Figure 4). On day 0, Mannheimia was numerically greater in PRECON steers (47%) than NONPRE (45%) or COMM (35%) steers. Pasteurella was more abundant in COMM steers (16%) than PRECON (0.82%) or NONPRE (4.4%) steers. On day 2, abundances shifted as Mannheimia was greater in COMM (33%) steers than PRECON (19%) or NONPRE (18%) steers. However, Pasteurella was greater in PRECON (4.3%) steers than NONPRE (3.6%) or COMM (3.2%) steers. The reduction of Mannheimia in PRECON steers from days 0 to 2 may provide preliminary evidence for the effects of PRECON on NP microbiota composition.

**IMPLICATIONS**

Changes in the composition of nasopharyngeal microbiotas of steers received on winter wheat pasture appear to be influenced by steer health status, prior health management strategies, and presence of M. haemolytica serotype A1, suggesting that multiple factors can cause changes in microbial composition. Understanding how these factors play a role in shifting the nasopharyngeal microbial community composition may allow researchers and producers to apply new methods for early detection of bovine respiratory disease.

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