Veterinary Microbiology 214 (2018) 140–147

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Injectable antimicrobials in commercial feedlot cattle and their effect on the nasopharyngeal microbiota and antimicrobial resistance

Devin B. Holman[a], Edouard Timsit[b,c], Calvin W. Booker[c], Trevor W. Alexander[d,*]

[a] Lacombe Research and Development Centre, Agriculture and Agri-Food Canada, Lacombe, AB, Canada
[b] Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada
[c] Feedlot Health Management Services, Okotoks, AB, Canada
[d] Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada

ARTICLE INFO

Keywords:
Bovine respiratory disease
Feedlot cattle
Antimicrobial resistance
Nasopharyngeal microbiota

ABSTRACT

Beef cattle in North America that are deemed to be at high risk of developing bovine respiratory disease (BRD) are frequently administered a metaphylactic antibiotic injection to control the disease. Cattle may also receive in-feed antimicrobials to prevent specific diseases and ionophores to improve growth and feed efficiency. Presently, attempts to evaluate the effects that these medications have on antibiotic resistance in the bovine nasopharyngeal microbiota have been focused on culturable bacteria that are associated with BRD. Therefore, we assessed the effects of injectable antibiotics on the nasopharyngeal microbiota of commercial feedlot cattle in Alberta, Canada, through the first 60 d on feed. Although all cattle in the study were also receiving in-feed chlortetracycline and monensin, the administration of a single injection of either oxytetracycline or tulathromycin at feedlot placement altered the nasopharyngeal microbiota in comparison with the cattle receiving only in-feed antibiotics. Oxytetracycline significantly ($P < 0.05$) reduced the relative abundance of *Mannheimia* spp. from feedlot entry to exit (≥ 60 d) and both oxytetracycline and tulathromycin treated cattle had a significantly lower relative abundance of *Mycoplasma* spp. at feedlot exit compared with the in-feed antibiotic only group. The proportion of the tetracycline resistance gene *tet*(H) was significantly increased following oxytetracycline injection ($P < 0.05$). Oxytetracycline also reduced both the number of OTUs and the Shannon diversity index in the nasopharyngeal microbiota ($P < 0.05$). These results demonstrate that in feedlot cattle receiving subtherapeutic in-feed antimicrobials, the administration of a single injection of either oxytetracycline or tulathromycin resulted in measurable changes to the nasopharyngeal microbiota during the first 60 d following feedlot placement.

1. Introduction

In North America, beef cattle are typically transported to a feedlot where they are finished on a high-grain diet until slaughter. Bovine respiratory disease (BRD), also called shipping fever, remains the most common cause of morbidity and mortality after feedlot placement (Booker et al., 2008), resulting in significant economic losses (USDA, 2013). It is a multifactorial disease but bacterial species, including *Histophilus somni*, *Mannheimia haemolytica*, *Mycoplasma bovis*, *Bibersteinia trehalosi,* and *Pasteurella multocida*, are frequently implicated (Confer, 2009).

Cattle that are deemed to be at high-risk of developing BRD early in the feeding period (recently weaned, light weighted, commingled, auction market derived, etc.) are often given, at feedlot placement, a single injection of an antibiotic such as florfenicol, tilmicosin, tulathromycin, or oxytetracycline to control BRD, i.e. metaphylaxis (DeDonder and Apley, 2015). In the most recent survey of antimicrobial use in feedlot cattle in the United States, tulathromycin was reported to be used for metaphylaxis in 45.3% of feedlots and oxytetracycline in 17.4% (USDA, 2013). In addition, 90.1% and 20.6% of cattle received an ionophore (e.g. monensin) and/or chlortetracycline in their feed (USDA, 2013). Ionophores are used to prevent coccidiosis and to improve feed conversion and weight gain, while chlortetracycline is used for disease prevention and control. Given that antimicrobial use can lead to the development of resistance (Cameron and McAllister, 2016; Portis et al., 2012), it is important to evaluate the effects of antimicrobials in order to maintain effective disease management.

To date, the effect of antimicrobial administration in feedlot cattle has only been assessed on a small number of bacterial species rather than the total nasopharyngeal (NP) microbiota (Zaheer et al., 2013).

* Corresponding author at: Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, 5403 – 1st Ave S, Lethbridge, AB, T1J 4B1, Canada.
E-mail address: trevor.alexander@agr.gc.ca (T.W. Alexander).

https://doi.org/10.1016/j.vetmic.2017.12.015
Received 23 November 2017; Received in revised form 19 December 2017; Accepted 21 December 2017

This despite the fact that antimicrobial agents act on non-target bacteria as well as pathogens (Looft and Allen, 2012) and that there is an increasing recognition of the respiratory microbiota in maintaining respiratory health of mammals (Man et al., 2017). Therefore, the objective of this study was to investigate the effect of a single antibiotic injection at feedlot placement on the NP microbiota of beef cattle in a commercial feedlot system during the first 60 d of production. We also determined the effect that these antimicrobials had on antimicrobial resistance determinants.

2. Materials and methods

2.1. Animals and sample collection

As part of a previous surveillance study to evaluate the antimicrobial susceptibility of *M. haemolytica*, NP swab samples were collected from cattle upon entry to feedlots and again from the same animals at ≥60 days on feed (Klima et al., 2011). The swabs were stored in a cryopreservative (brain heart infusion:glycerol, 0.8:0.2 mixture) at −80 °C. For the present study, NP samples from the collection were randomly chosen from three groups of cattle (n = 10 per group) originating from four feedlots. Cattle in all groups received in-feed chlortetracycline at 35 mg/kg feed and monensin sodium at 25 mg/kg feed during the study period, which was a common management practice. Thus the cattle groups were defined as animals that i) received in-feed chlortetracycline and monensin sodium only, without a metaphylactic antimicrobial at feedlot entry, or those that received in-feed chlortetracycline and monensin sodium in addition to ii) administration of a single injection of oxytetracycline (30 mg/kg body weight) at feedlot entry, and iii) administration of a single injection of tulathromycin (2.5 mg/kg body weight) at feedlot entry. None of the calves were treated with any additional antimicrobials throughout feedlot placement. Two swabs per animal were evaluated that were collected at feedlot entry (0 d) and after ≥60 d of placement (defined as exit sample) between September, 2009 and March, 2010.

2.2. 16S rRNA gene sequencing and analysis

Total DNA was extracted from NP swabs using a Qiagen DNeasy Tissue kit (Qiagen Inc., Mississauga, ON, Canada) as previously described (Holman et al., 2015). The generation of 16S rRNA gene libraries was conducted as detailed elsewhere (Holman et al., 2017). Briefly, the V4 hypervariable region of the 16S rRNA gene was PCR amplified using a two-step procedure and sequenced using an Illumina MiSeq (Illumina, Inc., San Diego, CA, USA) and the MiSeq Reagent Kit v2 (500 cycles; Illumina) according to manufacturer's instructions.

The 16S rRNA sequences were processed and analyzed using the QIIME software package v. 1.9.1 (Caporaso et al., 2010). Paired-end reads were merged using fastq-join with a minimum overlap of 200 bp and a maximum percent difference of 3 (Aronesty, 2013). These merged sequences were then quality filtered using the *split_libraries_fastq.py* script with reads being truncated following two consecutive base calls of a quality score of less than 25. Only sequences with 95% or more of the original sequence remaining following truncation were retained. The UCHIME algorithm (Edgar et al., 2011) implemented in VSEARCH v. 2.4.0 (Rognes et al., 2016) was used to remove chimeric sequences. The remaining sequences were then assigned to operational taxonomic units (OTUs) at 97% similarity using a *de novo* OTU picking method implemented in VSEARCH. Taxonomy was assigned to these OTUs using the UCLUST consensus taxonomy assigner (Edgar, 2010) and the SILVA database and the SILVA database v. 128 (Quast et al., 2013), with a minimum similarity of 0.8 and max accepts of 3. Representative sequences for each OTU were aligned using PyNast (Caporaso et al., 2010) and a phylogenetic tree was constructed using FastTree (Price et al., 2010). Operational taxonomic units containing fewer than 10 sequences were excluded from further analysis, as were those classified as chloroplasts and mitochondria. All 16S rRNA gene sequences were submitted to the NCBI short read archive under BioProject accession PRJNA394129.

2.3. Quantification of antibiotic resistance determinants

The quantities of 12 antimicrobial resistance determinants in the NP samples were assessed using real-time PCR as detailed previously (Holman et al., 2016). Briefly, the relative abundance of the rRNA methylase genes *erm*(A), *erm*(B), *erm*(F), and *erm*(X), the sulfonamide resistance genes *sul1* and *sul2*, the tetracycline efflux genes *tet*(B), *tet*(C), *tet*(H), *tet*(L), and the tetracycline ribosomal protection genes *tet*(M) and *tet*(W) were evaluated. These genes were reported as proportions of the 16S rRNA gene, which was also quantified by real-time PCR. The 16S rRNA gene was amplified using the same 515-F and 806-R primers that were also used to generate the 16S rRNA gene libraries.

2.4. Statistical analysis

The archaeal and bacterial community structure (beta-diversity) of each treatment group was assessed using the Bray-Curtis dissimilarity metric within the R-packages Phyloseq (McMurdie and Holmes, 2013) and vegan (Oksanen et al., 2017). The Bray-Curtis dissimilarities were compared by antibiotic treatment using PERMANOVA (adonis function) with 9999 permutations using vegan. Archaeal and bacterial richness (number of OTUs) and diversity (Shannon diversity index), as well as the relative abundance of BRD-associated genera, and the concentration of resistance determinants, were also compared by treatment group using a linear mixed model in R v. 3.4.1 (R Core Team, 2017) using lme4 v 1.1.12 (Bates et al., 2015) with animal as the random effect and antibiotic treatment, time, and feedlot as fixed effects, followed by Tukey's honestly significant difference (Lenth, 2016). For within-sample (alpha) and between-sample (beta-diversity) analyses, all samples were randomly subsampled to 18,500 sequences to account for uneven sequencing depth. Differentially abundant OTUs at feedlot exit between the two groups that received an injectable antimicrobial and the chlortetracycline-only cattle, were identified using DESeq2 (Love et al., 2014). For the DESeq2 analyses, samples were not randomly subsampled but only OTUs present in at least half of the samples were included.

3. Results and discussion

3.1. Nasopharyngeal microbiota of commercial feedlot cattle

Swabs were taken from the nasopharynx of cattle upon entry to and exit (≥60 d) from four commercial feedlots. A total of 1376 OTUs representing 266 genera were identified among all NP samples. Similar to previous 16S rRNA gene-based surveys of the NP microbiota of beef cattle, *Acinetobacter*, *Mannheimia*, *Moraxella*, *Streptococcus*, and *Psychrobacter* were among the more abundant genera [Fig. 1] (Hall et al., 2017; Holman et al., 2015; Holman et al., 2017; Timsit et al., 2016b). The number of OTUs and the Shannon diversity index of the NP samples (Fig. 2) in the present study were also similar to earlier studies with commercially sourced cattle (Holman et al., 2015; Timsit et al., 2017b; Timsit et al., 2016b).

3.2. Effect of antibiotic injection on the nasopharyngeal microbiota

Based on the analysis of the Bray-Curtis dissimilarities, the structure of the NP microbiota at feedlot exit was significantly affected by antimicrobial treatment ($P = 0.0014$; $R^2 = 0.22$; Fig. 3A). Samples taken from cattle injected with tulathromycin and those that received only the chlortetracycline and monensin-supplemented feed were most dissimilar from each other (Fig. S1). As the treatment groups did not differ significantly from each other at feedlot placement ($P = 0.35$), it may be