Rapid Communication: 16S ribosomal ribonucleic acid characterization of liver abscesses in feedlot cattle from three states in the United States¹

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ABSTRACT: Liver abscesses are a major economic burden to beef producers. Although a few causative organisms have been cultured from purulent material, the full polymicrobial diversity of liver abscesses has not been reported. The objective of this study was to characterize purulent material collected from liver abscesses in beef cattle in different production systems in 3 cattle producing states in the United States using 16S rRNA gene sequencing. Differences in purulent material microbial communities among geographic region of feeding and application of a common antimicrobial were also investigated. Cattle included in the study were fed in California (dairy type) and Colorado and Texas (both beef type). Liver abscesses from a cross section of feedlots, geographic areas, and tylosin phosphate–administered groups were collected at harvest; DNA from 34 liver abscess samples was extracted; and the V4 region of the 16S rRNA gene was amplified and sequenced. Sequences were classified into 5 phyla, 13 classes, and 17 orders in the domain Bacteria. The phyla identified included Bacteroidetes (35.2% of reads), Proteobacteria (28.6%), Fusobacteria (18.2%), Firmicutes (12.4%), and Actinobacteria (5.5%). Sequences matching the genera Fusobacterium and Trueperella, which have previously been identified as causative agents in liver abscesses, were both present in the abscess bacterial communities at a relative abundance of 15.1 and 3.2%, respectively, of the overall relative abundance. Furthermore, 3 of the most common phyla were Gram-negative bacteria. An analysis-of-similarities test was conducted on Euclidean distances to assess differences between cattle treated and not treated with tylosin as well as to assess differences between geographic regions. Geographical region and treatment with tylosin affected the microbiome (P = 0.002 and P = 0.026 respectively); however, a more robust sample scheme is needed to explore these differences. To our knowledge, this is the first publication describing the complex community of liver purulent material using next generation sequencing in cattle. These data provide a framework for research on a more targeted approach to liver abscess prevention and treatment.

Key words: liver abscess, microbiome, purulent material, 16S ribosomal ribonucleic acid, tylosin

INTRODUCTION

The occurrence of a liver abscesses in feedlot cattle is associated with negative performance and economic impacts. It is estimated that liver abscesses, observed at prevalence of 20.9% of fed cattle harvested in the United States, can decrease carcass value by US$20 to $80 (Brown and Lawrence, 2010; McKeith et al., 2012). Classically, the primary etiology of this disease is attributed to Fusobacterium necrophorum (Nagaraja and Chengappa, 1998). However, there has been some ambiguity in compositional differences of other microorganisms’ potential role in etiology that can be found in these abscesses, and these differences appear to systematically vary among feedlots and feeding strategies. Previous studies have indicated that liver abscesses are polymicrobial using anaerobic and aerobic culturing methods (Nagaraja and Chengappa, 1998) and whole genome sequencing of isolated bacteria (Amachawadi et al., 2016). Because reductions of liver abscesses remain a concern for the industry and the use of antimicrobial drugs used for prevention and treatment are increasingly scrutinized, a more thor-
ough understanding of the bacteriology of liver abscess- 
sation is warranted. Therefore, this study characterizes 
microbial communities in the purulent materials of liver 
abscesses using 16S rRNA gene amplicon sequencing.

MATERIALS AND METHODS

Cattle Population

Sixteen pens of feedlot cattle (average number of 
animals in pen: 141), from 5 different feedlots in the 
United States (1 feedlot in California, 1 feedlot in Texas, 
and 3 feedlots in Colorado) were used for this study. 
Cattle included in the study were a mix of dairy-type 
cattle (California) and beef-type cattle (Colorado and 
Texas). To investigate the effects of a commonly used 
abscess control strategy, one-half of the enrolled 
pens (8 pens) housed cattle that were supplemented 
with tylosin phosphate following label usage (Elanco, 
Greenfield, IN) for the duration of the feeding period, 
whereas the other 8 pens were not fed tylosin. The pens 
were identified prior to slaughter to facilitate sample 
collection at the time of harvest. More information on 
the cattle population can be found in Table 1.

Liver Abscess Collection

A sample of livers identified as having abscess (up 
to 5 per pen) were reserved for removal of the abscess. 
Liver abscess collection was performed by removing the 
abscess from the liver using a sterile scalpel, taking care 
to avoid puncturing the abscess during the collection 
process. When multiple abscesses were present in one liver, 
the abscess collected was the most convenient to collect 
that appeared to harbor the most purulent material. 
Liver abscess samples were placed in sterile bags (Whirl-Pak; 
Nasco Corp., Fort Atkinson, WI) and transported on ice 
to Colorado State University (Fort Collins, CO). An ali-
quot of the abscess purulent material was removed using 
stereile approaches from the abscess, placed in sterile 50-

mL conical tubes (Thermo Fisher Scientific, Waltham, 
MA), and frozen (−80°C) until the time of DNA extrac-
tion. Although the original sample plan called for the col-
lection of 80 liver abscess, due to logistical limitations 
(some pens of cattle did not have 5 abscessed livers to 

sample) and the limit on the amount of purulent material 
from some abscesses (resulting from the stage and size of 
the abscess), DNA from 34 liver samples was successful 
xtracted and used in downstream analysis.

Deoxyribonucleic Acid Extraction and Sequencing

Deoxyribonucleic acid was extracted from 0.021 
to 0.725 g of purulent material using the Mo-Bio 
PowerFecal DNA isolation kit (Mo Bio Laboratories, 
Inc., Solana Beach, CA) following the manufacturer’s 
protocols. Quality and concentration was evaluated 
using a NanoDrop spectrophotometer (Thermo Fisher 
Scientific, Waltham, MA). Deoxyribonucleic acid ex-
traction samples with a 260:280 mm ratio < 1.3 (lab 
average ratio 1.80) and a concentration < 20 ng/μL of 
DNA (concentration average 21.8 ng/μL) were concen-
trated using ethanol precipitation prior to sequencing.

Thirty-microliter aliquots of DNA from all liver 
samples were shipped to Novogene Bioinformatics 
Technology Company (Chula Vista, CA) for library pre-
paration and sequencing. The V4 region of the 16S rDNA 
subunit was amplified with the 515F/806R primer set. 
Paired-end sequencing (2 × 250 bp) was completed on 
an Illumina HiSeq 2500 (Illumina, Inc., San Diego, CA).

Bioinformatics and Statistical Analysis

Sequences and metadata for this research are retriev-
able from QIITA, study ID 10906. Reads from sequenc-
ing adaptors were trimmed from raw sequence data using 
cutadapt (Martin, 2011). Forward and reverse reads for 
each sample were merged using PEAR version 0.9.10 
(Zhang et al., 2013) with a minimum read length of 187 
bp and a maximum of 310 bp. Using QIIME version 
1.9 (Mercier, et al., 2013), raw sequencing reads were 
clustered into operational taxonomic units (OTU) using 
open reference methods at 97% similarity. De novo OTU 
were clustered using SUMACLUST whereas reference-
based clustering relied on SortMeRNA (Kopylova et al., 
2012) and the Greengenes 16S rRNA reference database 
(DeSantis et al., 2006). Taxonomy was assigned to OTU 
with UCLUST (Edgar, 2010) using the Greengenes da-
tabase. Operational taxonomic units assigned to mito-
ochondria and chloroplasts and singleton OTU were re-
moved. A rarefaction curve was constructed with using 
Chao1 measurements from biological observation matrix 
(BIOM) files to assess whether sequencing depth suffi-
ciently captured diversity. The OTU table was normal-
ized using cumulative sum scaling. Taxa present in all 
samples with a relative abundance of at least 1.0% in all 
samples were considered part of the “common micro-
biala.”

Nonmetric multidimensional scaling ordination us-
ing Euclidean distances was calculated on cumulative 
scale standardized counts, and the analysis of simi-
larities (ANOSIM) function in the vegan package in R 
(Oksanen, et al., 2017) was used to assess differences 
between cattle treated and not treated with tylosin as well 
as to assess differences between regions (Supplement 
File 1; see the online version of the article at http:// 
journalofanimalscience.org). For all comparisons in the 
study, α = 0.05 was used.
RESULTS AND DISCUSSION

Rarefaction Curve

The total number of reads considered in the analysis after quality control was 13,505,094. The mean number of reads in each sample was 350,716 (range 190,551 to 996,910 [SD 191,995]). The rarefaction curve (Fig. 1) shows a plateauing of reads mapped to novel OTU as the proportion of reads sampled increases, indicating that the microbial community was sampled to an appropriate depth to allow complete characterization of the bacterial community.

Summary Statistics

Through 16S characterization, 5 phyla, 13 classes, and 17 orders were identified in the DNA extracted among all sampled abscesses (Fig. 2). The phyla identified were Bacteroidetes (35.2% of reads), Proteobacteria (28.6%), Fusobacteria (18.2%), Firmicutes (12.4%), and Actinobacteria (5.5%). Of note, reads mapped to the 3 most predominant phyla, which made up 82% of the abscess microbial community, represent Gram-negative bacteria. This result is interesting because tylosin, a macrolide commonly used to prevent liver abscesses in feedlot cattle, is primarily active against Gram-positive bacteria with limited efficacy against Gram-negative bacteria. Although the isolation of Gram-negative bacteria from liver abscesses has been described (Nagaraja and Chengappa, 1998), the abundance of Gram-negative bacteria in the gut microbial community may explain why common prevention strategies (i.e., macrolide supplementation) have imperfect efficacy for prevention of liver abscessation. Instead, these results suggest that reduc-
Characterization of bovine liver abscesses

Strategies that provide more broad-spectrum action against the entire microbiome may be beneficial in more completely preventing this disease.

Identification of Bacteria of Interest and Common Microbiota

Previous literature has identified *F. necrophorum* as the primary causative organism for bovine liver abscesses (Nagaraja and Chengappa, 1998). *Fusobacterium* was found in all liver abscesses sampled in this study, and on average, reads mapped to this genus made up 15.1% of the microbial community when characterized using 16S rRNA amplicon sequencing (range 10.6 to 21.9%). Another common bacteria associated with liver abscesses, *Trueperella pyogenes*, was identified at genus level in all of the samples but at a lower overall community makeup of 3.2% (range 2.4 to 5.1%).

Ten other bacterial genera were present in all samples, 5 at a greater relative abundance than 3% of all classified reads: *Bacteroides* (17.6% of mapped reads), *Porphyromonas* (14.1%), *Pseudomonas* (5.7%), *Enterobacteriaceae* (3.7%; classified at the family level), and *Sneathia* (3.1%). The remaining 5 genera were present in 2.2 to 2.9% of the relative abundance of the community: *Parvimonas* (2.9%), *Helcococcus* (2.8%), *Psychrobacter* (2.6%), *Atopobium* (2.4%), *Campylobacter* (2.2%), and *Haemophilus* (2.2%). This bacterial community shares several genera (namely *Bacteroides*, *Enterobacteriaceae*, and *Fusobacterium*) with a characterization of human liver abscesses also characterized with 16S rRNA sequencing, although other genera, such as *Klebsilla*, were highly represented in the human abscesses and not characterized in this analysis (Song et al., 2014).

*Bacteroides* and *Porphyromonas* have both been previously described as being present in bovine liver abscess purulent material (Scanlan, and Hathcock, 1983; Nagaraja and Lechtenberg, 2007), whereas *Pseudomonas* has been reported in other ruminant abscesses (Tadayon et al., 1980) and *Sneathia* has been linked to abscesses in the cervical lymph nodes of other mammals (Eisenberg et al., 2016). Organisms from the *Enterobacteriaceae* family have also been cultured from liver purulent material such as *Salmonella enterica* (Amachawadi and Nagaraja, 2015).

Although there are no previous reports regarding the presence of *Parvimonas*, *Helcococcus*, *Psychrobacter*, *Atopobium*, *Campylobacter*, or *Haemophilus* in peer-reviewed literature as a causative agent of bovine liver abscesses, several of these species have been associated with other related disease. For examples, co-occurrence of *Parvimonas* and *Fusobacterium* has been...
found in human colorectal cancer (Nakatsu et al., 2015), *Helcococcus ovis* has been reported in association with bovine valvular endocarditis (Kutzer et al., 2008), and *Atopobium* has been found in purulent material of other mammals (Oyaert et al., 2014).

**Initial Evaluation of Differences Related to Geographic Region and Tylosin Exposure**

Although not the primary objective of the study and limited by sample size, the sampling structure used in this study provided an opportunity for an initial investigation of differences that might exist in the flora of liver abscesses based on comparison of geographic regions (8 in California, 9 in Texas, and 17 in Colorado) and tylosin exposure (18 non-tylosin fed and 16 tylosin fed). Sampling size and the partial confounding of region by cattle type (all the cattle in California were dairy-type cattle whereas the cattle in the other 2 regions were beef-type cattle) limit extensive formal comparison. However, in an observation of feedlot location and supplementation, the region where feedlots were located and supplementation with tylosin phosphate both affected liver abscess communities ($P = 0.002$ and $P = 0.026$, respectively). These comparisons among observed groups of cattle raise the possibility of different liver abscess rate composition by area and rearing methods, which, in turn, may lead to more targeted approaches to reduce abscess rates.

**Summary**

Management of liver abscesses in feedlot cattle continues to be an important priority for the North American beef cattle industry. Currently, the most common management strategies, which use treatment of cattle by including antimicrobial drugs in feed, are facing intensifying scrutiny. As such, a more thorough understanding...
of the microbial drivers of liver abscessation may lead to more efficient and sustainable management strategies, for example, the relationship between epimural bacteria and liver purulent material. The observed differences in region and rearing strategy provide further avenues for investigation and prevention of abscesses. We believe this characterization will allow for an ecological approach to treatment and prevention of liver abscesses.

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