"Microbiome of Healthy Beef Cattle Lymph Nodes and Digesta using 16S rRNA Gene Sequencing Analysis"

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I. INTRODUCTION

The process of bacterial cross-contamination during slaughter is intricate and difficult to identify foodborne pathogens due to their hidden, unseen, and intangible nature [1]. The digestive system of beef cattle and its associated organs are regarded as a primary source of microbial contamination of the final product [2]. While the digestive system is recognized as a primary source of microbial contamination [3], especially, in beef processing, fecal contamination of hides is recognized as the major source of contamination by enteric pathogens such as *E. coli* O157:H7 and *Salmonella* [4]. Mann et al. [1], noted that the risk associated with lymphatic organs is low as long as tissue integrity is maintained. However, the possibility of lymph nodes remaining intact during carcass processing and ground beef production is not feasible. Despite efforts, Webb, et al, reported a full characterization and description of the burden across multiple variables on the prevalence of *Salmonella* in the pre-scapular lymph nodes (PLN) from feedlot-fattened (FF) cattle from a region during the warmer season, the data reveal a significant prevalence rate of 31.1% [5]. Although recent progress in high-throughput sequencing, the bacterial microbiome composition in various animal niches has remained unexplored in beef cattle lymph nodes. We hypothesize that bacterial communities can be described in the lymph nodes of healthy slaughtered beef cattle. This study aims to estimate the bacterial biodiversity of each PLN and MLN comparing them with potential bacterial translocation from the digesta (fecal and cecal content) from beef cattle at harvest. This would constitute the first study to use DNA and RNA-based methods to investigate the diversity of the bacterial community in peripheral and mesenteric lymph nodes of beef cattle, offering insights pointing to pathogenic bacterial strains detected in the LNs derived from the Gastrointestinal Tract (GIT) and have therefore impact on risk assessments in slaughterhouses regarding a possible distribution of spoilage, foodborne and zoonotic pathogens.

II. MATERIALS AND METHODS

Beef lymph nodes (LNs) tissues were sampled at a commercial harvest facility in the Midwestern region of the USA. A total of 38 lymph nodes (LNs) were sampled: inferior-ileocecal (IILNs, n= 8), popliteal (PLNs, n= 8), subiliac (SLNs, n= 8), superior-ileocecal (SILNs, n= 8), and mandibular (MLNs, n= 6). After removal LNs were purified from loose tissue and fat residues, and the surface was disinfected by rigorously dipping into 70% ethanol. The digesta samples were constituted of 8 cecal content and 8 fecal samples. All beef cattle included in this study were market animals originating from commercial feedyards in the USA. DNA from LN tissues was extracted using Qiagen DNeasy blood and tissue kit. We utilized total genomic DNA extracted from bacterial samples as the template for polymerase chain reaction (PCR) amplification. Specifically, targeting the hypervariable regions within the V3-V4 region of the 16S ribosomal RNA (rRNA) gene, a widely used marker for bacterial identification and classification. Microbial abundance and taxonomic classification were evaluated using the DADA2 and QIIME2 Package in R version 4.3.2.

III. RESULTS AND DISCUSSION

We found a total of 14,115 amplicon variant sequences (ASVs) in fecal, cecal content and LNs samples, indicating a substantial diversity of microbial species present. Firmicutes, Bacteroidetes, and Proteobacteria were the most abundant phyla in all experimental groups, which has been previously described in beef fecal samples [6]. The taxonomic classification revealed the most abundant genus

according to the relative abundance composition of bacterial communities of the fecal samples belonged to *Rombutsia* 9.06%, *Bacteroides* 8.73%, and *Oscillospiraceae* 7.66%. This was followed by cecal content samples where the most abundant genus was represented by *Bacteroides* 11.09%, *Oscillospiraceae* 9.44%, and *Alloprevotella* 8.01%. The comparison with the most abundant genus in PLNs were represented by *Staphylococcus* 15.24%, *Enterococcus* 14.17%, and *Escherichia/Shigella* 6.87%. In contrast, MLNs most abunbdant genus were belonged to *Enterococcus* 45.75%, *Escherichia/Shigella* 26.73%, and *Bacillus* 4.79%. The alpha diversity comparison between groups LNs-BF and LNs-CC was according to the observed richness (p<1.075e-09), Chao1 Richness (p<1.091e-09), and Shannon diversity (p<8.177e-08). These results suggest variations in microbial diversity between different lymph node locations and digesta [7].

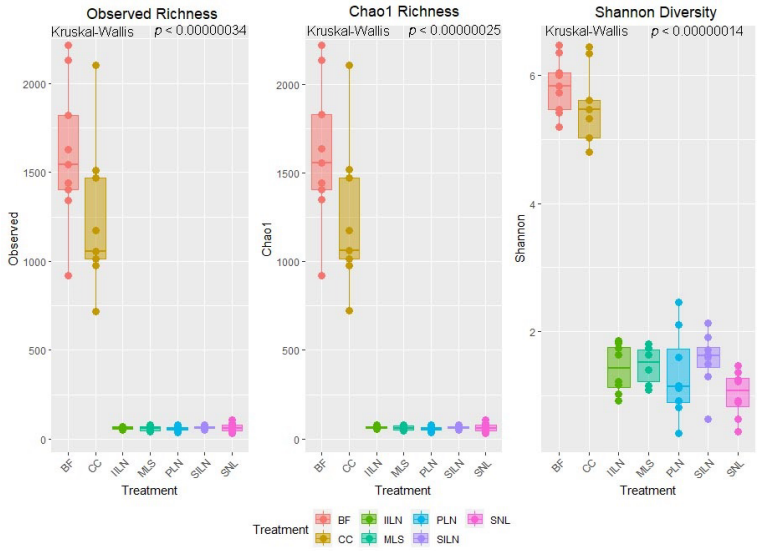

FIG 1. Alpha diversity indices of microbiome samples. Fecal and Cecal content were compared to LN groups significant differences were noted within the alpha diversity indexes (*P<0.05*).

IV. CONCLUSION

This pioneering study utilizes DNA-based methods to explore bacterial diversity in US beef cattle's peripheral and mesenteric lymph nodes. The presence of *Escherichia/Shigella* and *Clostridium* sensu stricto 1 genus and the powerful correlation of the bacterial relative abundance in the peripheral lymph nodes and digesta suggest a possible bacterial translocation and presence of potentially pathogenic bacteria from the Gastrointestinal Tract (GIT), highlighting the importance of risk assessments in slaughterhouses to prevent the spread of spoilage bacteria, foodborne, and zoonotic pathogens.

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