Description of the preputial microbiota of bulls using 16S r-DNA profiling

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Introduction

Worldwide there is approximately 971 million head of cattle, with 92 million head within the United States alone. The production of cattle is 1 of the most important industries in the United States, accounting for $78.2 billion in cash receipts during 2015. Despite the relevance of cattle economically, many aspects of their reproductive physiology and biology are still relatively unknown. Only recently has the vaginal microbiome been characterized in the cow and ewe, and the microbiota of the male urogenital system has yet to be determined. It is increasingly recognized that studying host-parasite-bacteria-virus complex interactions at mucosal or epithelial surfaces represents an important new paradigm to comprehend the impact, positive and negative, of these interactions on animal health and disease conditions. Therefore the objective of this study was to investigate and define the microbiota of the penis and prepuce of the post-pubertal bull using 16S r-DNA profiling as a proof-of-concept study. By establishing a baseline for the microbiota of pubertal bulls we can then begin to examine how certain disease conditions impact the epithelial surface of the penis and prepuce. The characterization of the urogenital microbiota coupled with knowledge about the immunology of the area can converge to improve our knowledge of how diseases affect the area, with *Tritrichomonas foetus* being an example.

Materials and Methods

Bulls presented to The Purdue University Large Animal Clinic for routine breeding soundness exams were utilized. Bulls were restrained in a livestock squeeze chute per routine standards for performing routine breeding soundness examinations. Each bull underwent a cursory physical exam in-line with normal practices. During the process of semen collection when the bull had fully extended his penis and prepuce a Dacron swab was used to collect samples from the epithelium of the penis and prepuce. Once taken, the sample was quickly placed in individually labeled vials containing RNAlater®.

Samples were transported to the lab, and DNA was extracted. Extracted DNA was used for the construction of a 16S rRNA gene library by amplifying the V3 to V4 region of bacterial 16S rRNA gene. Amplicons were sequenced using the Illumina MiSeq and the Illumina Nextera DNA library construction technology was used to create dual-indexed libraries that are pooled 100 to 300 deep with samples from other labs in a single Illumina MiSeq 300 cycle run (2x150 base paired-end reads). After demultiplexing, individual samples were de novo assembled using SPADES.

Results

Relative abundance was used for analysis. QIIME (Version 1.8.0) was used to analyze and compare microbial community characteristics between samples. All merged reads underwent operational taxonomic unit (OTU) picking, taxonomic assignment, and phylogenetic tree construction using Green Genes (13_5) as a reference database. Weighted UniFrac distance matrices, accounting for differences in both phylogeny and relative taxon abundance, were generated and compared. A minimum of 25% of the total number of samples per comparison that have at least 1 read per OTU was used for downstream analyses. The relative taxon and OTU abundances was compared pairwise between samples using the QIIME and Phyloseq software package, respectively, and a FDR (false discovery rate) of 0.05 was used to determine statistical significance. The results showed that the penis and prepuce of the bull consists of a rich and diverse microbial environment with over 300 different OTUs of organisms identified.

Significance

The microbiome of the penis and prepuce of the bull is extremely diverse. A comprehensive analysis of the microbiome represented across different age, breed, and diet groups as well as the core microbiome of the bull penis and prepuce will be provided.