

Profiling of Bacterial Communities Present in Feces, Milk, and Blood of Lactating Cows Using 16S rRNA Metagenomic Sequencing

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Abstract : Ecologically, the gut, mammary glands and bloodstream consist of distinct microbial communities of commensals, mutualists and pathogens, forming a complex ecosystem of niches. The by-products derived from these body sites i.e. faeces, milk and blood, respectively, have many uses in rural communities where they aid in the facilitation of day-to-day household activities and occasional rituals. Thus, although livestock rearing plays a vital role in the sustenance of the livelihoods of rural communities, it may serve as a potent reservoir of different pathogenic organisms that could have devastating health and economic implications. This study aimed to simultaneously explore the microbial profiles of corresponding faecal, milk and blood samples from lactating cows using 16S rRNA metagenomic sequencing. Bacterial communities were inferred through the Divisive Amplicon Denoising Algorithm 2 (DADA2) pipeline coupled with SILVA database v138. All downstream analyses were performed in R v3.6.1. Alpha-diversity metrics showed significant differences between faeces and blood, faeces and milk, but did not vary significantly between blood and milk (Kruskal-Wallis, $P < 0.05$). Beta-diversity metrics on Principal Coordinate Analysis (PCoA) and Non-Metric Dimensional Scaling (NMDS) clustered samples by type, suggesting that microbial communities of the studied niches are significantly different (PERMANOVA, $P < 0.05$). A number of taxa were significantly differentially abundant (DA) between groups based on the Wald test implemented in the DESeq2 package ($P_{adj} < 0.01$). The majority of the DA taxa were significantly enriched in faeces than in milk and blood, except for the genus *Anaplasma*, which was significantly enriched in blood and was, in turn, the most abundant taxon overall. A total of 30 phyla, 74 classes, 156 orders, 243 families and 408 genera were obtained from the overall analysis. The most abundant phyla obtained between the three body sites were Firmicutes, Bacteroidota, and Proteobacteria. A total of 58 genus-level taxa were simultaneously detected between the sample groups, while bacterial signatures of at least 8 of these occurred concurrently in corresponding faeces, milk and blood samples from the same group of animals constituting a pool. The important taxa identified in this study could be categorized into four potentially pathogenic clusters: i) arthropod-borne; ii) food-borne and zoonotic; iii) mastitogenic and; iv) metritic and abortigenic. This study provides insight into the microbial composition of bovine faeces, milk, and blood and its extent of overlapping. It further highlights the potential risk of disease occurrence and transmission between the animals and the inhabitants of the sampled rural community, pertaining to their unsanitary practices associated with the use of cattle by-products.

Keywords : microbial profiling, 16S rRNA, NGS, feces, milk, blood, lactating cows, small-scale farmers

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