TITLE: High-Throughput Sequencing of the 16S rRNA gene as a form of identification and analysis of four different types of mastitis in goats

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ABSTRACT:
Studies using 'Omic' technologies have modified the way in which many diseases have been diagnosed and the sequencing in high-throughput platforms of the 16S rRNA gene has been presented as a valuable tool of study of different microbiotas. Mastitis is a major problem for the losses generated in costs due to failure in treatment, excessive use of drugs such as antibiotics and also by concern the high number of different infectious pathogens involved. The goat mastitis is diagnosed from clinical, direct and indirect such as microbiological culture, and this may be differentiated in some types: subclinical mastitis (M1), clinical (M2), persistent (M3) and gangrenous (M4). The objective of this study was to identify the microbiota involved in the different types of mastitis and to determine the differences between communities together with clinically healthy animals (HO). 94 milk samples were collected and subdivided into 12 (HO), 42 (M1), 14 (M2), 22 (M3) and 2 (M4). The DNA was extracted with the QIAamp DNA Mini Kit (QIAGEN). Sequencing was carried out with an IluminaMiseq sequencing platform, targeting the V4 region (515f-806r) of the 16S rRNA ribosomal subunit gene. The bioinformatics analyzes were performed in package “DADA2” pipeline (version 1.8) in R program to infer the amplicon sequence variants (ASVs) present in each sample. Taxonomic assignment was performed against the Silva v132 database, using the implementation of the RDP naive Bayesian. The results showed 6089 different taxa and most abundant phylum among types of mastitis in HO was Firmicutes, followed by Bacteroides and Proteobacteria, indeed Tenericutes was highly prevalent for M2 and Actinobacteria in M3. In descending order, the most abundant genera in HO, M1, M2 and M3 were Staphylococcus, Bacteroides, Alkalibacterium. However, the differential abundance of M1 was genus Geobacillus, Mannheimia, Pseudomonas; M2 Streptococcus and Klebsiella; in M3 Truperella and Manheimnia; and M4 the sample were predominately dominated by Enterococcus, Escherichia/Shigella and Staphylococcus. When comparing microbial compositional based on beta diversity, the groups that presented significant dissimilarity were M1-M3, M1-M2, HO-M3 (P<0.001) and HO-M2 (P<0.01) (Adonis; R² = 0.12, PERMANOVA). 16S rRNA high-throughput sequencing gene revealed a large number of pathogenic bacteria, and showed how the microbiota in diagnosis of the major types of mastitis that affect goats can be complex.

Keywords: 16S rRNA, goat, mastitis, high-throughput, milk.

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