

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

AUTHORS: ¹POLVEIRO, R.C.; ²VIDIGAL, P. M. P.; ³YAMATOIGI R. S.; ⁴ MENDES, T.A.O.; ¹ LIMA, M. C.; ¹MOREIRA, M. A. S.

¹ LABORATORY OF BACTERIAL DISEASES (LDBAC), PREVENTIVE VETERINARY MEDICINE AND PUBLIC HEALTH SECTOR, VETERINARY DEPARTMENT; ²NUCLEUS OF ANALYSIS OF BIOMOLECULES (NUBIOMOL); ³INSPECTION LABORATORY OF ANIMAL PRODUCTS - INSPOA; ⁴DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY.

INSTITUTION: UNIVERSIDADE FEDERAL DE VIÇOSA; (P.H. ROLFS AVENUE, UNIVERSITY CAMPUS. 36570-900, VIÇOSA, MG, BRAZIL).

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 IlluminaMiseq 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 *Truuperella* and *Manheimmia*; and M4 the sample were predominantly 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; $R^2 = 0.12$, 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.

Development Agency: CAPES, CNPq and FAPEMIG.