

EAR MICROBIOTA IN HEALTHY DOGS

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The advent of next-generation sequencing (NGS) techniques allowed a better understanding of the human skin microbiota in healthy subjects and in people with skin disturbances. Few studies have investigated the canine skin and ear microbiota [1-2]. The aims of the present study are to characterize the ear canal microbiota in healthy dogs and to study the differences of its composition evaluated using the culture-dependent and the NGS approach. Twenty healthy dogs with no history of skin disease were enrolled. Clinical history and demographic data were recorded. Dogs treated with ear cleaners, antimycotics or antibiotics in the 2 and 4 weeks before sampling, respectively, were excluded. Ear cytology was performed in all dogs. Samples were obtained from the right vertical external ear canal of each dog by sterile swabs. Bacterial isolation was performed according to standard technique. Taxonomic identification was done by MALDI-TOF MS analysis. The DNA was extracted from each sample and quantified. After PCR amplification, V3-V4 16S-rDNA amplicon libraries were prepared and sequenced on Illumina MiSeq platform. The final feature table was obtained with QIIME2 pipeline [3] and the Greengenes database (<http://greengenes.lbl.gov>) was used for taxonomic assignments. Alpha diversity and beta diversity were calculated after rarefaction, as part of the QIIME2 analysis workflow. Bacteria cultures identified *Bacillus* spp. and *Staphylococcus* spp. in 65% and 40% of dogs, respectively. *Streptococcus* spp., *Clostridium* spp., *Micrococcus* spp. and *Exiguobacterium* spp. were isolated in 10% of cases. *Enterobacter* spp., *Acinetobacter* spp., *Ureibacillus* spp., *Kocuria* spp. and *Curtobacterium* spp. were isolated in 5% of dogs. Bacteria from 31 phyla were identified with NGS approach, with Proteobacteria being the most abundant phylum in all samples. *Photobacterium* was the prevalent genus in 95% of dogs, whereas *Bacillus* spp. and *Staphylococcus* spp. were identified in 75% and 90% of dogs, respectively. Diversity analysis showed a strong individual variability in terms of alpha and beta diversity. The Richness index showed large differences ranging from 30 to 449 observed features (mean: 159, SD: 96). The Pielou index values highlighted a good equitability in the population composition in most of the samples, with the exception of three dogs whose microbiota showed to be driven by the presence of some dominant species. In conclusion the NGS approach allowed to identify a very complex canine ear microbiota with great individual variability and partial agreement with the culture-dependent approach. Further studies may provide a clearer understanding of the clinical significance of these results.

[1] Ngo et al. Ear canal microbiota - a comparison between healthy dogs and atopic dogs without clinical signs of otitis externa. *Vet Dermatol*, 29:425-e140, 2018 [2] Korbelik et al. Characterization of the otic bacterial microbiota in dogs with otitis externa compared to healthy individuals. *Vet Dermatol* doi: 10.1111/vde.12734, 2019 [3] Bolyen et al. QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science. No. e27295v1. *PeerJ Preprints*, 2018.