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The human oral microbiome is known to play a signifcant role in human health and disease. While less well

K.w : Microbiotas; Conjunctivitis; micro ora; 16S rRNA

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Conjunctivitis, keratoconjunctivitis, and corneal sequestration are common clinical problems in cats. Based on research over the last few decades, characteristics of the bacterial ora in feline conjunctival sacs show a similar composition, and the occurrence of particular species of bacteria varies by frequency of their isolation [1]. However, Gelatt described the feline conjunctival and corneal surface as being generally colonized to a lower degree than in other domestic species. Among bacteria isolated from the conjunctiva, staphylococci are the most representative group [2].

A second group of frequently isolated microorganisms are hemolytic and no hemolytic streptococci. Previous studies based on the microbiological identi cation of bacteria or the sequencing of amplicons generated from microbial DNA have also been considered conjunctival commensals, which in some circumstances may be involved in conjunctival pathology. Chlamydophila felis has been identi ed as an indisputable pathogen of feline conjunctiva. is Gram-negative bacterium has already been isolated from a number of feline conjunctivitis cases. ere is also evidence that other Chlamydia-related microorganisms like Chlamydophila pneumoniae and Neochlamydia hartmannellae may be associated with conjunctiva. Investigating conjunctival infections in cats with lepromatous lesions [3]. Identi ed Mycobacterium spp. to have occurred. Based on a phylogenetic analysis, a novel species in the Mycobacterium simiae-related group was identi ed.

Most of the previous research has investigated feline ocular micro ora using a classical microbiology approach involving the culture and further characterization of isolates. e aim of the present study was to examine the suitability of the methodology which may disclose microbial diversity within feline conjunctivas of healthy cats and animals with conjunctivitis symptoms, using partial sequencing of the 16S rRNA gene [4]. To the best of our knowledge, it is a frontier research in the eld of veterinary ophthalmology and a preliminary study linked to our next project concerning next generation sequencing (NGS).

Conjunctival swabs obtained from three clinically healthy cats with no ocular disorders and from three cats with conjunctivitis symptoms were included in the study [5]. Based upon our own clinical experience with chronic conjunctivitis in cats and for the purpose of the study, sick animals comply with criteria such as manifestation of conjunctivitis lasting about six months and insu cient response to the standard ophthalmological treatment. Sick cats were also tested by PCR and RT-PCR to determine the presence of Chlamydia felis, feline herpesvirus-1 and Mycoplasma felis infections, according to published protocols by Chalker et al., Marsilio et al., and Helps et al., whereby speci c DNA was not detected [6]. Additionally, an ophthalmic examination was performed on each cat; eyelash and cartilage abnormalities and incorrect positioning of the eyelids were ruled out. Irregularities of the drainage system were eliminated with a 1% uorescein test and by irrigation via a 26 G catheter.

A total of 48 sequence reads were obtained in the study; only the 30 high-quality sequence reads were used in further analysis of the diversity

of bacterial ora in the feline conjunctiva. Eight genera were identi ed among the sequences from clinically healthy and diseased animals (Figure 1). Taking into consideration the maximal 16S rRNA distance scores <1%, the following species were recognized: Bacillus subtilis, Psychrobacter faecalis, Psychrobacter pulmonis [7], Propionibacterium acnes, Staphylococcus caprae, Staphylococcus capitis, Staphylococcus succinus, Streptococcus infantarius, and Streptococcus lutetiensis.

e low similarity in micro ora composition at the genus level was observed between diseased and healthy conjunctivas.

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e limited capacity of culture-based methods for the identi cation of bacteria from the feline conjunctiva makes standard procedures incomplete [8]. is is mainly due to the limited viability of some microbial species, coinfections, or the presence of uncultivable or as yet unknown species. e monitoring of feline conjunctiva using alternative methods is not commonly applied as a standard for analyzing the diversity of conjunctival micro ora in cats. DNA-based approaches were already used to assess the diversity of microbial communities or to monitor population dynamics. e analysis of bacterial taxa in conjunctival swabs by DNA sequencing provided evidence that feline conjunctiva may be settled by microorganisms not yet isolated. Our results, compared with those of culture-based studies [9], suggest that the diversity of bacterial ora within feline conjunctiva can vary more than previously believed. We found that our results based on sequence analysis methods were concordant with the culture-based analysis previously applied to the same material in terms of genera such as Bacillussp, Staphylococcus sp., and Streptococcus sp. Bacteria belonging to these genera had already been identi ed in cat conjunctivas [10]. A comparison of eye micro ora of clinically healthy animals and those with signs of conjunctivitis indicated no qualitative di erences. e results of our study revealed some species that had not been reported earlier in feline conjunctiva, including Bacillus subtilis, Staphylococcus caprae, Staphylococcus succinus, Streptococcus infantarius, Streptococcus lutetiensis, Psychrobacter faecalis, and Propionibacterium acnes.

Psychrobacter sp. belongs to the gamma Proteobacteria family and includes bacteria isolated from the skin of sh and chickens, meat products, clinical sources, and sea water [11]. In our study, bacteria from Psychrobacter taxon constituted a considerable subpopulation.

during a standard bacteriological investigation, for example, as detected in our study, actinomycetes which require customized incubation time. Furthermore, they belong to the leading producers of substances showing biological activity, which could interfere with a selection of antibiotic-resistant strains of other bacteria. As yet, the role of actinomycetes in feline conjunctivitis has not been established, but it is clear that other standards for cultivation or examination targeted at molecular detection should be taken into account. Clinical relevance of this microbiota requires further study.

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e authors declare that there are no con icts of interest regarding the publication of this paper.

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