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ISOLATION OF *Macrococcus brunensis* AND *Kocuria varians* IN DOGS SUFFERING FROM CHRONIC CONJUNCTIVITIS

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The Human Microbiome Project launched in 2008 by the National Institutes of Health, revealed a remarkably abundant and diverse community of microbial species inhabiting the human body, and the eye represents an emerging area of research [1] trying to understand possible relations between microbiota alterations and pathogenesis of ophthalmic diseases [2]. In veterinary medicine, there are only few paper focused on this topic [3,4,5]. The aim of the present study was to isolate and identify, by molecular techniques, several bacterial species from the conjunctival microflora in dogs with chronic conjunctivitis. Four owned dogs conducted at the UNICAM Veterinary Teaching Hospital for eye examination due to chronic and/or relapsing conjunctivitis, were included in the study. All subjects were submitted to complete ophthalmologic evaluation, including Schirmer Tear test and fluorescein test, slit lamp examination, direct ophthalmoscopy and tonometry, to rule out other causes of conjunctival disease. The administration of systemic antibiotic in the six months prior the visit was considered as an exclusion criteria. To obtain isolated bacterial colonies, conjunctival swabs from seven infected dog eyes were spread onto agar plates with selective and non-selective media. From each isolated colony, the bacterial DNA was extracted using Bacterial Genomic DNA Isolation kit (Norgen Biotek, Ontario, Canada); the 16S bacterial rRNA gene was amplified by PCR and purified by Nucleo Spin Extract kit (Macherey-Nagel, Dürham, Germany). Each purified DNA sample was prepared and sent to be sequenced by Eurofins MWG Operon (Martinsried, Germany). The sequences obtained from each bacterial strain were analyzed using BLAST[®] (Basic Local Alignment Search Tool, www.ncbi.nlm.nih.gov/BLAST). Enterococcus faecium, Kocuria varians, Macrococcus brunensis, Staphylococcus aureus and two strains of S. equorum were the six different bacterial strains isolated from 3 out of 7 samples (43%). Sensitivity of the isolated bacterial strains to the most frequently used antibiotics in veterinary ophthalmology: chloramphenicol, gentamycin, neomycin, and tobramycin was assessed using Kirby-Bauer method. All strains were resistant to chloramphenicol (all patients had previously been treated with it) and sensitive to tobramycin, neomycin, and gentamycin except E. faecium that was sensitive only to tobramycin. To the Authors' knowledge, this is the first isolation of Macrococcus brunensis and of Kokuria varians from the eyes of dogs suffering from chronic conjunctivitis. They had been previously isolated from animals (e.g. M. brunensis from the skin of llamas [6] and K. varians from the eyes of healthy donkeys [7]), but this is the first isolation from dog. Even if the low number of patients and the single isolates did not allow us to draw conclusion about the involvement of these bacteria in the disease, the isolation of K. varians is particularly relevant because Kokuria spp. is a well-known pathogen in humans, especially in compromised hosts [8].

[1] Dong et al. Invest Ophth Vis Sci. 2011;52(8):5408-13. [2] Lu et al. Yale Journal of Biology and Medicine. 2016;89:325-30. [3] Prado et al. Vet Ophthalmol. 2005;1:33-7. [4] Furiani et al. Vet Dermatol. 2011;22:490-6. [5] Verneuil et al. J Mycol Méd. 2014;24:25-8. [6] Mannerová et al. Int J Syst Evol Microbiol. 2003;53:1647-54. [7] Laus et al. Large Anim Rev. 2016;22:137-42. [8] Purty et al. Emerg Microbes Infect. 2013;2:e71.