

TITLE: Biofilm formation in *Moraxella bovis*, *Moraxella ovis* and *Moraxella bovoculi* from clinical keratoconjunctivitis

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Infectious keratoconjunctivitis is the most important ocular disease of cattle worldwide, leading to severe economic losses in affected herds. *Moraxella bovis*, *Moraxella ovis* and *Moraxella bovoculi* are the bacteria that cause the disease. These microorganisms may remain on the property for a long time in the animals carrying the infectious agent. Biofilm formation is an important viability maintenance tool for different microorganisms, especially on biotic and abiotic surfaces. In this study, the capacity of biofilm formation by clinical isolates of *M. bovis*, *M. bovoculi* and *M. ovis* was evaluated. We selected six isolates of each species of *Moraxella* pathogenic for ruminants. All isolates were isolated from ocular swabs from clinical cases in Southern Brazil, Argentina and Uruguay. In addition, standard strains were employed as controls, including: ATCC 19575 - *M. bovis*, ATCC 10900 - *M. ovis* and ATCC BAA1259 - *M. bovoculi*, totalizing 21 isolates. In biofilm formation assay, it was used a sterile 96-well polystyrene microplate, where was added 200 microlitres of Tryptone Soya Broth plus 1% glucose containing the *Moraxella* sp. isolate to be tested from a 24 hour culture. The plate was incubated in an incubator at 37°C for 48 hours and stained with 0.25% gentian violet. Soon after, the optical density reading was performed in a spectrophotometer. All assays were prepared in triplicate. All isolates of *Moraxella* spp. (21/21) presented as biofilm producers, with 47.6% (10/21) classified as poor biofilm forming, 42.9% (9/21) moderate biofilm forming and 9.5% (2/21) strong biofilm forming. Despite the known importance of biofilm in persistent infections, there are still few studies involving microorganisms of veterinary interest. In relation to *Moraxella* spp. pathogenic to animals, only *M. bovis* was previously studied for its biofilm formation ability. Bacteria in the form of biofilm exhibit greater resistance to host immune response and are much less susceptible to antimicrobials when confronted with the planktonic presentation. Complementary studies, with a greater number of isolates are being carried out by our research group. However, the results obtained so far are promising and contribute to the understanding of the pathogenesis, the process of colonization of the conjunctiva by *M. bovis*, *M. ovis* and *M. bovoculi*, as well as in the control measures in the cattle herds.

Key-Words: Pinkeye, Carrier animals, Polysaccharide matrix, Cell adhesion, Pathogenicity.