

TITLE: BIOFILM FORMATION BY NATIVE ISOLATES AND REFERENCE STRAINS OF *Leptospira* spp.

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ABSTRACT:

Leptospirosis is a spirochetal zoonosis of worldwide occurrence and great impact on public health. Biofilm formation in leptospires is an emerging field of study, and its role in the pathogenesis of infection has not been fully elucidated. The role of dogs as reservoir hosts and sentinels for human infection propels new research on isolation of autochthonous strains with potential for biofilm formation. In this study, we evaluated the ability of biofilm formation in 96-well plates of four *Leptospira interrogans* isolates of the Icterohaemorrhagiae serogroup (C20, C29, C51 and C82) obtained from naturally infected dogs, and of four reference strains of *Leptospira* spp. (L02 - *L. interrogans*, Sv. Hardjo; L15 - *L. borgpetersenii*, Sv. Ballum; L17 - *L. biflexa*, Sv. Patoc; L20 - *L. interrogans*, Sv. Copenhageni). *In vitro* biofilm formation was tested by incubation at 30°C for 21 days under static conditions. Biofilm growth quantification and characterization in low (OD₆₀₀: 0.200 to 0.299), medium (OD₆₀₀:0.300 to 0.699), and high (OD₆₀₀ ≥ 0.700) formation was performed in a spectrophotometer at 600 nm and as per the crystal violet staining methodology. Capacity of biofilm formation was variable between the studied strains. All isolates that formed aggregates produced suspended biofilms and adhered to the wall (at the air-liquid interface and at the bottom of the wells). Five strains were efficient in biofilm formation (C29 OD= 0.272±0.01, C51 OD= 0.397±0.02, L02 OD= 0.533±0.01, L15 OD= 0.587±0.01 and L17 OD= 0.480±0.02), while three other strains (L20, C20 and C82) did not form biofilm until the 21st incubation day. For the saprophytic strain L17 biofilm formation was observed within two days of incubation, while the pathogenic strains formed aggregates later, on days eight (L02, C29, C51) and 10 (L15). It has been proposed that biofilms play an important role in maintaining chronic carriage of *L. interrogans* in animal reservoirs. In this study, both saprophytic and pathogenic leptospires were capable of forming biofilm, highlighting the relevance of these findings to public health due to increased antimicrobial resistance, environmental survival, and potential to cause recurrent infections.

Keywords: biofilms, leptospirosis, *Leptospira interrogans*

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