TITLE: INVESTIGATION OF THE PRESENCE OF EXTRACELLULAR DNA IN LEPTOSPIRAL BIOFILMS

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ABSTRACT:

Leptospirosis is a zoonosis caused by pathogenic bacteria of the genus *Leptospira*. It is estimated that this disease causes 1.03 million cases in humans each year in the world. *Leptospira* form biofilms, which are characterized by aggregates of microorganisms surrounded by a self-synthesized extracellular matrix. The composition of the biofilm matrix produced by *Leptospira* is not yet fully known. Characteristically the biofilm consists of water, saccharides, proteins and extracellular DNA (eDNA). eDNA is involved in cell-cell and cell-surface adhesion in biofilms. Additionally, eDNA may be essential for biofilm formation by some bacteria. We aimed to investigate extracellular DNA as a matrix component of leptospiral biofilms. Saprophytic *Leptospira biflexa* was cultivated for 12 h, 48 h and 120 h, which corresponds to incubation times for initial, mature, and late stages of biofilm formation, respectively. Pathogenic *Leptospira interrogans* was cultivated for eight and 16 days, corresponding to mature and late stages of biofilm formation, respectively. Planktonic cells were cultivated as controls, for the same time-periods. Cells were fixed with paraformaldehyde 2%. We performed immunofluorescence using polyclonal antibodies anti-*Leptospira* and propidium iodide for eDNA staining, followed by confocal laser scanning microscopy observation. Biofilms of pathogenic *L. interrogans* and saprophytic *L. biflexa* presented eDNA in the matrix, while planktonic cells did not present eDNA, indicating a role for eDNA in the architecture and maintenance of leptospiral biofilms. In *L. biflexa* biofilms eDNA was not present in the beginning of biofilm formation at 12 h, suggesting that eDNA has a minor role in cell initial adhesion. eDNA staining was observed at 48h and was stronger at 120 h, indicating a time-dependent raise of eDNA during biofilm formation. Likewise, we observed for *L. interrogans* that eDNA staining was stronger in 16 days in comparison to eight days. eDNA appears to be a necessary component for the maintenance of mature leptospiral biofilms. However, the stronger staining of eDNA occurred in late biofilms, probably because in addition to eDNA release during biofilm formation, there is also cell death in response to nutrient paucity. Our study showed that eDNA is a component of leptospiral biofilm matrix. Further studies are needed to investigate its role in the phenotype.

Keywords: spirochetes; extracellular matrix; leptospirosis

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