Title: EXPRESSION OF IMPORTANT ANTIGENS DURING BIOFILM FORMATION BY *LEPTOSPIRA* SPP.

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Abstract:

Leptospirosis is an important zoonosis caused by pathogenic *Leptospira*. Affected humans may present severe disease as Weil Syndrome and Leptospirosis-associated Severe Pulmonary Hemorrhagic Syndrome, with 10% and 50% lethality rates, respectively. The major host reservoir of leptospires in urban areas is the brown rat, which is chronically colonized by leptospiral biofilms in the renal tubules. The molecular mechanisms of biofilm formation *in vitro* and *in vivo* are still largely unknown. The objective of this study is to evaluate *in vitro* the expression of the antigens LipL32 and Loa22 in biofilms of the pathogen *Leptospira interrogans* serovar Lai. Biofilms of *L. interrogans* were grown for eight days (mature stage), harvested, washed and lysed. Total protein extracts were precipitated in acetone and re-suspended in 4 M urea. Protein extracts were analyzed through 10% 1D SDS-PAGE followed by western blot using antibodies specific to the lipoproteins LipL32 and Loa22. We found that both proteins were expressed in mature biofilms of *L. interrogans*. LipL32 is exclusive of pathogenic *Leptospira* species, is the most abundant lipoprotein in leptospiral outer membrane, and is an immunodominant antigen during human leptospirosis. The Omp-A like Loa22 was the first genetically defined virulence factor of *L. interrogans* to be identified and is highly regulated during host infection. Our results evidence that these two proteins important for the virulence and immunogenicity of *Leptospira* are expressed in the biofilm. These molecules might play a role in biofilm formation or its maintenance in the reservoir host. Further studies need to be done to characterize the functional role of these proteins in leptospiral biofilms. Uncovering antigenic molecules expressed in biofilms is important to improve our knowledge of leptospiral biology and pathogenicity. We expect our findings to open new avenues for the development of leptospirosis control measures in reservoir hosts, using antigenic molecules of the biofilm as a target.

Keywords: leptospirosis, virulence factors, biofilms

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