## CHARACTERIZATION OF SURFACE PROTEINS FROM LEPTOSPIRA INTERROGANS

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Introduction: The whole-genome of L. interrogans serovar Copenhageni and the bioinformatic tools allow us to search for novel antigens suitable for improved vaccines against leptospirosis. Objectives: We focused on fifteen genes encoding for conserved hypothetical proteins that are predicted to be exported to the membrane. Methods: The chosen genes were amplified by PCR from six predominant pathogenic serovars, the DNA cloned into pDEST17<sup>™</sup> E. coli vector, the recombinant proteins expressed in fusion with 6xHis-tag at N-terminus and purified by metal-affinity chromatography. The ability of these proteins to mediate attachment to various ECM components was evaluated by ELISA or western blotting methods. Protective immunity assays of these antigens against challenge with L. interrogans virulent strain were evaluated in hamsters. **Results and Discussion:** Four proteins were shown to be surface-exposed by liquid-phase immunofluorescence assays with living organisms. We have identified a leptospiral protein encoded by LIC10368, named Lsa21, only present in virulent strains, that binds strongly to laminin, collagen IV and plasma fibronectin, in dose-dependent manner. By western blotting assay, we have further identified eight novel probable adhesins that interact with laminin and fibronectin. These adhesins, in addition to Lsa21, which is probably a novel virulence factor, might be involved on the leptospiral pathogenesis. The immunization/challenge assays showed that the rLIC12730 afforded protection against lethal leptospiral inoculation. The immunoprotection conferred is probable via Th2 response as revealed by the increase in antibody titers during subsequent boosters. Our data suggest that rLIC12730 is a promising candidate for prevention of leptospirosis.

Keywords: Leptospira, vaccine, recombinant protein.

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