Sub-area: Pathogenesis, virulence, and resistance

Leptospira interrogans biofilm formation in *Rattus norvegicus* (Norway rats) natural reservoirs

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Pathogenic *Leptospira* are the causative agent of leptospirosis, an infectious disease of global importance in public health. *Rattus norvegicus* (Norway rats) are the main reservoir hosts of leptospires in urban settings worldwide. Pathogenic *Leptospira* densely colonize and form aggregates in the kidneys' proximal tubules of reservoir host rats. In the environment, pathogenic *Leptospira* form biofilms, possibly contributing for bacterial survival and maintenance. Biofilms are communities of microorganisms embedded in an extracellular polysaccharidic matrix. This phenotype, ubiquitous in Bacteria, participates in the pathogenesis of several diseases. So far, *Leptospira* biofilms have not yet been observed in the kidneys of natural reservoir animals presenting leptospiral renal colonization. In this work, we described biofilm formation by pathogenic *Leptospira* inside the renal tubules of naturally infected *R. norvegicus*. We captured 87 rats from a Brazilian urban community endemic for leptospirosis. We used qPCR for *lipL32* gene to quantify pathogenic leptospires. We performed co-localization of pathogenic leptospires and biofilm extracellular matrix saccharides in the renal tubules, using immunohistochemistry anti-*Leptospira* and alcian blue staining, respectively. We characterized the biofilms using scanning electron microscopy and anionic dyes. We analysed demographic data to identify variables correlated with biofilm presence. We found that 78 rats were positive for *Leptospira* infection and 65 were positive for kidneys' colonization. From those, 24 (37%) were positive for biofilms inside the renal tubules (p=0.04). The intensity of leptospiral colonization in the renal tubules (OR: 1.00; 95% CI 1.05 – 1.1) and the type of occlusion pattern of the colonized renal tubules (OR: 3.46; 95% CI 1.20 – 9.98) were independently associated with the presence of *Leptospira* biofilm. Most of the rats with biofilms (n=15; 63%) were sub-adults (p=9.6e-05). We observed dense leptospiral colonization forming aggregates inside the renal tubules, with heavy deposition of amorphous extracellular matrix stained by ruthenium red between leptospires, compatible with biofilm morphology. Our work contributes with significant findings on the biology of pathogenic *Leptospira*, adding knowledge on leptospiral pathogenesis in animal reservoirs. The renal biofilm in *R. norvegicus* may impact the transmission cycle of leptospirosis and should be further investigated.

Keywords: Leptospirosis, host reservoir, renal carriage, immunohistochemistry, exopolymeric matrix, epidemiology.

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