

Title: KIDNEY COLONIZATION PATTERNS AND BIOFILM FORMATION BY *LEPTOSPIRA INTERROGANS* IN RATS, THE MAIN RESERVOIR HOSTS OF LEPTOSPIRES

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Abstract:

Leptospira interrogans is the main etiologic agent of severe leptospirosis in humans and animals. Leptospire chronically colonize *Rattus norvegicus* kidneys, the main natural reservoir worldwide. Leptospire are capable of forming biofilms; but it remained unknown whether they produce this phenotype during infection in mammalian natural reservoirs. The objective of this study was to identify leptospiral biofilm and to analyze renal pathology of naturally infected *R. norvegicus*. During 2013-14, we captured 154 *R. norvegicus* from a hyperendemic urban setting in Salvador, Bahia. Rats were euthanized and kidneys were processed for immunofluorescence anti-*L. interrogans* (IF) to screen for leptospiral infection. Then, kidney serial sections were processed for: (1) immunohistochemistry anti-*L. interrogans* (IH) to evaluate renal colonization; (2) Alcian Blue (AB) stain to identify biofilm exopolysaccharides; (3) periodic acid-Schiff; (4) PAS Silver Methenamine; (5) hematoxylin-eosin (HE); and (6) Picrosirius Red. IH-positive renal tubules were considered as colonized tubules (CTs). The number of CTs was counted by light microscopy, as a quantitative measurement of infection. The distribution of CTs was characterized, if they were found isolated or agglomerated in the tissue. Leptospiral biofilm in the renal tubules was identified by the co-localization of a CT and the same tubule positive in AB staining. 99 (64,3%) animals were positive and 55 (35,7%) were negative by IF and IH tests. 60 IF/IH-positive animals and 12 IF/IH-negative animals were selected for further histotechnology. We observed a wide range of variation for the CT count between individuals: from one CT/section to 654 CTs/section. CTs were found agglomerated in 37 (61,7%) animals and isolated in 23 (38,3%). From 46 colonized animals subjected to co-localization analysis, 19 (41,3%) presented positive co-localization results. Histopathology revealed mild chronic interstitial nephritis in positive and negative animals, what was expected according to previous literature. An expressive percentage (41,3%) of natural rat reservoirs presented co-localization, indicating the presence of biofilm. Biofilms might function in this context as a virulence factor, by which leptospire attach to the renal epithelium, generate high-grade carriage and evade the immune system. Additionally, biofilms may contribute to the survival of leptospire in the environment after urine excretion and to disease transmission.

Keywords: leptospirosis, biofilm, *Rattus norvegicus* natural reservoir.

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