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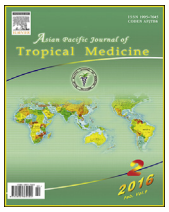

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First histopathological study in kidneys of rodents naturally infected with *Leptospira* pathogenic species from Yucatan, Mexico

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ABSTRACT

Objective: To report the renal histological lesions in synanthropic rodents, *Mus musculus* and *Rattus rattus*, naturally infected with *Leptospira* spp., captured in a rural community in Yucatan, Mexico.

Methods: Kidney samples of synanthropic rodents were collected from a rural community in Yucatan, Mexico. Polymerase chain reaction was used to detect *Leptospira* spp. infection. Tissue kidney was fixed in 10% buffered formalin, processed according to the usual techniques for paraffin inclusion, cut and stained with hematoxylin and eosin, and examined using a conventional electronic microscope.

Results: A total of 187 rodents were captured. Nine individuals (4.8%) were positive for *Leptospira* spp. in the molecular analysis. All renal lesions observed in the histopathological study had been reported previously for *Leptospira* spp. infection.

Conclusions: The histopathological lesions are present in the kidneys, plus the results of the polymerase chain reaction confirm that these rodents are true carriers of *Leptospira* spp.

1. Introduction

Leptospirosis is a zoonosis of worldwide importance caused by multi-host pathogens within the genus *Leptospira*. Renal carriage is the key to the persistence and epidemiology of leptospirosis; *Leptospira* spp. colonizes the renal tubules of animal kidneys and are released into the environment with urine [1,2]. The infection may be transmitted to human beings and other animals by direct contact or indirect exposure to urine from mammalian hosts such as synanthropic rodents and farms, wild and domestic animals [3]. It is well known that rodents are the main reservoir for *Leptospira* because they are persistent renal carriers, but rarely develop symptoms and are not impaired by the infection of their kidneys [4,5].

The kidney is a primary target of *Leptospira* during both acute and chronic infection, where conditions in the renal tubules favor *Leptospira* survival [6,7]. After infection, damage produced by spirochetes depends on several variables, such as pathogenicity and virulence of the serovar to the host and stage of the disease [8]. Histopathological lesions caused by *Leptospira* in naturally infected rodent reservoirs have been poorly described around the world [3,9].

2. Materials and methods

The mice for this study were collected from the rural community of Molas, Yucatan, Mexico (20°40′N, 89°38′W). The predominant vegetation type was tropical low deciduous forest and the climate was warm sub-humid with summer rains (Aw0). The site was divided into four grids, imagining two perpendicular axes that crossed at its center. In each grid, ten households were selected for convenience and sampled for three consecutive

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Table 1
Description of H-E histopathological lesions in kidneys of Rr and Mm positives by PCR for *Leptospira* spp.

Rodent identification number	Finding by H-E staining
Rr-161	Cell hyperplasia, moderate interstitial edema and severe lymphoplasmocitary inflammatory infiltrate
Mm-314	Severe glomerular atrophy and mild multifocal lymphoplasmocitary inflammatory infiltrate
Rr-87	No apparent histological lesions
Mm-223	Cell hyperplasia, mild interstitial edema, hydropic degeneration and mild focal lymphoplasmocitary inflammatory infiltrate
Rr-334	Hydropic degeneration, vacuolar degeneration, severe diffuse tubular necrosis and mild focal lymphoplasmocitary inflammatory infiltrate
Mm-117B	Mild multifocal lymphoplasmocitary inflammatory infiltrate
Rr-204B	No apparent histological lesions
Rr-54	Moderate multifocal lymphoplasmocitary inflammatory infiltrate and mild multifocal tubular hyperplasia
Rr-415	Moderate to diffuse tubular degeneration, severe focal necrosis, severe edema diffuse, glomerular atrophy and severe multifocal lymphoplasmocitary inflammatory infiltrate

nights each month. The sampling period was from October 2011 to March 2012. Twelve Sherman traps (7.5 cm × 23 cm × 9 cm, HB Sherman Traps Inc®, Tallahassee, Florida, USA) were set in each household. Traps were baited with oatmeal and vanilla flavoring, set in the morning and examined the following morning.

Capture, management and euthanasia of the rodents was conducted in compliance with the specifications of the American Society of Mammalogists. The captured rodents were transferred to the Zoology Laboratory of the Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán. After being anaesthetized with ether, the rodents were euthanized by cervical dislocation. Autopsies were conducted in order to collect both kidneys. One kidney was preserved to −70 °C until it was used in the Polymerase chain reaction (PCR) test, and the other was fixed in 10% buffered formalin and processed according to the usual techniques for paraffin inclusion. Next, 3 μm paraffin sections were cut and stained with hematoxylin and eosin (H-E) and examined using a conventional electric microscope with the lens 10×, 40× and 100×. The lesions were classified according to severity levels as mild (<30% affected), moderate (30%–50%) and severe (>50%). For the identification of *Leptospira* DNA in rodents, the kidneys preserved −70 °C were processed according the method established by Torres-Castro *et al*. [10].

3. Results

A total of 187 rodents were captured: 57 (30.48%) *Rattus rattus* (Rr) and 130 (69.51%) *Mus musculus* (Mm). All rodents appeared healthy under external physical examination. Nine (9/187, 4.81%) individuals were positive for *Leptospira* spp. in the PCR: seven Rr and two Mm. The H-E histopathological findings of the positive rodents to *Leptospira* spp. by PCR were described in the Table 1.

4. Discussion

A broad range of morphological alterations were detected in the kidneys of rodents naturally infected with *Leptospira* spp., captured in a rural community of Yucatan, Mexico. In the persistent colonization cases as in reservoir animals, *Leptospira* spp. cause systemic infection but are subsequently cleared from all organs except the renal tubules. The renal tubule is an immune-privileged site, a feature that may contribute to high-grade persistence of the pathogen [11].

The lesions observed in the kidneys of these rodents have been reported previously for *Leptospira* spp. infection [4]. The most common lesion we detected was the lymphoplasmacytic inflammatory infiltrate, which has been reported by Agudelo-Florez *et al*. [9], Athanazio *et al*. [12] and Monahan *et al*. [6]. The inflammatory infiltrate is a primary lesion during acute renal injury in leptospirosis and can be caused by direct damage to host tissue by *Leptospira* or the presence of leptospiral antigen, initiating a renal immune response [13]. However, this lesion cannot be attributed solely to *Leptospira* spp. since these rodents could also be exposed to other unknown environmental infectious agents that could cause renal lesions [9].

Other present lesions like edema and cell hyperplasia are also reported by others authors like Tucunduva de Faria *et al*. [3]. Also, tubular necrosis, have been reported in kidneys from human beings affected with *Leptospira* spp. infection [14,15], slaughtered pigs [16], and experimentally infected hamsters [17]; therefore our findings are most likely to be caused by the spirochete present in the kidneys of reservoir rodents. The histopathological lesions present in the kidneys of the rodents, plus the positive results of the PCR, confirm that these rodents are true carriers of *Leptospira* spp., and this make it a likely source of infection for the people in the study site.

This is the first study to describe and report the renal histological lesions in synanthropic rodents, *Mm* and *Rr* naturally infected with *Leptospira* spp, captured in a rural community in Yucatan, Mexico.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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