

Short Communication

Detection of antibodies against *Leptospira* spp in free-living marsupials caught in the Eastern Amazon

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Abstract

Introduction: Serological surveys are important to assess the health status of wild animals. In this study, antibodies against *Leptospira* spp, causal agents of leptospirosis, were detected in free-living marsupials in the State of Pará, Brazil. **Methods:** Nineteen blood samples collected from marsupials in the municipalities of Peixe-Boi, Viseu, and Castanhal were subjected to microscopic agglutination tests. **Results:** In total, 36.8% (7/19) of samples were positive, and two exhibited coagglutination. The most frequent serovars were Icterohaemorrhagiae (60%; 3/5), Panama (20%; 1/5), and Nupezo (20%; 1/5). **Conclusions:** Anti-*Leptospira* spp antibodies currently circulate in free-living marsupials in Northeastern Pará.

Keywords: Didelphidae. Wild animals. Microscopic agglutination test.

Leptospirosis is a disease caused by several species of *Leptospira*, which are spirochetes belonging to the order Spirochaetales of the family Leptospiraceae, and include more than 250 pathogenic serovars. Under favorable conditions and in the presence of suitable hosts, these organisms can survive for weeks or months. They are common in convalescent animals, and asymptomatic carriers contribute to the maintenance of leptospirosis hotspots¹.

Globalization and associated processes, such as urban demographic growth, forest clearing for the advancement of agriculture and livestock, and industrialization, have reduced the boundaries between populations of humans and domestic animals and those of wild animals, facilitating the spread of infectious diseases².

Marsupials in the family Didelphidae have distinct anatomical and physiological characteristics and extensive behavioral diversity. They inhabit forests, marshes, and semi-arid areas, at high or low altitudes, as well as aquatic environments; additionally, they are able to survive in

fluctuating environments. They may become synanthropic or live in areas of fragmented vegetation³.

Ecological disturbances cause marsupials to invade urban areas in search of food and shelter. Accordingly, they are common in cities, with substantial litter, which favors the spread of zoonoses, such as leptospirosis⁴. Therefore, in the present study, anti-*Leptospira* antibodies were examined in free-living marsupials in the municipalities of Peixe-Boi (01°11'31" S, 47°18'44" W), Castanhal (07°20'53" S, 50°23'45" W), and Viseu (01°11'48" S, 46°08'24" W) within the metropolitan mesoregion of Belém and Northeast Pará, Brazil.

A total of 19 marsupials (**Table 1**) were collected using Sherman, Pitfall, and Tomahawk bait traps. Traps were separated by 10m and remained open for at least ten consecutive nights, with daily morning inspections. The captured animals were anesthetized with a combination of xylazine hydrochloride (0.1mg/kg) and ketamine hydrochloride (5mg/kg) injected intramuscularly. Blood was collected by venipuncture at the base of the tail or by intracardiac puncture using sterile needles (0.45mm × 13mm), placed in tubes without an anticoagulant, and sent to the Laboratory of Zoonoses and Public Health (Federal University of Pará). Then, the serum was removed,

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Received 9 June 2017

Accepted 28 November 2017

TABLE 1: Distribution of captured marsupial species.

Species	Number
<i>Didelphis marsupialis</i>	10
<i>Caluromys</i> sp.	1
<i>Micoureus</i> cf. <i>demerarae</i>	1
<i>Philander opossum</i>	1
<i>Marmosa murina</i>	5
<i>Metachirus</i> cf. <i>nudicaudatus</i>	1
Total	19

transferred to 1.5-ml microtubes, and stored at -22°C until the serological test.

Antibodies were detected using the microscopic agglutination test (MAT) with antigens from 31 serovars of *Leptospira* spp Serovars Australis, Bratislava, Autmnalis, Butembo, Castellonis, Bataviae, Canicola, Whitcombi, Cynopteri, Djasiman, Sentot, Gryppotyphosa, Hebdomadis, Copenhageni, Icterohaemorrhagiae, Javanica, Panamá, Pomona, Pyrogenes, Hardjo, Wolffii, Shermani, Tarassovi, Andamana, Patoc, Guaricura (isolated at USP-São Paulo) Nupezo, Hardjo miniswajizak, Hardjo prajitno, Hardjo CTG, and Hardjo bovis were used as references.

Serum exhibiting an agglutination reaction equal to or greater than 50% on the 1:50 dilution were considered

positive. The cutoff point was a dilution of 1:100⁵. The most probable serovar was defined as the serovar with the highest titer and frequency. Samples that presented equal titers for two or more serovars were excluded from the analysis and considered only as reagents for *Leptospira* spp⁶.

Of the 19 samples collected, 36.8% (7/19) were positive for *Leptospira* spp according to the MAT. Among these, five samples were from the municipality of Peixe-Boi, and two were from the municipality of Viseu. Co-agglutination, i.e., reaction to more than one serovar, was observed in two samples; therefore, these were not considered in the analysis of the most frequent serovars. The most frequent serovars were Icterohaemorrhagiae, representing 60% (3/5) of positive samples, followed by Panama and Nupezo, each at 20% (1/5). The titers ranged from 100 to 400, and 100 and 200 were the most frequent titers (**Table 2**).

To our knowledge, this is the first report of the Icterohaemorrhagiae serovar in marsupials in the Brazilian Amazon. The serovar is considered pathogenic for various wild and domestic animals, as well as humans. We cannot confirm that the marsupials serve as a source of *Leptospira* infection, since the immune response in these animals is unknown. However, environmental disturbances can increase the proximity of these animals to urban areas and may promote the spread of pathogenic *Leptospira* to humans and domestic animals⁷.

In this study, one sample was reactive to the Panama serovar, with a titer of 200. Similarly, Paixão⁸ detected this serovar in free-living rodents at a conservation center of wild fauna in Ilha Solteira, SP. As rodents move freely in the forest in search of food, they acquire *Leptospira* and transmit

TABLE 2: Characterization of serovars in marsupial samples according to locality, frequency, and antibody titers.

Localities	Species	Serovars		
		Icterohaemorrhagiae	Panama	Nupezo
		titers		
Peixe-Boi	<i>Didelphis marsupialis</i>	100	–	–
Peixe-Boi	<i>Didelphis marsupialis</i>	200	–	–
Peixe-Boi	<i>Caluromys</i> sp.	100	–	–
Peixe-Boi	<i>Didelphis marsupialis</i>	–	–	400
Viseu	<i>Marmosa murina</i>	–	200	–
Total	5	3	1	1

it indirectly to animals of different species. This may explain why we found the Panama serovar in marsupials, since they live in the same habitat as several rodent species.

The Nupezo serovar (isolated from canine urine), belonging to the Canicola serogroup, was also identified in this study. Considering that marsupials are synanthropic and forage near human residences, it is possible that these wild animals had contact with domestic animals or environments containing the agent. A previous study of zoo animals involved 22 serovars and identified two samples that were reactive to the Patoc serovar⁹. However, we tested for 31 serovars in this study. The variation in the number and type of tested serovars may explain the differences in results. By including more serovars, we were able to obtain a more comprehensive evaluation of *Leptospira* circulation in the region.

In a study of domestic and wild animals at the university campus of *Faculdade de Ciências Agrárias e Veterinárias (FCAV), Universidade Estadual Paulista Julio de Mesquita Filho (UNESP), Jaboticabal*¹⁰, the detection rate (11/25; 44%) was higher than that in the present study. They examined 25 opossums (*Didelphis albiventris*) housed with horses, pigs, and dairy cattle and observed a higher frequency of reactive horses, pigs, and cattle during the period in which there was a high prevalence of reactive opossums, reinforcing the hypothesis that marsupials may be important reservoirs of *Leptospira* spp.

Lower detection rates have been observed for opossums (*Didelphis virginiana*) collected in peridomestic areas of Yucatán, Mexico¹¹, with a seropositivity of 4.9% (4/81) and a high prevalence of Pomona and Wolffi serovars. However, only 10 strains were considered, which may explain the low detection rate. Furthermore, a lack of knowledge of the circulating serovars in Mexico and Brazil may result in underestimates of seropositivity.

In a study of a farm area in the southern area of Lake Maracaibo, Mérida, Venezuela¹², serological tests were performed on small mammals (37 rodents and 2 marsupials) caught in pastures. No seroreactivity for *Leptospira* spp was detected, despite a high seropositivity rate in cattle in the area. Although we did not examine the seroprevalence in domestic animals in the study areas, the potential that they serve as a source of infection for wild animals cannot be ruled out.

We conclude that anti-*Leptospira* spp antibodies are currently circulating in free-living marsupials in the municipalities of Peixe-Boi and Viseu, Pará, suggesting that animals in these areas may be important sources of *Leptospira* infection.

Ethical considerations

The manipulation of wild animals followed the recommendations of the National Council for Control of Animal Experimentation (CONCEA), with authorization from the Ethics Committee on the Use of Animals of the Evandro Chagas Institute under protocol number 028/2014. Authorization for the collection of biological samples was obtained from the Biodiversity Information and Authorization System (SISBIO), under protocol number 37174-1.

Acknowledgements

The authors are grateful to the Laboratory of Mastozoology of the *Universidade Federal do Pará* and Evandro Chagas Institute for help in the capture of animals and collection of biological samples.

Conflict of interest

The authors declare that there is no conflict of interest.

Financial support

Institutional Scholarship Program of Scientific Initiation of the *Federal University of Pará* (PIBIC/UFPA) and *Projeto Pró-Amazônia* of the *Federal University of Pará*.

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