

The Contribution of Bats to Leptospirosis Transmission in São Paulo City, Brazil

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Abstract. The biodiversity of potential leptospiral reservoir hosts is lower in urban than in rural environments. Previous data indicate the potential for bats to act as carriers of *Leptospira* in regions such as the Amazon of South America and in Australia. Yet, little is known about the contribution of bats to leptospirosis in urban environments in South America. This study aimed to test the hypothesis that bats infected with *Leptospira* are sources of leptospirosis transmission to humans in São Paulo City, Brazil. Six of 343 bats caught in different districts within the city of Sao Paulo (182 insectivorous, 161 frugivorous or nectarivorous) were polymerase chain reaction (PCR) positive for pathogenic *Leptospira*; no seropositive bats were found. That few renal carriers of *Leptospira* were found in the city of Sao Paulo suggests that bats are not important in the transmission of leptospirosis to humans in this, and possibly other urban settings.

INTRODUCTION

Leptospirosis is a globally important zoonotic disease caused by pathogenic spirochetes of the genus *Leptospira*.¹ The highest number of human leptospirosis cases is found in the most populous city of Brazil, São Paulo. The annual incidence and case fatality in the city was 2.4, 2.7, and 1.7 per 100,000 inhabitants and 15%, 11%, and 18%, respectively from 2004 to 2006.² Severe leptospirosis has become a major urban health problem in São Paulo City and other regions due to people living in slums with lack of sanitation. Poor urban settings present potentially ideal ecological conditions for the transmission of leptospirosis.^{3,4} The source of leptospiral infection to humans is typically direct or indirect contact with the urine of infected animals. In most urban environments endemic for leptospirosis, including São Paulo City, the major sources of infection are urban rodents and sometimes dogs.³⁻⁵

One potential source of leptospiral infection is bats. Bats are known to carry some human pathogens, including *Leptospira*,⁶⁻⁹ as well as rabies¹⁰ and histoplasmosis.¹¹ Recent interest has emerged for investigating the potential role of bats as a source of infection of humans by pathogenic leptospires in forested areas such as the Amazon region.^{6,7} About 1200 species of bats have been described in the world, of which 167 are found in Brazil.¹² Bats present in São Paulo City are primarily insectivorous species¹³ that commonly feed on insects that concentrate around public lighting.¹⁴ The increased number of bats in some urban areas is at least in part explained by such anthropogenic changes in bat habitats. Landscape changes that facilitate bat migration include urban and suburban housing developments for expanding human populations, which alter roosting and foraging habitat for bats. Urban ecosystems present food and shelter favoring insectivorous bats. These facts associated with the absence of predators, have contributed to some species of bats using various building types as shelter, thus increasing the probability of contact of bats with people and domesticated animals.¹² Although insectivorous bats may control night-flying insect populations and frugivorous and nectarivorous bats play an important ecological role as pol-

linators and seed dispersal agents, people frequently complain about their presence, because of their noise, and the smell of their excrement.

Pathogenic *Leptospira* have been isolated from 22 species of bats.^{8,14} In the Peruvian Amazon region, a polymerase chain reaction (PCR)-based survey identified the presence of pathogenic *Leptospira* in 7 of 20 (35%) bats.⁶ Another larger survey based on PCR and culture of *Leptospira* showed a proportion of 23/589 (3.4%) positivity in Peruvian Amazonian bats. Phylogenetic analysis of 16S ribosomal sequences amplified by PCR reactions confirmed a diverse group of species present in these animals.⁷

The role of bats in leptospirosis transmission to humans in the urban environment in endemic areas has not previously been explored. The present study aimed to test the hypothesis that bats in the city of São Paulo, Brazil might contribute to *Leptospira* transmission to humans.

MATERIAL AND METHODS

This study was performed from January to November 2008. Three hundred and forty-three bats were trapped by mist netting or hand net in the city of São Paulo, Brazil, as part of the official Rabies Control Program. Also, bats were trapped in response to citizen complaints. São Paulo is the main economic center of Brazil with 10,886,518 inhabitants living in one area that is predominantly urban with 11,523 km² divided into 31 administrative areas. In São Paulo City bats were captured in buildings, houses, community squares, and in fruit trees.

Animals were identified according to Vizotto and Taddei.¹³ The Scientific Committee from Zoonosis Control Center of São Paulo City approved this work. The capture of bats was authorized by the Brazilian institution responsible for wild animal care (*Instituto Brasileiro do Meio Ambiente*).

Trapped bats were transported to the study laboratory where they were anesthetized with intramuscular ketamine; blood was collected by cardiac puncture. The animals were euthanized with CO₂ and transferred to a class II laminar flow hood for kidney removal under aseptic conditions. Bat sera were screened using the microscopic agglutination testing (MAT) with 20 leptospiral serovars comprising the panel¹⁴ (Table 1).

For molecular detection and speciation of bat kidney *Leptospira*, published methods were used. Total genomic DNA was extracted from the kidney as described.¹⁵ PCR

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TABLE 1
Leptospiral serovars used in microscopic agglutination testing of bats

Serogroup	Serovars	Reference strain
Icterohaemorrhagiae	Icterohaemorrhagiae	RGA
Canicola	Canicola	Hond Utrecht IV
Pyrogenes	Pyrogenes	Salinem
Australis	Australis	Ballico
Shermani	Shermani	1342 K
Andamana	Andamana	CH 11
Tarassovi	Tarassovi	Perepelitsin
Autumnalis	Autumnalis	Akiyami A
Djasiman	Djasiman	Djasiman
Javanica	Javanica	Veldrat Batavia 46
Ballum	Castellonis	Castellön 3
Panama	Panama	CZ 214
Sejroe	Wolffi	3705
Pomona	Pomona	Pomona
Icterohaemorrhagiae	Copenhageni	M 20
GrippotypHosa	GrippotypHosa	Moskva V
Autumnalis	Butembo	Butembo
Bataviae	Brasiliensis	An 776
Cynopteri	Cynopteri	3522 C
Sejroe	Hardjo	Hardjoprajitno

amplification was performed as described.¹⁶ Specific primers targeting the 16S rRNA region from *Leptospira interrogans* serovar canicola were used to amplify a 331 bp fragment: Lep 1:5' GGC GGC GCG TCT TAA ACA TG 3' e Lep 2:3' TTC CCC CCA TTG AGC AAG ATT. Amplification was carried out under the following conditions: denaturation at 95°C for 5 minutes, 29 cycles at 94°C for 1 minute, at 63°C for 1 minute and 30 seconds and at 72°C for 2 minutes with final extension at 72°C for 10 minutes. Simultaneous DNA extracts of *Leptospira interrogans* serovar Copenhageni and water were used as positive and negative controls, respectively. PCR products were visualized by ethidium bromide staining after 1.5% agarose gel electrophoresis.

PCR products were sequenced with an ABI3130 automated sequencer using ABI Big-Dye (Applied Biosystems/Hitachi, Foster City, CA) and the Lep1 primer described above. The nucleotide sequences for PCR products were aligned and compared with GenBank reference sequences to confirm their identification as *Leptospira* spp.

RESULTS

A total of 343 bats were captured from different districts within the city of Sao Paulo, of which 182 were insectivores of the family Molossidae, 131 were frugivores or nectarivores of the family Phyllostomidae, 29 were insectivores of the family Vespertilionidae, and 1 was an insectivore of the family Emballonuridae. Insectivorous bats constituted 62% of the sample, whereas frugivorous and nectarivorous bats represented 38%. A slight majority of bats were female (51%). Adults constituted the majority of the sample (81.6%); young, sub adults, and offspring represented 16%, 2%, and 1%, respectively.

Twenty-one species of bats were identified. The number of captured specimens are as follows: eight Molossidae: *Molossus molossus* (116), *Tadarida brasiliensis* (28), *Nyctinomops macrotis* (12), *Eumops glaucinus* (9), *Nyctinomops laticaudatus* (6), *Molossus rufus* (5), *Eumops* sp. (2), *E. auripendulus* (2), *Molossops neglectus* (1), and *Molossus* sp. (1); seven Phyllostomidae: *Glossophaga soricina* (82), *Platyrrhinus lineatus* (31), *Desmodus rotundus* (5), *Artibeus lituratus* (7), *A. fimbriatus* (2), *Sturnira lilium* (3), and *Micronycteris megalotis* (1); five

Vespertilionidae: *Myotis nigricans* (16), *Eptesicus furinalis* (4), *E. diminutus* (4), *Histiotus velatus* (2), *Lasiurus blossevillii* (2), and *Myotis* sp. (1) and one Emballonuridae: *Diclidurus scutatus*.

Evidence of leptospiral infection of kidney was found in six of 343 (2%) bats. The expected product size of PCR bands was observed. Sequence analysis confirmed the identities of the amplicons as belonging to pathogenic *Leptospira* spp. The six positive bats were members of the Phyllostomidae; four were nectarivorous identified as *Glossophaga soricina* (three adult males and one adult female), and two were frugivorous identified as *Platyrrhinus lineatus* (adult, males). Two *G. soricina* were trapped in the basement of a building and two were captured after entering houses. *P. lineatus* were caught in fruit trees. Among the 82 specimens of the *G. soricina*, 73% were captured in basements, garages, ceilings, and also abandoned buildings, whereas 21% were captured after entering houses. In relation to *P. lineatus*, 31 specimens, 48% were collected in garages, hanging in interspaces in homes and buildings or entering houses, whereas 52% specimens were capture in fruit trees, including the two *Leptospira* positive ones. The positivity rate among the Phyllostomidae was 5%, among *G. soricina* was 4/82 (5%), and *P. lineatus* was 2/31 (6%). Serum samples were only obtained from 169 bats. microscopic agglutination test (MAT) was negative for all.

DISCUSSION

The main conclusion from this study is that in the city of São Paulo, Brazil, where human leptospirosis is common, bats are not frequently infected with *Leptospira*. This major endpoint was determined by nucleic acid amplification testing of DNA extracted from bat kidneys obtained from bats trapped in various districts of the city of São Paulo.

In this study we found a discrepancy between positive kidney PCR (consistent with the chronic leptospiruric phase of infection) and negative MAT (*Leptospira* and immunity). This finding is consistent with previous studies of other mammals where only about half of animals with positive kidney culture or PCR were seropositive.^{17,18}

Based on the low frequency of pathogenic leptospiral infection in the Sao Paulo bats, we suggest that bats do not seem to be an important source of leptospirosis transmission to humans in this region. It is probable that *Leptospira* spp. harbored by these animals represents a crossing over from natural reservoir animals to the dead end host, bats. Given the burden of leptospirosis in Sao Paulo—comparable with highly affected regions around the world, and significantly more than in the United States and Europe—it is unlikely that bats represent major reservoirs of leptospirosis transmission in urban environments in general.

The present study extends previous studies performed by our group in the cities of Sao Paulo and Jundiá (State of São Paulo), in which 71 bats were captured and *Leptospira* infection was analyzed by MAT. In these studies, there were three positive results (2.8%): *M. molossus* (Sao Paulo City) and *Molossus ater* (Jundiá City) currently named *M. rufus* (Santos and others, unpublished data).

In this study, the bat species most commonly trapped was *M. molossus* (116), demonstrating a higher degree of adaptation of this species to the urban environment. *M. molossus* prefers buildings and residences as shelter, thus potentially exposing humans to their urine in this environment. Other bat species

favoring human environments in urban areas with facilities, but with less human use such as basements were *G. soricina* (82) and *P. lineatus* (31); these would have less potential for infecting humans but more possibility of interacting with rats.

Among frugivorous bats (131), 6.1% were positive. This observation is consistent with bat species-specific behavior and proclivity for specific types of shelters. *G. soricina* and *P. lineatus* in urban areas prefer to inhabit building types with little human activity. Matthias and others⁷ reported evidence of leptospiral infection in 2/22 *G. soricina*, whereas Bunnell and others⁶ reported 1/4 of the bats of the genus *Platyrrhinus* were leptospiral carriers. The frequency of *Leptospira*-positive bats found in this study was similar to that described previously.⁷

This paper reports the largest survey to date of urban bats in South America for chronic renal infection by *Leptospira*. Few such carriers of *Leptospira* in the city of São Paulo were found. Although the role of bats in leptospiral transmission to humans was not directly assessed in this study for humans, data from this study suggest that bats are not a key component of the epidemiology of leptospirosis in humans in this setting.

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