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Short Communication

Test for *Borrelia spp.* in bats in an urban area in the South of Brazil

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Abstract

Introduction: We investigated the occurrence of relapsing fever (RF) causing *Borrelia* genus spirochetes in synanthropic bats from the municipality of Maringá, Paraná, South of Brazil. **Methods:** Tissue samples from the wings of bats were collected monthly from April 2013 to February 2014 and extracted DNA was used to evaluate the presence of RF causing *Borrelia* spp. **Results:** All bat tissues tested negative for RF causing *Borrelia* spp. **Conclusions:** *Borrelia* spp. do not occur in chiropterans from Maringá.

Keywords: Urban bats. Chiropterans. Relapsing fever. *Borrelia* spp.

Numerous bat species currently live in urban environments; these animals, thus, participate in the epidemiological chain of several zoonoses, such as those caused by viruses, bacteria, fungi, protozoa, and ectoparasites[1].

Among the diseases caused by bacteria, borreliosis is an infectious illness caused by spirochetes from the genus *Borrelia*, transmitted by ticks to animals and/or humans[2]. Relapsing fever (RF), a type of borreliosis, is characterized by a severe disease that progresses rapidly with recurring cycles of fever. RF occurs worldwide and is endemic in 118 countries[3].

Studies about *Borrelia* spp. are still scarce in Brazil and are limited to the Lyme disease *Borrelia* group. There is also a lack of Brazilian studies on bats, which live in great proximity to humans[4] and have already been described as RF reservoirs in the United States, United Kingdom, and France[5-6]. Therefore, the present study investigated the occurrence of spirochetes of different RF-causing *Borrelia* ssp. in the DNA from bat wing tissue of synanthropic bats from the municipality of Maringá, State of Paraná, South of Brazil, using PCR employing specific primers.

The study was conducted in urban Maringá (location: 23° 25′ 30″ S, 51° 56′ 20″ W) from April 2013 to February 2014. Sampling was performed on four nights in each month, two in residential homes and two in areas of the native forest (**Figure 1**). Sampling on private properties was performed through voluntary scheduling through the Bats Project, run by the State University of Maringá. The method of sampling within homes varied depending on the structure of the house and involved nylon nets, fishing nets, and the direct capture of bats by entering the ceiling.

The forest areas in which sampling was performed included the Municipal Park of Ingá, Forest Garden Doutor Luiz Teixeira Mendes, and the Municipal Forest Park from the Palmeiras and Forest Park Pioneiros. Sampling within the forest started at sunset using eight nylon nets of 9 m length and 3 m height and was performed for 12 h per night.

Bats were captured and handled using leather gloves for data was collectioned such as sex, reproductive status and species. The animals were released after data collection. Bat tissue samples were collected through the non-lethal method described by Worthington Wilmer & Barratt[7]. After local asepsis, a piece of the bat-wing membrane (patagium) was removed with the aid of a disposable biopsy punch, being careful to not strike any blood vessels. The biopsy punch diameter used in this research was 1 mm (*Disposable Biopsy Punch* - Miltex®, Austin, EUA). At the end of the procedure, the bats were marked with an animal tattoer and released. The tissue samples were stored in numbered containers with 1 ml ethanol and analyzed at the Lyme Disease Lab at the Texas A&M University.

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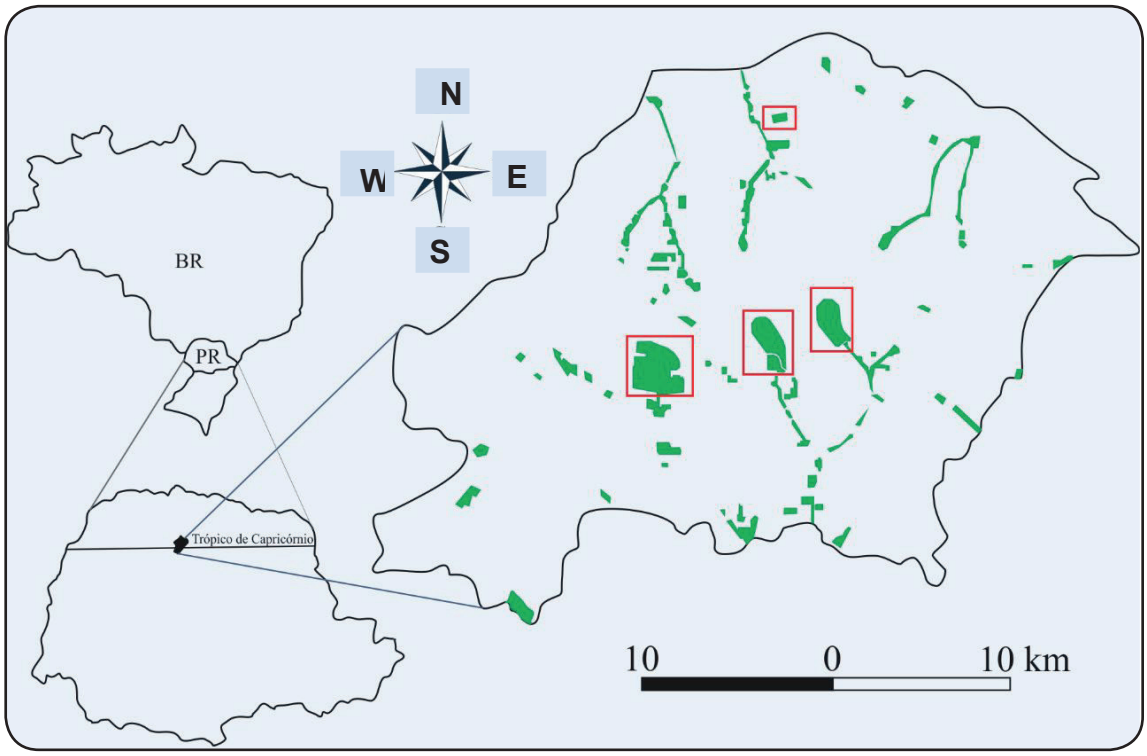

FIGURE 1: Area of study. The green areas represent the Municipality of Maringá, and the red boxes denote the parks sampled in the present research.

For DNA extraction from the tissue samples (1-mm skin biopsies), 50.0 µL of collagenase (Sigma®) and 50.0 µL of proteinase K (concentration of 200 µg/mL; Sigma®) were added to each sample-containing tube. Samples were incubated for 4 h in a water bath set at 57°C with manual shaking every 30 min to aid tissue digestion. The digested samples were transferred to clean PCR tubes, where they were incubated with equal volumes of the extraction buffer provided in the ForensicGen®Tissue DNA extraction kit (ZyGEM®, Charlottesville, EUA) for 30 min at 75°C, followed by a 5 min incubation at 95°C to inactivate enzymes. This protocol was performed in an Eppendorf™ thermocycler. DNA quantification and quality were evaluated through spectrophotometric measurement (NanoDrop®). This protocol was standardized to ensure the extraction of a sufficient amount of good quality DNA from small tissue biopsies. In this study, we considered a DNA quantity of 5 ng/µl satisfactory, to verify the occurrence of *Borrelia* ssp., considering the small size of the samples. Thirty nanograms of DNA per sample was used to carry out the PCR reaction.

PCR was performed using specific primers for detecting spirochetes of *Borrelia* spp., causing RF (5'ATGCTAGAAACTGCATGA-3' and 5'TCGTCTGAGTCCCCATCT-3') and amplifying a fragment of the 16S rRNA. These primers have been previously used successfully to detect the presence of DNA from *Borrelia crocidurae, Borrelia duttonii, Borrelia recurrentis, Borrelia hispanica, Borrelia coriaceae, Borrelia lonestari, Borrelia miyamotoi, Borrelia parkeri, Borrelia turicatae, Borrelia hermsii,* and *Borrelia anserine*[8]. As a positive control, we used DNA from a *B. turicatae* culture maintained in the Lyme Disease laboratory at Texas A&M University. The PCR products were separated via 1% agarose gel electrophoresis (Biolin® Scientific, Linthicum Heights, EUA) in TAE buffer, with the addition of 20 µl of ethidium bromide (10 mg/mL; Invitrogen®, Carlsbad, EUA) and using a molecular marker of 1 Kb (New England Biolabs, Rowley, EUA).

Licenses included the Permanent License to Collect Zoological Material from the Chico Mendes Institute of Biodiversity Conservation (ICMBio) number: 17869-2 (date of emission: 05/02/2009), certification of the Committee of Ethical Conduct in the Use of Animals in Experiments (CEAE) from the Internal Commission of Biosafety (CIBIO) of the State University of Maringá, and the Institutional Biosafety Committee permit 2013-039 from Texas A&M University. All sampling and laboratory procedures were performed in accordance with the ethical principles of the consulted entities.

A total of 337 bat-wing tissue samples were analyzed through PCR utilizing primers for the amplification of a fragment corresponding to the 16sRNA gene found in RF caused by bacteria from the *Borrelia* genus. Seven of these samples were from recaptured bats identified through tattoos, resulting in 330 individual bats of different species (**Table 1**). All the PCR results were negative for the 16SrRNA RF-causing spirochetes from the *Borrelia* genus. This indicates that chiropterans from the Maringá region are not carriers of any RF causing *Borrelia* ssp. described to date. The *Borrelia tunicatae* positive control showed positivity in all tests (**Figure 2**).

Borrelia spp. have been described in several animals in Brazil, such as dogs, cattle, and poultry; however, no study

TABLE 1: List of bats species captured.

Taxon	N	%	Provenience
Family Phyllostomidae			
Phyllostomus hastatus (Pallas, 1767)	4	1,2	Forest fragment
Platyrrhinus lineatus (E. Geoffroy, 1810)	7	2,1	Forest fragment (4) and Residence (3)
Sturnira lilium (E. Geoffroy, 1810)	52	15,8	Forest fragment
Sturnira tildae (de la Torre, 1959)	1	0,3	Forest fragment
Subfamily Carollinae			
Carollia perspicillata (Linnaeus, 1758)	5	1,5	Forest fragment
Subfamily Stenodermatinae			
Artibeus lituratus (Olfers, 1818)	142	43,0	Forest fragment
Artibeus obscurus (Schinz, 1821)	11	3,4	Forest fragment
Artibeus planirostris (Spix, 1823)	22	6,6	Forest fragment
Artibeus fimbriatus (Gray, 1838)	20	6,0	Forest fragment
Family Noctilionidae			
Noctilio leporinus (Linnaeus, 1758)	3	0,9	Forest fragment
Noctilio albiventris (Desmarest, 1818)	1	0,3	Forest fragment
Family Vespertillionidae			
Myotis nigricans (Schinz, 1821)	1	0,3	Forest fragment
Family Molossidae			
Molossus rufus (E. Geoffroy, 1805)	42	12,8	Residence
Molossus molossus (Pallas, 1766)	19	5,8	Residence
Total	**330**	**100**	

N: number of specimens captured**; %:** relative percentage of animals captured during the sampling period.

with chiropterans has been conducted in the country. Therefore, the present study represents the first evaluation of the potential occurrence of RF borreliosis in bats in Brazil.

A study performed in Jataizinho, Paraná, a municipality that is only 102 km from Maringá, found the presence of bacteria with 99.9% similarity to the B31 *Borrelia burgdorferi sensu stricto* in *Dermacentor nitens* ticks, acting as the first report suggesting the occurrence of these bacteria in Brazil[9]. Montadon et al. in 2004[13] found antibodies against *B. burgdorferi* in marsupials, horses, rodents, and dogs in two small towns in the state of Minas Gerais, providing evidence of infection with *Borrelia spp*. in both domestic and wild animals.

Studies in the United States and in Europe found RF-causing *Borrelia* bacteria in bats and their ticks[5-6]. Gill et al. in 2008[5] reported a partial molecular characterization of a new RF-causing spirochete found in a *Carios kelleyi* tick collected from the basement of a house inhabited by bats in Iowa, United States. In addition, Socolovschi et al. in 2010[6] collected *Argas vespertiolionis* ticks from a basement where bats used to live that was transformed into a dormitory. The ticks were found to be infected with *Borrelia sp*. CPB1, the same RF-causing agent found by Evans et al. in 2009[14]. In this study, CPB1 was found to be pathogenic for bats too. Moreover, recently, of *Borrelia garinii* DNA was found in a tick parasitizing a *Myotis daubentonii in a cave* in Poland[15].

These concerns, in addition to the finding of *B. burgdorferi* in the present study area[9] and the predisposition of bats to participate in several zoonotic cycles because of their lifestyle[1], were the reasons motivating our research. We were interested in monitoring the presence of RF *Borrelia* species in bats to determine whether or not there are favorable conditions for pathogenic RF species.

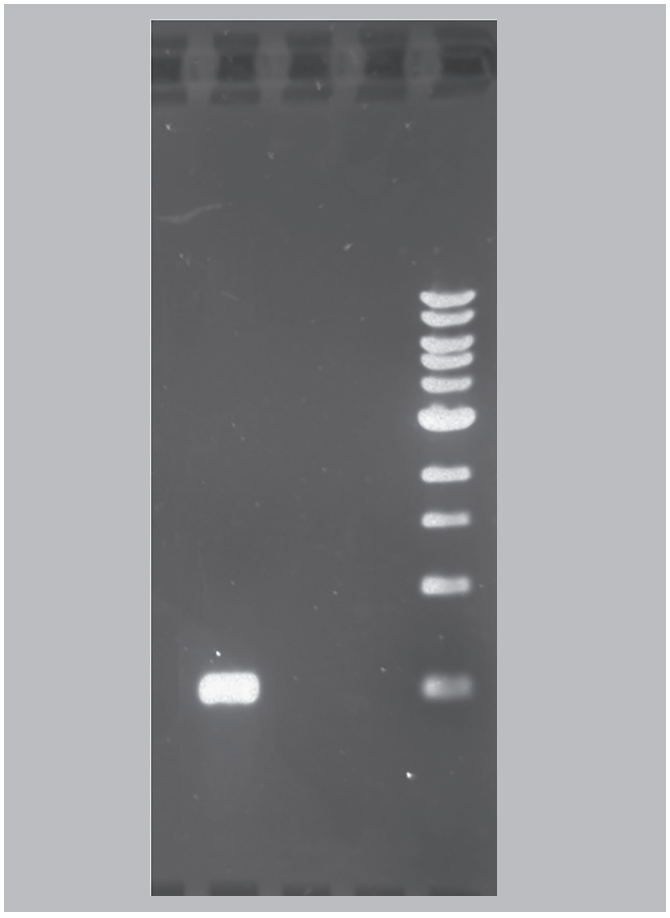

FIGURE 2: Agarose gel electrophoresis of the positive control. Example of the result obtained in this study. Lines MK: molecular marker of 1 Kb (New England Biolabs), 1: negative control; 2: negative control; 3: positive control *Borrelia turicatae* grown under laboratory conditions, in the Lyme Disease Laboratory at Texas A&M University, shown in the left.

The negative result for the presence of RF-causing *Borrelia* among bats found in the city of Maringá, both in the native forest fragments and residences, opposes those of research conducted in Europe and the United States, where these pathogens have been found in bats[14] or their ticks[5-6,15]. This can be attributed to the differences in temperature, habitat, and type of ticks that utilize the bats as hosts. Nevertheless, variations occur in *Borrelia* species, genospecies, and strains as well as in the vectors and vector-pathogen interactions according to the physiogeographic region, resulting in different clinical, epidemiologic, and pathologic disease manifestations[10-12].

In conclusion, the absence of the investigated bacteria in bats in Maringá indicates that these are not infected with RF *Borrelia* sp. There are no reports of the sampled bat species being infected by *Borrelia* spp. in other studies, so we cannot conclude that the bacteria or the vector have not been introduced into this area yet, without knowing if the sampled bat species is a competent reservoir. Overall, our findings indicate that endemic RF is not a city health concern at this point. Because of the significant habitat fragmentation of the area of study and the adaptability of bats to manmade constructions, more studies employing other molecular techniques, sampling different habitats from the region studied, evaluating the bats from other areas where *Borrelia* spp. have been found, and examining ticks that normally feed on chiropterans in this area are recommended.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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