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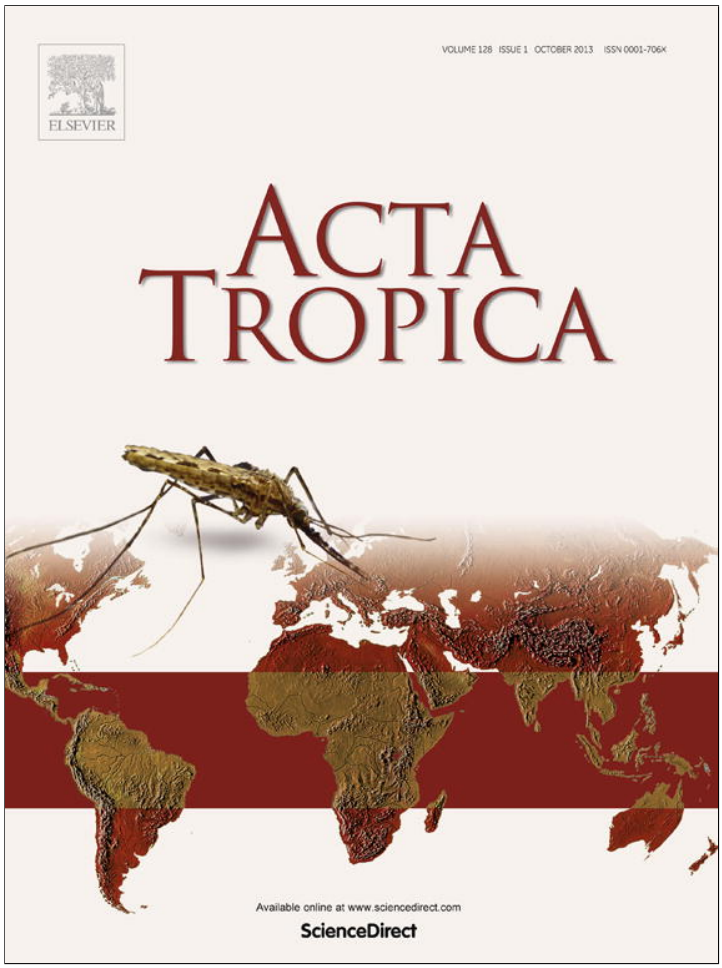

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First record of *Leishmania braziliensis* presence detected in bats, Mato Grosso do Sul, southwest Brazil

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ABSTRACT

Leishmaniasis, a zoonotic disease caused by parasites of the genus *Leishmania*, has expanded beyond its natural range and is becoming increasingly urban. Using PCR and PCR-RFLP, we detected *Leishmania* (*Viannia*) *braziliensis* in two bats (Chiroptera) in Mato Grosso do Sul, Brazil, an endemic area. This is the first record of *L.* (*V.*) *braziliensis* in bats. It is also the first record of any *Leishmania* sp. in bats in the state. The animals testing positive were found in both a rural site and an urban site. These results indicate the need for further research into the viability of *Leishmania* in bats and could potentially have implications for public health in Mato Grosso do Sul, given the large populations of urban bats, their mobility, and their ability to roost at close proximity to humans within residences and other buildings.

1. Introduction

Leishmaniasis is a "neglected tropical disease" (Ault, 2007; Maudlin et al., 2009) with several hundred thousand new cases reported per year worldwide (Alvar et al., 2012). The zoonosis is caused by parasites of the genus *Leishmania*, which are transmitted via sandflies (Diptera: Psychodidae: Phlebotominae). Although many carriers of the parasite have no symptoms, infections may result in lesions of the skin and mucosa (cutaneous or mucocutaneous form) or internal organ damage (visceral form), which is often fatal (Ministério da Saúde, 2010; WHO, 2010).

The disease is a serious public health issue in Brazil, which has the highest number of cases in the Americas (WHO, 2010). *Leishmania* (*Viannia*) *braziliensis*, which causes American cutaneous leishmaniasis (ACL), has been confirmed in all 26 Brazilian states. Approximately 25,000–35,000 infections are diagnosed per year throughout the country; in Mato Grosso do Sul, where the parasite is endemic, there are up to several hundred cases each year (Ministério da Saúde, 2010).

L. (*V.*) *braziliensis* has been detected in dogs, cats (Brandão-Filho et al., 2003; Schubach et al., 2004; Quaresma et al., 2011) and horses (Brandão-Filho et al., 2003). In some areas, there is no apparent correlation between infections in humans and domestic animals, pointing to the role of wild fauna reservoirs, possibly rodents or marsupials, in the maintenance and transmission of *Leishmania* sp. (Savani et al., 1999; Brandão-Filho et al., 2003; Quaresma et al., 2011).

The extent of *Leishmania* infection in bats remains unknown. In Central America the *Leishmania* vector *Lutzomyia vespertiolionis* prefers feeding on bats (Tesh et al., 1971; Christensen and Herrer, 1980) and in laboratory feeding tests, sandflies have been shown to consume bat blood (Lampo et al., 2000). They have also been observed in bat roosts (Tesh et al., 1971). *Leishmania chagasi* has been detected from a single specimen of *Carollia perspicillata* in

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Table 1
Species, capture location, and positive results for *Leishmania* in sampled bats.

Bat	Species	Location	Neighborhood	Result	Tissue type
1	*Carollia perspicillata*	Fazenda Piana	N/A	–	
2	*Glossophaga soricina*	Fazenda Piana	N/A	+	S, L
3	*Nyctinomops macrotis*	Campo Grande	Center	–	
4	*Molossus molossus*	Campo Grande	Center	+	B
5	*Glossophaga soricina*	Campo Grande	Arnaldo Figueiredo	–	

(+), positive; (–), negative; S, skin lesion; L, liver, B, blood; N/A, not applicable.

Venezuela (Lima et al., 2008). In São Paulo, 22 bats from eight species tested positive for *Leishmania amazonensis* using PCR or an indirect immunofluorescent antibody test and three bats of two species tested positive for *L. chagasi* using PCR (Savani et al., 2010).

The small number of studies on *Leishmania* in bats indicates a need for further research into the occurrence of the parasite in these mammals. The objective of this study was to investigate the presence of *Leishmania* sp. in bats captured in and around Campo Grande, Mato Grosso do Sul.

2. Materials and methods

2.1. Animals

Three bats were collected by the Center for the Control of Zoonoses (Centro de Controle de Zoonoses) within Campo Grande. Two of these were found in the Center district (20°27′S 54°36′W) and one in a peripheral neighborhood (20°28′S 54°33′W). Two other bats were collected during mist-net surveys conducted between April 2012 and November 2012 at Fazenda Piana, a forested, rural private reserve 40 km from Campo Grande (20°47′S 54°40′W) (Table 1). Bats were weighed and their species' were identified according to Vizotto and Taddei (1973) and Gregorin and Taddei (2002).

2.2. Collection of blood and tissue samples

The bats were anaesthetized with ketamine hydrochloride (dosage based on each animal's weight and volume) and then euthanized with CO_2.

Blood samples were obtained by cardiac puncture, placed in a tube with EDTA and stored at −20 °C. The liver and spleen were removed from all animals and imprinted on slides. Cutaneous lesions on the wing, ear, and nose, found on the two animals from Fazenda Piana, were also removed and imprinted on slides. Slides were fixed with methanol and then stained using the Instant-Prov kit (NewProv) following the manufacturer's instructions. They were examined for the presence of amastigotes under the microscope at 100× magnification.

2.3. DNA isolation

For the isolation of DNA, we used 400 μL of blood and the cutaneous lesion biopsies macerated in liquid nitrogen. To obtain DNA from the slides, 500 μL of SDS 20% was placed on each slide, which was then scraped with a sterile scalpel.

The following procedure was used to isolate DNA from each sample: 200 μL of lise buffer (NaCl 1 M; Tris-HCl 1 M pH 8.0; EDTA 0.5 M pH 8.0) was added to each sample, which was then vortexed and incubated at 65 °C for 5 min. 400 μL of chloroform was added to each tube. It was vortexed to completely homogenize the mixture and then centrifuged at 10,000 rpm. The liquid phase was transferred to a new 1.5 mL tube and 1 mL of 100% ethanol was added. The mixture was homogenized by inversion and centrifuged at 10,000 rpm for 5 min, after which the supernatant was removed. 1 mL of 70% ethanol was added, followed by 2 min of centrifuge at 10,000 rpm. The supernatant was then removed and this process was repeated twice more. The tubes were inverted in order to dry the sediment. The precipitate was resuspended with 100 μL of ultra-pure autoclaved water, incubated overnight at 4 °C and stored at −20 °C.

2.4. PCR and PCR–RFLP

The DNA target for PCR amplification was the ITS-1 region, following El Tai et al. (2000) with the primers LITSR: [5′-CTG GAT CAT TTT CCG ATG-3′] and L5.8S: [5′-TGA TAC CAC TTA TCG CAC TT-3′]. The mixture was incubated in a Bioer XP Cycler thermocycler.

PCR–RFLP was carried out according to Schönian et al. (2003). To confirm the *Leishmania* species, we used primers b_1 [5′-GGG GTT GGT GTA ATA TAG TGG-3] and b_2 [5′-CTA ATT GTG CAC GGG GAG G-3′] specific for *L.* (*V.*) *braziliensis* following Lima Junior et al. (2009). Each experiment included both negative and positive controls.

3. Results and discussion

The initial PCR with the primers LITSR and L5.8S indicated a positive result for *Leishmania* sp. in three samples from two individual bats: the blood sample from *Molossus molossus* and the cutaneous lesion and liver samples from *Glossophaga soricina* (Fig. 1, Table 1). The species was determined as *L. braziliensis* with RFLP for all three samples and confirmed by PCR using primers b_1 and b_2 (Fig. 2). No amastigotes were found in the slides.

Typically *L. braziliensis* initially causes dermal or mucosal lesions. It is also possible for infections that began as dermatrophic to become visceral (Ministério da Saúde, 2004), which may be the case in the bat whose liver sample tested positive. In addition,

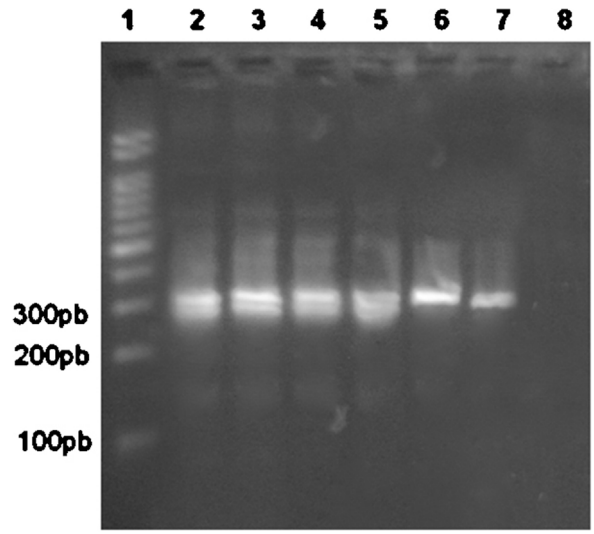

Fig. 1. Results of PCR for ITS-1. (1) 100pb marker; (2) skin, Bat 2; (3) liver, Bat 2; (4) blood, Bat 4; (5) *Leishmania* (*Viannia*) *braziliensi*; (6) *Leishmania* (*Leishmania*) *amazonensis*; (7) *Leishmania infantum*; (8) negative control.

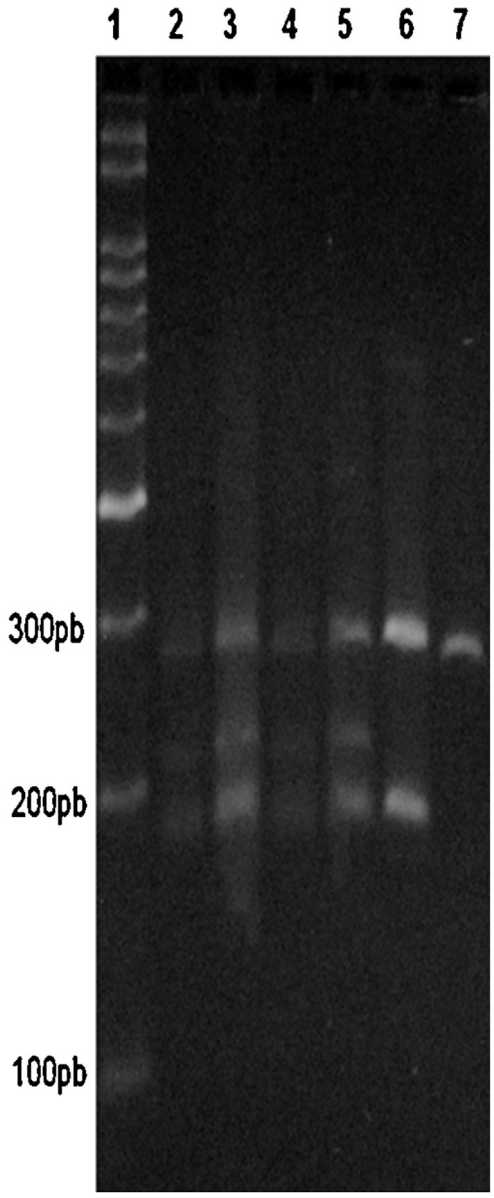

Fig. 2. Results of RFLP for ITS-1. (1) 100pb marker; (2) skin, Bat 2; (3) liver, Bat 2; (4) blood, Bat 4; (5) *Leishmania* (*Viannia*) *braziliensi*; (6) *Leishmania* (*Leishmania*) *amazonensis*; (7) *Leishmania infantum*.

the invasive capacity and virulence of *Leishmania* parasites may be highly variable (Ministério da Saúde, 2004).

This is the first record of the presence of *Leishmania* (*Viannia*) *braziliensis* detected in bats. In addition, it is the first record of any *Leishmania* sp. in bats in Mato Grosso do Sul. Previous studies have found *L. chagasi* and *L. amazonensis* in nine species, including *M. molossus* and *G. soricina* (Lima et al., 2008; Savani et al., 2010). Our results expand the number of known *Leishmania* parasites in bats.

In other studies only a small percentage of bats have tested positive for *Leishmania*, such as in São Paulo (3.66%) (Savani et al., 2010) and Venezuela (9.09%) (Lima et al., 2008) and the absence of *Leishmania* in 216 bats tested in French Guiana (Rotureau et al., 2006). Surprisingly, our findings yielded a higher percentage with two out of five bats (40%) testing positive, indicating the possibility of a potentially high prevalence.

In recent years, *Leishmania* has become increasingly urban, especially in Brazil, adapting to new, largely unknown hosts (WHO, 2010). The positive PCR reveals that bats could be a potential host for *Leishmania*. Our results indicate the need for further research examining the prevalence and viability of the parasites in bats, especially considering their ability not only to adapt to urban areas, but also to maintain relatively large colonies in residences and other buildings, putting them in close, frequent contact with humans and domestic animals (Bredt and Uieda, 1996; Avila-Flores and Fenton, 2005; Lima, 2008).

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