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Leishmania (*V.*) *braziliensis* infecting bats from Pantanal wetland, Brazil: First records for *Platyrrhinus lineatus* and *Artibeus planirostris*

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ABSTRACT

In the New World genus *Leishmania* parasites are etiological agents of neglected zoonoses known as leishmaniasis. Its epidemiology is very complex due to the participation of several species of sand fly vectors and mammalian hosts, and man is an accidental host. Control is very difficult because of the different epidemiological patterns of transmission observed. Studies about *Leishmania* spp. infection in bats are so scarce, which represents a large gap in knowledge about the role of these animals in the transmission cycle of these pathogens, especially when considering that Chiroptera is one of the most abundant and diverse orders among mammals. Leishmaniasis in Mato Grosso do Sul, Brazil are remarkably frequent, probably due to the abundance of its regional mastofauna. The recent record of *L. braziliensis* in bats from this state indicates the need to clarify the role of these mammals in the transmission cycle. In this study we evaluated the presence of *Leishmania* parasites in the skin of different species of bats, using PCR directed to *Leishmania* spp. kDNA for screening followed by PCR/RFLP analysis of the hsp70 gene for the identification of parasite species. *Leishmania* species identification was confirmed by PCR directed to the G6PD gene of *L. braziliensis*, followed by sequencing of the PCR product. Samples from 47 bats were processed, of which in three specimens (6.38%) was detected the presence of *Leishmania* sp. kDNA. PCR/RFLP and sequencing identified the species involved in the infection as *L. braziliensis* in all of them. This is the first report of *Leishmania braziliensis* in bats from Pantanal ecosystem and the first record of this species in *Platyrrhinus lineatus* and *Artibeus planirostris*, bats with a wide distribution in South America. These results reinforce the need to deepen the knowledge about the possibility of bats act as reservoirs of *Leishmania* spp. especially considering their ability of dispersion and occupation of anthropic environments.

1. Introduction

The tegumentary leishmaniasis (TL) is characterized by diverse clinical manifestations in humans, ranging from small nodules to the destruction of mucous membranes. It is endemic in the tropics, in four of the seven continents: Africa, America, Asia and Europe. It is present in at least 88 countries, with worldwide incidence of approximately 1.5 million cases per year (Alvar et al., 1997), 90% of them in seven countries: Brazil and Peru in the New World and Iran, Syria, Afghanistan, Algeria and Saudi Arabia in the Old World (Desjeux, 2004).

Seven out of 30 known *Leishmania* species are active in the etiology of TL in Brazil (Dedet, 1993; Grimaldi et al., 1987; Reithinger and

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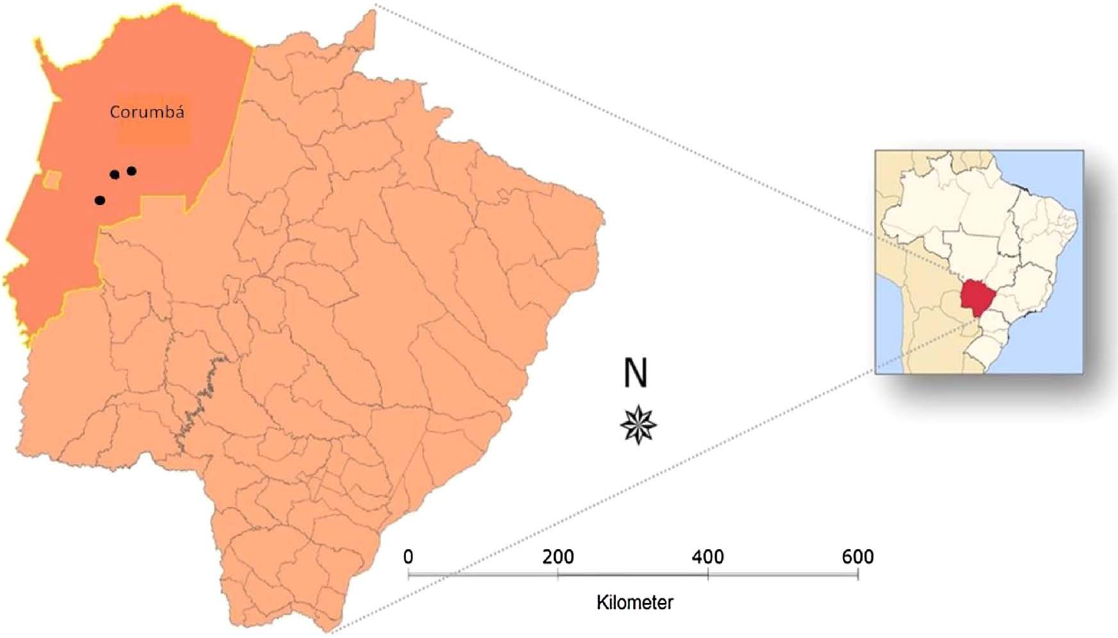

Fig. 1. Sites of bat collections in the Nhecolândia region, Pantanal wetland, Brazil. (1) Nhumirim farm (18° 59′ S, 56° 37′ W), (2) Alegria farm (19° 03′ S, 56° 47′ W) and (3) Arara Azul farm (19° 19′ S, 57° 01′ W).

Table 1
Bat individuals investigated for the presence of *Leishmania* spp., sex ratio, prevalence of *Leishmania braziliensis*, and positive/negative ratio for *L. braziliensis* in three sites of the Pantanal wetland, Brazil.

			Location			
Bat species	Sample size	Prevalence (%)	Fazenda Arara Azul (+/−)	Fazenda Nhumirim (+/−)	Fazenda Alegria (+/−)	Bat Gender (♂/♀)
Artibeus planirostris	29	3.45	0/12	0/12	1/4	15/14
Desmodus rotundus	1	–	–	–	0/1	0/1
Glosssophaga soricina	1	–	–	–	0/1	0/1
Noctilio albiventris	1	–	0/1	–	–	0/1
Platyrrhinus lineatus	15	13.33	2/11	–	0/2	6/9
Total	47	–	2/24	0/12	1/8	21/26

Dujardin, 2007; Akhoundi et al., 2016), where most cases are attributed to *L. braziliensis*, which is the widest distributed species of *Leishmania* in this country (Martins et al., 2010).

Leishmaniasis are endemic and an important public health problem in Mato Grosso do Sul, Brazil (Menezes Filho et al., 1986; Nunes et al., 1995, Brasil, 2014). Investigations on the transmission cycle of this disease at different sites in this state are still necessary for effective strategies of control, which depend on the nature of transmission in each outbreak of disease.

In Mato Grosso do Sul, the identification of *L. braziliensis* has already been described in humans (Nunes, 2001; Nunes et al., 1995), dogs (Savani, 2004), sandflies (Paiva et al., 2010) and bats (Shapiro et al., 2013). However, few studies are available on the specific identification of TL agents across their areas of occurrence in the region. Moreover, infection of *Leishmania* spp. into bats are scarcely investigated, thus the general importance of bats as reservoirs of leishmaniasis remains largely unknown (De Lima et al., 2008; Savani et al., 2010; Shapiro et al., 2013; Berzunza Cruz et al., 2015; Oliveira et al., 2015).

Here we evaluate the occurrence of infection of *Leishmania* spp. in pristine bat populations inhabiting the Pantanal wetland, Mato Grosso do Sul. Results raise new issues on the relevance of bats as hosts in the ecoepidemiology of Leishmaniasis.

2. Materials and methods

2.1. Samples

Bats were mistnetted in three sites in the Pantanal wetland, Nhecolândia region (Fig. 1). Fazenda Nhumirim (18° 59′ S, 56° 37′ W), Fazenda Alegria (19° 03′ S, 56° 47′ W) and Fazenda Arara Azul (19° 19′ S, 57° 01′ W). After inspection for identification, a small portion (approximately 0,5 mm^2) of epithelial tissue was taken from the bat's wing with sterile forceps and scissors, and then preserved in absolute ethanol at −20 °C. Bats were identified to species based on the known regional fauna (Alho et al., 2011; Munin et al., 2012) and through comparisons of voucher specimens with material in zoological collection (ZUFMS-M). Bat captures and collection were carried out under IBAMA license 377782-1.

2.2. DNA isolation, PCR and RFLP

The DNA of the bats skin samples was extracted using the Puregene Cell and Tissue kit – QIAGEN®, following the manufacturer's instructions. As a screening for the detection of *Leishmania* spp. parasites kDNA (*Leishmania* spp. kinetoplast DNA) PCR was performed by using the primers kDNA A: 5′(C/G) (C/G) (G/C) CC (C/A) ATC T (T/A) T TAC CCC AAC ACC 3′ and kDNA B: 5′ GGG GGG CGT TCT GAG GCG AA 3′, generating a fragment of 120 bp (Silva et al., 2000). The PCR reaction was prepared to a final volume of 25 μL using 5 μL of DNA template,