

***Leishmania* PRESENCE IN BATS IN AREAS ENDEMIC FOR LEISHMANIASIS IN CENTRAL-WEST BRAZIL**

Ludiele S. Castro^a; Maria E. C. Dorval^a; Larissa M. D. Matheus^a; Aline V. Bednaski^b; Gilberto G. Facco^b; Mauricio Silveira^c; Carolina F. Santos^c; Célia M. F. Gontijo^d; Ana Paula G. Oliveira^b; Eduardo C. Ferreira^{a,b,e}

^aPrograma de Pós-Graduação em Doenças Infecciosas e Parasitárias, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil.

^bUniversidade Anhanguera-UNIDERP, Campo Grande, MS, Brazil.

^cPrograma de Pós-graduação em Ecologia e Conservação, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil.

^dCentro de Pesquisas René Rachou, Fiocruz Minas, Fundação Oswaldo Cruz, Belo Horizonte, MG, Brazil.

^eFiocruz Mato Grosso do Sul, Fundação Oswaldo Cruz, Campo Grande, MS, Brasil

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Corresponding author: Ludiele Souza Castro - Email: tuticastro@hotmail.com or ludiele.castro@kroton.com.br

ABSTRACT

Leishmaniasis involves the participation of several species of both wild and domestic mammal hosts and sandfly vectors, which demonstrates the eco-epidemiological complexity observed in this disease. Bats are among the most abundant types of mammals and the scarcity of research on *Leishmania* infection in these animals gives evidence of the importance of new studies that aim to clarify this relationship. This study aimed to detect the *Leishmania* spp. in bats. 146 bats, representing 16 different species belonging to the Molossidae, Vespertilionidae, and Phyllostomidae families, were received and processed for collection of tissues. Skin samples were collected from 100% of the bats, and liver samples were collected from 87% (n=127). After evaluating the quality of the DNA extracted by means of PCR directed to the IRBP gene, the samples considered suitable for the *Leishmania* detection test were submitted for PCR directed to *Leishmania* kDNA, and to confirm positivity, were tested to the SSUrRNA gene-directed Nested-PCR. The *Leishmania* presence in the species *Molossus pretiosus*, *Nyctinomops macrotis*, and *Lasiurus cinereus* are the first reports this encounter in these species of bats in Brazil. Furthermore, new species of bats as possible hosts for *L. infantum* are reported, such as *Molossus pretiosus*, *Myotis nigricans*, *Nyctinomops laticaudatus*, *Nyctinomops macrotis*, and, for *L. braziliensis*, *Lasiurus cinereus* and *Cynomops planirostris*. These findings in

bats in an area endemic for leishmaniasis indicate that these animals may be involved in sustaining the disease cycle in this location.

Keywords: *Leishmania*; Bat; Chiroptera; reservoir; PCR.

INTRODUCTION

Leishmaniasis is an endemic zoonosis that is expanding in Brazil and is considered an important public health problem that is difficult to control (Brasil, 2014; OPAS, 2019). The disease is caused by obligate intracellular protozoa and presents a wide range of pathological processes, ranging from cutaneous to visceral, due to the multiplication of parasites within the cells of the monocytic phagocytic system of the vertebrate hosts (Desjeux, 2004; WHO, 2010).

The transmission of parasites between their numerous hosts, and, accidentally, to humans, is made during the hematophagy of different species of dipterans belonging to the Psychodidae family, subfamily Phlebotominae (Killick-Kendrick et al., 1991; Brandão-Filho, 2004). Akhoundi et al. (2016) in an historical overview proposed an updated list of possible and proven sandfly vectors for each *Leishmania* species in the Old and New World. These dipterans have been recorded in natural ecosystem and in rural and urban environments close to domestic animals and human habitations (Barata et al., 2004).

The emergence of new cases of leishmaniasis is strongly influenced by environmental, demographic, and human behavioral factors and is associated with changes in the habitat of vectors and natural hosts, which contributes to changes in the disease's eco-epidemiological profile (Gramiccia and Gradoni, 2005; Brasil, 2017). Moreover, the participation of several species of wild and domestic mammals and vector sandflies increases the eco-epidemiological complexity observed in this parasitosis, making it difficult to control due to the different epidemiological patterns observed in each transmission focus. The lack of knowledge about these aspects further aggravates the situation (Ferreira et al, 2010).

Bats are known to be hosts and possible reservoirs of various pathogens (Brown, 2003; Correa et al., 2013; Lei and Olival, 2014; Ferreira et al., 2017). The order Chiroptera has the second largest number of species, is characterized by high mobility, and is present in several environments, including urban and peri-urban

environments (Nowak, 1991; Simmons, 2005). These factors may increase the risk of *Leishmania* spp. transmission, since Chiroptera can act as pathogen hosts or reservoirs, thereby maintaining the protozoa in these different environments (Correa et al., 2013).

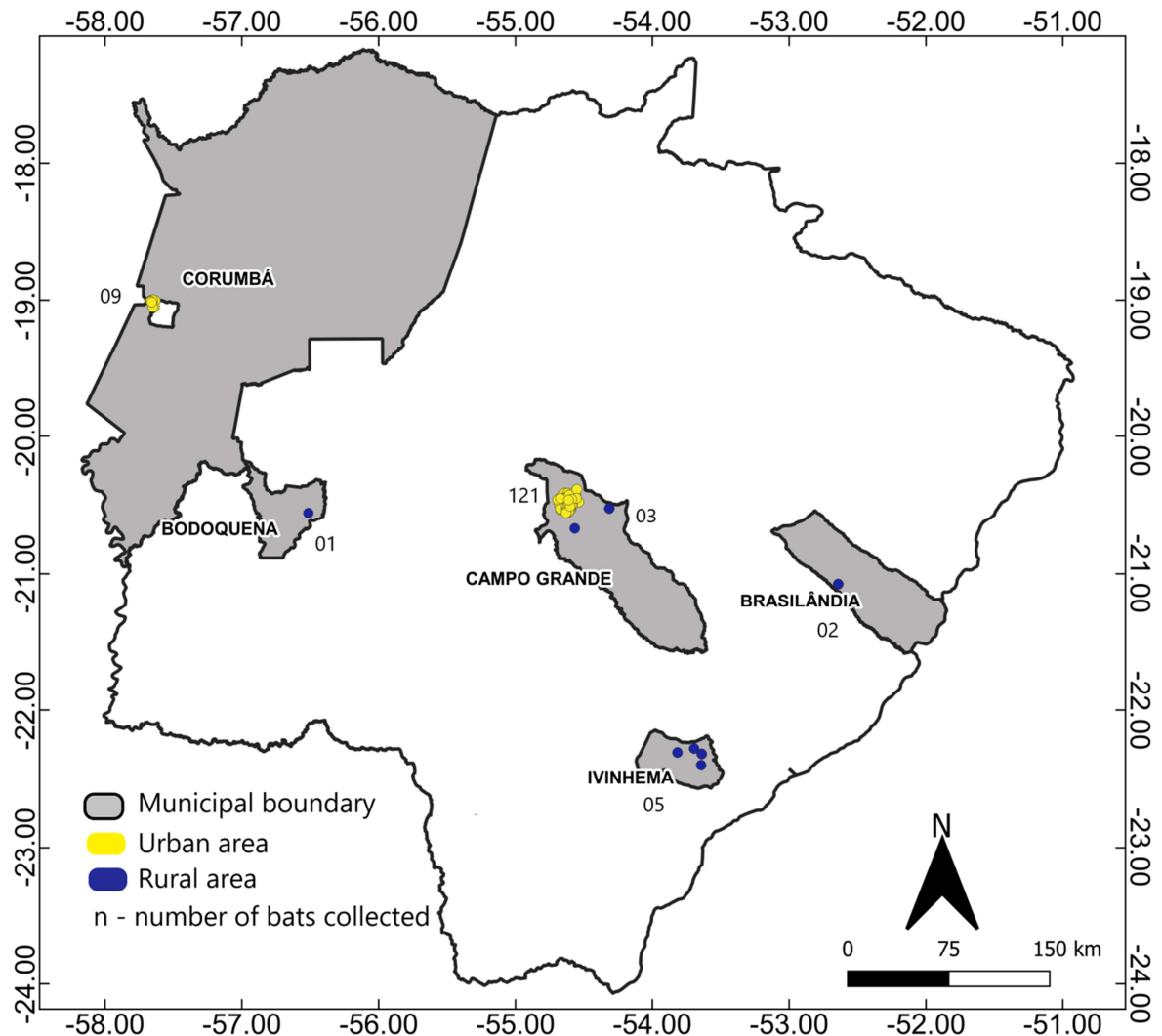
Leishmaniasis is an endemic and particularly important public health problem in Mato Grosso do Sul, Brazil (Nunes et al., 1995; Dorval et al., 2006; Reis et al., 2017; Castro et al., 2018; Mato Grosso do Sul, 2018). However, *Leishmania* spp. infection in bats has been subject to minimal investigation. Thus, the general importance of bats as reservoirs of *Leishmania* remains unknown. Therefore, the purpose of this study was to evaluate the occurrence of *Leishmania* spp. in bat populations inhabiting this region to obtain more knowledge about the participation of bats as hosts in endemic local for leishmaniasis as Mato Grosso do Sul.

MATERIAL AND METHODS

Bat Samples

The bats analyzed in this study were donated by the State Agency for Animal and Plant Health Defense (IAGRO/State Secretaria of Mato Grosso do Sul). These specimens were frozen at -20°C. At the time of processing of the biological material, the bats were classified according South American bat identification taxonomic key by Díaz et al. (2016), and the tissues of interest in the research (liver and skin) were duly identified and separated using a separate sterile scalpel for each tissue. These samples were frozen at -70 °C until were analyzed using molecular techniques.

One hundred and forty-six bats, collected in urban and rural areas in and surrounding five different cities in the state of Mato Grosso do Sul, Brazil, were used in this study. The majority of the animals had been collected in the municipality of Campo Grande (n=124), largely in urban areas (n=121) (Figure 1).



Note: was not possible to trace the collection information in five animals.

Figure 1. Bat collection sites in the state of Mato Grosso do Sul, Brazil.

Molecular tests

DNA Extraction

Approximately 10mg of bat tissues were separated and transferred to a sterile 1.5mL microtube. Subsequently, they were subjected to DNA extraction using a commercial kit (Promega Wizard® Genomic DNA Purification), following the manufacturer's instructions.

IRBP- PCR

IRBP-PCR was performed as a pre-diagnostic step to evaluate DNA quality for further testing for the presence of *Leishmania* spp. The PCR reaction was performed

using 5µl of target DNA, PCR buffer, 1.5mM MgCl₂, 200µM dNTP mix, 0.7U Taq DNA polymerase, and 15pmol of each primer: IRBPfwd: (5'-TCCAACACCACCACTGAGATCTGGAC-3') and IRBPrev: (5'-GTGAGGAAGAAATCGGACTGGCC-3'), flanking a 227 bp region. IRBP gene amplification followed the following conditions: denaturation at 95 ° C for 4 min, followed by 35 cycles at 94 ° C for 30 sec, 57 ° C for 30 sec, 72 ° C for 1 min and then a final extension step at 72 ° C for 5 min (Ferreira et al., 2010).

kDNA- PCR

The samples that were positive for the IRBP gene were submitted to the PCR amplifications of the variable region of the kinetoplastid DNA (kDNA) minicircle molecules to screen for the detection of *Leishmania* spp. The reactions were carried out using the specific primers A: 5'(C/G)(C/G)(G/C) CC(C/A) CTA T(T/A)T TAC ACC AAC CCC 3' and kDNA B: 5' GGG GAG GGG CGT TCT GCG AA 3', generating a fragment of 120bp (Silva et al., 2004).

The PCR reaction was prepared to a final volume of 25µL using 5µL of DNA template, 1.5mM MgCl₂, 200µM dNTP mix, 3.5U Taq DNA polymerase, and 10pmol of each primer. The reaction for kDNA gene amplification was based on the following conditions: denaturation at 94°C for 4 minutes followed by 35 cycles at 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and then a final extension step at 72°C for 5 minutes.

In all reactions, the DNA of the reference strains *Leishmania braziliensis* (MHOM/BR/75/M2903), *Leishmania infantum* (MHOM/BR/74/PP75), and *Leishmania amazonensis* (IFLA/BR/67/PH8) was used as a positive control. For the negative control, 5µL of ultra-pure water, DNase, and RNase free was added to a mix containing the reagents without DNA.

Nested PCR

Nested PCR for the gene small subunit ribosomal ribonucleic acid (SSUrRNA), LnPCR, was performed for the confirmation of the positive indicated by kDNA-PCR.

The first-stage PCR used the forward primer R221 (5' GGT TCC TTT CCT GAT TTA CG 3') with the reverse primer R332 (5' GGC CGG TAA AGG CCG AAT AG 3'). For the first amplification, 10µL of DNA solution were added to 40µL of PCR mix containing 3mM MgCl₂, 200µM dNTPs, 7.0U Taq DNA polymerase, and 10pmol

of each primer, generating a fragment of 603bp. The cycling conditions were 94°C for 5 minutes followed by 30 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and extension at 72°C for 30 seconds, followed by a final extension at 72°C for 10 minutes (Cruz et al., 2002).

The second amplification consisted of the reamplification of the previous SSU rRNA PCR product. Thus, 10µL of 1/40 dilution of the first PCR product were added to 25µL of PCR mix containing 3mM MgCl₂, 200µM dNTPs, 7.0U Taq DNA polymerase, and 10pmol of the *Leishmania*-specific primers R223 (5' TCC CAT CGC AAC CTC GGT T 3') and R333 (5' AAA GCG GGC GCG GTG CTG 3'), generating a fragment of 350bp. The cycling conditions were 94°C for 4 minutes followed by 30 cycles at 94°C for 30 seconds, 65°C for 30 seconds, and extension at 72°C for 30 seconds, followed by a final extension at 72°C for 10 minutes (Cruz et al., 2002).

Electrophoresis

The results were visualized on 2.0% agarose gel in 1x TBE buffer (tris base, boric acid and EDTA) in the presence of SYBR® Safe DNA gel stain (Invitrogen, Waltham, EUA) and examined on blue light exposure with a molecular weight marker of 100 bp.

Sequencing

All LnPCR-SSUrRNA PCR positive products were excised from the gel and purified using the PureLink® Quick Gel Extraction Kit (Invitrogen, Waltham, EUA) following the protocol provided by the manufacturer. Direct sequencing of the purified SSUrRNA positive products was performed with forward and reverse primers using the Big-Dye Terminator v3.1 Cycle Sequencing Kit and the automated ABI 3730 DNA Analyser (Applied Biosystems®, Foster City, EUA). Sequences obtained were analyzed and edited using the BioEdit Sequence Alignment Editor. The edited sequences were deposited at GenBank and compared to the others deposited by the BLAST tool.

Statistical analyses

To compare positivity ratios, 95% confidence intervals (CIs) were calculated using the Wald method. A chi-square test was used to verify statistical association

between variables. The statistical analysis was performed using Stata software, version 11.

RESULTS

We were able to identify 127/146 (87%) bats to the species level, representing 16 different species of bats. For 19 bats (13%), it was only possible to reach identification at the family level because the specimens were in an advanced state of decomposition. Skin samples were obtained from 100% (n = 146) of the bats, whereas liver samples were obtained from 87% (n = 127) of the specimens, because in 13% (n = 19) of them it was not possible to observe fundamental characteristics for organ identification or collection due to the animals' advanced states of decomposition.

In 100% (n = 146) of the specimens it was possible to collect skin samples, while that the collect of liver samples it was possible from 87% (n = 127) of the specimens, because in 13% (n = 19) of them it was not possible to observe fundamental characteristics for organ identification or collection due to the animals' advanced states of decomposition.

From the molecular tests and electrophoresis, it was possible to verify that 242 (89.3%) of the samples were positive for the IRBP target, of which 125 were skin samples and 117 were liver samples, were all the liver samples derive from bats included in the skin samples.

Only the samples that were positive for the IRBP target were submitted for kDNA PCR (n = 242). *Leishmania* spp. kDNA amplification by PCR was observed in 28.9% (70/242) of the total bat samples. Of the 125 skin samples positive for the IRBP target, six [4.8%; CI (95%) = 1.05%–8.5%] were positive for kDNA, and, of the 117 liver samples positive for IRBP, 64 [54.7%; CI (95%) = 45.7%–63.7%] were positive for kDNA (e.g., Figure 2).

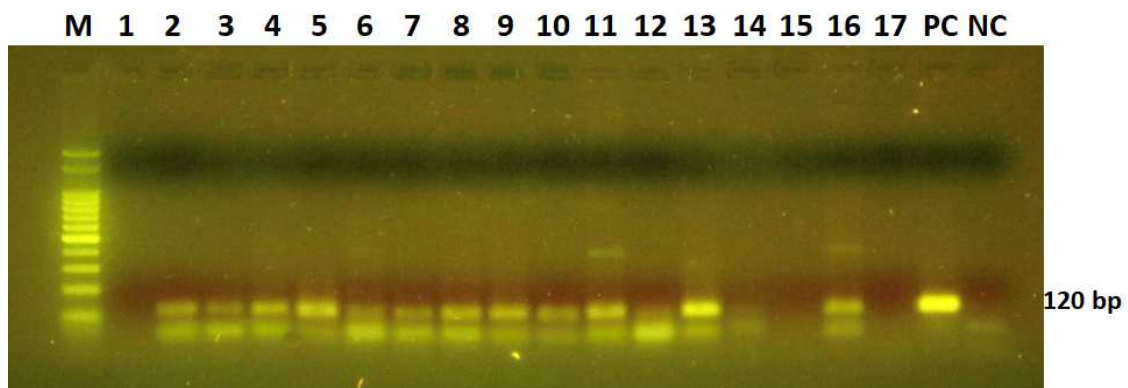


Figure 2. Agarose gel demonstrating kDNA PCR products (120bp). M: 100bp marker. 1-17: analyzed samples, of which 2-11, 13 and 16 were considered positive. PC: positive control of *Leishmania* spp., NC: negative control.

Subsequently, samples positive for the kDNA target were submitted to the nested-PCR for the gene SSU rRNA (LnPCR). Representative positive samples are shown in Figure 3.

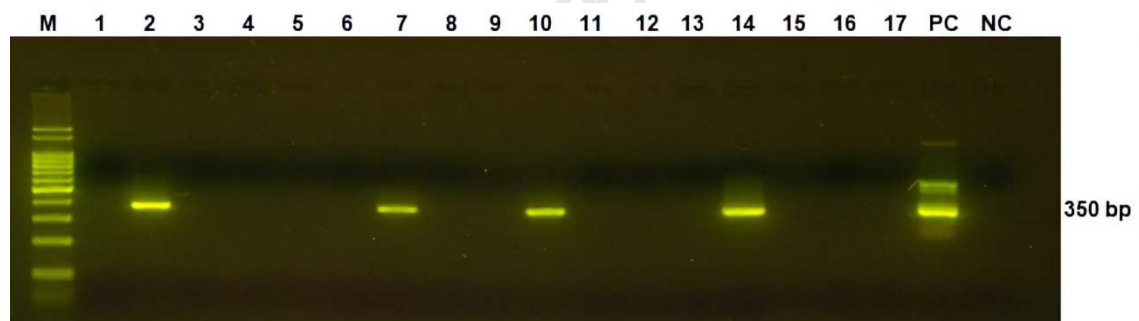


Figure 3. Agarose gel demonstrating LnPCR products (350bp). M: 100bp marker, 1-17: analyzed samples, of which 2, 7, 10 and 14 were considered positive. PC: positive control of *Leishmania* spp., NC: negative control.

The presence of *Leishmania* was confirmed via both techniques in 4 [3.2%; CI (95%) = 0.1%–6.3%] skin samples ($n = 125$) and 29 [24.8%; CI (95%) = 17.0%–32.6%] liver samples ($n = 117$) (Table 1). Significantly more liver samples were positive compared to skin samples ($p < 0.001$).

In terms of the bat collection locations, in Campo Grande, where the highest number of bats were collected, bats from the central and peripheral regions of the city were positive for the kDNA target whereas positivity for LnPCR was more concentrated in the central region, with some points on the periphery of the city. In the city of Corumbá, all animals were collected in urban areas, and bats that resulted in positive samples using both techniques were observed in the peripheral regions of the city. In Brasília and Ivinhema, all animals were collected in rural areas. The bat collected in the city of Bodoquena was considered negative for *Leishmania*.

Table 1 - Results from PCR testing of skin and liver of bats, collected in and around five cities in the state of Mato Grosso do Sul, 2019, for *Leishmania* using the kDNA and SSUrRNA targets.

| Family | Species | kDNA - Skin positive/n | SSUrRNA – Skin positive/n | kDNA – Liver positive/n | SSUrRNA – Liver positive/n |
|-------------------------|---------------------------------|---------------------------|------------------------------|----------------------------|-------------------------------|
| Phyllostomidae | <i>Artibeus planirostris</i> | 0/0 | - | 0/1 | - |
| | <i>Desmodus rotundus</i> | 0/3 | - | 3/6 | 3/3 |
| | <i>Glossophaga soricina</i> | 0/0 | - | 1/1 | 1/1 |
| | <i>Lonchophylla thomasi</i> | 0/1 | - | 1/1 | 0/1 |
| Total per Family | | 0/4 | - | 5/9 | 4/5 |
| Vespertilionidae | <i>Eptesicus furinalis</i> | 0/2 | - | 0/1 | - |
| | <i>Lasiurus blossevillii</i> | 0/1 | - | 0/0 | - |
| | <i>Lasiurus cinereus</i> | 0/2 | - | 4/5 | 2/4 |
| | <i>Myotis nigricans</i> | 0/2 | - | 2/3 | 1/2 |
| | Unidentified species | 0/8 | - | 1/3 | 0/1 |
| Total per Family | | 0/15 | - | 7/12 | 3/7 |
| Molossidae | <i>Cynomops planirostris</i> | 0/9 | - | 5/7 | 1/5 |
| | <i>Eumops glaucinus</i> | 0/4 | - | 1/4 | 0/1 |
| | <i>Molossus molossus</i> | 1/27 | 0/1 | 13/27 | 4/13 |
| | <i>Molossus pretiosus</i> | 2/19 | 2 ¹ /2 | 13/18 | 3 ¹ /13 |
| | <i>Molossus rufus</i> | 0/10 | - | 4/8 | 2/4 |
| | <i>Nyctinomops laticaudatus</i> | 2/10 | 2/2 | 3/10 | 2/3 |
| | <i>Nyctinomops macrotis</i> | 0/15 | - | 10/13 | 9/10 |
| | <i>Promops centralis</i> | 1/3 | 0/1 | 2/3 | 0/2 |
| Unidentified species | 0/9 | - | 1/6 | 1/1 | |
| Total per Family | | 6/106 | 4/6 | 52/96 | 22/52 |
| TOTAL | | 6/125 (4.8%) | 4/6 (66.7%) | 64/117 (54.7%) | 29/64 (45.3%) |

¹ One bat presented *Leishmania* DNA presence in both liver and skin.

Thus, 33 samples belonging to 32 different bats (one animal had positivity in the skin and the liver) were confirmed as positive. They were submitted to the sequencing technique to identify the *Leishmania* species involved.

After analysis of the sequences according to the scores obtained in the BLAST (supplementary material), it was possible to determine the species of *Leishmania* involved in 28 of the samples [84.8%; CI (95%) = 72.6% –97.1%]. Two of these samples (6.1%) were identified as having *Leishmania braziliensis*. The other 26 samples (78.8%) were confirmed as having *Leishmania infantum*. In 3 of them [9.1%; CI (95%) = 0%–18.9%], it was possible to conclude to the subgenus level, and 2 of them [6.1%; CI (95%) = 0%–14.2%] were categorized as only belonging to the genus *Leishmania* (Table 2). The sequences were deposited in GenBank, with accession numbers: MN852406 to MN852438.

Figure 4 shows the species of *Leishmania* identified according to the bat collection location. In Campo Grande there were different points of positivity for *L. infantum*, with 20 animals identified with this *Leishmania* specie from 26 positives [76.9%; CI (95%) = 60.7%–93.1%]. In Corumbá, Ivinhema and Brasilândia this same species was identified in positive animals, in both urban and rural areas.

Table 2 – Family and species of bats in relation to species of *Leishmania* found, in addition to the feeding habits and percentage of positive animals per species, Mato Grosso do Sul, 2019.

| Family | Bat Species (n) | Feeding habit | Positive n | Positive (%) | <i>Leishmania</i> identification | Tissue |
|----------------------------------|--------------------------------------|---------------|----------------|-----------------------|--|--------|
| Phyllostomidae | <i>Desmodus rotundus</i> (6) | Hematophagous | 3 | (50%) | <i>L. infantum</i> | Liver |
| | <i>Glossophaga soricina</i> (1) | Nectarivorous | 1 | (100%) | <i>L. infantum</i> | Liver |
| Vespertilionidae | <i>Lasiurus cinereus</i> (5) | Insectivorous | 1 | (40%) | <i>L. braziliensis</i> | Liver |
| | | | 1 | | <i>Leishmania</i> (<i>Viannia</i>) sp. | Liver |
| | <i>Myotis nigricans</i> (3) | Insectivorous | 1 | (33,3%) | <i>L. infantum</i> | Liver |
| Molossidae | <i>Cynomops planirostris</i> (9) | Insectivorous | 1 | (11,1%) | <i>L. braziliensis</i> | Liver |
| | | | 2 | | <i>L. infantum</i> | Liver |
| | <i>Molossus molossus</i> (27) | Insectivorous | 1 | (14,8%) | <i>Leishmania</i> (<i>Leishmania</i>) sp. | Liver |
| | | | 1 | | <i>Leishmania</i> sp. | Liver |
| | <i>Molossus pretiosus</i> (19) | Insectivorous | 2 | (26,3%) | <i>L. infantum</i> | Liver |
| | | | 2 ¹ | | <i>L. infantum</i> | Skin |
| | | | 1 ¹ | | <i>Leishmania</i> (<i>Leishmania</i>) sp. | Liver |
| | <i>Molossus rufus</i> (10) | Insectivorous | 2 | (20%) | <i>L. infantum</i> | Liver |
| | <i>Nyctinomops laticaudatus</i> (10) | Insectivorous | 2 | (40%) | <i>L. infantum</i> | Liver |
| | | | 2 | | <i>L. infantum</i> | Skin |
| <i>Nyctinomops macrotis</i> (15) | Insectivorous | 8 | (60%) | <i>L. infantum</i> | Liver | |
| | | 1 | | <i>Leishmania</i> sp. | Liver | |
| Molossidae ² (9) | - | 1 | (11,1%) | <i>L. infantum</i> | Liver | |

¹ Tissues of the same bat - *L. infantum* in the skin and *L. (Leishmania)* in the liver

² Species of bat not identified.

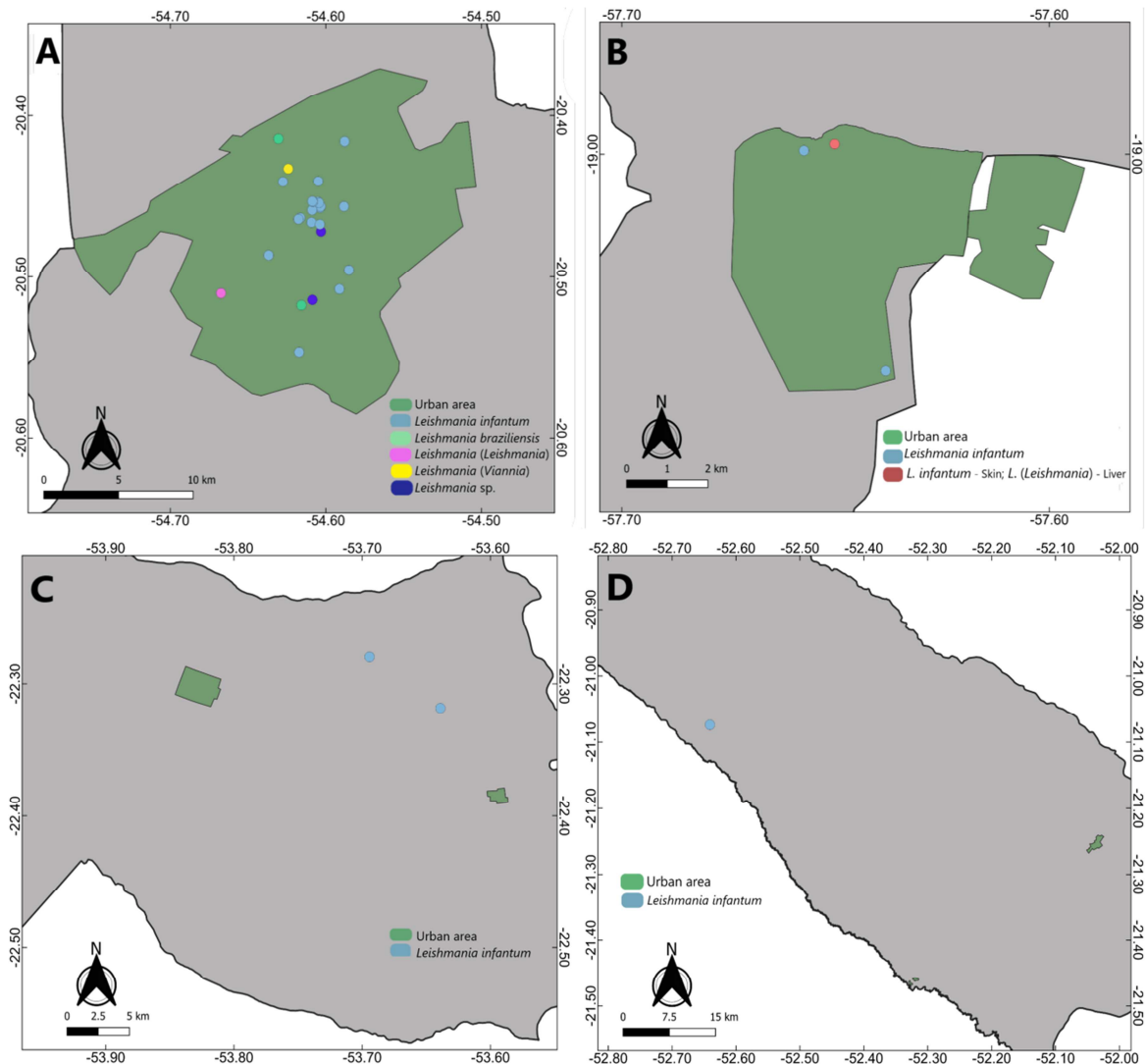


Figure 4 - Identification of *Leishmania* according to site of collect of the bats per city, Mato Grosso do Sul, Brazil; Grey area - municipal boundary; A – Campo Grande; B – Corumbá; C – Ivinhema; D – Brasilândia.

DISCUSSION

Leishmaniasis is present in the State of Mato Grosso do Sul in very significant numbers. Further, it is expanding and has widespread distribution and record in all municipalities, even with the implementation of control measures, such as the euthanizing of thousands of *L. infantum* seropositive dogs. It is clear that the existing control measures are not effective (Cunha et al., 2014). Therefore, there is the distinct possibility of the participation of other mammalian (wild or domestic) as possible reservoirs of the parasite in the transmission cycle of leishmaniasis in this area.

Of the total bat samples, *Leishmania* spp. kDNA amplification by PCR was observed in 28.9% (70/242). These results were similar to research conducted in the state of São Paulo, which found 23.9% (117/488) positivity in bat skin and spleen samples using qPCR for the same target (Oliveira et al., 2015).

This infection is considered an anthroponosis, in which more than 100 mammal species have been naturally infected with the parasites (Forattini et al., 1972; De Lima et al., 2002; Brandão-Filho et al., 2003; Ferreira et al., 2010; Roque and Jansen, 2014; Ferreira et al., 2015). Among them, bats have emerged as one of the most important supposed reservoirs of certain *Leishmania* species (Gómez-Hernández et al., 2017). The ability of bats to disperse and their longevity could facilitate the maintenance and dispersal of *Leishmania*, with considerable impacts on disease prevention and control measures (Lampo et al., 2000; Savani et al., 2010).

After the execution of the LnPCR, we observed positivity via both techniques for *Leishmania* in 33 samples from bats, of which, it was possible to determine the species of *Leishmania* involved in 28 of the samples. We detected the presence of *Leishmania infantum* in 26 samples (78.8%) and *Leishmania braziliensis* in two samples (6.1%). These two species are the most prevalent species in human cases of visceral and cutaneous leishmaniasis, respectively, in the Brazilian Midwest.

Some species of bats reported as being infected in the present study (*Molossus molossus*, *Molossus rufus*, *Myotis nigricans*, *Nyctinomops laticaudatus*, *Desmodus rotundus*, and *Glossophaga soricina*) have been similarly documented in studies carried out in Latin America, especially in Brazil, that recorded the occurrence of bats infected with *L. infantum* (De Lima et al., 2008; Oliveira et al., 2015; Gómez-Hernández et al., 2017; Rezende et al., 2017), *L. amazonensis* (Savani et al., 2010; Oliveira et al., 2015; Gómez-Hernández et al., 2017), and *L. braziliensis* (Shapiro et al., 2013; Ferreira et al., 2017; Gómez-Hernández et al., 2017).

In this study, we report new species of bats as possible hosts for *L. infantum*, including *Molossus pretiosus*, *Myotis nigricans*, *Nyctinomops laticaudatus*, and *Nyctinomops macrotis*. For *L. braziliensis*, the species *Lasiurus cinereus* and *Cynomops planirostris* were new species of bats

reported in the studied areas. These encounters demonstrate that bats could be contributing to maintaining *Leishmania* spp. in endemic areas.

In the cities of Campo Grande and Corumbá there were different points of positivity within the urban areas, scenario of occurrence of visceral leishmaniasis transmission, which corroborates with prior research that detected also *Leishmania* DNA presence in bats in urban areas (Oliveira et al., 2015; Rezende et al., 2017). On the other hand, it was also possible to detect *Leishmania* in rural areas, specifically in the cities of Ivinhema and Brasilândia, areas already been reported with of positive bats in other studies (Savani et al., 2010; Gómez-Hernández et al., 2017).

Leishmania infantum was the most widely identified species, both in rural and urban areas, this specie has already been identified from bats in other studies in Brazil, in different regions (Oliveira et al., 2015; Gómez-Hernández et al., 2017; Rezende et al., 2017). Moreover, higher prevalence identification of *L. infantum* in Campo Grande (76.9%) and Corumbá (100%) was expected, precisely because this species is responsible for the visceral manifestation, that is endemic in these cities (Botelho and Natal, 2009; Mato Grosso Do Sul, 2018). In this sense, although dogs are the main reservoir for humans, some wild species, such as bats, could be contributing to the maintenance of *Leishmania* spp. in these localities (Rezende et al., 2017). This could explain why traditional control measures do not appear to be efficient (Cunha et al., 2014).

Leishmania braziliensis, a species involved in cutaneous and mucosal leishmaniasis, was identified in an urban area of Campo Grande. This demonstrates that, although this species is involved in a pattern of rural and forest-area transmission, it may eventually circulate in cities, especially given bats' characteristic dispersion patterns. Moreover, to the best of our knowledge, the encounters of *Leishmania* in the species *Molossus pretiosus*, *Nyctinomops macrotis*, and *Lasiurus cinereus* are the first reports of *Leishmania* in these bat species in Brazil.

These findings of infected bats in areas endemic for leishmaniasis indicate that these animals may be participating in the maintenance of the disease cycle in these places. However, to validate the true role of these animals in the maintenance of leishmaniasis, further investigation is necessary. A reservoir for *Leishmania* spp. is required to ensure the subsistence and

transmission of the parasite, and extensive ecological studies are needed to define such a reservoir (Berzunza-Cruz et al., 2015). However, bats as possible reservoirs of the causative agents of leishmaniasis should not be disregarded.

Regarding samples, we detected *Leishmania* more often in liver versus skin samples, with statistical difference ($p < 0.001$). This difference should be better investigated to establish more clearly how the parasite behaves in this possible host. However, the lower presence of *Leishmania* DNA in the skin may suggest that bats are not the best source of infection for sandflies.

According to Shaw (2007), only when a better understanding of the genetic diversity of *Leishmania* species and the reservoirs involved in the enzootic cycle of each species is obtained will it be possible to evaluate what methods of control are most effective. It is important to carry out complementary surveys on all aspects involved in reservoir determination to propose more aggressive measures for prevention and control of the infection (De Lima et al., 2008; Savani et al., 2010; Shapiro et al., 2013; Roque and Jansen, 2014; Berzunza-Cruz et al., 2015; Ferreira et al., 2017).

CONSIDERATIONS

The use of animal samples donated by the IAGRO/MS, local service of the animal health, reinforces the importance of implementing sustainable projects, as it was not necessary to collect other animals exclusively for the development of this research.

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Table 1 - Results from PCR testing of skin and liver of bats, collected in and around five cities in the state of Mato Grosso do Sul, 2019, for *Leishmania* using the kDNA and SSUrRNA targets.

| Family | Species | kDNA - Skin positive/n | SSUrRNA – Skin positive/n | kDNA – Liver positive/n | SSUrRNA – Liver positive/n |
|-------------------------|---------------------------------|---------------------------|------------------------------|----------------------------|-------------------------------|
| Phyllostomidae | <i>Artibeus planirostris</i> | 0/0 | - | 0/1 | - |
| | <i>Desmodus rotundus</i> | 0/3 | - | 3/6 | 3/3 |
| | <i>Glossophaga soricina</i> | 0/0 | - | 1/1 | 1/1 |
| | <i>Lonchophylla thomasi</i> | 0/1 | - | 1/1 | 0/1 |
| Total per Family | | 0/4 | - | 5/9 | 4/5 |
| Vespertilionidae | <i>Eptesicus furinalis</i> | 0/2 | - | 0/1 | - |
| | <i>Lasiurus blossevillii</i> | 0/1 | - | 0/0 | - |
| | <i>Lasiurus cinereus</i> | 0/2 | - | 4/5 | 2/4 |
| | <i>Myotis nigricans</i> | 0/2 | - | 2/3 | 1/2 |
| | Unidentified species | 0/8 | - | 1/3 | 0/1 |
| Total per Family | | 0/15 | - | 7/12 | 3/7 |
| Molossidae | <i>Cynomops planirostris</i> | 0/9 | - | 5/7 | 1/5 |
| | <i>Eumops glaucinus</i> | 0/4 | - | 1/4 | 0/1 |
| | <i>Molossus molossus</i> | 1/27 | 0/1 | 13/27 | 4/13 |
| | <i>Molossus pretiosus</i> | 2/19 | 2 ¹ /2 | 13/18 | 3 ¹ /13 |
| | <i>Molossus rufus</i> | 0/10 | - | 4/8 | 2/4 |
| | <i>Nyctinomops laticaudatus</i> | 2/10 | 2/2 | 3/10 | 2/3 |
| | <i>Nyctinomops macrotis</i> | 0/15 | - | 10/13 | 9/10 |
| | <i>Promops centralis</i> | 1/3 | 0/1 | 2/3 | 0/2 |
| Unidentified species | 0/9 | - | 1/6 | 1/1 | |
| Total per Family | | 6/106 | 4/6 | 52/96 | 22/52 |
| TOTAL | | 6/125 (4.8%) | 4/6 (66.7%) | 64/117 (54.7%) | 29/64 (45.3%) |

¹ One bat presented *Leishmania* DNA presence in both liver and skin.

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Table 2 – Family and species of bats in relation to species of *Leishmania* found, in addition to the feeding habits and percentage of positive animals per species, Mato Grosso do Sul, 2019.

| Family | Bat Species (n) | Feeding habit | Positive | | <i>Leishmania</i> identification | Tissue |
|----------------------------------|--------------------------------------|---------------|----------------|-----------------------|---|--------|
| | | | n | (%) | | |
| Phyllostomidae | <i>Desmodus rotundus</i> (6) | Hematophagous | 3 | (50%) | <i>L. infantum</i> | Liver |
| | <i>Glossophaga soricina</i> (1) | Nectarivorous | 1 | (100%) | <i>L. infantum</i> | Liver |
| Vespertilionidae | | | 1 | | <i>L. braziliensis</i> | Liver |
| | <i>Lasiurus cinereus</i> (5) | Insectivorous | 1 | (40%) | <i>Leishmania</i> (<i>Viannia</i>) sp. | Liver |
| | <i>Myotis nigricans</i> (3) | Insectivorous | 1 | (33,3%) | <i>L. infantum</i> | Liver |
| Molossidae | <i>Cynomops planirostris</i> (9) | Insectivorous | 1 | (11,1%) | <i>L. braziliensis</i> | Liver |
| | | | 2 | | <i>L. infantum</i> | Liver |
| | <i>Molossus molossus</i> (27) | Insectivorous | 1 | (14,8%) | <i>Leishmania</i> (<i>Leishmania</i>) sp. | Liver |
| | | | 1 | | <i>Leishmania</i> sp. | Liver |
| | | | 2 | | <i>L. infantum</i> | Liver |
| | <i>Molossus pretiosus</i> (19) | Insectivorous | 2 ¹ | (26,3%) | <i>L. infantum</i> | Skin |
| | | | 1 ¹ | | <i>Leishmania</i> (<i>Leishmania</i>) sp. | Liver |
| | <i>Molossus rufus</i> (10) | Insectivorous | 2 | (20%) | <i>L. infantum</i> | Liver |
| | <i>Nyctinomops laticaudatus</i> (10) | Insectivorous | 2 | (40%) | <i>L. infantum</i> | Liver |
| | | | 2 | | <i>L. infantum</i> | Skin |
| <i>Nyctinomops macrotis</i> (15) | Insectivorous | 8 | | <i>L. infantum</i> | Liver | |
| | | 1 | (60%) | <i>Leishmania</i> sp. | Liver | |
| Molossidae ² (9) | | | 1 | (11,1%) | <i>L. infantum</i> | Liver |

¹ Tissues of the same bat - *L. infantum* in the skin and *L. (Leishmania)* in the liver

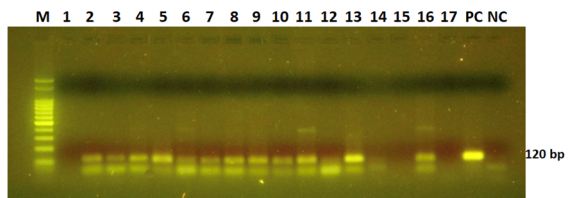
² Species of bat not identified.

Figure 1. Bat collection sites in the state of Mato Grosso do Sul, Brazil.

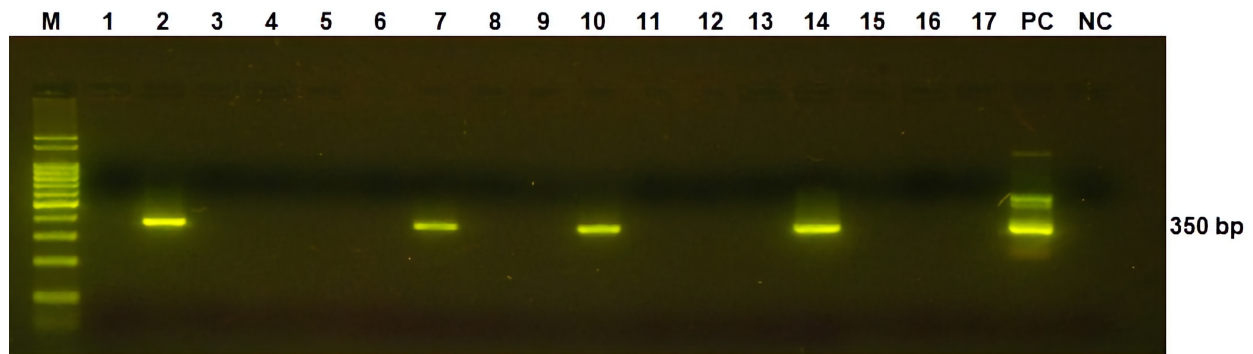
Figure 2. Agarose gel demonstrating kDNA PCR products (120bp). M: 100bp marker, 1-17: analyzed samples, of which 2-11, 13 and 16 were considered positive. PC: positive control of *Leishmania* spp., NC: negative control.

Figure 3. Agarose gel demonstrating LnPCR products (350bp). M: 100bp marker, 1-17: analyzed samples, of which 2, 7, 10 and 14 were considered positive. PC: positive control of *Leishmania* spp., NC: negative control.

Figure 4 - Identification of *Leishmania* according to site of collect of the bats per city, Mato Grosso do Sul, Brazil; Grey area - municipal boundary; A – Campo Grande; B – Corumbá; C – Ivinhema; D – Brasilândia.



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