



OPEN ACCESS

EDITED BY

Tania F. De Koning-Ward,
Deakin University, Australia

REVIEWED BY

Yasuyuki Goto,
The University of Tokyo, Japan
Iraj Sharifi,
Kerman University of Medical
Sciences, Iran
Sofia Cortes,
New University of Lisbon, Portugal

*CORRESPONDENCE

André Luiz Rodrigues Roque
✉ roque@ioc.fiocruz.br

SPECIALTY SECTION

This article was submitted to
Parasite and Host,
a section of the journal
Frontiers in Cellular and
Infection Microbiology

RECEIVED 21 September 2022

ACCEPTED 28 December 2022

PUBLISHED 13 January 2023

CITATION

de Macedo GC, Barreto WTG,
de Oliveira CE, Santos FM,
Porfírio GEO, Xavier SCdC, Alves FM,
da Silva AR, de Andrade GB, Rucco AC,
de Assis WO, Jansen AM, Roque ALR
and Herrera HM (2023) *Leishmania*
infantum infecting the carnivore *Nasua*
nasua from urban forest fragments in
an endemic area of visceral
leishmaniasis in Brazilian Midwest.
Front. Cell. Infect. Microbiol.
12:1050339.
doi: 10.3389/fcimb.2022.1050339

COPYRIGHT

© 2023 de Macedo, Barreto, de Oliveira,
Santos, Porfírio, Xavier, Alves, da Silva,
de Andrade, Rucco, de Assis, Jansen,
Roque and Herrera. This is an open-
access article distributed under the
terms of the [Creative Commons
Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use,
distribution or reproduction in other
forums is permitted, provided the
original author(s) and the copyright
owner(s) are credited and that the
original publication in this journal is
cited, in accordance with accepted
academic practice. No use,
distribution or reproduction is
permitted which does not comply with
these terms.

Leishmania infantum infecting the carnivore *Nasua nasua* from urban forest fragments in an endemic area of visceral leishmaniasis in Brazilian Midwest

Gabriel Carvalho de Macedo¹,
Wanessa Teixeira Gomes Barreto¹, Carina Elisei de Oliveira^{1,2},
Filipe Martins Santos¹, Grasiela Edith de Oliveira Porfírio¹,
Samanta Cristina das Chagas Xavier³,
Fernanda Moreira Alves^{3,4}, Alanderson Rodrigues da Silva²,
Gisele Braziliano de Andrade¹, Andreza Castro Rucco¹,
William Oliveira de Assis¹, Ana Maria Jansen³,
André Luiz Rodrigues Roque^{3*} and Heitor Miraglia Herrera^{1,2}

¹Post-Graduate Program in Environmental Sciences and Agricultural Sustainability, Dom Bosco Catholic University, Campo Grande, Brazil, ²Post-Graduate Program in Biotechnology, Dom Bosco Catholic University, Campo Grande, Brazil, ³Laboratory of Trypanosomatid Biology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, ⁴Post-Graduate Program in Parasite Biology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

Introduction: The aim of the present study was to investigate the occurrence of *Leishmania infantum* in South American coatis inhabiting two forest fragments in Campo Grande, Mato Grosso do Sul, Midwest region of Brazil, an endemic area of human and canine visceral leishmaniasis (VL).

Material and methods: A total of 110 South American coatis were sampled in the conservation unit “Parque Estadual do Prosa” (PEP) and in the residential area “Vila da Base Aérea” (VBA) from March 2018 to April 2019. As a longitudinal study that include up to six recaptures of the same individual, a total of 190 capture events were obtained. Blood, bone marrow and skin samples were obtained for parasitological (axenic culture), serological (Enzyme Linked Immunosorbent Assay – ELISA and Dual-path Platform immunoassay – DPP[®] CVL) and molecular diagnostic assays (targeting kDNA for *Leishmania* spp. and *L. infantum*; and HSP70 followed by sequence analysis).

Results: Seropositivity for *L. infantum* was found in 33 individuals, six in PEP and 27 in VBA. Furthermore, *L. infantum* was detected by molecular analysis in 16 individuals, seven from PEP and nine from VBA. We also isolated *L. infantum* from bone marrow of one individual and detected a single positive skin sample in molecular assay from other individual, both from VBA.

Discussion: An overall infection rate of 36.4% (40/110) was observed, significantly higher in the VBA (49.1%) than in the PEP (21.6%), probably because VBA presents: (i) a large number of resident dogs and chickens that would be attracting sandflies; (ii) a denser population of this wild mammal species; and (iii) physical barriers and a lack of functional connectivity in the surroundings, preventing these animals to disperse out. We conclude that South American coati populations living in urban forest fragments of Campo Grande are affected by the epidemiological scenario of VL, known to involve dogs, vectors and humans. We highlight the importance of investigate the parasitism by *L. infantum* in this and other potential *L. infantum* reservoirs that inhabit urbanized regions endemic to VL.

KEYWORDS

longitudinal study, South American coati, *Leishmania infantum*, urban fauna, visceral leishmaniasis

1 Introduction

The protozoan *Leishmania infantum* is the etiological agent of visceral leishmaniasis (VL), one of the most important parasitic diseases worldwide. Brazil ranks the top five countries with the highest number of human cases and the highest fatality rate (PAHO, 2020). Despite many control measures for VL in Brazil, the incidence of human cases is still rising, especially in urban areas. Indeed, the country shows an average of 3.404 new cases/100,000 people per year, and 23 out of 27 states notified human VL cases (MS-BR, 2019; MS-BR, 2021), indicating a widespread occurrence of this disease.

The adaptive plasticity of *Lutzomyia longipalpis* complex (the invertebrate vector of *L. infantum*) to urban environments (Salomón et al., 2015), and the presence of other host species than human and domestic dogs (the main vertebrate reservoir) in urban VL endemic areas contribute to the complexity of the enzootic scenarios. There is strong evidence that synanthropic species as rodents, bats and marsupials play a role in the maintenance of *L. infantum* in urban and peri-urban endemic areas in Brazil (Oliveira et al., 2005; Carreira et al., 2012; Lima et al., 2013; de Castro Ferreira et al., 2015; de Rezende et al., 2017; Castro et al., 2020; Vieira et al., 2022).

Wild carnivore species have been reported parasitized by *L. infantum* in Brazil since the half of the 20th century, when the Deane's couple described the infection in the crab-eating fox (*Cerdocyon thous*) (Deane and Deane, 1955), although the host was inaccurately reported as hoary fox (*Lycalopex vetulus*) (Courtenay et al., 1996). Indeed, crab-eating fox can be considered a putative *L. infantum* reservoir host (Roque and Jansen, 2014), as attested by parasitological assays in different studies (Lainson et al., 1990; Silva et al., 2000; Courtenay et al., 2002; Quinnell and Courtenay, 2009). Nevertheless, the free-

living crab-eating fox infected by *L. infantum* seems to be less competent to infect vectors than dogs (Courtenay et al., 2002). Apart from crab-eating fox, the role of other wild Carnivora in the epidemiology of VL is still unknown. It has been recorded the presence of *L. infantum* in intact skin of a captive bush dog (*Speothos venaticus*) (Figueiredo et al., 2008), although the potential of its transmissibility for the vector was reported to be low (Mol et al., 2015). The transmissibility shown for captive maned wolves (*Chrysocyon brachyurus*) was also very low (Mol et al., 2015). As these species do not live near human habitations, they are considered to have a secondary importance in epidemiology of VL in the urban areas (Dantas-Torres and Brandão-Filho, 2006).

The South American coati (Procyonidae: *Nasua nasua*; hereafter "coati") is a carnivore species well adapted to urban forest fragments in some regions of Brazil (Estevam et al., 2020; Barreto et al., 2021). To date, the only association between *L. infantum* and this taxon was reported by Paiz et al. (2015), who showed serological evidence of infection. Additionally, some reports have detected *Leishmania* spp. in this carnivore species through serological and molecular methods (Voltarelli et al., 2009; Porfirio et al., 2018; Reis et al., 2020), and *L. shawi* was isolated from this mammal (Lainson et al., 1989).

Campo Grande (CG), the capital of Mato Grosso do Sul (MS) state, Midwest Brazil, is considered an endemic area for human and canine VL (Botelho and Natal, 2009; Furlan, 2010; Brazuna et al., 2012; de Sousa et al., 2013; SES/MS, 2022). In this region, *L. infantum* has been detected in domestic cats (Antunes et al., 2016; Metzendorf et al., 2017) and wild mammal species as opossum and bats (Humberg et al., 2012; de Rezende et al., 2017; Castro et al., 2020). Moreover, *L. longipalpis* is present in this region (Cunha et al., 2014), indicating a high-risk area for *L. infantum* transmission (Falcão de Oliveira et al., 2020; SES/MS,

2022). Since, Barreto et al. (2021) reported that coati is a conspicuous species in the urban forest fragments at CG, we aimed to investigate the *L. infantum* infection in this mammal population. Our hypothesis is that this wild mammal species is affected by the local epidemiological scenario of *L. infantum* transmission.

2 Material and methods

2.1 Study areas and field procedures

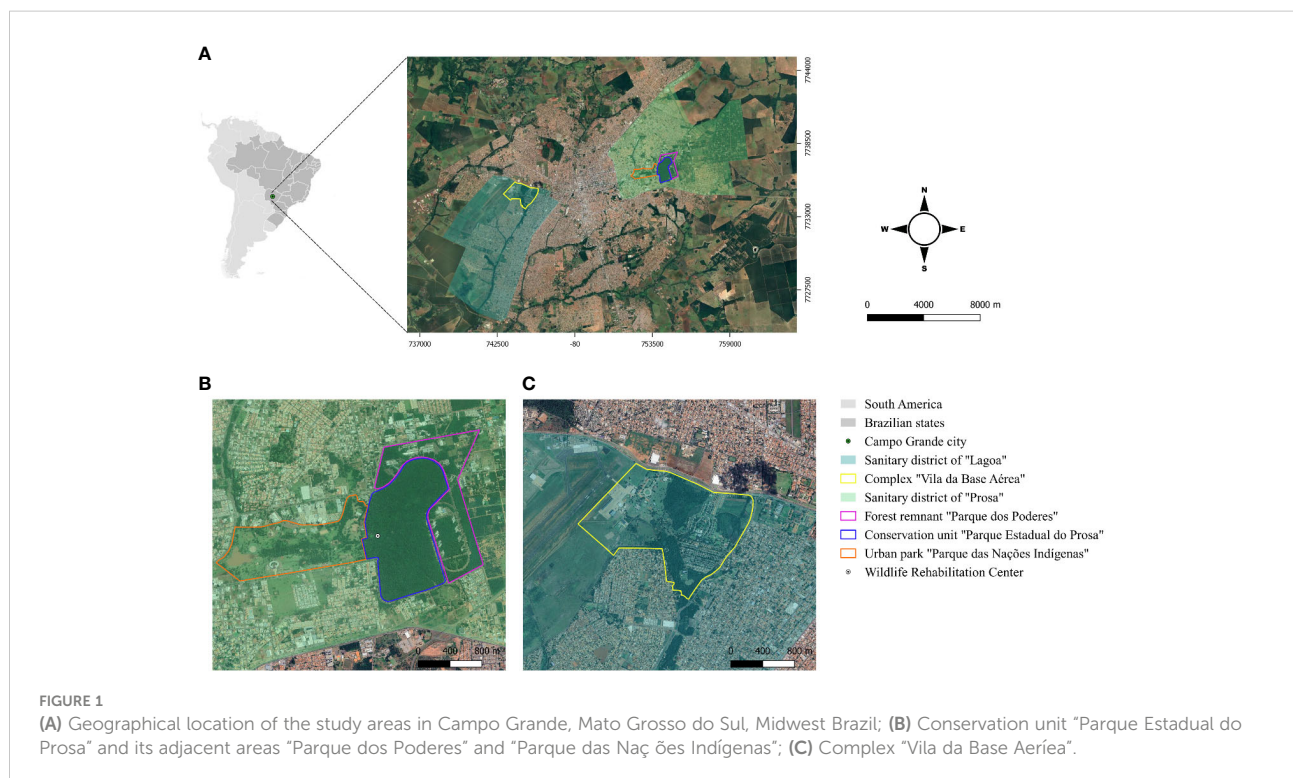
The study was conducted in two forest fragments “Parque Estadual do Prosa” (PEP) (20°26′59″S, 54°33′55″O) and “Vila da Base Aérea” (VBA) (20°28′17″S, 54°39′14″O), located in the urban area of CG/MS (Figure 1A). The population densities of coatis in these areas were estimated as 11.2 individuals/km² in PEP and 19.5 individuals/km² in VBA (Barreto et al., 2021). The fieldwork occurred every two months from March 2018 to May 2019 encompassing two weeks in each area.

The PEP is a conservation unit of 135 hectares composed of *cerradão* (tall savanna woodland) and riparian forest, and is located in the sanitary district of “Prosa” (Figure 1B). Human access is restricted in the PEP, but there is daily flow of people in the adjacent areas, the urban park “Parque das Nações Indígenas” and the public administration area “Parque dos Poderes” located in a forest remnant of Cerrado. Coatis have free access in all the three areas either by crossing the paved

streets or passing through the branches of the trees near the borders (Figures 2A, B). In addition, the PEP presents a diverse mammalian fauna and shelters a Wildlife Rehabilitation Center (CRAS) (<https://www.imasul.ms.gov.br/centro-de-reabilitacao-de-animaes-silvestres-cras/>) that receives and keeps several wild animals from all over the MS state.

The VBA, located in the sanitary district of “Lagoa”, is a complex of 197 hectares divided into a military operational area and a residential area surrounded by a dense vegetation that includes riparian forest and *veredas* (a plant formation with hydromorphic soil located in the vicinity of river springs) (Figure 1C). The residential portion is inhabited by at least 730 humans who raise domestic animals such as dogs, cats and chickens. The houses are not fenced and have many trash cans in front, which serve as food source for the coatis (Figures 2C, D). During fieldwork, few individuals of other wild mammal species were seen in the area, as the nine-banded armadillo (*Dasybus novemcinctus*), Azara’s agouti (*Dasyprocta azarae*), Brazilian guinea pig (*Cavia aperea*) and howler-monkey (*Alouatta caraya*), and other species such as the coati and Capybara (*Hydrochoerus hydrochaeris*) are abundant.

The coatis were captured and randomly recaptured in box traps (90 × 45 × 50; Equipos Fauna[®], Brazil) baited with 15 to 25 grams of bacon. All the captured coatis were sedated with an association of tiletamine hydrochloride and zolazepam hydrochloride (Telazol 100g; Zoetis[®], USA), tagged with subcutaneous transponders (Animal Tag[®], Brazil) and numbered colored ear-tags (Qualyplast[®] Brazil). Blood samples were collected through



femoral venipuncture using 0.25 x 0.8 mm needles (BD Vacutainer[®]) and deposited in clot activator tubes to obtain the serum. Bone marrow (BM) samples were collected from manubrium sterni of animals > 2.5 kg using 0.40 x 1.2 mm hypodermic needles and 10 mL syringes, and then placed in tubes containing Ethylenediamine Tetraacetic Acid (EDTA). These procedures were performed after appropriate asepsis of the collection site using bactericide soap, iodized ethanol and ethanol 70%. Ear skin fragments (0.4 to 30 mg) were obtained with sterilized material and stored in 99% ethanol (Sigma-Aldrich[®], United States). After total recovery from anesthesia, the animals were released at the capture site.

The field procedures were conducted in accordance with the *Instituto Chico Mendes de Conservação da Biodiversidade* (license number 56912-2), the *Instituto de Meio Ambiente de Mato Grosso do Sul* (license number 05/2017, process No.61/405959/2016), and Air force cooperation agreement (No.01/GAP-CG/2018). The present study was approved by the Ethics Committee for Animal Use of Universidade Católica Dom Bosco, Campo Grande, MS (license number 001/2017).

2.2 Serological detection of *Leishmania infantum*

A serological survey for the detection of anti-*L. infantum* IgG was performed through the Enzyme-Linked

Immunesorbent Assay (ELISA) (modified from EIE[®], BioManguinhos, Rio de Janeiro, Brazil) (Alves et al., 2016) (Figure 3). The sera of the coatis were tested with anti-Raccoon IgG HRP conjugated (Bethyl Laboratories[®], Inc., Montgomery, Texas, United States), diluted at 1:70,000, and each microtiter polystyrene plate contained positive and negative control samples in duplicates. The cut-off point was established by the mean Optical Density (OD) of the negative control \pm three standard deviations and the gray range adopted was 20% above the cut-off value. Serum samples were also submitted to a Dual Path Platform chromatographic immunoassay (DPP[®] CVL BioManguinhos, Rio de Janeiro, Brazil) for rapid detection of K26/K39 specific antibodies against *L. infantum* (da Costa et al., 2003; Rodrigues et al., 2022). To check the reliability of rapid test, the serum samples were tested for Protein A affinity, showing high affinity, as similar as the dog serum samples (data not shown). To improve the accuracy of the serological detection of *L. infantum*, the serum samples of coatis were also screened for *Trypanosoma cruzi* infection using ELISA according to Alves et al. (2016), and the Chagas/Bio-Manguinhos Lateral Flow Immunochromatographic Rapid Test (Chagas-LFRT), as described by Rodrigues et al. (2022). All the tests were performed according to the manufacturer's recommendations, and seropositivity was considered only when the samples presented positivity in both anti-*L. infantum* IgG ELISA and DPP[®] CVL.



2.3 Parasitological and molecular detection of *Leishmania infantum*

The axenic cultures were performed by placing 200 μ L of BM aspirates in duplicates in biphasic media, Novy-MacNeal-Nicolle (NNN) with Schneider's insect medium overlay supplemented with 10% fetal bovine serum. The tubes were incubated at 26°C – 28°C and weekly examined for 30 days. After the exponential growth phase of the flagellates, the positive culture was cryopreserved at – 196°C under liquid nitrogen. The promastigote forms of the isolated culture were deposited in the Collection of *Leishmania* (CLIOC) (clioc.fiocruz.br) of Instituto Oswaldo Cruz and characterized by them using isoenzyme electrophoresis of G6PDH and 6PGDH (Cupolillo et al., 1994).

Genomic DNA was extracted from 200 μ L of the BM samples through the phenol-chloroform method according to Sambrook and Russell (2001), and from the skin fragments using the DNeasy[®] Blood and Tissue Kit (Qiagen[®], Netherlands). The DNA was eluted in 50 μ L elution buffer and stored at –20°C until molecular analysis. The concentration and quality were assessed through spectrophotometry (Biodrop, Analítica[®], Brazil).

The BM and skin samples were firstly screened through Polymerase Chain Reaction (PCR) using a pair of primers (L1/L2) directed to the kinetoplastid DNA (kDNA) minicircle molecules of *Leishmania* spp. according to Schubach et al. (1998). Positive samples were then submitted to PCR targeting the heat-shock protein 70 gene (HSP70) and sequenced, as described by Graça et al. (2012) (Figure 3). Sanger sequencing was performed in the AB3500 platform (Applied Biosystems[®], United States) at ACTGene Molecular Analysis biotechnology company (<https://actgene.com.br/>). Negative samples at HSP70 PCR were submitted to *L. infantum*-specific kDNA PCR (MC1/MC2) according to Cortes et al. (2004) (Figure 3). All the PCRs were conducted in a thermocycler (GeneAmp PCR

System 9600, Applied Biosystems[®], United States), using positive controls of *L. infantum* (LHV14 strain) and *L. amazonensis* (IFLA/BR/1967/PH8 strain) and negative controls (ultrapure water). The PCR products were visualized on 1.5% agarose (Kasvi[®], Brazil), stained with Gel Red Nucleic Acid Stain (Biotium[®], EUA).

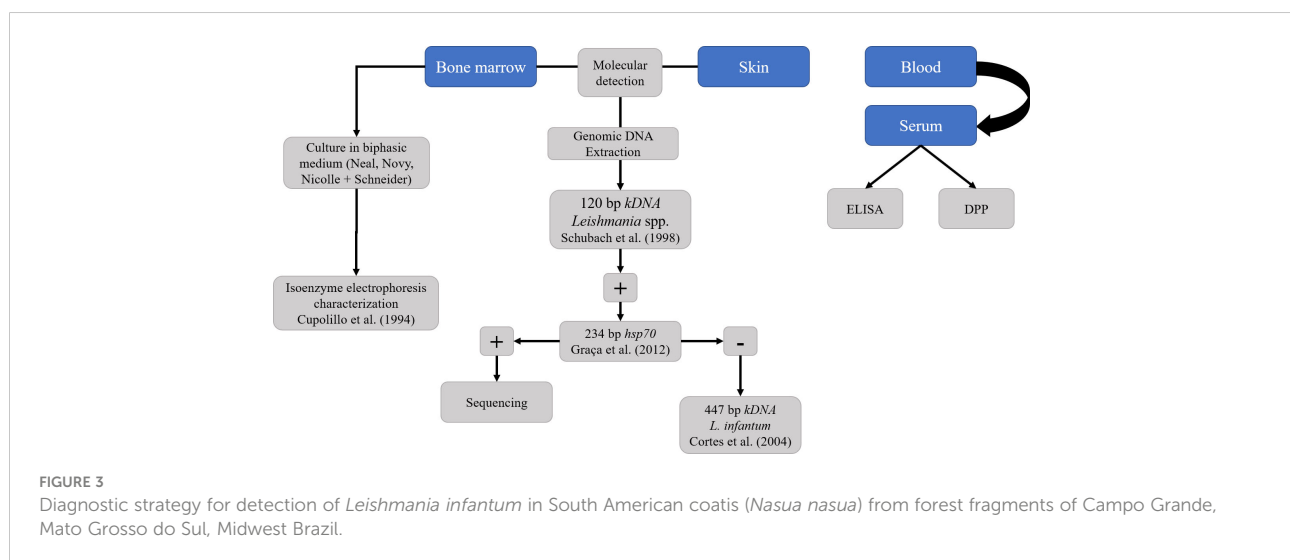
2.4 Statistical analysis

In order to observe the agreement between serological assays (ELISA and DPP[®] CVL), the Cohen's Kappa test was performed with a 95% confidence interval and interpreted as follows: poor agreement ($k = 0$), slight agreement ($0.20 \leq k < 0.40$), fair agreement ($0.40 \leq k < 0.61$), moderate agreement ($0.6 \leq k < 0.81$), substantial agreement ($0.80 \leq k < 0.91$), and almost perfect agreement ($1.0 \leq k < 1.0$). To check a possible influence of the studied areas and sex on *L. infantum* infection, a Chi-squared test ($p < 0.05$) was applied considering the individuals. We considered infection by *L. infantum* as positivity in any of the parasitological or molecular tests and/or positivity in both serological assays. Both Cohen's Kappa and Chi-squared tests were performed through the R software (R Development Core Team, 2015).

3 Results

3.1 Captures, recaptures and acquisition of samples

During the study, a total of 110 coatis were captured in the VBA and PEP. Blood was collected in all capture occasions corresponding to 21 males and 30 females from PEP, and 25 males and 34 females from VBA. The BM samples were collected



from 85 individuals corresponding to 16 males and 26 females from PEP, and 19 males and 24 females from VBA. Skin samples were collected from 65 ear-tagged animals corresponding to 10 males and 20 females from PEP, and 14 males and 21 females from VBA.

Throughout the study, we recaptured a total of 41 individuals (eight males and eight females from PEP, and 10 males and 15 females from VBA) in 80 occasions (Figure 4). Serum samples were collected, and serologic tests were performed in all recaptures, totalizing 190 samples (73 from PEP and 117 from VBA). Bone marrow samples were collected from 51 recaptures and submitted to molecular analysis (totalizing 136 samples = 63 from PEP and 73 from VBA). Skin samples were not collected during recaptures.

3.2 Serological detection of *Leishmania infantum*

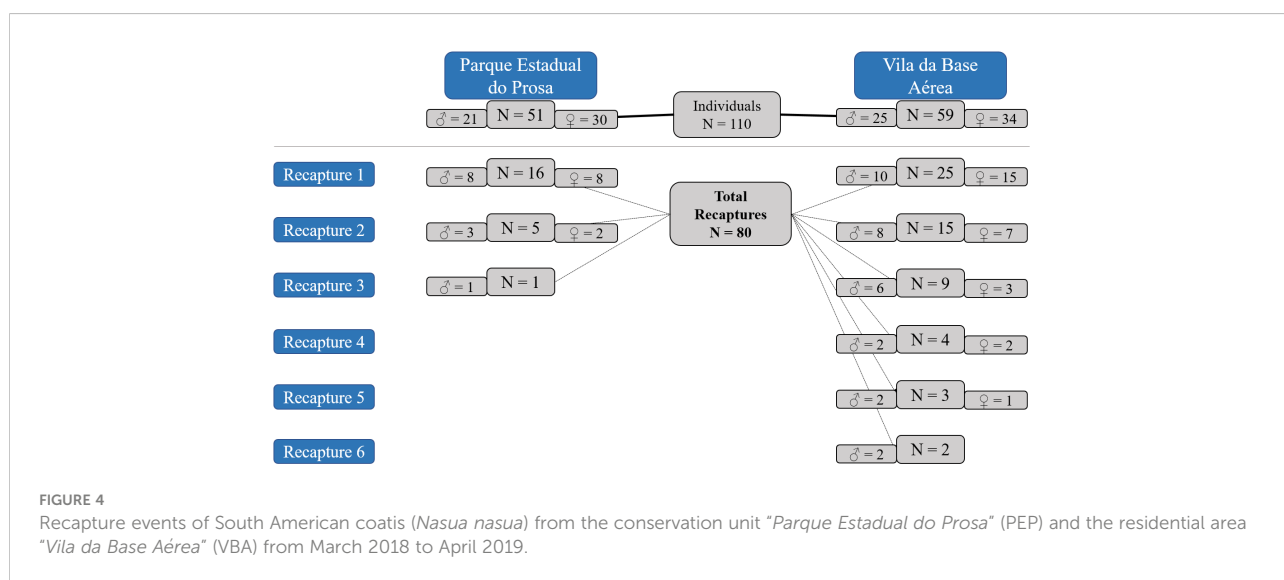
A total of 65 samples from 33 individuals were seropositive (ELISA+/DPP+) to *L. infantum*, indicating an overall seroprevalence of 30% (33/110). In PEP, the seroprevalence rate was 11.8% (6/51), corresponding to two males and four females, while in VBA the seroprevalence was estimated in 45.8% (27/59), corresponding to 11 males and 16 females. Despite the discordance observed in 19 samples (16 ELISA+/DPP- and three ELISA-/DPP+), the Cohen's kappa test attested a substantial agreement between ELISA and DPP ($k=0.78$; $CI_{95\%}=0.68-0.87$; $p=0.001$). Only one serum sample of a non-recaptured female coati from VBA, that was seropositive to *L. infantum*, presented seropositivity for *T. cruzi*, indicating a mixed infection.

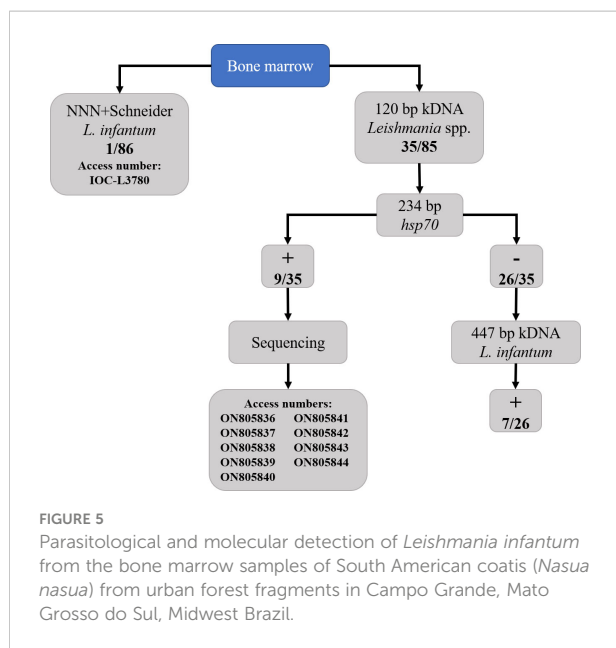
3.3 Parasitological and molecular detection of *Leishmania infantum*

During our study, we isolated *L. infantum* from one BM sample derived from a seropositive male captured in VBA (ID 11528) (Figure 5). This isolate was deposited in the CLIOC (access number: IOC-L 3780), but the molecular diagnosis was not performed in this individual due to insufficient amount of BM sample.

The screening kDNA PCR in BM samples showed an infection rate of 41.2% (35/85) for *Leishmania* sp. Posteriorly, *L. infantum* infection was confirmed in 16 individuals (nine by HSP70 sequencing and seven by MC1/MC2 PCR), seven from PEP (four males and three females) and nine from VBA (three males and six females). The results of parasitological and molecular assays performed in the coatis are presented in Figure 5. From the 19 individuals negative to *L. infantum* in molecular tests, four were classified as seropositive and 15 were seronegative. Only one skin sample was positive in kDNA PCR and *L. infantum* infection was confirmed by MC1/MC2 PCR. The same individual (ID 10826, a male from VBA) was seropositive and also positive in BM molecular assay (with *L. infantum* infection confirmed by HSP70 sequencing).

The BLASTn analysis of the nine HSP70 DNA sequences revealed identity of 100% with *L. donovani* HSP70 gene obtained from samples of humans from Ethiopia and China (accession numbers: FN669773.1 and JX970996.1 respectively). The obtained sequences were deposited in the GenBank database under the following accession numbers: ON805836 to ON805844 (Figure 5).





3.4 Data analysis

Together, the parasitological, serology and molecular tests showed a *L. infantum* infection rate of 36.4% (40/110). These 40 individuals correspond to 24 positive by serological tests (including an individual positive in parasitological test), seven positive by molecular tests, and nine positive by both tests. We observed through Chi-squared test that infection was significantly higher in VBA (49.1%, 29/59) than in PEP (21.6%, 11/51) ($Chi^2 = 90$; $df = 1$; $p = 0.003$). Our analysis did not show influence of sex in *L. infantum* infection ($Chi^2 = 1.580$; $df = 1$; $p = 0.208$).

When considering only individuals assessed by both serology and molecular tests ($N = 85$), we observed distinct *L. infantum* infection patterns (Table 1).

3.5 Longitudinal survey

Among the 40 coatis positive for *L. infantum*, 19 individuals were recaptured during the study at least on one occasion.

Eleven individuals remained positive by at least one diagnostic test throughout their recaptures (Figure 6). Of these, seven were only seropositive (IDs 10433, 10435, 10439, 10442, 10508, 10999 and 11522) and four presented positivity also in molecular tests (IDs 10443, 10444, 10507 and 10509). Five individuals (IDs 10320, 10322, 10323, 10440 and 10831) presented positivity (four by MC1/MC2 PCR and one by serological test) either in the capture or in the first recapture, and negativity in the subsequent recapture (Figure 6). We observed three seroconversion events, with individuals (IDs, 10441, 10511 and 10513) presenting positivity only in the subsequent recaptures (Figure 6). The remaining twenty-one positive animals were not recaptured: 13 were seropositive, five were positive in both serology and molecular tests and three were positive only in molecular assays.

4 Discussion

In this study we report serological, parasitological and molecular detection of *L. infantum* in coatis from forested fragments of an endemic region for VL, with an overall infection rate of 36.4% (40/110). Our results demonstrate that this wild carnivore species is somehow affected by the *L. infantum* epidemiological scenario at the studied area. Indeed, (i) the endemicity of VL in humans and dogs at CG (SES/MS, 2022), (ii) abundance of the main vector *L. longipalpis* s.l. (de Souza Fernandes et al., 2022), and (iii) the high density of coatis in forest fragments of CG (Barreto et al., 2021) may favor the *L. infantum* transmission to coatis. Future studies may indicate the genetic flow of *L. infantum* populations among the multiple hosts involved in this epidemiological scenario, which was not the aim of the present study. The evaluation of recaptured individuals was a particular aspect of the present study because it provided evidence of long-term exposure of *L. infantum* in the studied populations, including seroconversion events.

Our findings indicated that *L. infantum* infect coatis from both studied forest fragments in CG, but a higher rate of infection in VBA when compared to PEP was observed ($p = 0.003$), indicating a spatial influence. This finding may be related to the geographical localization of the studied areas, as well as differences

TABLE 1 South American coatis (*Nasua nasua*) infected by *Leishmania infantum* by both serological and molecular diagnosis at urban forest fragments in Campo Grande, Mato Grosso do Sul, Brazil.

Areas	Serology + Molecular -	Serology -Molecular +	Serology + Molecular +	Total
PEP	4/42 (9.5%)	5/42 (11.9%)	2/42 (4.8%)	11/42 (26.2%)
VBA	16/43 (37.2%)	2/43 (4.6%)	7/43 (16.3%)	25/43 (58.1%)
Total	20/85 (23.5%)	7/85 (8.2%)	9/85 (10.6%)	36/85 (42.3%)

PEP: conservation unit "Parque Estadual do Prosa"; VBA: residential área "Vila da Base Aérea". Four individuals from VBA (two male and two female) were excluded from this table because no molecular assay was performed.

in land use. For instance, VBA is a residential area with many dogs, the main domestic reservoir host of *L. infantum*, and chickens, which although refractory to *Leishmania* infection, are amplifiers and attractors of sandfly populations (Casanova et al., 2013). Even though maximal dispersion of *L. longipalpis* is reported to be 243 meters in urban areas (de Oliveira et al., 2013), Coatis at VBA often forage near houses looking for food in outdoor trash cans, which may favor their exposition to with *L. longipalpis* in this environment. As described by Barreto et al. (2021), VBA has a denser population of coatis than PEP, and in this area the animals are prevented from dispersing out due to the physical barrier and the lack of functional connectivity in the surroundings. In addition, VBA is in the sanitary district of “Lagoa”, a region that had the higher number of notified cases and cumulative incidence of human VL from 2002 to 2009 (Brazuna et al., 2012). This sanitary district also had the second highest number of canine VL from 2007 to 2009 in CG, and a large sandfly abundance (Brazuna et al., 2012; de Souza Fernandes et al., 2022).

Oppositely, the lower infection rate observed in PEP (21.6%) may be related to several factors including the absence or low abundance of competent reservoirs, a higher richness of mammal species than VBA, and the CRAS, that is located in this conserved area. Although the coatis have access to the adjacent areas *Parque das Nações Indígenas* and *Parque dos Poderes*, there are no resident dogs in these areas that could sustain high rates of *L. infantum* infection, as in VBA. Also, there are 17 mammal species listed in the PEP Management Plan, and this number can be higher if we consider the captive animals of the CRAS, while in VBA few species were sighted. Assuming that the PEP presents a higher diversity of wild mammal species in comparison to VBA, we can expect that the PEP has a higher

number of hosts not competent to be source of infection for the vector, which could reduce the risk of *L. infantum* infection in coatis through dilution effect (Keesing and Ostfeld, 2021). In addition, the CRAS, located inside the PEP, may influence the distribution of *L. longipalpis* due to the constant presence of captive animals and large amount of organic material from feces and concentrate food that could attract the vector and avoid its dispersion.

The detection of *L. infantum* in an ear skin fragment of an individual that was also seropositive and presented *L. infantum* DNA in BM sample (ID 10826), indicates that *L. infantum* is capable to colonize intact skin of coatis. In view of the importance for *L. infantum* transmission, domestic dogs are the main reservoir species mainly because they can present high parasitic loads in the skin, favoring the infection of the vectors (Dantas-Torres, 2007). Wild carnivores, as maned wolf, bush dog and crab-eating fox have been reported presenting *L. infantum* DNA in skin (Figueiredo et al., 2008; Souza et al., 2010; Jusi et al., 2011), although the first two demonstrated low competence to transmit the parasite to the vectors. Since the maintenance of a multi-host parasite as *L. infantum* in a given environment can involve different mammal species with diverse epidemiological competences (Roque and Jansen, 2014), efforts toward evaluation of blood meal source of sand flies in the forest fragments of CG, as well as isolation and quantification of parasite load from skin of different mammal species must be considered. Our results put the coati together with maned wolf and bush dog as carnivore species that may harbor parasite in intact skin. Nevertheless, their role in *L. infantum* epidemiology remains to be uncovered.

Our data showed no significant differences related to sex in the infection rate of *L. infantum* infection. The sex-related

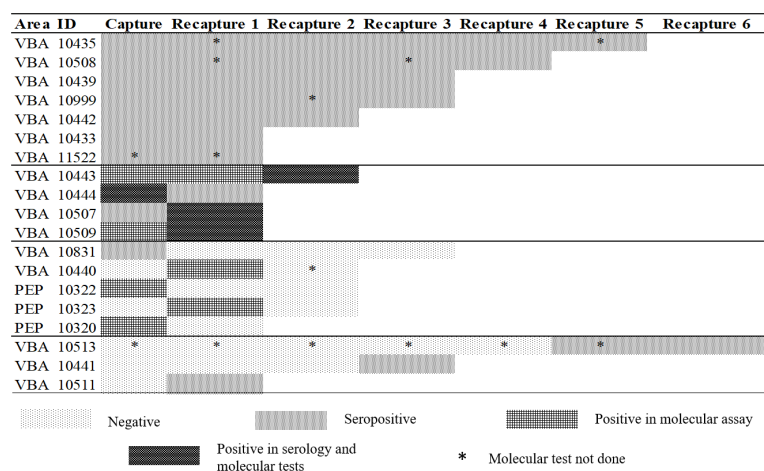


FIGURE 6 Longitudinal results of 19 South American coatis (*Nasua nasua*) infected by *Leishmania infantum* in urban forest fragments in Campo Grande, Mato Grosso do Sul, Midwest Brazil, from March 2018 to April 2019. ID: Identification number; PEP: conservation unit *Parque Estadual do Prosa*; VBA: residential rea *Vila da Base Aérea*. Positivity in molecular was obtained either by HSP70 sequencing or MC1/MC2 PCR. Seropositivity was considered when both ELISA and DPP® CVL tests were positive.

response to *L. infantum* infection includes a complex interaction among host, vector, and environment (Lockard et al., 2019), and therefore the sex differences in the prevalence of VL may not have a pattern. While higher VL prevalence in males have been recorded in humans and dogs naturally and experimentally infected (Guerra-Silveira and Abad-Franch, 2013; Lockard et al., 2019), studies with long-term data have shown no differences in the seroprevalence of *L. infantum* infection between male and female stray dogs (Miró et al., 2007; Müller et al., 2022). Risueño et al. (2018) found only a marginally greater *L. infantum* prevalence in female compared to male free-living rabbits. Given the nature of our data, we could not discriminate whether the absence of sex difference in the infection rate of *L. infantum* was due to biological differences or sex-related exposure. However, we can presume that social behavior of coatis in the urban matrix can contribute to a similar rate of infection between male and female. Coatis form social groups consisting of adult females and their offspring, with adult males joining the group only in the reproductive period (Gompper and Decker, 1998). However, Barreto et al. (2021) observed that males are cohesive to the group regardless of the reproductive period.

Our study showed that many individuals presented distinct infection patterns concerning their results in the serological and molecular assays. This dynamic, as well events of new infections observed in the longitudinal studies are expected in populations in endemic areas because individuals are constantly being exposed to infected vectors (Herrera et al., 2004; Oliva et al., 2006). As observed, seven seronegative coatis displayed positivity in the molecular assays suggesting recent infections, a common phenomenon found in populations inhabiting endemic areas (Herrera et al., 2004; Oliva et al., 2006). In dogs, the humoral response may take around three to five months after *L. infantum* infection to produce sufficient immunoglobulins to be detected by serological tests (Paranhos-Silva et al., 2003). It is important to mention that in endemic areas for leishmaniasis, about one fifth of *L. infantum* infected dogs presents low or undetectable anti-*Leishmania* antibodies (Ashford et al., 1995). On the contrary, seropositive animals with negative molecular results are expected because the serological assays detect antibodies that are maintained in serum for longer periods, while the molecular assays are positive only when parasites are present in the specific amount of analyzed sample. We highlight the importance of combine different diagnostic tests in order to improve the detectability of infected individuals.

The molecular diagnostic strategy used here allowed us to detect a greater number of positive samples. In fact, as the sensitivity of the HSP70 gene target is low when compared to the kDNA PCR assay (Graça et al., 2012), and the use of MC1/MC2 (*L. infantum* kDNA), as it is more sensitive and highly specific for *L. donovani* s.l., improved the detectability of molecular diagnostics. Concerning the recaptures, molecular negativity observed in the first recaptures of the individuals 10320 and 10322, and in the second recapture of the

individual 10323 (Figure 6) could be attributed to fluctuations in detectable DNA from the bone marrow. Transient PCR bone marrow positivity has been reported in dogs naturally and experimentally infected with *L. infantum* (Paranhos-Silva et al., 2003; Oliva et al., 2006). As discussed by these authors, this may also be attributed to limited sensitivity of specific molecular tests, in our case, MC1/MC2 and HSP70, especially in the first and second recaptures of the individuals 10320 and 10323 respectively, which were positive in the screening kDNA for *Leishmania* spp. A rare case of seroreversion was observed in one individual (10831) that was seropositive in the capture and seronegative in the three subsequent recaptures (Figure 6). Seroreversion was already reported in both canine and human VL (Ostyn et al., 2011; Maia et al., 2020), probably by decrease of antibody concentration over time, as observed in the ELISA OD from this individual (Capture: 0,168; First recapture: 0,149; Second recapture: 0,104; Third recapture: 0,075).

In contrast, we found evidence of active transmission of *L. infantum* in the area, evidenced by new infections occurring in coatis during the study period. Indeed, five individuals were seronegative in the capture or in a given recapture, and positive by serology or molecular diagnosis in subsequent recapture. Two individuals (IDs 10443 and 10509) remained positive in molecular test in consecutive captures, indicating that individuals may be capable of sustain BM parasitism for at least five months. At this point, the remaining question is whether these animals are capable of sustain high parasite loads in the skin and act as source of infection to sand flies.

Our results point to the occurrence of other *Leishmania* species besides *L. infantum* in the sampled coatis, since (i) 54.3% (19/35) of the infections detected by kDNA PCR were not confirmed by *L. infantum*-specific targets, and (ii) we found 16 samples ELISA positive and DPP negative that were also negative in the serological tests performed to detect other common trypanosomatids that infects wild mammals: *T. cruzi*. Geographical overlap among *Leishmania* species is reported (Talmi-Frank et al., 2010), and probably occurs in Brazil, which shelters the largest richness of *Leishmania* species among Latin American countries (Herrera et al., 2020). In Campo Grande municipality, Nantes et al. (2021) obtained DNA sequences with high similarity to *L. amazonensis* and *L. guyanensis* from blood samples of opossums, and Castro et al. (2020) reported *L. braziliensis* infection in the bats *Lasiurus cinereus* and *Cynomops planirostris*. In addition, the presence of other species of *Leishmania* than *L. infantum* circulating in coatis of Campo Grande is possible due to the occurrence of many phlebotomine species as *Nyssomyia whitmani*, *Ny. antunesi* and *Bichromomyia flaviscutellata*, that are involved in transmission of several species of *Leishmania* commonly reported in outbreaks of cutaneous leishmaniasis such as *L. braziliensis*, *L. shawi*, *L. guyanensis*, *L. lindenbergi* and *L. amazonensis* (Costa et al., 2007; Silva et al., 2008; Oliveira

et al., 2012; Vásquez Trujillo et al., 2013; Carvalho et al., 2018; de Souza Fernandes et al., 2022).

5 Conclusion

Coatis living in urban forested fragments at CG are affected by the epidemiological scenario of VL transmission that involves dogs, humans and *L. longipalpis* vectors, as demonstrated by their overall infection rate of 36.4%. The importance of this finding is highlighted by: (i) the high population densities of this mammal host in different urban forest fragments, (ii) their proximity to domiciliated areas and contact with human and dogs, (iii) the ability to maintain viable parasites in bone marrow, as demonstrated by *L. infantum* isolation and (iv) the possibility to maintain parasites in no lesioned ear skin, as demonstrated by *L. infantum* DNA detection. Given these findings, we highlight the importance to investigate the pattern of *L. infantum* infection in coatis inhabiting urban forest fragments to define their role as *L. infantum* reservoirs in endemic areas for VL.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article.

Ethics statement

The animal study was reviewed and approved by Ethics Committee for Animal Use of Universidade Católica Dom Bosco, Campo Grande, MS (license number 001/2017).

Author contributions

GCdeM, WTGB, ALRR and HMH wrote the manuscript. GCdeM, WTGB, ACR and WDeA collected biological samples. SCdasCX, FMA and ARdaS contributed in diagnostic tests execution. FMS performed statistical analysis. CEdeO, GEdeOP, SCdasCX, FMA, GBdeA, AMJ, ALRR and HMH critically reviewed the manuscript. All authors contributed to the article and approved the submitted version.

References

Alves, F. M., de Lima, J. S., Rocha, F. L., Herrera, H. M., Mourão, G., de, M., et al. (2016). Complexity and multi-factoriality of *Trypanosoma cruzi* sylvatic cycle in coatis, *Nasua nasua* (Procyonidae), and triatomine bugs in the Brazilian pantanal. *Parasit Vectors*. 9 (1), 378. doi: 10.1186/s13071-016-1649-4

Funding

This research was supported by the Foundation for Support to the Development of Education, Science and Technology of the State of Mato Grosso do Sul (FUNDECT) (Process 59/300.187/2016), Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) (Process E-26/202.994/2019 and E-26/010.002276/2019), the National Council for Scientific and Technological Development (CNPq) to HMH (Process 308768/2017-5), AMJ (PQ1-A) and ALRR (CNPq Process 425293/2018-1), and scholarship to FMS (process 88887.369261/2019-00), GCdeM (process 88887.149245/2017-00) and WTGB (process 150911/2021-1). The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Acknowledgments

The authors are grateful to the Base Aérea de Campo Grande (BACG) for the cooperation with the field works, and to the Dr. João Santana da Silva from the University of São Paulo (USP) and Dra. Carla Cardozo Pinto Arruda from the Federal University of Mato Grosso do Sul (UFMS), for providing the *L. infantum* and *L. amazonensis* positive controls respectively. The authors are especially thankful to the Insana Huna Research Group (www.insanahuna.com) for the fieldwork support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Antunes, T. R., Peixoto, R. A. V., Oliveira, B. B., Sorgatto, S., do Nascimento Ramos, C. A., and de Souza, A. I. (2016). Detection of *Leishmania infantum* in peripheral blood smear and lymph node of a domestic feline. *Acta Sci. Vet.* 44 (Suppl 1), 162. doi: 10.22456/1679-9216.83208

- Ashford, D. A., Bozza, M., Freire, M., Miranda, J. C., Sherlock, I., Eulalio, C., et al. (1995). Comparison of the polymerase chain reaction and serology for the detection of canine visceral leishmaniasis. *Am. J. Trop. Med. Hyg.* 53, 251–255. doi: 10.4269/ajtmh.1995.53.251
- Barreto, W. T. G., Herrera, H. M., de Macedo, G. C., Rucco, A. C., de Assis, W. O., Oliveira-Santos, L. G., et al. (2021). Density and survivorship of the south American coati (*Nasua nasua*) in urban areas in central–Western Brazil. *Hystrix It. J. Mamm.* 32 (1), 82–88. doi: 10.4404/hystrix-00386-2020
- Botelho, A. C., and Natal, D. (2009). First epidemiological description of visceral leishmaniasis in campo grande, state of mato grosso do sul. *Rev. Soc. Bras. Med. Trop.* 42 (5), 503–508. doi: 10.1590/s0037-86822009000500006
- Brazuna, J. C., Silva, E. A., Brazuna, J. M., Domingos, I. H., Chaves, N., Honer, M. R., et al. (2012). Profile and geographic distribution of reported cases of visceral leishmaniasis in campo grande, state of mato grosso do sul, Brazil, from 2002 to 2009. *Rev. Soc. Bras. Med. Trop.* 45 (5), 601–606. doi: 10.1590/s0037-86822012000500012
- Carreira, J. C., da Silva, A. V., de Pita Pereira, D., and Brazil, R. P. (2012). Natural infection of *Didelphis aurita* (Mammalia: Marsupialia) with *Leishmania infantum* in Brazil. *Parasitol. Vectors.* 5, 111. doi: 10.1186/1756-3305-5-111
- Carvalho, B. M., dos Santos, T. V., da R. Barata, I., Lima, J. A. N., Silveira, F. T., Vale, M. M., et al. (2018). entomological surveys of *Lutzomyia flaviscutellata* and other vectors of cutaneous leishmaniasis in municipalities with records of *Leishmania amazonensis* within the bragança region of par  state, brazil. *J. Vector Ecol.* 43 (1), 168–178. doi: 10.1111/jvec.12296
- Casanova, C., Andrighetti, M. T., Sampaio, S. M., Marcoris, M. L., Colla-Jacques, F. E., and Prado, A. P. (2013). Larval breeding sites of *Lutzomyia longipalpis* (Diptera: Psychodidae) in visceral leishmaniasis endemic urban areas in southeastern Brazil. *PLoS Negl. Trop. Dis.* 7 (9), e2443. doi: 10.1371/journal.pntd.0002443
- Castro, L. S., Dorval, M. E. C., Matheus, L. M. D., Bednaski, A. V., Facco, G. G., Silveira, M., et al. (2020). *Leishmania* presence in bats in areas endemic for leishmaniasis in central-west Brazil. *Int. J. Parasitol. Parasites Wildl.* 11, 261–267. doi: 10.1016/j.ijppaw.2020.02.008
- Cortes, S., Rol o, N., Ramada, J., and Campino, L. (2004). PCR as a rapid and sensitive tool in the diagnosis of human and canine leishmaniasis using leishmania donovani s.l.-specific kinetoplastid primers. *Trans. R. Soc. Trop. Med. Hyg.* 98 (1), 12–17. doi: 10.1016/s0035-9203(03)00002-6
- Costa, S. M., Cechinel, M., Bandeira, V., Zannuncio, J. C., Lainson, R., and Rangel, E. F. (2007). *Lutzomyia (Nyssomyia) whitmani* s.l. (Antunes & coutinho 1939) (Diptera: Psychodidae: Phlebotominae): geographical distribution and the epidemiology of American cutaneous leishmaniasis in Brazil – mini review. *Mem. Inst. Oswaldo Cruz.* 102, 149–153. doi: 10.1590/s0074-02762007005000016
- Courtenay, O., Quinnell, R. J., Garcez, L. M., and Dye, C. (2002). Low infectiousness of a wildlife host of *Leishmania infantum*: the crab-eating fox is not important for transmission. *Parasitol* 125 (Pt 5), 407–414. doi: 10.1017/s0031182002002238
- Courtenay, O., Santana, E. W., Johnson, P. J., Vasconcelos, I. A., and Vasconcelos, A. W. (1996). Visceral leishmaniasis in the hoary zorro *Dusicyon vetulus*: a case of mistaken identity. *Trans. R. Soc. Trop. Med. Hyg.* 90 (5), 498–502. doi: 10.1016/s0035-9203(96)90293-x
- Cunha, R. C., Andreotti, R., Cominetti, M. C., and Silva, E. A. (2014). Detection of *Leishmania infantum* in *Lutzomyia longipalpis* captured in campo grande, MS. *Rev. Bras. Parasitol. Vet.* 23 (2), 269–273. doi: 10.1590/S1984-29612014049
- Cupolillo, E., Grimaldi, G. Jr, and Momen, H. (1994). A general classification of new world *Leishmania* using numerical zymotaxonomy. *Am. J. Trop. Med. Hyg.* 50 (3), 296–311. doi: 10.4269/ajtmh.1994.50.296
- da Costa, R. T., Franca, J. C., Mayrink, W., Nascimento, E., Genaro, O., and Campos-Neto, A. (2003). Standardization of a rapid immunochromatographic test with the recombinant antigens K39 and K26 for the diagnosis of canine visceral leishmaniasis. *Trans. R. Soc. Trop. Med. Hyg.* 97 (6), 678–682. doi: 10.1016/s0035-9203(03)80102-5
- Dantas-Torres, F. (2007). The role of dogs as reservoirs of *Leishmania* parasites, with emphasis on *Leishmania (Leishmania) infantum* and *Leishmania (Viannia) braziliensis*. *Vet. Parasitol.* 149, 139–146. doi: 10.1016/j.vetpar.2007.07.007
- Dantas-Torres, F., and Brand o-Filho, S. P. (2006). Visceral leishmaniasis in Brazil: revisiting paradigms of epidemiology and control. *Rev. Inst. Med. Trop. Sao Paulo.* 48 (3), 151–156. doi: 10.1590/s0036-46652006000300007
- Deane, L. M., and Deane, M. P. (1955). Observa es preliminares sobre a import ncia comparativa do homem, do c o e da raposa (*Lycalopex vetulus*) como reservat rios da *Leishmania donovani* em  reas end micas de calazar, no cear . *Hospital* 48, 79–98.
- de Castro Ferreira, E., Cruz, I., Ca avate, C., de Melo, L. A., Pereira, A. A., Madeira, F. A., et al. (2015). Mixed infection of *Leishmania infantum* and *Leishmania braziliensis* in rodents from endemic urban area of the new world. *BMC Vet. Res.* 11, 71. doi: 10.1186/s12917-015-0392-y
- de Oliveira, E. F., Silva, E. A., Casaril, A. E., Fernandes, C. E., Paranhos Filho, A. C., Gamarra, R. M., et al. (2013). Behavioral aspects of *Lutzomyia longipalpis* (Diptera: Psychodidae) in urban area endemic for visceral leishmaniasis. *J. Med. Entomol.* 50 (2), 277–284. doi: 10.1603/me12082
- de Rezende, M. B., Herrera, H. M., Carvalho, C. M. E., Carvalho Anjos, E. A., Ramos, C. A. N., de Ara jo, F. R., et al. (2017). Detection of leishmania spp. in bats from an area of Brazil endemic for visceral leishmaniasis. *Transbound Emerg. Dis.* 64 (6), e36–e42. doi: 10.1111/tbed.12597
- de Sousa, K. C., Andr , M. R., Herrera, H. M., de Andrade, G. B., Jusi, M. M., dos Santos, L. L., et al. (2013). Molecular and serological detection of tick-borne pathogens in dogs from an area endemic for *Leishmania infantum* in mato grosso do sul, Brazil. *Rev. Bras. Parasitol. Vet.* 22 (4), 525–531. doi: 10.1590/S1984-29612013000400012
- de Souza Fernandes, W., de Oliveira Moura Infran, J., Falc o de Oliveira, E., Etelvina Casaril, A., Petilim Gomes Barrios, S., Lopes de Oliveira, S. L., et al. (2022). Phlebotomine sandfly (Diptera: Psychodidae) fauna and the association between climatic variables and the abundance of *Lutzomyia longipalpis* sensu lato in an intense transmission area for visceral leishmaniasis in central Western Brazil. *J. Med. Entomol.* 59 (3), 997–1007. doi: 10.1093/jme/tjac006
- Estevam, L. G. T. M., Fonseca Junior, A. A., Silvestre, B. T., Hemetrio, N. S., Almeida, L. R., Oliveira, M. M., et al. (2020). Seven years of evaluation of ectoparasites and vector-borne pathogens among ring-tailed coatis in an urban park in southeastern Brazil. *Vet. Parasitol. Reg. Stud. Rep.* 21, 100442. doi: 10.1016/j.vprsr.2020.100442
- Falc o de Oliveira, E., Oliveira, A. G., Arruda, C. C. P., Fernandes, W. S., and Medeiros, M. J. (2020). Spatio-temporal modeling of visceral leishmaniasis in Midwest Brazil: An ecological study of 18-years data, (2001–2018). *PLoS One* 15 (10), e0240218. doi: 10.1371/journal.pone.0240218
- Figueiredo, F. B., Gremi o, I. D., Pereira, S. A., Fedulo, L. P., Menezes, R. C., Balthazar, D. A., et al. (2008). First report of natural infection of a bush dog (*Speothos venaticus*) with *Leishmania (Leishmania) chagasi* in Brazil. *Trans. R. Soc. Trop. Med. Hyg.* 102 (2), 200–201. doi: 10.1016/j.trstmh.2007.10.001
- Furlan, M. B. G. (2010). Visceral leishmaniasis epidemic in campo grande, state of mato grosso do sul, Brazil, from 2002 to 2006. *Epidemiol. Serv. Sa de.* 19 (1), 16–25. doi: 10.5123/S1679-49742010000100003
- Gompper, M. E., and Decker, D. M. (1998). *Nasua nasua*. *Mammal Species*. 580, 1–9. doi: 10.2307/3504444
- Graça, G. C., Volpini, A. C., Romero, G. A., Oliveira Neto, M. P., Hueb, M., Porrozi, R., et al. (2012). Development and validation of PCR-based assays for diagnosis of American cutaneous leishmaniasis and identification of the parasite species. *Mem. Inst. Oswaldo Cruz.* 107 (5), 664–674. doi: 10.1590/s0074-02762012000500014
- Guerra-Silveira, F., and Abad-Franch, F. (2013). Sex bias in infectious disease epidemiology: patterns and processes. *PLoS One* 8 (4), e62390. doi: 10.1371/journal.pone.0062390
- Herrera, G., Barrag n, N., Luna, N., Mart nez, D., De Martino, F., Medina, J., et al. (2020). An interactive database of *Leishmania* species distribution in the Americas. *Sci. Data.* 7 (1), 110. doi: 10.1038/s41597-020-0451-5
- Herrera, H. M., D vila, A. M., Norek, A., Abreu, U. G., Souza, S. S., D’Andrea, P. S., et al. (2004). Enzootiology of *Trypanosoma evansi* in pantanal, Brazil. *Vet. Parasitol.* 125 (3–4), 263–275. doi: 10.1016/j.vetpar.2004.07.013
- Humberg, R. M., Oshiro, E. T., Cruz, M., do, S., Ribolla, P. E., Alonso, D. P., et al. (2012). *Leishmania chagasi* in opossums (*Didelphis albiventris*) in an urban area endemic for visceral leishmaniasis, campo grande, mato grosso do sul, Brazil. *Am. J. Trop. Med. Hyg.* 87 (3), 470–472. doi: 10.4269/ajtmh.2012.11-0534
- Jusi, M. M., Starke-Buzetti, W. A., Oliveira, T. M., Ten rio, M., da, S., de O., L., et al. (2011). Molecular and serological detection of leishmania spp. in captive wild animals from ilha solteira, SP, Brazil. *Rev. Bras. Parasitol. Vet.* 20 (3), 219–222. doi: 10.1590/s1984-29612011000300008
- Keesing, F., and Ostfeld, R. S. (2021). Dilution effects in disease ecology. *Ecol. Lett.* 24 (11), 2490–2505. doi: 10.1111/ele.13875
- Lainson, R., Braga, R. R., Souza, A. A., Povia, M. M., Ishikawa, E. A., and Silveira, F. T. (1989). *Leishmania (Viannia) shawi* sp.n., a parasite of monkeys, sloths and procyonids in Amazonian Brazil. *Ann. Parasitol. Hum. Comp.* 64 (3), 200–207. doi: 10.1051/parasite/1989643200
- Lainson, R., Dye, C., Shaw, J. J., Macdonald, D. W., Courtenay, O., Souza, A. A., et al. (1990). Amazonian Visceral leishmaniasis–distribution of the vector *Lutzomyia longipalpis* (Lutz & neiva) in relation to the fox *Cerdocoyon thous* (linn.) and the efficiency of this reservoir host as a source of infection. *Mem. Inst. Oswaldo Cruz.* 85 (1), 135–137. doi: 10.1590/s0074-02761990000100027
- Lima, B. S., Dantas-Torres, F., de Carvalho, M. R., Marinho-Junior, J. F., de Almeida, E. L., Brito, M. E., et al. (2013). Small mammals as hosts of leishmania spp. in a highly endemic area for zoonotic leishmaniasis in north-Eastern Brazil. *Trans. R. Soc. Trop. Med. Hyg.* 107 (9), 592–597. doi: 10.1093/trstmh/trt062

- Lockard, R. D., Wilson, M. E., and Rodríguez, N. E. (2019). Sex-related differences in immune response and symptomatic manifestations to infection with *Leishmania* species. *J. Immunol. Res.* 2019, 4103819. doi: 10.1155/2019/4103819
- Maia, C., Cristóvão, J., Pereira, A., Kostalova, T., Lestina, T., Sumova, P., et al. (2020). Monitoring *Leishmania* infection and exposure to *Phlebotomus perniciosus* using minimal and non-invasive canine samples. *Parasitol. Vectors.* 13 (1), 119. doi: 10.1186/s13071-020-3993-7
- Metzdorf, I. P., da Costa Lima, M. S. Jr., de Fatima Cepa Matos, M., de Souza Filho, A. F., de Souza Tsujisaki, R. A., Franco, K. G., et al. (2017). Molecular characterization of *Leishmania infantum* in domestic cats in a region of Brazil endemic for human and canine visceral leishmaniasis. *Acta Trop.* 166, 121–125. doi: 10.1016/j.actatropica.2016.11.013
- Miró, G., Montoya, A., Mateo, M., Alonso, A., García, S., García, A., et al. (2007). A leishmaniasis surveillance system among stray dogs in the region of Madrid: ten years of serodiagnosis, (1996–2006). *Parasitol. Res.* 101 (2), 253–257. doi: 10.1007/s00436-007-0497-8
- Mol, J. P., Soave, S. A., Turchetti, A. P., Pinheiro, G. R., Pessanha, A. T., Malta, M. C., et al. (2015). Transmissibility of *Leishmania infantum* from maned wolves (*Chrysocyon brachyurus*) and bush dogs (*Speothos venaticus*) to *Lutzomyia longipalpis*. *Vet. Parasitol.* 212 (3–4), 86–91. doi: 10.1016/j.vetpar.2015.08.024
- MS-BR (2019) *Vigilância em saúde no Brasil 2003/2019: da criação da secretaria de vigilância em saúde aos dias atuais. ministério da saúde (BR)*. Available at: <http://www.rets.epsviv.fiocruz.br/biblioteca/vigilancia-em-saude-no-brasil-20032019-da-criacao-dasecretaria-de-vigilancia-em-saude> (Accessed January 23, 2022).
- MS-BR (2021) *DATASUS: informações de saúde: informações epidemiológicas e morbidade (Ministério da Saúde (BR))*. Available at: <https://datasus.saude.gov.br/epidemiologicas-e-morbidade/> (Accessed January 25, 2022).
- Müller, A., Montoya, A., Escacena, C., de la Cruz, M., Junco, A., Iriso, A., et al. (2022). *Leishmania infantum* infection serosurveillance in stray dogs inhabiting the Madrid community: 2007–2018. *Parasitol. Vectors.* 15 (1), 96. doi: 10.1186/s13071-022-05226-6
- Nantes, W. A. G., Santos, F. M., de Macedo, G. C., Barreto, W. T. G., Gonçalves, L. R., Rodrigues, M. S., et al. (2021). Trypanosomatid species in *Didelphis albiventris* from urban forest fragments. *Parasitol. Res.* 120 (1), 223–231. doi: 10.1007/s00436-020-06921-y
- Oliva, G., Scalone, A., Foglia Manzillo, V., Gramiccia, M., Pagano, A., Di Muccio, T., et al. (2006). Incidence and time course of *Leishmania infantum* infections examined by parasitological, serologic, and nested-PCR techniques in a cohort of naive dogs exposed to three consecutive transmission seasons. *J. Clin. Microbiol.* 44 (4), 1318–1322. doi: 10.1128/JCM.44.4.1318-1322.2006
- Oliveira, A. G., Galati, E. A., Fernandes, C. E., Dorval, M. E., and Brazil, R. P. (2012). Ecological aspects of phlebotomines (Diptera: Psychodidae) in endemic area of visceral leishmaniasis, campo grande, state of Mato Grosso do Sul, Brazil. *J. Med. Entomol.* 49 (1), 43–50. doi: 10.1603/me11082
- Oliveira, F. S., Pirmez, C., Pires, M. Q., Brazil, R. P., and Pacheco, R. S. (2005). PCR-based diagnosis for detection of *Leishmania* in skin and blood of rodents from an endemic area of cutaneous and visceral leishmaniasis in Brazil. *Vet. Parasitol.* 129 (3–4), 219–227. doi: 10.1016/j.vetpar.2005.01.005
- Ostyn, B., Gidwani, K., Khanal, B., Picado, A., Chappuis, F., Singh, S. P., et al. (2011). Incidence of symptomatic and asymptomatic *Leishmania donovani* infections in high-endemic foci in India and Nepal: A prospective study. *PLoS Negl. Trop. Dis.* 5 (10), e1284. doi: 10.1371/journal.pntd.0001284
- PAHO (2020) *Leishmaniasis: epidemiological report of the Americas* (Pan American Health Organization). Available at: <https://iris.paho.org/handle/10665.2/53090> (Accessed December 15, 2021).
- Paiz, L. M., Fornazari, F., Menozzi, B. D., Oliveira, G. C., Coiro, C. J., Teixeira, C. R., et al. (2015). Serological evidence of infection by *Leishmania (Leishmania) infantum* (Synonym: *Leishmania (Leishmania) chagasi*) in free-ranging wild mammals in a nonendemic region of the state of São Paulo, Brazil. *Vector Borne Zoonotic Dis.* 15 (11), 667–673. doi: 10.1089/vbz.2015.1806
- Paranhos-Silva, M., Oliveira, G. G., Reis, E. A., de Menezes, R. M., Fernandes, O., Sherlock, I., et al. (2003). A follow-up of beagle dogs intradermally infected with *Leishmania chagasi* in the presence or absence of sand fly saliva. *Vet. Parasitol.* 114 (2), 97–111. doi: 10.1016/s0304-4017(03)00132-8
- Porfírio, G. E. O., Santos, F. M., de Macedo, G. C., Barreto, W. T. G., Campos, J. B. V., Meyers, A. C., et al. (2018). Maintenance of *Trypanosoma cruzi*, t. evansi and leishmania spp. by domestic dogs and wild mammals in a rural settlement in Brazil-Bolivian border. *Int. J. Parasitol. Parasites Wildl.* 7 (3), 398–404. doi: 10.1016/j.ijppaw.2018.10.004
- Quinnell, R. J., and Courtenay, O. (2009). Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. *Parasitol.* 136 (14), 1915–1934. doi: 10.1017/S0031182009991156
- R Development Core Team (2015) *R: a language and environment for statistical computing*. Available at: <http://www.R-project.org> (Accessed September 22, 2021).
- Reis, F. C., Minuzzi-Souza, T. T. C., Neiva, M., Timbó, R. V., de Moraes, I. O. B., de Lima, T. M., et al. (2020). Trypanosomatid infections in captive wild mammals and potential vectors at the Brasília zoo, federal district, Brazil. *Vet. Med. Sci.* 6 (2), 248–256. doi: 10.1002/vms3.216
- Risueño, J., Ortuño, M., Pérez-Cutillas, P., Goyena, E., Maia, C., Cortes, S., et al. (2018). Epidemiological and genetic studies suggest a common *Leishmania infantum* transmission cycle in wildlife, dogs and humans associated to vector abundance in southeast Spain. *Vet. Parasitol.* 259, 61–67. doi: 10.1016/j.vetpar.2018.05.012
- Rodrigues, E. S., Santos, G. Q., da Silva, M. V., Barros, J. H. S., Bernardo, A. R., Diniz, R. L., et al. (2022). Chagas immunochromatographic rapid test in the serological diagnosis of *Trypanosoma cruzi* infection in wild and domestic canids. *Front. Cell Infect. Microbiol.* 12. doi: 10.3389/fcimb.2022.835383
- Roque, A. L., and Jansen, A. M. (2014). Wild and synanthropic reservoirs of *Leishmania* species in the Americas. *Int. J. Parasitol. Parasites Wildl.* 3 (3), 251–262. doi: 10.1016/j.ijppaw.2014.08.004
- Salomón, O. D., Feliciangeli, M. D., Quintana, M. G., Afonso, M. M. S., and Rangel, E. F. (2015). *Lutzomyia longipalpis* urbanisation and control. *Mem. Inst. Oswaldo Cruz.* 110 (7), 831–846. doi: 10.1590/0074-02760150207
- Sambrook, J., and Russell, D. W. (2001). *Molecular cloning – a laboratory manual* (New York: Cold Spring Harbor Laboratory Press).
- Schubach, A., Haddad, F., Oliveira-Neto, M. P., Degraive, W., Pirmez, C., Grimaldi, G. Jr., et al. (1998). Detection of *Leishmania* DNA by polymerase chain reaction in scars of treated human patients. *J. Infect. Dis.* 178 (3), 911–914. doi: 10.1086/515355
- SES/MS (2022) *Boletim epidemiológico da leishmaniose visceral humana de 2021 (Secretaria do Estado de Saúde de Mato Grosso do Sul)*. Available at: <https://www.vs.saude.ms.gov.br/Geral/vigilancia-saude/vigilancia-epidemiologica/boletim-epidemiologico/leishmaniose/> (Accessed January 15, 2021).
- Silva, E. A., Andreotti, R., Dias, E. S., Barros, J. C., and Brazuna, J. C. (2008). Detection of *Leishmania* DNA in phlebotomines captured in campo grande, Mato Grosso do Sul, Brazil. *Exp. Parasitol.* 119 (3), 343–348. doi: 10.1016/j.exppara.2008.03.011
- Silva, E. S., Pirmez, C., Gontijo, C. M., Fernandes, O., and Brazil, R. P. (2000). Visceral leishmaniasis in the crab-eating fox (*Cerdoconyx thous*) in south-east Brazil. *Vet. Rec.* 147 (15), 421–422. doi: 10.1136/vr.147.15.421
- Souza, N. P., Almeida, A., do, B., Freitas, T. P., Paz, R. C., Dutra, V., et al. (2010). *Leishmania (Leishmania) infantum chagasi* in wild canids kept in captivity in the state of Mato Grosso. *Rev. Soc. Bras. Med. Trop.* 43 (3), 333–335. doi: 10.1590/s0037-86822010000300024
- Talmi-Frank, D., Nasereddin, A., Schnur, L. F., Schönián, G., Töz, S. O., Jaffe, C. L., et al. (2010). Detection and identification of old world *Leishmania* by high resolution melt analysis. *PLoS Negl. Trop. Dis.* 4 (1), e581. doi: 10.1371/journal.pntd.0000581
- Vásquez Trujillo, A., González Reina, A. E., Góngora Orjuela, A., Prieto Suárez, E., Palomares, J. E., and Buitrago Alvarez, L. S. (2013). Seasonal variation and natural infection of *Lutzomyia antunesi* (Diptera: Psychodidae: Phlebotominae), an endemic species in the orinoquia region of Colombia. *Mem. Inst. Oswaldo Cruz.* 108 (4), 463–469. doi: 10.1590/S0074-0276108042013011
- Vieira, T. M., Silva, S. O., Lima, L., Sabino-Santos, G., Duarte, E. R., Lima, S. M., et al. (2022). *Leishmania* diversity in bats from an endemic area for visceral and cutaneous leishmaniasis in southeastern Brazil. *Acta Trop.* 228, 106327. doi: 10.1016/j.actatropica.2022.106327
- Volterelli, E. M., Arraes, S. M. A. A., Perles, T. F., Lonardon, M. V. C., Teodoro, U., and Silveira, T. G. V. (2009). Serological survey for leishmania spp. infection in wild animals from the municipality of maringá, paraná state, Brazil. *J. Venom. Anim. Toxins Incl. Trop. Dis.* 15 (4), 732–744. doi: 10.1590/S1678-91992009000400011