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# *Leishmania* spp. EM HOSPEDEIROS RESERVATÓRIOS: META-ANÁLISE, ASPECTOS EPIDEMIOLÓGICOS E MOLECULARES

Santa Maria, RS
2023

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Tese apresentada ao Programa de Pós-graduação em Medicina Veterinária, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Doutor em Medicina Veterinária**.

Orientador: Prof. Dr. Luís Antônio Sangioni

Santa Maria, RS

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**Aprovada em 23 de janeiro de 2023:**

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**Luís Antônio Sangioni, Dr. (UFSM)**

(Presidente/Orientador)

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Santa Maria, RS,

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Aos meus pais Valdir José e Nelci Lorena Ratzlaff que com toda a certeza estão orgulhosos, à minha irmã Viviane Ratzlaff minha companheira de vida, ao meu marido Daniel Soares de Oliveira, uma pessoa ímpar que caminha firme ao meu lado e foi fundamental nesta difícil e longa jornada e ao meu "pacotinho", minha sobrinha e afilhada Manuela Ratzlaff Rosalino a alegria da minha vida durante esses anos do doutorado.

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“Por vezes sentimos que aquilo que fazemos não é senão uma gota de água no mar.
Mas o mar seria menor se lhe faltasse uma gota.”
(Madre Teresa de Calcutá)

## RESUMO

### *Leishmania* spp. EM HOSPEDEIROS RESERVATÓRIOS: UMA META-ANÁLISE, ASPECTOS EPIDEMIOLÓGICOS E MOLECULARES

AUTORA: Fabiana Raquel Ratzlaff
ORIENTADOR: Luís Antônio Sangioni

A leishmaniose é uma enfermidade infecciosa parasitária, causada por protozoários do gênero *Leishmania*, de manifestação clínica cutânea, mucocutânea e/ou visceral, com desenvolvimento agudo ou crônico, mantida entre reservatórios silvestres e urbanos, vetores e seres humanos. A enfermidade apresenta distribuição mundial, e tem apresentado mudanças importantes no padrão de transmissibilidade. O contato do homem com o ambiente e a fauna silvestres, ocasionados pela expansão das fronteiras agrícolas, das cidades ou turismo, aliado às alterações ambientais, propiciou uma maior adaptabilidade do parasito em infectar uma variedade de espécies animais, e uma perfeita adequação do vetor ao ambiente urbano, agravando o problema sanitário. O díptero pode infectar espécies mamíferas domésticas, silvestres e sinantrópicas, representando um grande risco para a população humana. Neste contexto, o Brasil vem apresentando um aumento expressivo no número de casos diagnosticados em seres humanos e animais. Desta forma, o objetivo deste trabalho foi identificar potenciais hospedeiros e/ou reservatórios de *Leishmania* spp. no país, diferentes do cão e do homem. Sendo assim, foi realizada uma revisão sistemática com meta-análise, buscando os estudos que identificaram as diferentes espécies de *Leishmania* circulantes no meio silvestre, peri-doméstico e doméstico, atuando diretamente na epidemiologia da enfermidade e na manutenção da endemicidade nas regiões brasileiras. Identificou-se 229 espécies de possíveis hospedeiros ou reservatórios, destacando-se os equinos com a maior ocorrência. Salienta-se que entre os animais estudados no Brasil, outras espécies infectadas nas regiões brasileiras tiveram destaque como potenciais reservatórios de *Leishmania* spp.: felinos domésticos, roedores, marsupiais e quirópteros. O método molecular foi o mais utilizado, identificando 25% dos indivíduos amostrados infectados com alguma espécie do protozoário, sendo: *Leishmania* spp. mais frequente, seguida de *Leishmania* (*Leishmania*) *infantum*, *Leishmania* (*Viannia*) *braziliensis* e *Leishmania* (*Leishmania*) *amazonensis*. A ordem Chiroptera, composta por diversas espécies de morcegos, mamíferos voadores com uma grande capacidade de deslocamento e adaptação a diversos ambientes e hábitos sinantrópicos, vem tornando-se importante para a pesquisa de *Leishmania* spp. no Brasil e no mundo. Identificou-se através da revisão sistemática, morcegos infectados por *Leishmania* spp. nos seguintes estados brasileiros: Maranhão, Mato Grosso do Sul, Minas Gerais, Espírito Santo e São Paulo. O papel destes animais no ciclo epidemiológico da leishmaniose tem sido reconhecido como sendo de potenciais reservatórios, e poderiam albergar o protozoário em áreas endêmicas e não endêmicas do Rio Grande do Sul. Sendo assim, identificou-se pela primeira vez, através de técnicas moleculares, sequenciamento e desenvolvimento da árvore filogenética, a presença de infecção natural por *Leishmania* spp. e *Leishmania* (*L*.) *infantum* em morcegos das espécies *Tadarida braziliensis* e *Molossus molossus*, provindos de diferentes localidades do estado. Esses animais podem servir como fonte de infecção para os insetos vetores que por consequência e proximidade, possam vir a infectar humanos e animais domésticos. No entanto, mais estudos são necessários para

determinar o papel desses mamíferos na epidemiologia e transmissão da leishmaniose. Desta forma, é fundamental conhecer as espécies de animais envolvidas no ciclo biológico do protozoário, a fim de constituir biomarcadores ambientais, bem como identificar as espécies de *Leishmania* para planejar ações e medidas de controle.

**Palavras-chave:** Leishmaniose. Doença transmitida por vetor. Revisão sistemática. Diagnóstico molecular. Morcego.

## ABSTRACT

### *Leishmania* spp. IN RESERVOIR HOST: META-ANALYSIS, EPIDEMIOLOGICAL AND MOLECULAR ASPECTS

AUTHOR: Fabiana Raquel Ratzlaff
ADVISOR: Luís Antônio Sangioni

Leishmaniosis is an infectious parasitic disease, caused by protozoa of the genus *Leishmania*, with cutaneous, mucocutaneous and/or visceral clinical manifestation, with acute or chronic development, maintained between wild and urban reservoirs, vectors, and humans. The disease has a worldwide distribution, and has shown important changes in the pattern of transmissibility. Human interaction with the wild environment and fauna, caused by the expansion of agricultural frontiers, cities, or tourism, combined with environmental changes, provided a greater adaptability of the parasite to infect a variety of animal species, and a perfect adaptation of the vector to the urban environment, aggravating the sanitary problem. Diptera can infect domestic, wild and synanthropic mammalian species, representing a great risk to the human population. In this context, Brazil has been showing a significant increase in the number of cases diagnosed in humans and animals. Thus, the objective of this work was to identify possible hosts and/or reservoirs of *Leishmania* spp. in the country, different from the dog and the man. Therefore, a systematic review with meta-analysis was carried out, seeking studies that identified the different species of *Leishmania* circulating in the wild, peri-domestic, and domestic environments, acting directly in the epidemiology of the disease and in the maintenance of endemicity in Brazilian regions. Therefore, 229 species of possible hosts or reservoirs were identified, highlighting the horses with the highest occurrence. It should be noted that among the animals studied in Brazil, other species infected in the Brazilian regions stood out as potential reservoirs of *Leishmania* spp.: domestic felines, rodents, marsupials, and bats. The molecular method was the most used, identifying 25% of the individuals sampled infected with some species of the protozoan, namely: *Leishmania* spp. most frequent, followed by *Leishmania* (*Leishmania*) *infantum*, *Leishmania* (*Viannia*) *braziliensis* and *Leishmania* (*Leishmania*) *amazonensis*. The order Chiroptera, composed of several species of bats, flying mammals with a great ability to move and adapt to different environments and synanthropic habits, has become important for research on *Leishmania* spp. in Brazil and in the world. Through systematic review, bats infected with *Leishmania* spp. in the following Brazilian states: Maranhão, Mato Grosso do Sul, Minas Gerais, Espírito Santo and São Paulo. The role of these animals in the epidemiological cycle of leishmaniasis has been recognized as being potential reservoirs and could harbor the protozoan in endemic and non-endemic areas of Rio Grande do Sul. Thus, it was identified for the first time, through molecular techniques, sequencing and development of the phylogenetic tree, the presence of natural infection by *Leishmania* spp. and *Leishmania* (*L.*) *infantum* in bats of the species *Tadarida braziliensis* and *Molossus molossus*, from different locations in the state. These animals can serve as a source of infection for insect vectors that, consequently and proximity, can infect humans and domestic animals. However, further studies are needed to determine the role of these mammals in the epidemiology and transmission of leishmaniasis. Therefore, it is fundamental to know the species of animals involved in the biological cycle of the protozoan, in order to constitute environmental biomarkers, as well as to identify the species of *Leishmania* to plan actions and control measures.



**Keywords:** Leishmaniosis. Vector-borne disease. Systematic review. Molecular diagnostics. Bat.

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**MANUSCRITO 1**

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**MANUSCRITO 1**



**ARTIGO 1**

**SUMÁRIO**

# 1 APRESENTAÇÃO

Esta tese de doutorado está composta pelos itens 2 INTRODUÇÃO, 3 REVISÃO BIBLIOGRÁFICA, 4 MANUSCRITO 1, 5 ARTIGO 1, 6 DISCUSSÃO e 7 CONCLUSÃO e REFERÊNCIAS. O manuscrito 1 e o artigo 1 compõem a íntegra deste estudo. Separadamente, no manuscrito 1 e no artigo 1, estão descritas as seções de Materiais e Métodos, Resultados, Discussão e Referências. As REFERÊNCIAS contêm as citações inseridas nos itens INTRODUÇÃO, REVISÃO BIBLIOGRÁFICA e DISCUSSÃO desta tese.

## 2 INTRODUÇÃO

A leishmaniose é uma enfermidade incluída no grupo das doenças tropicais negligenciadas (BRASIL, 2021). Estima-se que 700.000 a um milhão de novos casos humanos ocorram anualmente, na maioria das vezes associada a população em vulnerabilidade, à desnutrição, ao deslocamento populacional, à moradia precária, à fragilidade do sistema imunológico e à falta de recursos financeiros (WHO, 2022). A doença pode acometer seres humanos e animais sendo que a maioria dos infectados pode não desenvolver sintomas ou sinais clínicos, mas, se presentes, pode apresentar-se nas formas: cutânea (mais comum), muco-cutânea (desfigurante) ou visceral (mais grave), enquanto que nos animais pode apresentar sinais cutâneos e/ou viscerais (MAXFIELD e CRANE, 2022)

A enfermidade é endêmica e está em franca expansão na Ásia, Oriente Médio, Norte da África, Mediterrâneo e América do Sul e Central (MAXFIELD e CRANE, 2022). Nas Américas, a leishmaniose cutânea ou tegumentar (LT) e a visceral (LV) são endêmicas em 18 e 12 países respectivamente, ressaltando-se que mais de 90% dos casos de LV ocorrem no Brasil (BRASIL, 2014a; OPAS, 2019). A autoctonia da LV foi confirmada em 24 Unidades Federativas, distribuídas nas cinco regiões brasileiras, sendo a região Nordeste responsável pelo maior registro de casos do país (49,1%). Quanto a LT, se distribui por todo o território brasileiro, com maior registro de casos na região Norte (42,8%) (BRASIL, 2021).

O parasita causador da doença é o protozoário do gênero *Leishmania*, com diferentes espécies, de acordo com as várias formas de apresentações da doença (LANGONI, 2016), mantido entre humanos, animais silvestres e domésticos e os insetos vetores (BRASIL, 2014a). A transmissão do agente se dá pelo repasto sanguineo da fêmea hematófaga flebotomínea (ordem Diptera, família Psychodidae) que apresenta hábito alimentar crepuscular noturno, em hospedeiros vertebrados, havendo espécies mais “ecléticas” do que outras no que concerne às preferências alimentares (AGUIAR et al., 1987; MORRISON et al., 1993).

Nas últimas décadas, as análises epidemiológicas da leishmaniose, têm sugerido mudanças no padrão de transmissão da doença, inicialmente considerada zoonose de animais silvestres, que acometia ocasionalmente pessoas em contato com as florestas. A doença passou a ocorrer em zonas rurais, em locais praticamente desmatados, e em regiões periurbanas e urbanas, constituindo um grande problema de saúde pública (BRASIL, 2021).

A dinâmica da infecção advém das transformações no ambiente, ocasionadas pelo intenso fluxo migratório, expansão das fronteiras agrícolas e a consequente destruição de habitats silvestres, o aquecimento global, a popularização do ecoturismo, dentre outros fatores,

que levaram o homem a um contato maior com os ciclos de transmissão do protozoário, permitindo o intercâmbio de parasitos entre animais silvestres, domésticos e o homem (BRASIL, 2014a; CONCEIÇÃO-SILVA e ALVES, 2014).

A LT no Brasil é causada principalmente por *Leishmania* (*Viannia*) *braziliensis*, *Leishmania* (*Viannia*) *guyanensis, Leishmania* (*Viannia*) *naiffi*, *Leishmania* (*Viannia*) *shawi*, *Leishmania* (*Viannia*) *lainsoni* e *Leishmania* (*Leishmania*) *amazonensis*. Os principais animais reservatórios são: *Didelphis* (gambás), *Dasipodideos* (tatus), Primatas não humanos (macacos), *Bradypus variegatus* (bicho-preguiça), *Cuniculus paca* (paca) e outros roedores (GONTIJO e CARVALHO, 2003). O protozoário também foi identificado em animais domésticos como *Canis lupus familiaris* (cães), *Felis catus* (felinos) e Eqqus caballus (cavalos), entretanto, seu papel na manutenção do parasito no meio ambiente ainda não foi completamente esclarecido (BRASIL, 2016a).

Contudo, *Leishmania* (*Leishmania*) *infantum*, espécie de protozoário que causa a LV, no ambiente silvestre tem como reservatórios descritos as raposas (*Dusicyon vetulus* e *Cerdocyon thous*) e os marsupiais (*Didelphis albiventris*) (BRASIL, 2014a). No ambiente urbano, o cão doméstico configura um importante elo na transmissão da LV atuando como principal reservatório (MORENO e ALVAR, 2002; BRASIL, 2014a). O movimento de cães entre áreas endêmicas e não endêmicas, juntamente com as mudanças na ecologia do vetor, contribuem para a dispersão geográfica da doença. Uma vez o vetor estando disseminado pelo Brasil, e a introdução de cães infectados em áreas não endêmicas, podem resultar em novos focos da enfermidade (DANTAS-TORRES, 2009).

Devido a importância do cão no ciclo da leishmaniose, algumas estratégias de controle da enfermidade, até então foram dirigidas para este reservatório como: inquérito sorológico canino e eutanásia dos sororreagentes (BRASIL, 2016b). Entretanto, essas medidas, muitas vezes realizadas de forma isolada, não apresentaram efetividade para redução da incidência da doença, determinando a necessidade de reavaliação das ações propostas. Considerando que a eliminação dos cães soropositivos, não tem apresentado resultados satisfatórios como forma de controle da enfermidade (DANTAS TORRES e BRANDÃO-FILHO, 2006; QUINNELL e COURTENAY, 2009), são necessárias mais informações sobre a possibilidade de outras espécies de mamíferos infectados, domésticos e silvestres também servirem como fonte de infecção aos vetores e como mantenedores de *Leishmania* sp. no ambiente peridoméstico e doméstico (DANTAS TORRES e BRANDÃO-FILHO, 2006).

Novos comportamentos, transformações demográficas, novas situações de vida de segmentos populacionais, exposição à novos e inúmeros fatores de risco (GONTIJO e MELO, 2004; QUINNELL e COURTENAY, 2009), justificam mais estudos e vigilância desses animais que devem estar envolvidos no ciclo de transmissão destes parasitos, buscando o entendimento desse novo perfil epidemiológico de infecção nesses hospedeiros e/ou possíveis reservatórios (CONCEIÇÃO-SILVA e ALVES, 2014).

Sendo assim, diante do exposto acima e pela complexidade dos fatores envolvidos na transmissão e manutenção do gênero *Leishmania*, ressalta-se o pouco conhecimento sobre os hospedeiros e/ou reservatórios, constituindo um importante obstáculo para caracterização epidemiológica da leishmaniose. Este estudo, através do manuscrito 1, pretende expandir o conhecimento sobre a diversidade de hospedeiros ou reservatórios do protozoário no Brasil, que possam estar infectados e transmitindo o agente ao vetor próximo do ambiente domiciliar ou peridomiciliar. Também por este estudo, pretende-se sumarizar a quantidade de animais infectados tanto domésticos como silvestres com espécies de *Leishmania* nas diferentes regiões brasileiras, observando a presença ou não de sinais clínicos característicos da enfermidade e qual material é indicado para coleta e realização de testes diagnósticos.

Embora os morcegos sejam um dos mamíferos mais abundantes em nosso país e estejam presentes em praticamente todos os ecossistemas, incluindo ambientes silvestres, sinantrópicos e domésticos, eles ainda são pouco mencionados quando o assunto é leishmaniose, mesmo tendo-se a comprovação de que podem servir de fonte de alimentação aos vetores responsáveis pela transmissão de *Leishmania* sp. (LAMPO et al., 2000).

No Brasil desde 2010 (SAVANI et al., 2010), e em alguns países como: Venezuela (LIMA et al., 2008), Guiana Francesa (MEDKOUR et al., 2019), México (BERZUZA-CRUZ et al., 2015) e Espanha (AZAMI-CONESA et al., 2020), têm se mencionado a infecção dos morcegos por espécies de *Leishmania* causadoras tanto de LV quanto de LT. Juntamente com sua elevada abundância e adaptabilidade a ambientes peridomiciliares, reforçam e justificam a necessidade de se investigar o possível papel destes mamíferos como potenciais reservatórios do protozoário. Os estados brasileiros em que há registros de morcegos infectados com *Leishmania* spp são: Mato Grosso do Sul (CASTRO et al., 2020; FERREIRA et al., 2017; REZENDE et al., 2017; SHAPIRO et al., 2013), São Paulo (OLIVEIRA et al., 2015; PAIZ et al., 2015; SAVANI et al., 2010), Maranhão (COSTA et al., 2015) e Minas Gerais (GÓMEZ-HERNÁNDEZ et al., 2017).

Como não se tinha relato sobre a infecção dos morcegos por *Leishmania* spp. no estado do Rio Grande do Sul (RS), o artigo 1 identificou amostras de tecidos positivas para a presença do protozoário em morcegos provenientes de diferentes municípios do estado. Esses mamíferos podem estar participando do ciclo epidemiológico da leishmaniose como hospedeiros e/ou reservatórios circulando na região, dividindo o mesmo habitat com vetores, animais domésticos e seres humanos. A importância das espécies pertencentes a ordem Chiroptera no ciclo da leishmaniose, vem da sua grande longevidade, alta capacidade de dispersão e adaptabilidade a ambientes sinatrópicos, podendo estar desempenhando um papel na manutenção do ciclo de vida do protozoário.

# 3 REVISÃO BIBLIOGRÁFICA

## 3.1.IMPORTÂNCIA:

No ano de 2007, na 60ª Assembleia Mundial de Saúde reconheceu-se que a infecção por parasitos do gênero *Leishmania* causam uma das doenças tropicais mais negligenciadas no mundo. Assim, algumas resoluções foram tomadas no sentido de reforçar os programas nacionais de controle: melhorar o diagnóstico e a prevenção, conduzir estudos epidemiológicos mais precisos, melhorar o intercâmbio de informações e ações entre países a fim de promover a vigilância e o controle da doença. Também foram propostas ações prioritárias como: o desenvolvimento de novos medicamentos; o reforço nas pesquisas para controle de vetores e proteção dos seres humanos; melhoria dos métodos diagnósticos e acesso aos sistemas de saúde pela população sob risco (WHA, 2007).

No entanto, na atualidade, a população ainda se depara com os mesmos problemas, e a leishmaniose, sobretudo a LV, considerada endêmica no Brasil, tem se expandido para regiões antes consideradas indenes (BRASIL, 2016a). A exemplo disso refere-se o RS, que se manteve como área livre de transmissão da doença até o ano de 2008, quando foram relatados os primeiros casos autóctones em cães. No ano seguinte, foi registrado a primeira ocorrência do vetor e os primeiros casos humanos na cidade de São Borja. Diante disso, houve o desencadeamento de investigações epidemiológicas em vários municípios do RS, com realização de inquéritos sorológicos caninos, verificação da presença do vetor (*Lutzomyia longipalpis*) e detecção de *Leishmania* (*L.*) *infantum* em amostras biológicas caninas (RIO GRANDE DO SUL, 2011).

Em Porto Alegre (RS) o primeiro caso de leishmaniose visceral canina (LVC) foi registrado no ano de 2010 (TEIXEIRA et al., 2016). Entre os anos de 2008-2017 ocorreram no município dois óbitos humanos em decorrência desta enfermidade (RIO GRANDE DO SUL, 2017).

Em 2013, Mazaro et al. (2015) descreveram três casos caninos autóctones em Santa Maria, região central do RS. Os animais apresentaram manifestações clínicas, e o diagnóstico laboratorial e anatomopatológico compatíveis com LVC. Em um levantamento sorológico canino realizado entre novembro de 2014 e abril de 2016 no mesmo município, observou-se uma prevalência de 33% para o agente causador da leishmaniose, em animais assintomáticos (RATZLAFF et al., 2018). Em 2017 ocorreu o primeiro registro de LVC autóctone e em 2015,

2017 e 2021 ocorreram 1, 1 e 2 casos de leishmania visceral em humanos (LVH), respectivamente sendo que um evoluiu para óbito (SINAN, 2022). Entre janeiro de 2021 e janeiro de 2022, realizaram a primeira detecção de *Lutzomyia longipalpis*, caracterizando o município de Santa Maria como uma área da transmissão da enfermidade (OSMARI et al., 2022).

Casos autóctones reportados em cães e em seres humanos no RS reforçam a hipótese de que o vetor da doença está presente e mantém o ciclo epidemiológico do agente, oferecendo riscos de ocorrência de surtos em animais e seres humanos (BIANCHI et al., 2016). Normalmente, casos suspeitos ou clínicos em cães precedem os casos humanos (BRASIL, 2016), sendo que os cães são considerados os principais reservatórios domésticos e fundamentais na manutenção do ciclo da doença (KRAUSPENHAR et al., 2007).

## 3.2 ETIOLOGIA:

Os agentes etiológicos causadores da leishmaniose são protozoários Sarcomastigophoras pertencentes a classe Kinetoplastida, ordem Trypanosomatida, família Trypanosomatidae, do gênero *Leishmania,* subgêneros *Leishmania* e *Viannia* com diferentes espécies (Tab. 1), apresentando formas epidemiológicas e clínicas diferentes da doença, de acordo com a espécie do protozoário envolvida (TAYLOR et al., 2017). Atualmente são conhecidas cerca de 30 espécies de *Leishmania*, das quais 20 são confirmadas patogênicas para seres humanos (COSTA, 2005).

Esses tripanossomatídeos são organismos digenéticos, sendo a forma amastigota intracelular obrigatória, multiplicando-se no interior de macrófagos de hospedeiros vertebrados, enquanto a forma promastigota é encontrada no trato digestório do inseto vetor e constitui a forma infectante (LANGONI, 2016).

**Tabela 1** – Classificação taxonômica do protozoário do gênero *Leishmania*

| SUBGÊNERO | COMPLEXO | ESPÉCIE |
|---|---|---|
| *Leishmania* | *L.*(*L.*) *donovani* | *L.donovani*<br>*L.archibaldi*<br>*L.infantum*<br>*L.chagasi* |
| | *L.*(*L.*) *major* | *L.major*<br>*L.gerbilli* |
| | *L.*(*L.*) *tropica* | *L.tropica*<br>*L.killicki*<br>*L.aethiopica* |
| | *L.*(*L.*) *mexicana* | *L. enrietti*<br>*L. venezuelensis*<br>*L. foratinii*<br>*L.amazonensis*<br>*L. garnhani*<br>*L. mexicana*<br>*L. pifanoi*<br>*L. aritidesi* |
| | *L.*(*L.*) *arabica* | |
| | *L.*(*L.*) *turanica* | |
| | | |
| *Viannia* | *L.*(*V.*) *braziliensis* | *L. braziliensis*<br>*L. peruviana* |
| | *L.*(*V.*) *guyanensis* | *L. guyanensis*<br>*L. panamensis*<br>*L. shawi* |
| | *L.*(*V.*) *lainsoni,* | |
| | *L.*(*V.*) *utingensis* | |
| | *L.*(*V.*) *lindenbergi* | |
| | *L.*(*V.*) *naiffi* | |

Fonte:Adaptado de CUPOLILLO, Elisa; BOITÉ, Mariana Côrtes; PORROZZI, Renato. Considerações sobre a Taxonomia do Gênero Leishmania. *In* CONCEIÇÃO-SILVA, Fátima; ALVES, Carlos Roberto. **Leishmanioses do Continente Americano**. Rio de Janeiro: Editora Fio Cruz, p. 42, 2014.

## 3.3 CICLO E TRANSMISSÃO:

O protozoário possui um ciclo biológico heteroxênico, ou seja, necessita de dois hospedeiros, um flebotomíneo, o qual abriga a forma flagelada ou promastigota nas células intestinais, e um vertebrado, incluindo várias espécies de animais silvestres, domésticos, sinantrópicos, além do homem, o qual hospeda nos seus tecidos a forma aflagelada ou amastigota (SCHLEIN, 1993; ANTOINE, 1995).

A fêmea do inseto se infecta ingerindo macrófagos parasitados por formas amastigotas de *Leishmania* sp. no ato do repasto sanguíneo. No trato digestivo os macrófagos se rompem e liberam o parasito que se reproduz por divisão binária, diferenciando rapidamente em formas flageladas (promastigotas). Posteriormente os protozoários se multiplicam e colonizam o esôfago e faringe do vetor, onde se diferenciam em promastigotas metacíclicas. A forma infectante é liberada juntamente com a saliva do flebotomíneo quando realizam um novo repasto sanguíneo. Estas formas são fagocitadas por células do sistema mononuclear. No interior dos macrófagos, diferenciam-se em amastigotas e multiplicam-se intensamente até o rompimento do macrófago, ocorrendo desta forma a disseminação hematogênica para outros tecidos ricos em células do sistema mononuclear fagocitário, como linfonodos, fígado, baço e medula óssea (BRASIL, 2014a).

O protozoário possui mecanismos de adaptação para resistir ao efeito letal dos macrófagos no interior dos fagolisossomos, no entanto a intensa multiplicação do agente provoca a destruição celular com liberação das amastigotas e invasão de novos macrófagos (LANGONI, 2016).

Alguns autores admitem a hipótese da transmissão entre a população canina através da ingestão de carrapatos infectados, mordeduras, cópula (DINIZ et al., 2005; DUBEY, 2005; ROSYPAL et al., 2005), ingestão de vísceras infectadas, porém não há evidências sobre a importância epidemiológica destes mecanismos de transmissão para humanos ou na manutenção da enzootia (BRASIL, 2014a).

Não há transmissão direta de pessoa para pessoa. A infecção do protozoário ao vetor ocorre enquanto houver o repasto sanguineo na pele ou no sangue periférico no momento de uma alta parasitemia no hospedeiro (BRASIL, 2014a).

## 3.4 VETORES:

No Brasil, duas principais espécies de insetos flebotomíneos, são consideradas vetores da LV pelo Ministério da Saúde: *Lutzomyia longipalpis* e *Lutzomyia cruzi*, com ampla distribuição geográfica, popularmente conhecidos como mosquito palha, tatuquiras, birigui (BRASIL, 2016a).

Este vetor encontrava-se originalmente nas matas participando do ciclo primário da transmissão da doença. Posteriormente houve uma adequação do diptero ao ambiente rural, somando-se a presença de animais silvestres e sinantrópicos suscetíveis, que se tornaram reservatórios. Atualmente existe uma total adaptação deste artrópodo aos ambientes urbanos,

sendo capturado no peridomicílio e intradomicílio (BARATA et al., 2005; BARBOSA et al., 2008).

A atividade dos flebotomíneos é, usualmente, crepuscular e noturna, sendo as fêmeas hematófagas obrigatórias que realizam o repasto sanguíneo de mamíferos infectados com o protozoário (BRASIL, 2014a; BRASIL, 2016a). Os flebotomíneos infectam novos hospedeiros vertebrados mediante inoculação do protozoário pelo repasto sanguineo. Durante o crepúsculo estes insetos são atraídos pela luz, o que facilita a invasão dos domicílios e o contato com os animais e os seres humanos (REY, 2013).

*Lutzomya longipalpis* apresenta de 1 a 3 mm de comprimento e tem o corpo revestido de pelos e coloração clara. O voo é caracterizado por pequenos saltos e o pouso é feito com as asas entreabertas (asas de anjo). O ciclo de vida compreende 4 fases: ovo, larva (com 4 estádios), pupa e adulto. As fases larvárias imaturas se desenvolvem em ambiente terrestre úmido, rico em matéria orgânica, na camada superficial do solo, com baixa incidência luminosa e em árvores frondosas e encorpadas. Após a cópula as fêmeas fazem ovoposição no solo e as larvas eclodem entre 7 e 10 dias. As larvas se desenvolvem entre 20 e 30 dias, podendo entrar em diapausa dependendo das condições climáticas. A fase de pupa dura cerca de 1 a 2 semanas e as fêmeas adultas vivem em torno de 20 dias e são hematófagas obrigatórias, necessitando de sangue para o desenvolvimento dos ovos. A maior densidade de flebotomíneos é logo após a estação chuvosa, coincidindo com o período de maior transmissão da leishmaniose (RANGEL e VILELA, 2008).

As principais espécies envolvidas na transmissão da LT em nosso país são *Lutzomyia whitmani*, *Lutzomyia intermedia*, *Lutzomyia umbratilis*, *Lutzomyia wellcomei*, *Lutzomyia flaviscutellata* e *Lutzomyia migonei*. Sendo que a espécie envolvida capaz de transmitir *Leishmania* sp. varia conforme a região geográfica e suas características climáticas (BRASIL, 2014a).

### 3.5 APRESENTAÇÃO CLÍNICA:

Dependendo da localização da infecção, da espécie do parasito, reservatórios e vetores envolvidos, a leishmaniose nos humanos pode apresentar-se clinicamente sob a forma cutânea, muco-cutânea e/ou visceral (LANGONI, 2016).

### 3.5.1 Forma tegumentar (LT):

A LT apresenta-se como uma doença infecciosa, não contagiosa, manifestando-se de forma clínica cutânea ou muco-cutânea, variando sua patogenicidade e apresentação clínica conforme a espécie etiológica do protozoário envolvida, carga parasitária e estado imunológico do hospedeiro. No entanto, a importância da infecção na manifestação da forma muco-cutânea reside na alta incidência e ampla distribuição geográfica, sendo detectada em todos os estados brasileiros, resultando em grande impacto sanitário e social pelas lesões mutilantes que pode provocar (BRASIL, 2016; REY, 2013). Até o momento, sete espécies de *Leishmania*, pertencentes aos subgêneros *Leishmania* e *Viannia*, foram identificadas no Brasil como causadoras de LT: *L. (V) braziliensis, L. (V) guyanensis, L. (L) amazonensis, L. (V) naiffi, L. (V.) lainsoni, L. (V) shawi* e *L. (V) lindenbergi* (BRASIL, 2014a; GUERRA et al., 2015).

A lesão típica da LT é uma úlcera com bordas em moldura. Após a picada do flebotomíneo infectado, o período de incubação pode variar de 15 dias a 3 meses, surgindo primeiramente uma pápula eritematosa com poucos milímetros de diâmetro. Em alguns dias surge uma pápula, com crescimento progressivo, infiltração de polimorfonucleados e necrose central que se recobre por crosta. Esta forma ulcerosa típica com borda saliente também é conhecida no Brasil por úlcera de Bauru (CONCEIÇÃO- SILVA e ALVES, 2014).

Os cães, independentemente da espécie de *Leishmania* envolvida, frequentemente apresentam manifestações cutâneas e viscerais (OLIVEIRA et al., 2019). Os equinos apresentam lesões nodulares, as vezes crostosas ou ulceradas, principalmente nas orelhas. Entre os animais silvestres, geralmente a infecção é subclínica. Em roedores o complexo *L.*(*L.*) *mexicana* causam lesões na pele, com aumento de volume, alopecia, ou lesões na base da cauda, orelhas e patas, onde se pode identificar as formas amastigotas do protozoário. O complexo *L.*(*V.*) *braziliensis* produz infecção sistêmica nos animais silvestres, mas raramente se observa lesões na pele. Os agentes podem ser isolados a partir de amostras de sangue, baço, fígado ou da pele mesmo com aspecto normal (LANGONI, 2016).

### 3.5.2 Forma visceral (LV):

A LV também conhecida como calazar, febre dun-dun, esplenomegalia tropical, caracteriza-se por produzir febre irregular prolongada, hepatoesplenomegalia, anemia, caquexia (REY, 2013). É a apresentação mais grave da infecção, com grandes epidemias descritas no Sudão, na Índia, em Bangladesh e no Brasil (CONCEIÇÃO- SILVA & ALVES, 2014).

A enfermidade apresenta um índice de mortalidade em torno de 75 a 85% entre crianças e em torno de 90 a 95% entre adultos que não recebem tratamento. No Brasil, a doença em humanos apresentava um caráter inicialmente rural, se expandindo para áreas urbanas, acometendo frequentemente crianças menores de 10 anos (54,4%), sendo o sexo masculino mais prevalente (60%). A maior susceptibilidade das crianças ocorre pela imaturidade imunológica celular agravada pela desnutrição, além da maior exposição ao vetor no peridomicílio. Contudo o envolvimento do adulto assume um papel importante na epidemiologia da enfermidade devido as formas assintomáticas (BRASIL, 2014a).

No Brasil, *L.*(*L.*) *infantum* é o principal agente causador desta enfermidade, tanto nos cães como nos humanos, sendo uma zoonose transmitida principalmente pelo flebotomíneo *Lutzomyia longipalpis*. São vários os reservatórios para a LV, no entanto, na área urbana, o cão doméstico é considerado a fonte de infecção mais importante. A incidência tanto no cão quanto em humanos sempre foi maior na Região Nordeste, principalmente no Ceará, Bahia, Piauí e Maranhão, sendo o baixo poder aquisitivo, a vulnerabilidade social, a alta densidade de flebotomíneos e cães infectados e os fatores climáticos que propiciam a proliferação dos vetores; os principais fatores predisponentes para o estabelecimento da doença (DANTAS-TORRES, 2009; DANTAS-TORRES e BRANDÃO-FILHO, 2006).

Os inquéritos epidemiológicos em diferentes regiões onde a LVC é endêmica, têm registrado prevalências variáveis, e indicam franca expansão da doença tanto em cães como em gatos, dados que revelam sérios riscos para saúde pública, uma vez que, além da presença do protozoário, estão disponíveis reservatórios e condições ambientais propícias em várias cidades para a transmissão do patógeno (BRASIL 2014; CAMPRIGHER et al. 2019; GONTIJO e MELO, 2004).

No cão a doença geralmente apresenta-se de forma sistêmica, de evolução crônica e lenta, variando de animais assintomáticos a quadros graves com extrema debilidade e caquexia (LANGONI, 2016). Até o momento em cães, não foi verificada predisposição racial, sexual ou etária (SOUSA e ALMEIDA, 2008). Após a infecção da pele ocorre a disseminação do parasita por todo o organismo, sendo que a resposta dos linfócitos T exerce grande influência sobre a infecção. Portanto o aparecimento dos sinais clínicos depende da imunocompetência do animal (BRASIL, 2020).

Os cães sintomáticos podem apresentar lesões cutâneas, com descamação e eczema, especialmente no espelho nasal e orelhas, pequenas úlceras rasas nas orelhas, focinho, cauda e articulações, hiperqueratose, onicogrifose, emagrecimento, esplenomegalia, linfoadenopatia,

alopecia, ceratoconjuntivite, coriza, apatia, diarreia, hemorragia intestinal, edema de patas, vômito, paresia dos membros posteriores. Esses sinais clínicos podem ser confundido com outras enfermidades que acometem os cães, mascarando e dificultando ou retardando o diagnóstico clínico de LVC (BRASIL, 2014a).

## 3.6 RESERVATÓRIOS, HOSPEDEIROS E MANTENEDORES:

Em 1908, Charles Nicolle descrevia a participação dos cães como reservatórios da leishmaniose através de estudos experimentais (REY, 2013). No Brasil, o primeiro caso de LVC descrito ocorreu na cidade de Araçatuba/SP no ano de 1998 (FUNASA, 2001). Em áreas urbanas, o cão doméstico é considerado o reservatório mais importante para LV, uma vez que, tanto no homem como no cão, *Leishmanias* são raras no sangue, mas encontram-se em 16,3% e 77,6% das biópsias de pele humanas e caninas respectivamente, apresentando-se portanto de forma muito mais abundante na derme dos cães, sendo, desta forma, um potencial muito maior de infecção para os flebotomíneos (REY, 2013). A enzootia canina, tem precedido a ocorrência de casos humanos e a infecção tem sido mais prevalente quando comparado ao homem (LANGONI, 2016).

Vários fatores associados às alterações ambientais promoveram a dispersão destes protozoários para novas áreas, proporcionando o contato destes com novas espécies animais, as quais poderão atuar como reservatórios ou hospedeiros, amplificando a área geográfica de ocorrência por dispersão do parasito (BRASIL, 2014a). Os carnívoros selvagens, são também geralmente considerados como reservatórios de diferentes espécies de *Leishmania*. Alguns estudos mostram que as populações de raposas carnívoras não mantêm um ciclo de transmissão de forma independente, diferentemente de cães domésticos. É improvável que elas introduzam o parasita em populações de cães livres de *Leishmania.* A infectividade para o vetor da raposa é menor se comparada ao cão (COURTENAY et al., 2002).

No ambiente silvestre, as raposas, marsupiais e roedores foram encontrados naturalmente infectados (BRASIL, 2014a; LANGONI, 2016; REY, 2013). Contudo, as espécies de *Leishmania* presentes no continente americano associadas a zoonoses podem infectar um amplo espectro de espécies de mamíferos silvestres. Ainda são restritos os dados sobre o papel desempenhado pelos diferentes mamíferos nos ciclos de transmissão, sendo na maioria relatos de achados pontuais de infecção. São escassos também os estudos sobre

reservatórios de *Leishmania* mantenedores e amplificadores (CONCEIÇÃO-SILVA e ALVES, 2014).

Os morcegos (Ordem Chiroptera), são comumente encontrados infectados por diversos tripanossomatídeos, principalmente do gênero *Trypanosoma.* Em relação à infecção por *Leishmania* spp., há poucos estudos relatando a ocorrência e as características moleculares deste agente. Esses hospedeiros são os únicos mamíferos capazes de voar e algumas espécies realizam migração sazonal. Essas características lhes confere uma elevada capacidade de dispersão por diferentes ambientes (CONCEIÇÃO-SILVA e ALVES, 2014).

Lampo et al. (2000) demonstraram que morcegos constituem fonte alimentar de *Lutzomyia longipalpis*, em cavernas na Venezuela. Em 2008, isolou-se *L.* (*L.*) *infantum* em sangue de morcegos frugívoros neste mesmo país (LIMA et al., 2008). Na Etiópia, KASSAHUN et al., (2015) descreveram infecções por *L.* (*L.*) *tropica* e *L.*(*L.*) *major*, representando a primeira observação confirmada de infecção natural em morcegos do Velho Mundo, com espécies que causam LVC no país.

Segundo Berzunza-Cruz et al. (2015), no México, a leishmaniose é considerada uma zoonose endêmica, sendo a forma cutânea, causada pela *L.*(*L.*) *mexicana*, encontrada em 9,7% dos morcegos, sem no entanto evidenciar lesões cutâneas. Nesse estudo foi infectado experimentalmente dois morcegos (*Tadarida brasiliensis*) em cativeiro, demonstrando que esta espécie é capaz de abrigar parasitas infecciosos como *L.*(*L.*) *mexicana.* Após a inoculação e posterior obtenção do agente em meio de cultura celular, se obteve promastigotas de *L.* (L.) *mexicana* para inocular em camundongos BALB/c demonstrando que conservaram a sua infecciosidade, sugerindo a viabilidade de transmissão.

Os estudos no Brasil investigando o papel dos morcegos na cadeia epidemiológica da leishmaniose são escassos. Savani et al. (2010) identificaram *L.(L) infantum e L.(L) amazonenses* em macerado de baço e fígado através de PCR em morcegos do Estado de São Paulo. Oliveira et al. (2015) desenvolveram outro estudo no Estado de São Paulo, onde detectaram a presença de *Leishmania* spp. em 23,9% dos morcegos capturados exclusivamente em áreas urbanas. Através da diferenciação das espécies, identificaram a presença de *L.(L) amazonenses*, *L.(L) infantum*, incluindo também a espécie *Desmodus rotundus*, morcego hematófago infectado por estas espécies de *Leishmania*. Em estudos anteriores, não havia sido detectado a presença de *Leishmania* spp. em exemplares de *D. rotundus* na Guiana Francesa e na Bahia (ROTUREAU et al., 2006; CUNHA et al., 2014).

No Estado do Mato Grosso do Sul, foi encontrado *L*.(*V*.) *braziliensis* em morcegos capturados na área rural e urbana, ao qual dois morcegos apresentavam lesões cutâneas, reforçando o potencial desta espécie em servir como reservatório de *Leishmania* spp. (SHAPIRO et al., 2013). Adicionalmente, na região do pantanal mato-grossense foi identificado a presença de *L*.(*V*.) *braziliensis* nesta espécie de mamífero (FERREIRA et al., 2017). Em Campo Grande (MS), 30/80 morcegos (das espécies *Platyrrhinus incarum, Phyllostomus discolor, Platyrrhinus lineatus, Artibeus planirostris* e *Artibeus lituratus*) foram positivos para *Leismania* spp. Destes animais, 18 foram positivos para *L*.(L.) *infantum*, demonstrando que estes morcegos podem estar infectados e talvez devam ser levados em consideração nas operações de controle de doenças pelas autoridades de saúde pública. A eutanásia de milhares de cães soropositivos nesta cidade, não parece estar sendo eficaz para controlar a doença, pois é crescente a incidência de LVC, podendo desta forma, possivelmente espécies silvestres e sinantrópicas estarem contribuindo para manutenção e circulação da leishmaniose (REZENDE et al., 2017).

Estes quirópteros representam 39% das 560 espécies de mamíferos viventes nas florestas tropicais sul-americanas. É importante não excluir a possibilidade de considera-los como potenciais hospedeiros e/ou reservatórios de *Leishmania* spp., dado o pequeno número de estudos que envolvem leishmaniose. Além disso, esses animais são encontrados em ambientes silvestre, doméstico e sinantrópico, sendo capazes de colonizar hábitats variados. Seus refúgios incluem ocos de árvores, folhas de bananeiras, forros de habitações humanas e construções rurais, locais onde também pode haver o vetor transmissor da leishmaniose (CONCEIÇÃO-SILVA e ALVES, 2014).

## 3.7 DIAGNÓSTICO:

A LV requer um diagnóstico preciso e o mais precocemente possível (BRASIL, 2014a). Diferentes técnicas diretas e indiretas são utilizadas no diagnóstico, com base em métodos sorológicos, parasitológicos e/ou moleculares, sendo muitas vezes necessária a associação de mais de um teste, pois há limitações de sensibilidade e especificidade inerentes a cada técnica (MEGID et al., 2016).

A problemática para os serviços de Saúde Pública, quanto ao diagnóstico da LVC, se deve a três fatores: variedade de sinais clínicos semelhantes a outras enfermidades infecto-contagiosas; alterações histopatológicas inespecíficas e a inexistência de um teste diagnóstico

altamente específico e sensível. O diagnóstico laboratorial da enfermidade no cão e no homem são semelhantes assim como muitos sinais clínicos (KRAUSPENHAR et al., 2007).

O método sorológico é a técnica de escolha para utilização em inquéritos epidemiológicos, pela praticidade, rapidez, facilidade de conservação da amostra e por não ser invasivo. Atualmente, está disponível pelo Ministério da Saúde (MS), a técnica de ELISA que apresenta alta sensibilidade e especificidade, a qual expressa os níveis de anticorpos circulantes. O único laboratório de referência no RS, que tem seus resultados validados pelo MS, é o Instituto de Pesquisas Biológicas – Laboratório Central de Saúde Pública (IPB-LACEN/RS), o qual envia os laudos diretamente ao Centro Estadual de Vigilância em Saúde (CEVS). Este por sua vez encaminha os dados para a Coordenadoria Regional de Saúde ao qual o município solicitante pertence (RIO GRANDE DO SUL, 2011).

Até o ano de 2011, o MS recomendava o teste ELISA como triagem (*screening*) e a RIFI como confirmatória, com uma sensibilidade entre 98 a 99,2% e especificidade de 70 a 90%. Os cães com títulos iguais ou superiores a 40 na RIFI são considerados positivos. Em humanos títulos maiores de 80 são avaliados como positivos. A RIFI pode apresentar reações cruzadas entre espécies de *Leishmanias* e outros Tripanosomatideos (BRASIL, 2006).

A partir de 2012, após a emissão de uma Nota Técnica Conjunta 01/2011, orientou-se para a utilização de um teste rápido imunocromatográfico (TR DPP®-Leishmaniose Visceral Canina – Bio- Manguinhos, RJ) como triagem e o ELISA como confirmatório da doença (BRASIL, 2011; BRASIL, 2014a). No DPP é possível utilizar soro, plasma ou sangue total. No método ELISA, a amostra será considerada reagente quando o valor da densidade óptica for igual ou superior a três desvios padrões do ponte de corte.

Em decorrência das reações cruzadas e possíveis falsos diagnósticos, deve-se observar os sinais clínicos se houverem, aliados aos aspectos epidemiológicos como: procedência do animal, presença de vetores (flebotomíneos e triatomíneos), contato com animais silvestres, dentre outros. O diagnóstico definitivo deve ser confirmado por outra técnica como a PCR (LUCIANO et al., 2009).

O diagnóstico parasitológico é o método direto que permite a visualização do parasito obtido de material biológico de punções: hepática, linfonodos, esplênica, medula óssea e biópsia ou escarificação da pele. É considerado um procedimento simples, porém invasivo. Este procedimento oferece risco ao animal, sendo impraticável em programas de Saúde Pública, quando um grande número de animais deve ser avaliado em um curto espaço de tempo. A sensibilidade do teste é de 80% em cães sintomáticos e abaixo de 80% nos casos soropositivos

assintomáticos e dependerá do grau de parasitemia do hospedeiro, tipo do material coletado, o tempo disponível para realização da leitura da lâmina e a experiência do técnico, (WHO, 2010).

O isolamento do protozoário em meio de cultura (*in vitro*) é realizado em meio contendo ágar acrescido de sangue de ovino desfibrinado, onde ocorre a transformação da forma amastigota em promastigota. O meio de rotina utilizado é denominado de *Novy-MacNeal-Nicolle* (NNN). O cultivo deve ser examinado semanalmente, durante 4 a 6 semanas. Em caso de suspeita de diagnóstico que apresentou cultura positiva, encaminha-se o material para o laboratório de referência, para se realizar a identificação da espécie de *Leishmania* envolvida por PCR.

A inoculação do parasito em animais suscetíveis (*in vivo*) como o hamster (*Mesocricetus auratus*) é utilizado de forma experimental para a obtenção do protozoário, pois tem um período longo de observação que varia entre 1 a 3 meses (REITHINGER et al., 2007; WHO, 2010).

As técnicas biomoleculares para detecção do material genético (DNA) do parasita, têm demonstrado boa perspectiva, principalmente a Reação da Polimerase em Cadeia (PCR), pois apresenta sensibilidade e especificidade próximas a 100%. Entretanto, o custo inicial pode ser elevado, e desta forma, constituir um fator limitante para utilização na rotina de diagnósticos. Avanços dos métodos moleculares permitem a utilização do PCR em tempo real (*real-time* PCR), que possibilita a quantificação da carga parasitária e a identificação da espécie envolvida (LANGONI et al., 2016).

A identificação exata das espécies de *Leishmania* é de grande importância no que diz respeito a epidemiologia, incluindo o diagnóstico, prognóstico da doença, vigilância epidemiológica e os estudos clínicos. As técnicas moleculares têm grande utilidade para a identificação de agentes infecciosos em tecidos, secreções, excreções e fluidos corporais de animais, trazendo informações que não podem ser obtidas por meio dos testes imunológicos ou morfológicos (SINGH, 1997).

A detecção de DNA do parasito em amostras de sangue, medula óssea ou produtos de biópsias através do PCR é promissora, mas a técnica ainda não está padronizada. O teste apresenta sensibilidade superior à pesquisa de amastigotas em esfregaços de medula óssea, mas a sensibilidade de detecção de DNA *Leishmania* no sangue periférico foi de 70% (OSMAN et al., 1997). O método de PCR em tempo real apresenta as mesmas dificuldades da técnica convencional, com sensibilidade comparável, podendo ser útil no acompanhamento da carga parasitária durante o tratamento, na definição da cura da infecção e na identificação de recidivas (CONCEIÇÃO-SILVA & ALVES, 2014).

## MANUSCRITO 1

**Identification of infection by *Leishmania* spp. in wild and domestic animals in Brazil: a systematic review with meta-analysis (2001–2021)**

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# Identification of infection by *Leishmania* spp. in wild and domestic animals in Brazil: a systematic review with meta-analysis (2001–2021)


## Abstract

Leishmaniosis is a protozoonosis caused by *Leishmania* spp. causing different clinical manifestations in humans and animals, and presenting multiple hosts. The transmission of the agent is carried out by sandfly vectors. The main objectives of this systematic review were to identify the host or reservoir animal species of *Leishmania* spp., with the exception of domestic dogs, that were recorded in Brazil, including diagnostic methods, and the species of protozoa. For this purpose, a literature search was conducted across index journals. This study covered the period from 2001 to 2021, and 124 studies were selected. Eleven orders of possible hosts were identified, with 229 species of mammals. Perissodactyla order having the highest number of infected individuals, 30.69% (925/3014), emphasizing the highest occurrence in horses. In Brazil, the main infected species were horses, domestic cats, rodents, and marsupials. Bats were identified as new potential reservoirs of *Leishmania* spp. infection by one or more species of the protozoan. Molecular tests are the most commonly used diagnostic method (94 studies). Many studies detected: *Leishmania* spp. (n=1422), *Leishmania* (*Leishmania*) *infantum* (n=705), *Leishmania* (*Viannia*) *braziliensis* (n=319), and *Leishmania* (*Leishmania*) *amazonensis* (n=141). Therefore, it is fundamental to know the species of animals involved in the epidemiology and biological cycle of the protozoan in order to identify environmental biomarkers, as well as *Leishmania* species to control this relevant zoonosis.


**Keywords**: leishmaniosis, reservoir, host, protozoan, mammals

## 1. Introduction

Leishmaniasis are reemerging diseases with worldwide distribution, and constitute a public health problem. Brazil is considered endemic for the three forms of presentation of the disease: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL) and visceral leishmaniasis (VL) (WHO 2021). Leishmaniasis in Brazil are included in the National Information System on Compulsory Notification of Diseases of the Ministry of Health, with case records in all Federated Units, affecting mainly the poorest populations (WHO 2010).

Among the countries in the Americas, Brazil has more than 97% of cases of VL in humans, with a high lethality rate, where cases of comorbidity with HIV contribute to the spread of infection. Considering CL and MCL, the country presented, in the 2018–2020 period, a very intense level of transmission of the disease, with 50.5% of its population living in transmission zones. Regarding VL in the Americas, in Brazil, 16 municipalities were classified as very high risk and 63 as high risk of transmission (WHO 2021; PAHO 2021).

Leishmaniasis is a group of infectious diseases caused by flagellated protozoans of the genus *Leishmania*, maintained among multiple hosts, wild, synanthropic, and domestic reservoirs, vectors, and humans (Quinnell and Courtenay 2009). *Leishmania* spp. is transmitted by blood meal of the female of several sandflies species (order Diptera, family Psychodidae, subfamily Phlebotominae). The transmission and dissemination of the disease are complex because of the ecological relationships between vertebrate hosts and vectors, which are linked to environmental and socioeconomic factors (Hong et al. 2020).

The disease in hosts is asymptomatic or symptomatic, depending on the parasite species involved and the host's immune response (BRASIL 2014). The difficulty in diagnosing leishmaniasis arises from the different clinical manifestations of the disease in humans and animals, diverse species of *Leishmania* as etiological agents, and the variability of the diagnostic tests used. In addition, the disease control measures recommended by the Brazilian Ministry of Health are complex, ineffective and generalists (Machado et al. 2016), including euthanasia of untreated infected dogs because they are considered the main reservoirs of *L.* (*L.*) *infantum*, which causes VL in Brazil (BRASIL 2014).

The lack of impact of these control measures raises the need to understand the triggering factors of the territorial spread of leishmaniasis, as the disease was considered wild, now it has been adapted to the urban environment. Machado et al. (2016) found that mass euthanasia of dogs did not decrease the prevalence of this disease in humans or the incidence of canine diseases.

Such evidence suggests that other mammals may be involved in maintaining this protozoan under conditions of natural infections close to human houses. It is assumed that when infected foxes and skunks feed close to human dwellings, they contribute to the transmission of the parasite to the vector, and consequently to dogs or humans, which makes these animals an important peridomestic reservoir (Machado et al. 2016). These factors, added to the changes that influence the abundance of vectors, such as deforestation, climate change, or new urbanized areas, as well as the investigation of the role of other animals in the epidemiology of the disease, are essential in monitoring new outbreaks (BRASIL 2014; Oliveira et al. 2005).

The objective of this study was to summarize information from scientific studies addressing leishmaniasis in different regions of Brazil, carried out between 2001 and 2021, including wild and domestic hosts and/or potential reservoirs, with the exception of domestic dogs. Compared to dogs and sandflies, the importance of other hosts and/or reservoirs in the transmission and maintenance of *Leishmania* spp. is poorly understood (Quinnell and Courtenay 2009).

Thus, we sought to verify the main epidemiological aspects, including the main animal species involved in the biological cycle, the presence or absence of clinical signs, and the Brazilian regions and states with scientific studies identifying the protozoan, sample tissue collected, diagnostic methods used, and etiological agents circulating in different Brazilian regions.

## 2. **Materials and Methods**

This study was characterized as a systematic review with an meta-analysis. The systematic review method was used to highlight the species of wild and domestic animals where the protozoan *Leishmania* spp. was identified in Brazil. After this review, meta-analysis was used to summarize the studies carried out in different locations by diverse researchers, using varied experimental designs, which, if considered individually, would not provide conclusive data on a major effect.

Four researchers independently, following the guidelines for systematic reviews and meta-analyses (PRISMA) (Page et al. 2021), carried out analyses of scientific articles published in indexed journals to compose a database with studies that evaluated the presence of *Leishmania* spp. in different naturally infected animal species, which acted as hosts and/or reservoirs in the wild or domestic environment during 2001–2021.

2.1 Information source

In this study, the following databases were used: PubMed (Medline), Science Direct, SciELO, and Web of Science (WoS), in addition to Google Scholar. The search terms for the research were: "*Leishmania*" AND "host" OR "reservoir" AND "Brazil".' Each author reviewed a database and selected studies carried out according to the eligibility criteria in the stipulated period.

2.2 Inclusion criteria

To compose the sample, studies involving various animal species naturally infected with the genus *Leishmania* were selected, including scientific articles, notes, and case reports. The criteria considered for inclusion of the studies were: the date of publication of the work between January 2001 and December 2021, all wild and domestic animal species involved, and studies carried out only in Brazil with positive results for the protozoan *Leishmania* spp.

2.3 Exclusion Criteria

Studies published in books, book chapters, reviews, meta-analyses, annals, theses, and dissertations were excluded. The exclusion criteria were as follows: research with human beings, domestic dogs as VL reservoirs, vaccine and immunological tests, drug tests, other trypanosomatids, vectors or food sources of sandflies, and publications outside the stipulated period. Additionally, articles that presented inconsistent data, duplicated publications, experimental infections, or negative results were rejected.

2.4 Extraction of information

The systematic review was conducted in three stages: identification, screening, and inclusion. All titles and abstracts of the papers obtained were analyzed, and when the information provided was insufficient for inclusion, the text was read in full.

Subsequently, the selected publications were read according to the inclusion criteria (n=124), and information (Fig. 1) was extracted from the material and methods sections and the results of each study. Data were tabulated in an electronic spreadsheet (Excel 2013), and the following variables were analyzed: year of publication, type of publication, Brazilian geographic region of capture of the animal or collection of biological material, animal species, sample number, order and species, wild or domestic, number of infected animals, presence of clinical signs, type of biological sample collected, diagnostic test, and the species of protozoan found. As the main

objective of the systematic review was to verify Brazilian animals identified with one or more species of the protozoan, studies with a sample size equal to one were also considered.

### 2.5 Data analysis

All relevant information was tabulated and submitted to three sequential analyses. Publication bias was assessed using funnel plots. To assess heterogeneity, the Higgins and Thompson $I^2$ test was used, where an $I^2$ value close to 0% indicated that there is no heterogeneity among the studies. A scale close to 25% indicated low heterogeneity, close to 50% indicated moderate heterogeneity, and close to 75% indicated high heterogeneity. A graphical representation of the meta-analysis was generated using a forest plot. Analyses were performed using the statistical program R, version 3.4.3.

## 3. **Results**

The database search included 3856 scientific studies, of which 1541 were selected from Science Direct, 1120 from Google Scholar, 446 from PubMed, 390 from Web of Science (WoS), and 359 from SciELO. From these studies, articles were excluded because they were characterized according to the criteria, resulting in 336 eligible studies (Fig. 1).

During the screening process, 103 and 83 studies were excluded because of duplicity and inconsistent data, respectively, resulting in 150 studies for further evaluation. Subsequently, 26 articles were eliminated after complete reading as follows: negative results for *Leishmania* spp. (n=6), describing only the vector (n=2), reviews (n=2), experimental infections (n=3), studies carried out in another country (n=1), publication outside the stipulated period (n=1), study carried out only with another protozoan (n=2), and inconclusive results (n=9).

The systematic review and meta-analysis comprised 124 studies, including 81 articles, 29 brief communications, and 14 case reports (Online Resource 1).

### 3.1 Animals evaluated

A total of 13,905 animals were evaluated for the presence of protozoan belonging to the genus *Leishmania*. These domestic and wild animals belong to 11 orders and 229 species: Artiodactyla (8 species), Carnivora (24 species), Cetartiodactyla (1 species), Chiroptera (63 species), Cingulata (4 species),

Didelphimorphia (23 species), Lagomorpha (2 species), Perissodactyla (4 species), Pilosa (2 species), Primate (32 species), and Rodentia (66 species). The meta-analytical study showed that of all animals evaluated, 25% ($I^2$=94%, $\tau^2$=3.2837, p=0) were infected with one or more species of *Leishmania* according to the forest plot (Online Resource 2). Considering the infection of animals by the protozoan, it was observed that of the total number of domestic animals evaluated, 26% ($I^2$=95%, $\tau^2$=4.5041, p<0.01) were infected, and of the total number of wild animals surveyed, 24% ($I^2$=93%, $\tau^2$=2.4932, p<0.01) were infected with one or more species of *Leishmania*, as shown in the forest plot graphs (Online Resource 3 and 4).

The main orders and species infected with *Leishmania* spp. in this study are described in Tab. 2 (Online Resource 5). The order Perissodactyla, including domestic horses, donkeys, mules, and ponies, had the highest number of individuals infected with one or more species of *Leishmania* spp. (33.3%). Domestic horses (*Eqqus caballus*) were researched in 17 studies for the meta-analysis, with 889 animals infected by the protozoa *L.* (*L.*) *infantum*, *L.* (*V.*) *braziliensis* and *Leishmania* spp. in different states of the country: Rio Grande do Sul, Paraná, Mato Grosso do Sul, Mato Grosso, Minas Gerais, São Paulo, Rio de Janeiro, Goiás, Pernambuco, Sergipe and Tocantins.

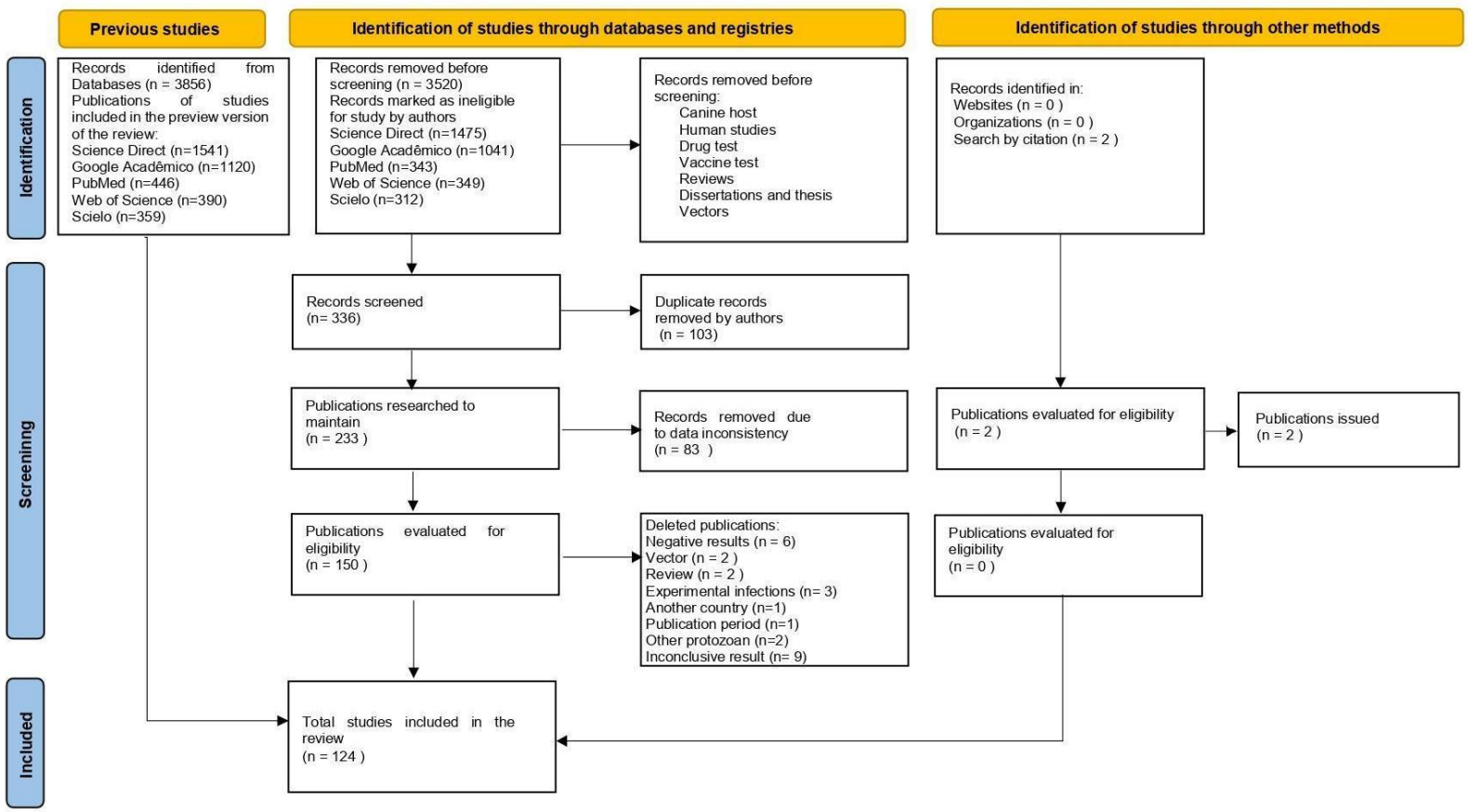


**Fig. 1** Flowchart (PRISMA 2020) demonstrating the phases of the systematic review, including the number of papers included and excluded, from 2001 to 2021, and the studies selected for final analysis (Page et al. 2021)

The order Carnivora (25.2%) included domestic animals, standing out the cats, present in 38 studies, where they obtained a positivity of 13.9% (557/3,983) for *Leishmania* spp. In wild animals, positivity was observed in the following species: bush dog (*Spheotos venaticus*) (65.2%, 15/23), (*Cerdocyon thous*) (31.3%, 63/201), maned wolf (*Chysocyon brachyurus*) (24.7%, 19/77), ring-tailed coati (*Nasua nasua*) (21.1%, 15/71), and Brazilian fox (*Lycalopex vetulus*) (15.4%, 8/52), among others.

Animals of the Rodentia order accounted for 16% of the total infected species in Brazil, described in 28 scientific studies, which evaluated 1893 animals, belonging to 66 species, detecting *Leishmania* spp. in 446 individuals (23.5%) of 39 species, obtaining greater emphasis on *Rattus rattus,* where 397 individuals were evaluated, with 117 infected (29.5%) with *L.* (*L.*) *infantum*, *L.* (*L.*) *amazonensis*, *L.* (*V.*) *braziliensis*, *L.* (*L.*) *mexicana*, *L.* (*V.*) spp., and *Leishmania* spp., *Nectomys squamipes* with an infection rate of 30.5% (65/213), where they identified *L.* (*L.*) *infantum*, *L.* (*V.*) *braziliensis*, and *L.* (*V.*) spp. and *Necromys lasiurus* with 24.5% (27/110) of individuals infected with *L.* (*L.*) *infantum*, *L.* (*L.*) *amazonensis*, *L.* (*V.*) *braziliensis*, *Leishmania* spp., among others.

In the systematic review constituting the order Didelphimorphia, it was observed that 12.3% (342/2779) were infected with *Leishmania* spp. of the total number of infected animals. The order Didelphimorphia was researched in 28 studies, where the protozoan was identified in 20.3% (342/1680) of the animals, including mainly the species, such as common opossum (*Didelphis marsupialis*) (32%, 50/156), black-eared opossum (*Didelphis aurita*) (21.9%, 34/155), white-eared opossum (*Didelphis albiventris*) (17.7%, 183/1033), among others.

In the order Chiroptera, the bats were present in 10 studies (9%, 251/2779) analyzed. However 11.6% (251/2167) of the individuals were infected with the genus *Leishmania* among the total number of bats evaluated. In 63 species of these mammals, *Leishmania* spp. was mostly found in the species, *Molossus rufus* (77.3%, 34/44), *Desmodus rotundus* (22.7%, 15/66), *Artibeus planirostris* (18.7%, 12 /64), *Carollia perspicillata* (16.9%, 12/71), *Artibeus lituratus* (16.7%, 20/120), *Molossus molossus* (14.9%, 53/356), and *Glossophaga soricina* (8.6%, 31/361), among others.

### 3.2 Presence of clinical signs

Regarding the presence of clinical signs, 11% ($I^2$=6%, $\tau^2$=121.9466, p=0.30) of the analyzed animals presented some type of clinical manifestation. When analyzed separately, domestic animals and wild animals presented 14% ($I^2$=36%, $\tau^2$= 157.7120, p<0.01) and 6% ($I^2$=0%, $\tau^2$=15.8338, p=1.00) of individuals with clinical signs, respectively (Online Resource 6, 7 and 8).

3.3 Sample for diagnosis

In order to evaluate the positivity of the protozoan *Leishmania* in the animals investigated in the scientific journals comprising this review, different organs and tissues were sampled and tested by various methods. Whole blood, serum, and skin samples were used in 86, 65, and 42 studies, respectively. The spleen (29 studies), liver (23 studies), bone marrow (27 studies), and lymph nodes (16 studies) were posthumously collected. Other organs have also been used in the study, however, with less frequency, such as kidneys (7), lungs (5), heart (4), intestine (3), central nervous system (2), adrenal gland (1), trachea (1), esophagus (1), stomach (1), tongue (1), ocular conjunctiva (2), and scent gland (1).

In studies that used live animals, the collected samples were blood (76), lymph node aspirate (13), aspirate of lesions or nodules (6), spleen (2) or liver aspirate (2), conjunctival swab (8), oral swab (1), and aqueous humor collection (1). Also in two studies, ectoparasites were collected to assess the presence of protozoan DNA (Morais et al. 2013; Pedrassani et al. 2019).

3.4 Diagnostic tests

Serological, parasitological and molecular tests are used for diagnosing leshmaniasis. In this systematic review, it was observed that diagnostic tests are used alone or in combination to determine the presence of the protozoan and the species involved in animal infection. In 59.7% (74/124) of the studies, indirect serological tests were performed to detect the presence of antibodies, 39.5% (49/124) used direct parasitological tests, 75.8% (94/124) used molecular techniques for diagnosis. In addition, 4% (5/124) of the studies associated xenodiagnosis. The diagnostic tests used in each sample collected (tissue/organ) from the respective animals of each order and the identified protozoan species can be verified in the Online Resource 1.

3.5 Brazilian regions showing the states with scientific studies included in this systematic review:

The Northern region of the country comprises the states of Amazonas, Acre, Rondônia, Roraima, Pará, Tocantins, and Amapá. However, seven studies included in this review encompassed the states of Amazonas (1), Tocantins (2), and Pará (4), identifying *L.* (*L.*) *infantum* (62.6%), *Leishmania* spp. (3.9%), *L.* (*V.*) *lainsoni* (1.3%), *L.* (*L.*) *amazonensis* (0.4%), *L.* (*V.*) *braziliensis* or *L.* (*V.*) *guyanensis* (20.9%), *L.* (*L.*) *amazonensis* or *mexicana* (10.9%), and *L.* (*L.*) *major* or *tropica* (0.4%) (Online Resource 9).

The Northeast region has nine states; however, in Ceará, there were no studies about leishmaniasis in animals meeting the criteria. Six publications were regarding Pernambuco. In Bahia and Piauí, four studies were conducted and in Maranhão and Rio Grande do Norte three publications in each state were selected. Paraíba and Sergipe contained one publication from each state. The protozoan species that was most frequently described in this region was *Leishmania* spp. (32.7%), followed by *L.* (*L.*) *infantum* (29.7%), *L.* (*V.*) *braziliensis* (27.2%), *L. Viannia* spp. (9.4%), and finally, coinfection with *L. Viannia* spp. and *L.* (*L.*) *infantum* (1.0%) (Online Resource 10).

The Southeast region had the highest number of publications that met the inclusion criteria, with 52 studies being selected: 26 from the state of São Paulo, 20 from Minas Gerais, five from Rio de Janeiro, and one from Espirito Santo. In this region, 1059 infected individuals were identified and determined: *Leishmania* spp. (48.5%), *L.* (*L.*) *infantum* (21.3%), *L.* (*L.*) *amazonensis* (12.3%), *L.* (*V.*) *braziliensis* (10.3%), *Leishmania* spp. (2.7%), *Leishmania donovani* (2.2%), *L.* (*L.*) *mexicana* (0.8%), *L.* (*V.*) *guyanensis* (0.4%), *L. Viannia* (0. 1%) and co-infection with *L.* (*L.*) *amazonensis* and *L.* (*L.*) *infantum* (0.5%), *L.* (*V.*) *braziliensis* and *L.* (*L.*) *infantum* (0.3%), *L. donovani*, and *L.* (*L.*) *infantum* (0.3%) (Online Resource 11).

In the Midwest region, comprising three states and the Federal District, 13 studies were published in the state of Mato Grosso do Sul, seven in Mato Grosso, and six in Goiás, including *Leishmania* spp. (72.5%), *L.* (*L.*) *infantum* (22.1%), *L.* (*V.*) *braziliensis* (3.2%), *L.* (*L.*) *amazonensis* (0.9%), *Leishmania* spp. (0.7%), *L.* (*Viannia*) spp. (0.2%), *L.* (*V.*) *guyanensis* (0.2%), and *L.* (*L.*) *hertigi* (0.2%) (Online Resource 12).

In the Southern region of Brazil, six publications were obtained from the state of Paraná, one from Santa Catarina, and four from Rio Grande do Sul, with 40.7% of *Leishmania* spp. in infected animals, 27.3% *L.* (*L.*) *infantum*, 22.8% *L.* (*V.*) *braziliensis*, 7.3% *L.* (*L.*) *enrietii*, and 1.9% *L.* (*L.*) *amazonensis* (Online Resource 13).

Among the selected articles, six involved data collected in multiple Brazilian states or in more than one region. Almeida et al. (2018) investigated the presence of the protozoan *Leishmania* in wild carnivores (*Cerdocyon thous*) in the Northeast region, including the states of Alagoas, Bahia, Paraíba, and Pernambuco, identifying *L.* (*L.*) *infantum* in an individual in captivity in Bahia. Cássia-Pires et al. (2014) detected positivity for *Leishmania* spp. in 3.9% (13/373) of rodents: *Clyomys laticeps* (*L.* (*L.*) *infantum*), *Dasyprocta azarae* (*L.* (*L.*) *infantum*), *Thrichomys fosteri* (one *L.* (*V.*) *naiffi* and one *Leishmania* spp.) captured in the state of Mato Grosso do Sul. *Thrichomys inermis* (*L.* (*V.) shawi*) was captured in Bahia. *Thrichomys laurentius* (*L.* (*V.*) *guyanensis*, *L.* (*L.*) *infantum*, *L.* (*V.*) *guyanensis* and *L.* (*V.*) *braziliensis*, four individuals with *Leishmania* spp. in the state of Piauí. In the state of Mato Grosso, rodents captured did not presented *Leishmania* sp.

Evers et al. (2017) detected serologically *Leishmania* spp. (46%, 183/398) in horses from the South (Paraná), Southeast (Minas Gerais and Rio de Janeiro), and Midwest regions (Goiás, Mato Grosso, and Mato Grosso do Sul). Ferreira et al. (2013) obtained samples of carnivores from Minas Gerais and Bahia, where *Leishmania* sp. was detected in *Chrysocyon brachyurus* using serology. Mol et al. (2015) used molecular, serological, and xenodiagnostic tests to detect *L.* (*L.*) *infantum* in *Chrysocyon brachyurus* and *Speothos venaticus* captured in Minas Gerais, Goiás, and the Federal District. Silva et al. (2016) collected blood samples from *Didelphis albiventris* and *Didelphis aurita* in Alagoas and Pernambuco and, using molecular techniques, identified the DNA of *L.* (*V.*) *braziliensis* in *Didelphis albiventris* (17.4%, 4/23).

## 4. **Discussion**

Successful attempts to prevent and control leishmaniasis depend on a combination of intervention strategies, one of which is the identification of hosts and reservoir animals. However, control is complex and must be adapted to local situations. Changes in temperature, precipitation and humidity can affect hosts, reservoirs and vectors, altering their distribution and influencing their survival and population size (WHO 2022). In this study, we were able to gather information from publications that described *Leishmania* spp. in domestic and wild animals in Brazil, classified into 11 orders, of which only Cetartiodactyla had no species (*Odocoileus virginianus* = white-tailed deer) infected with this protozoan.

Domestic animals that were positive for one or more parasite species belonged to three orders: Artiodactyla (cattle and pigs), carnivores (domestic cats) and Perissodactyla (horses, donkeys and mules). In the wild animals evaluated in Brazil, positivity was detected for one or more species of protozoan comprising the following orders: Didelphimorphia, Carnivore, Chiroptera, Cingulata, Lagomorpha, Pilosa, Primate, and Rodentia (Online Resource 5).

In this study, the similarity in occurrence of infection by the protozoan in domestic (26%) and wild (24%) animals was observed, with *Leishmania* species causing both VL and CL. The role of wild animals in maintaining the circulation of the agent between the vector, animals, and humans has been highlighted, contributing to the increase in the occurrence of the disease in humans and animals throughout Brazil. Hence, it becomes difficult to promote intervention actions to control the disease because the biological cycle is maintained in a wild environment (Campbell-Lendrum et al. 2001). The disease shows some epidemiological changes arising from the expansion of agricultural frontiers and their consequent destruction of wild habitats, global warming and the popularization of ecotourism, among other factors, which have led to greater contact with protozoan transmission

cycles, allowing the exchange of parasites between wild and domestic animals and humans (BRASIL 2014; Conceição-Silva and Alves 2014).

Therefore, it is essential to evaluate the role of naturally infected animal species, which can circulate and share these spaces with humans and domestic animals. The protozoa that cause leishmaniasis have great genetic heterogeneity and eclecticism regarding the different orders of mammals that they are capable of infecting, resulting in a complex epidemiology, where only the detection of infection by *Leishmania* spp. in a mammal is not sufficient to incriminate it as a reservoir (Chagas et al. 1938). Therefore, for its characterization, it is necessary to associate it with certain conditions; for example, the parasite population depends on the infected mammal for its multiplication and maintenance of its cycle, the survival time of the host, the animal susceptibility to infection by the parasite, and the presence of protozoa in the peripheral circulation, where they are available for infection by sandflies (WHO 2010).

The order Artiodactyla presented *L.* (*L.*) *infantum* only in domestic species, such as domestic cattle (*Bos taurus*) and pigs (*Sus scrofa domesticus*) (Moraes-Silva et al. 2006; Vioti et al. 2019). Data on the effects of cattle and other large animals on the risk of VL acquisition are contradictory. In a study carried out in Nepal by Bern et al. (2010), an entomological survey was conducted, and the blood present in the vectors was exclusively bovine. However, in shed of both cattle and humans, insects feed on both species. In Colombia, cows and pigs are the preferred hosts for *Lutzomyia longipalpis* to feed on in an endemic area for VL. The vector is an opportunistic feeder that is not highly anthropophilic, and domestic dogs are not preferred (Morrison et al. 1993).

According to Ferro et al. (1995), after cattle, the vector's preferred food source is pigs, and pigsties are an important environment for the development, mating, and oviposition of *Lutzomyia longipalpis*. Pigs are often present in the vicinity of households in endemic areas, with a high prevalence of VL. Moreira (2003) suggested that dogs that live in these habitats have a 4.1 times high risk of getting infected *L.* (*L.*) *infantum*. Brazil et al. (1987) reported that in regions associated with the clearing of primary forests in Maranhão, where there were autochthonous cases of human CL, amastigotes of *Leishmania* spp. in a pig with an ulcerated lesion on the ear and the animal could be a probable source of infection for the vector.

Moraes-Silva et al. (2006) showed that *Sus scrofa domesticus* responded to the inoculation of *L.* (*L.*) *infantum* by producing circulating antibodies. Subclinical infections were verified; however, it was not possible to detect the parasite in the organs of these experimentally infected pigs, and using *in vivo* tests and xenodiagnosis. Pigs do not seem to act as reservoirs for *L.* (*L.*) *infantum*; however, they could play an important role in attracting

and maintaining the vector in the peridomicile area. More studies involving the order Artiodactyla are needed to elucidate the role of these animals as reservoirs for the VL and CL.

CL caused by *L.* (*V.*) *braziliensis* has been reported in all regions of Brazil (BRASIL 2005). In recent decades, changes in CL epidemiology have been understood in the context of deforestation, promoting the adaptation of parasites and vectors to modified and domestic environments, and the inclusion of new animal species acting as reservoirs (Vale and Furtado 2005). The occurrence of CL in horses has been described with greater frequency since the 1980s (Aguilar et al. 1986). The disease has endemic and zoonotic characteristics and should therefore be considered by veterinarians in the differential diagnosis of equine diseases when they present with papular and/or ulcerated lesions (Soares et al. 2013).

The first evidence of autochthonous infection of three horses with *L.* (*L.*) *infantum* was reported in Belo Horizonte/Minas Gerais, which was the first report from the Americas, and as a co-infection with *L.* (*V.*) *braziliensis*. Diagnosis was performed using serology (IFAT and ELISA), molecular techniques, and parasitological testing of skin lesions and bone marrow aspirates (Soares et al. 2013). However, the importance of horses in maintaining the parasite cycle as a reservoir needs to be further investigated, mainly through xenodiagnosis (Vedovello Filho et al. 2008).

Escobar et al. (2019) demonstrated in studies in Rio Grande do Sul that horses infected with *L.* (*L.*) *infantum* showed clinical changes similar to dogs with VL. The main clinical signs manifested are alopecia, nasal discharge, cutaneous nodules, and lymphadenopathy. These animals are a source of food for vectors in endemic areas. Moreover, *Lutzomyia intermedia* may be related to the transmission of the protozoan in horses and may also feed on humans, rodents, birds, and dogs (Afonso et al. 2005). This can pose risks to public health because many of these animals live close to humans, especially in peri-urban areas (Meneses et al. 2002).

The following species were identified in the order Carnivora in domestic cats: *L.* (*L.*) *infantum* (Batista et al. 2020), *L.* (*L.*) *amazonensis* (Carneiro et al. 2020), *L.* (*V.*) *braziliensis* (Costa-Val et al. 2020), and *Leishmania* spp. (Bezerra et al. 2019). The number of pets is increasing in Brazilian homes and according to Instituto Brasileiro de Geografia e Estatistica (IBGE 2013) 22.1 million domestic cats share the same environment with humans and other domestic animals. These animals may represent an important source of infection for sandflies, because cats have a high level of protozoan parasitism in the skin (Vides et al. 2011) demonstrated by xenodiagnosis (Silva et al. 2010), and in the field situation sandflies feed on feline, among other species (Sales et al. 2015). In the last decade, an increase in cases of infection with *L.* (*L.*) *infantum* in cats has been observed, suggesting that these animals may be alternative reservoirs for this protozoan in urban areas, in addition to the participation of

synanthropic mammals, such as opossums and rats (Oliveira et al., 2005; Schallig et al., 2007). This increase in infections in other animal species may be due to the use of sandfly repellents on dogs, forcing the parasite to search for other hosts (Ahuir-Baraja et al. 2021).

Symptomatic domestic felines show nonspecific clinical signs, including cutaneous and mucocutaneous alterations (Souza et al. 2005), lymphadenopathy, splenomegaly, weight loss, anorexia, and ocular lesions (Sobrino et al. 2008) which may occur in any age group, and the possibility of vertical transmission (Sousa et al. 2014). Thus, it is recommended that leishmaniasis be included in the differential diagnosis of diseases involving dermatopathies, ocular secretion, or lymph node enlargement (Berenguer et al. 2021).

The transmission of the protozoan to cats can occur close to residential areas, where there are degraded secondary forests due to the habit of hunting or wandering far from the residence, exposing the animal to contact with sandflies. This increases the risk of infection in humans because cats co-inhabit urban and wild environments (Souza et al. 2005; Maia and Campino 2011). However, Pennisi et al. (2018) considered that knowledge of many aspects of feline leishmaniasis is still incipient. Few studies have defined the course of the disease in felines, and there is no consensus on the best sample tissue for diagnosis. The underestimation of feline leishmaniasis is mainly due to low seroprevalence titles, asymptomatic cases, or neglect in the diagnosis of the disease that affects animals (Soares et al. 2016).

In Brazil, some wild canids are considered wild reservoirs of VL, such as the tarantula fox (*Cerdocyon thous*), gray fox (*Lycalopex vetulus*), and maned wolf (*Chrysocyon brachyurus*) (Luppi et al. 2008). VL in endemic areas represents a threat to the conservation of some canid species kept in captivity. Currently adopted prevention measures are considered ineffective; however, the use of antiparasitic collars in captive wild canids has been recommended (Fornazari and Langoni 2014).

In the order Rodentia, synanthropic animals, such as *Rattus rattus*, *L.* (*L.*) *infantum*, and *L.* (*L.*) *amazonensis* were identified, demonstrating the urbanization of these protozoans, especially *L.* (*L.*) *amazonensis*, which causes CL, as described for the first time in urban areas in the Paraná State (Caldart et al. 2017). Rodents, such as *Necromys lasiurus* and *Rattus rattus* are considered reservoirs of *L.* (*V.*) *braziliensis* in an endemic region of Northeastern Brazil. Other species, such as *Agouti paca*, *Oryzomys subflavus*, *Nectomys squamipes*, *Bolomys lasiurus*, *Proechimys* spp., *Ripidomys* spp., *Akodon* spp., and *Trichomys aperoides* are reservoir rodents (Basano and Camargo 2004; Silveira et al. 1991; Brandão-Filho et al. 1994; 2003). Black rats (*Rattus rattus*) are potential sources of infection for peridomestic sandflies, such as *Lutzomyia intermedia* and *Lutzomyia whitmani*, as demonstrated by Brandão-Filho et al. (2003).

Humans create ecologically simplified landscapes that favor some wild species that tolerate human-modified environments and become synanthropic. Rodents are a source of infectious diseases, such as leishmaniasis, when compared to wild hosts, and this association is independent of the taxonomy of the animal species (McFarlane et al. 2012). Many species of the order Rodentia living close to human habitats should be monitored for the presence of *Leishmania* spp. in wild and synanthropic reservoirs, which constitute an important risk factor for humans and domestic animals (Achilles et al. 2021).

In the order Didelphimorphia, adult opossums probably acquired infection with *Leishmania* spp. by close contact with humans and domestic dogs as a result of its territorial expansion. Wild species can also serve as biomarkers, reflecting the impact of environmental changes, acting as sentinel animals for environmental health (Berzunza-Cruz et al. 2015). Santiago et al. (2007) observed that the presence of opossums in the same canine living area is a predisposing factor for infection in dogs, although the role of these animals as reservoirs and their impact on the transmission of *L.* (*L.*) *infantum* in urban areas is not fully understood. Deforestation, the habit of people transforming their backyards into "rural areas" raising subsistence animals, such as chickens and pigs, maintaining a variety of fruit trees, offer conditions that contribute to the maintenance and proliferation of the vector and animals, such as those of the order Didelphimorphia (Humberg et al. 2012).

Considering the order Chiroptera, the first identification of a protozoan of the genus *Leishmania* in bats in Brazil was carried out by Savani et al. (2010), where they identified *L.*(*L.*) *amazonensis* in 18 individuals, of the species *Molossus molossus* (n=4), *Molossus rufus* (n=1), *Nyctinomops laticaudatus* (n=1), *Eumops glaucinus* (n=1), *Eumops auripendulus* (n=1), *Glossophaga soricina* (n=6), *Artibeus lituratus* (n=1), *Sturnira lilium* (n=1), *Myotis nigricans* (n=2), and *L. (L.) infantum* in three animals of the species *Molossus molossus* (n=2), *Glossophaga soricina* (n=1) from the state of São Paulo. It is noteworthy that in this study, 14 bats were captured in urban shelters, such as roofs, attics, basements, and garages, four of which were caught inside houses, and three were found motionless on the ground (alive or dead). All cohabiting humans and domestic animals can serve as a food source for the vector, and infection for VL and CL.

Lampo et al. (2000) demonstrated that bats can be a food source for *Lutzomyia longipalpis* in caves in Venezuela. These animals are nocturnal mammals and are only capable of flying, and may share environments and nocturnal habits with dipteran vectors of leishmaniasis. Some species migrate seasonally, which is an important feature that can result in the spread of parasitic species (Jones and Teeling 2006).

Berzunza-Cruz et al. (2015) infected bats with *L.* (*L.*) *mexicana* and demonstrated their ability to infect and transmit the agent to the vector. In Brazil, studies involving bats infected with *Leishmania* spp. were performed

in Minas Gerais, São Paulo, Rio de Janeiro, Espírito Santo, Mato Grosso do Sul and Maranhão. Based on our results, it can be inferred that bats in nature are infected with protozoa of the genus *Leishmania*. Further studies are needed to elucidate whether this species has the potential to infect the vector in nature and participate as a reservoir in the epidemiological cycle of leishmaniasis without showing clinical signs of the disease.

The appearance of clinical signs depends on the immunocompetence of the animal (BRASIL 2014). Most wild animals are considered reservoirs of *Leishmania* spp. that are asymptomatic for the manifestations of leishmaniasis, serving as a source of infection for the vector (Laison and Shaw 1987). Our review corroborates this fact, where 95% of infected wild animals did not show any type of clinical signs, indicating the presence of the protozoan. Infections in animals that present with asymptomatic VL and CL are common in endemic countries such as Brazil (Fornazari and Langoni 2014).

The high number of asymptomatic domestic or wild animals within an infected population, serving as a source of infection for the vector, often fails to be identified due to the absence of clinical signs or even due to presenting false-negative results in serological tests (Baneth et al. 2008). This represents a great risk to public health and even to the conservation of endangered species, in the case of those kept in captivity (Fornazari and Langoni 2014).

In general, the symptomatic form of leishmaniasis is associated with a variety of clinical signs that result from infected organs, depending on the parasite species involved. In humans, CL is characterized using processes that vary from benign, self-resolving lesions to ulcerated, edematous skin lesions at the site of the vector bite or disseminated throughout the body. In VL we observed fever, splenomegaly and/or hepatomegaly, cutaneous mucous pallor, diarrhea and/or non-productive cough, edema of the lower limbs, hemorrhages, jaundice, and ascites (BRASIL 2014).

Wild animals show clinical signs that are similar to those of domestic dogs. Clinical signs are rarely observed in free-living animals, and when present, they are of little severity (Fornazari and Langoni 2014). In a study of 41 captive primates in Belo Horizonte, an endemic region for VL, *L.* (*L.*) *infantum* was detected in 18 animals. Only one primate showed clinical signs compatible with the disease and died of cardiorespiratory arrest. At necropsy, marked weight loss, pulmonary edema, petechial hemorrhage in the lungs, epicardium, duodenum, and jejunum, liver enlargement with color changes, splenomegaly, and lymphadenopathy were observed. In the lymph nodes, liver, kidneys, lungs, and intestine, macrophages with intracytoplasmic amastigotes are present, resulting in a diagnosis of VL (Malta et al. 2010).

The protozoan that causes VL has a preference for lymphoid organs; therefore, these tissues are widely used for diagnostic tests, where amastigotes can be seen within monocytes and macrophages of tissues, such as the spleen, liver, lymph node, and bone marrow (Grimaldi and Tesh 1993). With regard to blood, parasite DNA detection may be lower than that in other organs due to low parasitemia (Lopes et al. 2017; Riboldi et al. 2018), or because blood tissue contains PCR inhibitors, which can interfere with DNA amplification (Al-Soud and Radstrom 2001). The detection of amastigotes in peripheral blood can also be difficult because it characterizes transitory passage tissue (Oliveira et al. 2015a).

However, the skin is the place where the first interaction between the parasite and the immune system takes place, in addition to being the place where large amounts of amastigotes can be found, depending on the animal species. Even in this clinically healthy tissue, parasites may be present, demonstrating the importance of these animals in the disease transmission cycle (Solano-Gallego et al. 2004). The sample tissues used for the detection of *Leishmania* spp. in the studies that comprise this review are described in more detail in the Online Resource 1.

The diagnosis of CL can be based on clinical examinations and epidemiological data; however, laboratory diagnosis and demonstration of the parasite must be performed (Gontijo and Carvalho 2003).

In relation to the diagnostic techniques used in the studies that made up this systematic review, it was observed that many studies used a combination of laboratory techniques, which varied according to the animal species studied, the number of samples used, the form of restraint required, and whether it was performed. Animals were euthanized for material collection, or if less invasive collection techniques were necessary. According to the Ministry of Health, to guide the choice of diagnostic techniques, it is important to know the limitations of each technique and their clinical interpretations (BRASIL 2014).

Serology has been widely used in studies on the order Carnivores. When sample is of greater magnitude, a serological technique is usually indicated owing to its practicality, which is usually used in epidemiological surveys in public health. The tests available and recommended by the Ministry of Health are TR-DPP and ELISA (BRASIL 2016). The serological techniques used in the systematic review included indirect immunofluorescence (IFAT), immunoenzymatic assay (ELISA), Direct Agglutination Test (DAT), immunohistochemistry, dot blot, and rapid tests, such as the Dipstick Test rk39, TR-DPP, and Kalazar Detect.

IFAT is widely used in studies, however, the diagnosis may interfere and present cross-reactions between VL, LC, and Chagas disease (BRASIL 2014). In addition, the use of the specific conjugate in wild species in immunomediated tests is restricted (Ferreira et al. 2013) and sometimes adapted techniques are used, constituting

a limiting factor, making it difficult to diagnose the agent. DAT with Leishmania promastigotes can be used in epidemiological studies because of its ease of use compared with other serological techniques (Vedovello Filho et al. 2008).

Many epidemiological studies have been conducted based on serological evaluations. However, many infected dogs do not seroconvert, hence, disease prevalence is always higher than seroprevalence (Baneth et al. 2008). In a study carried out with 55 domestic cats from a shelter in an endemic area for VL, with clinical dermatological signs, 49% of the animals were diagnosed with the disease using direct parasitological examination of lymphoid organs, serology, and immunohistochemistry (IHC) of skin lesions. However, it is noteworthy that only 55% of the evaluated cats had a positive diagnosis using serology, even with visible clinical signs, demonstrating the need for the use of other diagnostic techniques (Vides et al. 2011).

Parasitological diagnosis is a direct technique that demonstrates the protozoan in biological materials, such as liver punctures, lymph nodes, spleen, bone marrow, and skin biopsy. This technique is simple, however invasive and impractical in public health programs when it is necessary to evaluate a large number of animals. Specificity is approximately 100%, and sensitivity depends on the degree of parasitemia, decreasing in asymptomatic cases (BRASIL 2014).

Molecular techniques for detecting a parasite's genetic material (DNA) have demonstrated good applicability. Owing to its greater sensitivity, it reduces the possibility of false negatives compared with isolation methods or direct exams (Brandão-Filho et al. 2003). However, the cost of the test can be high, requiring specific laboratory equipment, qualified technicians, and a longer time to perform the technique, which limits its use in routine diagnosis. In the systematic review, PCR and some variations, such as n-PCR, multiplex-PCR, q-PCR, and RFLP-PCR were the diagnostic techniques used in the largest number of studies. The main genomic targets used in the molecular techniques were kDNA, ITS-1, HSP70, and SSU-rDNA, associated with sequencing (Achilles et al. 2021; Araujo et al. 2013; Brandão et al. 2020; Savani et al. 2010) (Online Resource 1).

The use of real-time PCR makes it possible to quantify the parasite load and identify the species of protozoan involved, and may also be useful in monitoring the parasite load during treatment, in defining the cure of the infection and in identifying relapses (Conceição-Silva and Alves 2014).

PCR is a valuable tool in epidemiological studies, allowing the detection of DNA from *Leishmania* spp. in different clinical samples of humans, animal hosts, and vectors without the need for exhaustive parasite isolation (Oliveira et al. 2005). However, the difficulty that often arises when dealing with the genus *Leishmania* is the inability to determine the species involved, due to lack of standardization of molecular technique. Furthermore,

low species specificity of kDNA and rDNA primers for members of the subgenus *L.* (*Viannia*) was observed (Brandão-Filho et al. 2003). In our study, most of the diagnoses in four of the five Brazilian regions were *Leishmania* spp., regardless of animal species. *Leishmania* causes a wide spectrum of diseases that are distinguished by different parasitic and host factors. The detection and characterization of the infecting species is essential for confirming the diagnosis, establishing the clinical prognosis, and initiating an adequate therapeutic approach (Marfurt et al. 2003).

In Brazil, the protozoan species described in this review are widely distributed in all regions, and are observed in both domestic and wild animals, offering risks of infection to humans (Santos et al. 2021). The transmission of CL and VL to humans has been described in several municipalities across all federal units (BRASIL 2014). Epidemiological data provided by the identification of parasite species in a given region are fundamental for the design of disease control measures (Bañuls et al. 2007).

*L.* (*L.*) *infantum* was detected in all Brazilian regions, with the highest number of infected animals identified in the northern region presented in this systematic review (62.6%, 143/230). Tocantins represent the state with the highest number of animals infected by this species within this region. This state has a high incidence of VL in humans, and the disease is expanding rapidly. The results obtained from the municipality of Araguaína suggest that horses from urban and rural areas, together with domestic cats, are in contact with sandflies that transmit the disease. The role of these animals in the parasite cycle needs to be elucidated so that more effective VL prevention actions can be instituted (Alencar et al. 2020; Sousa et al. 2019).

CL is an infectious, non-contagious disease caused mainly by *L.* (*L.*) *amazonensis*, *L.* (*V.*) *guyanensis*, and *L.* (*V.*) *braziliensis* (BRASIL 2016). An increased incidence of CL associated with *L.* (*V.*) *braziliensis* has been reported in all the Brazilian regions. In the state of Pernambuco, Northeastern region of the country, water rats (*Nectomys squamipes*), grass rats (*Bolomys lasiurus*) and black rats (*Rattus rattus*) were identified *L.* (*V.*) *braziliensis zymodeme* IOC/Z74. Diagnosis was performed using PCR using kDNA and SSurDNA primers, which commonly infect humans, reinforcing the hypothesis that small wild and synanthropic rodents are the main reservoirs of this parasite species. It should be noted that black rats are sources of infection for peridomestic sandflies, such as *Lutzomyia intermedia* and *Lutzomyia whitmani*. CL presents several regional patterns and reveals different aspects regarding the vectors involved, possible reservoirs, accidental hosts, transmission patterns, and eco-epidemiological traits (Brandão-Filho et al. 2003).

*L.* (*L.*) *amazonensis* was not identified in the northeastern region presented in this study. Eleven works identified this species of parasite, four involving the order Carnivora, three in the order Didelphimorphia and also

in Chiroptera, two in Rodentia and one in Primata, using molecular, serological, and parasitological techniques. In these studies, samples, such as the liver, spleen, blood, serum, skin, and aspirate of cutaneous nodules were used, indicating that the *Leishmania* species, even causing the cutaneous form of the disease, the visceralization of the protozoan was observed (Achilles et al. 2021; Caldart et al. 2017; Cândido et al. 2021; Cardoso et al. 2015; Carneiro et al. 2020; Gómez-Hernández et al. 2017; Oliveira et al. 2015b; Quintal et al. 2011; Savani et al. 2010; Souza et al. 2005; Souza et al. 2009).

In the southern region, we detected the DNA of the main species found in other Brazilian regions. This regionis endemic to CL and VL in several municipalities, however, *L.* (*L.*) *enriettii*, is outstanding. In Brazil, *L.* (*L.*) *enriettii* was first described in guinea pigs (*Cavia porcellus*) in the city of Curitiba in 1944 (Medina 1946-2001).

The strain was isolated and distributed to laboratories worldwide, and is currently marketed under ATCC® Number:50120™. It is used as a model organism for several studies, including experimental infections, cell-mediated immune studies, intracellular parasite survival, research on the spread of CL, treatment, and protozoan genome (Soccol et al. 2021). In 1967, there was again a report of a natural infection in Curitiba, with the described vectors *Lutzomyia monticola* and *Lutzomyia correalimai* (Luz et al. 1967). *L.* (*L.*) *enriettii* exists and survives in guinea pigs in Brazil; however, data on natural infections have not yet been explored. Infect humans but is a non-pathogenic species in this case. In Australia it has been identified in red kangaroo and in bovine in Switzerland (Paranaíba et al. 2017).

Soccol et al. (2021) evaluated 26 guinea pigs from eight cities in Paraná, with skin nodules or ulcers on the extremities of the body compatible with leishmaniasis, and 77 healthy captured wild animals in an attempt to identify natural hosts of *L.* (*L.*) *enriettii*. In the lesions of 100% of the guinea pigs, they found amastigotes of *Leishmania.* They also presented dyspnea, epistaxis, and died due to respiratory failure, and all others wild animals were negative for the genus *Leishmania*. The strains were confirmed as *L.* (*L.*) *enriettii*, causing diffuse cutaneous leishmaniasis, spreading via lymphatic and hematogenous routes, which needs to be considered in the differential diagnosis of dermatological diseases of guinea pigs, such as neoplasms and scabies. So far, the guinea pig is the only reservoir in Brazil, however, it develops a severe form of the disease and does not represent a good reservoir according to WHO criteria (WHO 2010).

One of the main problems that limits the understanding of the epidemiology of leishmaniasis is the identification of a vertebrate host as a natural reservoir. Natural reservoirs are largely unknown because of the

difficulties in capturing a sufficient number of wild animals and the techniques used to isolate and identify the parasites (Oliveira et al. 2005).

## 4. Conclusion

Studies carried out in different states of Brazil have reported different rates of positivity for infection by *Leishmania* spp. in diverse animal species, being responsible for VL and CL. The knowledge of role of these animals as reservoirs or hosts is essential for the adoption of control measures to prevent the expansion of transmission areas in the country. Although dogs are considered the main reservoirs of *L.* (*L.*) *infantum* in Brazil, the evidence found in our meta-analysis suggests that many other apparently healthy free-ranging mammals harbor *Leishmania* spp. and should be included in epidemiological studies on leishmaniasis, as cities expand and encompass forest remnants. It should be noted that horses and domestic cats were the most infected species in this systematic review. These animals usually share wild and domestic environments and may help maintain the protozoan cycle, increasing the risk of this zoonosis. The small number of animals showing clinical signs may indicate that several species can serve as hosts and/or reservoirs in different environments and close to human residences, favoring infection of the vector and, consequently, the emergence of the disease in humans and animals.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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**Author's contributions**

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Supplementary Material

**Online Resource 1- Table 1:** Studies carried out in Brazil between 2001 and 2021, identifying *Leishmania* spp. in animals belonging to different orders through molecular, serological and/or parasitological methods from collected organs and tissues.

**Online Resource 5- Table 2:** Description of the orders of mammals surveyed for the presence of the protozoan *Leishmania* sp., and the main host animal species where there was identification of one or more species of the agent.

**Online Resource 2- Fig. 2:** Forest Plot indicating the total number of animals infected with the protozoan *Leishmania* spp. (0.25 = 25%) in relation to the total number surveyed (13905 animals) selected by the authors in the systematic review.

**Online Resource 3- Fig. 3:** Forest Plot indicating the frequency of domestic animals infected with one or more species of *Leishmania* (0.26=26%).

**Online Resource 4- Fig. 4:** Forest Plot indicating the frequency of wild animals infected with one or more species of *Leishmania* (0.24 = 24%).

**Online Resource 6- Fig. 5:** Forest Plot indicating the total number of animals that showed clinical signs compatible with leismaniosis (0.11 = 11%).

**Online Resource 7- Fig. 6:** Forest Plot indicating the total number of domestic animals that showed clinical signs compatible with infection by *Leishmania* spp. in relation to the total number of domestic animals infected by the protozoan (0.14 = 14%).

**Online Resource 8- Fig. 7:** Forest Plot indicating the total number of wild animals that showed clinical signs compatible with infection by *Leishmania* spp. in relation to the total number of wild animals infected by the protozoan (0.06 = 6%).

**Online Resource 9- Fig. 8:** Number of mammals infected with *Leishmania* spp., demonstrating the percentage of protozoan species circulating in the states of Amazonas, Pará, and Tocantins in the North region of Brazil.

**Online Resource 10- Fig. 9:** Number of mammals infected with *Leishmania* spp., demonstrating the percentage of protozoan species circulating in the states of Pernambuco, Piauí, Bahia, Maranhão, Rio Grande do Norte, Paraíba and Sergipe in the Northeast region of Brazil.

**Online Resource 11- Fig. 10:** Number of mammals infected with *Leishmania* spp., demonstrating the percentage of protozoan species circulating in the states of São Paulo, Minas Gerais, Rio de Janeiro and Espírito Santo in the Southeast region of Brazil.

**Online Resource 12- Fig. 11** Number of mammals infected with *Leishmania* spp., demonstrating the percentage of protozoan species circulating in the states of Mato Grosso, Mato Grosso do Sul, Goiás and Distrito Federal in the Midwest region of Brazil.

**Online Resource 13- Fig. 12** Number of mammals infected with *Leishmania* spp., demonstrating the percentage of protozoan species circulating in the states of Paraná, Santa Catarina and Rio Grande do Sul in theSouth region of Brazil.

. **Online Resource 14 -** Search references in the databases to compose the systematic review (124 studies).

Table 1. Studies carried out in Brazil between 2001 and 2021, identifying *Leishmania* spp. in animals belonging to different orders through molecular, serological, and/or parasitological methods from collected organs and tissues.

| Authors | Study type | Animal order | Sample tissue | Diagnostic method | Protozoan species |
|---|---|---|---|---|---|
| 1. Achilles et al., 2021 | A | R/D | liver | molecular | *L.*(*V.*) *braziliensis* or *guyanensis*<br>*L.*(*V.*) *lainsoni*<br>*L.*(*L.*) *amazonensis*<br>*L.*(*L.*) *major* or *tropica*<br>*Leishmania* spp. |
| 2. Alencar et al., 2020 | A | P | serum | serological | *L.*(*L.*) *infantum* |
| 3. Almeida et al., 2018 | A | C | blood/serum | molecular/serological | *L.*(*L.*) *infantum* |
| 4. Araújo et al., 2013 | A | Pi | BM | molecular | *L.*(*L.*) *infantum* |
| 5. Barbosa et al., 2020 | SC | Ci | serum | serological | *Leishmania* spp. |
| 6. Batista et al., 2020 | A | C | serum | serological | *L.*(*L.*) *infantum* |
| 7. Benassi et al., 2017 | A | C | bood/CS | molecular | *L.*(*L.*) *infantum* |
| 8. Benassi et al., 2018 | A | P | blood/serum/CS | molecular/serological | *L.*(*L.*) *infantum*<br>*Leishmania* spp. |

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|---|---|---|---|---|---|
| 9. Berbigier et al., 2021 | A | R/D | blood/serum<br>skin/liver/spleen | molecular/serological/<br>parasitological | *Leishmania* spp. |
| 10. Berenguer et al., 2021 | SC | C | blood/CS<br>lymph node aspirate | molecular/parasitological | *L.*(*L.*) *infantum* |
| 11. Bezerra et al., 2019 | SC | C | blood/serum | molecular/serological | *Leishmania* spp. |
| 12. Biral et al., 2021 | A | P | serum | serological | *Leishmania* spp. |
| 13. Braga et al., 2014 | SC | C | serum | serological | *Leishmania* spp. |
| 14. Brandão et al., 2019 | A | R/D | blood/skin/spleen/liver | molecular/serological/<br>parasitological | *L.*(*V.*) *braziliensis* or *guyanensis*<br>*L.*(*V.*) *guyanensis*<br>*Leishmania* spp. |
| 15. Brandão et al., 2020 | A | C | blood/ BM/skin | molecular/serological/<br>parasitological | *L.*(*L.*) *infantum*<br>*Leishmania* spp. |
| 16. Brandão-Filho et al., 2003 | A | P/R/D | spleen/skin/lesion aspirate | molecular/parasitological | *L.*(*V.*) *braziliensis*<br>*L. Viannia* spp. |
| 17. Bresciani et al., 2010 | SC | C | blood/serum/lymph node | serological/parasitological | *Leishmania* spp. |
| 18. Cabrera et al., 2003 | A | D | blood/serum/liver/spleen/<br>lymph node/BM/ear skin | serological/parasitological | *L.*(*L.*) *infantum* |
| 19. Caldart et al., 2017 | A | R | blood/serum | molecular/serological | *L.*(*L.*) *amazonensis* |

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|---|---|---|---|---|---|
| | | | | | *L.*(*L.*) *infantum* |
| | | | | | *Leishmania* spp. |
| 20. Camprigher et al., 2019 | A | C | serum | serological | *Leishmania* spp. |
| 21. Candido et al., 2021 | SC | Pr | blood | molecular | *L.*(*L.*) *infantum* |
| | | | | | *L.*(*L.*) *amazonensis* |
| 22. Cardia et al., 2013 | SC | C | serum | serological | *Leishmania* spp. |
| 23. Cardoso et al., 2015 | A | C/R/D | blood | molecular | *L.*(*L.*) *amazonensis* |
| | | | ear skin | | *L.*(*V.*) *braziliensis* |
| | | | | | *Leishmania* spp. |
| 24. Carneiro et al., 2020 | CR | C | ear injury skin | molecular/parasitological | *L.* (*L.*) *amazonensis* |
| 25. Carreira et al., 2012 | A | D | serum/liver/spleen/lymph | molecular/serological | *L.*(*L.*) *infantum* |
| | | | node/ear/abdominal skin/scent | | |
| | | | glands/BM | | |
| 26. Cássia-Pires et al., 2014 | A | R | spleen/serum | molecular/serological | *L.* (*V.*)*shawi*,*L.*(*V.*)*guyanensis* |
| | | | | | *L.*(*V.*) *naiffi*,*L.*(*V.*)*braziliensis* |
| | | | | | *L.*(*L.*) *infantum*, |
| | | | | | *Leishmania* spp. |
| 27. Castro et al., 2020 | A | Ch | skin/liver | molecular | *L.*(*L.*) *infantum* |
| | | | | | *L.*(*V.*) *braziliensis* |

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|---|---|---|---|---|---|
| | | | | | *Leishmania* spp. |
| | | | | | *L.*(*Viannia*)spp. |
| 28. Coelho et al., 2011 | A | C | serum | serological | *Leishmania* spp. |
| 29. Costa et al., 2014 | A | C/D | serum/lymph node aspirate | molecular/serological/ parasitological | *L.*(*L.*) *infantum* |
| 30. Costa et al., 2015 | A | Ch/R/D | blood/serum/spleen/liver | molecular/serological/ parasitological | *L.*(*L.*) *infantum* |
| 31. Costa et al., 2021 | A | P | blood/serum | molecular/serological | *L. (V.) braziliensis* |
| 32. Costa-Val et al., 2020 | A | C | blood/serum | molecular/serological | *L.*(*V.*) *braziliensis* |
| | | | CS and oral swab | | *L.*(*L.*) *infantum* |
| | | | | | *Leishmania* spp. |
| 33. Coura et al., 2018 | SC | C | blood/serum/BM aspirate/ear | molecular/serological | *Leishmania* spp. |
| | | | biopsy | /parasitological | |
| 34. Courtenay et al., 2002 | A | C | blood/serum/BM | molecular/serological/ parasitological | *L.*(*L.*) *infantum* |
| 35. Curi et al., 2006 | SC | C | blood/serum | serological | *Leishmania* spp. |
| 36. Curi et al., 2012 | SC | C | blood/serum | serological/parasitological | *Leishmania* spp. |
| 37. Dahroug et al., 2010 | SC | C | blood/lymph node aspirate | molecular | *L.*(*L.*) *infantum* |
| 38. Dahroug et al., 2011 | CR | C | blood | molecular | *L.*(*L.*) *infantum* |

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| 39. | Donalisio et al., 2017 | A | Pr/Ar R/D | blood | molecular | *L.*(*L.*) *infantum* L. Viannia |
| 40. | Escobar et al., 2019 | A | P | blood/serum | molecular | *L.*(*L.*) *infantum* |
| 41. | Escobar et al., 2019 2 | SC | P | blood | molecular | *Leishmania* spp. |
| 42. | Escobar et al., 2020 | A | P | blood | molecular | *L.*(*L.*) *infantum* |
| 43. | Evers et al., 2017 | A | P | serum | serological | *Leishmania* spp. |
| 44. | Ferreira et al., 2013 | A | C | serum/plasma | serological | *Leishmania* spp. |
| 45. | Ferreira et al., 2015 | A | R/D | liver/spleen/skin(tail,ear)/ BM | molecular | *L.donovani, L.*(*V.*) *braziliensis Leishmania* sp |
| 46. | Ferreira et al., 2017 | A | Chi | skin(wing) | molecular | *L.*(*V.*) *braziliensis* |
| 47. | Ferreira et al., 2018 | A | P | blood/serum | serological | *Leishmania* spp. |
| 48. | Figueiredo et al., 2008 | SC | C | intact skin of the scapula region | parasitological | *L.*(*L.*) *infantum* |
| 49. | Freitas et al., 2012 | CR | R | skin ear/bone marrow femur | molecular | *L.*(*V.*) *braziliensis* |
| 50. | Gomes et al., 2007 | A | C | blood/serum/BM | molecular/serological/ parasitological xenodiagnosis | *Leishmania* spp. |

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| 51. Gómez-Hernández et al., 2017 | A | Chi | blood | molecular | *L.*(*L.*) *infantum*<br>*L.*(*L.*) *amazonensis*<br>*L.*(*V.*) *braziliensis* |
| 52. Gonçalves et al., 2020 | CR | P | facial lump biopsy/serum | serological/parasitological | *L.*(*L.*) *infantum* |
| 53. Headley et al., 2019 | CR | C | facial nodule biopsy/fine needle aspirates | serological<br>parasitological | *Leishmania* spp. |
| 54. Humberg et al., 2012 | SC | D | blood/BM | molecular/parasitological | *L.* (*L.*) *infantum* |
| 55. Jusi et al., 2011 | A | C | blood/serum/tissues (skin lesions, spleen, liver, lymph nodes, kidneys)/skin/BM | molecular/serological | *L.*(*L.*) *infantum* |
| 56. Leonel et al., 2020 | A | C | blood/serum/CS/entomological survey=1 male *L. longipalpis* | molecular/serological | *Leishmania* spp. |
| 57. Leonel et al., 2021 | A | P | blood | molecular | *L.*(*L.*) *infantum* |
| 58. Lima et al., 2009 | CR | C | blood/serum/lymph node biopsy/female death (skin/spleen/lymph node/liver) | molecular/serological/ parasitological | *Leishmania* spp. |
| 59. Lima et al., 2013 | A | R/D | blood/serum/spleen/ear skin | molecular/serological/ parasitological | *Leishmania* spp.<br>*L.*(*V.*) *braziliensis*<br>*L.*(*L.*) *infantum* |

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| 60. | Limeira et al, 2021 | A | P | blood | molecular | *L.*(*L.*) *infantum* |
| 61. | Lombardi et al., 2014 | A | R/D/C/ Pr/Ar/P i/P | blood | molecular | *L.*(*L.*) *infantum* |
| 62. | Luppi et al., 2008 | SC | C | blood/serum/organs dead animal(lymph nodes, spleen, liver, kidneys, lung, intestines, ulcerated skin) BM, lymph node aspirate | molecular/serological/ parasitological | *L.*(*L.*) *infantum* |
| 63. | Madruga et al., 2018 | CR | C | aqueous humor/ocular conjunctiva/lid and lymph node biopsy/blood/BM | molecular/serological/ parasitological | *L.*(*L.*) *infantum* |
| 64. | Malta et al., 2010 | SC | Pr | blood/tissues (necropsy) | molecular/serological/ parasitological | *L.*(*L.*) *infantum* |
| 65. | Marcelino et al., 2011 | A | R | skin/blood/BM/spleen/tail | molecular | *L.*(*V.*) *braziliensis* |
| 66. | Matos et al., 2018 | SC | C | serum | serological | *Leishmania* spp. |

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| 67. Mendonça et al., 2017 | A | C | blood/serum/lymph node aspirate | molecular/serological/ parasitological | *L.*(*L.*) *infantum* |
| 68. Mendonça et al., 2020 | A | C | blood | molecular/xenodiagnosis | *L.*(*L.*) *infantum* |
| 69. Metzdorf et al., 2017 | A | C | blood/lymph node aspirate/BM | molecular/parasitological | *L.*(*L.*) *infantum* |
| 70. Mol et al., 2015 | A | C | blood/serum | molecular/serological/ xenodiagnosis | *L.*(*L.*) *infantum* |
| 71. Moraes-Silva et al., 2006 | A | Ar | serum | molecular/serological/ parasitological *in vivo* inoculation | *L.*(*L.*) *infantum* |
| 72. Morais et al., 2013 | A | C | blood/ectoparasites | molecular | *L.*(*L.*) *infantum* |
| 73. Nantes et al., 2021 | A | R/D | blood | molecular/parasitological | *Leishmania* spp. |
| 74. Oliveira et al., 2005 | A | R | tail blood/ear skin | molecular | *L* (*V.*) *braziliensis* *L.* (*L.*) *mexicana* *L.donovani* *Leishmania* spp. |
| 75. Oliveira et al., 2015b | A | Chi | spleen/epigastric skin | molecular | *L.*(*L.*) *amazonensis* *L.*(*L.*) *infantum* *L.*(*L.*) *amazonensis and* |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | *L.*(*L.*) *infantum* |
| 76. | Oliveira et al., 2015 | SC | C | CS | molecular | *Leishmania* spp. |
| 77. | Oliveira et al., 2015 | SC | C | blood/serum | serological | *L.*(*L.*) *infantum* |
| 78. | Oliveira et al., 2017 | A | P | blood/serum | serological | *Leishmania* spp. |
| 79. | Oliveira et al., 2019 | A | Pr | blood before exposure to the vector/serum | molecular/serological/ xenodiagnosis | *L.*(*L.*) *infantum* |
| 80. | Paiz et al., 2015 | A | Pr/Ar/Ci/Chi/Pi/C/R/D/L | blood/serum | serological | *L.*(*L.*) *infantum* |
| 81. | Paiz et al., 2016 | SC | Pr/Ar/R/D | blood | molecular | *L.*(*L.*) *infantum*<br>*L.* (*V.*) *guyanensis* |
| 82. | Paiz et al., 2018 | A | Pr | blood/tail skin/serum | molecular/serological | *L.*(*L.*) *infantum*<br>*L.*(*L.*) *donovani* |
| 83. | Pedrassani et al., 2019 | A | C | blood/serum/ectoparasites | molecular/serological/ parasitological | *L.*(*L.*) *infantum* |
| 84. | Pereira et al., 2017 | A | R/D | spleen/liver/skin(tail and ear)/BM | molecular/parasitological | *L.*(*L.*) *infantum*<br>*L.*(*V.*) *braziliensis* |

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| 85. | Porfirio et al., 2018 | A | Pr/Ci/C/R | blood | molecular/serological/ parasitological | *Leishmania* spp. |
| 86. | Pradella et al., 2020 | A | P | blood | molecular | *L.*(*L.*) *infantum* |
| 87. | Quaresma et al., 2011 | A | R/D | blood/BM/skin tail and ear/spleen/liver/lymph node | molecular | *L.*(*L.*) *infantum* *L.*(*V.*) *braziliensis;* *L.*(*V.*) *guyanensis* |
| 88. | Quintal et al., 2011 | A | D | ear tip skin | molecular | *L.*(*L.*) *amazonensis* *L.*(*V.*) *braziliensis* |
| 89. | Reis et al., 2019 | A | R/Pe/C Pi/Ce/ Pr | blood | molecular | *Leishmania* sp |
| 90. | Rezende et al., 2017 | SC | Chi | blood | molecular | *L.*(*L.*) *infantum,* *Leishmania* spp. |
| 91. | Richini-Pereira et al., 2014 | A | D/R/C/ Pi Ci/Ar/P r | lung/liver/spleen/kidney/heart lymph node/adrenal gland | molecular | *L.*(*L.*)*infantum* *Leishmania* spp. |
| 92. | Riva et al., 2021 | A | Chi | BM/skin/spleen | molecular | *L.*(*V.*) *braziliensis* |
| 93. | Rocha et al., 2019 | A | C | blood/serum/CS | molecular/serological | *L.*(*L.*) *infantum* |

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| 94. Santiago et al., 2007 | A | D | tail blood/BM | molecular/serological/ parasitological | *Leishmania* spp. |
| 95. Santos et al., 2018 | CR | C | blood/serum/popliteal lymph node aspirate | molecular/serological/ parasitological | *Leishmania* spp. |
| 96. Santos et al., 2021 | A | C | blood/BM/lymph node aspirate or skin lesion/ CS | molecular/serological/ parasitological | *L.*(*L.*) *infantum* |
| 97. Savani et al., 2004 | SC | C | serum/liver/spleen/skin (nose lump) | molecular/serological/ parasitological | *L.*(*L.*) *infantum* |
| 98. Savani et al., 2010 | A | Chi | blood/serum/spleen/liver | molecular/serological/ parasitological | *L.*(*L.*) *infantum*<br>*L.*(*L.*) *amazonensis* |
| 99. Schallig et al., 2007 | A | D | blood/serum | molecular/serological | *L.* (*L.*) *donovani*<br>*L.* (*V.*)*braziliensis*<br>*Leishmania* spp. |
| 100. Shapiro et al., 2013 | A | Chi | blood/skin lesions (wing, ear, nose, farm bats)/liver/spleen | molecular/parasitological | *L.* (*V.*) *braziliensis* |
| 101. Silva et al., 2008 | SC | C | blood/serum | molecular/serological | *L.*(*L.*) *infantum* |
| 102. Silva et al., 2010 | SC | C | blood/serum/BM/after euthanasia: | molecular/serological/ xenodiagnosis | *L.*(*L.*) *infantum* |

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| | | | liver/spleen/kidney/skin/lymp h node/heart | | |
| 103.Silva et al., 2013 | SC | R | intact skin/spleen | molecular/parasitological | *L.(L) hertigi* |
| 104.Silva et al., 2013 | A | C | blood/serum | serological | *L.(L)infantum* |
| 105.Silva et al., 2016 | SC | D | blood | molecular | *L.(V)braziliensis* |
| 106.Silva et al., 2020 | CR | C | blood/serum/skin lesions/lymph node aspirate /necropsy: skin; lymph node; CNS; thoracic and abdominal organs | molecular/serological/ parasitological | *L.(L)infantum* |
| 107.Soares et al., 2013 | CR | P | blood/serum/skin lesion biopsy/BM | molecular/serological/ parasitological | *L.(L) infantum* *L.(V) braziliensis* |
| 108.Sobrinho et al., 2012 | SC | C | blood/BM/lymph node aspirate | molecular/serological/ parasitological | *L.(L)infantum* |
| 109.Soccol et al., 2021 | A | R | skin (lesion: scraping;puncture;biopsy)/ liver/ lymph node/spleen | molecular/parasitological | *L. enrietii* |
| 110.Sousa et al., 2014 | A | C | blood/serum | serological | *L.(L)infantum* |

| | | | | | |
|---|---|---|---|---|---|
| 111.Sousa et al., 2019 | CR | C | blood/serum/ conjunctiva/spleen/ liver/ lymph node/skin/ lung/kidney | molecular/serological/ parasitological | *L.(L)infantum* |
| 112.Souza et al., 2005 | CR | C | skin (injuries, nodules) | serological/parasitological | *L.(L)amazonensis* |
| 113.Souza et al., 2009 | CR | C | aspiration puncture nodules/serum | serological/parasitological/ in vivo inoculation | *L.(L)amazonensis* |
| 114.Souza et al., 2010 | SC | C | skin/BM aspirate and lymphnode | molecular | *L.(L)infantum* |
| 115.Tenório et al., 2011 | CR | C | blood/serum/lymph node and BM aspirate/skin/lymph nodes/spleen/liver/kidneys/ lungs/brain/ heart/tongue/ trachea/esophagus/ stomach/small intestine | molecular/serological/ parasitological | *L.(L)infantum* |
| 116.Teodoro et al., 2019 | A | D | blood/serum/ear skin/tissue biopsy | molecular | *Leishmania* spp. |

| | | | | | |
|---|---|---|---|---|---|
| 117. Tolentino et al., 2019 | A | C | blood/serum | serological | *Leishmania* spp. |
| 118. Tonelli et al., 2017 | A | R/D | spleen/liver/skin(tail and ear) | molecular parasitological | *L.(V)braziliensis* |
| 119. Trüeb et al., 2018 | A | Pr/Ci/C /R/D | blood/spleen and liver aspirate | molecular/parasitological | *Leishmania* spp. |
| 120. Truppel et al., 2014 | A | Pe | blood/serum | molecular/serological | *L.(V)braziliensis* |
| 121. Vedovello Filho et al., 2008 | A | Pe | blood/serum | molecular/serological/ parasitological | *L.(V)braziliensis* |
| 122. Vides et al., 2011 | A | C | blood/serum/fine needle aspirate (BM, lymph node, spleen, liver) | molecular/serological/ parasitological | *L.(L)infantum* |
| 123. Vioti et al., 2019 | SC | Ar | blood (red blood cells) | molecular | *L.(L)infantum* |
| 124. Voltarelli et al., 2009 | A | Pr/Ci/C /R/D | blood/serum | serological/parasitological | *Leishmania* spp. |

Study type: A (Article), SC (Short Communication) or CR (Case Report)

Animal order: D (Didelphimorphia), R (Rodentia), P (Perissodactyla), Pi (Pilosa), C (Carnivore), Ch (Chiroptera), Ci (Cingulata), Ce (Cetartiodactyla), Ar (Artiodáctyla), Pr (Primate) and L (Lagomorpha)

Sample: CS (conjunctival swab), BM (bone marrow) and CNS (central nervous system)

Search references in the databases to composse the systematic review (124 studies) (Online Resource 14).

Table 2. Description of the orders of mammals surveyed for the presence of the protozoan *Leishmania* spp., and the main host animal species where one or more species of the agent were identified

| ORDER | INFECTED ANIMAL SPECIES - COMMON NAME | IDENTIFIED PROTOZOA |
|---|---|---|
| 1. ARTIODACTYLA | *Bos taurus* - catle | *L.*(*L*) *infantum* |
| | *Sus scrofa domesticus* – swine | *L.*(*L*) *infantum* |
| 2. CARNIVORA | *Cerdocyon thous* – bush dog | *Leishmania* spp.; *L.*(*L*) *infantum* |
| | *Chysocyon brachyurus* – guara wolf | *Leishmania* spp.; *L.*(*L*) *infantum* |
| | *Felis catus* – cat | *Leishmania* spp.; *L.*(*L*) *infantum*; *L.* (*L*) *amazonensis* |
| | *Lycalopex vetulus* – brazilian fox | *Leishmania* spp.; *L.*(*L*) *infantum* |
| | *Nasua nasua* – ring-tailed coati | *Leishmania* spp.; *L.*(*L*) *infantum* |
| | *Spheotos venaticus* – vinegar dog | *Leishmania* spp.; *L.* (*L*)*infantum* |
| 3. CINGULATA | *Dasypus septemcinctus* – little armadillo | *Leishmania* spp. |
| | *Dasypus sp*. | *Leishmania* spp. |
| | *Euphractus sexcinctus* – armadillo; hock armadillo | *Leishmania* spp. |
| 4. CHIROPTERA | -*Artibeus lituratus* | -*L.* (*L*) *amazonensis*; *Leishmania* spp.; *L.* (*L*)*infantum*; *L.* (*V*) *braziliensis.* |
| | -*Artibeus planirostris* | -*L.*(*V*) *braziliensis*; *L.*(*L*) *infantum*; *L.* (*L*) *amazonensis*; *Leishmania* spp.. |
| | -*Carollia perspicillata* | -*Leishmania* spp.; *L.*(*L*) *infantum.* |
| | -*Desmodus rotundus* – vampire bat | -*L.* (*L*) *infantum*; *L.* (*V*) *braziliensis*; *L.* (*L*) *amazonensis*; *Leishmania* spp.. |
| | -*Glossophaga soricina*. | -*L.*(*L*) *infantum*; *L.*(*L*) *amazonensis*; *L.*(*V*) *braziliensis*; *Leishmania* spp. |
| | -*Lasiurus cinereus* | -*L.*(*V*) *braziliensis*; *L.* (*Viannia*) spp.. |
| | -*Molossus molossus* - thick tailed bat | -*L.* (*L*) *infantum*; *L.* (*Leishmania*) spp.; *L.* (*L*) *amazonensis*; *L.*(*V*) *braziliensis; Leishmania spp*. |
| | -*Molossus rufus* | -*L.*(*L*) *infantum*; *L.* (*L*) *amazonensis*; *L.*(*V*) *braziliensis*; *Leishmania* spp . |

| | | | |
|---|---|---|---|
| 5. | DIDELPHIMORPHIA | -*Didelphis albiventris* – white ear possum | -*Leishmania* spp.; *L.*(*Viannia*) spp.; *L.*(*L*) *infantum*; *L.*(*V*) *braziliensis*; *L.* (*V*) *guyanensis* |
| | | -*Didelphis aurita* – black ear possum | -*Leishmania* spp.; *L.* (*L*) *infantum* |
| | | -*Didelphis marsupialis* – common skunk | -*L.*(*L*) *infantum*; *L.* (*V*) *braziliensisis*; *Leishmania* spp. |
| | | -*Gracilinanus agilis* – opossum; graceful opossum | -*L.* (*V*) *braziliensis*; *L.* (*V*) *guyanensis*; *Leismania* spp. |
| | | -*Marmosa paraguayana* - quaint-grey; gray guaiquica; opossum | -*L.* (*L*) *amazonensis*; *L.*(*V*) *braziliensis*; *Leishmania* spp. |
| 6. | LAGOMORPHA | *Lepus europaeus* – common hare; European hare | *L.* (*L*) *infantum* |
| 7. | PERISSODACTYLA | *Eqqus caballus* - domestic horse | *L.* (*L*) *infantum*; *Leishmania* spp.; *L.*(*Viannia*) spp.; *L.* (*V*) *braziliensis* |
| | | *Equus asinus africanus* - dumb | *L.* (*L*) *infantum*; *L.* (*V*) *braziliensis* |
| | | *Equus asinus caballus* – mule | *L.* (*L*) *infantum*; *Leishmania* spp; *L.* (*V*) *braziliensis* |
| 8. | PILOSA | *Myrmecophaga tridactyla* – giant anteater | *Leishmania* spp. |
| | | *Tamandua tetradactyla* - lesser anteater; giant anteater | *L.* (*L*) *infantum*; *Leishmania* spp. |
| 9. | PRIMATE | *Alouatta guariba* – red howler | *L.* (*L*) *infantum* |
| | | *Aotus azarae* – night monkey | *L.* (*L*) *infantum* |
| | | *Callicebus nigrifrons* – monkey monkey | *L.* (*L*) *infantum* |
| | | *Leontopithecus rosalia* – golden lion tamarin | *L.* (*L*) *infantum* |
| | | *Mico melanurus* – cerrado marmoset | *L.* (*L*) *infantum*; *L.* (*L*) *amazonensis* |
| | | *Sapajus apela* – capuchin monkey | *L.* (*L*) *infantum*; *L.* (*L*) *amazonensis* |
| 10. | RODENTIA | -*Rattus rattus* – common mouse; house mouse; black mouse | -*Leishmania* spp.; *L.* (*L*) *amazonensis*; *L.* (*L*) *infantum*; *L.* (*V*) *braziliensis*; *L.*(*Viannia*) spp.; *L.* (*L*) *mexicana* |
| | | -*Necromys lasiurus* – cerrado mouse; pixuna; hairy tail mouse | -*L.* (*L*) *amazonensis*; *L.* (*V*) *braziliensis*; *Leishmania* spp.; *L.* (*L*) *infantum* |
| | | -*Nectomys squamipes* - water rat | -*L.*(*Viannia*) spp.; *L.* (*L*) *infantum*; *L.* (*V*) *braziliensis* |

**Fig. 2:** Forest Plot indicating the total number of animals infected with the protozoan *Leishmania* spp. (0.25 = 25%) in relation to the total number surveyed (13905 animals) selected by the authors in the systematic review.

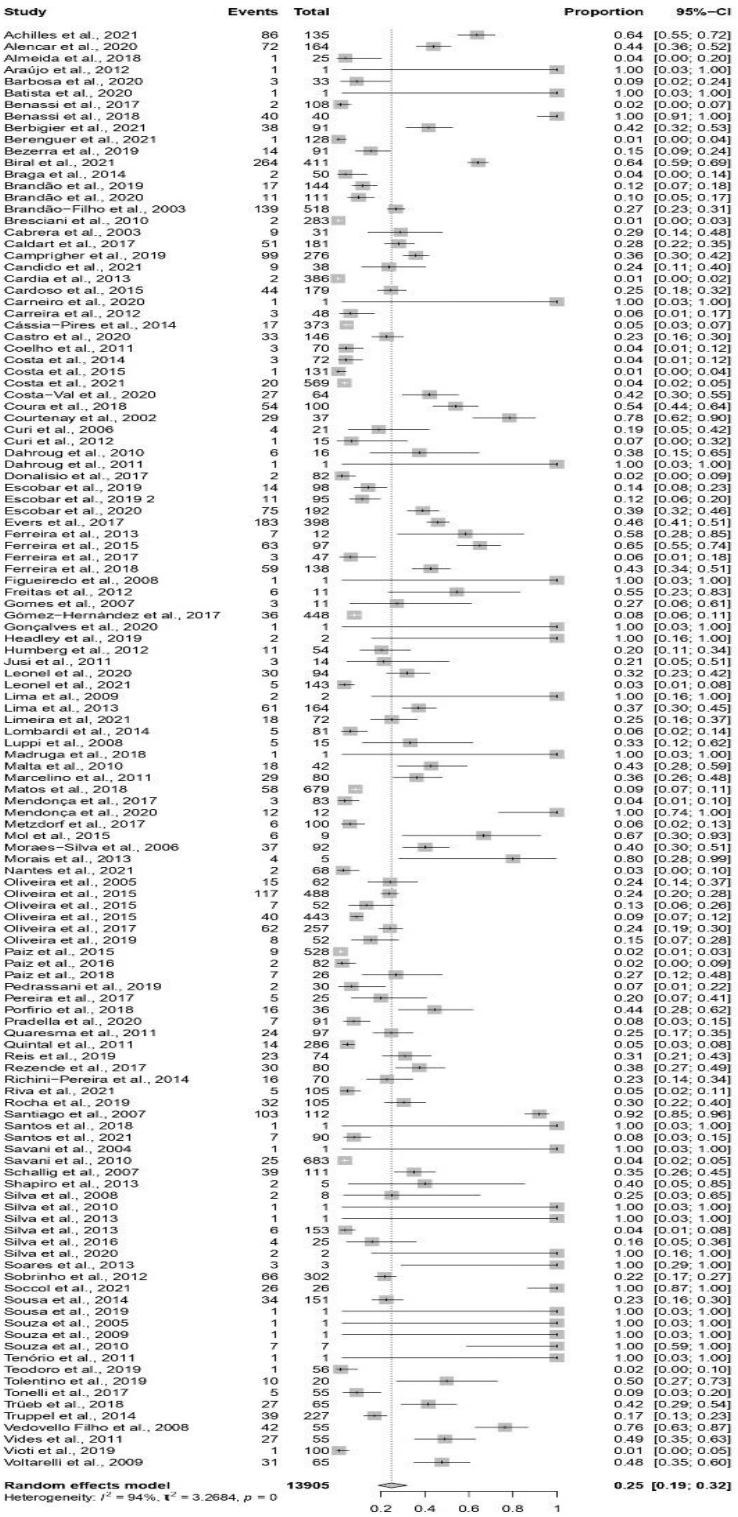


**Fig. 3:** Forest Plot indicating the frequency of domestic animals infected with one or more species of *Leishmania* (0.26=26%).

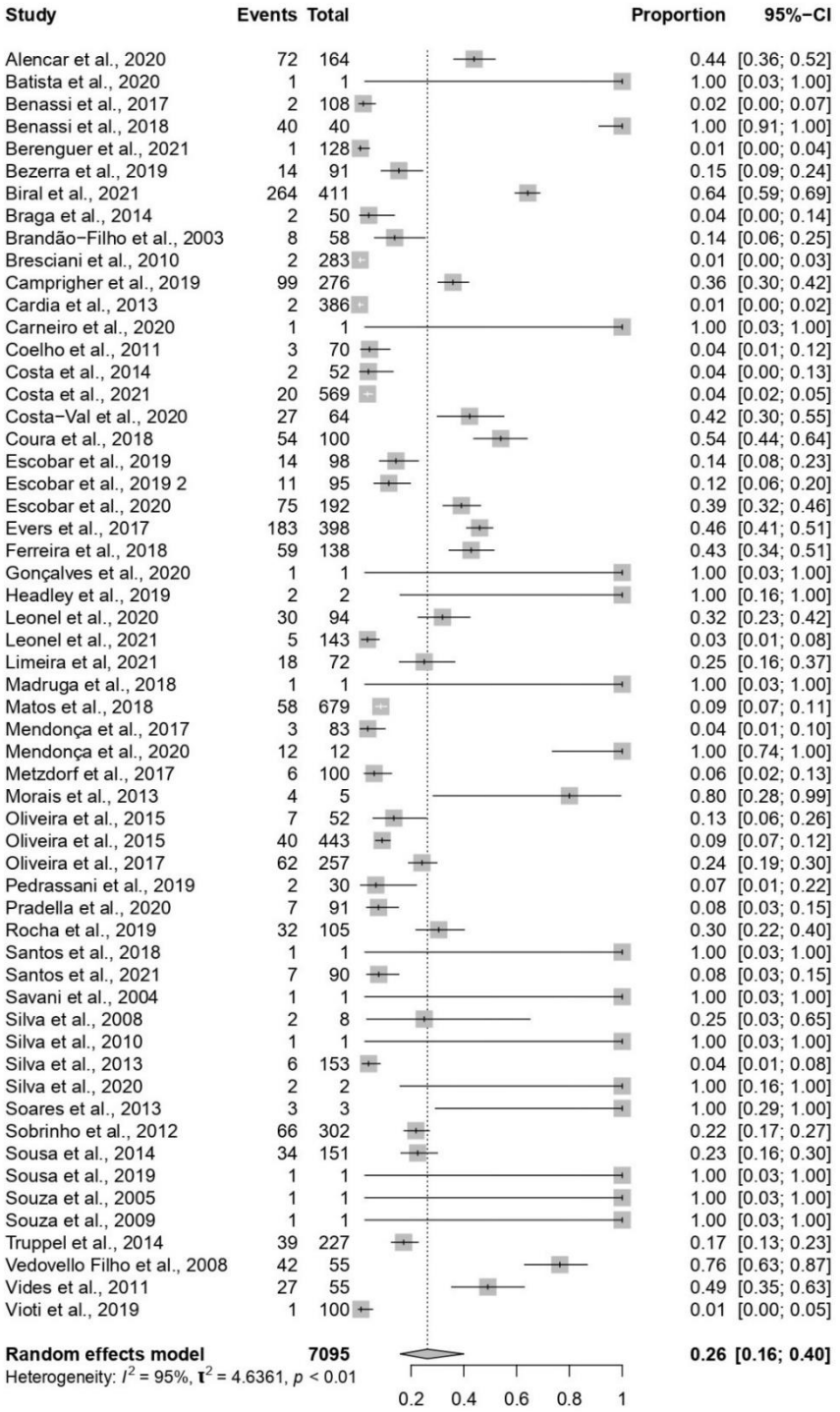


**Fig. 4:** Forest Plot indicating the frequency of wild animals infected with one or more species of *Leishmania* (0.24 = 24%).

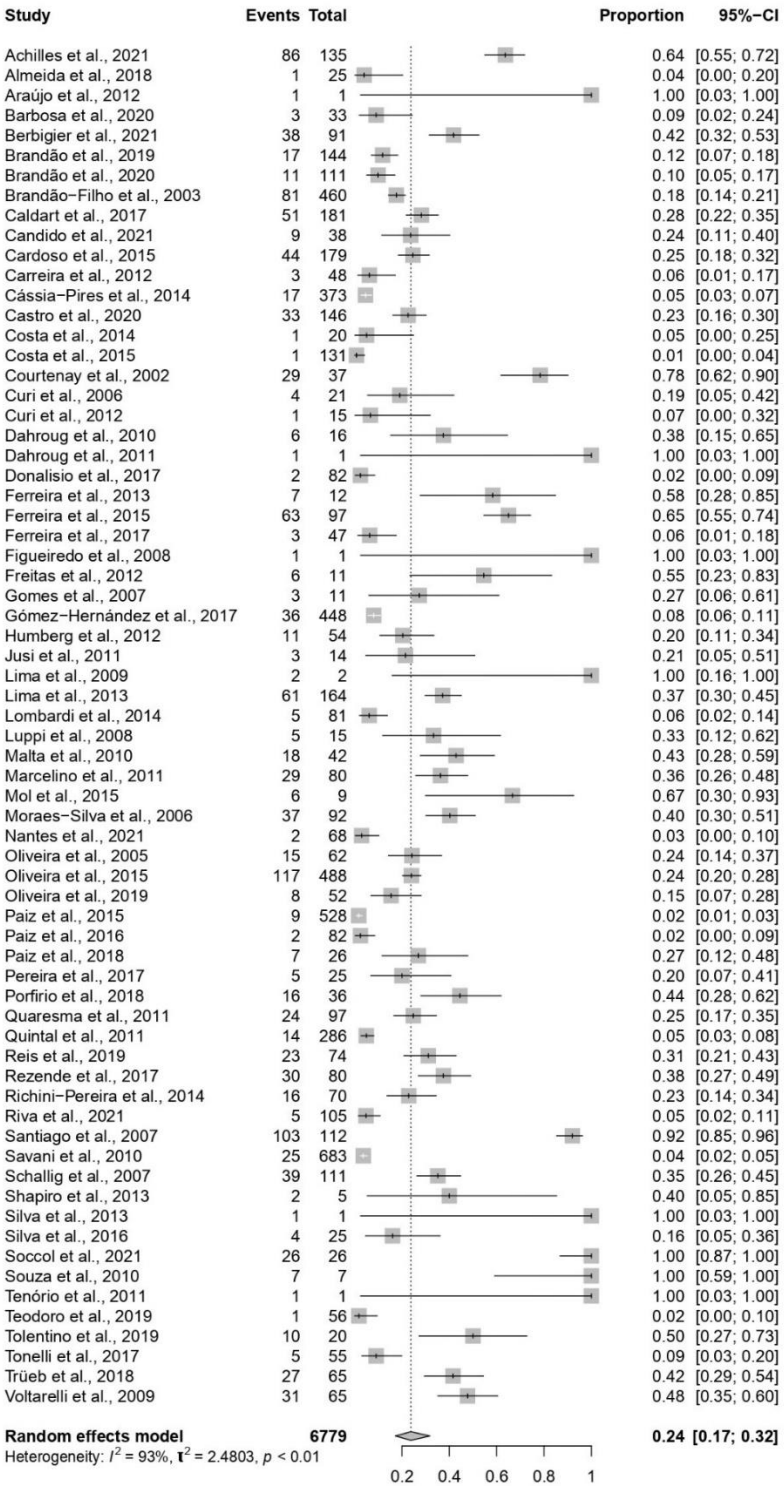


**Fig. 5:** Forest Plot indicating the total number of animals that showed clinical signs compatible with leismaniasis (0.11 = 11%).

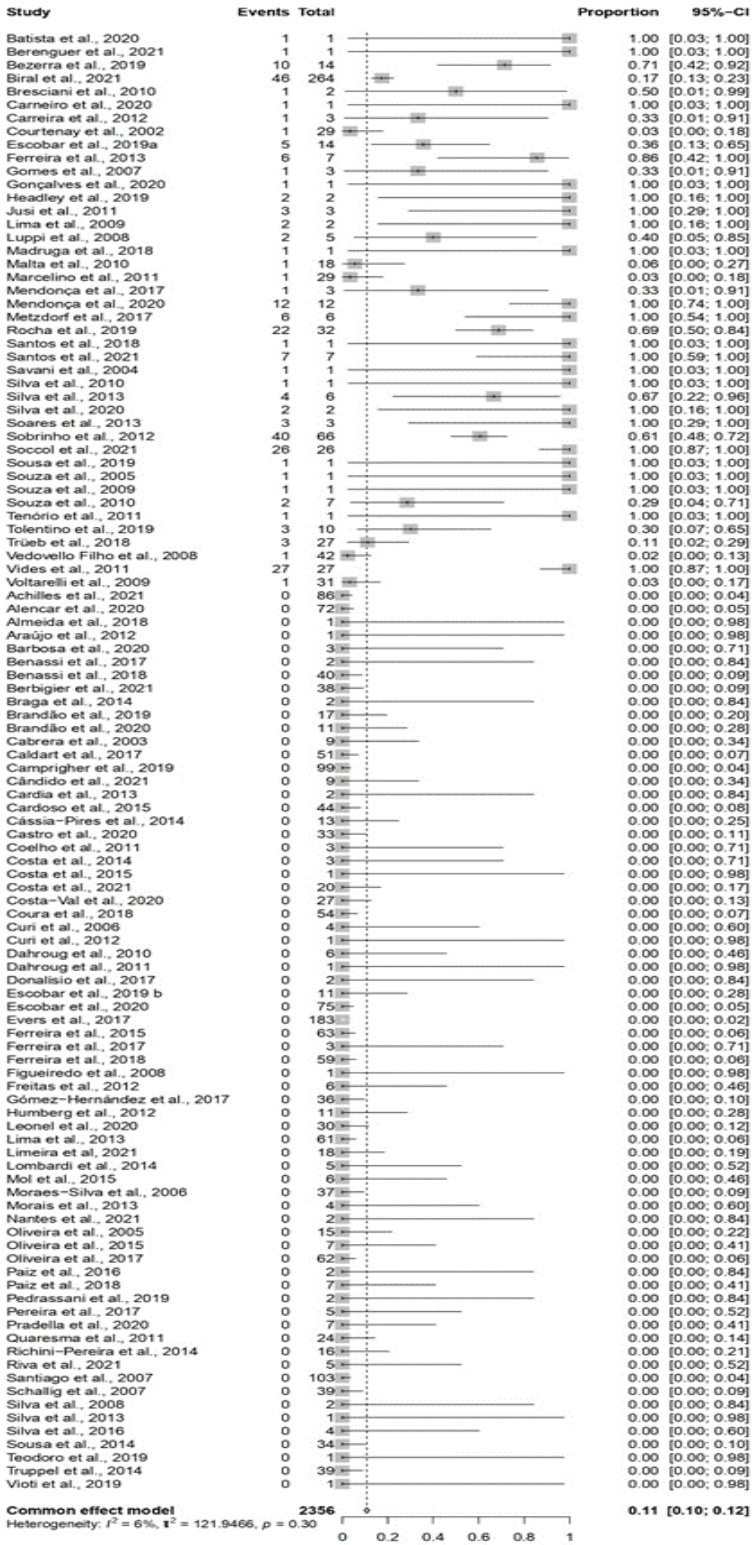


**Fig 6:** Forest Plot indicating the total number of domestic animals that showed clinical signs compatible with infection by *Leishmania* spp. in relation to the total number of domestic animals infected by the protozoan (0.14 = 14%).

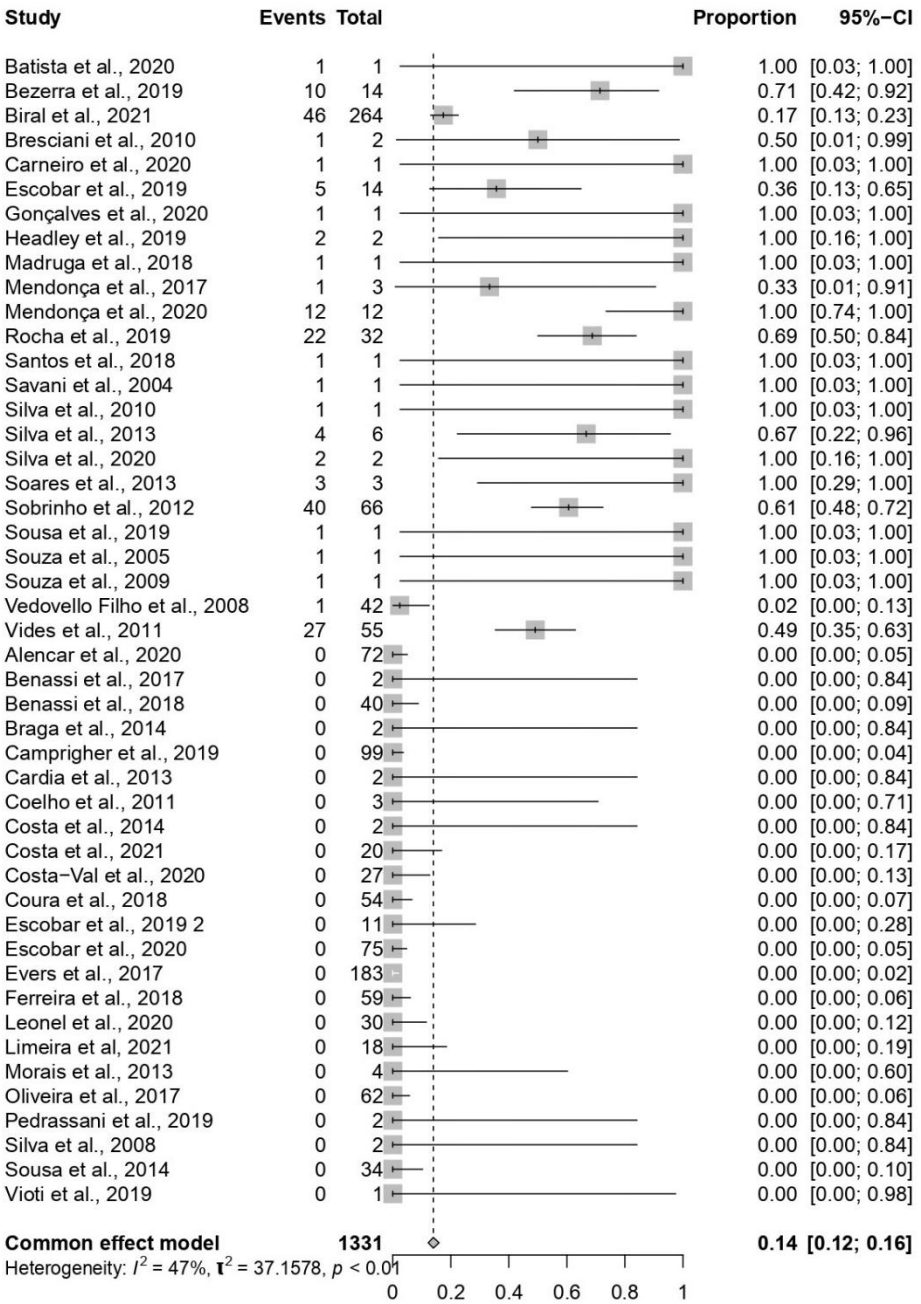


**Fig 7:** Forest Plot indicating the total number of wild animals that showed clinical signs compatible with infection by *Leishmania* spp. in relation to the total number of wild animals infected by the protozoan (0.06 = 6%).

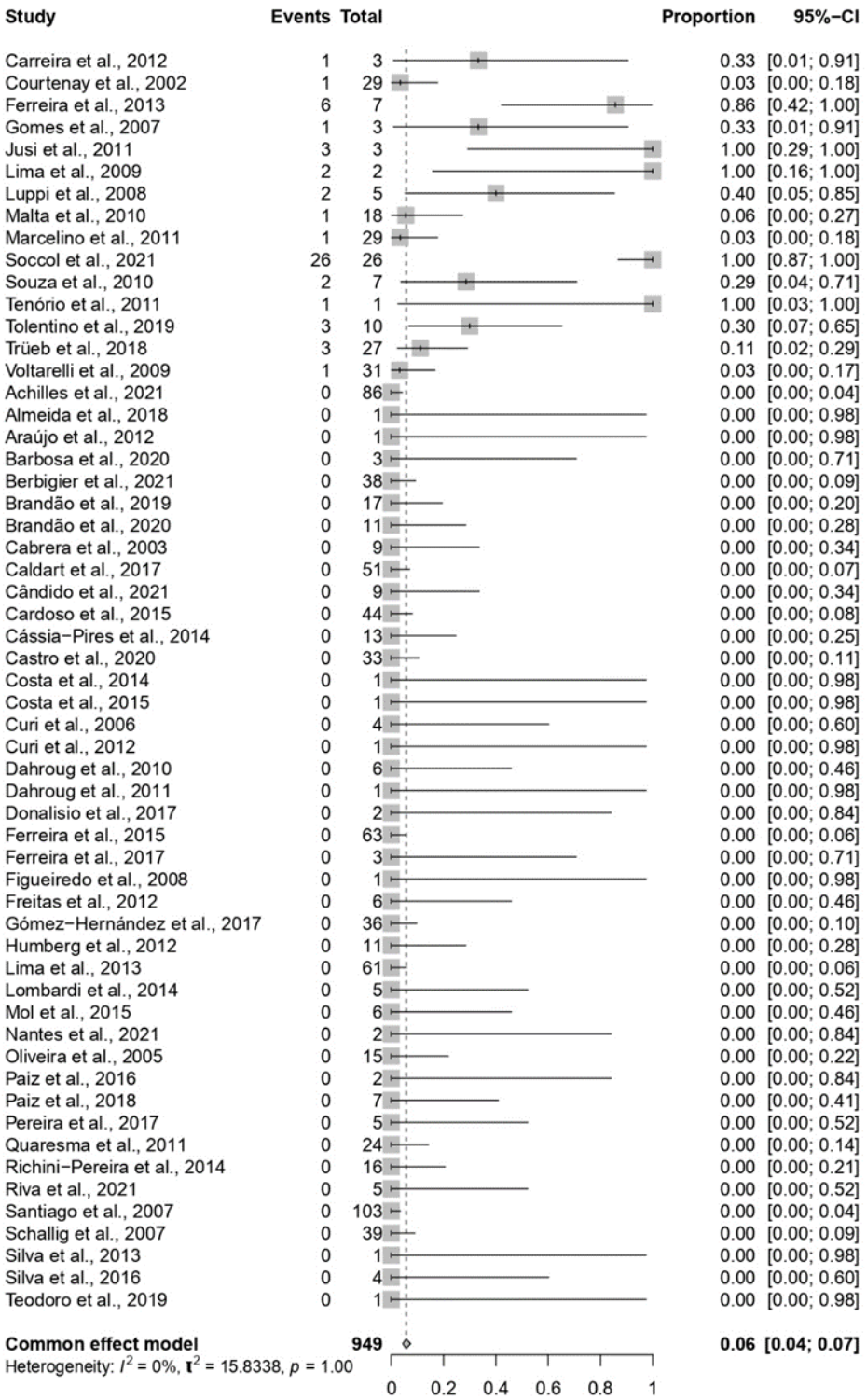


**Fig. 8:** Number of mammals infected with *Leishmania* spp., demonstrating the percentage of protozoan species circulating in the states of Amazonas, Pará, and Tocantins in the North region of Brazil

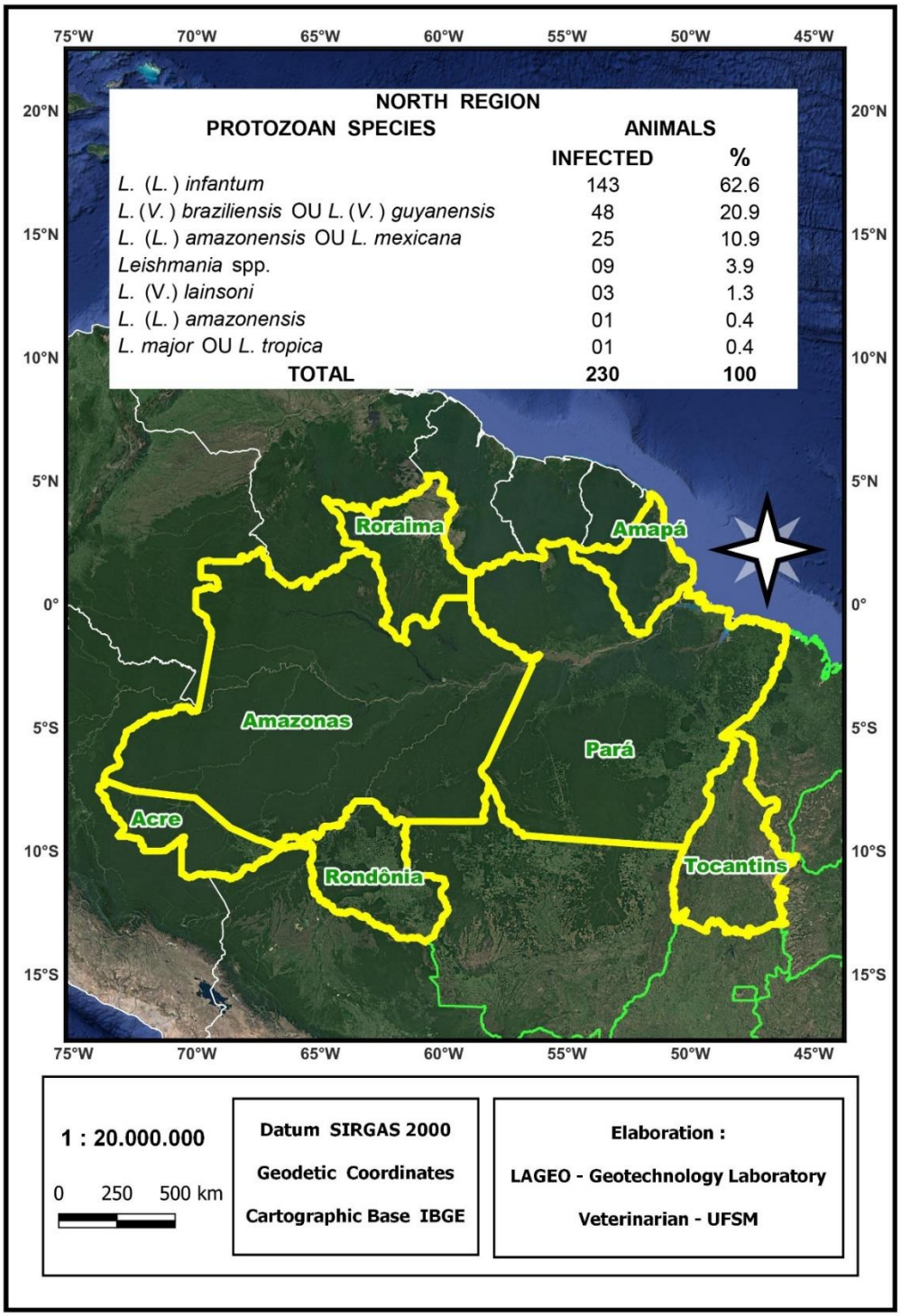


**Fig. 9:** Number of mammals infected with *Leishmania* spp., demonstrating the percentage of protozoan species circulating in the states of Pernambuco, Piauí, Bahia, Maranhão, Rio Grande do Norte, Paraíba and Sergipe in the Northeast region of Brazil.

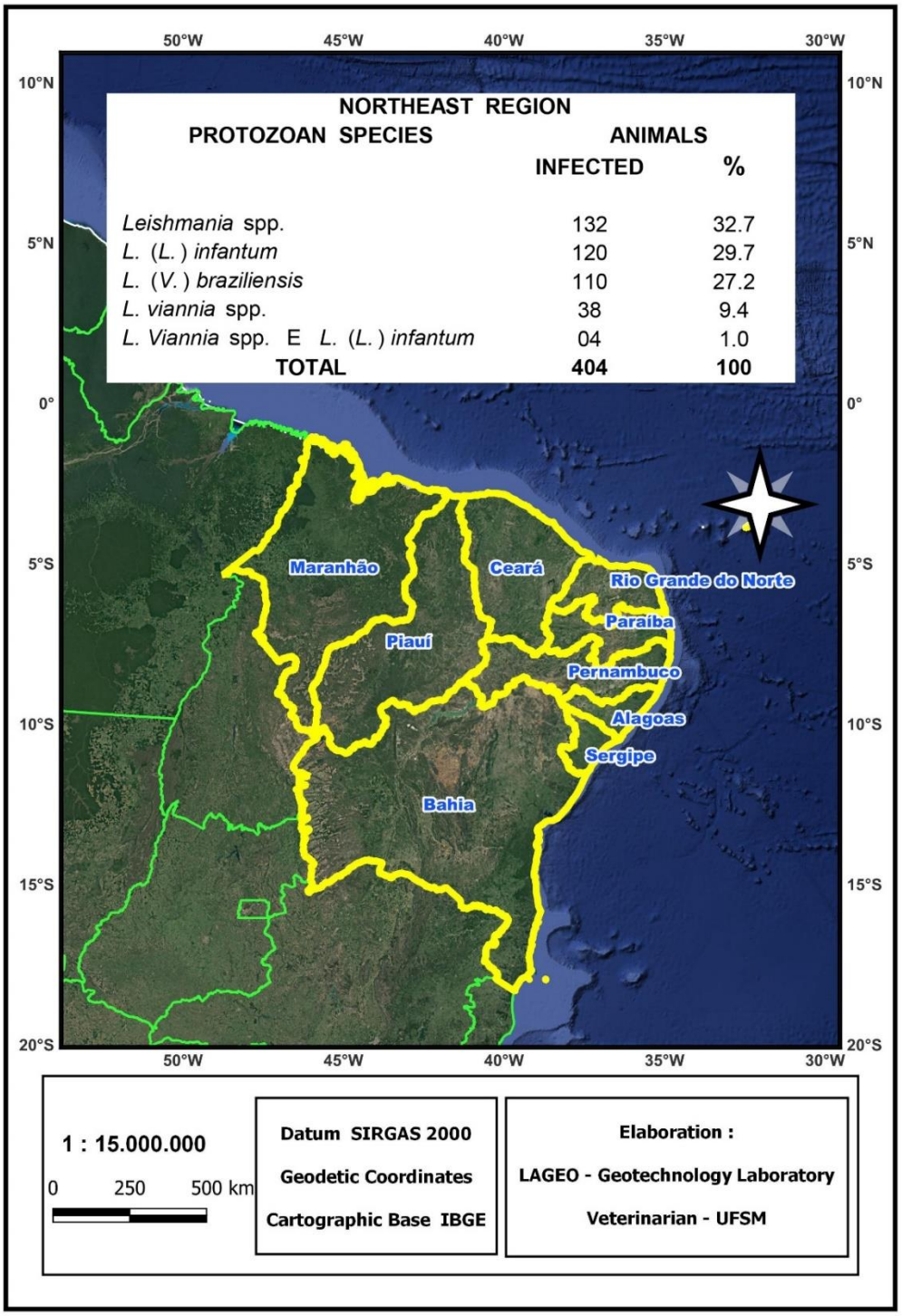


**Fig. 10:** Number of mammals infected with *Leishmania* spp., demonstrating the percentage of protozoan species circulating in the states of São Paulo, Minas Gerais, Rio de Janeiro and Espírito Santo in the Southeast region of Brazil

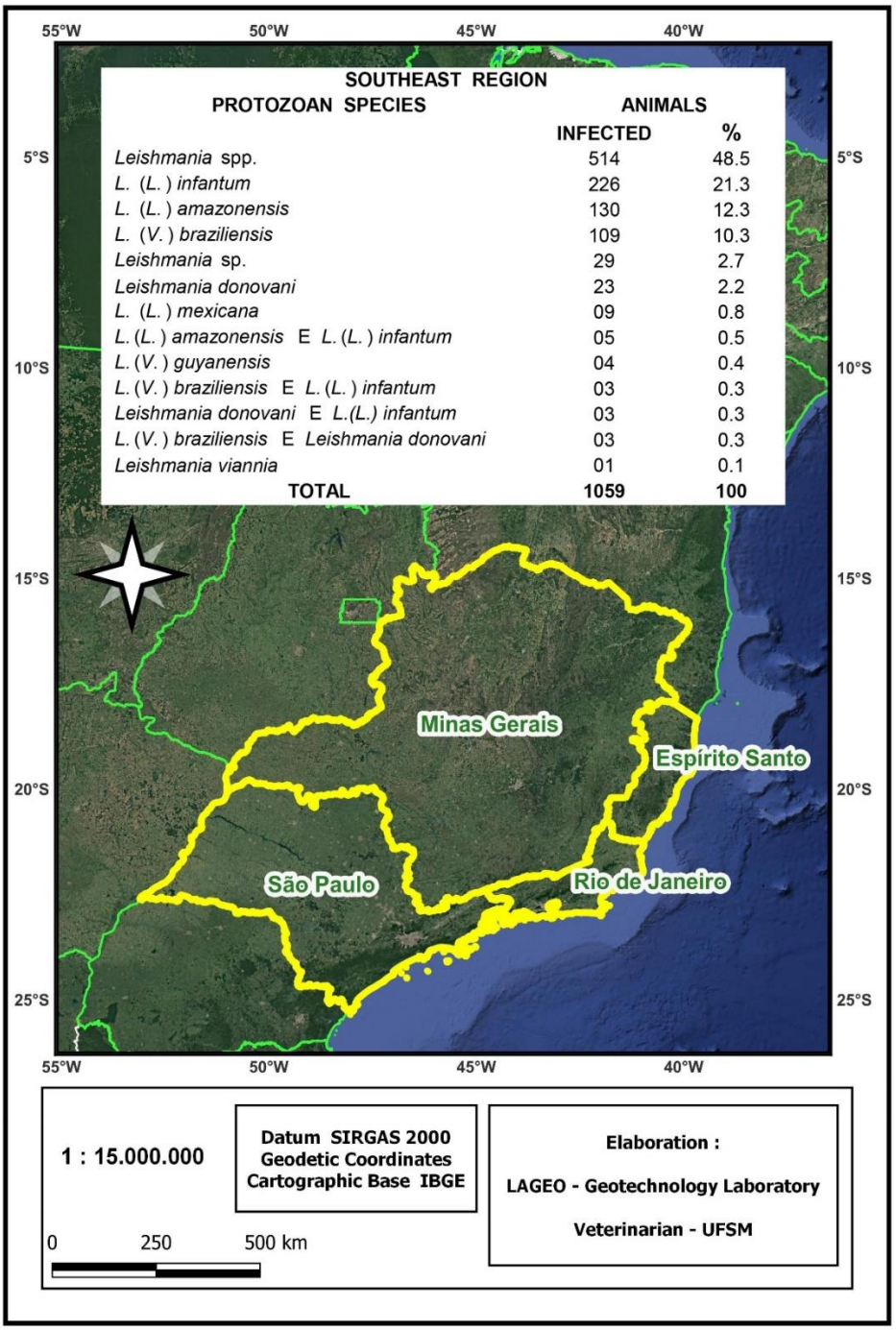


**Fig. 11** Number of mammals infected with *Leishmania* spp., demonstrating the percentage of protozoan species circulating in the states of Mato Grosso, Mato Grosso do Sul, Goiás and Distrito Federal in the Midwest region of Brazil

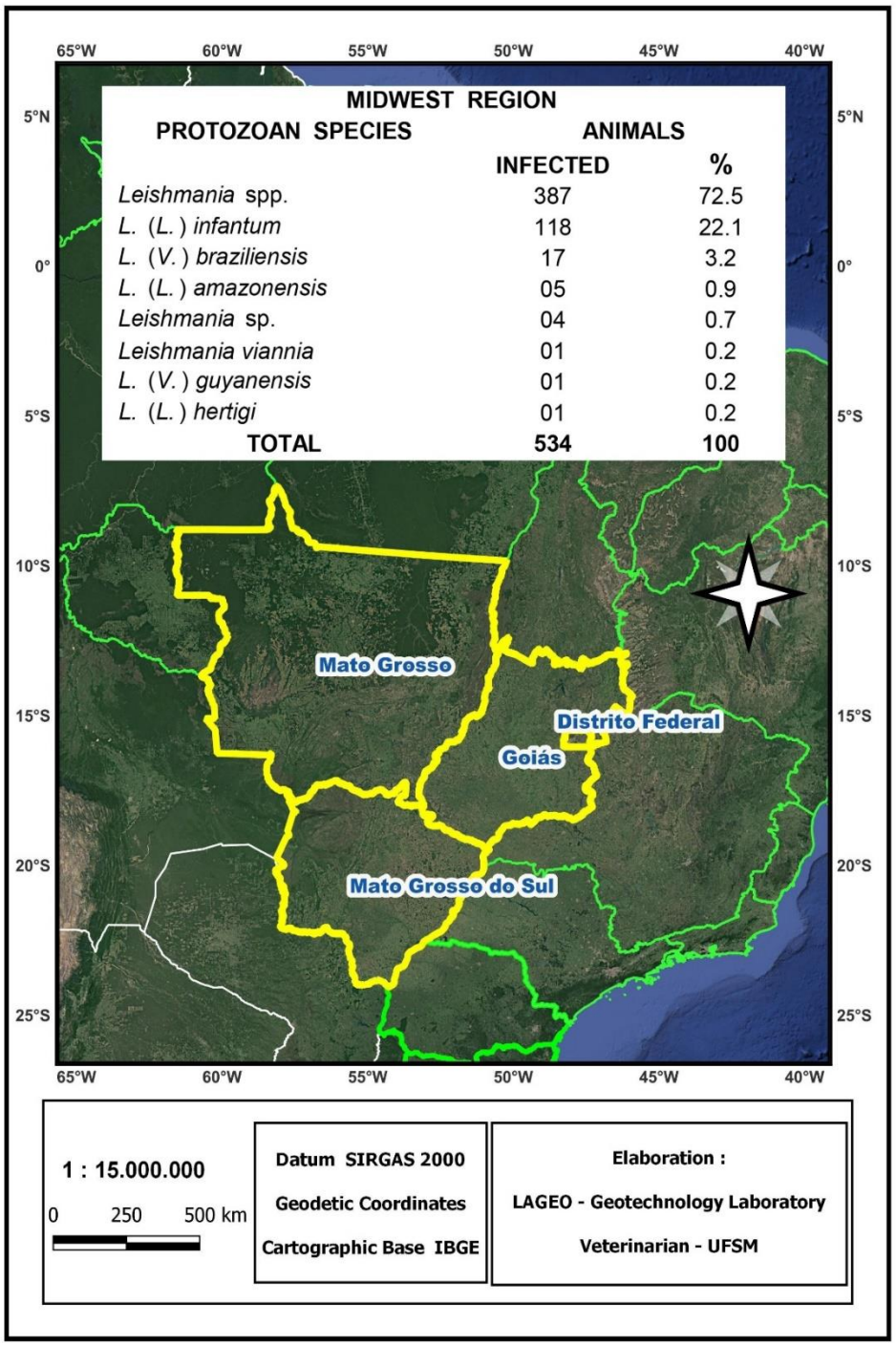
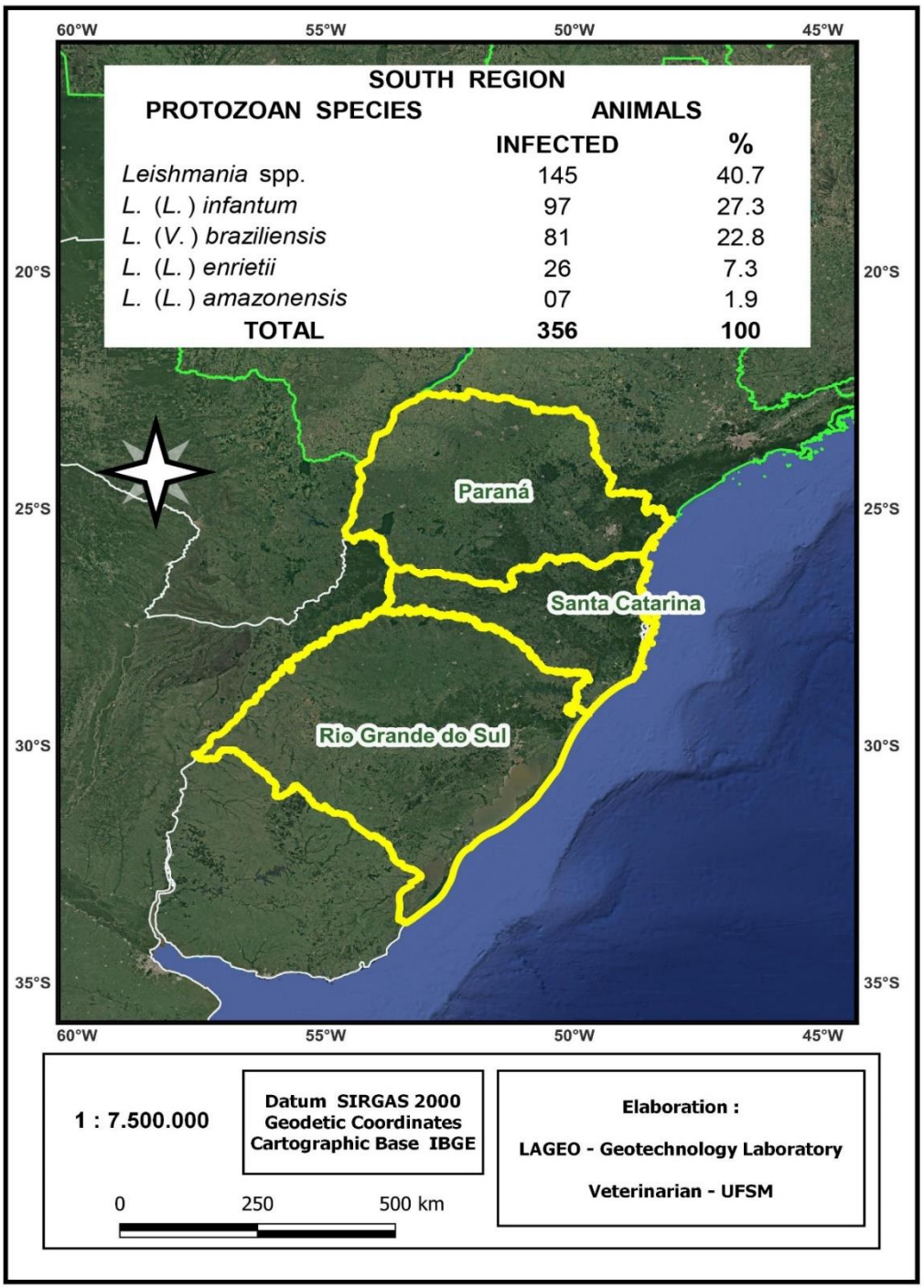


**Fig. 12** Number of mammals infected with *Leishmania* spp., demonstrating the percentage of protozoan species circulating in the states of Paraná, Santa Catarina, and Rio Grande do Sul in theSouth region of Brazil.



Online Resource - 14

Search references in the databases to composse the systematic review (124 studies)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**ARTIGO 1**

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RESEARCH

# Prevalence and molecular detection of *Leishmania* spp. in bats from Rio Grande do Sul state, Brazil

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**Abstract**
This study aimed to detect the occurrence of infection by *Leishmania* spp.in bats from 34 municipalities of Rio Grande do Sul state (RS; southern Brazil) from 2016 to 2021. A total of 109 bats were provided by the Centro Estadual de Vigilância em Saúde of RS, including six species belonged to Molossidae family, six to Vespertilionidae family, and two to Phyllostomidae family. *Leishmania* spp. was identified using the nested-PCR method by amplifying the SSU rDNA ribosomal subunit gene into four organ pools: (1) the liver, spleen, and lymph node; (2) heart and lungs; (3) skin; and (4) bone marrow of each bat. Three (3/109, 2.7%) animals tested positive for *Leishmania* spp. The respective PCR-positive organs came from pools 1 and 3. Two bats (*Tadarida brasiliensis*) were from the municipality of Canoas, and sequences analysis confirms the species identification as *Leishmania infantum*. In the third bat (*Molossus molossus*), from Rio Grande, it was not possible to determine the protozoa species, being considered *Leishmania* spp. Our results indicate that bats can participate in the biological cycle of *Leishmania* spp. and perform as host, reservoir, and/or source of infection of the protozoa in different areas of RS. More studies will be needed to elucidate the role of these Chiropteras in the circulation of *Leishmania* spp. This is the first study reporting the occurrence of *Leishmania* spp. in bats in Rio Grande do Sul state, southern Brazil.

**Keywords** Bat · *Leishmania infantum* · Molecular diagnosis · SSU rDNA · Protozoan

## Introduction

Leishmaniasis is a complex tropical disease with three main forms of infection, cutaneous, mucocutaneous, or visceral, and has One Health impact. It is among the most neglected diseases in the world, with a worldwide occurrence of new cases estimated in 50,000 to 90,000 of visceral leishmaniasis (VL) and 600,000 to 1 million of cutaneous leishmaniasis (CL) in humans per year (WHO 2021).

The different forms of leishmaniasis are characterized by the etiology of several species of *Leishmania*, maintained by multi-host mammals, and transmitted by several species of phlebotomine sand flies, which are hematophagous insects active during twilight hours that require blood intake to complete their reproductive cycle, in different ecotopes (Dantas-Torres 2018; Roque and Jansen 2014).

The visceral form caused by *Leishmania infantum* is the most severe and can be fatal in humans and animals untreated, and the main vector species is *Lutzomyia longipalpis* with wide geographic distribution in Brazil (Dantas-Torres 2009), and the domestic dog is considered the main reservoir in urban areas (Gramiccia 2011).

Nevertheless, roughly 70 species animals have been found to be natural reservoir or hosts of *Leishmania* spp. (WHO 2021). Bats (order Chiroptera) are recognized as reservoirs of trypanosomatids, and some *Leishmania* spp. found in these animals make them hosts and/or possible reservoirs (Roque and Jansen 2014). Corrêa et al. (2013) reported 40 bat species belonging to the families Emballonuridae, Molossidae, Mormoopidae, Noctilionidae, Phyllostomidae,

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Thyropteridae, and Vespertilionidae involved in zoonoses caused by protozoa, especially Chagas disease and leishmaniasis.

Bats inhabit the entire Brazilian territory and are the second-largest animal order of the class (Reis et al. 2007). In Rio Grande do Sul state (southern Brazil), bats are widely distributed, with over 40 native species recognized (Pacheco et al. 2001, 2010; Passos et al. 2010); nonetheless, few studies have investigated the role of these animals in the epidemiological chain of leishmaniasis (Savani et al. 2010).

Chiropteras have great functional diversity and develop fundamental ecology role in rural and urban areas. However, these animals host several zoonotic agents, and, by performing seasonal migration, they can spread relevant infectious agents (Weber et al. 2013). Additionally, bats cohabit with other wild, domestic, and synanthropic species, further exacerbating the exchange of pathogens between animals and humans (Roque and Jansen 2014). This approximation of bats to the human results from climate change, urban sprawl, and deforestation (Pacheco et al. 2010; Weber et al. 2013).

This study aimed to investigate the prevalence of *Leishmania* spp.in bats from different municipalities of Rio Grande do Sul state (RS) in southern Brazil and phylogenetically characterize the species of *Leishmania* spp.in the cities studied.

## Materials and methods

### Study area

Rio Grande do Sul state is located in southernmost Brazil (32° 02′ 06″ S and 52° 05′ 55″ W); it has an average altitude of 950 m and comprises 281,707,156 km$^2$, and the capital is Porto Alegre (POA). In 2021, the population was estimated at 11,466,630 inhabitants, distributed in 497 municipalities, predominately in urban areas (IBGE, 2021) (Fig. 1).

This study was carried out with 109 bats from 34 municipalities in RS, which were collected between 2016 and 2021 and provided by the State Health Surveillance

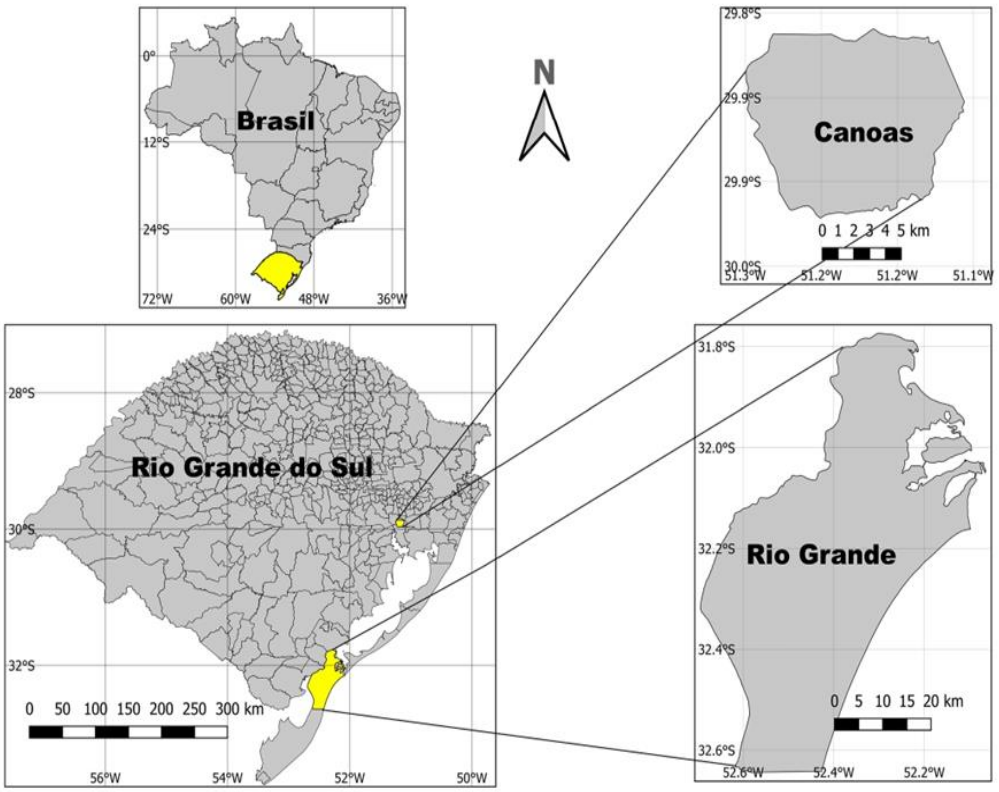


**Fig. 1** State of Rio Grande do Sul, located in southern Brazil. The bats used in this study were collected in the municipalities of the state of Rio Grande do Sul and were evaluated for the presence of *Leishmania* spp. Highlighted on the map, the municipalities of Canoas and Rio Grande, where there was the identification of *Leishmania infantum* and *Leishmania* spp., respectively, in bats

Center (Centro Estadual de Vigilância em Saúde (CEVS)), to veterinary research institute; Instituto de Pesquisas Veterinárias Desidério Finamor (IPVDF) linked to the Department of Agriculture, Livestock, and Irrigation in the municipality of Eldorado do Sul (RS) to research and identify the rabies virus.

In the IPVDF, bats were identified and measured using standard measures and taxonomically classified into the family, genus, and species (Díaz et al. 2016). The animals were kept frozen at – 20 °C and later transported in isothermal boxes to the Laboratório de Doenças Parasitárias (LADOPAR) at Universidade Federal de Santa Maria (UFSM). Then, the following characteristic eating habits, sex and clinical examination were verified. Later, a necropsy was performed for the collection of organs of each animal. The tissues were individually separated, identified, macerated, aliquoted, and stored in freezers at – 20 °C until DNA extraction. For this procedure, an aliquot of each organ was collected, totaling 20 mg for pool, and grouped into four different samples: lymph node, spleen, and liver (pool 1); heart and lungs (pool 2); skin of the epigastric region, ears, and wings (pool 3); and bone marrow (pool 4).

Total DNA was extracted from the samples using the Wizard Genomic DNA Purification Kit (A1125, Promega, USA) following the manufacturer's instructions. Differently, in the extraction of DNA from the skin, samples consisted incubated overnight at 55 °C with the addition of proteinase K (17.5 µL) at a concentration of 20 mg/mL (Ludwig Biotec, Brazil). The quantity and quality of DNA extraction were evaluated using a NanoDrop 1000 Spectrophotometer at an absorbance rate of 260/280 nm (Thermo Fisher Scientific). Subsequently, DNA samples were stored at – 20 °C until molecular testing.

*Leishmania* spp. was detected by amplifying the total DNA extracted from the four pools of each animal sample by nested polymerase chain reaction (n-PCR) assay and targeting the ribosomal subunit gene (SSU rDNA). In the first phase of the reaction, external primers S4 (5′-GATCCAGCT GCAGGTTCACC-3′) and S12 (5′-GGTTGATTCCGTCAA CGGAC-3′) were used, amplifying a fragment of 520 bp (Uliana et al. 1994). For the second phase, internal primer pairs S17 (5′-CCAAGCTGCCCAGTAGAAT-3′) and S18 (5′-TCGGGCGGATAAAACCC-3′) were used, resulting in the amplification of a fragment of approximately 490 bp (Savani et al. 2009). Positive and negative controls were included in all reactions and consisted of DNA extracted from *L. infantum* culture (MHOM/BR/1974/PP75) and Milli-Q water, respectively.

The PCR reaction was prepared to a final volume of 25 µL containing 1 × buffer (Promega, USA), 2 mM of $MgCl_2$, 0.2 mM of dNTPs (Ludwig Biotec, Brazil), 0.2 µM of each primer (Exxtend Biotecnologia, São Paulo, Brazil), 2 U of Taq DNA polymerase (Promega, USA), and ~ 20 ng of the extracted DNA sample. The amplification was processed in an automatic thermal cycler (T100, Bio-Rad, Singapore) following the recommendations of Savani et al. (2009) with the following modifications: 45 s at 72 °C in the extension phase in the 30 cycles of the second phase of n-PCR. The products of the amplification reactions were subjected to electrophoresis in multipurpose agarose gel (Ludwig Biotecnologia, Brazil) 2% stained with SYBR safe DNA staining (Invitrogen, USA) visualized in the UV transilluminator and photographed for documentation and analysis of results.

For DNA sequencing, the products of the amplified samples from the second round of n-PCR assay were submitted to purification using a PCR purification kit (PureLink, Invitrogen, Thermo Fisher Scientific) and following the manufacturer's instructions. Subsequently, the DNA was analyzed in a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, USA) to determine the concentration. In addition, a known positive sample from *L. infantum* culture was purified for sequencing and comparison with the samples of this study.

Due to the low *Leishmania* spp. concentrations extracted with the Wizard genomic DNA purification kit (A1125, Promega, USA) or the possible presence of PCR inhibitors in the samples, the detection threshold and PCR reaction inhibition tests were performed to eliminate possible PCR amplification failures using DNA samples extracted from bat organs. The reaction detection threshold was performed using the positive control diluted in Milli-Q water at the following serial dilutions from 1:10 to $1:10^6$ and tested by PCR. Additionally, the positive samples named 1.1 and 2.1 were also diluted in the serial dilutions from 1:10 to $1:10^4$ and retested. Inhibition of the PCR reaction was performed with sample named 105.1 (pool 1) (considered negative), which was diluted with the positive control of *L. infantum* in the proportion of 1:1; the n-PCR reaction was then performed as described previously.

Each sample to be sent for DNA sequencing was prepared with 50 ng of purified PCR product, Milli-Q water, and 5 pmol of each internal primer (S17 and S18) separately, totaling a final volume of 6 µL. Dehydration was then performed at 60 °C for 2 h for subsequent referral for sequencing using the Sanger method. Generated DNA sequences were analyzed using the Staden software package (http://staden.sourceforge.net/) and compared using the GenBank NCBI BLAST database (http://www.ncbi.nlm.nih.gov/BLAST).

The phylogenetic analysis was performed on the Phylogeny.fr platform (Dereeper et al. 2008). The phylogenetic tree was constructed using the Bayesian inference method implemented in the MrBayes program v3.2.6 (Huelsenbeck and Ronquist 2001). The number of substitution types was fixed to 6. The standard (4×4) model of nucleotide substitution was used, while rates variation across sites were fixed

to "invgamma." Four Markov chain Monte Carlo (MCMC) chains were run for 10,000 generations, sampling every 10 generations, with the first 250 sampled trees discarded as "burn-in." Finally, a 50% majority rule consensus tree was constructed (Dereeper, et al. 2010). Graphical representation and edition of the phylogenetic tree were performed with TreeDyn v198.3 (Chevenet et al. 2006).

This study received institutional authorization to carry out the research, including CEVS (SES/RS 334/2019), Ethics Committee on the Use of Animals/UFSM (n° 7,576,231,019), and National Genetic Heritage Management System (SISGEN A308EE6).

## Results

Among the 109 bats studied, 36 (33%) were collected in 2016, 33 (30.3%) in 2018, two (1.8%) in 2019, 31 (28.4%) in 2020, and 7 (6.4%) in 2021, from 34 municipalities in RS. Three families, 10 genera, and 14 species were identified (Table 1), with predominantly insectivorous feeding habits in a total of 101/109 (92.6%) animals, except for bats 01/109 (0.9%) of the species *Sturnira lilium*, which feeds on fruits, and 02/109 (1.8%) *Desmodus rotundus*, which are hematophagous (05/109, 4.6%), were not identified. Males 68/109 (62.4%) constituted most of the sample.

It was not possible to collect the lymph nodes in 11 bats, due to autolysis and the small size of the organ. The amount of bone marrow in the animals was low or non-existent at the time of necropsy, and it was not possible to collect this organ from 22 animals. In addition, from three animals, it was not possible to collect organs from pool 1, and in one animal, it was not possible to collect organs from pool 2 due to tissue autolysis.

Of the 410 samples processed, *Leishmania* spp. DNA was detected in five (1.2%) tissue pools, corresponding to three animals (3/109, 2.7%) captured in 2016. In pool 1 (lymph node, spleen, and liver), DNA was amplified in three animals (3/3, 100%); in the pool 3, containing skin, the presence of DNA was observed in two samples (2/3, 66.7%).

The three bats that tested positive for *Leishmania* spp. were females, and two (*Tadarida brasiliensis*) were from the municipality of Canoas (2/3, 66.7%), and one (*Molossus molossus*) was from the municipality of Rio Grande (1/3, 33.3%) (Fig. 1). No skin lesion was observed on external physical examination in any animal, including bats that tested positive for *Leishmania* spp.

By analyzing the amplified DNA sequences, we identified similarity (> 99%) of sequences of *L. infantum* deposited in the GenBank in two bats of the species *T. brasiliensis* from the municipality of Canoas under accession numbers OL614781 and OL614783. However, in samples of the bat from the municipality of Rio Grande (*Molossus molossus*), it was not possible to determine the protozoan species by sequencing; thus, it was considered *Leishmania* spp. The DNA sequencing of the positive control of *L. infantum* culture was deposited under accession number OL614754. By phylogenetic analysis, using sequences of the ribosomal subunit gene (SSU rDNA), the DNA sequences obtained from the samples were grouped with *L. infantum* (GenBank access OL616091) (Fig. 2).

In evaluating the threshold of DNA detection of the parasite, the positive control amplified until the dilution $1:10^4$, and the positive samples 1.2 and 2.1 were of 1:10.The test to evaluate the PCR inhibition amplified the negative sample 105.1 when in the presence of the DNA of *L. infantum*.

## Discussion

Leishmaniasis has shown important changes in the pattern of transmissibility, with an alternation of prevalence, as previously detected in rural and peri-urban environments and currently in urban centers. These constant changes have increased human and animal exposure to infected sand flies (Dantas-Torres 2009; Hong et al. 2020; Tafuri et al. 2001).

The adaptation of the vector in urban areas occurs due to deforestation and the expansion of agricultural areas, as well as the uncontrolled growth of cities, providing favorable conditions for the development of the vector in these domestic environments, as well as domestic and synanthropic animal shelters (Hong et al. 2020). In these places, there is availability of food and organic matter, and they favor the breeding of the vector, besides attract domestic, wild, and synanthropic animals, increasing the transmission of *Leishmania* spp. between hosts.

Berzunza-Cruz et al. (2015) infected bats with *Leishmania mexicana* and demonstrated the ability to infect and transmit the agent to the vector. Therefore, with our results, it can be inferred that bats in nature can be infected with *L. infantum* and *Leishmania* spp. becoming a source of infection for vectors that share the same habitat.

This study demonstrated the occurrence of *Leishmania* spp.in two bat species of the Molossidae family considered to be adapted inhabitants in urban areas of the municipalities of Canoas and Rio Grande (Weber et al. 2013). The importance of our findings may influence the occurrence of leishmaniasis, since these animals can cohabit with humans and act as maintainers of the protozoan circulation. Phylogenetic analysis separated *Leishmania* species in clades corresponding to the subgenera and supported that the samples detected in the present study clustered with *L. infantum*. Therefore, phylogenetic tree inferred from the ribosomal subunit gene (SSU rDNA) sequences classified the samples from bats as *L. infantum*.

**Table 1** Distribution of bats by family (feeding habits), genus, and species, municipality of capture, and frequency of molecular detection of *Leishmania* spp. DNA, between 2016 and 2021 in the state of Rio Grande do Sul (RS), Brazil

| Family (feeding habits) | Genus/species | County | No. of bats county/family % | Sex M/F | Positive DNA (N/%) | Positivity per species (%) | Positivity per family (%) |
|---|---|---|---|---|---|---|---|
| Molossidae (insetivorous) | *Tadarida brasiliensis* | Pelotas | 14/14.9 | 07/07 | - | 0.0 | |
| | | Canoas | 11/11.7 | 05/06 | 02/66.7 | 3.9 | |
| | | Porto Alegre | 07/7.4 | 04/03 | - | 0.0 | |
| | | Rio Grande | 05/5.3 | 04/01 | - | 0.0 | |
| | | Caxias do Sul | 05/5.3 | 05/00 | - | 0.0 | |
| | | Camaquã | 01/1.1 | 00/01 | - | 0.0 | |
| | | Capão do Leão | 01/1.1 | 01/00 | - | 0.0 | |
| | | Gramado | 01/1.1 | 01/00 | - | 0.0 | |
| | | Gravataí | 01/1.1 | 00/01 | - | 0.0 | |
| | | Ivoti | 01/1.1 | 01/00 | - | 0.0 | |
| | | Passo Fundo | 01/1.1 | 01/00 | - | 0.0 | |
| | | Santa Maria | 01/1.1 | 01/00 | - | 0.0 | |
| | | São Gabriel | 01/1.1 | 00/01 | - | 0.0 | |
| | | São Leopoldo | 01/1.1 | 01/00 | - | 0.0 | |
| | *Molossus* spp. | Santa Maria | 03/3.2 | 02/01 | - | 0.0 | 3.2 |
| | *Molossus correntium* | Pelotas | 05/5.3 | 03/02 | - | 0.0 | |
| | | Capivari do Sul | 01/1.1 | 01/00 | - | 0.0 | |
| | | Eldorado do Sul | 01/1.1 | 01/00 | - | 0.0 | |
| | | Guaíba | 01/1.1 | 01/00 | - | 0.0 | |
| | | Nova Alvorada | 01/1.1 | 00/01 | - | 0.0 | |
| | | Porto Alegre | 01/1.1 | 01/00 | - | 0.0 | |
| | | Rio Grande | 01/1.1 | 00/01 | - | 0.0 | |
| | | São José do Norte | 01/1.1 | 01/00 | - | 0.0 | |
| | *Molossus molossus* | Rio Grande | 04/4.2 | 00/04 | 01/33.3 | 7.2 | |
| | | Bento Gonçalves | 02/2.1 | 02/00 | - | 0.0 | |
| | | Caxias do Sul | 02/2.1 | 00/02 | - | 0.0 | |
| | | Pelotas | 02/2.1 | 01/01 | - | 0.0 | |
| | | Campo Bom | 01/1.1 | 00/01 | - | 0.0 | |
| | | Canoas | 01/1.1 | 01/00 | - | 0.0 | |
| | | São Leopoldo | 01/1.1 | 01/00 | - | 0.0 | |
| | | Sapucaia do Sul | 01/1.1 | 01/00 | - | 0.0 | |
| | *Molossus rufus* | Porto Alegre | 04/4.2 | 03/01 | - | 0.0 | |
| | | Ijuí | 03/3.2 | 01/02 | - | 0.0 | |
| | | Cachoeirinha | 01/1.1 | 01/00 | - | 0.0 | |
| | | Campo Bom | 01/1.1 | 01/00 | - | 0.0 | |
| | | São Leopoldo | 01/1.1 | 01/00 | - | 0.0 | |
| | | Taquari | 01/1.1 | 01/00 | - | 0.0 | |
| | | Tiradentes do Sul | 01/1.1 | 01/00 | - | 0.0 | |
| | *Nyctinomops laticaudatus* | Alegrete | 01/1.1 | 01/00 | - | 0.0 | |
| | *Promops nasutus* | Toropi | 01/1.1 | 01/00 | - | 0.0 | |
| Subtotal Molossidae | | | 94/86.2 | 58/36 | | | |
| Vespertilionidae (insetivorous) | *Eptesicus furinalis* | Nova Boa Vista | 02/28.6 | 02/00 | - | 0.0 | |
| | *Eptesicus brasiliensis* | Igrejinha | 01/14.3 | 00/01 | - | 0.0 | |
| | *Histiotus velatus* | Sertão Santana | 01/14.3 | 01/00 | - | 0.0 | 0.0 |
| | *Lasiurus blossevillii* | Humaitá | 01/14.3 | 00/01 | - | 0.0 | |
| | *Myotis levis* | Rio Grande | 01/14.3 | 01/00 | - | 0.0 | |
| | *Myotis nigricans* | Pelotas | 01/14.3 | 01/00 | - | 0.0 | |

**Table 1** (continued)

| Family (feeding habits) | Genus/species | County | No. of bats county/family % | Sex M/F | Positive DNA (N/%) | Positivity per species (%) | Positivity per family (%) |
|---|---|---|---|---|---|---|---|
| Subtotal Vespertilionidae | | | 07/6.4 | 05/02 | | | |
| Phyllostomidae (hematophagous or frugivorous | *Desmodus rotundus* | Santiago | 02/66.7 | 02/00 | - | 0.0 | |
| | *Sturnira lilium* | Canoas | 01/33.3 | 01/00 | - | 0.0 | 0.0 |
| Subtotal Phyllostomidae | | | 03/2.7 | 03/00 | | | |
| Not identified | | Santa Maria | 02/40.0 | 00/02 | - | 0.0 | |
| | | Unknown | 02/40.0 | 01/01 | - | 0.0 | 0.0 |
| | | Agudo | 01/20.0 | 01/00 | - | 0.0 | |
| Unidentified subtotal | | | 05/4.6 | 02/03 | | | |
| Total | | | 109/100 | 68/41 | 3/100 | | |

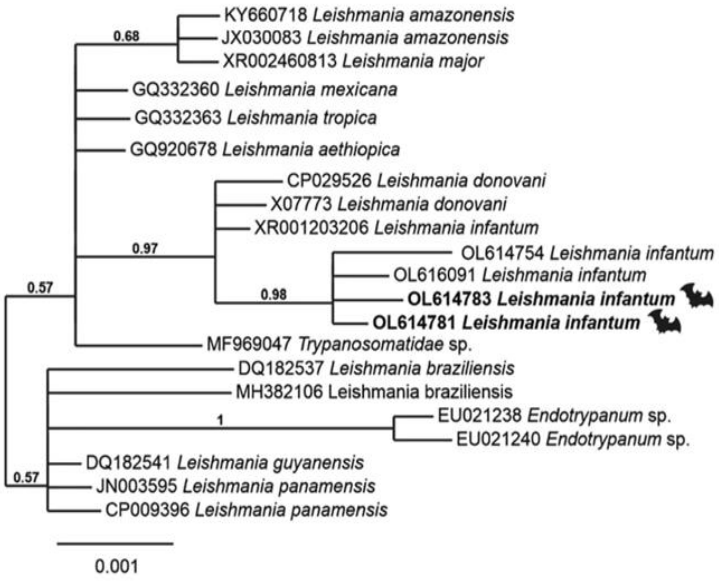


**Fig. 2** Phylogenetic analysis of SSU rDNA ribosomal subunit sequences obtained from various species of *Leishmania* spp. The phylogenetic tree was constructed using the Bayesian inference method implemented in the MrBayes program v3.2.6. The phylogenetic tree was performed with TreeDyn v198.3

*L. infantum* and *L. braziliensis* have been reported to cause autochthonous cases of VL in humans, during 2016 to 2017, in the metropolitan region of Porto Alegre, which includes the municipality of Canoas; also in this case, multiple vectors were involved in the biological cycle, including *Lutzomyia longipalpis*, *Lutzomyia gaminarai*, *Pintomyia fischeri*, *Lutzomyia neivai*, *Migonemyia migonei*, and *Nyssomyia neivai* (Pita-Pereira et al. 2009; Rêgo et al. 2019, 2020).

The phlebotomine sand flies that transmit leishmaniasis, as well as bats, tend to have nocturnal behavior (Rebêlo 2001); however, these insects travel short distances. The transmission of the protozoan from the vector to the bat may allow the agent to multiply, maintaining infectivity and disseminating into different regions due to the capacity of displacement and seasonal migration characteristic of some bats species (Berzunza-Cruz et al. 2015; Pacheco et al. 2010).

Synanthropic animals collected and sent to reference laboratories can be of great use for the sanitary monitoring of several diseases, which is similar to what occurs in rabies surveillance (Kotait et al. 2007). Thus, some studies in Brazil identified *L. braziliensis*, *Leishmania amazonensis*, and *L. infantum* infecting bats in Brazilian states such as Mato Grosso do Sul, São Paulo, and Mato Grosso as an attempt to help investigate the epidemiological chain of leishmaniasis (Ferreira et al. 2017; Oliveira et al. 2015; Rezende et al. 2017; Savani et al. 2010; Shapiro et al. 2013).

In this study, different organs of bats were used for the molecular detection of the etiological agents of leishmaniasis successfully, identifying the protozoan in macerated organs (lymph node, spleen, liver, and skin). Savani et al. (2010) described 3.2% (21/659) of bats infected with *Leishmania* using molecular tests in macerated spleen and liver samples, identifying *L. amazonensis* and *L. infantum* in *M. molossus*, among other bat species. The findings are similar to those found in the cities of Canoas and Rio Grande, where protozoan DNA was detected in the spleen, liver, and lymph node of chiropterans of the *Tadarida brasiliensis* species and in a *M. molossus* specimen, respectively.

Oliveira et al. (2015) carried out a study using real-time PCR with bats from 21 VL endemic municipalities in São Paulo state, where they were identified the DNA of *L. amazonensis* and *L. infantum* in 23.9% (117/488) of the animals in which 47.9% (56/117) tested positive in the skin, 37.6% (44/117) in the splenic tissue, and 14.5% (17/117) in both tissues. Unlike this study, the highest positivity rate for the DNA of the protozoan was found in the macerated spleen, liver, and lymph node samples, followed by the skin and in both tissues, although the organs used for the research were similar.

*Leishmania* spp.is an obligate intracellular protozoan present in all vertebrate tissues (BRASIL 2014). In VL, histopathology is mainly associated with hypertrophy and hyperplasia of the cells of the phagocytic mononuclear system (PMS). Thus, the liver, spleen, lymph nodes, and skin of bats can be considered the organs of choice to investigate the presence of *Leishmania* spp. as they are rich in PMS cells (Shapiro et al. 2013; Alcover et al. 2020). The option to obtain a skin sample from the epigastric region, wings, and ears was because these areas have good vascularization and the possibility of greater contact with the insect vector of leishmaniasis (Oliveira et al. 2015). Regarding the protozoan species that are considered dermotropic, it is known that they can also lead to the development of visceral disease with the involvement of lymph nodes, spleen, and liver (Oliveira et al. 2019).

Savani et al. (2010) found a higher occurrence of the protozoan in female bats, corroborating this study; however, the authors emphasized that the sex variable was irrelevant in the epidemiology of the disease. Further research is needed to clarify the influence of sex on the prevalence of infection in female bats as the sample of this study was predominantly male. In addition, it should be noted that the behavioral biology of this mammal is different between both sexes, which form groups segregated by sex and age, which may influence exposure to sand flies and blood meals (Weber et al. 2013).

In the present study, most bats that tested positive for *Leishmania* spp. were insectivores. This fact can be explained by the abundance of bat species that have this feeding habit in the studied regions (Pacheco et al. 2010). In addition, it is possible to attribute the finding of organs infected by the protozoan in bats to ingesting infected phlebotomine sand flies, as well as similarly described for *Trypanosoma cruzi* in other mammals and humans, in which the transmission of the agent occurs through the ingestion of sugarcane juice or crushed "açaí" fruit with the vector (Oliveira et al. 2015). Future studies are needed to verify this hypothesis.

The two bat species that tested positive for the protozoan *Leishmania* spp. were *Tadarida brasiliensis* and *Molossus molossus*, which were captured in 2016. The species *T. brasiliensis*, from the municipality of Canoas, was detected with *L. infantum*, the causative agent of VL. The municipality of Canoas can be considered a transmission area for the disease, making up the metropolitan region of the capital Porto Alegre, which is only 14 km away (Fig. 1). Canoas is the largest municipality in the metropolitan area and an important industrial, university, and military center with a large flow of individuals (IBGE 2010). *T. brasiliensis* is the most abundant bat species in RS and adapted to urbanized areas, taking shelter in urban and peri-urban residential and commercial environments (Weber et al. 2013), thus likely maintaining the VL-causing protozoan in nearby areas of humans and domestic animals.

Likewise, the species *Molossus molossus* was identified with the infection of the agent *Leishmania* spp. in the municipality of Rio Grande, as it cohabits in the urban environment (Weber et al. 2013). From a sanitary point of view, this is the oldest municipality in RS (IBGE 2010); it is located in the south of the state and an important urban center and port region where the Port of Mercosur is located, which is responsible for importing and exporting products from all continents. Thus, it has a significant influx of individuals and products and the conditions for maintaining and spreading pathogenic agents such as *Leishmania* spp. and their vectors to various regions.

Between 2008 and 2021, 43 autochthonous cases of human VL (HVL) were confirmed in RS. From 2016 to 2021, in the Porto Alegre region, 24 cases of VL were reported, resulting in four deaths. CL has also been reported in humans in Canoas, with one case confirmed in 2014 and two cases in 2018 (DATASUS 2021; RIO GRANDE DO SUL 2017). The positivity of *L. infantum* in bats in Canoas in 2016 alerts to the circulation of the agent in the area, corroborating the endemicity of the region.

According to reports of leishmaniasis cases in the municipality of Rio Grande, in 2012 and 2015, there were confirmed cases of CL in humans (HCL), and in 2019, there was a notification and confirmation of HVL (DATASUS 2021). The identification of *Leishmania* spp. in a bat captured in 2016 in this region can also corroborate in epidemiology of the disease because the parasite was detected in the region.

Recognizing the animal species that make up the epidemiological cycle of leishmaniasis is vital to expand control actions. In addition, we emphasize the role of these animals as biomarkers of a certain region. Therefore, it is necessary to carry out more studies on wild and synanthropic animals that may be contributing and maintaining the circulation of *L. infantum* in the state of RS (Caldart et al. 2021; Azami-Conesa et al. 2020).

Strategies for disease control and prevention are especially focused on surveillance, notification, and monitoring of cases in dogs and humans and entomological investigation (BRASIL 2014; RIO GRANDE DO SUL 2017), not involving synanthropic animals. However, these isolated measures have not reduced the incidence of the disease, and the cases of CL and VL in RS have increased considerably in recent years (RIO GRANDE DO SUL, 2017), resulting in some deaths. Health success requires effective measures according to the epidemiological situation of each region, giving rise to different actions in search of one health. The importance of controlling the disease in border or port regions, where there is an international movement of individuals, is highlighted, enabling the spread of the disease to several countries.

## Conclusions

This is the first study reporting the occurrence of *Leishmania* spp. in bats in Rio Grande do Sul, southern Brazil. *Leishmania infantum* DNA was detected in different organs of insectivorous bats from Canoas and Rio Grande. The present study contributes to the growing literature on the participation of bats in the biological cycle of leishmaniasis. Therefore, it is suggested to monitor *Leishmania* spp. in different animal species, including bats, in order to mitigate the occurrence of cases in humans. However, more studies will be needed to elucidate the participation of this Chiropteran in the circulation of *Leishmania* to better understand the potential of bats as hosts and reservoirs of infection.

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## Declarations

**Competing interests** The authors declare no competing interests.

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# 6 DISCUSSÃO

A leishmaniose está presente em todos os continentes, com exceção da Antártida e da Austrália. Mais de 90% dos casos de LV ocorrem em Bangladesh, Brasil, Etiópia, Índia, Sudão e Sudão do Sul. E o Brasil, bem como Afeganistão, Argélia, Colômbia, Irã, Síria, Etiópia, Sudão do Norte, Costa Rica e Peru, respondem por 70 a 75% da incidência global de LT (ALVAR et al., 2012).

A enfermidade é um problema de saúde pública, de difícil erradicação por apresentar transmissão vetorial, consequentemente atrelada a fatores ambientais e mudanças socioeconômicas e manutenção por multi-hospedeiros mamíferos em diferentes ambientes: silvestres, peri-domésticos e domésticos (HONG et al., 2020; ROQUE e JANSEN, 2014). As preocupações em relação a leishmaniose, dizem respeito a várias lacunas desconhecidas sobre a epidemiologia do ciclo de transmissão no ambiente doméstico e silvestre, sobre os hospedeiros que são fontes de infecção ao vetor (HONG.et al., 2020).

No presente estudo, verificamos que no Brasil existem muitas espécies de mamíferos envolvidos no ciclo da leishmaniose, tanto domésticos como silvestres. Por meio da revisão sistemática confirmamos a endemicidade da doença no País, na qual o protozoário infecta animais silvestres e domésticos em todas as regiões brasileiras. Evidencia-se que é necessário investigar a relação das espécies animais identificados com o protozoário na epidemiologia da enfermidade, para que se tenha a possibilidade de minimizar a sua expansão, uma vez que, regiões onde não há registros autóctones humanos ou caninos, pode ocorrer a circulação de espécies de *Leishmania* entre outros mamíferos (PAIZ et al., 2015).

As espécies animais infectadas com *Leishmania* spp., não significam que são responsáveis pela transmissão ao homem ou a outros animais presentes no ambiente (LUPPI et al., 2008). O conceito de reservatório ainda pode ter uma visão antropocêntrica, definido como a espécie animal que é fonte de infecção de um parasito para o homem. No entanto, considera-se como reservatório um sistema que pode incluir mais de uma espécie de animal responsável pela manutenção de um parasito na natureza, mantendo circulante em vários hospedeiros. Um animal infectado é, portanto, um hospedeiro do parasito. O que definirá seu papel como

reservatório é a competência da espécie na manutenção e/ou transmissão do protozoário ao vetor (ROQUE e JANSEN, 2014).

Desta forma, salienta-se que são necessários mais estudos que identifiquem quais espécies são reservatórios e qual o real impacto no ciclo da enfermidade no Brasil. Observa-se que a maioria das pesquisas determinam a infecção focal nos hospedeiros. No entanto, na grande maioria dos trabalhos não é identificado se a espécie animal é reservatório e corrobora para manutenção do protozoário na região.

Os resultados da revisão sistemática demonstraram o baixo índice de apresentação de sinais clínicos nos animais, característicos da enfermidade. Essa é uma condição controversa na determinação de animais reservatórios, principalmente quando se tenta definir essa característica em animais silvestres. Esse fato é relevante na cadeia epidemiológica, enquadrando muitos desses animais em uma das características esperadas para um reservatório (SOUZA et al., 2010). A característica de animais assintomáticos reservatórios foi por muito tempo considerada uma condição essencial, entretanto, uma relação parasito-hospedeiro nem sempre evolui de forma harmônica, ocasionando a doença. Contudo, o essencial para se considerar um animal reservatório, é a condição de possibilitar a maior transmissibilidade do agente para o vetor (ROQUE e JANSEN, 2014). A presença ou não de sinais clínicos ou a gravidade do quadro clínico, depende da imunocompetência do hospedeiro ou da espécie de *Leishmania* responsável pela infecção (CUPOLILLO et al., 2014).

Animais que possam ser considerados sentinelas podem auxiliar no âmbito da saúde pública, uma vez que são indicadores da saúde ambiental para essa zoonose (PADILHA et al., 2021). Caldart et al. (2017) relataram *L.* (*L.*) *amazonensis*, em roedores sinatrópicos (*Rattus rattus*) evidenciando a urbanização desse agente etiológico e a disseminação da enfermidade no sul do Brasil. Esses animais convivem muito próximos dos seres humanos e animais domésticos, no peri ou intradomicílio, configurando um grande risco, uma vez que, existe negligência com relação a leishmaniose por parte do poder público.

A vigilância da LV, no contexto da saúde única, é um desafio global e contínuo, que objetiva reduzir os níveis de transmissão do agente, a ocorrência de casos e mortes de seres humanos e os níveis de infecção animal, envolvendo o controle do vetor, o manejo dos cães, dos casos humanos e do ambiente. O Ministério da Saúde preconiza, que em áreas endêmicas a suspeita de LV deve estar entre os diagnósticos possíveis, uma vez que os sintomas são

comuns aos de outras doenças. Em áreas não endêmicas a enfermidade não deve ser ignorada, sob risco de agravamento ou óbito por diagnóstico tardio. A notificação é compulsória para casos em humanos e em animais (BRASIL, 2020).

O cão é o principal reservatório urbano do parasito por apresentar elevado parasitismo cutâneo. A presença de cães positivos requer atenção quanto a saúde ambiental e às condições em que são mantidos os animais (BRASIL, 2014a). É importante orientar os tutores quanto à limpeza dos locais, com relação à presença de matéria orgânica que favorece a reprodução dos flebotomíneos, bem como, a circulação de animais silvestres onde ficam os animais domésticos. As medidas de controle ambiental incluem: realizar a limpeza de quintais, terrenos e praças públicas, eliminar resíduos sólidos e orgânicos, retirar entulhos e materiais inservíveis e dar o destino adequado e realizar o esclarecimento da população (RIO GRANDE DO SUL, 2011). O controle vetorial pode ser realizado por meio do controle químico, com aplicação de inseticidas de ação residual, direcionada aos insetos adultos e de forma integrada com manejo ambiental a fim de se reduzir condições propícias à proliferação do vetor, porém pode provocar impactos ambientais (BRASIL, 2020). Além disso, o emprego de coleiras impregnadas com deltametrina a 4% para cães, pode reduzir a prevalência e a incidência de LVC (KAZIMOTO et al., 2018). Essas medidas são fundamentais para que o vetor não permaneça na região e simplesmente mude seus hábitos alimentares, infectando outros hospedeiros e reservatórios.

No caso da LVC, sendo a notificação também compulsória, é direcionada a Secretaria da Agricultura (BRASIL, 2014b). Havendo suspeita ou diagnóstico clínico confirmado é recomendável que os médicos-veterinários, comuniquem à autoridade sanitária local, principalmente em áreas livres ou silenciosas para a doença. A eutanásia é, atualmente, uma das medidas de controle previstas para os cães infectados/doentes ou, ainda, aqueles que não serão tratados por opção do tutor, bem como os animais errantes, sob a forma de medida de política pública. Portanto, a utilização da coleira, associada às demais medidas de saúde pública atualmente propostas, podem ser consideradas como medida de controle da LVC no Brasil (KAZIMOTO et al., 2018).

Os protozoários causadores da enfermidade vêm apresentando multipilicidade genética e ecletismo quanto às diferentes ordens de mamíferos que são capazes de infectar. Os equinos e mais especificamente os felinos domésticos são espécies de convívio próximo aos seres humanos, e de acordo com nossos resultados estão albergando *Leishmania* sp..

Devido os equinos serem infectados por *L*. (*L*.) *infantum* apresentarem alterações clínicas semelhantes aos cães com LV, deve-se realizar vigilância com essa espécie, estabelecendo o diagnóstico diferencial em animais suspeitos, pois esses animais estão em contato próximo ao homem, especialmente em área peri-urbana, onde geralmente são mantidos os animais de tração, podendo servir de reservatórios aos vetores. (SOARES et al. 2013). Essa condição deve ser melhor investigada por meio de estudos empregando o xenodiagnóstico.

Em gatos domésticos, as principais manifestações clínicas da leishmaniose são lesões cutâneas, o que pode ser confundida com várias outras doenças, mesmo em casos de infecção por *L*. (*L*.) *infantum*, uma espécie viscerotrópica. Os gatos comVírus da Imunodeficiência Felina (FIV) são mais predispostos a desenvolver leishmaniose assim como humanos com HIV (PENNISI e PERSICHETTI, 2012).

Por meio de xenodiagnóstico, demonstraram que gatos domésticos podem transmitir *Leishmania* (*L*.) *infantum* aos flebotomíneos (MAROLI et al., 2007; SILVA et al., 2010) e apresentam alto parasitismo na pele avaliada por imuno-histoquímica (VIDES et al., 2011), podendo portanto servirem como reservatórios urbanos de LV. Embora os gatos são relativamente resistentes à infecção por *Leishmania* sp., principalmente devido ao fato de que eles produzem uma resposta imune celular, com baixos títulos de anticorpos (SOLANO-GALLEGO et al., 2007). PENNISI e PERSICHETTI (2012) conseguiram amplificar através de PCR o DNA do parasita de sangue de gatos com resultados negativos na Reação de Imunofluorescência Indireta (RIFI), sugerindo a existência de um equilíbrio entre o hospedeiro e o parasita alcançado por essa resposta imune celular.

Durante muito tempo, os gatos foram considerados hospedeiros acidentais das espécies de *Leishmania* causadoras de LT, provavelmente porque a maioria dos gatos infectados apresentam exclusivamente alterações cutâneas. No diagnóstico da doença eram utilizadas a citologia ou detecção de anticorpos, identificando somente o gênero do parasito (SOUZA et al., 2005, 2009; FIGUEIREDO et al., 2009; SILVEIRA NETO et al., 2011). Atualmente, os trabalhos demonstraram o amplo emprego da PCR e sequenciamento e/ou uma associação de técnicas de diagnóstico, com o propósito de demonstrar a espécie de *Leishmania* envolvida, comprovando que gatos no Brasil podem ser reservatórios do agente causador da LV apesar de apresentarem sinais clínicos cutâneos (COELHO et al., 2011; SAVANI et al., 2004; SOBRINHO et al., 2012; VIDES et al., 2011).

A escolha do tecido amostral para determinação do diagnóstico é um fator importante uma vez que pode variar conforme a espécie animal a ser testada. Em cães, foi demonstrado que a medula óssea apresenta a maior densidade de *Leishmania* sp. durante o curso clínico da doença (REIS et al., 2009), no entanto para felinos, há discordância entre os autores de qual é o melhor órgão linfóide para realizar um aspirado por agulha fina, entre medula óssea (VIDES et al., 2011) ou linfonodo (COSTA et al., 2010). Contudo, é de consenso que se deve combinar técnicas de diagnóstico como: citopatológico do órgão linfóide, imuno-histoquimica da pele e PCR. As técnicas de PCR são geralmente consideradas mais específicas do que a sorologia para diagnosticar a leishmaniose (AYLLON et al., 2008; MAIA et al., 2008; MARTÍN-SÁNCHEZ et al., 2007; TABAR et al.,2008). No entanto, dependendo do estágio da doença (parasitemia), pode ser necessário realizar um PCR em órgãos linfóides ou pele e não somente em amostras de sangue.

No nosso estudo, em morcegos provenientes do RS, realizando PCR em diversos órgãos, obtivemos os melhores resultados nas amostras de fígado, baço, linfonodo e pele, ressaltando que nenhum animal apresentou lesão cutânea compatível com leishmaniose, corroborando com nossos achados, CASTRO et al. (2020) identificaram DNA de *L.* (*L.*) *infantum* e *L.* (*V.*) *braziliensis* em fígado e pele, *L.* (*V.*) *braziliensis* foi detectada na pele (FERREIRA et al., 2017). Além disso, no baço e na pele também detectaram DNA de *L.* (*L.*) *amazonensis* e *L.* (*L.*) *infantum* (OLIVEIRA et al., 2015).

A detecção da infecção de quirópteros, através da revisão sistemática, por algumas espécies de *Leishmania* em algumas regiões brasileiras, indica que esses mamíferos estão envolvidos nos ciclos da LV e LC (CASTRO et al., 2020; COSTA et al., 2015; CUNHA et al., 2014; FERREIRA et al., 2017; OLIVEIRA et al., 2015; REZENDE et al., 2017; RIVA et al., 2021; ROQUE e JANSEN, 2014, SAVANI et al., 2010; SHAPIRO et al., 2013).

É importante que se defina o papel epidemiológico dos quirópteros no ciclo do protozoário, uma vez que, eles têm uma grande distribuição, sendo no mundo, mais de 1400 espécies, ocorrendo cerca de 181 no Brasil e reconhecidas mais de 40 espécies no RS (PACHECO et al., 2001; 2010; PASSOS et al., 2010; SBEQ, 2022). Esses animais possuem grande plasticidade comportamental e diversidade funcional, e podem ser hospedeiros de diversos agentes zoonóticos. Algumas espécies apresentam deslocamento sazonal, e poderiam disseminar vários agentes infecciosos de importância epidemiológica (WEBER et al., 2013)

assim como *Leishmania* sp.. Morcegos habitam ambientes diversos e, eventualmente podem coabitar com outras espécies silvestres, domésticas e sinantrópicas. Neste caso, poderia haver intercâmbio de enfermidades entre os animais e os humanos (ROQUE & JANSEN, 2014).

Em decorrência principalmente da expansão urbana e do desflorestamento desordenado, esses animais se aproximaram do domicílio humano (PACHECO et al., 2010; WEBER et al., 2013). Nosso estudo identificou pela primeira vez em quirópteros das espécies *Tadarida brasiliensis* e *Molossus molossus*, a presença de *L.(L) infantum* e *Leishmania* spp. em dois municípios do RS. Esse resultado pode levar a inferência de que espécies de morcegos podem estar participando do ciclo da leishmaniose, pois há relação trófica entre o diptero, vetor da doença e o morcego predador, uma vez que já há relato de que o vetor se alimenta em morcegos e o quiróptero tem a capacidade de se infectar com *Leishmania* sp. e se tornar reservatório (BERZUNZA-CRUZ et al., 2015; LAMPO et al., 2000).

Ressalta-se que as relações de proximidade entre hospedeiros, reservatórios humanos e/ou animais domésticos ou silvestres, protozoários e vetores flebotomíneos potencializam a ocorrência de *Leishmania* spp. em diversos ambientes (HONG et al., 2020). Observa-se que diferentes espécies silvestres, incluindo os morcegos, vêm sendo deslocadas para as proximidades das residências humanas, devido a oferta de alimentos e água ou pela busca de abrigo em decorrência de alterações ambientais, o que também vem ocorrendo com os vetores pelo desequilíbrio ambiental e a aproximação da população humana do habitat de florestas remanescentes.

# 7 CONCLUSÃO

A complexidade e as lacunas ainda existentes na epidemiologia da leishmaniose, dificultam a adoção de medidas eficazes para implementação de protocolos específicos regionais para evitar a expansão territorial da doença e alcançar a identificação precoce de novos casos. A negligência por parte do poder público também é um fator que vem propiciando o agravamento da situação da enfermidade principalmente na população em vulnerabilidade. A reemergência da leishmaniose vem ocorrendo a nível mundial, com a perfeita adaptação do vetor ao ambiente urbano e quase nada ou nada sendo feito para combater essa questão. Os resultados obtidos nesse trabalho, além de evidenciar a grande variedade de espécies mamíferas infectadas com *Leishmania* sp., demonstraram que diversos animais podem atuar como hospedeiros e/ou reservatórios sem a presença de sinais clínicos, no peri-domicílio ou intradomicílio e no ambiente silvestre, em todas as regiões brasileiras, favorecendo a sobrevivência e infecção do vetor, colocando em risco a população humana e animal. Além disso, a detecção de equinos, animais de produção como suínos e bovinos e felinos domésticos infectados com protozoários causadores de LV e LT é relevante do ponto de vista epidemiológico e requer mais estudos para que se defina o papel de reservatórios. As espécies animais infectadas identificadas neste estudo além de servirem como fonte de alimentação para o vetor próximo das residências humanas, podem auxiliar como biomarcadores de uma região, para que os dirigentes de saúde possam aplicar medidas de prevenção e controle mais eficazes.

A manutenção do ciclo de *Leishmania* sp. se dá através de multi-hospedeiros, portanto consegue manter seu ciclo de vida tanto em animais domésticos como silvestres. Nosso estudo identificou pela primeira vez, quirópteros provenientes de dois municípios do RS, Canoas e Rio Grande, infectados por *Leismania* (*L*.) *infantum* e *Leishmania* spp.. Os morcegos identificados com o DNA do protozoário não podem ser caracterizados como reservatórios, mas podem ser um indicativo de que esses animais estão compartilhando o mesmo ambiente com vetores infectados no estado e podem estar participando do ciclo desta enfermidade. Os animais usualmente são coletados para verificação da presença da raiva urbana. Portanto, deve-se considerar o emprego para o monitoramento da presença de *Leishmania* sp em uma região, sem prejuízo para as comunidades dos morcegos e sem a necessidade de capturas específicas. Além disso, os morcegos insetívoros também poderiam auxiliar no combate ao vetor, realizando um

controle biológico do ciclo desse protozoário. Assim é almejado que os dados obtidos com esse presente estudo, sirvam para impulsionar novos estudos que estabeleçam o verdadeiro papel das diferentes espécies animais no ciclo epidemiológico do protozoário *Leishmania* sp., não somente a identificação da infecção, mas a possibilidade de manutenção do agente, replicação e capacidade de infectar o vetor, para que se estabeleçam estratégias eficazes de controle e principalmente prevenção da expansão da enfermidade para novas áreas.

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