UNIVERSIDADE FEDERAL DE CAMPINA GRANDE
CENTRO DE SAÚDE E TECNOLOGIA RURAL
UNIDADE ACADÊMICA DE MEDICINA VETERINÁRIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E SAÚDE ANIMAL

Laysa Freire Franco e Silva

Leishmaniose felina: revisão sistemática com meta-análise, aspectos clínico-epidemiológicos e de diagnóstico

Patos/PB
2022

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Tese submetida ao Programa de Pós-Graduação em Ciência e Saúde Animal, da Universidade Federal de Campina Grande, como requisito parcial para obtenção do grau de Doutora em Ciência e Saúde Animal.

Profª. Drª. Marcia Almeida de Melo

Patos/PB
2022

S586l Silva, Laysa Freire Franco e.
Leishmaniose felina: revisão sistemática com meta-análise, aspectos clínico-epidemiológicos e de diagnóstico / Laysa Freire Franco e Silva. – Patos, 2022.
85 f. : il. color.

Tese (Doutorado em Ciência e Saúde Animal) – Universidade Federal de Campina Grande, Centro de Saúde e Tecnologia Rural, 2022.
"Orientação: Profa. Dra. Marcia Almeida de Melo".
Referências.

1. Epidemiologia. 2. Gatos. 3. Leishmaniose Felina – Sinais Clínicos – Diagnóstico. 4. *Leishmania spp*. 5. Zoonose. 6. Controle das Doenças de Animais. I. Melo, Marcia Almeida de. II. Título.

CDU 614.449:636.09:636.8(043)

FICHA CATALOGRÁFICA ELABORADA PELA BIBLIOTECÁRIA SEVERINA SUELI DA SILVA OLIVEIRA CRB-15/225

MINISTÉRIO DA EDUCAÇÃO

UNIVERSIDADE FEDERAL DE CAMPINA GRANDE

PÓS-GRADUACÃO EM CIÊNCIA E SAÚDE ANIMAL

Rua Aprígio Veloso, 882, - Bairro Universitário, Campina Grande/PB, CEP 58429-900

FOLHA DE ASSINATURA PARA TESES E DISSERTAÇÕES

LAYSA FREIRE FRANCO E SILVA

LEISHMANIOSE FELINA: REVISÃO SISTEMATICA COM METANÁLISE, ASPECTOS CLÍNICO- EPIDEMIOLÓGICOS E DE DIAGNÓSTICO

Tese apresentada ao Programa de Pós-Graduação em Ciência e Saúde Animal como pré-requisito para obtenção do título de Doutora em Ciência e Saúde Animal.

Aprovada em: 31/08/2022

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Ao meu anjo forte e amoroso, minha abuelita.

Dedico

AGRADECIMENTOS

Os verdadeiros primeiro e último capítulos dessa tese não possuem dados estatísticos e respostas para comunidade científica e sociedade. Eles contêm sonhos, lutas, vontades perdidas pelo caminho e um amor e saudade que dilaceram o peito.

Quero, de antemão, pedir desculpas aos religiosos mais fervorosos, mas meu primeiro agradecimento não vai para Deus (com a plena certeza de que Ele compreende tudo que passa aqui dentro) e, sim, para minha abuelita, Lourdes Brito, o amor da minha vida.

Mas, antes, preciso contextualizar um pouco...

O primeiro ano de doutorado foi um ano tão difícil, repleto de rejeições e de uma sensação de fracasso que eu nunca havia sentido antes e que, infelizmente, perdurou durante os anos seguintes. Mas eu tinha um alívio, uma força que era enviada para mim todos os dias, nas primeiras horas da manhã (antes mesmo que eu tivesse acordado), vindos de uma esquina humilde e de uma senhora de coração ainda mais simples, descalça, debaixo da árvore de casa (ou, como sempre dizíamos: debaixo do pé de pau) rezando, incansavelmente, por mim. Bom, do que mais eu iria precisar para enfrentar o dia? Mais nada.

E assim eu fui seguindo, assim eu fui fazendo o que tinha de ser feito ao longo dessa trajetória tão dura. Acontece que minha fonte de força foi embora no finalzinho dessa caminhada e com ela se foi também a minha fé de que tudo daria certo. Mas, deu, estou aqui na linha de chegada com a capacidade de ver mais claramente que a reza dela é forte, que o zelo dela por mim ainda me alcança. Não mais debaixo do pé de pau, naquela esquina humilde, mas lá de cima, para sempre.

Obrigada por acreditar em mim, Vó, obrigada por me fazer acreditar, por ser você além e acima de tudo, obrigada por permanecer. Eu te amo, abuelita, sempre e sempre e sempre e sempre!

Agradeço aos meus pais, Maria do Socorro e Cícero Antônio, como eu agradeço! Vocês me ensinam tanto. Como é bonita a força das atitudes, vocês não falam, simplesmente são e fazem, vocês agem, e eu aprendo. Onde eu estaria sem a senhora, mãe? Como eu entenderia a importância do conhecimento, de aprender, de questionar, de buscar sempre algo, o tempo todo? A senhora me ensinou a ter respeito, a ser leal, me fez entender o poder do merecimento, me fez acreditar nisso e lutar pelo que é meu, de direito. Como a senhora é extraordinária! E tu,

painho? Não há no mundo alguém mais honesto e bondoso que o senhor, me ensinou a ter caráter e humildade, a tentar fazer as coisas com tranquilidade, dando sempre o melhor de mim. Com o senhor, aprendi sobre honestidade, sobre humildade, servidão. Que bonito o senhor é! Que orgulho e sorte de ter vocês por mim!

Aos meus irmãos, Lindsay e Líneker. Vocês foram, antes de mim, em busca desse caminho de conhecimento, foram e não voltaram mais. Continuam seguindo em frente, vencendo os obstáculos que aparecem e me mostrando que eu também posso, que também sou capaz. Ah, eu devo tanto do que me tornei a vocês. Amo muito vocês dois! Preciso agradecer, também, a minha irmãzinha, Lela, que viveu comigo durante 13 anos e, agora, vive dentro de mim! Nunca vou esquecer o seu focinho gelado me acordando toda manhã na hora da escola, às vezes, ainda sinto você aqui. Obrigada por me ensinar a amar os animais. Você sempre vai ser insubstituível para mim, meu amor!

Ao meu marido, Rafael, por todo amor, dedicação, cumplicidade. Obrigada por me amar como você ama, por não ir a lugar algum, por segurar minha mão e não soltar, nunca.

Agradeço aos meus tios e tias, primos e primas, toda a minha família e a família do meu marido, pelo suporte, carinho, acolhimento. Amo todos vocês!

A todos os amigos que a vida colocou nesse caminho difícil da pós-graduação, para me dar apoio e carinho! Vocês são incríveis, agradeço a compreensão, o ombro amigo, os conselhos, as risadas, os dramas rsrs. Em especial a Sóstenes, Luanna e Saul. Obrigada, Arthur, por ter sido como um irmão para mim, especialmente nos últimos tempos, você me trouxe segurança e paz quando precisei. Lulu, obrigada por ter me ouvido nos meus momentos de tristeza, por me apoiar e me fazer rir o tempo todo. Saulzinho, obrigada por toda energia boa e positividade que me passa! Que bom ter vocês na minha vida!

Agradeço aos amigos do BiolMol pelas horas de trabalho e aprendizado, pelo suporte e colaboração. Em especial à Luana que me incentivou e apoiou tanto na etapa final, quando eu mais precisei de ajuda, te agradeço demais; e à Raizza, que bom ter você para me ajudar, aconselhar, apoiar e me servir de exemplo, amiga. Minha gratidão, orgulho e carinho por você só crescem, a cada dia, você foi essencial para que eu chegasse até aqui, muito obrigada!

À minha orientadora, Márcia Almeida de Melo, pela confiança em meu trabalho, pelo incentivo para meu crescimento e por compartilhar seus conhecimentos. Agradeço-te por tudo, Profa!

A todos os docentes e funcionários pela dedicação às suas profissões, pelo auxílio e palavras de conforto, sempre que necessário. Em especial ao professor Fernando Vaz que é a personificação de calmaria na tempestade; obrigada, professor, por sempre me receber com um olhar de ternura e compreensão, por ter me tirado de situações difíceis, em prazos curtíssimos, simplesmente por ter uma generosidade que não cabe no senhor, por ser um exemplo incrível de ser humano e professor. Saiba que eu não teria conseguido sem o senhor.

Por fim, agradeço ao senhor, Deus, o grande maestro de tudo! O maior motivo para eu ter tanto pelo que ser grata. Obrigada por me dar toda força, pessoas e instrumentos dos quais eu precisava para conquistar meus objetivos. Obrigada por me provar todos os dias a força do seu amor e da sua bondade, por me mimar tanto assim e por ser por mim, agora e sempre.

SUMÁRIO

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RESUMO

A leishmaniose visceral (LV) é uma zoonose causada pela *Leishmania infantum*, havendo relatos da participação cada vez mais relevante dos felinos domésticos no ciclo da doença. Diante disso, o presente estudo teve como objetivo avaliar o perfil epidemiológico e identificar as alterações clínicas, hematológicas e bioquímicas em gatos naturalmente infectados por *L. infantum* e avaliar as técnicas de diagnóstico para leishmaniose felina (LF). Para tanto, foi realizada uma revisão sistemática, seguida de meta-análise dos dados quantitativos obtidos sobre a LF no Brasil, além de coleta de sangue de 91 gatos do município de Mãe d'Água e de 427 gatos atendidos no Hospital Veterinário Universitário Professor Doutor Ivon Macedo Tabosa, da Universidade Federal de Campina Grande (HVU/UFCG), situado no município de Patos, Estado da Paraíba, Brasil. Os exames hematológicos, sorológicos (teste rápido DPP® LVC, ELISA S7® e VETLISA Leishmaniose Felina IgG (Bioclin) e moleculares (qPCR), foram realizadas a partir da coleta de sangue venoso. Os animais foram considerados positivos quando reagentes em dois ensaios sorológicos ou quando positivos na qPCR. Os fatores associados à doença foram determinados a partir dos dados levantados no questionário epidemiológico e, para a avaliação da distribuição da doença no município, foi empregado o programa QGIS. As amostras de sangue provenientes dos animais atendidos no HVU/UFCG foram utilizadas para análises hematológicas e bioquímicas e testadas pelo DPP® e ELISA/S7®. Além disso, os dados das fichas de atendimento clínico veterinário foram analisadas para obtenção dos dados gerais dos animais e dos aspectos clínicos sugestivos de LF. Na revisão sistemática, 31 trabalhos foram analisados na íntegra e, na meta-análise, observou-se uma prevalência combinada de 11%, com alta heterogeneidade entre os estudos, que foi atribuída às diferenças nos métodos diagnósticos e no desenho amostral utilizados; não foi detectado viés de publicação e os métodos moleculares se mostraram mais eficazes para o diagnóstico da doença em gatos (I^2 = 57%). Os estudos identificaram o uso potencial da sorologia em levantamentos epidemiológicos da doença em felinos, especialmente em áreas endêmicas. Os artigos avaliados descreveram, predominantemente, sinais clínicos dermatológicos nos gatos infectados e a maioria identificou *L. infantum* como agente etiológico. A prevalência de LF em Mãe d'Água foi de 10,9%, sem identificação de fatores de risco, e os casos se concentraram em uma área de expansão urbana. Os animais infectados apresentaram número de hemácias, concentração de hemoglobina, hematócrito, albumina e ureia inferiores e número de monócitos superior aos animais negativos para *L. infantum*. Houve concordância pobre entre os testes sorológicos e a qPCR; nos animais atendidos no HVU/UFCG a prevalência da doença foi de 2,1%. A maioria dos animais sororreagentes era do sexo masculino, tinha 1 (um) ano de idade e todos apresentavam, pelo menos, um sinal clínico sugestivo da LF. A eritrocitose e trombocitopenia acometeram 28,6% dos animais positivos. O leucograma apresentou, na maioria dos casos, leucocitose por neutrofilia. A necessidade de padronização das técnicas de diagnóstico em felinos e da inclusão da LF como diagnóstico diferencial nas doenças em gatos, principalmente as que cursam com sinais dermatológicos, é ratificada.

PALAVRAS-CHAVE: dermatologia, diagnóstico, doença negligenciada, felídeos, parasitologia.

ABSTRACT

Visceral leishmaniasis (VL) is a zoonosis caused by *Leishmania infantum*, with increasing reports of the relevant participation of domestic cats in the disease cycle. Therefore, the present study aimed to evaluate the epidemiological profile and identify clinical, hematological and biochemical alterations in cats naturally infected with *L. infantum* and evaluate diagnostic techniques for feline leishmaniasis (FL). For this purpose, a systematic review was carried out, followed by a meta-analysis of the quantitative data obtained on FL in Brazil, in addition to the collection of blood from 91 cats in the municipality of Mãe d'Água and from 427 cats attended at the University Veterinary Hospital Professor Doutor Ivon Macedo Tabosa, at the Federal University of Campina Grande (HVU/UFCG), located in the municipality of Patos, State of Paraíba, Brazil. Hematological, serological (DPP® LVC rapid test, ELISA/S7® and VETLISA Leishmaniasis Feline IgG (Bioclin) and molecular (qPCR) tests were performed from blood samples. Animals were considered positive when either reactive in two serological assays or positive in qPCR.. The factors associated with the disease were determined from the data collected in the epidemiological questionnaire and, to assess the distribution of the disease in the municipality, the QGIS program was used. The samples from the animals attended at the HVU/UFCG were used for hematological and biochemical analyzes and tested by the DPP® and ELISA/S7®. In addition, data from veterinary clinical records were analyzed to obtain general data on the animals and clinical aspects suggestive of FL. In the systematic review, 31 studies were analyzed in full and, in the meta-analysis, a combined prevalence of 11% was observed, with high heterogeneity between the studies, which was attributed to differences in the diagnostic methods and sample design used; no publication bias was detected and molecular methods were more effective for the diagnosis of the disease in cats (I^2 = 57%). Among the articles that used this technique, serology showed potential for epidemiological studies of the disease in felines, especially in endemic areas. The articles evaluated predominantly described clinical dermatological signs in infected cats and most identified *L. infantum* as the etiologic agent. The prevalence of FL in Mãe d'Água was 10.9%, with no identification of risk factors, and the cases were concentrated in an area of urban expansion. Infected animals had lower numbers of red blood cells, hemoglobin concentration, hematocrit, serum albumin and urea, and higher monocyte numbers than the reference values, as well as when compared to negative animals for *L. infantum*. There was poor agreement between serological tests and qPCR; among the animals attended at the HVU/UFCG the prevalence of the disease was 2.1%. Most seroreactive animals were male, 1 (one) year old and all had at least one clinical sign suggestive of FL. Erythrocytosis and thrombocytopenia affected 28.6% of positive animals. The leukogram showed, in most cases, leukocytosis due to neutrophilia. The need for standardization of diagnostic techniques in felines and the inclusion of FL as a differential diagnosis in diseases in cats, especially those with dermatological signs, is ratified.

KEY-WORDS: dermatology, diagnosis, felids, neglected disease, parasitology.

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LISTA DE ABREVIATURAS E SIGLAS

ALB	Albumina
ALT	Alanina Aminotransferase
AST	Aspartato Aminotransferase
BiolMol	Laboratório de Biologia Molecular do Semiárido
CA	Califórnia
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CEUA	Comissão de Ética no Uso de Animais
CHCM	Concentração de hemoglobina corpuscular média
CMPA	Clínica Médica de Pequenos Animais
CRD	Com raça definida
CRE	Creatinina
CSTR/UFCG	Centro de Saúde e Tecnologia Rural/Universidade Federal de Campina Grande
DI	Desvio interquartílico
DP	Desvio padrão
DPP	*Dual path platform*
EDTA-K3	Anticoagulante ácido etilenodiaminotetracético potássico
ELISA	Enzyme Linked Immunosorbent Assay
EUA	Estados Unidos da América
GGT	Gamaglutamiltransferase
GN	Grupo negativo
GNB	Grupo negativo bioquímica
GNH	Grupo negativo hematologia
FAL	Fosfatase Alcalina
FELV	Vírus da leucemia felina
FIV	Vírus da imunodeficiência felina
GP	Grupo positivo
GPB	Grupo positivo bioquímica
GPH	Grupo positivo hematologia

GPS	*Global Position System*
Hb	Concentração de hemoglobina
Ht	Hematócrito
HVU/UFCG	Hospital Veterinário Universitário Prof. Dr. Ivon Macedo Tabosa da Universidade Federal de Campina Grande
L. chagasi	*Leishmania chagasi*
Leuc T.	Leucócitos totais
L. infantum	*Leishmania infantum*
LF	Leishmaniose felina
LV	Leishmaniose visceral
LVC	Leishmaniose visceral canina
MAPA	Ministério da Agricultura Pecuária e Abastecimento
MG	Minas Gerais
MPE	Membro pélvico esquerdo
ND	Não determinado
NI	Não informado
PB	Paraíba
PCR	Reação em cadeia da polimerase
PRISMA	Itens de Relatório Preferenciais para Revisões Sistemáticas e Meta-Análise
qPCR	Reação em cadeia da polimerase em tempo real
Ref	Referencial
RIFI	Reação de imunofluorescência indireta
SRD	Sem raça definida
SIG	Sistemas de Informações Geográficas
SP	São Paulo
spp.	Várias espécies
VCM	Volume corpuscular médio
URE	Ureia
UFCG	Universidade Federal de Campina Grande
Th1	Linfócito T auxiliar 1
M-CSF	Fator estimulador de colônia de macrófagos

LISTA DE SÍMBOLOS

%	Percentual
<	Menor que
>	Maior que
≤	Menor ou igual que
=	Igualdade
ºC	Graus Celsius
g/dL	Grama por decilitro
Nº	Número
m²	Metro quadrado
mg/dL	Miligrama por decilitro
mm	Milímetros
mL	Mililitro
Rpm	Rotações por minuto
U/L	Unidade por litro

INTRODUÇÃO GERAL

A leishmaniose visceral (LV) é uma zoonose sistêmica crônica, muitas vezes fatal, de grande impacto na Saúde Pública e ampla distribuição geográfica, ocorrendo na Europa, Oriente Médio, África, Ásia e Américas (BANETH et al., 2008; WHO, 2010; BRASIL, 2014). O agente causador da LV é *Leishmania infantum*, um protozoário que acomete o sistema fagocítico mononuclear de mamíferos e é transmitido, principalmente, através do repasto sanguíneo de insetos flebotomíneos infectados (MELO, 2004; WHO, 2010).

Nos últimos anos, estudos têm relatado a infecção em gatos domésticos o que tem gerado hipóteses sobre uma possível participação desta espécie no ciclo epidemiológico da doença em áreas endêmicas, atuando como fonte alternativa de infecção para os flebotomíneos (SILVA et al., 2010; MAIA; CAMPINO, 2011; VIDES et al., 2011; BATISTA et al., 2020).

A infecção por *L. infantum* em gatos domésticos pode transcorrer com sinais clínicos associados a um padrão sistêmico e/ou dermatológico, sendo este último mais prevalente (LEIVA et al., 2005; MARCOS et al., 2009; VIDES et al., 2011; SILVEIRA-NETO et al., 2015; FERNANDEZ-GALLEGO et al., 2020; SILVA et al., 2020).

Estudos realizados em gatos domésticos demonstram que a resposta imunológica destes animais difere da relatada em cães. Enquanto estes últimos apresentam uma elevada resposta humoral, a resposta nos felinos parece ser predominantemente celular, devido à produção de IFN-γ após exposição ao antígeno da *L. infantum*, característica que pode lhes conferir resistência natural à leishmaniose, com anticorpos específicos anti-*Leishmania* ausentes ou em níveis muito baixos. A baixa produção de anticorpos também pode ser justificada por, quando do surgimento de manifestação clínica, a forma mais comum ser a cutânea (SIMÕES-MATTOS et al., 2005; SOLANO-GALLEGO et al., 2007; MAIA; CAMPINO, 2011; PRIOLO et al., 2019).

Em relação ao diagnóstico, a maioria das técnicas disponíveis para os cães também é empregada em felinos, como as parasitológicas, sorológicas e moleculares (MAIA et al., 2008; PENNISI et al., 2015; CHATZIS et al., 2014ab), porém, divergências na padronização das técnicas sorológicas apontam para dificuldades na aplicação destes métodos, ressaltando a necessidade da validação e padronização de novas técnicas de diagnóstico para os felinos (METZDORF, 2015). Por isso, os métodos parasitológicos e moleculares têm se demonstrado vantajosos, principalmente pela alta especificidade que apresentam (COSTA et al., 2010; MAIA et al., 2010; PENNISI et al., 2015). No entanto, alguns autores alertam para menor

sensibilidade do método parasitológico, sendo importante a associação de técnicas para o diagnóstico da doença (VIDES et al., 2011; CHATZIS et al., 2014b).

Os gatos domésticos podem atuar como fonte de infecção para vetores transmissores de *Leishmania* spp. (SIMÕES-MATTOS, 2005; MAROLI et al., 2007; SILVA et al., 2010; BATISTA et al., 2020; MENDONÇA et al., 2020) e o estreito contato destes animais com cães e seres humanos se configura como um risco adicional para a ocorrência da doença (AKHTARDANESH et al., 2018; BATISTA et al., 2020). No estado da Paraíba foi identificada a infecção por *L. infantum* em gatos domésticos provenientes do município de Sousa; onde os animais apresentavam lesões nodulares em áreas da cabeça e lesões ulceradas nos membros (SILVA et al., 2020).

Os escassos relatos a respeito dos aspectos clínico-patológicos e epidemiológicos da doença nos felinos dificultam o entendimento sobre o verdadeiro papel destes animais no ciclo da LV e a identificação das melhores condutas para diagnóstico e tratamento dos gatos infectados. Diante disso, o objetivo da presente tese foi avaliar o perfil epidemiológico da leishmaniose felina (LF), além de identificar as alterações clínicas e laboratoriais nos gatos naturalmente infectados com *L. infantum*. Para tanto, o corpo estrutural do trabalho foi divido em três capítulos, que são descritos a seguir.

O **capítulo I** consistiu em uma revisão sistemática da literatura sobre a LF, com posterior meta-análise dos dados quantitativos disponíveis em artigos de periódicos indexados. A revisão objetivou reunir informações sobre a prevalência da doença, os tipos de diagnósticos empregados, manifestações clínicas dos animais e características individuais dos gatos que pudessem exercer influência na epidemiologia da LF.

O **capítulo II** consistiu em um estudo da LF no município paraibano de Mãe D'Água, localizado na região metropolitana de Patos. O objetivo do trabalho foi avaliar a prevalência, distribuição espacial e fatores de risco associados à doença em felinos domésticos, além de avaliar alterações clínicas/laboratoriais nos gatos infectados e o desempenho das técnicas sorológicas utilizadas no diagnóstico da LVC (DPP® [*Dual Path Platform*] e ELISA/S7®), do VETLISA Leishmaniose Felina IgG (Bioclin) e da qPCR para a detecção da doença em gatos.

No **capítulo III** foi avaliada a ocorrência da LF em animais atendidos no Hospital Veterinário Professor Doutor Ivon Macedo Tabosa, da Universidade Federal de Campina Grande (UFCG), situado no município de Patos, semiárido paraibano, visando estimar a prevalência da doença na região e identificar características clínicas e laboratoriais nos animais com LF, auxiliando, assim, no diagnóstico e tratamento mais adequado dos animais infectados.

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CAPÍTULO I:

Leishmaniose felina no Brasil: uma revisão sistemática com meta-análise

Trabalho a ser submetido à Revista Brasileira de Parasitologia Veterinária (ISSN: 1984-2961) Jaboticabal /SP, Qualis A2.

Feline leishmaniasis in Brazil: a systematic review with meta-analysis

Leishmaniose felina no Brasil: uma revisão sistemática com meta-análise

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ABSTRACT

Robust and substantiated information on feline leishmaniasis (FL) remains scarce. In view of this, the aim of the present study was to carry out a systematic review with meta-analysis on the occurrence of leishmaniasis in felines in Brazil. Therefore, 31 studies were analyzed in full (31 qualitatively and 23 quantitatively). Through the meta-analysis, a combined prevalence of 11% was observed, with high heterogeneity between studies attributed to differences in diagnostic methods and in the sample design used, since publication bias was not detected. Research has predominantly described dermatological clinical signs in infected cats, but alterations such as lymphadenomegaly, weight loss, ocular alterations, gingivitis and dehydration have also been reported. In 19 out of 31 studies, *L. infantum* was identified as the etiologic agent of FL. Molecular methods proved to be more effective for the diagnosis of the disease in cats (I^2=57%), however, serological methods have potential for epidemiological studies of FL, especially in endemic areas. The need for standardization of diagnostic techniques for felines and the inclusion of FL as a differential diagnosis in diseases in cats, especially those with dermatological signs, is confirmed.

Keywords: Cats, clinical signs, diagnosis, epidemiology, *Leishmania* spp.

RESUMO

Informações fundamentadas e robustas sobre a leishmaniose felina (LF) continuam escassas. Diante disto, o objetivo do presente estudo foi realizar uma revisão sistemática com meta-análise sobre a ocorrência de leishmaniose em felinos no Brasil. Para tanto, 31 trabalhos foram analisados na íntegra (31 de forma qualitativa e 23 de forma quantitativa). Através da meta-análise, observou-se uma prevalência combinada de 11%, com alta heterogeneidade entre os

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estudos atribuída às diferenças nos métodos diagnósticos e no desenho amostral utilizados, visto que viés de publicação não foi detectado. As pesquisas descreveram, predominantemente, sinais clínicos dermatológicos nos gatos infectados, mas alterações como linfadenomegalia, emagrecimento, alterações oculares, gengivite e desidratação também foram relatadas. Em 19, dos 31 estudos, *L. infantum* foi identificada como agente etiológico da LF. Os métodos moleculares se mostraram mais eficazes para o diagnóstico da doença em gatos (I^2=57%), porém, métodos sorológicos apresentam potencial para estudos epidemiológicos da LF, especialmente em áreas endêmicas. Ratifica-se a necessidade de padronização das técnicas de diagnóstico para felinos e da inclusão da LF como diagnóstico diferencial nas doenças em gatos, principalmente as que cursam com sinais dermatológicos.

Palavras-chave: Diagnóstico, epidemiologia, gatos, *Leishmania* spp., sinais clínicos.

INTRODUCTION

Visceral leishmaniasis (VL) is a systemic and chronic infectious-parasitic zoonosis caused by protozoa of the genus *Leishmania* that affect the mononuclear phagocytic system of mammals and is transmitted mainly through the blood meal of infected sandfly insects (Melo, 2004; WHO, 2010).

Although, in the domestic cycle of the disease, the dog is considered the main link in the chain of transmission, with reports of its infection in foci of the disease in humans (Melo, 2004; Ursine et al., 2016), the occurrence of leishmaniasis caused by *L. infantum* in cats in Brazil has been increasingly reported (Antunes et al., 2018, Berenguer et al., 2021, Coelho et al., 2010, Pennisi et al., 2015). In addition, the potential of the species to infect *Lutzomyia longipalpis* (Silva et al., 2010), the main vector of the disease in Brazil, and the transmission through sandflies to dogs (Batista et al., 2020), generating hypotheses about the participation of felines in the epidemiological cycle of the disease in endemic areas (Silva et al., 2010; Maia & Campino, 2011; Vides et al., 2011; Batista et al., 2020).

Despite the significant increase in research on the disease in cats in recent years, robust information on feline leishmaniasis (FL) remains scarce. Thus, the present study aims to gather information on the prevalence of the disease in this species, the types of diagnosis used, clinical manifestations of animals and individual characteristics of cats, which may influence the epidemiology of FL.

MATERIAL AND METHODS

Study design

This research consisted of a systematic review of the literature on leishmaniasis in cats infected with *Leishmania infantum*, with subsequent meta-analysis of the quantitative data available in articles from indexed journals, being conducted in accordance with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (Liberati et al., 2009).

Eligibility Criteria

Studies were considered appropriate for data extraction when they had the following inclusion criteria: full articles, brief communications and case reports with information on the prevalence of the disease in this species, clinical manifestations of the animals, individual characteristics of the cats that could influence the epidemiology of FL caused by *L. infantum* (race, sex and age) and the diagnosis of leishmaniasis using at least one of the following techniques: cytology, isolation by culture, serological examination and molecular diagnostic methods.

The exclusion criteria included: studies that were not on the frequency/prevalence/incidence of leishmaniasis caused by either *L. infantum* or *Leishmania* spp. in felines in Brazil, using any of the aforementioned diagnostic methods, or that did not address the clinical manifestations of the disease, and literature reviews and books or book chapters (for titles); researchs with laboratory data/experimental tests, abstracts of articles presented in proceedings of symposia or conferences; studies with inappropriate design (unrepresentative sample size, incorrect sampling approach, inappropriate diagnostic method) (for abstracts) or containing confusing text and incomprehensible analyzes (for full-text researchs).

Information sources and search strategies

Considering the pre-established inclusion and exclusion criteria, the search was carried out in the following electronic databases: PubMed, Scopus, Web of Science, Science Direct and Scielo using combinations of the keywords: feline OR cat AND leishmaniasis OR *Leishmania* AND Brazil.

Study selection and data extraction

The data obtained were saved in BibTex format and exported to Mendeley's bibliographic manager, with subsequent rigorous removal of duplicates, followed by screening

of titles and abstracts. The screening and selection of studies was performed by two researchers and differences between them were resolved by discussion and consensus.

After this step, the studies considered eligible had their text analyzed in full and the data recorded in an Excel spreadsheet. For the qualitative evaluation of the data from the articles, the following information was used: name of the authors, year of publication, area of study, clinical manifestations, particularities of the animals (race, sex and age), diagnostic method used and identification or not of the species from *Leishmania*. For the analysis of quantitative data, the authors' name, year of publication, study area, sample size and number of positive animals were used.

Data analysis

In the analysis of qualitative data, descriptive statistics were used, in order to characterize the clinical aspects of leishmaniasis in felines and factors that influence the occurrence of the disease. And, based on the different regions evaluated, a distribution map for FL was generated.

For quantitative data, a meta-analysis was performed to assess the prevalence of leishmaniasis in felines, using a 95% confidence interval. A Forest plot was made to visualize the estimated measure of effect of each study and the aggregate effect.

Statistical heterogeneity was determined from visual analysis of Forest plot results and using Cochran's Q statistical test (p-value <0.1). As a complement, the Higgins and Thompson inconsistency test (I^2) was also used.

The meta-analysis was calculated using a random or fixed effects model whether or not heterogeneity was identified, respectively. When necessary, to correct possible sources of heterogeneity, the articles were stratified into subgroups, according to the characteristics of their data. Funnel plot and Egger's statistical test were used to verify the existence of publication bias. All statistical analyzes were performed using RStudio software.

RESULTS

During the data search, 5407 articles were found in the bases used in the research. Of these, 31 met the eligibility criteria and were evaluated by qualitative and/or quantitative analysis. A flowchart with the steps involved in carrying out the systematic review is shown in Figure 1.

In the studies included in the qualitative analysis, 730 were diagnosed with *Leishmania* spp. or *L. infantum* and reports of positive animals occurred in the states of Bahia, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Pará, Paraíba, Paraná, Pernambuco, Rio de Janeiro, Rio Grande do Norte, Santa Catarina, São Paulo and Tocantins. The map presented in Figure 2 demonstrates the distribution of the species found. In the studies in which this information was included, there was a predominance of positive mixed-breed animals (80/82), a slightly higher number of infected females (79/149) and animals aged between 2 months and 12 years. The main clinical signs, when apparent, are shown in Table 1, with a predominance of dermatological alterations.

For the meta-analysis, 23 articles were included, however, as in ten of them different diagnostic techniques were used, the total number of studies counted for the quantitative analysis was 36 (Figure 3).

Analyzing the results of Cochran's I^2 and Q tests, high heterogeneity was observed between the studies ($I^2 = 95\%$, $p<0.01$), thus, a random effects model was used and the studies were divided into subgroups, according to the diagnostic tests used (serological, parasitological or molecular). Even so, heterogeneity was high in the serology ($I^2 = 96\%$) and parasitological ($I^2 = 77\%$) subgroups, and moderate in the molecular methods subgroup ($I^2 = 57\%$). The combined prevalence of FL was 11% (CI: 6-18%). The main characteristics of the studies included in the meta-analysis are shown in Figure 3.

In the funnel plot (Figure 4), an asymmetric distribution between studies was identified, demonstrating the possibility of publication bias, which was discarded by the application of the Egger test ($p = 0.20$).

DISCUSSION

The first report of infection by *L. infantum* in a domestic cat, in Brazil, occurred in the year 2000 (Savani et al., 2004) and, although studies on FL are increasingly frequent, there are still deficiencies regarding robust information on epidemiology, clinical features and the best diagnostic methods used for the species.

Regarding the risk factors associated with the disease, race and age do not seem to influence the occurrence of FL (Coelho et al., 2011; Spada et al., 2013; Bresciani et al., 2010). Sousa et al. (2019) also state that there is no minimum or maximum age for infection and warn of the need to investigate the possibility of vertical transmission.

With regard to the sex of the animals, Sobrinho et al. (2012) demonstrated a statistical association between males and the occurrence of FL, a fact that may be related to male behavior in spending more time in an open environment, increasing exposure to sandflies.

In 13 of the 17 studies in which there was information on clinical signs, dermatological alterations were present (such as skin lesions, ulcers and alopecia), a common feature of infection in domestic cats (Marcos et al., 2009; Pennisi et al., 2013). The other alterations that were commonly found (lymphadenomegaly and weight loss [47.1%; 8/17], ocular alterations [29.4%; 5/17), gingivitis [23.5%; 4/17] and dehydration [17] .6%; 3/17]) demonstrate the potential for more systemic alterations in *Leishmania* spp. in felines and alert to the need for more accurate investigations during the clinical examination of animals, including FL in the differential diagnosis when similar clinical pictures are observed.

Due to the fact that cats have a certain resistance to infection by *Leishmania* spp. because they mount an effective cellular response (Solano-Gallego et al., 2007; Pirajá et al., 2013; Akhtardanesh et al., 2018), the presence of a greater number of asymptomatic animals in relation to animals with some clinical symptom is a finding common (Chatzis et al., 2010; Soares et al., 2015; Akhtardanesh et al., 2017), which justifies the results obtained in several studies in which clinical signs suggestive of FL were not observed in positive animals (Benassi et al. ., 2017; Braga et al., 2014ª; Coelho et al., 2011; Coura et al., 2018; Da Silva et al., 2008; De Morais et al., 2013).

In our study, the global prevalence of leishmaniasis in felines was 11%, but the high heterogeneity of the analyzed studies may have interfered with the results of the combined prevalence. A publication bias would justify this heterogeneity between studies, but it was not detected by the Egger test. Thus, the difference in the methodologies used for the diagnosis of the disease and the variations in the sample number between studies seem to have been responsible for the high heterogeneity observed here.

Worldwide, the prevalence rates of *L. infantum* infection in felines, in serological or molecular studies, range from zero to more than 68.5% (Pennisi et al., 2015). This difference is expected and reflects the divergences in the diagnostic methodologies used, a situation evident in the results of the meta-analysis by subgroups, where the animals showed greater positivity in serology (19%), compared to parasitological (3%) and molecular tests (11%).

Despite the higher percentage of positive animals in the serology (19%), there are studies that indicate that felines have natural resistance to leishmaniasis (Alves et al., 2022;

Pirajá et al., 2013), which leads to a lower production of antibodies in this species (Maia et al., 2010; Solano-Gallego et al., 2007). However, as many of the studies evaluated in the present research used total soluble antigens in the performance of serological techniques (Alves et al., 2022; Alves-Martin et al., 2017; Benassi et al., 2017; Bezerra et al., 2019; Braga et al., 2014ab; Bresciani et al., 2010; Coelho et al., 2011; Costa et al., 2010; Costa-Val et al., 2020; Coura et al., 2018; De Matos et al., 2018; Leonel et al., 2020; Oliveira et al., 2015; Pedrassani et al., 2019; Rocha et al., 2019; Sobrinho et al., 2012; Sousa et al., 2014; Vioti et al., 2021), the occurrence of cross-reaction with other agents cannot be discarded (Braga et al., 2014b; Spada et al., 2013; Costa-Val et al., 2020).

In contrast, Alves et al. (2022) report the possibility that exposed cats develop, simultaneously, a significant cellular response against the infection and humoral, which culminates in a negativity in the molecular and parasitological tests, but allows the detection of antibodies against *Leishmania* spp. Thus, a residual antibody response may indicate only a transient exposure to the parasite (Santos et al., 2021).

Coura et al. (2018) suggest that cats respond to *Leishmania* infection by producing antibodies, even though there are few or no parasites in bone marrow and tissue samples. Thus, serology should be used in epidemiological investigations, especially in endemic areas (Da Silva et al., 2008; Oliveira et al., 2015; Alves-Martin et al., 2017).

Through parasitological techniques, although many animals presented at least one clinical sign suggestive of FL, which could increase the chances of observing amastigotes in the parasitological evaluation (Assis et al., 2010), the percentage of positives in the technique was low. Coura et al. (2018) claim that the feline immune system can eliminate the infection or contain the parasites within an organ, decreasing the levels of parasitaemia. According to Santos et al. (2021), lower tissue parasitism in cats compared to dogs may make the cytological examination less accurate during diagnosis.

Although it has a specificity of 100%, confirmation of infection by parasitological methods can be difficult, since its sensitivity varies according to the degree of parasitism, the way in which the samples are processed and the type of biological material used (Laurenti, 2009), since the parasites are not evenly distributed across the tissues (Costa et al., 2010; Mello et al., 2016), which may also influence the heterogeneity observed in this subgroup.

Reflecting the moderate heterogeneity found in the subgroup of molecular methods, studies employing PCR have, in fact, demonstrated the effectiveness of this method for the diagnosis of infection in felines (Alves-Martin et al., 2017; Benassi et al., 2017; Antunes et al.,

2018), but the need to combine diagnostic methods for the safe identification of positive animals is strongly recommended (Oliveira et al., 2015; Alves-Martin et al., 2017; Santos et al., 2021).

Regarding the identification of the etiologic agent of the disease, *L. infantum* was detected in 19 of the 31 studies evaluated (Table 2). The presence of *L. infantum* in domestic cats, especially in endemic areas for visceral leishmaniasis, highlights the potential role of these animals in the disease cycle, since *Lu. longipalpis* can become infected when taking a blood meal from cats (Silva et al., 2010) and transmit the parasite to dogs (Batista et al., 2020).

CONCLUSION

The high heterogeneity between the studies reflects the differences in the diagnostic methods used and demonstrates the need to standardize these techniques for felines. The presence of dermatological alterations was a frequent finding in studies on FL, emphasizing the importance of including FL as a differential diagnosis in diseases in cats, especially those with dermatological signs.

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Legends of Tables and Figures

Figure 1. Flowchart of the steps of the systematic review on feline leishmaniasis in Brazil.

Figure 2. Choropletic map of the distribution of selected studies on feline leishmaniasis in Brazil.

Table 1. Characteristics of the studies included in the qualitative analysis on feline leishmaniasis in Brazil.

Table 2. *Leishmania* species found in the studies included in the qualitative analysis on feline leishmaniasis in Brazil.

Figure 3. Meta-analysis of studies on feline leishmaniasis in Brazil.

Figure 4. Funnel plot showing the distribution of studies on the prevalence of feline leishmaniasis in Brazil.

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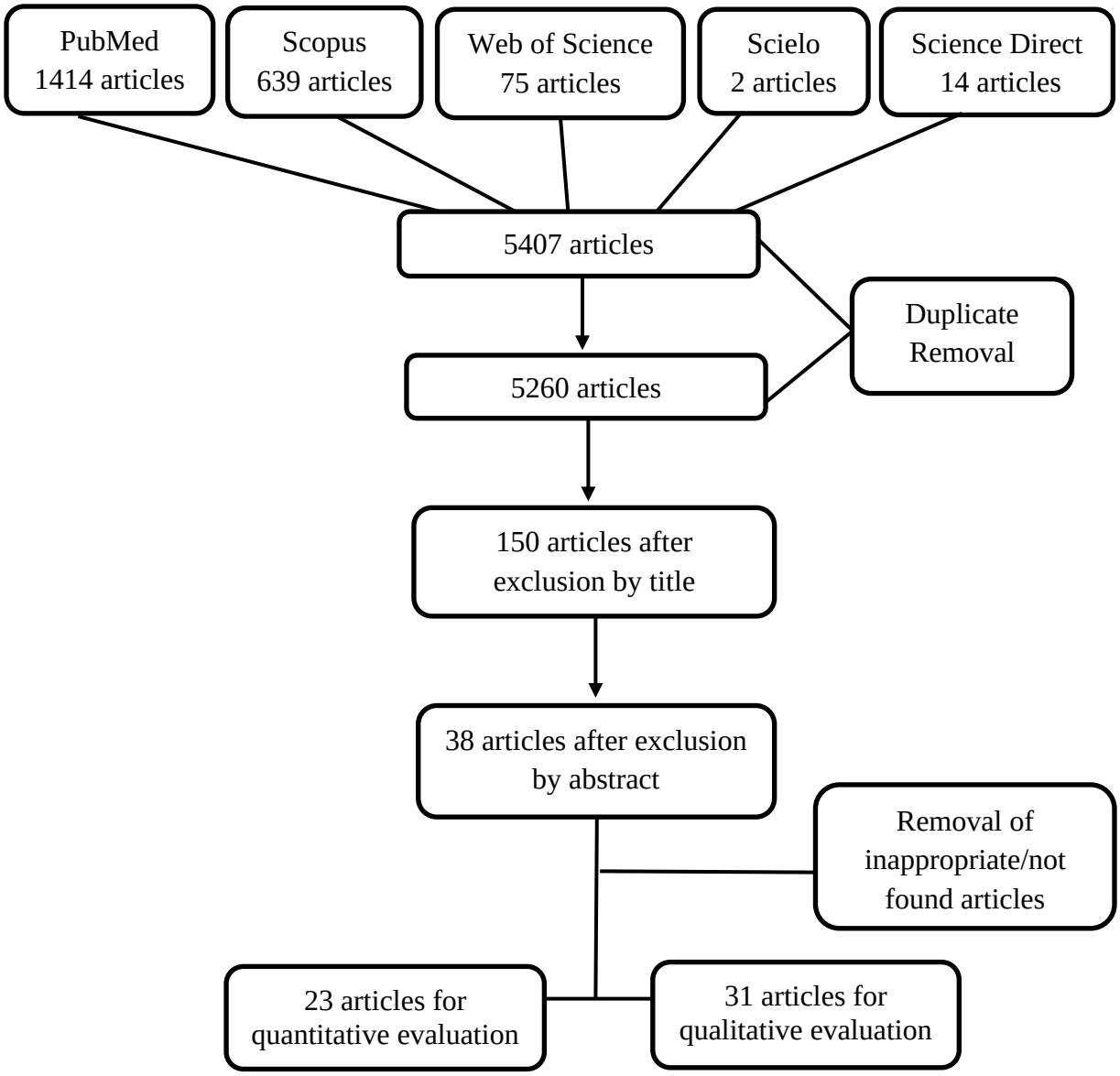


Figure 2. Choropletic map of the distribution of selected studies on feline leishmaniasis in Brazil.

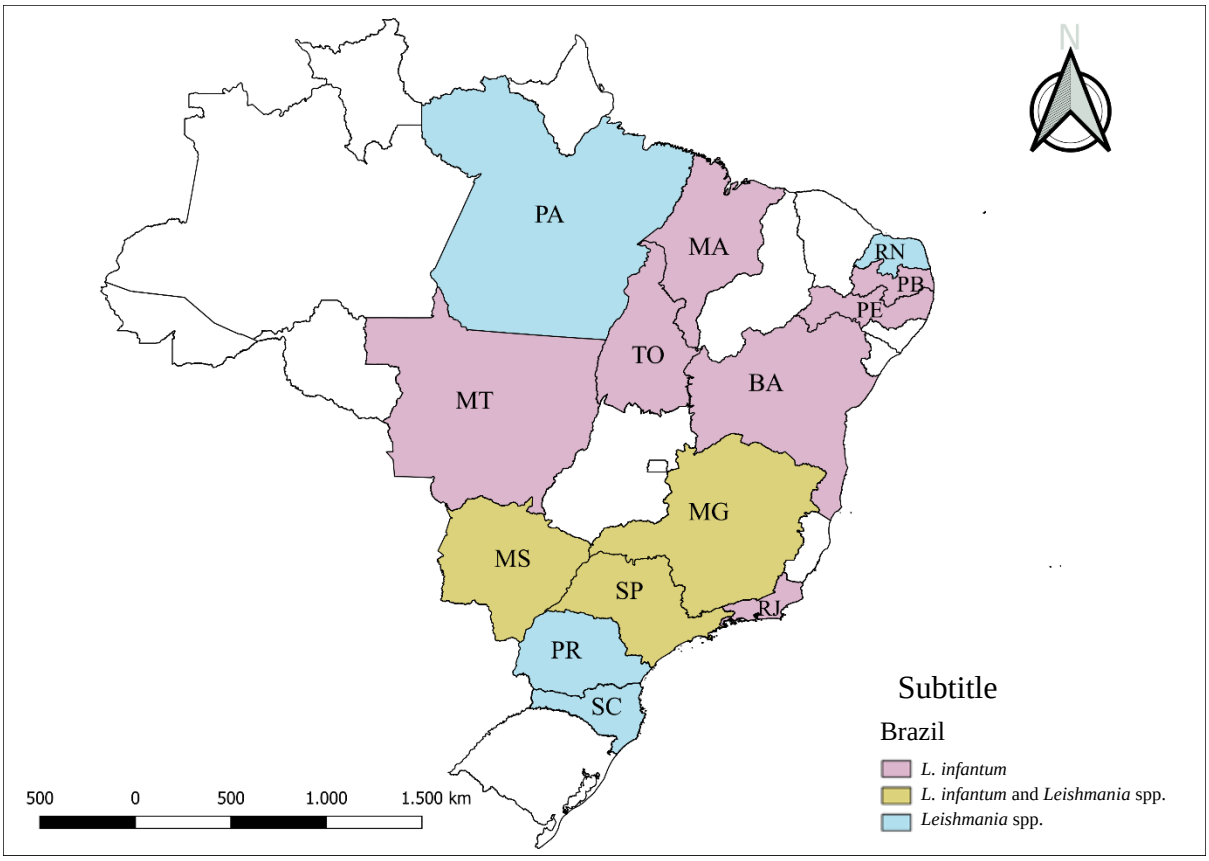


Table 1. Characteristics of the studies included in the qualitative analysis on feline leishmaniasis in Brazil

References		Clinical signs	Diagnosis	
Authors	Study area		Test	Species
Alves et al., 2022	São Paulo	Skin lesions, weight loss, lymph node enlargement, alopecia	IFA, ELISA, PCR and Cytology	*L. infantum*
Alves-Martin et al., 2017	São Paulo	Alopecia, weight loss and skin lesions	IFA, ELISA, PCR and Blood culture	*Leishmania* spp.
Antunes et al., 2018	Mato Grosso do Sul	Presence of a lump near the breast	Cytology and PCR	*L. infantum*
Berenguer et al., 2021	Pernambuco	Lymphadenopathy	Cytology and PCR	*L. infantum*
Bezerra et al., 2019	Rio Grande do Norte	Gingivostomatitis complex, skin lesions, chronic rhinitis, bronchopneumonia, chronic kidney disease, lymphadenomegaly, blepharitis	IFA and PCR	*Leishmania* spp.
Bresciani et al., 2010	São Paulo	Dermatological alterations	Cytology and IFA	*Leishmania* spp.
Costa et al., 2010	São Paulo	Dermatological alterations and hepatosplenomegaly	Cytology and ELISA	*Leishmania* spp.
Madruga et al., 2018	Mato Grosso	Anterior uveitis	PCR, Culture and Histopathology	*L. infantum*
Metzdorf et al., 2017	Mato Grosso do Sul	Alopecia, oral ulcers, gingivostomatitis complex, weight loss, mucopurelent nasal and eye discharge	Cytology and PCR	*L. infantum*
Rocha et al., 2019	Maranhão	Alopecia, thinning hair, lacerations, and ulcerative dermatites	IFA and PCR	*L. infantum*
Santos et al., 2021	Bahia	Skin lesions, alopecia, weight loss, gingivitis, oral ulcers, sneezing, dehydration, and lymphadenopathy	Cytology, DPP® and PCR	*L. infantum*
Savani et al., 2004	São Paulo	Nodular lesion in the nose, weight loss, dehydration and lymphadenopathy	Cytology and PCR	*L. infantum*
Sousa et al., 2019	Tocantins	Diarrhea, pale mucous membranes, and apathy	IFA, PCR and Parasitological	*L. infantum*
Silva et al., 2014	Pernambuco	Skin lesions	ELISA/S7	*L. infantum*
Silva et al., 2020	Paraíba	Skin lesions (nodular and ulcerated), lymphadenopathy, weight loss, dehydration,	Serology, Cytology and PCR	*L. infantum*

		difficulty breathing and damaged coat		
Sobrinho et al., 2012	São Paulo	Lymphadenopathy, weight loss, alopecia and eye discharge	Serology, Cytology and PCR	*L. infantum*
Vioti et al., 2021	São Paulo	Skin lesions, alopecia, weight loss, eye lesions, lymphadenopathy, gingivitis and diarrhea	IFA, ELISA and PCR	*L. infantum*

IFA: Indirect immunofluorescence technique; ELISA: Enzyme-Linked Immunosorbent Assay; PCR: Polymerase Chain Reaction; DPP: Dual-path Platform chromatographic immunoassay

Table 2. *Leishmania* species found in the studies included in the qualitative analysis on feline leishmaniasis in Brazil.

Authors	Study area	Species
Alves et al., 2022	São Paulo	*L. infantum*
Alves-Martin et al., 2017	São Paulo	*Leishmania* spp.
Antunes et al., 2018	Mato Grosso do Sul	*L. infantum*
Benassi et al., 2017	São Paulo	*L. infantum*
Berenguer et al., 2021	Pernambuco	*L. infantum*
Bezerra et al., 2019	Rio Grande do Norte	*Leishmania* spp.
Braga et al., 2014a	Mato Grosso do Sul	*Leishmania* spp.
Braga et al., 2014b	Mato Grosso do Sul e São Paulo	*Leishmania* spp.
Bresciani et al., 2010	São Paulo	*Leishmania* spp.
Coelho et al., 2010	São Paulo	*L. infantum*
Coelho et al., 2011	São Paulo	*Leishmania* spp.
Costa et al., 2010	São Paulo	*Leishmania* spp.
Costa-Val et al., 2020	Minas Gerais	*L. infantum*
Coura et al., 2018	Minas Gerais	*Leishmania* spp.
Da Silva et al., 2008	Rio de Janeiro	*L. infantum*
De Matos et al., 2018	Paraná	*Leishmania* spp.
De Morais et al., 2013	Pernambuco	*L. infantum*
Leonel et al., 2020	São Paulo	*Leishmania* spp.
Madruga et al., 2018	Mato Grosso	*L. infantum*
Metzdorf et al., 2017	Mato Grosso do Sul	*L. infantum*
Oliveira et al., 2015	Pará	*Leishmania* spp.

Pedrassani et al., 2019	Santa Catarina	*Leishmania* spp.
Rocha et al., 2019	Maranhão	*L. infantum*
Santos et al., 2021	Bahia	*L. infantum*
Savani et al., 2004	São Paulo	*L. infantum*
Sousa et al., 2014	Mato Grosso do Sul	*L. infantum*
Sousa et al., 2019	Tocantins	*L. infantum*
Silva et al., 2014	Pernambuco	*L. infantum*
Silva et al., 2020	Paraíba	*L. infantum*
Sobrinho et al., 2012	São Paulo	*L. infantum*
Vioti et al., 2021	São Paulo	*L. infantum*

Figure 3. Meta-analysis of studies on feline leishmaniasis in Brazil.

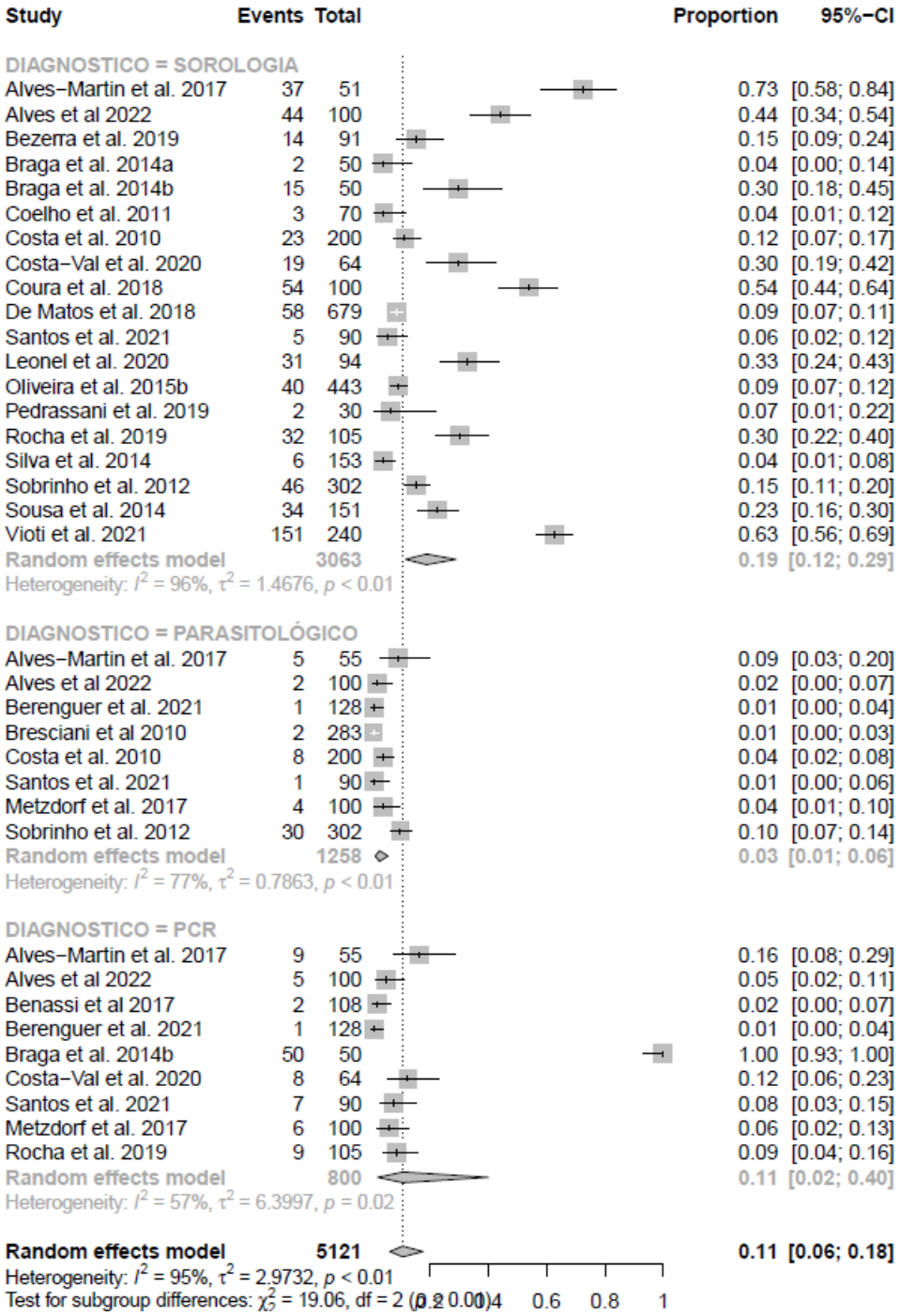
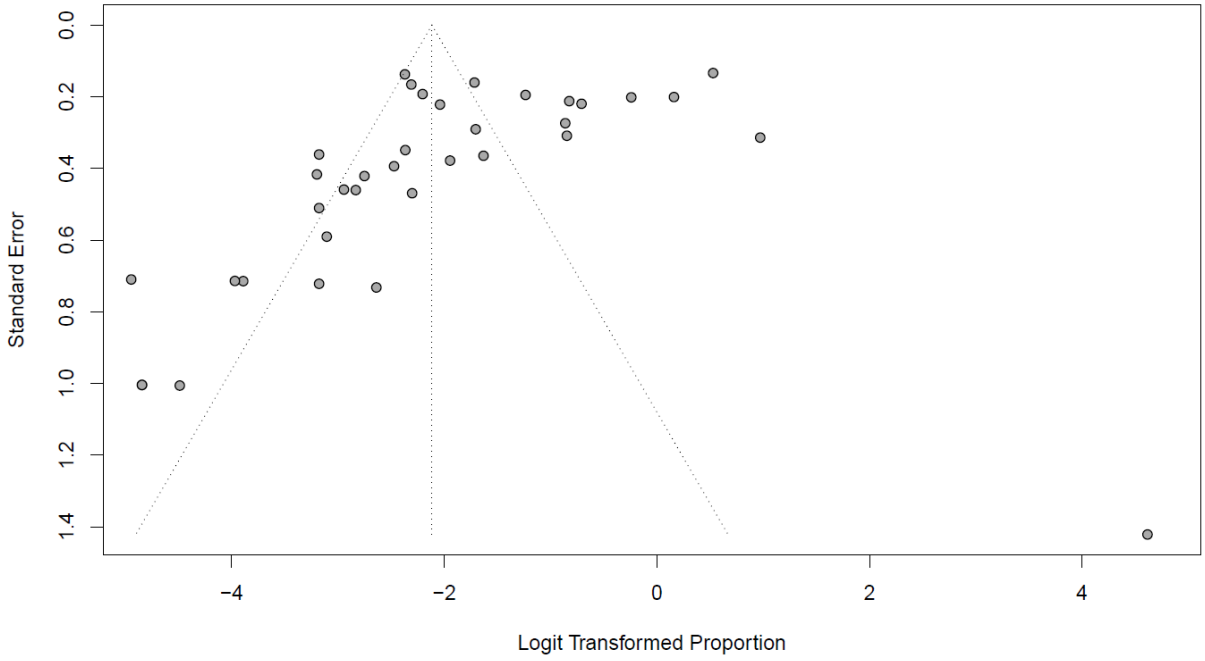


Figure 4. Funnel plot showing the distribution of studies on the prevalence of feline leishmaniasis in Brazil.



CAPÍTULO II:

Aspectos epidemiológicos e alterações hematológicas e bioquímicas em gatos naturalmente infectados com *Leishmania infantum* no Nordeste brasileiro

Trabalho submetido à Revista Brasileira de Parasitologia Veterinária (ISSN: 1984-2961) Jaboticabal /SP, Qualis A2.

Epidemiological aspects and hematological and biochemical alterations in cats naturally infected with *Leishmania infantum* in Northeast Brazil

Aspectos epidemiológicos e alterações hematológicas e bioquímicas em gatos naturalmente infectados com *Leishmania infantum* no Nordeste brasileiro

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ABSTRACT

Visceral leishmaniasis (VL) is a zoonosis of great impact on Public Health and, in endemic regions, infection in felines has been reported. The aim of this research was to investigate the epidemiological, hematological and biochemical profile of feline leishmaniasis (FL) in the municipality of Mãe d'Água and to evaluate diagnostic tests for FL. Between 2021 and 2022, 91 blood samples were collected from cats in the municipality. The prevalence of FL was determined using DPP®, ELISA/S7®, VETLISA Feline Leishmaniasis IgG (Bioclin) and qPCR. Risk factors were determined from data obtained from the epidemiological questionnaire. Spatial analysis was performed in the QGIS program. The prevalence of FL was 10.9%, no risk factors were identified and the cases were concentrated in an urban sprawl area of the city. The infected animals had lower numbers of red blood cells, hemoglobin concentration, hematocrit, albumin and urea and a higher number of monocytes than the negative animals. There was poor agreement between serological tests and qPCR. The data obtained demonstrate the distribution of FL throughout the municipality, however presenting areas with higher concentrations, suggesting the implementation of control measures that prioritize them. Continuous efforts should be made to standardize diagnostic techniques for FL.

Keywords: Animal pathology, felides, georeferencing, neglected disease, One Health.

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RESUMO

A leishmaniose visceral (LV) é uma zoonose de grande impacto na Saúde Pública e, em regiões endêmicas, a infecção em felinos tem sido relatada. O objetivo desta pesquisa foi investigar o perfil epidemiológico, hematológico e bioquímico da leishmaniose felina (LF) no município de Mãe d'Água e avaliar testes de diagnóstico para LF. Entre 2021 e 2022, foram coletadas 91 amostras de sangue em gatos do município. A prevalência de LF foi determinada através de DPP®, ELISA/S7®, VETLISA Leishmaniose Felina IgG (Bioclin) e qPCR. Fatores de risco foram determinados através dos dados obtidos no questionário epidemiológico. Análise espacial foi realizada no programa QGIS. A prevalência de LF foi de 10,9%, nenhum fator de risco foi identificado e os casos se concentraram em uma área de expansão urbana da cidade. Os animais infectados apresentaram número de hemácias, concentração de hemoglobina, hematócrito, albumina e ureia inferiores e número de monócitos superior aos animais negativos. Houve concordância pobre entre testes sorológicos e qPCR. Os dados obtidos demonstram a distribuição da LF em todo município, porém apresentando áreas com maiores concentrações, sendo sugerida a realização de medidas de controle que as priorizem. Esforços contínuos devem ser empregados para padronização de técnicas de diagnóstico para LF.

Palavras-chave: Doença negligenciada, felídeos, georreferenciamento, patologia animal, Saúde Única.

INTRODUCTION

Visceral leishmaniasis is a zoonosis of great importance for public health and has a wide geographic distribution (Baneth et al., 2008; WHO, 2010; Brazil, 2014). In felines, the prevalence rates of infection by *L. infantum*, in serological or molecular studies, range from zero to 68.5% (Pennisi et al., 2015), with a previous report of infection by *L. infantum* in domestic cats in the municipality of Sousa, in the state of Paraíba, northeast region of Brazil (Silva et al., 2020).

Most of the diagnostic techniques available for dogs are also used in felines, such as parasitological, serological and molecular ones (Maia et al., 2008; Pennisi et al., 2015; Chatzis et al., 2014ab), however, divergences in the standardization of serological techniques point to difficulties in the application of these methods, highlighting the need for validation and standardization of new diagnostic techniques for felines (Metzdorf, 2015).

The ability of domestic cats to act as a source of infection for vectors transmitting *L. infantum* has already been reported (Silva et al., 2010) and the lack of early diagnosis of FL in endemic areas represents a potential risk of transmission of the disease to others mammals, including man (Alves-Martin et al., 2017; Akhtardanesh et al., 2018; Batista et al., 2020).

Therefore, the aim of this research was to investigate the epidemiological, hematological and biochemical profile of FL in the municipality of Mãe d'Água, an endemic area for VL, as well as to evaluate diagnostic tests for FL.

MATERIAL AND METHODS

The methodological protocols adopted in this research were approved by the Ethics Committee on the Use of Animals (CEUA) of the Universidade Federal de Campina Grande (UFCG), under protocol number 07/2021 and signature by the tutors of the Free and Informed Consent Term (TCLE).

The study was carried out in the urban area of the municipality of Mãe d'Água (latitude: 7°15'25" South, longitude: 37°25' 40" West), located in the intermediate and immediate geographic region of Patos (Fig. 1). The sample size was calculated considering the number of cats (113) counted in the urban area of Mãe d'Água and made available by the Municipal Health Department, a confidence level of 95% and a sampling error of 5% (Thursfield, 2007), which resulted in a sample population of at least 88 cats.

At the time of collection of biological material, the clinical alterations observed in the animals were recorded and the tutors answered an epidemiological questionnaire in order to verify socio-environmental factors that could act as possible risk factors for the disease under study (Table 1).

To obtain blood, cephalic venipuncture was performed with the aid of a 5mL syringe and 25x8mm needles, sterile and for individual use. The collected blood was, immediately, transferred to tubes with the anticoagulant ethylenediaminetetraacetic acid potassium (EDTA-K - Vacutte do Brasil Ltda) and tubes containing clot separator gel, stored under refrigeration and sent to the Laboratory of Molecular Biology of the Semiarid Region (UFCG - Patos/ BP).

For serum separation, blood collected without anticoagulant was centrifuged at 2000 rotations per minute (rpm) for 5 minutes, stored in 1.5mL microtubes, identified and stored at -20ºC until serological and biochemical assays were performed. The blood collected with EDTA-K3 was stored in 1.5mL microtubes, identified and sent to the Clinical Pathology Laboratory (UFCG - Patos/PB) for a hemogram and, later, stored at -20ºC until the molecular assays were carried out.

Hemogram and biochemistry analyzes were performed at the Clinical Pathology Laboratory (UFCG – Patos/PB). For the hemogram, an automated and hematoscopic evaluation was performed. In the first analysis, an electrical impedance hematology counter (pocH-100 iV

Diff, Sysmex) was used to obtain the total red blood cell and leukocyte count, hemoglobin concentration, hematocrit, mean corpuscular volume and mean corpuscular hemoglobin concentration. For the blood smears, a kit of dyes was used for rapid differential staining in hematology (Instant Prov from New Prov, Produtos para Laboratório Ltda., Pinhais – PR), proceeding to the differential count of leukocytes and evaluation of morphotintorial characteristics of erythrocytes, leukocytes and platelets, which are quantified and qualified in terms of shape, size and presence of parasites. Biochemical analyzes (urea, creatinine, alanine aminotransferase, aspartate aminotransferase, gammaglutamyltransferase, alkaline phosphatase, albumin and total protein) were performed using a colorimetric kinetic process in an automatic analyzer (Cobas C 111, Roche) using commercial kits. The reference values for species were those described by Kaneko et al. (2008) and Jain (1986).

Serological and molecular tests were performed at the Laboratory of Molecular Biology of the Semiarid Region (UFCG – Patos/PB). The serum of the sampled animals was used to perform the DPP® (Dual Path Platform - Chembio Diagnostic Systems, INC.), ELISA/S7® (Biogene Indústria e Comércio Ltda.) and VETLISA Feline Leishmaniasis IgG assays (Bioclin - Quibasa - Química Básica Ltda.) according to the manufacturer's instructions. The quantitative PCR (qPCR) was performed as described by Silva et al. (2016), from the extraction of DNA from 100 μL of blood, using the DNeasy blood and tissue kit (Qiagen®, Hilden, Germany), following the manufacturer's recommendations, and using the Linf kDNA-F primers 5' GGCGTTCTGCAAAATCGGAAA -3', Linf kDNA-R 5' CCGATTTTTGGCATTTTTGGTCGAT-3' and Linf kDNA_FAM-5'-TTTTGAACGGGATTTCTG-3' to amplify the *L. infantum* kinetoplast minicircle gene (kDNA). A culture of *L. infantum* was used as a positive control and ultrapure water as a negative control.

The prevalence of VL was determined from the samples that reacted in the two serological tests commercialized for canine VL, in the VETLISA Feline Leishmaniasis IgG and/or in the qPCR and evaluated through descriptive statistics.

For the statistical analysis of hematological and biochemical alterations in animals with FL, two groups were formed: animals positive for *L. infantum* (GPH [positive hematology group], n = 6, and GPB [positive biochemistry group], n = 5) and animals negative for *L. infantum* in all the techniques used (GNH [negative hematology group], n = 6, and GNB [negative biochemistry group], n = 6), identified in the serological and molecular tests. Statistical analysis was performed using the BioEstat 5.3 program at a 5% significance level

($p<0.05$). The data obtained were initially submitted to the Shapiro-Wilk normality test. Parametric data are presented as mean ± standard deviation and non-parametric data as median ± interquartile deviation. Comparison between groups and data analysis were performed using Student's t test (parametric distribution) or U-Mann-Withney test (nonparametric distribution).

The analysis of possible risk factors associated with the positivity of the animals was carried out in two stages (univariate analysis and multivariate analysis) through the data collected with the questionnaires. Independent variables (possible risk factors) were categorized and coded (Latorre, 2004). The variables that presented a value of $p \leq 0.20$ by the chi-square test or Fisher's exact test (Zar, 1999) were selected and used in the multivariate analysis, using multiple logistic regression (Hosmer & Lemeshow, 2000). The significance level adopted in the multiple analysis was 5%. The analyzes were performed using the SPSS 20.0 for Windows program.

The agreement of the results obtained in the techniques used was evaluated through the Kappa (k) indicator, sensitivity and specificity, with a confidence interval of 95%, through the Dag Stat program (Mackinnon, 2000). Agreement in serological diagnoses was determined according to Thursfield's classification (2007), according to the values of the Kappa index (k), considering the levels of comparison: 0.81 to 0.99: almost perfect agreement; 0.61 to 0.8: substantial agreement; 0.41 to 0.6: moderate agreement; 0.21 to 0.4: reasonable agreement; 0.1 to 0.2: weak agreement; <0: poor agreement. The gold standard was the true status of the animal (ill or not sick) which, in this case, was confirmed by the positivity in qPCR from the blood samples.

For the analysis and spatial representation of the cases of FL in Mãe d'Água, the geographic coordinates were marked, obtaining the location of each participating property through the GPS receiver (Global Position System - Garmin eTrex 30), at the time of collection of biological material. The georeferenced data were entered into the digitized cartographic database of the municipality. The maps and the spatial visualization of FL cases were performed by QGIS (Quantum Geographic Information System), a free and an open-source software.

The analyzes of the spatial distribution of the FL were carried out through a descriptive analysis of the epidemiological situation of the disease in the municipality. A heatmap was made through the quartic function of the Kernel density estimation to observe clusters (hot areas) of cases of the disease, using QGIS software.

RESULTS AND DISCUSSION

For the serological tests, 91 samples were evaluated and for the qPCR, 89 samples were used. The DPP® rapid test resulted in 8 reactive samples (8.8%), the ELISA/S7® test in 4 (4.4%) positive samples, the VETELISA in 1 reactive sample (1.09%) and the qPCR in 8 (9.0%) positive samples. Considering the results, the prevalence of FL in Mãe d'Água was 10.9% (Table 1).

The observed prevalence is close to those found in Araçatuba-São Paulo (14.5%) (Costa et al., 2010), based on parasitological tests and ELISA, and in Mossoró – Rio Grande do Norte (15.38%) (Bezerra et al., 2019), using IFA; but differing from that observed in Pernambuco (3.9%) (Silva et al., 2014), through ELISA/S7®, and in São Luís – Maranhão (30.48%) (Rocha et al., 2019), using IFA.

In addition to the diagnostic techniques used, several factors influence the variation in prevalence observed in different studies, such as the region studied and, also, climatic factors, since the population of sandflies shows seasonal variation, and its dynamics are affected by rainfall and humidity (Cockroach et al., 2004). The scarcity of standardized/commercialized techniques for felines and the fact that cats have a certain natural resistance to leishmaniasis, leading to a lower production of antibodies, may also be associated with the difference in prevalence in endemic areas, due to failure to detect antibodies (Maia et al. al., 2010; Solano-Gallego et al., 2007; Pirajá et al., 2013).

The discrepancies between the results of the serological tests observed in the present study can also be justified by the fact that ELISA assays using protein A conjugated to peroxidase detect more antibodies than those using anti-dog IgG conjugated to peroxidase, being able to detect animals in the acute phase of infection by the reaction with IgM antibodies (Lima et al., 2005).

Comparing the diagnostic techniques, poor agreement ($p<0.01$, kappa=0) was observed between qPCR and serological methods, due to low (ELISA/S7®) or absence (DPP® and VETLISA Feline Leishmaniasis IgG) of simultaneous reaction between tests. Sensitivity and specificity were, respectively, 0% and 90% for DPP®; 25% and 97% for ELISA/S7®; and 0% and 99%, for VETLISA Feline Leishmaniasis IgG.

These results are similar to those observed by Chatzis et al. (2014a), who identified a discrepancy between the results of serological tests (RIFI and ELISA) and PCR, with diagnostic sensitivity considered very low in all serological tests, but with a specificity of 100%.

Akhtardanesh et al. (2017), considering PCR as the gold standard, found sensitivity and specificity of the ELISA method of 44.4% and 100%, respectively, and a slight agreement (Kappa= 0.368) between the diagnostic methods; the authors also suggested the use of ELISA to discard the disease in cases of suspected felines.

In cats experimentally infected with *L. infantum* promastigotes, antibody titers were not significantly elevated during 16 weeks of evaluation, making it impossible to diagnose animals by ELISA (Akhtardanesh et al., 2018). Thus, qPCR positive cats could be in earlier stages of infection where there was not enough antibody production to be detected in the serological tests used, or even positive animals were not able to generate a cellular response intense enough to prevent the replication and maintenance of the parasite (Alves et al., 2022).

Our results suggest the need to combine diagnostic methods for the safe identification of positive animals, as already recommended in different studies (Oliveira et al., 2015; Alves-Martin et al., 2017; Costa-Val et al., 2020; Santos et al., 2021).

The characteristics of the population studied can be seen in Table 2. All positive animals had access to the street, most had short hair length (7/10), with 60% (6/10) males and 40% (4/10) of females.

As reported by Berenguer et al. (2021), even though the animals were considered domiciled, they had free access to outdoor areas, which increases the risk of infection due to the possibility of contact with other animals and with sand fly vectors, and in animals with short-haired dogs, the blood meal by the sand fly is facilitated. Regarding the highest percentage of positive males, Sobrinho et al. (2012) observed a higher risk of infection in males, however, other authors do not consider sex a predisposing factor for FL (Spada et al., 2013; Akhtardanesh et al., 2017).

The analysis of risk factors did not point to any variable associated with FL. Bezerra et al. (2019) also did not observe a statistical association between seropositivity for *Leishmania* spp. and sex, age and presence of suggestive clinical signs. Despite this, Oliveira et al. (2015) demonstrated that younger animals were slightly more likely to test positive than older animals, Sobrinho et al. (2012) demonstrated a higher risk of infection in males and Rocha et al. (2019) identified risk factors such as free access to the streets, cohabitation with dogs previously affected by VL and lack of yard cleaning and garbage collection.

In the hematological evaluation (Table 3), statistically significant differences were observed between the control (n=6) and sick (n=6) groups in the variables: red blood cells,

hemoglobin, hematocrit and monocytes; even though the observed values were within the reference range for the species (Jain, 1986).

In cats infected with *L. infantum*, there may be a reduction in the number of circulating red blood cells, as a consequence of the decrease in erythrocyte precursors in the bone marrow and due to immune-mediated hemolysis secondary to leishmaniasis (Marcos et al., 2009). There are even frequent reports on the occurrence of anemia in cases of FL (Noé et al., 2015; De Mendonça et al., 2017; Sousa et al., 2019).

As *Leishmania* spp lacks the ability to synthesize heme and does not contain cytosolic iron storage proteins, to develop inside macrophages, it needs nutritional iron and heme from the host (Laranjeira-Silva et al., 2020). Even in mice infected with *L. donovani*, increased hemophagocytosis was demonstrated, especially in heavily infected macrophages (Morimoto et al., 2016). *Leishmania* spp. it can also acquire hemoglobin via endocytosis, through the hemoglobin receptor located in its flagellar pouch (Sengupta et al., 1999). Thus, the association of these factors not only interferes with the number of red blood cells, but also with the concentration of hemoglobin in infected animals.

The observation of monocytosis in cats with FL has been demonstrated previously (Sousa et al., 2019). In dogs, macrophage activation is an important mechanism involved in the protective immune response against *Leishmania* spp. (Holzmuller et al., 2005; Zafra et al., 2008).

According to Menezes et al. (2016), the promastigote forms are phagocytosed by macrophages recruited to the sand fly bite site (Menezes et al., 2016). In this case, the efficient Th1 immune response in cats (Day, 2016), may favor the action of macrophages at the site of infection (Holzmuller et al., 2005) and increase the rate of monocyte release from the bone marrow to the bloodstream, due to the release of macrophage colony stimulating factor (M-CSF) (Chitu & Stanley, 2006).

Leishmania spp. was not identified in any of the smears evaluated and, in fact, the observation of amastigote forms in the feline peripheral blood is a rare finding (Antunes et al., 2018).

In the biochemical evaluation (Table 4), statistically significant differences were observed between the groups in the variables: albumin and urea.

Albumin is considered an acute phase protein that has its levels decreased during infectious and/or inflammatory processes (Ceron et al., 2005; Kann et al., 2012). In relation to urea, feeding management with the provision of feed and home-cooked food in most of the

positive animals may justify the findings observed in our study, since diets rich in protein tend to increase serum urea values (Backlund et al., 2011; Ephraim & Jewell, 2021).

In the spatial evaluation, the presence of positive cats was observed in different areas of the municipality of Mãe d'Água, but a cluster was located in the northwest region (Figure 2), in an area of recent urban expansion. In the urban area of the same municipality, in a study on canine VL, clusters of cases of the disease were also identified (Braz et al., 2021) that covered the area identified in the present study and, according to Rocha et al. (2019), cohabitation with dogs previously affected by VL is a risk factor for FL.

The environmental dynamics of the vectors are affected by the deforestation of native vegetation, which facilitates the colonization of the periurban environment and increases the risk of VL transmission (Ximenes et al., 2007; Almeida et al., 2012; Marcondes & Rossi, 2013). In addition, the disorderly occupation of the territory associated with poor sanitation conditions also justifies the increase in the occurrence of the disease in urban areas (Cesse et al., 2001; Teles et al., 2015; Who, 2010).

CONCLUSION

The present research demonstrates the presence of FL in areas of urban expansion in the municipality of Mãe d'Água and, although the prevalence is not considered high, as cats mount a less significant humoral response than dogs, the importance of this species in the life cycle transmission of VL in the region should not be discarded. An area with the highest concentration of cases was identified and should be prioritized for the control of the disease in the municipality, intensifying vector control measures. It is also suggested the development of awareness campaigns in the municipality to alert tutors about ways to prevent the disease and manage positive animals. It is also alerted to the need to standardize FL diagnostic techniques.

ACKNOWLEDGEMENT

To the Coordination for the Improvement of Higher Education Personnel (CAPES) for the scholarship granted and to Bioclin for providing the VETLISA Leishmaniose Felina IgG kits.

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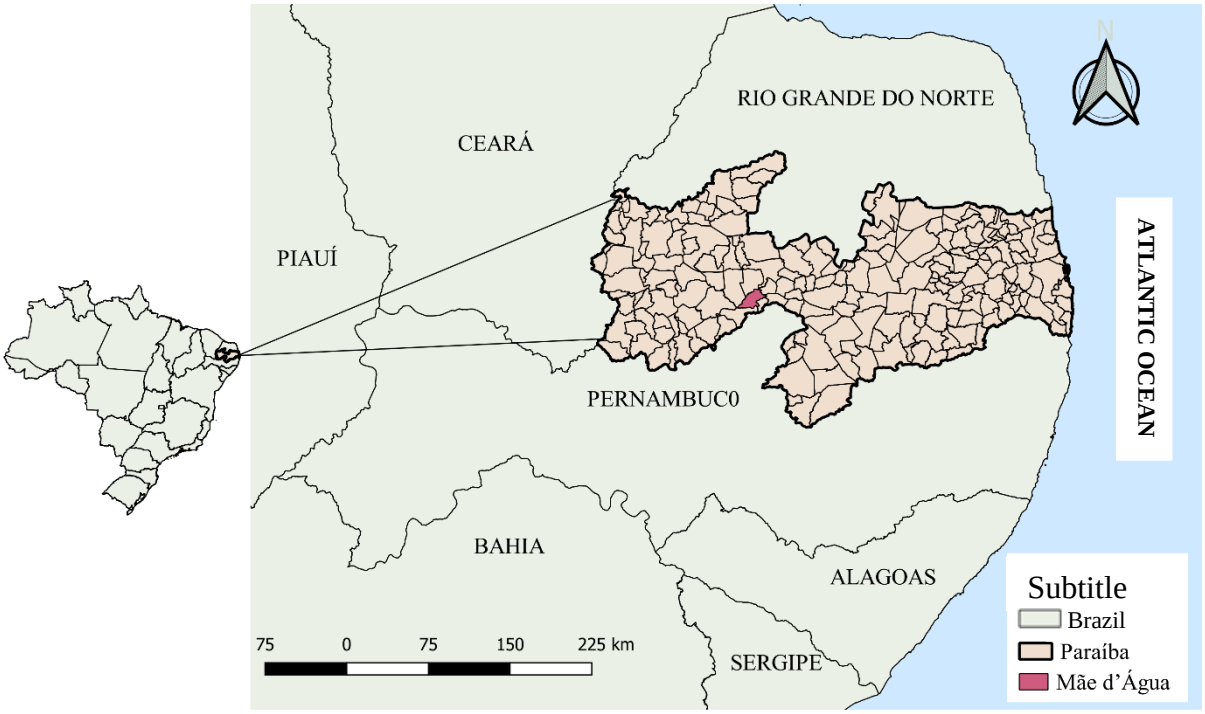


Tabela 1. Prevalence, by diagnostic methods and total, of feline visceral leishmaniasis in the municipality of Mãe d'Água, Paraíba, northeastern Brazil, in the period 2021-2022.

Test	ID of the positive animals	Prevalence per test	Total prevalence
DPP®	32, 35, 44, 50, 73, 77, 82 e 92	8.8%	10.9%
ELISA/S7®	15, 32, 40 e 43	4.4%	
VETLISA	27	1.09%	
qPCR	16, 28, 40, 43, 53, 57, 63 e 76	9.0%	

ID: identification

Tabela 2. Univariate analysis of possible risk factors associated with feline visceral leishmaniasis in Mãe d'Água, Paraíba, northeastern Brazil, 2021-2022.

Variable	Total number of animals	Positive animals (%)	p value
Schooling Degree			
Unlettered	1	0 (0.0%)	
Complete/incomplete elementary school	18	2 (11.1%)	0.939
Completed/incomplete high school	72	8 (11.1%)	
University education	0	0 (0.0%)	
Family income			
Less than 2 minimum wages	89	9 (10.1%)	
2 to 4 minimum wages	2	1 (50.0%)	0.209
5 to 6 minimum wages	0	0 (0.0%)	
More than 6 minimum wages	0	0 (0.0%)	
Sex			
Male	52	6 (11.5%)	1.000
Female	39	4 (10.3%)	
Age (months)			
6 – 24	29	2 (6.9%)	
25 – 72	57	7 (12.3%)	0.604
> 72	5	1 (20.0%)	
Breed			
Defined breed dog	2	0 (0.0%)	1.000
Mongrel dog	89	10 (11.2%)	
Fur color			
White	8	0 (0.0%)	
Black	3	0 (0.0%)	
Grey	3	1 (33.3%)	0.561
Caramel	7	1 (14.3%)	
More than 1 color	70	8 (11.4%)	
Fur length			
Short	65	7 (10.8%)	
Long	26	3 (11.5%)	1.000
Type of confinement			
Resident dogs	9	0 (0.0%)	
Semi-resident dogs	74	9 (12.2%)	0.540
Free-roaming dogs	8	1 (12.5%)	
Food			
Commercial food	25	2 (8.0%)	
Homemade food	19	3 (15.8%)	0.711
Both	47	5 (10.6%)	
Contact with animals			
No	16	2 (12.5%)	1.000
Yes	75	8 (10.7%)	
Contact with felines			
No	21	2 (9.5%)	1.000
Yes	70	8 (11.4%)	
Contact with dogs			

No	42	5 (11.9%)	1.000
Yes	49	5 (10.2%)	
Contact with birds			
No	83	10 (12.0%)	0.591
Yes	8	0 (0.0%)	
Place where the dog is created			
Without pavement	0	0 (0.0%)	
With pavement	20	2 (10.0%)	1.000
Both	71	8 (11.3%)	
Cleanliness of the place			
No	2	0 (0.0%)	1.000
Yes	89	10 (11.2%)	
Cleaning frequency			
None	2	0 (0.0%)	0.500
Daily or weekly	81	10 (12.3%)	
Biweekly or monthly	8	0 (0.0%)	
Deworming			
No	79	10 (12.7%)	0.348
Yes	12	0 (0.0%)	
Vaccination			
No	25	2 (8.0%)	0.721
Yes (anti-rabic)	66	8 (12.1)	
Presence of ticks			
No	88	10 (11.4%)	
Yes	2	0 (0.0%)	0.826
Already had	1	0 (0.0%)	
Always lived with the owner			
No	2	1 (50.0%)	0.209
Yes	89	9 (10.1%)	
Always lived in this place			
No	4	0 (0.0%)	1.000
Yes	87	10 (11.5%)	
Place where sleep			
Inside home	36	4 (11.1%)	
Peridomicile	44	5 (11.4%)	0.977
On the street	11	1 (9.1%)	
Surroundings of the house			
No organic or inorganic matter	1	0 (0.0%)	
Stones	10	2 (20.0%)	
Plants	8	1 (12.5%)	0.863
Stones and plants	70	7 (10.0%)	
Stones, plants and animal husbandry	2	0 (0.0%)	
General appearance of the animal			
Good	80	10 (12.5%)	
Skinny	5	0 (0.0%)	
Skin injury	3	0 (0.0%)	0.819
Hair failure	2	0 (0.0%)	
Pale mucosa	1	0 (0.0%)	

* Variables used in multiple logistic regression.

Tabela 3. Statistical analysis of hematological data from cats in the municipality of Mãe d'Água, Paraíba, northeastern Brazil, infected and not infected by *Leishmania infantum*, in the period 2021-2022.

Hematological Evaluation			
Variables	GNH (N=6)	GPH (N=6)	Referential interval *
RBC ($x10^6$/L)	10.21±1.14°[a]	8.20±1.83•[b]	5-10
Hb (g/dL)	14.20±2.45°[a]	11.90±2.00•[b]	8-15
Ht (%)	41.65±7.43°[a]	33.72±4.49•[b]	24-45
VCM (fL)	41.05±1.48•[a]	42.27±2.63•[a]	39-55
CHCM (g/dL)	33.58±1.00•[a]	33.68±1.30•[a]	31-35
Total LEUK ($X10^3$/uL)	12.91±14.95•[a]	15.81±3.53•[a]	5.50-19.50
Rod Neutrophils ($X10^2$/uL)	0•[a]	0•[a]	0-3.00
Segmented Neutrophils ($X10^2$/uL)	67.90±20.66•[a]	100,16±37.22•[a]	25.00-125.00
Lymphocytes ($X10^2$/uL)	49.40±16.29•[a]	41.66±17.52•[a]	15.00-70.00
Monocytes ($X10^2$/uL)	0.54±1.23°[a]	4.56±2.14•[b]	0-8.50
Eosinophils ($X10^2$/uL)	11.90±3.22°[a]	11.77±6.28•[a]	0-15.00
Basophils ($X10^2$/uL)	0•[a]	0•[a]	Rare
Platelets($X10^3$/uL)	480±90.75•[a]	460±223,75°[a]	300-800

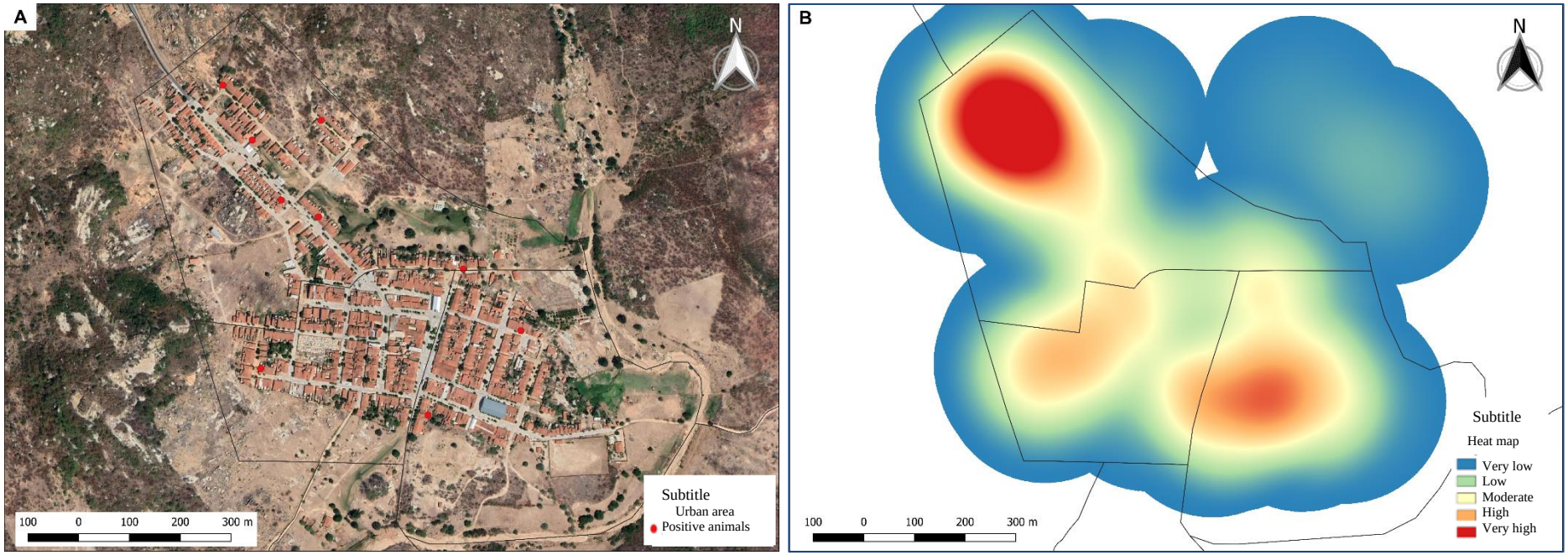
Different letters indicate statistical difference ($p<0.05$). RBC: Red blood cells; Hb: Hemoglobin; Ht: Hematocrit; VCM: Mean Corpuscular Volume; CHCM: Mean Corpuscular Hemoglobin Concentration; LEUK: Leukocytes; • Normally distributed variables expressed as mean and standard deviation. ° Non-normally distributed variables expressed as median and interquartile deviation. * Jain (1986).

Tabela 4. Serum biochemistry of *Leishmania infantum* infected and non-infected cats in the municipality of Mãe d'Água, Paraíba, northeastern Brazil, in the period 2021-2022.

Biochemical Assessment			
Variables	GNB (N=6)	GPB (N=5)	Referential interval *
PT (g/dL)•	7.62±0.91[a]	7.48 ± 0.34[a]	5.4-7.8
ALB (g/dL)•	3.73±0.38[a]	3.20±0.29[b]	2.1-3.3
ALT (U/L)•	40.97±17.85[a]	24.12±10.45[a]	6-83
AST (U/L)•	25.57±11.43[a]	21.04 ±6.12 [a]	26-43
PAL (U/L)•	24.93±6.78[a]	32.52±11.55[a]	25-93
GGT (U/L)•	0[a]	0[a]	1.3-5.1
CRE (mg/dL)◦	1.30±0.09a	1.26 ±0.39[a]	0.8-1.8
URE (mg/dL)•	62.73±11.55[a]	40.66±10.23[b]	20-30

PT: Total Protein; ALB: Albumin; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; PAL: Alkaline Phosphatase; GGT: Gammaglutamyltransferase; CRE: Creatinine; URE: Urea; SD: Standard Deviation; ID: Interquartile Deviation. Ref: Reference. • Normally distributed variables expressed as Mean and SD. ° Variables with non-normal distribution expressed as Median and ID. * Kaneko et al. (2008).

Figura 2. A. Distribution of feline visceral leishmaniasis cases. B. Heatmap with clusters of feline visceral leishmaniasis in the municipality of Mãe d'Água, Paraíba, northeastern Brazil, 2022.



CAPÍTULO III:

Aspectos clínicos e alterações hematológicas e bioquímicas em gatos domésticos soropositivos para *Leishmania infantum* na região nordeste do Brasil

Trabalho submetido à Revista Pesquisa Veterinária Brasileira (ISSN: 1678-5150), Qualis A4.

Aspectos clínicos e alterações hematológicas e bioquímicas em gatos domésticos soropositivos para *Leishmania infantum* na região nordeste do Brasil[1]

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ABSTRACT. – Franco-Silva L.F., Silva R.B.S., Araújo Junior D.S., Silva R.C., Fonseca S.S., Vaz A.F.M. & Melo M.A. 2021. **Clinical aspects and hematological and biochemical alterations in domestic cats seropositive for *Leishmania infantum* in northeastern Brazil.** *Pesquisa Veterinária Brasileira 00(0):00-00*. Laboratório de Biologia Molecular do Semiárido, Universidade Federal de Campina Grande (UFCG), Av. Universitária s/n, Patos, PB 58708-110, Brazil. E-mail: laysafrfranco@gmail.com; marcia.melo@ufcg.edu.br

Visceral leishmaniasis (VL) is a parasitic zoonosis of importance to Public Health and the dog is considered the main link in the chain of transmission in the domestic cycle of the disease, however, in endemic regions, infection in felines has been reported. Therefore, the present study aimed to evaluate the prevalence of feline leishmaniasis (FL) in animals attended at the Hospital Veterinário Universitário Prof. Dr. Ivon Macedo Tabosa from the Universidade Federal de Campina Grande (HVU/UFCG), as well as to analyze the clinical signs and hematological and biochemical alterations of seropositive cats. Blood samples from 427 domestic cats attended between March 2018 and December 2019 were used to identify anti-*Leishmania infantum* antibodies by serological tests, DPP® (Dual Path Platform) and ELISA/ S7®, to identify *L. infantum* DNA by qPCR and for hematological and biochemical analyses. The veterinary clinical records were analyzed to obtain both general data and clinical aspects of the animals. The prevalence of the disease was 2.1%. Most of the seroreactive animals were male and one year old. In all seroreactive animals, at least one clinical sign suggestive of FL was observed. Erythrocytosis and thrombocytopenia affected 28.6% of positive animals. The leukogram showed, in most cases, leukocytosis due to neutrophilia; eosinophilia, monocytosis and lymphopenia were also observed. In the evaluation of the biochemical profile, the most relevant result was the increase in the serum levels of urea. Our results reinforce the presence of FL in VL endemic areas and point to clinical, hematological and biochemical characteristics that should be taken into account in the differential diagnosis of the disease in cats.

INDEX TERMS: Animal disease, animal health, felines.

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RESUMO.- [**Aspectos clínicos e alterações hematológicas e bioquímicas em gatos domésticos soropositivos para *Leishmania infantum* na região nordeste do Brasil**]
A leishmaniose visceral (LV) é uma zoonose parasitária de importância para a Saúde Pública e o cão é considerado o principal elo na cadeia de transmissão no ciclo doméstico da doença, porém, em regiões endêmicas, a infecção em felinos tem sido relatada. Diante disso, o presente estudo teve como objetivo avaliar a prevalência da leishmaniose felina (LF) nos animais atendidos no Hospital Veterinário Universitário Prof. Dr. Ivon Macedo Tabosa da Universidade Federal de Campina Grande (HVU/UFCG), bem como analisar os sinais clínicos e alterações hematológicas e bioquímicas dos gatos soropositivos. Amostras de sangue de 427 felinos domésticos atendidos entre março de 2018 e dezembro de 2019 foram utilizadas para identificação de anticorpos anti-*Leishmania infantum* por testes sorológicos, DPP® (Dual Path Platform) e ELISA/S7®, para identificação de DNA de *L. infantum* por qPCR e para análises hematológicas e bioquímicas. As fichas de atendimento clínico veterinário foram analisadas para obtenção dos dados gerais e dos aspectos clínicos dos animais. A prevalência da doença foi de 2,1%. A maioria dos animais sororreagentes era do sexo masculino e tinha um ano de idade. Em todos os animais sororreagentes, foi observado pelo menos um sinal clínico sugestivo da LF. A eritrocitose e trombocitopenia acometeram 28,6% dos animais positivos. O leucograma apresentou, na maioria dos casos, leucocitose por neutrofilia; eosinofilia, monocitose e linfopenia também foram observados. Na avaliação do perfil bioquímico, o resultado mais relevante foi o aumento nos níveis séricos de ureia. Nossos resultados reforçam a presença de LF em áreas endêmicas para LV e apontam características clínicas, hematológicas e bioquímicas que devem ser levadas em consideração no diagnóstico diferencial da enfermidade em gatos.

TERMOS DE INDEXAÇÃO: Doença animal, saúde animal, felinos.

INTRODUCTION

Visceral leishmaniasis is a chronic systemic zoonosis caused by *Leishmania infantum*, which has a wide geographic distribution and a great impact on Public Health (Baneth et al. 2008, WHO 2010, Brazil 2014). In the domestic cycle, the dog is considered the main link in the chain of transmission, with reports of infection in outbreaks of the disease in humans (Melo 2004, Ursine et al. 2016). However, studies have reported the infection in domestic cats, which has raised questions about a possible participation of this species in the epidemiological cycle of the disease in endemic areas (Silva et al. 2010, Maia & Campino 2011, Vides et al. 2011, Batista et al. 2020).

In felines, prevalence rates of *L. infantum* infection, in serological or molecular studies, range from zero than 68.5% (Pennisi et al. 2015). In this species, the clinical signs associated with the infection may present a systemic and/or dermatological pattern, the latter being more prevalent (Leiva et al. 2005, Marcos et al. 2009, Vides et al. 2011, Silveira-Neto et al. 2015, Fernandez-Gallego et al. 2020, Silva et al. 2020).

The close contact of these animals with other mammals represents an additional risk for the occurrence of the disease (Akhtardanesh et al. 2018, Batista et al. 2020), since domestic cats can act as a source of infection for vectors transmitting *Leishmania*. spp. (Maroli et al. 2007, Silva et al. 2010, Batista et al. 2020, Mendonça et al. 2020). Therefore, the present study aimed

to evaluate the prevalence of FL in animals attended at the HVU/UFCG, as well as to analyze the clinical signs and hematological and biochemical alterations of seropositive cats.

MATERIAL AND METHODS

This study was approved by the Ethics Committee on the Use of Animals (CEUA), Universidade Federal de Campina Grande (UFCG), under protocol number CEUA 325/2015.

Considering that, on average, 1,589 cats were attended per year, from 2015 to 2017, at the Small Animal Medical Clinic (CMPA) of the HVU/UFCG, Patos campus, Paraíba, the sample size was estimated using the formula for simple random samples, based on 50% prevalence, 95% confidence level and 5% margin of error (Thrusfield 2007), determining a minimum sample population of 310 cats. However, due to the demand for assistance from the CMPA/HVU/UFCG, 427 cats were analyzed.

Blood samples came from animals attended at the HVU, from March 2018 to December 2019, regardless of the clinical complaint, and sent to the Clinical Pathology Laboratory of the HVU/UFCG. The blood was aliquoted into microtubes containing the anticoagulant ethylenediaminetetraacetic acid potassium (EDTA-K3) to perform the blood count and into a tube containing clot separator gel to obtain serum for serological and biochemical tests (urea, creatinine, alanine aminotransferase, aspartate aminotransferase, gammaglutamyltransferase, alkaline phosphatase, albumin and total protein). The reference values for species were those described by Jain (1986) and Kaneko et al. (2008).

The hemogram was performed by automated and hematoscopic evaluation. In the first analysis, an electrical impedance hematology counter (pocH-100 iV Diff, Sysmex) was used to assess the total red blood cell and leukocyte count, hemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular volume (VCM) and mean corpuscular hemoglobin concentration (MCHC). As for blood smears, a kit of dyes was used for rapid differential staining in hematology (Instant Prov from New Prov, Produtos para Laboratório Ltda., Pinhais - PR), proceeding to the differential count of leukocytes and evaluation of morphotintorial characteristics of erythrocytes, leukocytes and platelets, which were quantified and qualified as to the shape, size and presence of parasites. Biochemical analyzes were performed using a colorimetric kinetic process in an automatic analyzer (Cobas C 111, Roche) using commercial kits.

Serological and molecular tests were performed at the Laboratory of Molecular Biology of the Semiarid Region of the HVU/UFCG.

The investigation of anti-*Leishmania infantum* antibodies was performed by means of the ELISA/S7® (Biogene Ind. e Com. Ltda), which consists of a recombinant antigen formed by the carboxy-terminal region of the HSP70 of *L. infantum*. The kit is authorized by the Ministry of Agriculture, Livestock and Supply (MAPA) for the diagnosis of canine VL and has 97% sensitivity and 90% specificity. In addition to this, the DPP® Rapid Test (Dual Path Platform, Bio-Manguinhos), an immunochromatographic test for the diagnosis of canine VL, was also performed. Both tests were performed according to the manufacturers' recommendations.

The quantitative PCR (qPCR) was performed on the animals that reacted in the two serological tests, following the protocol described by Silva et al. (2016), from the extraction of DNA from 100μL of blood, using the DNeasy blood and tissue kit (Qiagen®, Hilden, Germany), following the manufacturer's recommendations, and using the Linf kDNA-F primers 5' GGCGTTCTGCAAAATCGGAAA -3', Linf kDNA-R 5' CCGATTTTTGGCATTTTTGGTCGAT-3' and Linf kDNA_FAM-5'-TTTTGAACGGGATTTCTG-3' to amplify the *L. infantum* kinetoplast minicircle gene

(kDNA). A culture of *L. infantum* was used as a positive control and ultrapure water as a negative control.

The prevalence of FL was determined by descriptive statistics, considering as positive only the samples that reacted in the two serological tests used and/or in the qPCR. Clinical records of positive cats were analyzed to collect data such as origin, age, sex and clinical signs suggestive of FL.

For the statistical analysis of hematological and biochemical alterations in animals with FL, two groups were formed: animals positive for *Leishmania* spp. (GP, n = 7) and animals negative for *Leishmania* spp., considered healthy by clinical and laboratory parameters of health (GN, n = 11). Hematological and biochemical variables were analyzed for normality using the D'Agostino & Pearson test with a significance level of 5%. Comparison of means between the GP and GN groups was performed by ANOVA analysis of variance (Two-way) followed by the post hoc Bonferroni test using the GraphPrism® software (GraphPad Software Inc., San Diego, CA, USA) for Windows, with significance of $p<0.05$.

RESULTS AND DISCUSSION

Among the 427 cats analyzed, 10.1% (43/427) were reactive in the DPP® test and 10.5% (45/427) in the ELISA/S7®. As only the samples that reacted in the two serological tests were considered positive, the prevalence of FL was 2.1% (9/427), close to those found in Teresina-Piauí (4%), through parasitological tests and ELISA (Mendonça et al. 2017) and in Pernambuco (0.8%), through parasitological test and PCR (Berenguer et al. 2021), but very different from what was observed in Araçatuba-São Paulo (14.5%), through parasitological test and ELISA (Costa et al. 2010).

Differences in prevalence occur as a result of the region studied, as well as due to the diagnostic techniques used. In felines, it is not known whether the difference in prevalence in endemic areas is associated with the failure to detect antibodies or because they present some natural resistance to leishmaniasis (Pirajá et al. 2013) and the development of weak humoral immunity in this species (Solano- Gallego et al. 2007, Maia et al. 2010).

In eight of the nine animals considered positive, qPCR was performed but there was no amplification of the fragment corresponding to the kDNA. According to Coura et al. (2018), cats respond to *Leishmania* infection by producing antibodies, even though there are few or no parasites in tissue samples. In addition, cats exposed to *L. infantum* infection develop both a significant cellular and humoral response against the infection, which may explain the negativity in the molecular test, although it is possible to detect antibodies against *Leishmania* spp. in the serum of animals (Alves et al. 2022).

From the survey of clinical care records, it was possible to obtain information on seven of the nine positive animals: six lived in Paraíba (in the municipality of Patos) and one of the cats was from the municipality of Itapetim, state of Pernambuco (Fig.1).

All animals evaluated were mixed breed (SRD), with 57.1% (4/7) males and 42.8% (3/7) females. The age range of seropositive animals ranged from one to four years, with a predominance of one year old (42.8%; 3/7). All animals had access to the street and 57.1% (4/7) had not received any type of vaccine or deworming.

Despite the higher percentage of positive males in this research, and the higher risk of infection in males having been previously reported (Sobrinho et al. 2012), studies show that sex does not appear to be a predisposing factor for FL (Spada et al. 2013). Akhtardanesh et al. 2017). Regarding age, Chatzis et al. (2014) also observed many positive animals (44.1%) with the same age group reported in the present study, suggesting that cats living in endemic regions

can be infected at a relatively young age. On the other hand, Akhtardanesh et al. (2017) associated the prevalence of the disease with the fact that the animals were elderly. However, some authors report that there is no relationship between age and FL (Bresciani et al. 2010, Silva et al. 2014), showing that the association of the disease with this variable needs further investigation.

As shown in Table 1, at least one clinical sign suggestive of FL was observed in all positive cats. Among the clinical manifestations recorded in the records of the animals were: cachexia/thinness, areas of ulceration/wound, gingivitis and lymphadenomegaly.

In other studies, clinical signs similar to those found in this research were also observed, such as ulcers (Pennisi et al. 2015, Silva et al. 2020), cachexia/thinness (Savani et al. 2004, Pirajá et al. 2013, Mendonça et al. 2013, Mendonça et al. 2017), lymphadenomegaly (Pennisi et al. 2015, Fernandez-Gallego et al. 2020, Berenguer et al. 2021) and gingivitis (Leva et al. 2005, Spada et al. 2020).

Due to the fact that cats are resistant to infection by *Leishmania* spp. (Pirajá et al. 2013, Akhtardanesh et al. 2018), many authors report that asymptomatic animals present in greater numbers compared to animals with some clinical sign (Chatzis et al. 2010, Soares et al. 2015, Akhtardanesh et al. 2017). In our study, all animals showed clinical signs suggestive of the disease, but it should be noted that the population studied consisted of animals attended at a veterinary hospital, whose tutors had some clinical complaint. On the other hand, despite the clinical signs being suggestive of FL, the fact that most animals have access to the street, are not vaccinated or dewormed and because they are non-specific clinical manifestations, the possibility of concomitant diseases involved in the clinical conditions observed cannot be discarded, since among the diagnoses suggested for the attended animals were: gingivitis-stomatitis complex, FIV, FELV, hepatic lipidosis, pancreatitis, platynosomiasis and pyometra.

In hematological and biochemical analysis, no statistically significant differences were observed when data from positive and healthy animals were compared.

The analysis of the erythrogram of the positive animals showed that 28.6% (2/7) had erythrocytosis (Table 2). As the elevation of the levels of He, Hb and/or Ht was slight, and considering that the animals did not present clinical signs corresponding to absolute erythrocytosis, it is believed that the erythrocytosis observed is relative. One of the possible explanations for this result is the occurrence of splenic contraction with consequent increase in circulating erythrocyte mass, a situation commonly observed in excitable animals such as cats (Thrall et al. 2015). Several studies show results contrary to those observed here, with the presence of anemia in animals positive for VL, both in dogs (Reis et al. 2006, Braz et al. 2015) and in cats (Pennisi et al. 2004, Noé et al. 2015, De Mendonça et al. 2017). In cats infected with *L. infantum*, anemia may result from a decrease in erythrocyte precursors in the bone marrow and from immune-mediated hemolysis secondary to leishmaniasis (Marcos et al. 2009).

In the microscopic analysis of the blood smear, the erythrocytes did not present any alteration in terms of morphology, color and size in most animals, in addition to being negative in the search for hemoparasites. Among those that showed alterations, the only one observed was anisocytosis 42.8% (3/7), which is consistent with the results of VCM in some animals (Table 2). According to Thrall et al. (2015), in the presence of inflammatory diseases, red blood cells may be moderately microcytic. Furthermore, as the animals had erythrocytosis, probably due to splenic contraction, the release of old (microcytic) red blood cells (Cordero et al. 2004) influences the observation of anisocytosis.

From the total leukocyte count, it was possible to verify that 57.1% of the seropositive cats (4/7) had leukocytosis. This type of assessment, by itself, has limited value, making it necessary to assess the concentration of each type of cell (Stockham & Scott 2011). Thus, in the differential analysis of leukocytes, the most frequent alteration was neutrophilia (4/7;

57.1%), but lymphopenia (1/7; 14.28%), monocytosis (1/7; 14.28%) and eosinophilia (1/7; 14.28%) was also observed (Table 3).

Neutrophilia is a frequent finding in cats with VL (Silva et al. 2010, Mendonça et al. 2017, Chatzsis et al. 2020) and, in fact, the increase in neutrophil production can be stimulated by infections and tissue injury (Vaden et al. 2013). In 50% (2/4) of the animals, neutrophilia showed a shift to the left, a common alteration in dogs with leishmaniasis (Schultze 2010) and indicates an increased concentration of non-segmented neutrophils (usually rods), suggesting an increased tissue demand for neutrophils in response to a relatively intense and usually acute inflammatory stimulus (Stockham & Scott 2011). In 25% (1/4) of the animals, neutrophilia with a shift to the right was observed, which occurs when there is an increase in the concentration of hypersegmented neutrophils, usually indicating the presence of aged neutrophils. Causes for this type of neutrophilia include chronic inflammatory diseases (the pattern of leishmaniasis) and longer retention time of neutrophils in the circulation as a result of steroid action (Stockham & Scott 2011, Thrall et al. 2015).

Eosinophilia in cats with FL has also been reported by other authors (Antunes et al. 2018, Mendonça et al. 2017). In our study, 50% of the positive animals were not dewormed and the cat that presented eosinophilia was among these animals. However, in addition to parasitism, hypersensitivity reaction or unusual injury, which produces chemotactic agents for eosinophils, are also involved in the occurrence of this alteration. Although tissue-invading parasites are often associated with eosinophilia, organisms that infect blood cells are not expected to cause eosinophils to increase above reference levels (Stockham & Scott 2011, Thrall et al. 2015).

The occurrence of lymphopenia and monocytosis has been reported in cats (Chatzsis et al. 2020) and in dogs (Braz et al. 2015, Lacerda et al. 2017) with leishmaniasis. The presence of lymphopenia is generally attributed to a response to steroids, which can induce apoptosis of lymphocytes and promote alterations in their recirculation patterns (Thrall et al. 2015). In dogs with VL, increased expression of the programmed death receptor PD-1 in lymphocytes, with consequent exhaustion of this cell type, has already been mentioned (Esch et al. 2013). Monocytosis is associated with acute and chronic inflammation, tissue necrosis and stress (Stockham & Scott 2011, Vaden et al. 2013).

Thrombocytopenia was observed in 28.6% (2/7) of the animals (Table 2), an alteration also verified by Chatzsis et al. (2020) in FL positive cats and by Braz et al. (2015) in dogs with VL.

The pathogenesis of this alteration in association with infections, including leishmaniasis, is multifactorial, but often includes decreased platelet production (Stockham & Scott 2011). In fact, in a case report of a cat infected with *L. infantum*, Marcos et al. (2009) observed a decrease in megakaryocyte precursors in the bone marrow. Furthermore, in dogs with VL, it has already been shown that there is an association between the occurrence of thrombocytopenia and the presence of antibodies against the platelet membrane (Terrazzano et al. 2006) and that the chronic inflammatory process, associated with liver and kidney damage, may influence platelet function and the synthesis and metabolism of clotting factors (Ciaramella et al. 2005).

In 28.6% (2/7) of the seropositive animals, analyzes of serum biochemical markers were performed, where it was observed that the urea values were above the maximum limit for the species (Table 4).

The increase in serum urea level was also reported in cats with leishmaniasis by Silva et al. (2010), Mendonça et al. (2017) and Chatzis et al. (2020). According to Thrall et al. (2015), failures in the blood supply to the kidneys (caused by dehydration, heart failure and shock), in

the kidneys themselves (due to various pathologies) or in eliminating urine (due to obstruction or rupture of the urinary system) can result in an increase in serum urea.

In cats with leishmaniasis, glomerulonephritis and interstitial nephritis have been observed (Marcos et al. 2009). According to Rigo et al. (2013), renal involvement is very common in dogs with VL due to the deposition of immune complexes in this organ. Probably, in some infected cats, the presence of *L. infantum* may also contribute to the pathogenesis of kidney disease, however, further studies are needed to confirm this hypothesis (Chatzis et al. 2020), since, apparently, cats do not mount a potent humoral response (Solano-Gallego et al. 2007, Maia et al. 2010).

Our data also indicate that, probably, the evaluated cats did not have liver damage or the lesions were not extensive enough to generate an elevation in the serum levels of AST and GGT (Table 5).

CONCLUSION

The percentage of positive animals for FL in the animals attended at the HVU/UFCG in the municipality of Patos was low in the studied period, however, as cats mount a less significant humoral response than dogs, the importance of cats in the VL transmission cycle in region should not be discarded. FL should be taken into account in the differential diagnosis of diseases in cats, especially when dermatological signs are present.

Acknowledgement.- To the Coordination for the Improvement of Higher Education Personnel (CAPES) for the scholarship granted.

Declaration of conflict of interest.- The authors have no conflict of interest.

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Legends of Tables and Figures

Table 1

Clinical Signs					
Animals	**Body score**	**Evaluation of mucous membranes**	**Alterations in skin and mucous membranes**	**Lymphadenopathy**	**Gingivitis**
1	2.5	Normocolored	Wound in the LPL and close to the labial commissure	Present	NI
2	2.5	Mildly hyperemic	NI	Present	Present
3	NI	Normocolored	NI	Absent	Present
4	1.5	Icteric	NI	Absent	NI
5	NI	Normocolored	NI	Present	NI
6	2.5	Mildly hyperemic	Ulcer near the molars	Present	Present
7	2.5	Normocolored	NI	Present	Present

NI: not informed. LPL: left pelvic limb.

Table 2

Hematological evaluation						
Animals	**RBC ($X10^6$/µL)**	**Hb (g/dL)**	**Ht (%)**	**VCM (fL)**	**CHCM (g/dL)**	**Platelet Count ($X10^3$/uL)**
1	10.24	12.9	46	44.9	28	384
2	8.21	11.5	33.8	41.2	34	361
3	8.49	12.8	39.8	46.9	32.2	332
4	8.04	12.1	38.3	47.6	31.6	366
5	11.46	13.2	44.2	38.6	29.9	100
6	8	10.8	31.7	37.6	34.7	67
7	7.69	10.4	29.9	38.9	34.8	356
Mean ± SD•/	8.2 ±	12.0 ±	37.7 ±	42.2 ±	32.2 ±	356 ± 266°
Median ±ID°	2.2°	1.1•	6.2•	4.2•	2.6•	
Reference range*	5-10	8-15	24-45	39-55	31-35	300-800

RBC: Red blood cells; Hb: Hemoglobin; Ht: Hematocrit; VCM: Mean Corpuscular Volume; CHCM: Mean Corpuscular Hemoglobin Concentration; SD: Standard Deviation; ID: Interquartile Deviation; • Normally distributed variables expressed as Mean and SD. °Variables with non-normal distribution expressed as Median and ID. * Jain (1986).

Table 3

Leukogram							
Animals	**Total Leuk ($X10^3$/uL)**	**Rod ($X10^2$/ uL)**	**Seg ($X10^2$/ uL)**	**Lymp ($X10^2$/ uL)**	**Mon ($X10^2$/ uL)**	**Eos ($X10^2$/ uL)**	**Bas ($X10^2$/ uL)**
1	24.20	4.84	174.24	36.30	0	26.62	0
2	11.00	ND	ND	ND	ND	ND	ND
3	21.30	0	178.92	25.56	8.52	0	0
4	14.80	0	97.68	38.48	0	11.84	0
5	19.70	0	161.54	25.61	7.88	1.97	0
6	7.30	ND	ND	ND	ND	ND	ND
7	29.80	38.74	241.38	8.94	2.98	5.96	0
Mean ± SD•/ Median ± ID°	18.30 ± 7.79•	0 ± 21.79°	170.75 ± 51.21•	26.97 ± 11.70•	3.87 ± 4.13•	9.27 ± 10.69•	0±0•
Reference range *	5.00-19.50	0-3.00	25.00-125.00	15.00-70.00	0-8.50	0-15.00	Rare

Leuk: Leukocytes; Rod: rod neutrophils; Seg: Segmented neutrophils; Lymp: Lymphocytes; Mon: Monocytes; Eos: Eosinophils; Bas: Basophils; ND: not determined; SD: Standard Deviation; ID: Interquartile Deviation; • Normally distributed variables expressed as Mean and SD. °Variables with non-normal distribution expressed as Median and ID.* Jain (1986).

Table 4

Serum Biochemistry							
Animals	**ALB (g/dL)**	**ALT (U/L)**	**AST (U/L)**	**PAL (U/L)**	**GGT (U/L)**	**CRE (mg/dL)**	**URE (mg/dL)**
5	ND	ND	25.5	17.2	0	0.4	33.9
7	3.29	55.7	29.5	ND	ND	1	53.83
Reference range *	2.1-3.3	6-83	26-43	25-93	1.3-5.1	0.8-1.8	20-30

ALB: Albumin; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; PAL: Phosphatase alkaline; GGT: Gammaglutamyltransferase; CRE: Creatinine; URE: Urea; ND: not determined; * Kaneko et al. (2008).

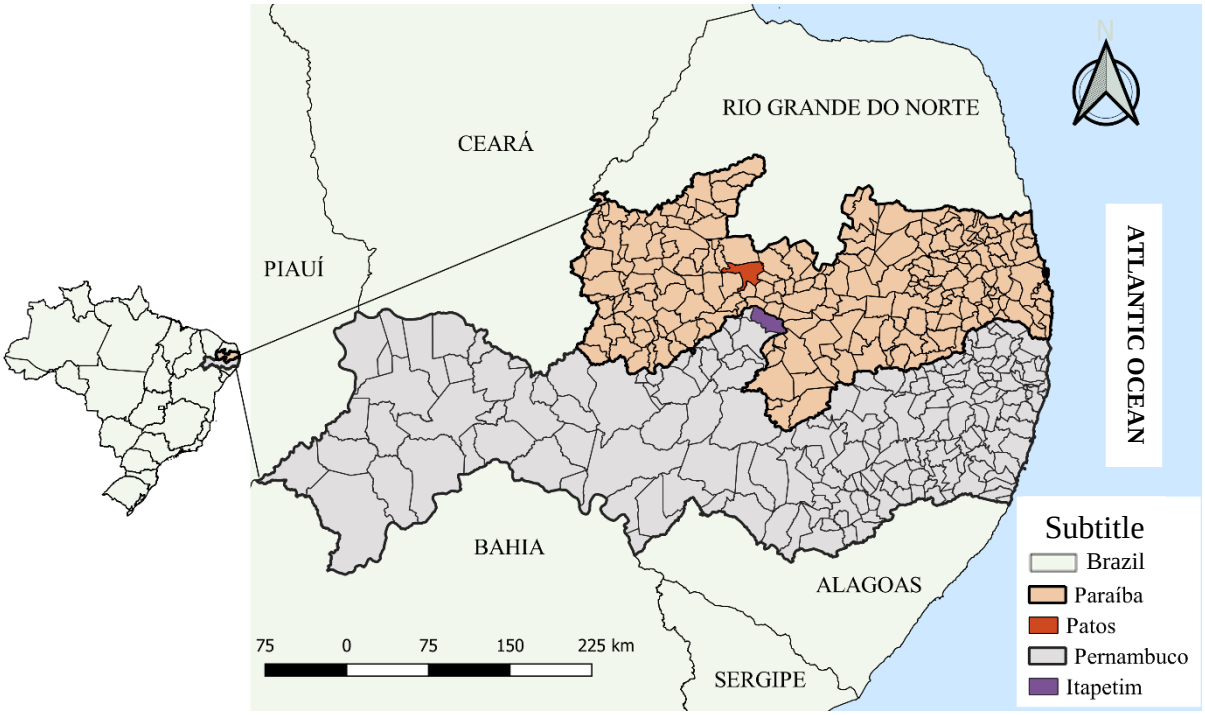
RIO GRANDE DO NORTE
CEARÁ
PIAUÍ
BAHIA
ALAGOAS
SERGIPE
ATLANTIC OCEAN
N
75 0 75 150 225 km
Subtitle
Brazil
Paraíba
Patos
Pernambuco
Itapetim

Fig.1.

CONCLUSÃO GERAL

A alta heterogeneidade entre os estudos sobre LF identificada na meta-análise reflete as diferenças nos métodos diagnósticos utilizados, que pode também ter refletido nos resultados dos estudos apresentados nos capítulos 2 e 3, e demonstra a necessidade de padronização dessas técnicas no diagnóstico da leishmaniose em felinos.

A presença de alterações dermatológicas foi um achado frequente na revisão sistemática dos estudos sobre LF no Brasil e nos gatos atendidos no HVU/UFCG do município de Patos, enfatizando a importância da inclusão da leishmaniose felina como diagnóstico diferencial nas doenças em gatos, principalmente as que cursam com sinais dermatológicos.

No município de Mãe d'Água, os casos de LF se concentraram em uma área de expansão urbana que deve ser priorizada para o controle da doença, sobretudo vetorial. Apesar da prevalência não ser considerada alta, como os gatos montam uma resposta humoral menos significativa que os cães, a importância desta espécie no ciclo de transmissão da LV na região estudada não deve ser descartada.