

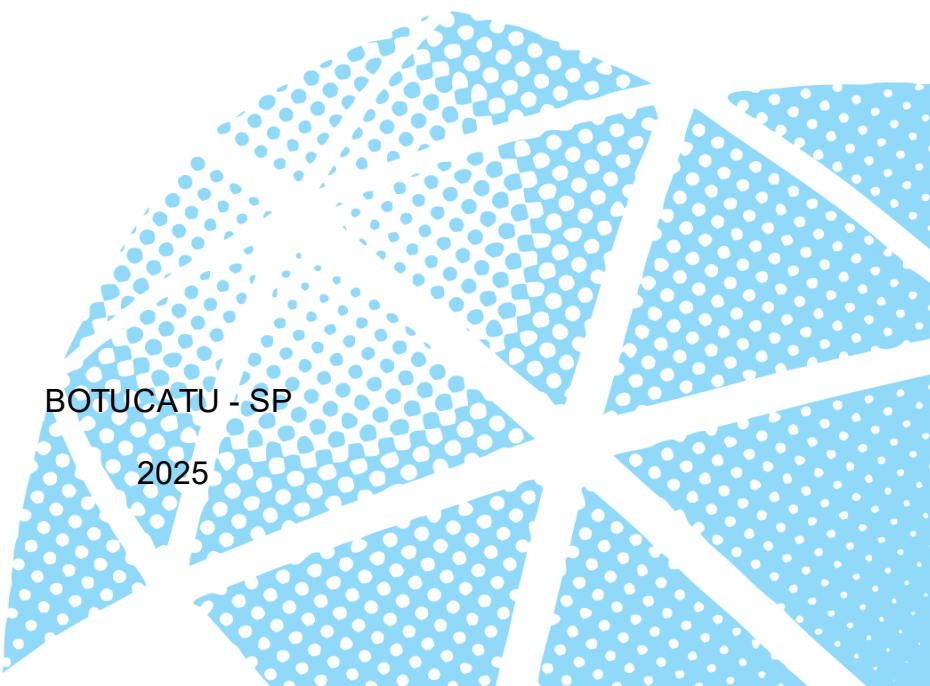
UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA
PROGRAMA DE PÓS GRADUAÇÃO EM MEDICINA VETERINÁRIA
ÁREA DE MEDICINA VETERINÁRIA PREVENTIVA

INVESTIGAÇÃO MOLECULAR DE AGENTES ZOONÓTICOS EM
MORCEGOS DO ESTADO DE SÃO PAULO DURANTE A
PANDEMIA DA COVID-19

DANILO ALVES DE FRANÇA

BOTUCATU - SP

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Tese apresentada ao Programa de Pós-
Graduação em Medicina Veterinária para
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Orientador: Prof. Dr. Helio Langoni

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IMPACTO SOCIAL E ECONÔMICO

Esta tese aborda a importância dos morcegos como potenciais reservatórios de agentes zoonóticos no Estado de São Paulo. Socialmente, a pesquisa alerta para a necessidade de vigilância epidemiológica e estratégias de controle mais efetivas, visando reduzir o risco de transmissão zoonótica e proteger a saúde das populações mais vulneráveis. Economicamente, a prevenção de zoonoses através da compreensão dos reservatórios naturais pode reduzir custos de saúde pública associados ao diagnóstico e tratamento de doenças emergentes e reemergentes, além de mitigar perdas na pecuária e em outras atividades econômicas que podem ser impactadas por esses patógenos.

COMISSÃO EXAMINADORA

Prof. Dr. Helio Langoni

Presidente e Orientador

Faculdade de Medicina Veterinária e Zootecnia

Universidade Estadual Paulista (UNESP de Botucatu)

Profa. Dra. Leila Sabrina Ullmann

Membro Titular

Faculdade de Medicina Veterinária e Zootecnia

Universidade Federal de Mato Grosso do Sul (UFMS)

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Membro Titular

Centro de Medicina Veterinária do Coletivo

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Profa. Dra. Christina Pettan-Brewer

Membro Titular

School of Medicine

University of Washington (UW)

Prof. Dr. Felipe Fornazari

Membro Titular

Faculdade de Medicina Veterinária e Zootecnia

Universidade Estadual Paulista (UNESP de Botucatu)

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Fiquei alegre olhando o livro e disse: ‘O que eu sempre invejei nos livros foi o nome do autor’. E li o meu nome na capa do livro. ‘Carolina Maria de Jesus. Diário de uma favelada. Quarto de despejo’. Fiquei emocionada. É preciso gostar de livros para sentir o que eu senti

Carolina Maria de Jesus

DEDICATÓRIA

Aos meus pais, Antônia e José, por toda luta, carinho e proteção,

Aos meus filhos, Madalena e Zion, por serem companhias essenciais em dias intensos de trabalho,

E a todos os professores que passaram pela minha vida, que de alguma forma, contribuíram para o meu caráter e serviram de inspiração para o caminho que escolhi.

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A Ygor Ferreira Lopes (in memoriam), um grande amor que passou pela minha vida. Vítima da depressão e da homofobia, um ser justo e amoroso que me ensinou sobre resiliência, força e coragem.

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RESUMO

As zoonoses são uma ameaça crescente à saúde pública, particularmente em regiões tropicais como o Brasil, onde os morcegos atuam como reservatórios milenares de patógenos. Este estudo teve como objetivo investigar a presença de agentes zoonóticos em diferentes espécies de morcegos do Estado de São Paulo, utilizando técnicas moleculares para detecção de *Leishmania* spp., *Trypanosoma cruzi*, *Toxoplasma gondii*, *Bartonella* spp., *Coxiella burnetii* e *Rickettsia rickettsii* e *Leptospira* spp. A pesquisa envolveu morcegos urbanos e rurais, abrangendo diversas espécies com diferentes hábitos alimentares. Foram coletados 203 morcegos de diferentes cidades do Estado, e foram amostrados fragmentos de fígado, baço, rins, intestinos, pulmões, placenta e encéfalo para a extração total do DNA. As amostras foram analisadas por meio de protocolos moleculares, com posterior confirmação por sequenciamento genético. Análises estatísticas, georeferenciais e filogenéticas foram aplicadas adicionalmente. A prevalência molecular de *Leptospira* spp. foi alta em morcegos hematófagos (43%), indicando seu papel na transmissão da leptospirose em áreas rurais. *Leishmania* spp. foi detectada em morcegos urbanos (6%) de áreas endêmicas e não endêmicas. *Toxoplasma gondii* e *Trypanosoma cruzi* foram detectados em morcegos urbanos e rurais (15% e 3%, respectivamente), sugerindo sua participação em diferentes ciclos. *Bartonella* spp. foi detectada em morcegos urbanos (4%), enquanto *Coxiella burnetii* e *Rickettsia rickettsii* não foram detectadas. Um caso inédito de coinfeção entre vírus rábico, *B. koehlerae* e *L. infantum* foi observado em um *Myotis nigricans*. Estes resultados destacam a necessidade de monitoramento contínuo desses animais para fins de vigilância epidemiológica e proteção da saúde pública, oferecendo subsídios para a formulação de estratégias de controle e prevenção de zoonoses sob uma perspectiva de Saúde Única.

Palavras-chave: Saúde Única, chiroptera, reservatório, zoonoses, vigilância.

FRANÇA, D.A. **Molecular investigation of zoonotic agents in bats from São Paulo state during the COVID-19 pandemic.** Botucatu, 2025. 159p. Thesis (PhD) – School of Veterinary Medicine and Zootechny, Botucatu Campus, São Paulo State University.

ABSTRACT

Zoonoses are a growing threat to public health, particularly in tropical regions such as Brazil, where bats act as millennial reservoirs of pathogens. This study aimed to investigate the presence of zoonotic agents in different bat species in the São Paulo state, using molecular techniques to detect *Leishmania* spp., *Trypanosoma cruzi*, *Toxoplasma gondii*, *Bartonella* spp., *Coxiella burnetii* and *Rickettsia rickettsii* and *Leptospira* spp. The research involved urban and rural bats, including several species with different feeding habits. 203 bats were collected from different cities in the state, and fragments of liver, spleen, kidneys, intestines, lungs, placenta and brain were sampled for total DNA extraction. The samples were analyzed using molecular protocols, followed by confirmation through genetic sequencing. Statistical, georeferential and phylogenetic analyses were also applied. The molecular prevalence of *Leptospira* spp. was high in hematophagous bats (43%), indicating their role in the transmission of leptospirosis in rural areas. *Leishmania* spp. was detected in urban bats (6%) from endemic and non-endemic areas. *Toxoplasma gondii* and *Trypanosoma cruzi* were detected in urban and rural bats (15% and 3%, respectively), suggesting their participation in different cycles. *Bartonella* spp. was detected in urban bats (4%), while *Coxiella burnetii* and *Rickettsia rickettsii* were not detected. An unprecedented case of coinfection involving rabies virus, *B. koehlerae*, and *L. infantum* was observed in a *Myotis nigricans*. These results highlight the need for continuous monitoring of these animals for the purposes of epidemiological surveillance and public health protection, providing input for the formulation of zoonosis control and prevention strategies from a One Health perspective.

Keywords: One Health, chiroptera, reservoir, zoonoses, surveillance.

CAPÍTULO 1: CONSIDERAÇÕES GERAIS

1.1. INTRODUÇÃO

As zoonoses emergentes e reemergentes representam um desafio significativo para a saúde pública global, especialmente em regiões biodiversas e tropicais como o Brasil. A interação crescente entre humanos e a vida selvagem, motivada pela expansão urbana, desmatamento e desequilíbrios ambientais, tem sido identificada como um dos principais fatores que promovem o surgimento e a disseminação de doenças zoonóticas. Os morcegos, devido à sua ampla diversidade genética e ecológica, desempenham um papel importante como reservatórios de diversos patógenos, incluindo vírus, bactérias e protozoários, que podem causar infecções importantes em humanos e animais domésticos. No entanto, ainda há lacunas consideráveis no conhecimento sobre o papel específico desses animais na epidemiologia de diversas doenças zoonóticas no Brasil.

Este estudo investigou a presença de agentes zoonóticos em morcegos coletados em diferentes municípios do Estado de São Paulo, com foco em agentes causadores da leishmaniose, doença de Chagas, toxoplasmose, leptospirose, bartonelose, febre Q e da febre maculosa brasileira. Para a investigação, foram utilizados métodos estabelecidos de PCR, PCR em tempo real e sequenciamento genético, bem como o uso de análises estatísticas, georreferenciamento e filogenia para uma melhor compreensão do tipo de patógeno detectado e de sua distribuição pelo Estado de São Paulo. Foi possível detectar a presença de patógenos em morcegos insetívoros, frugívoros e hematófagos, urbanos e rurais, contribuindo para a compreensão de seu papel como potenciais reservatórios e amplificadores dessas zoonoses. Os resultados obtidos aqui reforçam a importância da vigilância epidemiológica em áreas de risco e destacam a relevância dos morcegos na manutenção de agentes infecciosos no ecossistema.

Ao explorar a relação entre esses animais e a saúde pública sob a perspectiva de Uma Só Saúde, esta tese buscou fornecer subsídios científicos para o desenvolvimento de estratégias de controle e prevenção das zoonoses.

1.2. REVISÃO DE LITERATURA

1.2.1. Do *spillover* ao reservatório de agentes infecciosos

As zoonoses são doenças transmissíveis entre animais e humanos. Muitas doenças hoje conhecidas como restritas a humanos já foram estudadas em sua origem e identificadas como tendo uma origem animal. É o caso da AIDS, do Ebola, e de inúmeras doenças virais causadoras de gripe como a Influenza. Pensar nos seres vivos como um grande grupo reafirma a lógica darwiniana de que a humanidade é um tipo de animal intrinsecamente ligado aos demais animais, em sua origem, na saúde e na doença (QUAMMEN, 2012).

Com os avanços no campo da biologia molecular, foi possível perceber por meio de análises do perfil genético de microrganismos e da elaboração de árvores filogenéticas que as enfermidades infecciosas têm aparecido e reaparecido ao longo de períodos, e tais ressurgências são motivadas por uma ampla gama de influências, como: adaptação gênica do agente, doenças polimicrobianas, susceptibilidade do hospedeiro humano, aspectos demográficos e comportamento humano, comércio e viagens transfronteiriças, guerras e bioterrorismo, exposição ocupacional, uso inadequado de antimicrobianos, alterações climáticas, desenvolvimento econômico, uso da terra, pobreza e desigualdade social, ausência de serviços públicos de saúde, populações animais, e a fome. Cerca de sessenta a oitenta por cento das recentes infecções em seres humanos se originaram de animais, com uma ênfase maior em roedores e morcegos (MORENS e FAUCI, 2013).

A doença infecciosa é uma espécie de argamassa natural que liga um animal a outro dentro dos elaborados edifícios biofísicos a que chamamos ecossistemas. Os agentes patogénicos são como predadores que comem as suas presas, sendo tão naturais quanto um leão que ataca uma zebra. Assim como os predadores têm as suas presas habituais, os seus alvos preferidos, os agentes patogénicos também possuem. E da mesma forma que um leão pode ocasionalmente afastar-se do seu comportamento natural e atacar uma vaca que invadiu seu habitat, um agente patogénico também pode mudar para um novo hospedeiro alvo. As circunstâncias mudam e, com elas, as exigências e as oportunidades também mudam. Quando um agente patogénico passa de um

animal não humano para uma pessoa e consegue aí estabelecer-se, temos o que chamamos de *spillover* e o surgimento de uma zoonose (QUAMMEN, 2012).

Esse processo ocorreu na história de inúmeras doenças, dentre elas algumas pandêmicas, como a peste, a gripe espanhola, a varíola dos macacos, a tuberculose bovina, a febre do Nilo, a raiva, a hantavirose, a COVID-19, dentre outras. Suas origens animais foram diversas, desde porcos e aves aquáticas selvagens, até primatas não humanos e morcegos, assim como suas origens territoriais, passando tanto pela América e Eurásia, como pela África e Oceania. Esta forma de *salto interespecies* é comum, não rara (ROBBINS, 2012). Cerca de sessenta por cento das doenças infecciosas emergentes que afetam os seres humanos são zoonóticas, tendo sua origem em animais, e mais de dois terços delas têm origem na vida selvagem. De acordo com o International Livestock Research Institute (ILRI), mais de dois milhões de pessoas são mortas por ano por doenças que se espalham para os seres humanos a partir de animais selvagens e domésticos (ROBBINS, 2012). Algumas doenças podem ser mais complexas de combater do que outras, como a febre amarela que possui além de primatas não humanos e humanos envolvidos na cadeia epidemiológica, os mosquitos, que podem fazer surgir inúmeros novos tipos de novas espécies fontes de infecção do agente, uma vez que o controle dos mosquitos é extremamente mais difícil do que controlar focos selvagens ou domésticos de doenças (MORENS e FAUCI, 2013).

Frequentemente os microrganismos se “escondem” no que chamamos de hospedeiro reservatório. Um reservatório é um organismo vivo que transporta o agente patogénico, abrigando-o cronicamente, enquanto sofre pouca ou nenhuma doença. Em situações onde agentes infecciosos parecem não ocorrer mais em certos territórios, na verdade eles podem estar residindo em um local onde a diversidade biológica é elevada e o ecossistema é relativamente pouco perturbado. Quando ocorre a perturbação ecológica, esses agentes se revelam (ALEXANDER et al., 2018). As doenças emergentes consistem em patógenos presentes em um território que sofreram mutações, resultando em variantes que se comportam de maneira diferente no novo alvo. Um exemplo disso é a

influenza, que sofre mutações regularmente, levando à necessidade de atualizações anuais das vacinas (MORENS e FAUCI, 2013).

Essa compreensão de como as zoonoses surgem e como se dá o *spillover*, faz com que as agências internacionais avancem no combate a novas doenças de potencial pandêmico e epidêmico, identificando-as nos locais antes mesmo que elas atinjam humanos e se espalhem (ALEXANDER et al., 2018). Embora tenha sido viável erradicar algumas doenças infecciosas e controlar efetivamente muitas outras nas últimas décadas, parece improvável que consigamos eliminar a grande maioria das enfermidades infecciosas emergentes no futuro próximo. Isso se deve ao fato de que os microrganismos patogênicos têm a capacidade de passar por mudanças genéticas rápidas, resultando em novas características fenotípicas que se aproveitam das alterações no hospedeiro e das condições ambientais disponíveis (ROBBINS, 2012). As vantagens conferidas pelos microrganismos só podem ser enfrentadas e superadas por meio de uma vigilância rigorosa, de pesquisas contínuas e dedicadas, bem como da rápida elaboração e implementação de contramedidas, tais como instrumentos de monitorização, diagnósticos, fármacos e vacinas. Identificar, diagnosticar, sequenciar e tipificar novos microrganismos em novas localidades são ferramentas importantes no combate à novos surtos. A luta contra as enfermidades infecciosas emergentes precisa ser um esforço constante (MORENS e FAUCI, 2013).



Fonte: Olaf Hajek, The New York Times, 2012

1.2.2. Importância da vida selvagem na emergência e reemergência de moléstias infecciosas

As enfermidades historicamente têm tido origem nas florestas e na fauna selvagem, antes de se propagarem às comunidades humanas. Dois exemplos notáveis são a peste e a malária (QUAMMEN, 2012). Os especialistas afirmam que nos últimos cinquenta anos, o número de doenças emergentes aumentou quatro vezes, em grande parte devido à expansão constante das áreas habitadas por seres humanos em habitats animais, especialmente em regiões tropicais identificadas como pontos críticos de disseminação de enfermidades em todo o mundo. Além disso, com a mobilidade global facilitada pelas viagens aéreas modernas e o comércio ilícito de animais selvagens, existe um potencial significativo para a ocorrência de surtos graves em áreas densamente povoadas (MORENS e FAUCI, 2013).

As mudanças nos ecossistemas, como desmatamento, urbanização, mudanças climáticas e degradação ambiental, podem levar a um aumento nos encontros entre a vida selvagem e seres humanos. Isso pode resultar em maior risco de transmissão de doenças infecciosas (DASZAK et al., 2000). A vida selvagem, devido à sua ampla diversidade genética, pode ser um ambiente propício para a mutação e evolução de patógenos, e essas mudanças genéticas, podem dar origem a variantes mais virulentas ou mais transmissíveis que representam uma ameaça para a saúde global (ROBBINS, 2012). A detecção de doenças em animais selvagens pode servir como um indicador de alerta precoce sobre a presença de patógenos perigosos. Isso pode fornecer informações valiosas para a prevenção e controle de surtos de doenças infecciosas em humanos. A conservação da vida selvagem está intrinsecamente ligada à saúde humana, e, portanto, a manutenção de ecossistemas saudáveis e da biodiversidade pode reduzir o risco de emergência dessas doenças (MORENS e FAUCI, 2013).

A dinâmica das populações de vida selvagem e suas migrações sazonais ou movimentos de longa distância podem desempenhar um papel importante na disseminação de patógenos (ALEXANDER et al., 2018). Por exemplo, a migração de aves pode transportar patógenos de uma região para outra, ou a

migração de mamíferos marinhos para áreas costeiras, contribuindo para a dispersão geográfica de doenças (ROBBINS, 2012). A degradação do habitat e o desmatamento muitas vezes levam a uma maior interação entre a vida selvagem, animais domésticos e seres humanos, aumentando o risco de transmissão de doenças infecciosas. À medida que os humanos se expandem para áreas anteriormente intocadas, podem ocorrer encontros mais frequentes com a vida selvagem, aumentando as chances de exposição a patógenos. Essa mesma exposição pode ocorrer em hábitos recorrentes de ecoturismo ou de migração de povos indígenas. O comércio ilegal de animais selvagens também é uma preocupação significativa, pois pode introduzir patógenos em novas regiões (QUAMMEN, 2012).

De acordo com especialistas, a chave para prever e prevenir a próxima pandemia reside na compreensão dos chamados *efeitos protetores* proporcionados pela natureza intocada. Um exemplo disso é evidenciado na Amazônia, onde um estudo revelou que um aumento de aproximadamente quatro por cento na desflorestação resultou em um aumento de quase cinquenta por cento na incidência da malária. Isso ocorreu devido ao ambiente propício ao desenvolvimento dos mosquitos, transmissores da doença, que prosperam em áreas recém-desmatadas que oferecem a combinação adequada de luz solar e água (ALEXANDER et al., 2018).

O desequilíbrio ecológico provoca sem sombra de dúvidas o surgimento de novas condições de infecção, onde microrganismos encontram espécies que não estão adaptadas a seus mecanismos de patogenicidade (ROBBINS, 2012). Mais um exemplo disso foi a migração do vírus do Nilo Ocidental da África para a América do Norte, que encontrou no novo habitat um novo hospedeiro susceptível, o tordo americano, que se tornou uma fonte de infecção e amplificadora do agente para mosquitos e humanos dos Estados Unidos. Outro exemplo foi a difusão da doença de Lyme que evoluiu em meio aos desmatamentos responsáveis por diminuir a população de predadores, aumentando a população de roedores amplificadores da doença. Essa difusão se deu em parte pela perda de gambás e esquilos que se alimentavam de carrapatos vetores no ambiente de mata (DASZAK et al., 2000).

Muitos dos patógenos que se deslocam entre a vida selvagem, animais domésticos e seres humanos existem nos animais selvagens há um longo período de tempo (DASZAK et al., 2000). Originalmente, esses patógenos não necessariamente causavam doenças, eles evoluíram junto com seus hospedeiros naturais ao longo de milhões de anos (ROBBINS, 2012). Ao longo desse tempo, eles estabeleceram uma espécie de equilíbrio, replicando-se de forma lenta e constante, passando discretamente pela população de hospedeiros. Isso lhes permitiu garantir a segurança a longo prazo, evitando o sucesso a curto prazo na forma de replicação máxima em cada indivíduo hospedeiro. Essa estratégia tem sido bem-sucedida, entretanto, quando os seres humanos perturbam esse equilíbrio, seja invadindo as populações de hospedeiros, caçando para consumo de carne, deslocando ou destruindo ecossistemas, suas ações aumentam o risco, proporcionando oportunidades para que esses patógenos saltem de seus hospedeiros naturais para novos hospedeiros. Esses novos hospedeiros podem ser qualquer animal, mas com frequência são humanos, devido à sua presença intrusiva e abundante nos ambientes, oferecendo um local rico em oportunidades para esses patógenos (QUAMMEN, 2012).

O estudo das doenças em animais selvagens é fundamental para a compreensão e prevenção da emergência de doenças infecciosas. A pesquisa sobre a ecologia de patógenos e a dinâmica das populações de vida selvagem pode levar a estratégias mais eficazes de prevenção e controle. De acordo com especialistas, a abordagem mais eficaz para prevenir futuros surtos em seres humanos é a chamada abordagem de Uma Só Saúde. Este é um programa global que envolve uma comunidade de mais de seiscentos cientistas e outros profissionais, promovendo a noção de que a saúde humana, animal e ambiental estão intrinsecamente ligadas e devem ser abordadas e gerenciadas de maneira holística (MORENS e FAUCI, 2013).

1.2.3. Papel dos morcegos para a manutenção de agentes infecciosos nas populações: uma abordagem de Uma Só Saúde

A ordem Chiroptera é o táxon de mamíferos mais diversificado e geograficamente distribuído (MÜHLDORFER, 2012). Já foi provado que os morcegos fazem parte da nossa antiga fauna nativa. Registos fósseis de Queensland, Austrália, mostram que os pequenos morcegos existem lá há pelo menos 55 milhões de anos, enquanto que a presença humana é muito mais recente, datando apenas de dezenas de milénios (MÜHLDORFER, 2012).

Os morcegos são considerados reservatórios milenares de doenças infecciosas. Por serem animais com histórico evolutivo de milhões de anos, eles passaram por um processo de coevolução com diversos microrganismos, tornando-os resistentes a maioria das doenças causadas por eles. Isso é diferente do que acontece quando esses microrganismos atingem espécies que não evoluíram com eles, o ser humano por exemplo, e sem dúvidas o impacto vai ser grave uma vez que este novo hospedeiro não tem defesas imunológicas específicas para combatê-los (QUAMMEN, 2012). Um exemplo desse impacto negativo foi o que ocorreu na Malásia com a emergência do henipahvirus, que infectava morcegos frugívoros, e em dado momento, pelo constante contato dos porcos domésticos com a saliva de morcegos presente nas frutas caídas das árvores, o vírus saltou para uma nova espécie, e, em seguida, chegou ao ser humano, provocando 106 mortes por distúrbios neurológicos e incapacitantes na região rural do país (CARROLL et al., 2018). Algo parecido ocorreu com o hendravírus na Austrália, porém no ambiente urbano. Com a urbanização, os morcegos mudaram de habitat em busca de alimento, e passaram a transmitir o vírus a cavalos e humanos, com uma mortalidade de aproximadamente 70% (CARROLL et al., 2018).

Acredita-se que o SARS-CoV-2 também tenha surgido dessa forma, pela interação entre humanos, porcos e morcegos na China, entretanto mais estudos são necessários, pois os vírus SARS são complexos em sua história natural (HU et al., 2015). Outras duas epidemias, uma na China e uma na Arábia Saudita, foram causadas por coronavírus que se assemelham muito ao SARS-CoV-2, o SARS-CoV e o MERS-CoV. A SARS surgiu na província de Guangdong no sul

da China e era marcada por febre, dor de cabeça e subsequente aparecimento de sintomas respiratórios, incluindo tosse, dispneia e pneumonia. A doença causada por este vírus era altamente transmissível entre humanos, se espalhando por toda a China e para outros 28 países, provocando 774 casos fatais da doença (HU et al., 2015). A MERS surgiu como uma síndrome respiratória semelhante à causada pelo SARS, e embora menos transmissível, apresentou uma letalidade 15% maior. As descobertas sobre o vírus nipah e hendra levaram os pesquisadores a considerarem os morcegos como possíveis reservatórios desses vírus mortais (HU et al., 2015). Em 2005, um avanço foi feito quando dois grupos de pesquisa independentes relataram, quase que simultaneamente, a descoberta de novos coronavírus relacionados ao SARS-CoV em morcegos-ferradura do gênero *Rhinolophus* na China, que foram denominados coronavírus semelhantes ao SARS (SL-CoV). Estes SL-CoVs de morcegos apresentaram identidade de sequência genômica de 88-90% entre si e 87-92% de identidade com isolados de SARS-CoV humanos. Outros estudos posteriores com morcegos de outras regiões demonstravam essa mesma similaridade, e também, demonstraram que ambos os vírus eram capazes de usar o mesmo receptor para funções proteicas específicas. Havia de fato estreita relação filogenética entre esses dois vírus, sugerindo fortemente que ambos tiveram a mesma origem advinda dos morcegos (HU et al., 2015). No caso do MERS-CoV, após os casos humanos registrados, ele foi identificado em espécies diversas de morcegos. A análise de ancestrais mais recente especulou que o MERS-CoV pode ter saltado de morcegos para camelos há aproximadamente 20 anos na África, com camelos sendo então importados para a Península Arábica (HU et al., 2015). Acredita-se que a maioria, senão todos, os alfacoronavírus e betacoronavírus que circulam atualmente em diferentes mamíferos estão evolutivamente ligados a coronavírus ancestrais originados de morcegos (HU et al., 2015).

A transmissão de microrganismos de morcegos para humanos e outros animais requer o alinhamento de vários fatores, incluindo os determinantes ecológicos, epidemiológicos e comportamentais da exposição a patógenos, e fatores humanos que podem afetar a suscetibilidade à infecção (MÜHLDOERFER,

2012). Acredita-se, por exemplo, que os morcegos urbanos possuem um potencial maior de transmitir doenças do que os demais, pois eles tendem a permanecer no mesmo local e experimentam uma redução na exposição regular a microrganismos que costumavam adquirir na natureza. Essa exposição anterior ajudava a manter a infecção em níveis mínimos. Como resultado, mais morcegos, devido a fatores como má nutrição, perda de habitat e outros elementos, acabam sendo infectados e, conseqüentemente, propagam uma maior quantidade de microrganismos nas áreas urbanas (MÜHLDOERFER, 2012). Outro exemplo é o hábito alimentar, pois morcegos hematófagos possuem uma grande facilidade de transmitir vírus pela saliva, como o vírus da raiva, assim como morcegos insetívoros têm maior chance de portar agentes infecciosos transmitidos por artrópodes. Além disso, a fisiologia dos morcegos, evoluída para as condições de voo, permite uma regulação sanguínea compatível e propícia para o desenvolvimento e manutenção de diversas famílias de patógenos (WANG et al., 2019).

As habilidades de voo desse mamífero influenciam, além de fisiologicamente, geograficamente na ocorrência de novas doenças, pois eles são capazes de voar por longas distâncias em busca de alimento, a depender das condições de relevo da região, e conseqüentemente funcionar como agente migratório (WANG et al., 2019). Os morcegos são conhecidos por suas habilidades de voo excepcionais, e sua capacidade de voo pode variar dependendo da espécie. Em média, a maioria das espécies de morcegos pode voar por distâncias que variam de 20 a 50 quilômetros em uma única noite (WANG et al., 2019). Algumas espécies de morcegos migratórios podem voar muito mais longe em suas migrações sazonais. Um exemplo notável é o morcego-de-cauda-livre, *Tadarida brasiliensis*, que é amplamente encontrado nas Américas. Essa espécie é conhecida por suas longas migrações e pode voar centenas de quilômetros em uma única noite durante suas migrações de primavera e outono. Em algumas regiões, esses morcegos foram registrados voando até 1.600 quilômetros em migrações sazonais (SPEER et al., 2017). Alguns morcegos, como *Desmodus rotundus*, são conhecidos por serem hematófagos e transmitirem raiva para rebanhos animais e populações

ribeirinhas. Pelo fato de se alimentarem excepcionalmente de sangue, eles são muito menos resistentes ao jejum, em função da ausência de células β pancreáticas que é característica desses tipos de morcegos animais (DI TOSTO et al., 2006). Essa ausência impede que o fígado do morcego produza reservas de glicogênio e faz com que a busca por alimento precise ser diária, tornando o seu contato com outros animais maior ainda, aumentando também as possibilidades de interação entre animais e novos patógenos. O potencial de voar longas distâncias, hábitos migratórios, hábitos gregários e longevidade, os tornam um veículo com mais potencial de transmissão de vírus zoonóticos do que outros animais (DI TOSTO et al., 2006).

A relação entre os morcegos e a manutenção de doenças infecciosas nas populações é um tópico complexo que requer uma abordagem abrangente de Uma Só Saúde (DI TOSTO et al., 2006). Os morcegos desempenham vários papéis nesse contexto, pois além de funcionarem como reservatórios e amplificadores de doenças infecciosas, eles têm papel fundamental para o ecossistema dos locais que habitam (SPEER et al., 2017). Um dos papéis mais importantes dos morcegos é na dispersão de sementes, contribuindo para a biodiversidade e a restauração de ecossistemas. Outros dois papéis fundamentais são no controle biológico de insetos e pequenas aves e na polinização, havendo a necessidade desses tipos de animais em certos locais para que não haja o surgimento de pragas e não ocorra a diminuição das áreas verdes (SPEER et al., 2017). Distúrbios ambientais, como o desmatamento, alterações climáticas, construção de barragens e urbanização de áreas florestais podem levar à migração de morcegos e à interação mais próxima com seres humanos e animais domésticos, aumentando o risco de transmissão de doenças e processos de mutação genética. Mudanças naturais no ambiente, bem como aquelas produzidas pelas atividades humanas, promovem novas interações entre microrganismos, vetores e hospedeiros mamíferos (DASZAK et al., 2000).

O campo de pesquisa sobre morcegos e doenças infecciosas envolve diversas disciplinas, como ecologia, epidemiologia, medicina veterinária e saúde pública. Uma abordagem de Uma Só Saúde promove a colaboração entre essas disciplinas, permitindo a compreensão e o gerenciamento dos riscos de doenças

zoonóticas associadas aos morcegos e o reconhecimento da necessidade de se pensar nesses fatores associados para que seja possível alcançar a saúde humana. Essa abordagem é crucial para prevenir surtos de doenças e promover a coexistência sustentável entre morcegos e sociedade (DI TOSTO et al., 2006).

1.2.4. Função dos vetores em contato com os morcegos

A importância dos vetores de morcegos na transmissão de doenças é um tópico de grande relevância na pesquisa em saúde pública e epidemiologia. Vetores, como carrapatos, pulgas e mosquitos, desempenham um papel fundamental na disseminação de patógenos de morcegos para outros animais, incluindo seres humanos. Vetores podem transportar patógenos de morcegos para áreas distantes, ampliando a geografia das doenças. Isso pode ser particularmente relevante em contextos de mudanças climáticas, que podem expandir as áreas de distribuição de vetores.

1.2.4.1. Carrapatos (Ixodoidea)

Os carrapatos são artrópodes que se alimentam obrigatoriamente de sangue. São encontrados em todo o mundo e compreendem mais de novecentas espécies conhecidas por parasitar vertebrados terrestres. A história evolutiva desse grupo remonta a cerca de cem milhões de anos, quando ocorreu uma ampla diversificação genética e dispersão por territórios. Os carrapatos se dividem em duas categorias principais: carrapatos duros, representados pela família Ixodidae, que é a mais numerosa, e carrapatos moles, pertencentes à família Argasidae (SÁNDOR et al., 2021).

Os morcegos, por sua vez, têm uma distribuição geográfica ampla, uma grande diversidade de espécies e também apresentam diversas adaptações evolutivas, e isso os torna hospedeiros ideais para o parasitismo destes artrópodes (SÁNDOR et al., 2021). Os carrapatos moles, embora minoria, possuem distribuição mundial com maior enfoque em regiões tropicais, estando relacionados com a maioria das doenças humanas, e além disso, possuem grande resistência ao jejum extremo e têm maior facilidade de transferir patógenos para outros animais, tornando-os grandes vetores em potencial.

Alguns outros pontos favorecem esse parasitismo, como as adaptações morfológicas para o voo, dificultando a eliminação dos carrapatos pelo morcego; o descanso em cavernas e abrigos diversos, locais com ampla quantidade de carrapatos; e a sociabilidade, aumentando a proximidade de animais livres de ectoparasitas com outros animais infestados (SÁNDOR et al., 2021).

Os dados disponíveis na literatura de um estudo que avaliou morcegos e seus carrapatos na Europa e no Norte da África demonstram que: existem 44 espécies de morcegos infectadas por carrapatos moles atualmente; 76% das espécies de morcegos vivem em fendas de pedras e de árvores; a maioria são tidos como hospedeiros secundários; os estágios mais infectantes são os estágios larvais em 93% dos registros; os patógenos mais detectados nesses morcegos eram *Bartonella* spp., *Borrelia* spp., *Coxiella burnetii* e *Rickettsia* spp. (SÁNDOR et al., 2021).

No Brasil, estudos apontam para essa relação intrínseca na região do Pantanal, onde 1.028 morcegos coletados apresentavam infestações por diferentes espécies de carrapatos, e na região de Caatinga, onde 409 morcegos coletados apresentavam infestações. Os carrapatos mais frequentes eram *Ornithodoros hasei* em ambos os estudos (MUÑOZ-LEAL et al., 2016; LUZ et al., 2016). Nesta espécie de carrapato, um grande estudo da Guiana Francesa, vizinha do Brasil, encontrou uma positividade de 29% (31/354) para espécies do gênero *Rickettsia*. Para *Bartonella*, *Borrelia* e *C. burnetii* não houveram detecções (TAHIR et al., 2016). Entretanto, embora estes agentes não tenham sido descritos em carrapatos no Brasil, essa interação não se exclui, uma vez que são quase inexistentes os estudos com este foco de investigação e uma vez que os morcegos comprovadamente podem abrigar esses agentes no sangue e em órgãos (TAHIR et al., 2016).

1.2.4.2. Pulgas (Siphonaptera)

As pulgas são parasitas hematófagos obrigatórios, mais abundantes e diversos em mamíferos de pequeno e médio porte, com mais de duas mil espécies conhecidas atualmente (KRASNOV et al., 2007). A maioria das espécies de pulgas parasitam roedores, entretanto não são específicas do

hospedeiro e geralmente exploram outros grupos de animais mamíferos, digerindo muito bem o sangue dos morcegos. Por outro lado, morcegos são frequentemente parasitados por pulgas da família *Ischnopsyllidae*, que não exploram outros hospedeiros além dos morcegos. Assim, os morcegos são vistos como alvos potenciais para as pulgas em geral. Isso faz com que, embora não haja descrição de morcegos infestados por pulgas de gatos, isso seja possível, principalmente em ambientes urbanos (KRASNOV et al., 2007).

Em Madagascar e na Argélia, morcegos eram infestados por pulgas *Thaumapsylla* e *Ischnopsyllus intermedius*, respectivamente. Nos Estados Unidos, *Sternopsylla texanus* infestam morcegos, e um genótipo de *Bartonella* já foi descrito (STUCKEY et al., 2017). Uma grande investigação pelo território da Rússia identificou diversas espécies da família *Ischnopsyllidae* em morcegos (ORLOVA et al., 2022). O parasitismo por pulgas de espécies prevalentes de morcegos, assim como a presença de agentes infecciosos nesses parasitas já foram descritas em todo o mundo, e nas Américas tem sido bastante documentada (CHILTON et al., 2000; BERRIZBEITIA et al., 2017; PEARCE et al 2017). Estudo no Canadá observou que o parasitismo tende a ocorrer em poleiros e outros locais de descanso, principalmente em fêmeas reprodutoras que passam a maior parte do tempo nesses locais (CHILTON et al., 2000).

Embora não seja tão comum quanto a transmissão de patógenos por outros vetores, como mosquitos ou carrapatos, houveram relatos de casos em que pulgas estavam envolvidas na transmissão de doenças de morcegos para outros animais (KORINE et al., 2012). Um exemplo notável é a transmissão de *Yersinia pestis*, a bactéria responsável pela peste, de morcegos para outros hospedeiros por meio de pulgas. A relação vetorial entre pulgas e morcegos é um campo de pesquisa em andamento, e os cientistas estão investigando as espécies de pulgas associadas a morcegos, os patógenos que podem ser transmitidos e os mecanismos de transmissão (KORINE et al., 2012).

1.2.4.3. Mosquitos (Culicidae)

Os mosquitos são insetos hematófagos amplamente reconhecidos por sua importância tanto nos ecossistemas naturais quanto na saúde humana.

Quando um mosquito se alimenta de um morcego infectado, ele pode adquirir o microrganismo e, subsequentemente, transmiti-lo a outros animais ou seres humanos. Existem milhares de espécies em todo mundo, cada uma com adaptações específicas ao seu ambiente e ciclo de vida. Regiões tropicais tendem a ter maior diversidade desse tipo de inseto (LAMPO et al., 2000).

Estudos têm revisado casos na literatura em que é possível identificar que mosquitos se alimentaram de sangue de morcegos. No entanto, as informações sobre os mosquitos vetores primários que se alimentam de morcegos ainda são poucas. Alguns flavivírus, como o vírus da dengue (DENV), o vírus Koyose (YOKV), o vírus Tamana (TABV), o vírus Rio Bravo (RBV), o vírus da encefalite japonesa (JEV) e o vírus do Nilo Ocidental (WNV), já foram detectados em morcegos, porém as taxas de viremia e sua função na transmissão de patógenos ainda é pouco definida (MARRERO et al., 2022). Acredita-se que essas infecções tenham ocorrido devido à transmissão por mosquitos vetores, que desempenham um papel importante na disseminação desses vírus entre morcegos e, potencialmente, para outras espécies, incluindo seres humanos (MARRERO et al., 2022). Em relação aos flebotomíneos, os vetores das Leishmanioses, os morcegos demonstram ser uma fonte natural de alimento para eles, e isso ocorre pois eles compartilham os mesmos habitats em cavernas e abrigos, além dos morcegos serem vistos como excelentes reservatórios de espécies de tripanossomatídeos (LAMPO et al., 2000).

De fato, a relação entre morcegos e mosquitos pode ser bidirecional, uma vez que muitos morcegos têm uma dieta que inclui insetos, os insetívoros. Por exemplo, no sudeste dos Estados Unidos, foi observado que morcegos como *Culex quinquefasciatus*, *Culex coronator*, *Culiseta melanura*, *Culex salinarius*, *Culex erraticus* e *Coquillettidia perturbans* foram consumidos entre 12 e 50% dos 180 morcegos amostrados. Essa variação na ingestão de mosquitos pode ser explicada por diversas variáveis, como a prevalência larval dos mosquitos na área, o tamanho e a espécie dos morcegos, a temperatura e as várias condições de estresse presentes. Os morcegos também desempenham um papel importante no controle da população de mosquitos, provando que o contato com

agentes infecciosos transmitidos por eles, podem também ser adquiridos por outras vias (HUGHES et al., 2022).

1.2.5. O Impacto da pandemia da COVID-19 no diagnóstico e no controle de doenças importantes no Brasil

A COVID-19 teve um impacto significativo no diagnóstico e controle de outras doenças infecciosas importantes no Brasil (BRASIL, 2021). Alguns dos principais impactos envolvem os seguintes pontos: (I) No início da pandemia, houve um redirecionamento significativo de recursos de saúde para o combate à COVID-19, incluindo pessoal médico, equipamentos de proteção individual (EPIs) e financiamento. Isso pode ter afetado a capacidade de diagnóstico e tratamento de outras doenças infecciosas; (II) A pandemia levou à interrupção temporária de serviços de saúde em muitas áreas, incluindo consultas médicas de rotina, exames de diagnóstico e programas de vacinação. Isso pode ter levado ao aumento da incidência de outras doenças infecciosas que normalmente seriam controladas ou tratadas a tempo; (III) Durante a pandemia, muitas pessoas ficaram com medo de buscar atendimento médico por receio de contrair a COVID-19 em hospitais e clínicas. Isso pode ter levado a diagnósticos tardios e ao agravamento de doenças infecciosas existentes; (IV) A sobrecarga do sistema de saúde devido ao aumento do número de casos de COVID-19 pode ter afetado a capacidade de resposta a outras doenças infecciosas. Hospitais e unidades de saúde podem ter ficado sobrecarregados, resultando em atrasos no diagnóstico e tratamento de outras doenças; (V) A atenção significativa dada à COVID-19 pode ter afetado a vigilância epidemiológica e o monitoramento de outras doenças infecciosas. Isso pode ter levado a subnotificação e atrasos na detecção de surtos de outras doenças; (VI) Além disso, o impacto econômico e social da pandemia, como o desemprego e a redução de recursos para programas de saúde pública, pode ter tido efeitos indiretos no controle de outras doenças infecciosas, uma vez que medidas de prevenção e controle podem ter sido prejudicadas.

É importante observar que os impactos específicos podem variar dependendo da região e da evolução da pandemia em diferentes momentos. À

medida que uma pandemia evolui e medidas de controle são implementadas, é fundamental que os sistemas de saúde e as autoridades de saúde pública trabalhem para minimizar o impacto negativo nas outras doenças infecciosas importantes, retomando serviços essenciais, promovendo a conscientização e mantendo a vigilância epidemiológica (OPAS, 2020).

Durante a pandemia, surgiu um desafio considerável relacionado à distribuição de tratamentos para a tuberculose, a principal causa de morte por uma única doença infecciosa em todo o mundo, afetando aproximadamente 80% dos países na região da América Latina e do Caribe (MCQUAID et al., 2020). Existe atualmente o Plano Nacional pelo Fim da Tuberculose no Brasil, que previa a redução da incidência para menos de dez casos por cem mil habitantes, bem como a redução da mortalidade para menos de um indivíduo por cem mil habitantes até 2035, entretanto, essa previsão foi prejudicada e provavelmente isso não será possível (HINO et al., 2021). As pessoas com tuberculose ou que tiveram a doença são consideradas vulneráveis à COVID-19, e isso gerou medo da população em buscar tratamento, além da dificuldade do sistema de saúde em providenciar tratamento para todos. A pandemia levou à implementação de medidas essenciais de distanciamento social, as quais impactaram negativamente o acesso ao diagnóstico precoce, o acompanhamento e a adesão ao tratamento da tuberculose (HINO et al., 2021). Além disso, observou-se uma deterioração nas condições de vida e de trabalho das populações mais pobres, aumentando significativamente a vulnerabilidade social, que implica diretamente na epidemiologia da doença (MCQUAID et al., 2020). Outro ponto relevante diz respeito às questões relacionadas à vacinação com *Bacillus Calmette-Guérin* (BCG), especialmente em relação à sua eficácia protetora, a disponibilidade da vacina e a redução de doses aplicadas por conta do negacionismo científico de parte da população (HINO et al., 2021). Esse impacto se deu não somente na tuberculose, mas também em outras doenças respiratórias, onde a redução das internações ocorreu em 45% durante a pandemia, mas a letalidade subiu para 60% (MAURICI, 2023). Os indivíduos com doenças respiratórias crônicas podem ter adiado sua busca por atendimento médico de emergência devido ao receio de contrair o vírus SARS-CoV-2, por serem um importante grupo de risco,

resultando em atrasos no tratamento farmacológico de suas condições. Isso, por sua vez, levou a casos mais graves no momento da hospitalização. Os pesquisadores identificam esse temor como uma das principais razões para a demora na procura por cuidados médicos, o que, por sua vez, está associado a um aumento na taxa de mortalidade (MAURICI, 2023).

O controle de doenças transmitidas por mosquitos também foi fortemente afetado. As informações sobre doenças transmitidas por mosquitos, como a malária, diminuíram em mais de 40% em termos de notificações. No Acre, Estado brasileiro que concentra a maior parte dos casos de malária, a pandemia não afetou o tratamento da doença, no entanto, foi necessário interromper a realização do controle de vetores em residências, o que intensificou o número de casos posteriormente (BRASIL, 2021). Nos primeiros dois meses de 2020, houve um aumento de 139% nos casos de dengue nas Américas em comparação com o mesmo período de 2019. No entanto, após a chegada da COVID-19 ao continente em março, o número de casos notificados de dengue começou a diminuir (OPAS, 2020). Para a febre amarela, o maior impacto se deu na suspensão da vacinação em diversas regiões, além da suspensão da vigilância ativa de focos selvagens da doença nos Estados e municípios, fazendo com que a doença reaparecesse em locais onde há anos já não haviam mais registros (BRASIL, 2021).

Um estudo analisou a evolução dos casos de leishmaniose visceral, leptospirose e malária durante o ano de 2020 no Brasil, com base em dados epidemiológicos registrados no Sistema de Informações Hospitalares do Sistema Único de Saúde (SIH/SUS) (DIAS et al., 2020). Segundo essa pesquisa, no período de 2017 a 2019, a média mensal de internações nos meses de janeiro a agosto foi de 222,25 para leishmaniose visceral, 220,5 para leptospirose e 160,67 para malária. Por outro lado, no mesmo período de 2020, a média mensal de internações foi de 125,38 para leishmaniose visceral, 155,87 para leptospirose e 113,25 para malária. Esses números representam uma redução na média de internações de aproximadamente 43,59%, 29,31% e 29,51%, respectivamente, quando comparados à média dos três anos anteriores à pandemia (DIAS et al., 2020). O que ocorreu não somente para essas doenças,

mas também para as hantavirose e rickettsioses foi uma redução nas notificações acompanhada da redução do número de casos confirmados e do crescimento da taxa de letalidade (DIAS et al., 2020).

A acurácia e confiabilidade dos indicadores de saúde estão intimamente ligadas à qualidade dos registros, ou seja, à precisão das informações coletadas. Em situações em que os sistemas de saúde estavam sobrecarregados, com escassez de profissionais de saúde e a impossibilidade de realizar testes laboratoriais para todos os casos, pode ter ocorrido uma superestimação dos diagnósticos de COVID-19 (SOUZA et al., 2020). Isso afetou a qualidade dos registros e resultou na subnotificação de doenças respiratórias crônicas, doenças febris e doenças com sintomas inespecíficos semelhantes à da influenza, o que implica no alto erro diagnóstico de doenças zoonóticas já subdiagnosticadas corriqueiramente no país (OJETTI et al., 2020). No caso de doenças infecciosas e parasitárias que causam febre, é evidente que a pandemia teve um impacto significativo na coleta de dados e nos registros dessas enfermidades. As estatísticas disponíveis, na verdade, representam apenas uma pequena parte do problema, deixando a maior parte não registrada e, portanto, não documentada (OJETTI et al., 2020).

Entende-se que os recursos são limitados e que as pandemias podem sobrecarregar o sistema de saúde, no entanto, é fundamental manter as operações de rotina para os programas de vigilância em saúde. A Vigilância Epidemiológica, que tem como um de seus principais objetivos a realização de ações preventivas e de controle, requer um compromisso contínuo e sistemático (COSTA et al., 2020). Isso implica que os serviços de saúde devem estar prontos para manter de forma consistente as ações de vigilância, prevenção e controle para todas as doenças e problemas de saúde que fazem parte do escopo da Vigilância em Saúde. A pandemia intensifica as disparidades na área da saúde (MOTA e TEIXEIRA, 2020).

Em nações em desenvolvimento, como o Brasil, a desigualdade social cria um ambiente propício para a propagação de doenças infecciosas de potencial pandêmico. Isso torna desafiador seguir as orientações de saúde, limita o acesso a itens essenciais de higiene e equipamentos de proteção individual, e impacta

negativamente os serviços de cuidados de saúde disponíveis na rede de atenção à saúde. Em soma, doenças zoonóticas importantes passam a ser mais subestimadas e esquecidas do que já eram nos Estados (COSTA et al., 2020).

1.2.6. Zoonoses ocorrentes no Estado de São Paulo e a relação dos morcegos com os agentes etiológicos

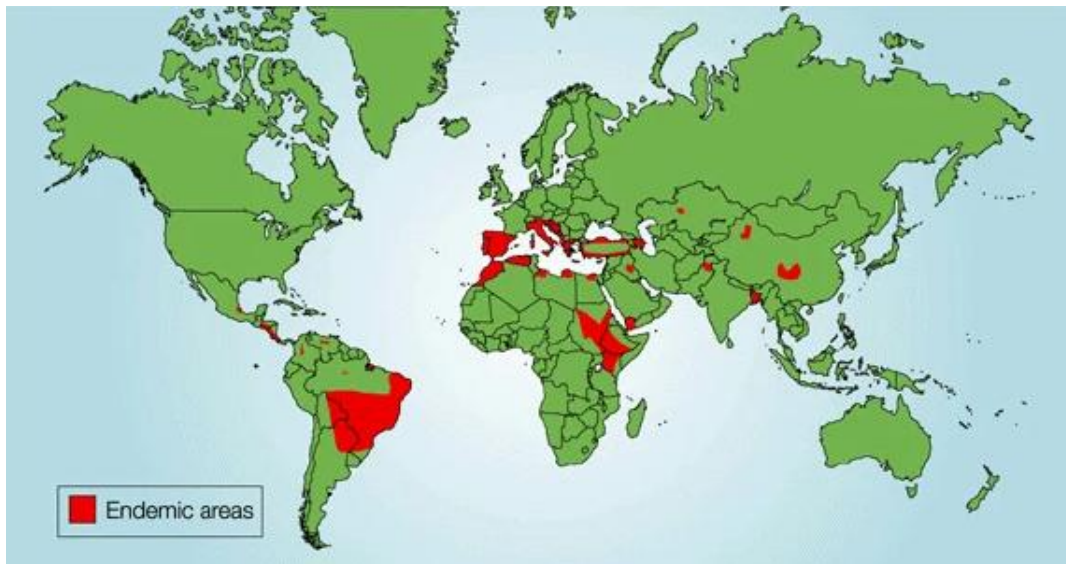
Muitas zoonoses são classificadas como sérios problemas de saúde pública no Estado de São Paulo. Dentre as zoonoses de maior importância, pela sua prevalência, gravidade e letalidade, estão a leishmaniose, a leptospirose e a raiva. Logo atrás, temos a doença de Chagas e a toxoplasmose, que também são prevalentes e em casos específicos também podem levar ao óbito. Algumas outras zoonoses também têm um impacto significativo em gravidade e letalidade, todavia são altamente subdiagnosticadas, e estudos recentes têm demonstrado que estão circulantes em animais e humanos do Estado, como a bartonelose, a febre Q e a febre maculosa brasileira. Os dados e informações sobre estas enfermidades estão descritas a seguir.

1.2.6.1 Leishmaniose Visceral e Cutânea (LV e LC)

As leishmanioses são causadas por protozoários flagelados do gênero *Leishmania* spp. Há cerca de 1,5 milhões de casos de LC e 500 mil casos de LV a cada ano. Uma população de 350 milhões está em risco, com foco em populações de países tropicais e em desenvolvimento (DESJEUX, 2004). Noventa por cento dos casos mundiais de LV ocorrem em cinco países, sendo o Brasil um deles. O mesmo ocorre na LC, onde sete países, incluindo o Brasil, representam 90% dos casos globais da doença (DESJEUX, 2004). Existem atualmente 20 espécies de *Leishmania* e 30 espécies de flebotomíneos vetores. Os principais vetores transmissores da doença são os mosquitos do gênero *Lutzomyia*, conhecidos popularmente como mosquito-palha ou birigui. No Brasil, eventos associados à desmatamento, intrusão em florestas primárias, migração maciça de populações rurais para áreas urbanas e a construção de barragens elevaram a incidência da doença nos últimos anos, pois permitiu o maior desenvolvimento dos mosquito vetores, associado sem dúvidas à situação de

desigualdade econômica encontrada aqui (DESJEUX e ALVAR, 2003). Como ilustrado na Figura 2, a Leishmaniose Visceral apresenta uma distribuição concentrada em áreas tropicais e subtropicais, refletindo a presença dos vetores e condições socioambientais favoráveis à transmissão.

Figura 2. Distribuição mundial da Leishmaniose Visceral



Fonte: Nature Reviews, 2020

A leishmaniose pode atingir vários tipos de animais, como domésticos, sinantrópicos e selvagens, no entanto, os cães configuram a principal fonte de infecção do ponto de vista de saúde pública (ALVAR et al., 2004). Na LC, o animal geralmente não desenvolve sinais graves da doença, porém se torna um risco muito maior uma vez que sua função como reservatório acaba passando despercebida e provavelmente dura muito mais tempo (ALVAR et al., 2004). Na LV, o cão desenvolve sinais graves, gerando perda acentuada de peso e lesões generalizadas pela pele, culminando muitas vezes com o óbito. Até chegar ao óbito, este cão se torna uma potencial fonte de infecção para humanos, podendo acarretar na forma mais grave da leishmaniose para nós (ALVAR et al., 2004).

A principal espécie causadora da LV no Brasil é *L. infantum* (sin. *chagasi*), enquanto que na LC são *L. amazonensis* e *L. braziliensis* com distribuição em todo país, e *L. guyanenses* concentrada na região Norte (ANVERSA et al., 2018). Em humanos, os sintomas da LV vão desde febre, perda de peso e anemia, até

complicações mais graves como esplenomegalia e hepatomegalia. A LC apresenta-se com lesões ulcerativas pela pele e mucosas, coriza, sangramentos nasais e dores de garganta. A letalidade da leishmaniose varia muito a depender da endemicidade da área, que consequentemente influencia na qualidade do atendimento médico e na disponibilidade de tratamento (ANVERSA et al., 2018).

No Estado de São Paulo, durante o período de 1999 a 2019, foram notificados 8.553 casos. Dentre esses casos, 3.046 (35,6%) foram confirmados como autóctones. Desses casos, 266 resultaram em óbito, com uma taxa de mortalidade global de 8,7%. Dos 645 municípios, 107 (16,6%) relataram transmissão humana autóctone durante o período. No triênio de 2017-2019, 14% dos casos ocorreram em pacientes com HIV (SUCEN, 2020). Na Figura 3, 107 municípios paulistas notificaram casos humanos autóctones de LV em 2019.

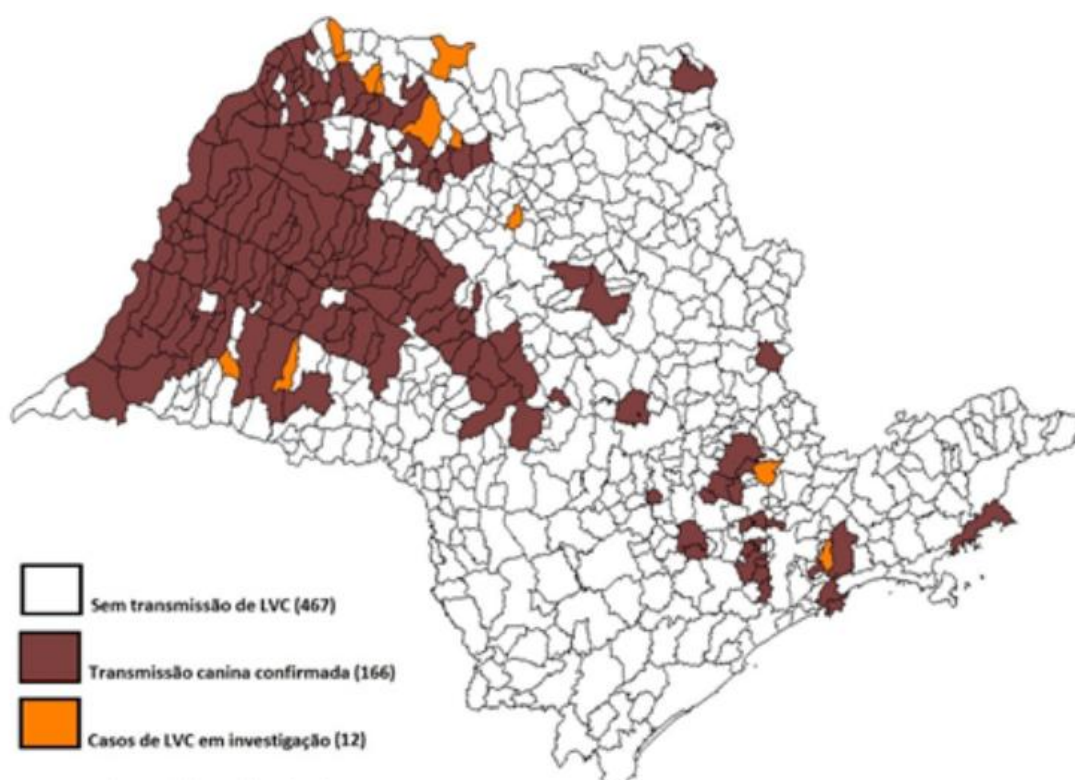
Figura 3. Municípios com casos humanos autóctones em São Paulo, 2019



Fonte: Sinan, Divisão de Zoonoses/CVE/CCD/SES-SP

Durante todo período, 25,7% dos municípios apresentaram transmissão canina confirmada (LCV), e a presença da *Lutzomyia longipalpis* também foi confirmada (SUCEN, 2020). Na Figura 4, A distribuição dos casos caninos em 2019 mostra maior concentração de LVC no oeste do estado.

Figura 4. Municípios com casos caninos (LVC) em São Paulo, 2019



Fonte: Instituto Adolfo Lutz/CCD/SES-SP

Os morcegos são uma fonte de alimento natural para os flebotomíneos, compartilham os mesmos habitats em cavernas e abrigos, além de serem reservatórios de várias espécies de tripanossomatídeos (LAMPO et al., 2000). No Brasil, existem fatores ambientais que favorecem o desenvolvimento desses vetores, por tratar-se de um país tropical, com altas temperaturas e excesso de matéria orgânica (LORD e BROOKS, 2014).

Um estudo brasileiro recente avaliou a detecção de espécies do gênero *Leishmania* em espécies de morcegos desde sua primeira detecção até o presente momento. Do total de morcegos avaliados, 11,6% (251/2167) estavam infectados. Foram analisadas 63 diferentes espécies de morcegos, e dessas, *Leishmania* spp. foram encontradas principalmente em *Molossus rufus* (77,3%, 34/44), *Desmodus rotundus* (22,7%, 15/66), *Artibeus planirostris* (18,7%, 12/64), *Carollia perspicillata* (16,9%, 12/71), *Artibeus lituratus* (16,7%, 20/120), *Molossus molossus* (14,9%, 53/356) e *Glossophaga soricina* (8,6%, 31/361) (RATZLAFF et al., 2023).

L. (L.) amazonensis já foi identificada em diversas espécies de morcegos, assim como *L. (L.) infantum* e *L. (Vianna) braziliensis*. Achados do protozoário em morcegos de áreas endêmicas sugerem uma relação direta com o ciclo da doença nos locais, como é o caso de estudos no Mato Grosso com 28,9% de positivos (70/242), município de Belo Horizonte com 1,67% (2/120), município de Montes Claros com 4,4% (11/247), Espírito Santo com 4,8% (5/105), município de Campo Grande com 22,5 % (18/80) e município de São Paulo com 3,1% (21/683). Os métodos moleculares mais usados consistiam na detecção de genes específicos por PCR convencional e Nested-PCR, com apenas um estudo testando PCR em tempo real nas amostras. Pele e baço eram os tecidos mais avaliados (CASTRO et al 2020, ARAÚJO et al., 2022, DUARTE et al., 2022, RIVA et al., 2021, REZENDE et al., 2017, SAVANI et al., 2010). Curiosamente, em áreas não endêmicas, a positividade também foi encontrada, como no Rio Grande do Sul com 1,2% (5/402) e nas áreas do Triângulo Mineiro e Alto da Paranaíba com 8% (36/448) (RATZLAFF et al., 2022, GÓMEZ-HERNÁNDEZ et al., 2017).

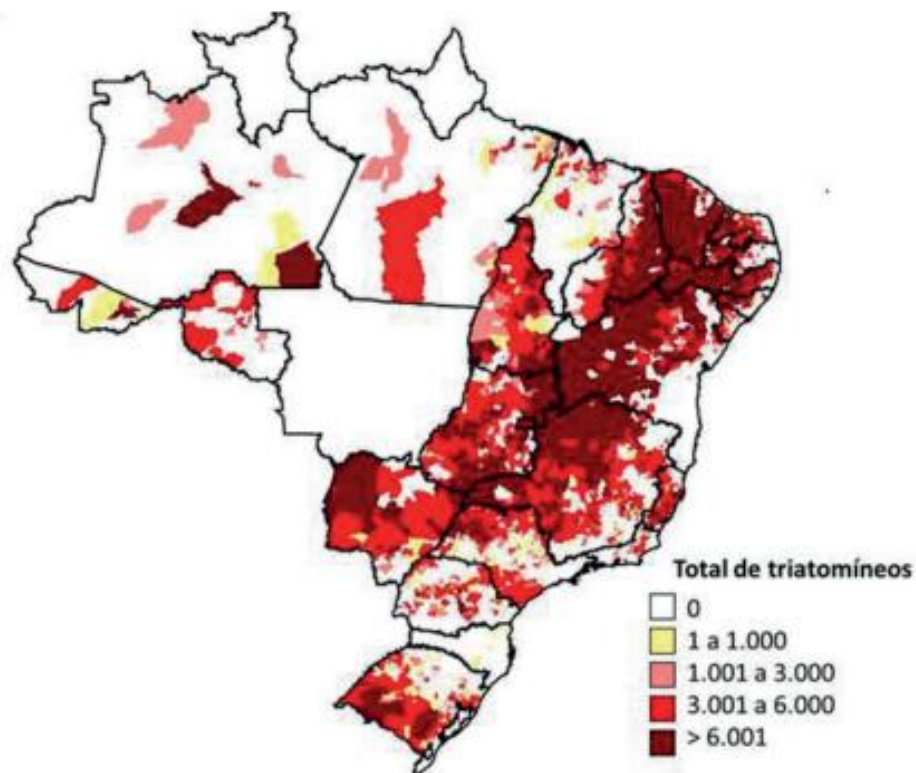
No Estado de São Paulo há carência de investigações em morcegos, considerando o panorama da doença e o número de casos autóctones anuais. Na região central de São Paulo, nenhum estudo foi publicado até o momento. São necessárias mais investigações para doenças negligenciadas, como no caso da leishmaniose. A OMS, juntamente com várias outras instituições, tem trabalhado para estabelecer parcerias público-privadas para atrair mais financiamento para a investigação da leishmaniose e o controle da doença. A disponibilidade de um pacote econômico para a gestão de casos e o controle de vetores deverá proporcionar uma oportunidade para implementar programas de eliminação passo a passo nos principais focos. O principal desafio na investigação da leishmaniose é traduzir o conhecimento derivado da investigação em ferramentas de controle econômicas e acessíveis que melhorem os resultados para as populações susceptíveis a esta doença (DAVIES et al., 2003). O controle da doença baseado na eutanásia animal já se mostrou ineficiente, exigindo das autoridades a implementação de novas alternativas (DANTAS-TORRES et al., 2019).'

1.2.6.2. Doença de Chagas (DC)

A DC é uma enfermidade resultante da infecção causada pelo protozoário *Trypanosoma cruzi* e é uma enfermidade característica do continente americano. Existem cerca de 10 milhões de pessoas infectadas, 25 milhões de pessoas sob risco de contrair a infecção e cerca de 20 mil óbitos anuais por conta da doença (PÉREZ-MOLINA, 2007). A DC ocorre em toda América Latina, mas as manifestações da doença e as características epidemiológicas variam de uma área endêmica para outra, e as taxas de prevalência, as características do parasita, a patologia clínica, os vetores e os hospedeiros reservatórios também diferem amplamente (PÉREZ-MOLINA, 2007). Os vetores do *T. cruzi* são percevejos hematófagos popularmente conhecidos como barbeiros, e tecnicamente chamados de triatomíneos. São descritas atualmente três espécies de importância vetorial, que são *Triatoma infestans*, *Rhodnius prolixus* e *Triatoma dimidiata*, todos amplamente distribuídos pela América Latina (GUARNER, 2019). As espécies selvagens de triatomíneos têm uma distribuição ainda mais ampla, chegando ao norte do continente americano, e são importantes para o ciclo selvagem da doença. Esses vetores possuem hábito noturno e transmitem o *T. cruzi* por meio das fezes durante o repasto sanguíneo (GUARNER, 2019).

No Brasil, estimou-se para a população rural brasileira uma prevalência global de 4,2% de infecção pelo *T. cruzi*, com maiores taxas de prevalência em Minas Gerais e Rio Grande do Sul (8,8%), Goiás (7,4%), Distrito Federal (6,1%), Sergipe (6,0%) e Bahia (5,4%). Foram registrados, de 2000 a 2013, 1.570 casos da doença aguda ou crônica pelos órgãos de saúde dos municípios, enquanto que no Estado de São Paulo, foram somente quatro casos confirmados durante todo o período (BRASIL, 2015). Em 2011, a captura de triatomíneos concentrou-se nas regiões Nordeste e Centro-Oeste do país (Figura 5).

Figura 5. Distribuição de triatomíneos capturados nos municípios, 2011



Fonte: Boletim Epidemiológico/SVS/MS

O protozoário já foi descrito em mais de 100 espécies de mamíferos, entre cães, gatos, roedores, animais selvagens, e, eventualmente, aves e répteis, funcionando como reservatórios importantes do *T. cruzi* (NUNES et al., 2013). A maioria das pessoas infectadas permanecem assintomáticas por toda vida, entretanto, em pessoas imunocomprometidas e idosas a forma aguda e crônica da doença podem se expressar. A forma aguda envolve sinais brandos como febre, mal estar e náuseas, enquanto que a forma crônica envolve complicações graves como cardiomiopatia, megaesôfago, megacólon, distúrbios locomotores e neurológicos (NUNES et al., 2013).

Os vetores do *T. cruzi* estão amplamente presentes nos domicílios e peridomicílios, bem como cavernas e abrigos, podendo ter um contato próximo com morcegos, seja pela alimentação no caso de morcegos insetívoros, como pelo contato desses morcegos com as fezes do percevejo. Um estudo brasileiro no Estado do Acre, região amazônica, analisou 367 morcegos de diferentes espécies, detectando o gênero *Trypanosoma* em 81 amostras. Destas, 16,5%

(60/367) eram *T. cruzi* (SANTOS et al., 2017). O Acre, assim como São Paulo, possui poucos casos humanos registrados da DC na última década. Outro estudo, ainda na região amazônica, detectou 20,3% (15/74) positivos (COURA e JUNQUEIRA, 2015). Um grupo de pesquisa avaliou a presença do *T. cruzi* em morcegos de diferentes regiões do país, incluindo Pará, Rio de Janeiro, Mato Grosso do Sul e Piauí, objetivando espécies de todos os biomas, e o agente foi detectado em morcegos de todas as localidades, resultando em 14% (13/93) de positividade. Tanto espécies de morcegos hematófagos como não hematófagos já foram descritas no Brasil (LISBOA et al., 2008).

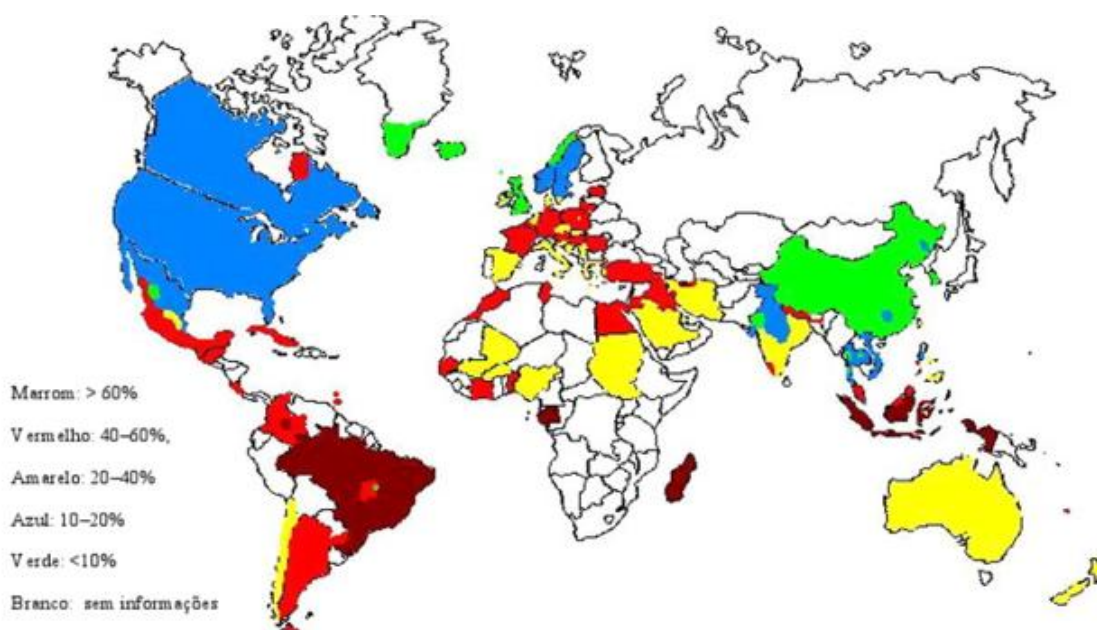
Nos estudos desenvolvidos no Brasil, sangue e hemoculturas foram selecionados para as investigações, e o método de PCR convencional foi utilizado por eles. Algumas cepas apresentam um tropismo preferencial por macrófagos no baço, no fígado e na medula óssea, enquanto outras são muito escassas nesses órgãos. A seleção do melhor tecido para a investigação varia bastante (NUNES et al., 2013). Há carência de investigações nas populações de morcegos brasileiras, uma vez que são espécies que possuem habilidades infinitas de abrigar microrganismos patogênicos e uma vez que estão presentes em todas as extensões territoriais onde os triatomíneos vivem. Em São Paulo, nenhum estudo até a presente data havia sido conduzido.

Para além do triatomíneo, outros mecanismos de transmissão podem contribuir para a produção de novos casos, especialmente através de transfusões de sangue, transfusão de órgãos e da transmissão vertical mãe infectada/filho, durante a gestação (WHO, 2010). Mais do que qualquer outra doença parasitária, a DC está intimamente relacionada a fatores sociais, econômicos, políticos e culturais (VENTURA-GARCIA et al., 2013). Os esforços para interromper a transmissão da doença de Chagas foram bem-sucedidos em vários países e devem continuar: métodos econômicos de controle químico dos vetores e triagem de bancos de sangue estão entre os mais efetivos métodos de combate à doença, e, sobretudo, a melhoria das condições de vida dos mais pobres, por meio de medidas de promoção da saúde (WHO, 2010).

1.2.6.3. Toxoplasmose

A toxoplasmose é uma enfermidade parasitária causada pelo protozoário *Toxoplasma gondii*. Este protozoário está disseminado por todo o mundo, infectando cerca de um terço da população mundial (DUBEY e JONES, 2008). A infecção ocorre principalmente pelo consumo de alimentos ou água contaminados por oocistos liberados nas fezes de hospedeiros animais, como os felídeos que são hospedeiros definitivos e eliminam grandes quantidades de oocistos no ambiente. A ingestão de carne crua ou mal cozida contendo cistos do parasita também é uma possibilidade de infecção (MONTROYA e LIESENFELD, 2004). Como dito anteriormente, muitas pessoas são infectadas pelo parasita, mas não desenvolvem sinais clínicos. Muito se discute sobre complicações que ocorrem quando gestantes adquirem a infecção durante a gestação, e os fatores de risco associados a essa infecção (MONTROYA e LIESENFELD, 2004). O controle da toxoplasmose é extremamente complexo, e exige atenção a todas as variáveis, incluindo o papel da vida selvagem na manutenção de sua endemidade (DUBEY e JONES, 2008). A Figura 6 mostra a variação da soroprevalência de *T. gondii* no mundo, com destaque para regiões da América Latina, África e Ásia.

Figura 6. Soroprevalência global de *Toxoplasma gondii*



Fonte: Pappas et al., 2009.

Cerca de metade das crianças que frequentam a escola primária e a grande maioria das mulheres em idade fértil possuem anticorpos contra o *T. gondii* em seus sistemas. As mulheres que ainda não foram infectadas têm um risco significativo de contrair a toxoplasmose durante a gravidez, uma vez que o ambiente está amplamente contaminado com oocistos do parasita. Além disso, a carga da toxoplasmose em crianças que nascem com a infecção congênita é muito alta (DUBEY et al., 2012). Com base em dados limitados sobre a triagem de recém-nascidos para a presença do *T. gondii*, estima-se que de 5 a 23 crianças nascem com essa infecção a cada 10 mil nascimentos no Brasil. Isso significa que provavelmente nascem anualmente cerca de 2.649 crianças com toxoplasmose congênita no país. A maioria dessas crianças provavelmente apresentará sintomas ou sinais de toxoplasmose clinicamente observável. Entre as crianças infectadas congenitamente, várias não sobrevivem por muito tempo após o nascimento, 35% delas enfrentam problemas neurológicos, como hidrocefalia, microcefalia e retardo mental, 80% têm lesões oculares e 40% sofrem perda auditiva. A gravidade da toxoplasmose clínica em crianças brasileiras pode estar relacionada às características genéticas das cepas de *T. gondii* predominantes em animais e seres humanos no Brasil (DUBEY et al., 2012).

Nos humanos, a infecção tende a ser subclínica, porém quando ela se torna clínica, febre e linfadenopatia são comuns. Sobre tudo, distúrbios reprodutivos em gestantes como abortamentos e nascimentos prematuros podem ocorrer. Além disso, hepatoesplenomegalia e lesões oculares também podem ocorrer em pacientes predispostos (AGUIRRE et al., 2019). A reativação de infecções latentes em pacientes imunodeficientes também preocupa, podendo gerar encefalite grave já diagnosticada em pacientes com HIV (AGUIRRE et al., 2019). Os gatos domésticos são assintomáticos na maioria das vezes, podendo raramente ter febre, inflamação ocular, anorexia, pneumonia e distúrbios neurológicos. Em animais de produção, atenção clínica especial é dada a pequenos ruminantes que costumam apresentar distúrbios reprodutivos graves, como abortamentos e natimortos (AGUIRRE et al., 2019).

A quantidade de espécies selvagens infectadas por *T. gondii* é extensa. As vias de infecção estão associadas à predação de hospedeiros intermediários, consumo de vegetais ou água contaminados por fezes de felídeos infectados, ou por transmissão congênita de taquizoítos (AGUIRRE et al., 2019). Em relação aos morcegos, algumas suspeitas são levantadas, como o consumo de frutas ou água contaminados com oocistos, ou pela transmissão vertical, que embora pouco proposta é uma possibilidade real de acordo com a patogenia do protozoário. O parasita parece ter preferência por se alojar no fígado, cérebro e baço dos morcegos (YANG et al., 2021). Estudo com morcegos da cidade de São Paulo verificou uma soropositividade de 32,6% (201/616) para *T. gondii*, demonstrando exposição dos morcegos locais ao agente (CABRAL et al., 2014). Outros estudos sugerem um papel ativo dos morcegos no ciclo da toxoplasmose no Brasil, como estudo na cidade de Natal que avaliou amostras de tecido de morcegos por PCR convencional (*gene B1*), encontrando 21,6% (11/51) positivos em diferentes órgãos, e estudo na Bahia que investigou amostras de cérebro de morcegos, encontrando 2,1% (2/97) de positivos também pelo PCR convencional (FOURNIER et al., 2014, JESUS et al., 2016). *T. gondii* já foi genotipada em espécies de morcegos da cidade de São Paulo, em morcego não-hematófago e hematófago, apontando a presença desses genótipos anteriormente em outras espécies animais, domésticas e selvagens, sugerindo que este mesmo genótipo esteja circulando por outras regiões brasileiras (CABRAL et al., 2012).

1.2.6.4. Bartonelose

A bartonelose é uma enfermidade bacteriana causada pelas espécies do gênero *Bartonella*, e apresenta-se com diferentes formas clínicas, diferentes hospedeiros alvo e diferentes espécies patogênicas. No Brasil, a sua forma zoonótica mais ocorrente, conhecida como doença da arranhadura do gato (DAG), é causada pelas espécies *B. henselae*, *B. koehlerae* e *B. clarridgeiae* (GUPTILL, 2010). Nos gatos, a prevalência da infecção é muito alta, chegando a 90% em certas localidades, o que dificulta a diferenciação entre infecção e doença em alguns casos (SOLANO-GALLEGO et al., 2004). Essa alta

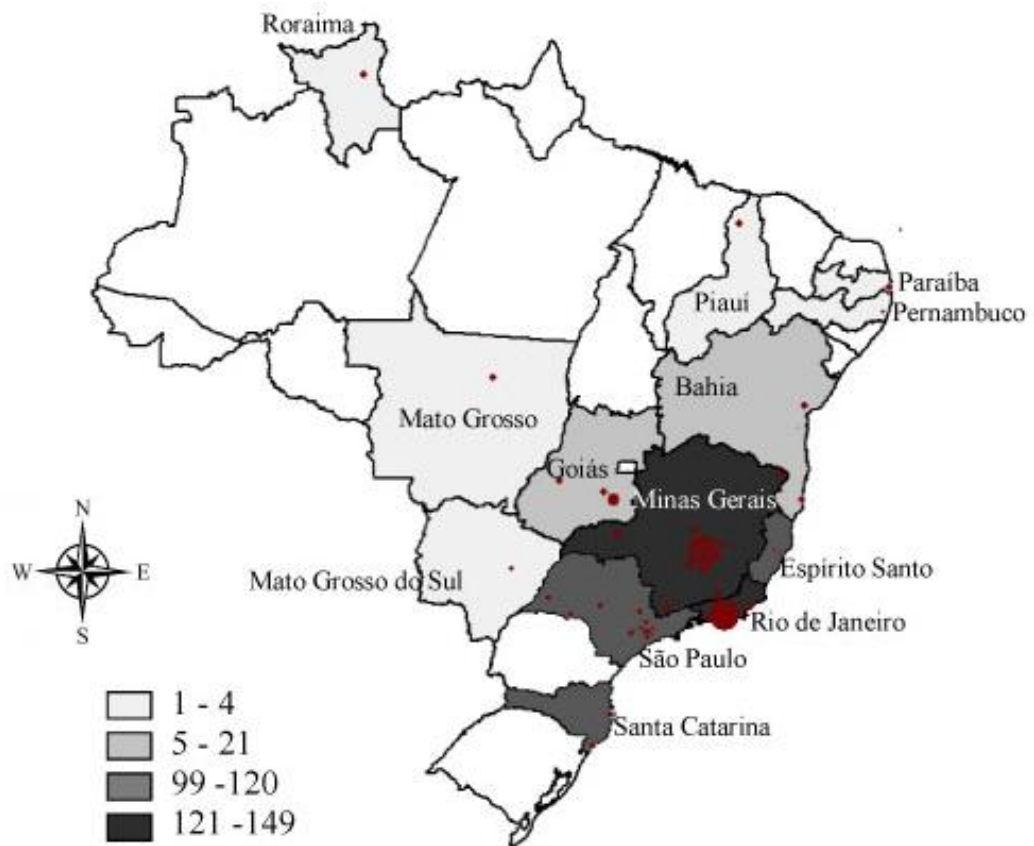
prevalência pode ser justificada pela bacteremia persistente em hospedeiros reservatórios assintomáticos, que é comum e pode facilitar o processo de transmissão do agente (CHOMEL et al 2003). Os gatos aparecem como uma grande preocupação para a epidemiologia da bartonelose, uma vez que na próxima década, estima-se que a população de gatos nos domicílios superará a de cães, e possivelmente a de crianças (CHOMEL et al 2003). A transmissão bacteriana entre os gatos ocorre por meio de pulgas da espécie *Ctenocephalides felis*, via inoculação, enquanto a transmissão entre gatos e humanos se dá a partir de arranhadura ou mordidas. Quanto mais profunda a lesão, maior a possibilidade de transmissão, e é claro, a depender da cepa e da imunidade do indivíduo. Climas tropicais favorecem o desenvolvimento do vetor (GUPTILL, 2010). Além dessa forma, os carrapatos tem sido estudados como potenciais transmissores de *B. henselae* para humanos (GUPTILL, 2010). Adicionalmente, *B. henselae* pode infectar cães, porém as taxas de prevalência são bem menores, até 35%, e não costumam conferir risco de transmissão para humanos (JAMESON et al., 1995).

Nos gatos, a doença é geralmente assintomática, e quando clinicamente notável, febre, uveíte, linfadenomegalia e endocardite podem ser percebidas (SOLANO-GALLEGO et al., 2004). Em humanos, a DAG também tem caráter assintomática, porém, ao persistir no organismo, e mesmo em situações de imunodeficiência ou predisposição, ela pode evoluir para febre recorrente, endocardite, encefalopatia em crianças, linfadenomatia, granulomatose, osteomielite, entre outras complicações crônicas graves (GUPTILL, 2010). Entretanto, vale afirmar, que o potencial de virulência e os efeitos clínicos da infecção em detalhes seguem sem respostas definitivas, além da compreensão de sua epidemiologia (GUPTILL, 2010).

Nos EUA, país onde a doença é frequentemente investigada, são relatados mais de 40 mil casos DAG e cerca de 2 mil hospitalizações por ano (MAGUIÑA et al., 2009). No Brasil há poucos dados disponíveis sobre a ocorrência de casos em nível populacional. A distribuição de amostras reativas à bartonelose em humanos no Brasil, considerando local de ocorrência, data e perfil demográfico dos pacientes, entre os anos de 2011-2017 apontam para

37,94% (480/1265) amostras positivas para anticorpos anti-*B. henselae*. De acordo com a região, o Sudeste foi a região mais reagente com 85,42% (410/480) dos casos, estando o Rio de Janeiro 31,04% (149/480) e Minas Gerais 29,38% (141/480), liderando as prevalências. A população urbana, quando comparada à população rural, foi a mais afetada (SILVA et al., 2019). A Figura 7 mostra maior ocorrência de *B. henselae* no Sudeste, especialmente em São Paulo e Minas Gerais.

Figura 7. Distribuição das amostras positivas para *B. henselae*, 2011-2017



Fonte: Sistema GAL, Ministério da Saúde

Esses dados sugerem a necessidade das autoridades sanitárias se atentarem ao real impacto da doença no país, bem como a influência de reservatórios selvagens na cadeia epidemiológica.

Um estudo realizado com amostras de morcegos de cinco Estados do Brasil (Mato Grosso, São Paulo, Paraná, Pará e Tocantins), visando a detecção do gene *nuoG* por qPCR, revelou uma positividade de *Bartonella* em 5,3%

(17/322) das amostras de fígado, baço e/ou sangue. Nenhuma das amostras sequenciadas se tratavam de *B. henselae* (IKEDA et al., 2017). O mesmo ensaio de qPCR foi usado para investigar a bactéria em morcegos do Maranhão, e das 29 amostras testadas, nenhuma foi positiva (BRAGA et al., 2020). Um outro estudo, este nos Estados do Rio de Janeiro, Santa Catarina e Bahia, avaliaram a presença da *Bartonella* em 18,5% (22/119) amostras de baço por meio de PCR convencional (gene *gltA*), somente a nível de gênero (FERREIRA et al., 2018). De acordo com os achados, espécies de *Artibeus*, *Carollia* e *Desmodus* têm sido identificadas como hospedeiras da bactéria no Brasil. Em outro estudo com amostras do Rio de Janeiro, 3,6% (4/110) de amostras de fígado e/ou baço foram positivas pela PCR convencional (gene *gltA*) (OLIVEIRA et al., 2020). Ao avaliar a presença da *Bartonella* em ectoparasitas de morcegos, 19,8% (40/202) das moscas foram positivas, enquanto nenhum ácaro de 100 foi positivo, ambos por qPCR (gene *nuoG*) (AMARAL et al., 2018). Um estudo amplo com amostras de quinze Estados do Brasil investigou a presença da bactéria em fígado de morcegos hematófagos por meio da PCR em tempo real ITS combinada à PCR convencional *gltA*. Foram encontradas 24,5% (51/208) de amostras positivas, e após sequenciamento, as únicas espécies encontradas foram *B. bacilliformis* e *B. ancachensis* (ANDRÉ et al., 2019). Nenhum dos estudos citados sequenciou ou detectou *B. henselae* ou qualquer outra espécie relacionada à doença da arranhadura do gato em tecidos de morcegos.

1.2.6.5. Febre Q

A febre Q é uma doença humana de caráter febril causada pela bactéria *Coxiella burnetii*. Este patógeno pode infectar animais vertebrados e invertebrados e causar nestes uma doença caracterizada por distúrbios reprodutivos denominada coxielose (FRANÇA et al., 2023). O aumento do conhecimento sobre a doença nas últimas décadas levou à identificação de surtos em todo o mundo, sugerindo que a bactéria está dispersa por todos os continentes. Esta é uma bactéria altamente transmissível e infecciosa, e sua principal e mais importante via de transmissão é a aérea, onde após o parto ou episódios de abortamento animal, a bactéria é dispersa no ambiente,

contaminando-o e tornando susceptíveis os animais e humanos que estejam próximos do local (ANDERSON et al., 2013). A partir do seu local de aerossolização, a bactéria consegue ser carregada pelo vento por até 30km de distância, atingindo populações que não necessariamente têm contato direto com animais infectados, que geralmente são pequenos ruminantes e bovinos, além de uma única célula ser o suficiente para infectar uma pessoa, o que a coloca na categoria B do Centers for Disease Control and Prevention (CDC) como potencial arma de bioterrorismo (ANDERSON et al., 2013).

Nos animais, na maioria das vezes é assintomática, sendo eliminada pelo animal no momento do parto. Por vezes, complicações reprodutivas como abortos, natimortos e partos prematuros podem ocorrer (FRANÇA et al., 2023). Em humanos, os casos assintomáticos são maioria, no entanto, cerca de 35 a 39% dos pacientes podem desenvolver febre, dores pelo corpo e dores de cabeça, sendo associada no momento da avaliação clínica com uma série de doenças infecciosas como primeira suspeita e subestimando completamente a febre Q, e, cerca de 1 a 5% dos pacientes, costumam desenvolver complicações mais sérias, consequência da persistência bacteriana no organismo, como pneumonia, hepatite e endocardite (FRANÇA et al., 2023). Embora a porcentagem de infecções persistentes seja mínima, a prevalência da doença no Brasil não parece ser, e casos de endocardite por *C. burnetii* já foram diagnosticados em hospitais brasileiros, o que sugere uma quantidade muito maior de doenças crônicas que ainda não foram associadas ao agente etiológico (SICILIANO et al., 2008).

São Paulo compreende o maior Estado brasileiro com estudos e relatos da doença, incluindo um surto em trabalhadores de abatedouro do município de Barbosa (21°16'00"49°56'57"), e uma soropositividade de 21,5% (129/604) em pacientes febris suspeitos de dengue em municípios do interior (FRANÇA et al., 2022). Estudos e dados hospitalares sobre a Febre Q foram registrados em diferentes estados brasileiros, com destaque para a região Sudeste e para o Estado de São Paulo (Figura 8).

Figura 8. Relatos hospitalares e estudos envolvendo a Febre Q no Brasil



Fonte: França et al., 2023

Os morcegos são considerados potenciais reservatórios da doença, uma vez que são mamíferos que possuem um ciclo aéreo e muitas vezes tenham ambientes rurais como habitats naturais. O único estudo com morcegos no Brasil foi realizado com amostras dos Estados do Rio de Janeiro, Santa Catarina e Bahia, sendo avaliadas 119 amostras de baço por PCR convencional (gene IS1111) e tendo 3,4% (4/119) positivos. As espécies de morcego positivas pertenciam ao gênero *Artibeus* spp. A prevalência da *C. burnetii* nesses Estados ainda não foi estabelecida e por esse motivo segue-se sem qualquer tipo de possibilidade de associar a infecção em morcegos com a infecção em humanos. Em nosso país vizinho, a Colômbia, um grupo pesquisou a presença da bactéria no sangue de morcegos capturados de cavernas, detectando 6,3% (8/126) de

positivos por qPCR (gene IS1111). A ausência de casos humanos no país também dificulta possíveis associações (SILVA-RAMOS et al., 2022).

O controle da febre Q em humanos está diretamente ligada ao controle da doença em animais, portanto, a avaliação e o diagnóstico sanitário da pecuária por meio da vigilância de abortos, o trânsito de animais sob intenso controle sanitário e, não menos importante, o monitoramento da vida selvagem, incluindo os morcegos, são essenciais para o combate à doenças emergentes (FRANÇA et al., 2023).

1.2.6.6. Febre Maculosa Brasileira (FMB)

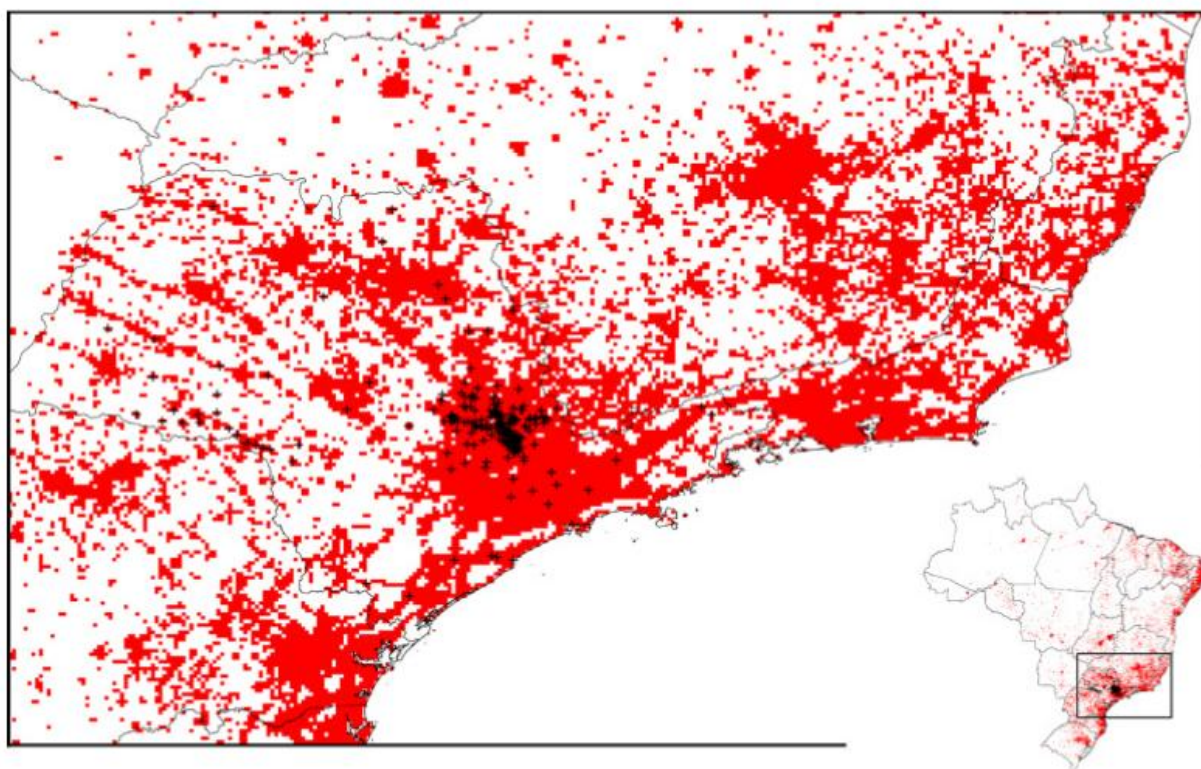
A FMB é uma doença febril grave causada pela bactéria *Rickettsia rickettsii* e transmitida por carrapatos do gênero *Amblyomma*, especialmente *A. cajennense*, popularmente conhecido como carrapato-estrela (LABRUNA et al., 2017). Este carrapato pode estar presente em bovinos, equinos, cães, aves domésticas e alguns animais sinantrópicos, entretanto, um dos seus alvos e o principal reservatório da *Rickettsia* é a capivara (LABRUNA et al., 2017). As capivaras são animais selvagens, mas que possuem uma proximidade grande com humanos, principalmente em locais com coleções hídricas extensas, como lagos e lagoas. Essa proximidade associada a infestação de carrapatos-estrela, propicia uma condição favorável para que a infecção humana ocorra (POLO et al 2017). O controle da doença está sobretudo associado ao controle de carrapatos e medidas de prevenção em locais arborizados ou rurais, principalmente locais onde vivam reservatórios animais (POLO et al 2017).

Em humanos, a doença pode gerar febre, manchas avermelhadas pelo corpo, principalmente na palma das mãos e planta dos pés, dores musculares e articulares, dor de cabeça intensa, náuseas, lesões maculopapulares e distúrbios neurológicos (COUTO et al., 2005). Nos animais, a doença vem acompanhada de febre, perda acentuada de peso, hemorragias, dificuldade respiratória e convulsões. As capivaras, em especial, são resistentes à doença, e por esse motivo se tornam alvos preferidos para os carrapatos e conseqüentemente se tornam um perigo epidemiológico para seres humanos (GRECA et al., 2008). Embora isso ocorra, vale ressaltar que métodos mais

eficazes de controle da doença têm sido estudados, como o controle populacional de capivaras, pelo controle da natalidade e da migração (POLO et al 2017, ROSENFELD et al., 2019).

No Brasil, desde sua primeira descrição em 1929 até 1980 casos esporádicos de FMB haviam sido relatados. De 2000 a 2018, após um grande esforço por parte do Ministério da Saúde, 2090 casos foram confirmados, e deles, 55% resultaram em óbito dos pacientes. De todos os Estados brasileiros, São Paulo foi líder de casos com 982 casos (47%). A região central-leste de São Paulo é considerada uma área endêmica para FMB (DONALISIO et al., 2020). A Figura 9 evidencia a concentração de casos confirmados de febre maculosa brasileira na região central-leste do estado de São Paulo entre 2011 e 2014.

Figura 9. Distribuição de casos de FMB entre 2011-2014 em São Paulo. As cruzes pretas representam os casos confirmados e as áreas em vermelho representam áreas previstas para ocorrência.



Fonte: Sistema de Vigilância Epidemiológica/CVE/SP

Muitas infestações por carrapatos já foram descritas em morcegos, inclusive a presença de infecção mútua de agentes zoonóticos em morcegos e seus parasitas, como no caso de rickettsias patogênicas para humanos encontradas em morcego e seus carrapatos na Suécia (TOMPA et al., 2023). Estudo com morcegos na cidade de São Paulo avaliou a soropositividade de *R. rickettsii*, mostrando que 8,6% (39/451) deles eram soropositivos (D'AURIA et al., 2010). Entretanto, por métodos diretos de diagnóstico, como PCR de tecidos, a positividade não é tão aparente. Nos Estados do Rio de Janeiro, Santa Catarina e Bahia, um estudo com 119 amostras totais de baço não revelou nenhum positivo (FERREIRA et al., 2018). Ainda na cidade de São Paulo, um relato interessante de infecção de carrapatos que se alimentam de morcegos e humanos foi publicado, entretanto, trata-se de uma espécie *Candidatus R. paranaensis*. (DANTAS-TORRES et al., 2022). O mesmo ocorreu no Rio de Janeiro com o sequenciamento de uma amostra positiva para *Rickettsia* spp. em moscas de morcegos, onde encontrou-se similaridade com *Candidatus R. andaenae* (AMARAL et al., 2018). Evidências moleculares de espécies de *Rickettsia* spp. em tecidos de morcegos ainda não foram descritas no Brasil, mas é claro, são poucos os estudos realizados até o momento. Há a necessidade de mais estudos com amostras de outras localidades, inclusive de áreas endêmicas do interior de São Paulo, para se ter mais conclusões da importância dos morcegos no ciclo da FMB.

1.2.6.7. Leptospirose

A leptospirose é enfermidade causada por bactérias do gênero *Leptospira* spp. e possui ampla distribuição mundial. A principal espécie associada a casos clínicos no Brasil é a *L. interrogans* e suas sorovariedades (GUERRA, 2009). A leptospirose é tratada como uma doença de importância global, atingindo em maior proporção populações urbanas superpopulosas, mas também ocorrendo em populações rurais que lidem diretamente com animais de produção (BHARTI et al., 2003). A mortalidade por essa doença é significativa e está associada não só as cepas patogênicas e à resposta imunológica do indivíduo, como também às condições de infraestrutura precárias e com a ausência de um diagnóstico

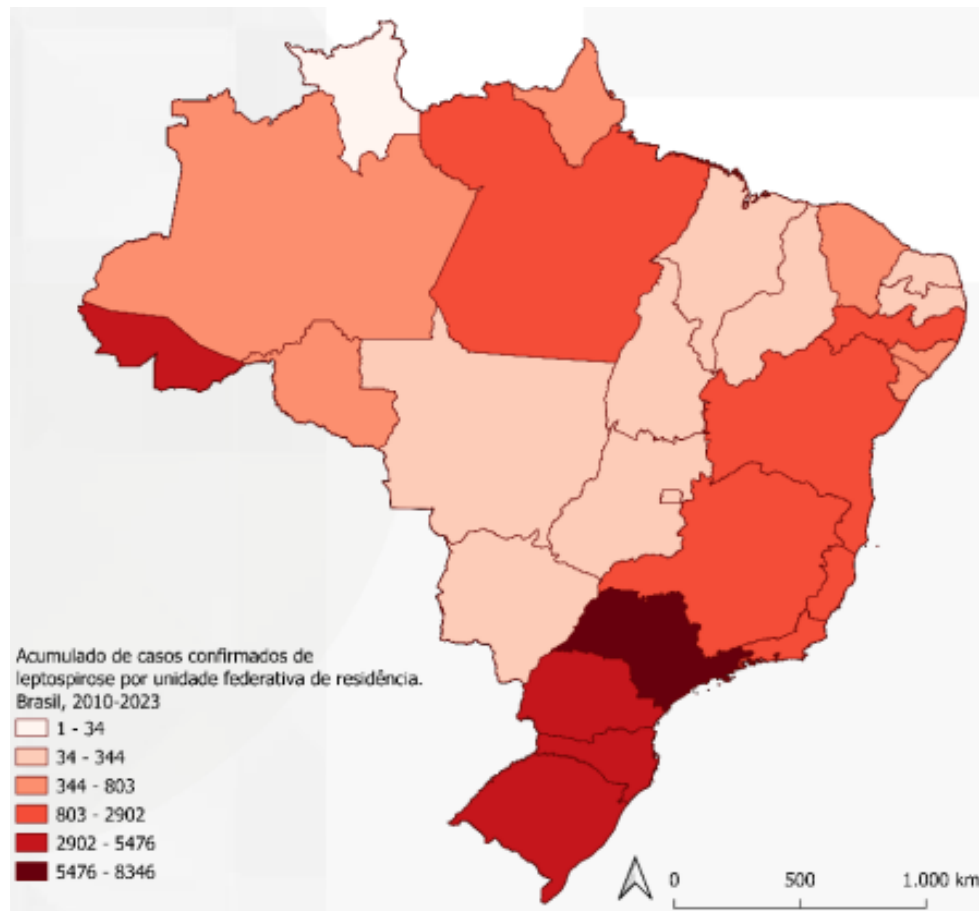
clínico precoce (BHARTI et al., 2003). Existem atualmente descritas quinze espécies patogênicas e 6 não patogênicas, subdivididas em mais de 300 sorovares, que vão variar entre si dependendo da localização na qual elas se encontram (GUERRA, 2009). Os animais domésticos funcionam como reservatórios temporários dessa doença, sendo os ratos urbanos os principais reservatórios, pois além de serem resistentes à doença, se reproduzem e transmitem o agente pela urina com grande facilidade, principalmente em ambientes com excesso de matéria orgânica e coleções hídricas. *Rattus rattus* (ratos de casa), *Mus musculus* (camundongo) e *Rattus norvegicus* (ratazana) são espécies de ratos presentes em praticamente qualquer grande cidade do planeta, e o controle dessas populações segue sendo um desafio (GUERRA, 2009).

Os cães são as espécies animais mais afetadas pela doença e a que com maior mortalidade. Os sintomas nos cães variam de febre, anorexia, gastroenterites e icterícia, até sintomas mais graves como dores abdominais, hemorragias internas e dificuldade respiratória intensa. A insuficiência renal aguda é a consequência mais grave que pode ocorrer nesses animais e geralmente é o motivo dos óbitos (ELLIS, 2015). Nos animais de produção, especialmente os bovinos, a leptospirose possui uma caráter mais reprodutivo, levando à anorexia, abortamentos, baixa produção de leite e desidratação intensa (ELLIS, 2015). Nos humanos, a leptospirose se comporta como uma doença aguda, levando a sinais inespecíficos como febre e dores pelo corpo, mas também a um rápido dano renal, levando a uma insuficiência renal aguda, que na maioria das vezes também é a causa-morte dos pacientes (GUERRA, 2009). Em casos graves, a dificuldade respiratória é tão intensa pelo comprometimento pulmonar que a parada respiratória ocorre, além de outros quadros como meningite e erupções cutâneas pela pele (GUERRA, 2009).

No Brasil, as áreas urbanas são as mais afetadas, principalmente as localizadas nas regiões Sul e Sudeste. De acordo com os últimos censos, os ambientes domiciliares estão mais associados aos casos confirmados e a presença de ratos o maior fator de risco relacionado (BRASIL, 2023). A Figura

10 apresenta a distribuição dos casos confirmados de leptospirose no Brasil entre 2010 e 2023.

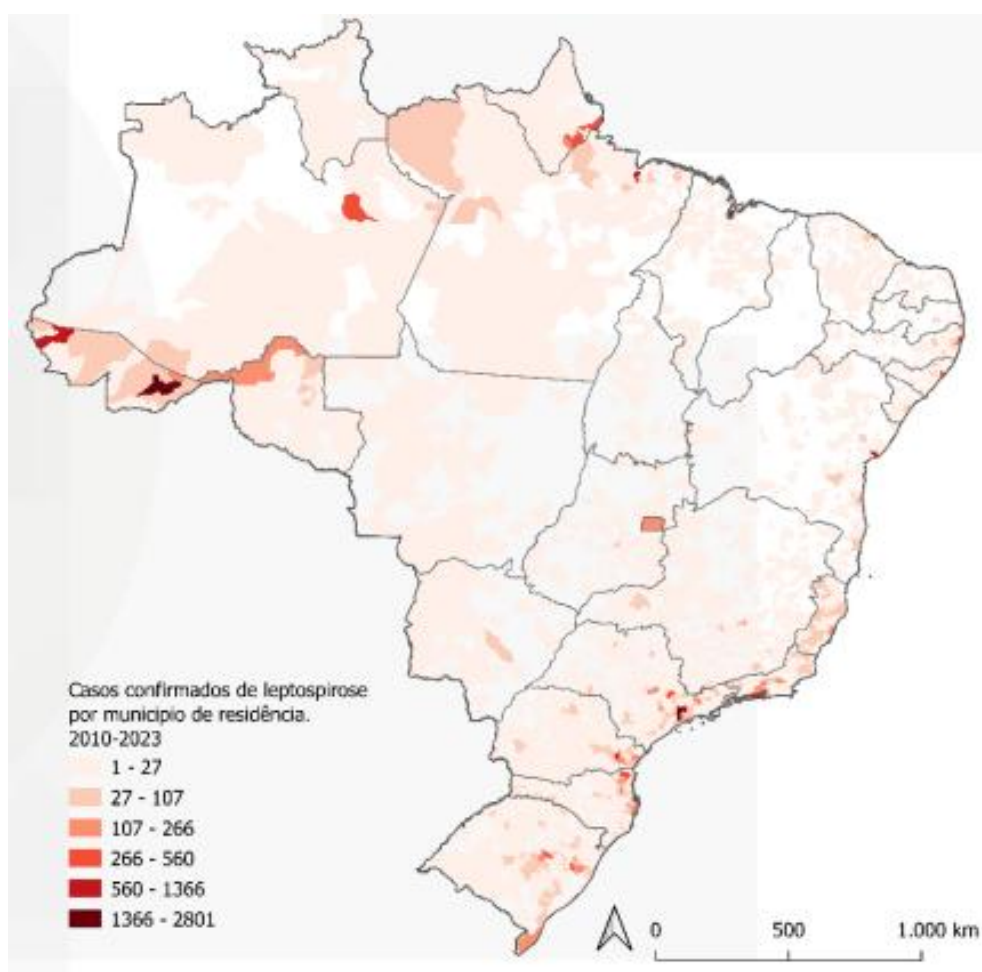
Figura 10. Casos confirmados no Brasil por Estado, 2010-2023



Fonte: Sinan/e-SUS-VS

O mapa a seguir mostra que o Brasil é endêmico para a leptospirose, entretanto o que geralmente acentua o número de casos nos municípios são os surtos epidêmicos que ocorrem em épocas de chuvas e inundações, como o verão, por exemplo, redobrando as cobranças por melhores condições de infraestrutura dos municípios, saneamento básico, controle de roedores e medidas de proteção individual por parte dos cidadãos (BRASIL, 2023). Casos de leptospirose entre 2010 e 2023 foram registrados principalmente em municípios do Sul e Sudeste (Figura 11).

Figura 11. Casos confirmados no Brasil por município, 2010-2023



Fonte: Sinan/e-SUS-VS

Os reservatórios animais são sem dúvidas essenciais para a ocorrência da leptospirose. Assim como no caso dos ratos urbanos, os morcegos carregam inúmeros agentes infecciosos. Já se tem bem definido o papel dos ratos na epidemiologia da doença, entretanto, não se tem definido o papel dos morcegos na manutenção e/ou transmissão da *Leptospira* spp., seja para outros animais, seja para humanos (ESTEVES et al., 2022). Alguns estudos têm começado a pesquisar a influência da vida selvagem nessa cadeia, mas são poucos os estudos envolvendo quirópteros. Um estudo avaliou a presença da bactéria com PCR convencional (gene *LipL32*) em rins de morcegos da cidade de São Paulo, encontrando 1,7% (6/343) de leptospiros patogênicas (BESSA et al., 2010). Estudo no município de Montes Claros realizado com 163 amostras de sangue,

fígado e baço de morcegos da região não encontrou nenhuma amostra *Leptospira* positiva pelo PCR (FERREIRA et al., 2021). Em contraste, no Rio Grande do Sul, dois estudos obtiveram maiores positividade. Um dos estudos avaliou amostras de rim por PCR em tempo real e obtiveram 39% (36/92) de leptospiros patogênicos (MAYER et al., 2017). O outro avaliou amostras de rim por PCR convencional (gene *LipL32*) e 22,5% (23/102) delas foram positivas para leptospiros do grupo *L. interrogans* (ULSENHEIMER et al., 2022). Ainda não sabemos como os morcegos se infectam pela bactéria ou como eles funcionam na epidemiologia da leptospira, e para isso mais estudos são necessários para permitir comparações robustas com dados humanos e avaliar os hábitos dos morcegos. Comparando com estudos de outros países, as positivities encontradas nos nossos morcegos ainda são baixas, principalmente quando comparamos com as dos morcegos asiáticos. Há falta de estudos que avaliassem morcegos de ambientes rurais e que têm contato com animais de produção, como é o caso dos morcegos hematófagos (HAN et al., 2015, RAMOS et al., 2022, ESTEVES et al., 2022, MCCUTCHAN et al., 2023).

1.2.7. Aplicação da biologia molecular no diagnóstico e na determinação de zoonoses nas populações

A biologia molecular desempenha um papel importante no diagnóstico, monitoramento e prevenção de zoonoses nas populações. A capacidade de identificar rapidamente os agentes patogênicos, rastrear sua evolução genética e entender as dinâmicas de transmissão é essencial para proteger a saúde pública e minimizar o impacto zoonótico (TRAUB et al., 2005).

As ferramentas moleculares permitem que o agente etiológico dentro de uma população seja diagnosticado com um maior grau de eficiência e precisão do que as ferramentas diagnósticas convencionais (HIDE e TAIT, 1991). Elas aumentaram a compreensão da relação com o hospedeiro, da patogenicidade e da virulência dos agentes etiológicos, forneceram informações sobre a estrutura genética e taxonomia dos deles e permitiram a determinação do potencial zoonótico de agentes anteriormente não identificados (HIDE e TAIT, 1991). As ferramentas moleculares forneceram uma dimensão à epidemiologia das

doenças que seria inalcançável utilizando ferramentas de diagnóstico convencionais (HIDE e TAIT, 1991). A epidemiologia molecular foi definida por Dorman (1992) como uma ciência que se concentra na contribuição de potenciais fatores de risco genéticos e ambientais, identificados a nível molecular, para a etiologia, distribuição e prevenção de doenças dentro das famílias e entre populações.

Com a biologia molecular é possível a detecção sensível e específica de agentes patogênicos. Isso é feito por meio de técnicas como a reação em cadeia da polimerase (PCR), que amplifica o material genético do patógeno, tornando-o mais fácil de ser identificado (HIDE e TAIT, 1991). O desenvolvimento de técnicas de amplificação de ácidos nucleicos permitiu melhorias adicionais na identificação e taxonomia dos microrganismos. As principais vantagens destas técnicas são a rapidez, o aumento do poder discriminatório e a capacidade de analisar pequenas quantidades de amostra (HIDE e TAIT, 1991). Uma vantagem importante dos procedimentos baseados em PCR sobre os métodos mais tradicionais de técnicas de diagnóstico é a capacidade da PCR para detectar e caracterizar a variabilidade genética de agentes infecciosos, particularmente a nível intraespecífico. Além disso, os testes de PCR, se devidamente concebidos, podem permitir a detecção direta de DNA do parasita a partir de modelos mistos, tais como amostras clínicas ou ambientais, rapidamente e com elevados níveis de sensibilidade e especificidade (TRAUB et al., 2005).

Somada à PCR, o sequenciamento dos ácidos nucleicos permite não só confirmar a presença do agente, como também identificar mutações e variações genéticas em patógenos que podem afetar a transmissão e a virulência. Ele fornece rapidamente grandes quantidades de informação genética e tende a se tornar um dos pilares mais importantes na rotina dos laboratórios de saúde pública (RILEY e BLANTON, 2018). O sequenciamento foi inicialmente caro e demorado, especialmente quando ele era executado de forma manual, porém com o desenvolvimento de sequenciamento de ciclo e dos sistemas automatizados esse custo e tempo reduziram bastante, tornando o sequenciamento em grande escala mais rápido e acessível (RILEY e BLANTON, 2018). O sequenciamento direto de um produto de PCR, é uma abordagem

comumente utilizada para detectar agentes patogênicos, no entanto, se existirem múltiplas sequências relacionadas em um produto de amplificação, quer devido a infecção mista com genótipos semelhantes quer devido a alelos polimórficos, pode ser impossível decifrar a sequência ou o modelo misto pode ser completamente perdido por amplificação preferencial (TRAUB et al., 2005). A clonagem do modelo pode superar este problema, no entanto, este processo é trabalhoso e moléculas variantes ainda podem ser perdidas se existirem em baixas frequências (TRAUB et al., 2005).

Pequenas quantidades de informações resultantes do sequenciamento podem ser utilizadas para a identificação de patógenos a nível de espécie, mesmo que sejam utilizados primers a nível de gênero ou de família, por outro lado, grandes quantidades de informações são essenciais para performar uma análise filogenética (TRAUB et al., 2005). A análise filogenética tem uma grande importância para o estudo da epidemiologia molecular, pois permite uma compreensão sólida da taxonomia e da estrutura populacional do microrganismo, o que é essencial para estudarmos aspectos da biologia e epidemiologia do patógeno, incluindo a sua especificidade ao hospedeiro, diversidade genética e potencial zoonótico (RILEY e BLANTON, 2018). O sequenciamento por PCR em combinação com a análise filogenética tem sido usado para investigar a biologia de uma variedade de agentes, bem como identificar a relação de agentes detectados em diferentes áreas geográficas e a proximidade genética entre eles (RILEY e BLANTON, 2018).

Outro avanço na aplicação de técnicas moleculares à epidemiologia tem sido o desenvolvimento de tecnologia para medir a amplificação de ácidos nucleicos em tempo real, como a qPCR por exemplo, a fim de quantificar o número de parasitas em uma determinada amostra ou medir os níveis de expressão gênica (TRAUB et al., 2005). Uma variedade de produtos químicos pode ser usada para detectar a amplificação de ácidos nucleicos em tempo real, incluindo corantes SYBR Green e sondas Taqman. A amplificação em tempo real tem diversas vantagens em comparação com a amplificação convencional, incluindo quantificação simples de DNA, análise de alta velocidade e análise de alto rendimento (TRAUB et al., 2005). A PCR em tempo real é muito mais

sensível que a PCR convencional, detectando fragmentos que não são possíveis de serem detectados pelos equipamentos convencionais (TRAUB et al., 2005). A capacidade que a qPCR tem de realizar análises em tubos fechados elimina a necessidade de manuseio pós PCR, como eletroforese em gel de agarose, reduzindo o risco de contaminação da amostra e reduzindo o manuseio da amostra, reduzindo também os custos da análise (SCHNEIDER et al., 2005). No caso dos sistemas baseados em sondas é possível detectar polimorfismos de nucleotídeo único, o que aumenta muito a sensibilidade e confirmando o agente a nível de espécie sem a necessidade de sequenciamentos genéticos (SCHNEIDER et al., 2005). Com abordagens moleculares em tempo real, é possível obter mais informações na genotipagem porque é teoricamente possível detectar e diferenciar todos os mutantes em diferentes posições de base dentro da região de ligação da sonda, sendo possível rastrear a origem e a propagação zoonoses nas populações (SCHNEIDER et al., 2005).

A biologia molecular permitiu substituir o PCR pelos isolamentos, que além de serem caros e demorados, eram muito perigosos à saúde dos técnicos (HIDE e TAIT, 1991). Essa substituição influenciou fortemente no diagnóstico precoce de zoonoses em humanos, o que é crucial para evitar surtos e permitir um tratamento direcionado de pacientes em fases agudas de suas doenças. O diagnóstico sorológico, além de não poder discriminar muito bem infecções antigas de recentes, só é possível de ser executado após duas semanas de sintomas, com necessidade de sorologia pareada em alguns casos, prolongando mais ainda o diagnóstico e agravando o quadro clínico dos pacientes (HIDE e TAIT, 1991). Testes moleculares podem, portanto, detectar a presença do patógeno antes que os sintomas clínicos se desenvolvam. Isso se aplica, pensando neste contexto de identificação de reservatórios de doenças, no monitoramento eficiente de focos de doenças e antecipação a possíveis saltos e surtos em humanos (TRAUB et al., 2005). A partir dos tecidos animais, como os dos morcegos, é possível detectar pequenos fragmentos de DNA que não seriam possíveis por outras técnicas que necessitam de condições patológicas específicas, sendo possível a identificação de patógenos importantes nas

regiões. Para o monitoramento de reservatórios, essa é a única técnica capaz de detectar a presença de agentes infecciosos (TRAUB et al., 2005).

As técnicas baseadas em PCR podem ser o único meio de diagnóstico para desvendar o papel dos animais como transmissores mecânicos ou zoonóticos de doenças (RILEY e BLANTON, 2018). É imperativo que em situações como estas, sejam realizados inquéritos simultâneos em populações humanas e animais para correlacionar dados tanto a nível epidemiológico como molecular e para descartar as possibilidades de variação geográfica (TRAUB et al., 2005). Uma boa escolha de marcadores genéticos de acordo com a questão epidemiológica que está sendo investigada é essencial para uma maior sensibilidade de detecção do agente presente na área do estudo, tornando a técnica mais robusta e mais adequada ao contexto epidemiológico. Uma PCR robusta e bem desenhada é uma ferramenta de diagnóstico ideal para estudos epidemiológicos que visam desvendar relações zoonóticas (TRAUB et al., 2005). Para ser utilizada de forma económica e eficiente, uma técnica de PCR deve ser reproduzível entre vários laboratórios, rápida, com um elevado grau de sensibilidade e especificidade, e deve ser capaz de alcançar este objetivo quando aplicada a modelos mistos de várias amostras biológicas, tais como fezes, água, sangue, tecidos, amostras ambientais e vetores. No entanto, mesmo que isto seja alcançado, o sucesso da aplicação de métodos baseados em PCR no estudo da epidemiologia depende da compreensão das limitações e pressupostos das técnicas utilizadas (RILEY e BLANTON, 2018).

A superior precisão dos testes diagnósticos resulta invariavelmente em maior grau de confiança para as estatísticas epidemiológicas, e proporciona uma outra dimensão ao estudo da epidemiologia microbiana (HIDE e TAIT, 1991; TRAUB et al., 2005; RILEY e BLANTON, 2018).

1.2.8. Erradicar sem matar: Em busca de estratégias para mitigar o ciclo das doenças sem afetar o meio ambiente

É importante ressaltar que a maioria das espécies de morcegos não representa uma ameaça significativa à saúde pública e desempenha papel importante no equilíbrio do ecossistema. Em geral, eles apresentam uma notável

diversidade de hábitos alimentares, desempenhando papéis ecológicos importantes (MIKICH e BIANCONI, 2005).

Algumas famílias e espécies são especializadas em diferentes tipos de alimentos. Algumas famílias, como Vespertilionidae e Molossidae, consistem em morcegos que se alimentam exclusivamente de insetos, desempenhando um papel crucial no controle das populações de insetos e contribuindo para a regulação de pragas. Morcegos da família Phyllostomidae exibem uma ampla diversidade em suas dietas, algumas são frugívoras, se alimentando de frutos, enquanto outras são nectarívoras, consumindo néctar, pólen e partes de flores. Esses morcegos desempenham um papel essencial na dispersão de sementes e na polinização de várias espécies vegetais, contribuindo para a regeneração de áreas florestadas (REIS, 2007).

Existem apenas três espécies de morcegos hematófagos, e todas elas são encontradas em regiões neotropicais, incluindo o Brasil. Elas se alimentam de sangue de vertebrados e atuam no controle das populações de suas presas, um papel também importante para o equilíbrio ecológico. Alguns morcegos caçam pequenos vertebrados, como roedores, aves, anfíbios, répteis e até mesmo outros morcegos, reconhecidos, portanto como morcegos carnívoros. Um exemplo de morcego carnívoro é o morcego *Chrotopterus auritus*, desempenhando o mesmo papel de controle populacional que os hematófagos desempenham. Morcegos da família Noctilionidae, como o morcego pescador *Noctilio leporinus*, são piscívoros e têm uma dieta que consiste principalmente de peixes e outros animais marinhos. Eles desempenham um papel importante na ecologia de ambientes aquáticos (REIS, 2007).

Os mitos populares fazem com que as pessoas enxerguem os morcegos como pragas perigosas à nossa saúde, e isso leva à morte de diversas espécies importantes. Muito disso se deve ao período alarmante que o Brasil viveu com a raiva nos anos 90 e do impacto que os morcegos ainda têm na ocorrência de casos humanos descritos nos últimos anos. Existem atualmente cerca de 178 espécies de morcegos no Brasil e apenas três se alimentam de sangue, e mesmo assim, ainda prevalece a ideia de que todos os morcegos são vampiros e capazes de transmitir a raiva, mesmo que a maioria dos morcegos hematófagos

vivam em ambientes rurais próximos a criações de gado e sejam minoria entre os morcegos. A extensão universitária e discussão científica sobre o papel dos morcegos na ocorrência de doenças infecciosas deve ser feita com cautela e responsabilidade (JACOBI, 2003).

Além da caça aos morcegos, a destruição florestal também tem impactado na diminuição das populações de morcegos e na migração destes para áreas urbanas. Claro que a maioria das espécies adaptadas ao ambiente urbano migram por conta de alimento e condições favoráveis para sobrevivência, entretanto o desmatamento tem influenciado nessa migração e na morte de muitas espécies (JACOBI, 2003).

Existem estratégias não-destrutivas para o controle de populações de morcegos em áreas urbanas, que sem dúvida impactam no ciclo das doenças zoonóticas. Estas estratégias podem ser aplicadas em qualquer contexto, seja em pequenas ou grandes cidades, em áreas menos ou mais desfavorecidas, e dependem sem dúvidas do investimento em saúde e de iniciativas políticas municipais (KOTAIT, 2003). Dentre elas, temos: (I) Exclusão Física: Identificar os locais de entrada e saída dos morcegos em edifícios e estruturas e usar barreiras físicas, como redes ou dispositivos de exclusão, para impedir que os morcegos acessem esses locais; (II) Mudança de Habitat: Remover fontes de abrigo e alimentos próximos, como árvores frutíferas e vegetação densa, para desencorajar os morcegos a permanecerem na área; (III) Iluminação: Iluminar áreas propensas a abrigar morcegos, pois eles preferem locais escuros. Luzes brilhantes podem desencorajá-los a se estabelecerem nessas áreas; (IV) Atuação Especializada nos Municípios: Consulta e engajamento de especialistas em vida selvagem ou agências ambientais locais para as melhores práticas e regulamentações específicas da região; (V) Selagem de Aberturas: Fechar todas as aberturas e fendas nos edifícios depois de ter certeza de que os morcegos não estão mais presentes; (VI) Repelentes Sonoros e Visuais: Usar dispositivos que emitem sons de alta frequência ou luzes piscantes para afastar os morcegos, embora seja um método de eficácia limitada a algumas espécies; (VII) Educação Pública: Sensibilizar a comunidade local sobre a importância dos morcegos e promover práticas que minimizem conflitos. O entendimento público

pode ser crucial para o sucesso a longo prazo; (VIII) Monitoramento Contínuo: Após a implementação das medidas, é importante monitorá-las continuamente para garantir que os morcegos não voltem à área protegida e ajustar as estratégias conforme necessário.

O controle populacional deve ser realizado com sensibilidade e respeito ao papel desses animais no ecossistema. Buscar a orientação de profissionais qualificados é fundamental para garantir que as práticas adotadas estejam em conformidade com as leis locais e padrões éticos (KOTAIT, 2003).

ORIGINAL ARTICLE

Detection of *Leishmania infantum* and *Leishmania amazonensis* in Bats From Endemic and Non-endemic Areas of São Paulo State, Brazil

Danilo Alves de França¹  | Sara Zúquete^{2,3} | Mariana Louro^{2,3}  | Maíra Guimarães Kersul⁴ | Benedito Donizete Menozzi¹ | Felipe Fornazari¹ | Gabriela Santos-Gomes⁵ | Isabel Pereira da Fonseca^{2,3} | Helio Langoni¹

¹Department of Animal Production and Preventive Veterinary Medicine, São Paulo State University, Botucatu, Brazil | ²Faculty of Veterinary Medicine, Centre for Interdisciplinary Research in Animal Health, University of Lisbon, Lisbon, Portugal | ³Associate Laboratory for Animal and Veterinary Sciences (AL4Animals), Lisbon, Portugal | ⁴Department of Agricultural and Environmental Sciences, University State of Santa Cruz, Ilhéus, Brazil | ⁵Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade Nova de Lisboa, UNL, Lisbon, Portugal

Correspondence: Helio Langoni (helio.langoni@unesp.br)

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ABSTRACT

Aims: Leishmaniasis is an endemic disease in several regions of Brazil, a tropical country that presents specific environmental conditions that contribute to the development of phlebotomine vectors. This study aimed to detect *Leishmania* species in naturally infected bats from 17 municipalities in the São Paulo state.

Methods and Results: Spleen and liver samples from 203 bats were analysed by real-time PCR and confirmed by conventional PCR followed by gene sequencing. *Leishmania* DNA was amplified by real-time PCR in 6.4% of the bats and by conventional PCR followed by sequencing in 3.4% of the bats. Positive samples were characterised and included in GenBank. *Leishmania* species were confirmed in *M. molossus*, *M. nigricans* and *E. glaucinus* bats. *Leishmania* (*Leishmania*) *amazonensis* and *L. infantum* (syn. *L. chagasi*) were identified. This is the first detection of *Leishmania* spp. in bats in the studied areas. All the positive bats came from urban areas. Insectivorous bats were statistically more positive. There was similarity between our sequences and those of a human isolate and a phlebotomine from the region.

Conclusions: This result points to bats as important possible reservoir of *Leishmania* in Brazil and guides the country's health authorities towards epidemiological surveillance, control and prevention actions in endemic areas.

1 | Introduction

Leishmaniasis are caused by flagellated protozoa of the genus *Leishmania*. Cutaneous leishmaniasis (CL) is a widely geographical distributed disease and endemic in 19 countries; however, of

the nine countries with the highest number of cases, three are from South America, Brazil being the main one (PAHO 2022). This disease occurs in all states of Brazil and human cases has been steadily increasing over the last 20years, with an average of 26,402 cases per year (SINAN/SVS/MG 2010). Visceral

Isabel Pereira da Fonseca and Helio Langoni contributed equally to this study.

Summary

- This study evaluates the bats as possible wild reservoirs for visceral and cutaneous leishmaniasis in the studied area.
- *L. amazonensis*, which causes cutaneous leishmaniasis, and *L. infantum*, which causes visceral leishmaniasis, were detected in spleen and liver samples from these bats.
- The discovery of *Leishmania*-positive bats in urban areas reinforces the importance of epidemiological surveillance to investigate bat community.

leishmaniasis (VL) is widely distributed throughout the Americas, but around 93% of global cases occur in Brazil. It is a systemic disease that mainly affects the most vulnerable age groups, such as children under five and adults over 50, as well as adults with comorbidities or diseases that suppress immunity. If not treated promptly, it can lead to death in more than 90% of cases. In the period 2001 to 2021, there were 69,665 new cases of VL in the Region of the Americas (PAHO 2022). In Brazil, the annual average is around 3500 human cases (SINAN/SVS/MG 2010).

In São Paulo state, 8553 human cases were reported between 1999 and 2019. Of these cases, 3046 (35.6%) were confirmed as autochthonous and 266 resulted in death, with an overall mortality rate of 8.7%. Of the 645 municipalities, 107 (16.6%) reported autochthonous human transmission. In the 3-year period 2017 to 2019, 14% of cases occurred in HIV patients. In general, 25.7% of the municipalities had confirmed canine transmission, and the presence of *Lutzomyia longipalpis* was also confirmed (SUCEN 2020).

The main causative agents of VL in Brazil is *L. infantum* (syn. *L. chagasi*), while in CL is *L. (Viannia) braziliensis* and *L. (Leishmania) amazonensis* with distribution in territory, and *L. (Viannia) guyanenses* concentrated in the northern (Anversa et al. 2018). The causative agents of leishmaniasis have been identified in several types of animal reservoirs, such as dogs, synanthropic animals and wild animals (Brandão-Filho et al. 2003; Dantas-Torres, de Brito, and Brandão-Filho 2006; Marcelino et al. 2011). Some studies have been published on Brazilian bats, but in São Paulo state there has only been one focal study in the capital, and one in the northwest region, while information remains scarce in other parts of the state (Savani et al. 2010; Oliveira et al. 2015).

Bats are a natural source of blood for phlebotomines, as they share the same habitats and are reservoirs for several species of trypanosomatids (Lampo et al. 2000). Brazil is a tropical country with an environment and climate suitable for the growth of phlebotomines, as well as having an important socioeconomic contact that directly influences the maintenance of endemic areas of leishmaniasis throughout the country (Buzanovsky et al. 2020). Knowing that bats are important reservoirs of infectious diseases and that there are scarce studies in São Paulo state evaluating their potential as transmitters or amplifiers of the leishmaniasis, we aimed to detect *Leishmania* species by

molecular method in bats from endemic and nonendemic areas from São Paulo state and to evaluate them as possible wild carriers of leishmaniasis in the state.

2 | Materials and Methods

2.1 | Ethics Statement

Ethics Committee for the Use of Animals, Faculty of Veterinary Medicine and Zootechny, UNESP Botucatu number 0259/2022. Environmental authorisation, ICMBio number 85973-1.

2.2 | Study Area

This study was conducted in 17 municipalities within the São Paulo state, Brazil, an area characterised by significant ecological and urban diversity. São Paulo encompasses a range of habitats, including remnants of the Atlantic Forest biome, which is one of the world's most biodiverse regions. This area is also home to over 70% of Brazil's human population, intensifying the interaction between wildlife, domestic animals and humans due to urbanisation and habitat fragmentation (Oliveira 2007). The study timeline coincided with the COVID-19 pandemic, which presented logistical challenges but also highlighted the importance of zoonotic disease surveillance during times of public health crisis. The COVID-19 has intensified the country's efforts to control the disease, leaving the surveillance of endemic zoonoses, such as leishmaniasis, in the background. There have been 37 million cases and 700,000 deaths by illness in Brazil. There were 6 million cases and 181 deaths in São Paulo alone (Secretariat of Health Surveillance, Ministry of Health, Department of Health Analysis and Surveillance of Noncommunicable Diseases 2021). To ensure representativeness, collection sites were chosen in urban and rural settings across municipalities with varying levels of endemicity for leishmaniasis. Spatial data, including latitude, longitude and habitat characteristics, were recorded for each collection point. These data were later integrated into a geographic information system to analyse the spatial distribution of positive cases. The map of collection sites is shown in Figure 1 (Global Ecoregions n.d.; World Bank Official Boundaries n.d.; Continuous Cartographic Bases n.d.).

2.3 | Sample Collection

A total of 203 bats were collected between 2020 and 2022, with sampling occurring in endemic and nonendemic areas for leishmaniasis. The identification of bat species followed preestablished criteria (Reis et al. 2013). The bat species collected and their respective sample numbers are shown in Table 1.

The collection of vampire bats was conducted by the Agricultural Defense Office as part of the rabies epidemiological surveillance programme in the state of São Paulo. The capture took place directly in the natural and artificial shelters where the bats inhabit, including manholes, tunnels, abandoned houses and caves. These locations were previously identified based on information from local residents,

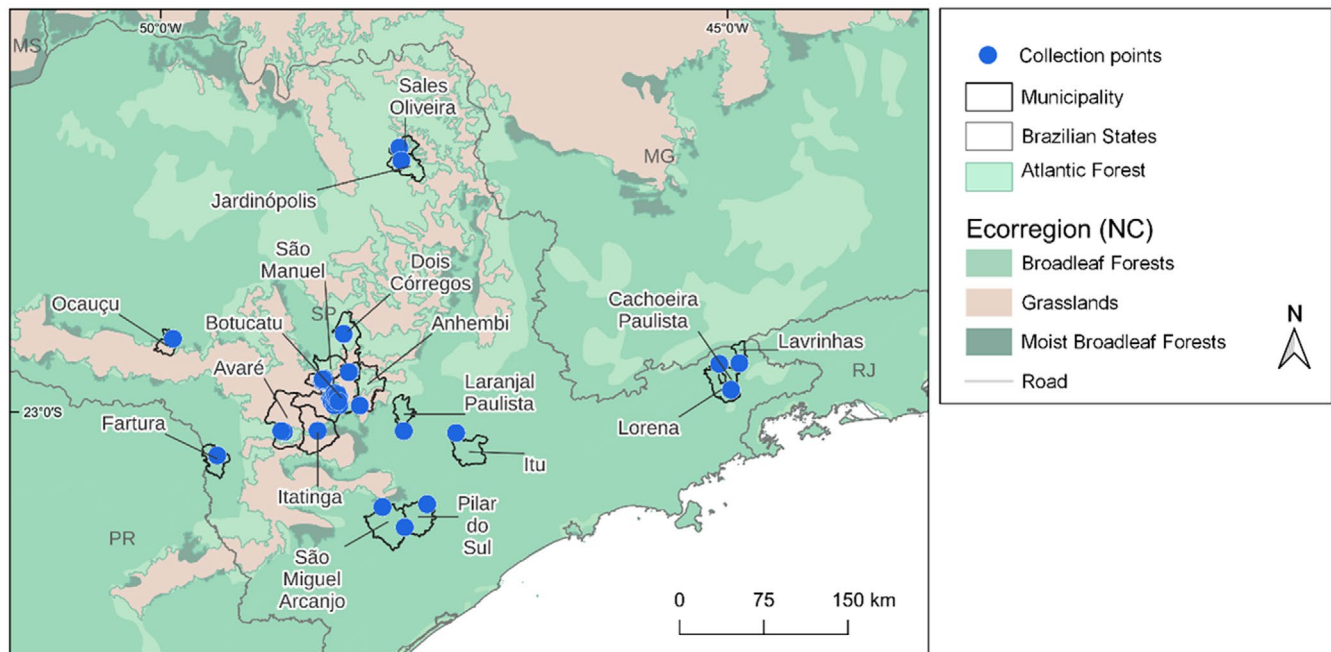


FIGURE 1 | Map of the study's bat collection areas (2020 to 2022). São Paulo state and the municipalities are delimited by dots and dashes. The Brazilian Atlantic Forest is represented in green tones, and the ecoregions are represented in different colours to facilitate evaluation. The bat collection point is characterised by blue markers.

TABLE 1 | Bat species, feeding habits and number of animals per species collected in the São Paulo state, Brazil (2020–2022).

Bat Species	Eating habit	Sampling
<i>Desmodus rotundus</i>	Hematophagous	101 (49.7%)
<i>Molossus molossus</i>	Insectivorous	41 (20.1%)
<i>Molossus rufus</i>	Insectivorous	2 (1%)
<i>Myotis nigricans</i>	Insectivorous	13 (6.4%)
<i>Eumops glaucinus</i>	Insectivorous	24 (11.8%)
<i>Eumops auripendulus</i>	Insectivorous	4 (2%)
<i>Eumops perotis</i>	Insectivorous	2 (1%)
<i>Eptesicus brasiliensis</i>	Insectivorous	3 (1.5%)
<i>Eptesicus furinalis</i>	Insectivorous	2 (1%)
<i>Artibeus lituratus</i>	Frugivorous	4 (2%)
<i>Lasiurus blossevillii</i>	Insectivorous	3 (1.5%)
<i>Nyctinomops laticaudatus</i>	Insectivorous	3 (1.5%)
<i>Histiotus velatus</i>	Insectivorous	1 (0.5%)

environmental studies and continuous monitoring carried out by the surveillance team. Before the collection, an initial inspection was carried out to confirm the presence of bat colonies and assess the safety conditions for the team and the animals. Personal protective equipment (PPE) was used by all professionals involved, including leather gloves, PFF2 respirator masks and impermeable coveralls, to minimise the risk of exposure to zoonotic pathogens. In manholes, entry was made during the daytime, when the bats were resting. Capture nets

were installed near the manhole openings, blocking potential escape routes. Collection was facilitated by careful handling techniques to avoid stressing the animals. In tunnels, a similar method was employed, but with controlled artificial lighting to locate the bats without disturbing them excessively. Mist nets were strategically installed in the passages most used by the bats to ensure efficient capture. In abandoned houses, the team carefully inspected the rooms and access areas where the bats could be sheltering, such as attics, roofs and basements. Portable nets were used to capture the bats in flight, while equipment such as red-light flashlights were used to reduce the impact of direct lighting on the animals. In caves, the approach was more meticulous due to the closed environment and the need to avoid damaging the local ecosystem. Mist nets were placed at the cave entrances and exits before dusk, allowing bats to be captured as they moved to feed. After capture, bats were placed in individually labelled cloth bags for transport to the initial analysis site. Throughout the process, care was taken to minimise stress to the animals, ensuring that handling conditions were compatible with animal welfare. The bats were safely transported to the laboratory.

Bats collected from other species were animals that had fallen into occupied homes in urban areas. These collections were conducted by local city hall teams in response to reports from residents. These events were treated as part of the rabies surveillance programme, ensuring safety for both residents and animals. When a bat was sighted inside a home or occupied area, the team was called to capture it. The process began with an initial inspection of the location to identify where the bat was roosting. The team used appropriate equipment, such as hand nets or capture boxes, to capture the animal without disturbing it or exposing it to unnecessary risk. Preliminary species identification was made on site, based on morphological characteristics,

such as wing shape, body size and facial structure. Captured bats were kept in a cool, ventilated environment to avoid heat stress and taken directly to the nearest laboratory or analysis centre. During transport, the samples were handled with extreme care to ensure that the bats arrived alive at the site where they would be euthanised and necropsied for tissue collection. The handling strictly followed ethical and health guidelines, prioritising the safety of the operators, the quality of the samples and the well-being of the animals.

2.4 | Necropsy Procedures

Upon arrival at the laboratory, the bats were euthanised using isoflurane in accordance with ethical guidelines for animal research. After euthanising the bats with isoflurane, the animals underwent necropsy in a controlled environment for tissue extraction. The entire procedure was performed under aseptic conditions to ensure the integrity of the samples and avoid cross-contamination. The workspace was previously sanitised with a 1% sodium hypochlorite solution and 70% alcohol, and the instruments used, such as scalpels, tweezers and scissors, were sterilised in an autoclave and kept in sterile packages until use. Operators wore personal protective equipment, such as sterile gloves, aprons and masks, and work surfaces were covered with disposable surgical drapes. Brain tissue was removed for rabies testing, and liver and spleen tissues were collected for *Leishmania* DNA analysis. The bats were placed in dorsal decubitus on a sterile dissection table, and an incision was made in the abdominal region along the midline, extending to the sternum to expose the thoracic and abdominal cavity. The ribs were carefully retracted to facilitate access to the internal organs. The spleen was identified and removed using sterile forceps and scalpels, being handled with care due to its fragile consistency. Next, the liver was extracted, prioritising the obtaining of representative portions of the organ. After collection, each tissue was immediately placed in sterile tubes and identified with an individual code. The selection of liver and spleen tissues was based on their high vascularisation, which increases the probability of detecting protozoan DNA. Tissue handling was performed under sterile conditions to avoid contamination. All samples were immediately frozen at -80°C for later processing.

2.5 | DNA Extraction and Quality Assessment

The Illustra Tissue Kit (GE Healthcare Life Sciences, Marlborough, MA, USA) was used, according to its protocol. Subsequently, the purified DNA samples were eluted in $100\mu\text{L}$. To assess DNA quality and ensure suitability for downstream molecular analyses, the concentration of each sample was adjusted to $20\text{ng}/\mu\text{L}$. The DNA purity was evaluated by measuring absorbance ratios at $260/280\text{nm}$ and $260/230\text{nm}$ using a Nanovue spectrophotometer (GE Healthcare Life Sciences, Chicago, IL, USA). An $A_{260/280}$ ratio within the range of 1.8–2.0 was considered indicative of high-quality DNA, free from significant protein contamination. Similarly, an $A_{260/230}$ ratio above 2.0 confirmed minimal presence of residual salts and organic solvents. The extracted DNA samples were stored at -20°C until further use.

2.6 | *Leishmania* DNA Detection by Real-Time PCR

For real-time PCR, a protocol was optimised using 1:10 to 1:10⁷ serial dilutions of a positive DNA sample and, based on cycle threshold (Ct) and the resulting curves, the 1:10³ dilution was selected as positive control standard for subsequent reactions. The extracted material was tested in a real-time PCR assay targeting the putative spermidine synthase 1 gene of 204bp, with the primers CL-SPD forward (5' AGA TCA TTG CGT ACT TGA C 3') and CL-SPD reverse (5' TCA TCG ACA CAA CAG ACC 3') (Wu et al. 2022). The reaction was performed in duplicate in a total volume of $20\mu\text{L}$, containing $1\mu\text{L}$ of each primer, $10\mu\text{L}$ SYBR Green qPCR Master Mix (Promega, Madison, WI, USA), $7\mu\text{L}$ of water and $2\mu\text{L}$ of extracted DNA. The real-time PCR assay was performed (Biosystems™ Step-One model ABI 7500 Fast) in the cycle $1\times$ (95°C for 3'), $40\times$ (95°C for 20", 60°C for 20", 72°C for 20'), $1\times$ (72°C for 10'). The negative and positive controls for the reactions consisted of ultrapure water and DNA from the MHOM/BR/IOC/L2906 strain of *Leishmania infantum*, respectively. Fluorescence was recorded at the end point of each cycle.

2.7 | *Leishmania* DNA Detection by Conventional PCR

The positive samples by real-time PCR have been tested to cPCR reaction flanking the end of the 18S gene and the beginning of the ITS1 ribosomal RNA gene, using primers Leish_18S/ITS1 forward (5' CTG GAT CAT TTT CCG ATG ATT A 3') and Leish_18S/ITS1 reverse (5' AAC ACA AAC ACA CGC CTC CT 3'), primers were manually designed after multiple *Leishmania* sequence alignment on Mafft (<https://mafft.cbrc.jp/alignment/server/index.html>) and visualised on Jalview V2.11.3.3. All sequences used were retrieved from NCBI. The reaction occurred in a total volume of $25\mu\text{L}$, containing $1\mu\text{L}$ of each primer, $12.5\mu\text{L}$ Master Mix (Promega, Madison, WI, USA), $8.5\mu\text{L}$ of water and $2\mu\text{L}$ of extracted DNA. The PCR assay was performed (Applied Biosystems, Foster City, CA, USA), in the cycle $1\times$ (94°C for 5'), $35\times$ (94°C for 30", 61°C for 1', 72°C for 1'), $1\times$ (72°C for 5'). The same negative and positive controls as in the previous reaction were used. Ten microliters of product was run on a $30\text{cm}\times 20\text{cm}$ 1.5% agarose gel at 100 V. To establish molecular weight standards, a Ladder 100bp (Invitrogen, Waltham, MA, USA) was employed to visually assess the amplified fragment sizes. Gel visualisation was performed by transilluminator equipment (Bio-Rad, Hercules, CA, USA).

2.8 | Gene Sequencing

The PCR products were purified using the NZYGelpure Kit (NZYTech, Lisbon, Portugal), following the manufacturer's protocol. The purified products were quantified to confirm adequate DNA concentrations before sequencing. Subsequently, the samples were sent for Sanger sequencing at a certified sequencing facility. The sequences obtained were analysed using the BLASTn algorithm to assess similarity with sequences deposited in the GenBank database, providing taxonomic confirmation and genetic characterisation. Sequence quality was rigorously evaluated

using the BioEdit Sequence Alignment Editor software (version 7.2). The assessment criteria included the absence of overlapping peaks, adequate DNA quantity to generate high-quality reads, smooth peak morphology and uniform base spacing. Sequences with inconsistencies or low-quality regions were either trimmed or excluded from further analyses to ensure reliability.

2.9 | Georeferencing

To characterise the bat collection areas, QGIS software was used to georeference the sampling points and the places where the bats tested positive. The data were collected in the field and entered into a geographic coordinate system, ensuring the accuracy of the location of the samples. The ecoregions were integrated into the map, allowing for a more comprehensive spatial analysis. The data were processed and visualised using layers, making it possible to identify the relationships between the distribution of bats and the characteristics of the ecoregions. This georeferencing process was fundamental for interpreting the results and identifying relevant spatial patterns.

2.10 | Phylogeny

To construct the phylogenetic tree, the sequence data obtained in this study were combined with reference sequences retrieved from the GenBank database. The data set included sequences of *Leishmania infantum* and *Leishmania amazonensis* detected in various hosts, such as dogs, cats, rodents, phlebotomines and humans from the region, to provide a comprehensive representation of genetic relationships among strains. The phylogenetic analysis was conducted using MEGA11 software (version 11.0.13). The tree was constructed employing the neighbour-joining method, a distance-based approach well-suited for analysing genetic relationships. The robustness of the inferred topologies was evaluated through 1000 bootstrap resamplings, ensuring statistical reliability and confidence in the branching patterns. To root the tree and provide an evolutionary context, a sequence from a *Leishmania donovani* isolate was included as the outgroup (Kumar et al. 2018).

2.11 | Statistical Analysis

To explore the associations between *Leishmania* positivity and the variables collected, statistical analyses were performed using SAS Studio (version 3.81, SAS Institute Inc., Cary, NC, USA). The chi-squared test was employed to evaluate the significance of associations between categorical variables, with a significance level set at 0.05. This threshold ensured that observed relationships were statistically meaningful while minimising the likelihood of false positives. The analysis focused on assessing potential correlations between *Leishmania* infection and various factors, such as eating habit, sex, area's endemicity to LC, area's endemicity to LV, collection points, year and area typology.

3 | Results

Leishmania DNA was amplified by real-time PCR in samples of 13 bats, representing 6.4% [CI (95%) = 3.8%–10.7%] of the total of

samples. The positive samples had their dissociation temperature (Melt) vary by a maximum of 1°C compared with the positive control. The Cycle threshold (Ct) of the samples ranged from 28 to 32, indicating good amplification. Of the 13 positive samples, 5 (38.5%) were from *E. glaucinus*, 4 (30.7%) from *M. molossus*, 3 (23.1%) from *M. nigricans* and 1 (7.7%) from *E. perotis*. *Leishmania* was detected in the spleen of all the positive bats, but was only detected in the liver of two *M. molossus* bat. When submitted to conventional PCR, only nine samples were amplified, generating products of approximately 520bp. Genetic sequencing allowed the identification of *Leishmania* in seven samples, including *L. amazonensis* and *L. infantum*. Therefore, *Leishmania* species were identified in 3.4% [CI (95%) = 1.7%–6.9%] of the bat samples. Figure 2 shows the positive bats according to where they were collected. On the map, the collection municipalities are represented in dark lines and the bats sequenced for *Leishmania* spp. are marked in red.

According to the spatial analysis, the São Manuel city, which is endemic for visceral and cutaneous leishmaniasis, had bats infected with *L. infantum* and *L. amazonensis*. On the other hand, Botucatu city, which is endemic only for cutaneous leishmaniasis, also had both species detected. Dois Córregos city, which is not endemic for cutaneous leishmaniasis, had two bats infected with *L. amazonensis*. These cities belong to the same region and are within a 50 km radius of each other.

Statistical analysis revealed an association between insectivore feeding habits and positivity. These bats were 0.7 and 16.6 times more positive than frugivores and haematophages, respectively (Table 2).

The similarity assessment with the sequences available in the database revealed an identity with species of the genus *Leishmania* of over 93.91%. The accession numbers of the sequences obtained in this study are presented in Table 3. The table shows the main information about the samples, including the percentage of identity with deposited sequences.

Figure 3 shows the bootstrap consensus phylogenetic tree. There was similarity between our *L. amazonensis* sequence PP953498 and the *L. amazonensis* sequence from the phlebotomine OK481174. There was also similarity between our *L. amazonensis* sequence PP953508 and the cat *L. amazonensis* sequence OP876785.

4 | Discussion

In this study, we report the detection of *Leishmania* spp. DNA in bats captured in endemic and nonendemic urban areas of São Paulo that have not yet been investigated.

The *Leishmania* species detected in this study have been detected previously in São Paulo (Savani et al. 2010). The findings focused on insectivorous urban bats, while vampire bats were considered free of the pathogen. Insectivorous bats were statistically associated with *Leishmania* positivity. In an overview of infected bat species in Brazil, insectivorous bats are in fact the leaders in detection. This may be because insectivorous bats feed on insects, which are often carriers of *Leishmania*, although the intestinal

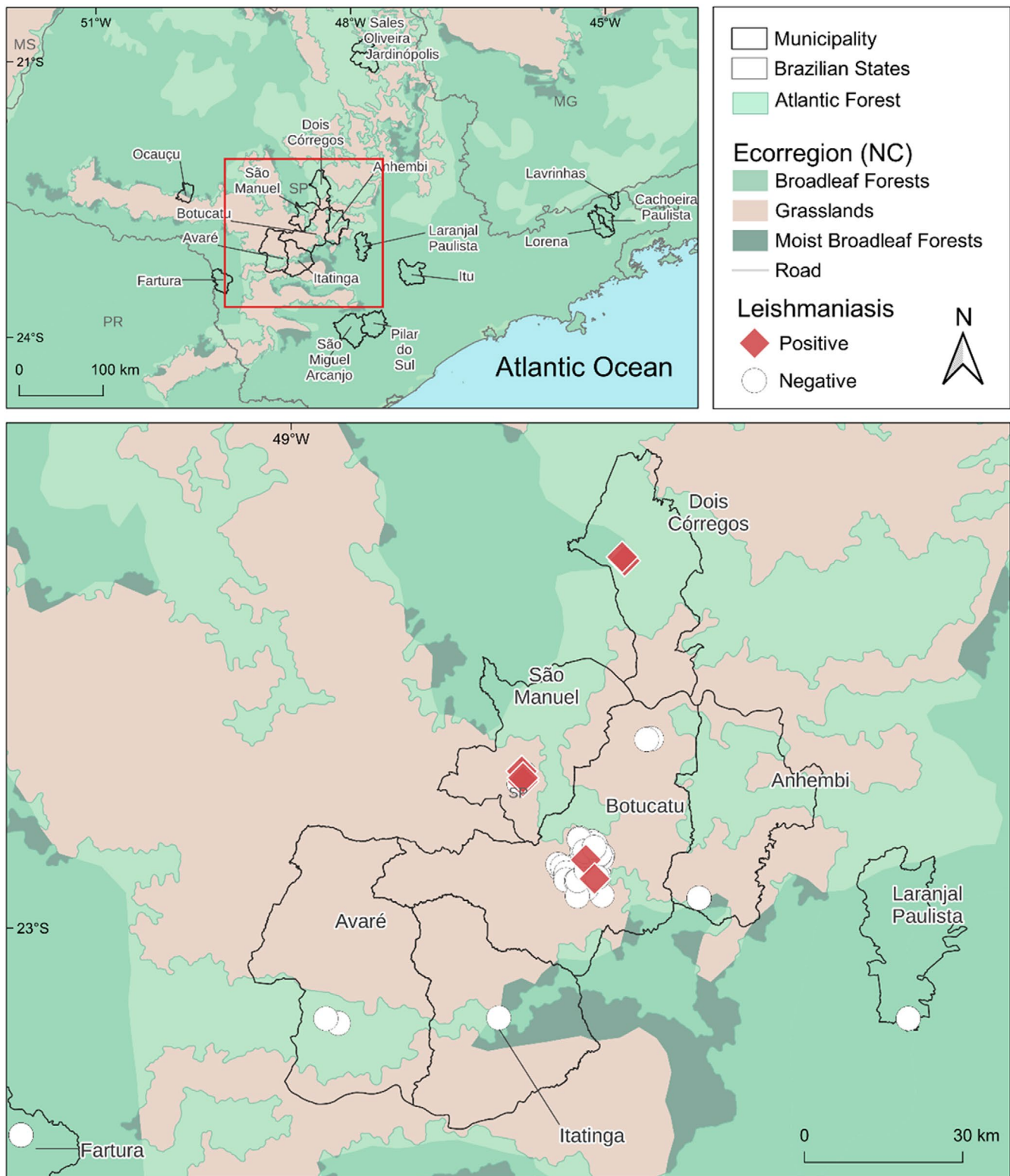


FIGURE 2 | Map of *Leishmania* spp. infection in Brazilian bats from 2020 to 2022. Approximate representation of the areas where *Leishmania*-positive bats were detected, according to the collection site. The red markings represent the points where the seven positive bats were collected.

environment of bats can make it difficult for parasites to survive. On the other hand, fruit bats and hematophagous bats have more specialised diets, which can limit their exposure. In addition, insectivorous bats can exploit a broader range of environments to capture insects, which exposes them to different sources of infection, including bites from phlebotomines (Andriollo, Michaux,

and Ruedi 2021). There are also variations in the susceptibility of different bat species to *Leishmania* infection, influenced by immunological and genetic factors, which are still unknown. It is important to note that in the endemic areas there was a higher concentration of insectivorous bats, and this may have influenced the results.

TABLE 2 | Analysis of variables in relation to the positivity of *Leishmania* infection.

Variables	<i>Leishmania</i> Positive	<i>Leishmania</i> Negative	OR (95% CI)	<i>p</i>	Total population
Eating habit					
Insectivorous	7 (7.1%)	91 (92.9%)	1.0 (ref)	0.0235	98
Frugivorous	0 (0%)	4 (100%)	0.7 (0.03–15.0)		4
Haematophagous	0 (0%)	101 (100%)	16.6 (0.93–295.4)		101
Sex					
Male	5 (5.0%)	95 (95.0%)	1.0 (ref)	0.2494	100
Female	2 (1.9%)	101 (98.1%)	2.7 (0.50–14.0)		103
Area's endemicity (LV)					
Endemic	3 (8.3%)	33 (91.7%)	1.0 (ref)	0.0962	36
Nonendemic	4 (2.4%)	163 (97.6%)	3.7 (0.79–17.3)		167
Area's endemicity (LC)					
Endemic	5 (5.1%)	93 (94.9%)	1.0 (ref)	0.2302	98
Nonendemic	3 (2.9%)	103 (97.1%)	2.7 (0.52–14.6)		105
Collection point					
Occupied houses	7 (6.1%)	108 (93.9%)	1.0 (ref)	0.2023	115
Manholes	0 (0%)	35 (100%)	4.9 (0.27–88.1)		35
Tunnels	0 (0%)	13 (100%)	1.9 (0.10–34.5)		13
Abandoned houses	0 (0%)	38 (100%)	5.3 (0.30–95.4)		38
Cave	0 (0%)	2 (100%)	0.3 (0.01–7.9)		2
Year					
2022	3 (7.5%)	37 (92.5%)	1.0 (ref)	0.3222	40
2021	4 (2.7%)	145 (97.3%)	3.9 (0.63–13.7)		149
2020	0 (0%)	14 (100%)	2.7 (0.13–55.7)		14
Area					
Urban	7 (6.9%)	95 (93.1%)	1.0 (ref)	0.0592	102
Rural	0 (0%)	101 (100%)	15.94 (0.89–282.9)		101

Note: *p* value <0.05 indicates statistical difference within the categories.

Abbreviations: 1.0 (ref.), reference category; OR (95% CI), odds ratio (95% confidence interval).

TABLE 3 | Study sequences generated by amplification of the 18S rDNA gene.

Bat sample	Bat species	Tissue	Bat sex	Ct	<i>Leishmania</i> specie	Closest GenBank match
PP939070	<i>M. nigricans</i>	Spleen	Female	30	<i>L. infantum</i>	94.02% (OR073760)
PP939071	<i>M. molossus</i>	Liver	Male	32	<i>L. infantum</i>	95.90% (OM033710)
PP939072	<i>M. molossus</i>	Spleen	Male	31	<i>L. amazonensis</i>	93.91% (MT940889)
PP953498	<i>M. molossus</i>	Spleen	Male	32	<i>L. amazonensis</i>	99.45% (MT606271)
PP953508	<i>E. glaucinus</i>	Spleen	Male	32	<i>L. amazonensis</i>	99.11% (OP876785)
PP955180	<i>M. nigricans</i>	Spleen	Male	28	<i>L. amazonensis</i>	100.00% (AB709806)
PP955190	<i>E. glaucinus</i>	Spleen	Female	32	<i>L. infantum</i>	94.51% (PP002182)

Abbreviation: Ct, cycle threshold.

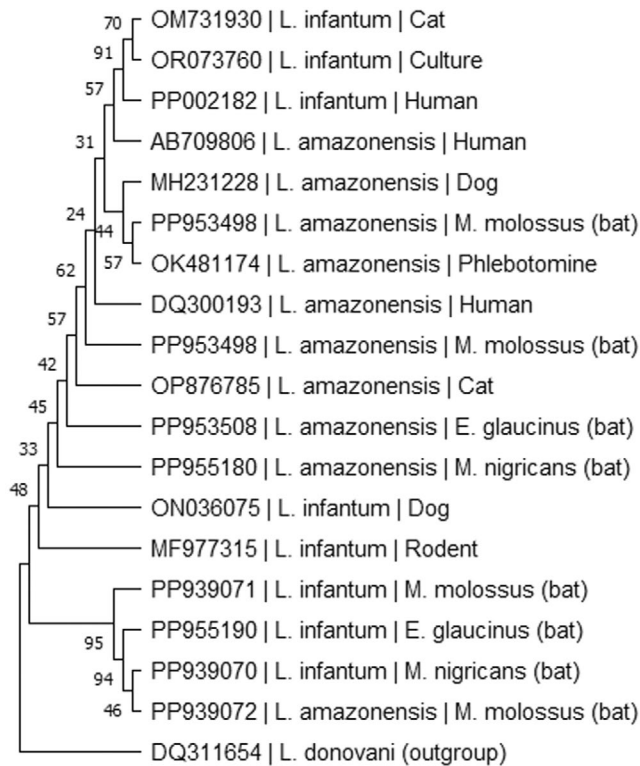


FIGURE 3 | Comparison of the reference sequences of *Leishmania infantum* and *Leishmania amazonensis* from the region with the sequences obtained in this study. The phylogenetic tree was created by the neighbour-joining method in Mega11.

Our study found a considerable difference in the detection of pathogens by the different molecular techniques. The greater sensitivity of real-time PCR for detecting *Leishmania* species was also noted in another Brazilian study, which obtained 23.9% (117/488) positivity using the technique, while conventional PCR followed by sequencing obtained only 4.7% (23/488) (Oliveira et al. 2015).

A recent Brazilian study evaluated the detection of species of the genus *Leishmania* in bat species from their first detection until the conclusion of the study. Of the total number of bats evaluated, 11.6% (251/2167) were infected. Sixty-three different bat species were analysed, and of these, *Leishmania* spp. were found mainly in *Molossus rufus* (77.3%, 34/44), *Desmodus rotundus* (22.7%, 15/66), *Artibeus planirostris* (18.7%, 12/64), *Carollia perspicillata* (16.9%, 12/71), *Artibeus lituratus* (16.7%, 20/120), *M. molossus* (14.9%, 53/356) and *Glossophaga soricina* (8.6%, 31/361) (Ratzlaff et al. 2023). With the results of our study, *M. molossus* continues to be one of the most positive species in Brazil.

In the Espírito Santo state, *L. braziliensis* was detected in 4.76% (5/105) of bats (Riva et al. 2021). In the south region, in the Rio Grande do Sul state, *L. infantum* was detected in 2.7% (3/109) bats (Ratzlaff et al. 2022). In the centre-west region, in the state of Mato Grosso do Sul, *Leishmania* species were detected in 40% (2/5) of the bats in a first study, in 22.5% (18/80) of the bats in a second study and in 20.3% (48/237) of the bats in a third study (Souza et al. 2023; Rezende et al. 2017; Torres et al. 2024). 146 In Triângulo Mineiro and Alto da Paranaíba, *L. infantum*, *L. braziliensis* and *L. amazonensis* were detected in 8% (36/448) of

bats (Gómez-Hernández et al. 2017). In Belo Horizonte, *L. infantum* was detected in 1.67% (2/120) of bats (Araújo et al. 2022). In Montes Claros, in the north of Minas Gerais state, 4.4% (11/247) of the bats were positive for *Leishmania* (Vieira et al. 2022). In this sense, it is difficult to establish an overall picture with few results, but it is possible to observe that the species that occur most frequently in dogs and humans are also those found in bats. Further studies need to be carried out in the northeast and north of the country, which will help to understand bat role in leishmaniosis epidemiology.

Findings of *Leishmania* protozoan in bats from endemic areas suggest a direct relationship with the disease cycle in these places, as is the case in studies from the states of Mato Grosso do Sul (40%, 22.5% and 20.3%) and Espírito Santo (4.8%), and in the cities of Belo Horizonte (1.67%), Montes Claros (4.4%) and São Paulo (3.2%) (Savani et al. 2010; Riva et al. 2021; Souza et al. 2023; Rezende et al. 2017; Torres et al. 2024; Araújo et al. 2022; Vieira et al. 2022). Curiously, in nonendemic areas, positivity was also found, such as in the state of Rio Grande do Sul (1.2%) and in the Triângulo Mineiro and Alto da Paranaíba areas (8%) (Ratzlaff et al. 2022; Gómez-Hernández et al. 2017). According to our study, there were positive bats in both endemic and nonendemic areas, which reinforces previous findings that the confirmation of autochthonous cases in the region and its endemicity may not be related to the infection of bats or any other wild animal. We know about the flight abilities of some bat species and their ability to migrate from one region to another in search of shelter and food, which can easily happen in areas with positive bats, due to their proximity. In the statistical evaluation, there was no association between positivity and the endemicity of the area. There was also no association between positivity and the urban area, although all the positive bats were from urban areas. This may be due to the greater proximity to the canine population and possible infected phlebotomines, which is greater than in rural environments.

The sequences detected in this study were compared with *L. infantum* and *L. amazonensis* sequences from other animal species in the region, revealing interesting points. The similarity between the *L. amazonensis* sequence from a bat and an *L. amazonensis* sequence from a phlebotomine indicates a possible relationship between these two animals, either because the phlebotomine feeds on the bat's blood and transfers the pathogen, or for another reason. The similarity between the sequence of *L. amazonensis* from a bat and a sequence of *L. amazonensis* from a cat alerts the authorities to possible transmission between animals.

The bats in this study were previously evaluated for the presence of *Trypanosoma cruzi*, and detection occurred in 2.97% (6/203) of the bats, including the vampire bat *D. rotundus*. Complementarily, these studies show that bats in the region are susceptible to trypanosomatid infection and may have some impact on the maintenance of leishmaniasis and Chagas disease in São Paulo (França et al. 2024).

The collections took place from 2020 to 2022, a period in which the COVID-19 pandemic was severely affecting the Brazilian population. The allocation of resources to its control prevented efforts to control leishmaniasis from continuing, causing setbacks in the fight against the disease. In

addition, the socioeconomic impact of the pandemic, such as increased unemployment and reduced resources for public health programmes, may have indirectly influenced the control of leishmaniasis, since this disease is linked to important socioeconomic factors (Souza et al. 2020). During this period, it is believed that many febrile illnesses were confused with COVID-19 and resulted in a significant increase in underdiagnosis and underreporting, hindering the control of leishmaniasis at a national level (Secretariat of Health Surveillance, Ministry of Health, Department of Health Analysis and Surveillance of Noncommunicable Diseases 2021).

Health authorities must act to investigate these wild outbreaks of infection and work to investigate potential reservoirs of *Leishmania* in São Paulo. Both VL and CL have autochthonous cases in the cities, and the concern of zoonotic transmission is a serious public health situation. It was important to evaluate bats from other areas of São Paulo, contributing to the situation of bat infection and understanding how they can act in the disease cycle.

5 | Conclusion

This is the first detection of *Leishmania* species in bats in the studied areas. Insectivorous bats were statistically more positive. Real-time PCR was more sensitive than conventional PCR in detecting the pathogen. All the positive bats came from urban areas. Spleen tissue was considered better for detecting *Leishmania* than liver tissue. There was similarity between our sequences and those of a cat and a phlebotomine from the region. This result points to bats as important potential reservoirs of *Leishmania* in Brazil, and guides the country's health authorities towards epidemiological surveillance, control and prevention actions in endemic areas.

Ethics Statement

Ethics Committee for the Use of Animals (number 0259/2022), Faculty of Veterinary Medicine and Zootecny, UNESP Botucatu, Brazil. Environmental authorisation (number 85973-1).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Brief Report

Trypanosoma cruzi in Bats (Chiroptera; Mammalia) from the Brazilian Atlantic Forest, São Paulo State

Danilo Alves de França ¹, Mariana Louro ^{2,3} , Sara Zúquete ^{2,3}, Dayane da Silva Zanini ¹ , Gustavo Nunes de Moraes ¹, Gabrielle dos Santos Rocha ¹ , Leandro Meneguelli Biondo ^{4,5} , Felipe Fornazari ¹, Benedito Donizete Menozzi ¹, Isabel Pereira da Fonseca ^{2,3,†} and Helio Langoni ^{1,*,†}

¹ Department of Animal Production and Preventive Veterinary Medicine, School of Veterinary Medicine and Animals Science, São Paulo State University, Botucatu 18618-681, SP, Brazil; danilo.franca@unesp.br (D.A.d.F.); f.fornazari@unesp.br (F.F.)

² Department of Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal; ifonseca@fmv.ulisboa.pt (I.P.d.F.)

³ CIISA—Centre for Interdisciplinary Research in Animal Health, Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Faculty of Veterinary Medicine, 1300-477 Lisbon, Portugal

⁴ National Institute of the Atlantic Forest (INMA), Brazilian Ministry of Science, Technology and Innovation, Santa Teresa 29650-000, ES, Brazil

⁵ Interdisciplinary Graduate Studies, University of British Columbia, Kelowna, BC V1V 1V7, Canada

* Correspondence: helio.langoni@unesp.br

† These authors contributed equally to this work.

Abstract: The causative agent of Chagas disease is *Trypanosoma cruzi*, which is widely distributed throughout the South American continent and extends into North America. Its occurrence in bats is poorly described and may impact the disease's maintenance and epidemiology. The aim of this study was to detect the agent by PCR assays targeting kDNA and nuclear DNA in the organs of 203 urban bats and rural vampire bats from the Brazilian Atlantic Forest, São Paulo state, during the pandemic period from 2020 to 2022. In total, 6 of the 203 bats (2.97%) were positive for *T. cruzi*. Infection was detected in 2% (2/101) of *Desmodus rotundus*, 33% (1/3) of *Nyctinomops laticaudatus*, 25% (1/4) of *Artibeus lituratus*, 4% (1/24) of *Eumops glaucinus* and in 2% (1/41) of *Molossus molossus*. The gene sequences obtained were assessed for quality and deposited in a public repository. Fruit bats were statistically associated with positivity for *T. cruzi*. To our knowledge, this study detected *T. cruzi* for the first time in bats from São Paulo state and in *N. laticaudatus* and *E. glaucinus* species.

Keywords: emerging disease; vampire bats; urban bats; molecular epidemiology; PCR; phylogeny



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1. Introduction

Around sixty percent of emerging infectious diseases affecting humans are zoonotic, and more than two thirds of them originate in wildlife [1]. Microorganisms often hide in reservoir hosts, sheltering them chronically while they suffer little or no illness. When an ecological disturbance occurs, these agents reveal themselves [2]. Experts say that in the last fifty years, the number of emerging diseases has increased fourfold, mainly due to the constant expansion of areas inhabited by humans into animal habitats, especially in tropical regions identified as hotspots for the spread of diseases around the world [3].

Bats, due to their wide genetic diversity, can be a favorable environment for the mutation and evolution of pathogens, and these genetic changes can give rise to more virulent or more transmissible variants that pose a threat to global health [4]. The detection of diseases in bats can serve as an early warning indicator of the presence of dangerous pathogens. This can provide valuable information for preventing and controlling infectious disease outbreaks in humans. In addition to human invasion of their natural habitats, ecological imbalance and the process of migration in search of food also favor the spread of pathogens [4].

Chagas disease is a disease characteristic of the American continent and is the result of infection caused by the protozoan *Trypanosoma cruzi*. The vectors of *T. cruzi* are *Triatoma infestans*, *Rhodnius prolixus*, and *Triatoma dimidiata*, all widely distributed throughout South America. The presence of infected reduviids extends into North America [5].

Triatomines are widely present in homes and peridomestic areas, as well as in caves and shelters, and can have close contact with bats, mainly by feeding on these bats, in the case of insectivorous bats. There are currently around 10 million people infected, 25 million people at risk of contracting the infection and around 20,000 deaths a year from the disease [5]. In Brazil, the largest country in South America, 1570 cases of the disease were recorded by health agencies between 2000 and 2013. In the state of São Paulo, there were only four confirmed cases during the entire period [6].

In 2018, a Brazilian study in the state of Acre, in the Amazon region, analyzed 367 bats of different species, detecting the genus *Trypanosoma* in 81 samples. Of these, 16.5% (60/367) were *T. cruzi* [7]. It is worth noting that Acre, like São Paulo, has had few recorded human cases of Chagas disease in the last decade. Another study in the Amazon region detected 20.3% (15/74) of *T. cruzi* in bat samples [8].

There is a lack of research on Brazilian bat populations. According to our knowledge, no studies on bats have been carried out in the state of São Paulo, Brazil's most populous region and home to an extensive area of Atlantic Forest, a biome where there is a great diversity of urban bat species and vampire bats in rural areas. In order to understand the dynamics of *T. cruzi* infection in bats in the state of São Paulo and expand research into the role of bats in the epidemiology of Chagas disease, a molecular investigation was carried out using organs of bats of different species and feeding habits from different regions of the state, as well as phylogenetic analysis of the sequenced agents.

2. Materials and Methods

2.1. Ethics Statement

The Ethics Committee for Animal Use of the School of Veterinary Medicine and Zootechnics, São Paulo State University, Brazil (protocol number 0259/2022) approved this study. We obtained environmental authorization through the Biodiversity Authorization and Information System (SISBIO) from the Chico Mendes Institute for Biodiversity Conservation (ICMBio), protocol number 85973-1.

2.2. Study Design and Sampling

A total of 203 bat samples were obtained from São Paulo state, where part of the Brazilian Atlantic Forest is located. There were samples from nine bat genera and fourteen species: *Desmodus rotundus* (n = 101); *Molossus molossus* (n = 41); *Molossus rufus* (n = 2); *Myotis nigricans* (n = 13); *Eumops glaucinus* (n = 24); *Eumops auripendulus* (n = 4); *Eumops perotis* (n = 2); *Eptesicus brasiliensis* (n = 3); *Eptesicus furinalis* (n = 2); *Artibeus lituratus* (n = 4); *Lasiurus blossevillii* (n = 3); *Nyctinomops laticaudatus* (n = 3); *Histiotus velatus* (n = 1).

The Atlantic Forest is made up of several forests and ecosystems of its own, with more than 2000 native animal species, including birds, mammals, reptiles, amphibians and fish, covering 15% of Brazil's territory. More than 70% of the Brazilian population lives in this area, so the biodiversity of the forests is very close to human beings. The forests of this region have suffered from deforestation and human invasion over the last few centuries, and currently, only 7% of the original forest remains [9]. The samples were obtained in the years 2020 to 2022, a pandemic period that resulted in around 37 million confirmed human cases of COVID-19 and 700,000 deaths in Brazil. In the state of São Paulo alone, where this study was carried out, there were more than 6 million confirmed cases and 181,000 deaths, which led to a prioritization of dealing with the disease in the country and a consequent deficiency in the control of other prevalent zoonotic diseases [10]. Figure 1 shows the study area [11–13].

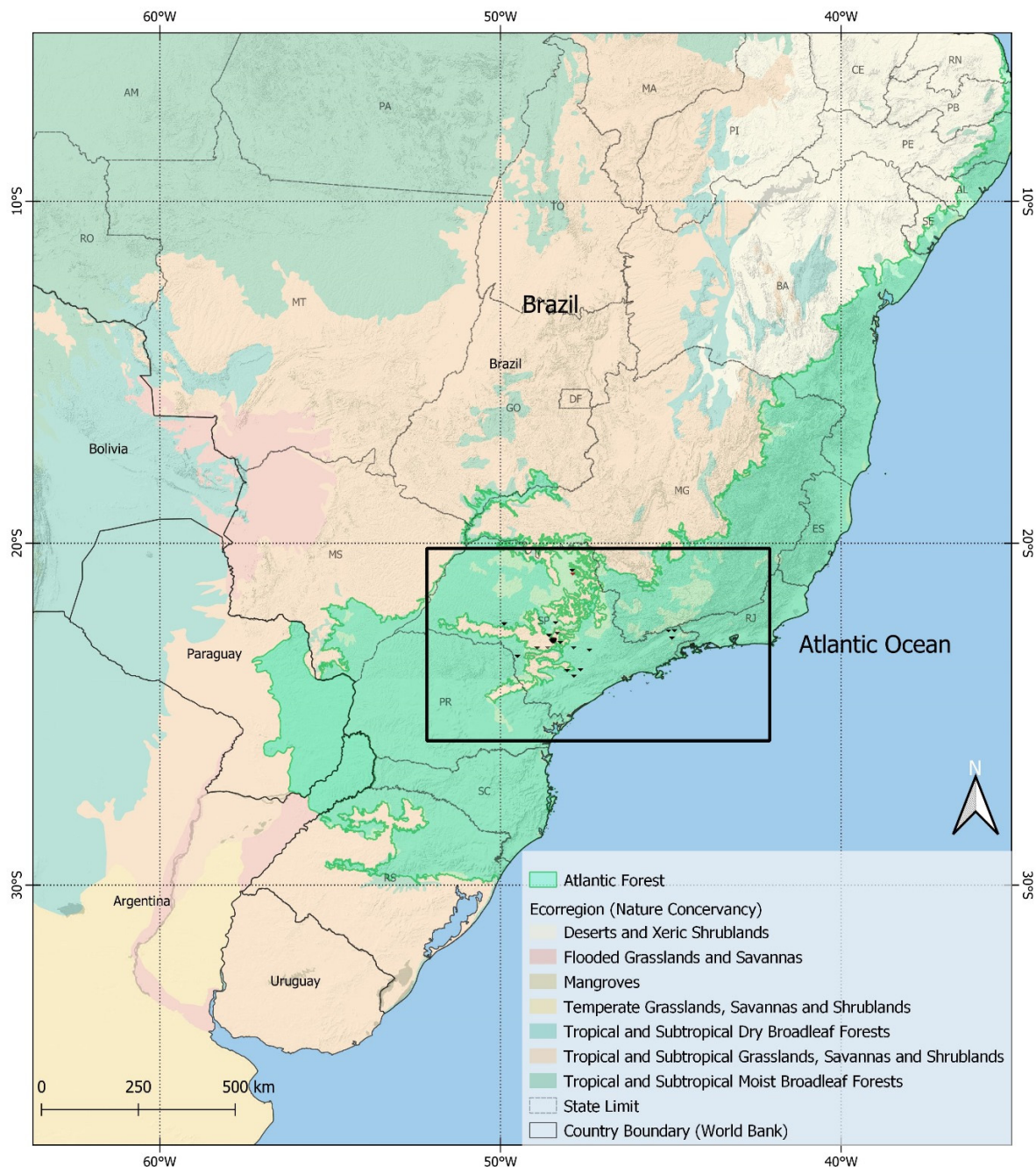


Figure 1. The study area's map showing the Brazilian Atlantic Forest located in South America and the state of São Paulo inside the square. The bat collection areas are marked with bat flags.

The samples of fruit bats and insectivorous bats came from animals that had fallen into people's homes in urban areas and were sent alive by the municipalities to the regional Zoonosis Diagnostic Service for rabies diagnosis. The samples of vampire bats were obtained in conjunction with the Agricultural Defense Office (EDA), which carries out active rabies surveillance work in the state of São Paulo, aiming to diagnose rabies and monitor cases in the region.

The bats received alive were euthanized with a supra dose of isoflurane inhalation anesthetic soaked in absorbent cotton, in a closed container with a physical separation between the absorbent cotton and the animal, and kept there until the animal stopped moving. After removal of the brain for rabies diagnosis, fragments of the heart, spleen, lungs, liver, intestines and kidneys were collected aseptically for investigation of *T. cruzi*.

The animals' hearts and kidneys were screened, and in positive cases, the other organs were tested. The collected material was stored in DNA-free microtubes at -80°C . The taxonomic identification of the bat species was made using the criteria described for determining Brazilian chiroptera [14]. Data on the sex and collection site of the animals was also recorded.

2.3. DNA Extraction and Quality Assessment

DNA was extracted individually from each animal sample using the Illustra Tissue and Cells Genomic Prep Mini Spin Kit (GE Healthcare Life Sciences, Marlborough, MA, USA), following the manufacturer's instructions. The purified DNA samples were eluted in 100 μL . The quality of the DNA was assessed by the concentration, which was then adjusted up to 100 ng/ μL , and by the absorbance ratios of 260/280 and 260/230 nm using a Nanovue spectrophotometer (GE Healthcare Life Sciences, Chicago, IL, USA).

2.4. *Trypanosoma cruzi* DNA Detection

All DNA samples were submitted to a cPCR assay targeting the *T. cruzi* kinetoplast, using primers 121 (5' AAA TAA TGT ACG GGT GAG ATG CAT GA 3') and 122 (5' GGG TTC GAT TGG GGT TGG TGT 3') [15]. All positive samples were subjected to a second cPCR reaction targeting *T. cruzi* nuclear DNA, using the primers TCZ1 (5' CGA GCT CTT GCC CAC ACG GGT GCT 3') and TCZ2 (5' CCT CCA AGC AGC GGA TAG TTC AGG 3') [16]. In both reactions, the final volume was 25 μL and the mixtures contained 2 μL of DNA template, 1.5 mM of MgCl_2 (50 mM/1 mL), 200 μM of dNTP mixture, 3.5 U of Taq DNA polymerase, 10 pmol of each primer and the rest of the volume made up of ultrapure water. Sterile milli-Q water was used as a negative control and the products of DNA extraction from strains maintained in NNN-LIT medium, under cultivation in a B.O.D. oven at 25°C , repeated every two weeks, of *T. cruzi* ("Y" strain) were used as a positive control. The amplifications were performed in a thermal cycler (Applied Biosystems, Foster City, CA, USA).

Reaction first round: denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, 62°C for 60 s, 72°C for 60 s and then a final extension step at 72°C for 10 min. Reaction second round: denaturation at 94°C for 30 s, followed by 25 cycles at 94°C for 30 s, 66°C for 30 s, 72°C for 30 s and then a final extension step at 72°C for 30 min. The products of all the cPCR assays were separated by electrophoresis on 1.5% agarose gel, stained with Sybr Safe (Invitrogen, Waltham, MA, USA) 0.1 $\mu\text{L}/\text{mL}$, in a horizontal vat containing TBE $1\times$ (0.1 M Tris, 0.09 M boric acid and 0.001 M EDTA) and a voltage of 100 V/150 mA for 40 min. For the molecular weight standard, the DNA Ladder 100 bp (Invitrogen, Waltham, MA, USA) was used in order to visually compare the size of the amplified fragments. The gels were visualized under ultraviolet light (Bio-Rad, Hercules, CA, USA) using Image Lab software version 4.1.

2.5. Sequencing and Phylogenetic Analyses

The amplified products were purified using the NZYGelpure Kit (NZYTech, Lisbon, Portugal) according to the manufacturer's instructions and sequenced with 7 μL of the purified sample added to a solution containing 3 μL of each primer (3.2 pmol). Sequencing took place in the forward and reverse directions with the TCZ1 and TCZ2 primers used in PCR. Sequencing took place using the BigDyeTM Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The sequences obtained from the positive samples were submitted to the BLASTn program for nucleotide analysis in comparison with sequences from the international database (GenBank). The quality of the sequences was analyzed using BioEdit Sequence Alignment Editor summer 7.2. The absence of overlapping peaks, sufficient amount of DNA, smoothness of the peaks and uniform spacing were evaluated for the selection of viable sequences.

A phylogenetic tree was constructed including all the sequences generated in this study together with representative reference sequences from GenBank. South American

sequences of *T. cruzi* reference strains that have recently been published were added to build the phylogenetic tree. Phylogenetic analysis was carried out using distance methods in MEGA11 version 11.0.13. The Neighbor-Joining method was applied using Kimura's two-parameter model. The topologies of the inferred trees were evaluated by 1000 bootstrap resamplings.

2.6. Data Analysis

The Chi-square test was used to compare the molecular prevalence of *T. cruzi* for the epidemiological variables. The significance level was 0.05. All tests were performed using SAS Studio version 3.81 (SAS Institute Inc., Cary, NC, USA).

3. Results

DNA of *T. cruzi* was amplified in samples from six bats, representing 3% [CI (95%) = 1.4–6.3%] of the total number of samples. Of the positives, there were 33% (1/3) of *N. laticaudatus*, 25% (1/4) of *A. lituratus*, 4% (1/24) of *E. glaucinus*, 2% (1/41) of *M. molossus* and 2% (2/101) of *D. rotundus*. Amplification occurred in the heart samples of four animals, kidney samples of three animals, liver samples of three animals and lung samples of two animals. There was no amplification in the intestine and spleen tissues. Figure 2 shows the distribution of infection in bats according to the study area [11–13].

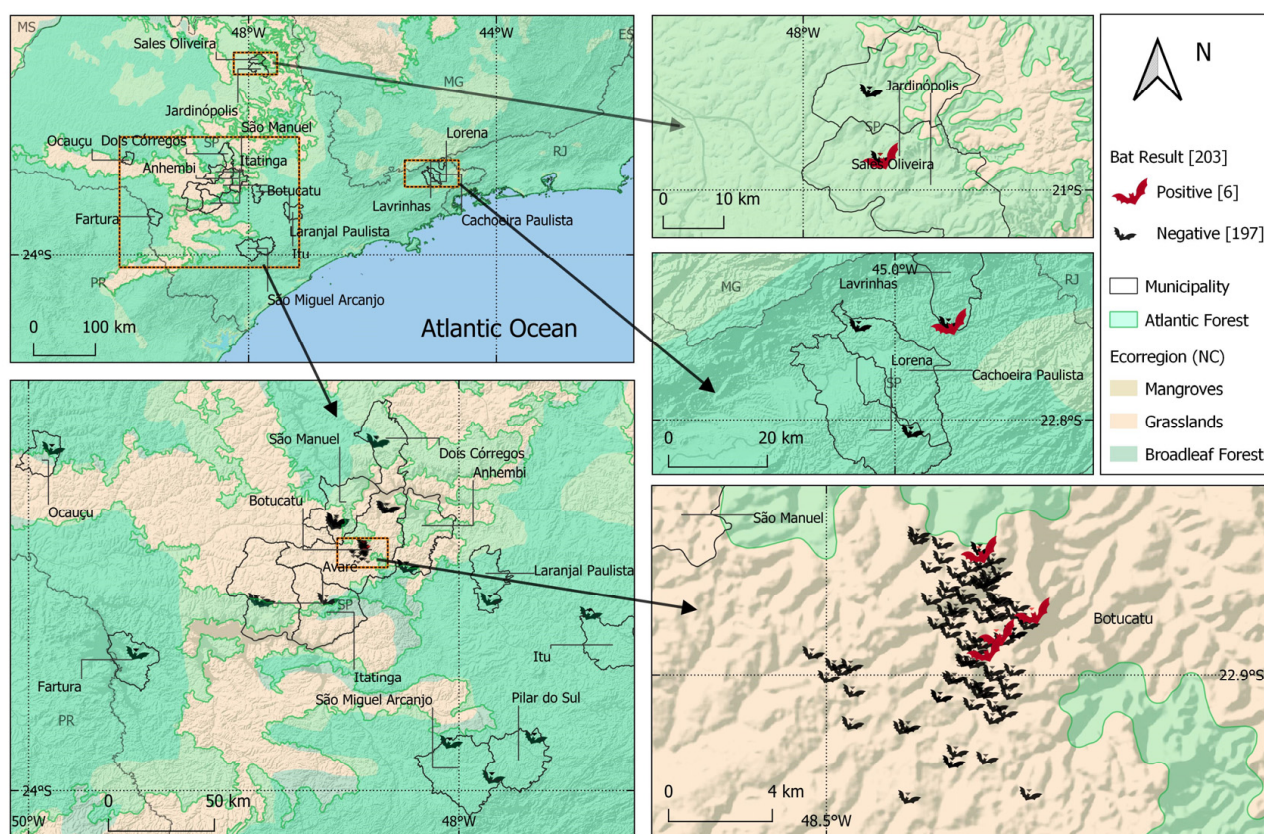


Figure 2. Distribution map of *Trypanosoma cruzi* infection detected in bats from Brazilian Atlantic Forest, São Paulo state (2020–2022).

The risk assessment showed that the bats' feeding habits were statistically associated with *T. cruzi* positivity. Fruit bats were 10.5 and 16.5 times more likely to be positive than insectivorous and hematophagous bats, respectively. For the other variables, there was no statistical difference (Table 1).

Table 1. Statistical associations between bat demographic variables and *Trypanosoma cruzi* positivity in the Brazilian Atlantic Forest, São Paulo state (2020–2022).

Variables	<i>T. cruzi</i> Positive	<i>T. cruzi</i> Negative	OR (95% CI)	<i>p</i> Value	Total Population
Eating habit				0.0285	
Frugivorous	1 (25.0%)	3 (75.0%)	1.0 (ref)		4
Insectivorous	3 (3.1%)	95 (96.9%)	10.5 (0.83–133.6)		98
Haematophagous	2 (2.0%)	99 (98.0%)	16.5 (1.15–236.2)		101
Sex				0.4283	
Female	4 (3.9%)	99 (96.1%)	1.0 (ref)		103
Male	2 (2.0%)	98 (98.0%)	1.97 (0.35–11.1)		100
Collection point				0.7342	
Occupied houses	4 (3.5%)	111 (96.5%)	1.0 (ref)		115
Manholes	1 (2.9%)	34 (97.1%)	1.23 (0.13–11.3)		35
Tunnels	1 (7.7%)	12 (92.3%)	0.43 (0.04–4.19)		13
Abandoned houses	0 (0.0%)	38 (100.0%)	3.10 (0.16–59.1)		38
Cave	0 (0.0%)	2 (100.0%)	0.20 (0.01–4.85)		2
Year				0.3867	
2020	1 (7.1%)	13 (92.9%)	1.0 (ref)		14
2021	3 (2.0%)	146 (98.0%)	3.74 (0.36–38.6)		149
2022	2 (5.0%)	38 (95.0%)	1.46 (0.12–17.5)		40
Area				0.4141	
Urban	4 (3.9%)	98 (96.1%)	1.0 (ref)		102
Rural	2 (2.0%)	99 (98.0%)	2.02 (0.36–11.3)		101

p-value in bold indicates statistically significant association ($p > 0.05$). 1.0 (ref.): reference category. OR (95% CI): odds ratio (95% Confidence Interval).

The standard sequencing results showed over 97.4% identity with *T. cruzi* sequences in all samples. The sequences obtained were deposited in GenBank: accession numbers OR736031–OR736036. The full results of the BLAST analysis, including score, query coverage and identity percentage, can be found in Table 2.

Table 2. DNA sequencing analysis of the PCR product with specific primers for a region of *Trypanosoma cruzi* nuclear DNA and characteristics of positive samples.

Bat Sample	Bat Specie	Tissue	Bat Sex	Score	Query Coverage	Closest GenBank Match
OR736031	<i>A. lituratus</i>	Kidney	Female	279	100%	98.12% (XM800623.1)
OR736032	<i>E. glaucinus</i>	Heart	Female	276	96%	98.72% (AY520070.1)
OR736033	<i>M. molossus</i>	Heart	Female	246	100%	97.39% (ON872176.1)
OR736034	<i>N. laticaudatus</i>	Kidney	Male	261	98%	98.54% (AY520077.1)
OR736035	<i>D. rotundus</i>	Heart	Male	237	95%	98.55% (DQ914519.1)
OR736036	<i>D. rotundus</i>	Heart	Female	265	100%	98.67% (DQ914484.1)

In the phylogenetic tree shown (Figure 3), represented by the bootstrap consensus tree, we observed a similarity between the sequences OR736036 from *D. rotundus* and OR532282.1 from a *Phyllostomus discolor* bat from São Luís, Bahia. Both sequences were genetically close to the OR736034 sequence from *N. laticaudatus*. The second *D. rotundus* sequence generated in this study, OR736035, was similar to the X83599.1 sequence of the Argentine strain, and had very low proximity to the first *D. rotundus* sequence. The OR736032 and OR736033 from *E. glaucinus* and *M. molossus*, respectively, sequences were genetically similar. The OR736031 sequence from *A. lituratus* was relatively close to Y08881.1, an Argentine isolate from *Mus musculus*, and somewhat close to the two bat sequences mentioned above.

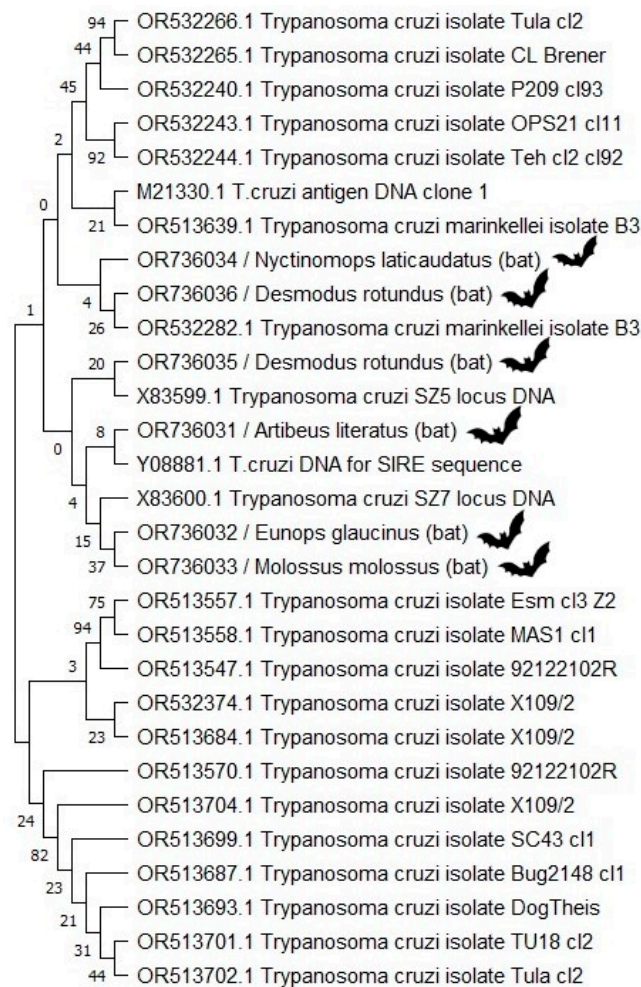


Figure 3. Phylogenetic tree comparing 23 *Trypanosoma cruzi* nuclear DNA reference segments with the sequences generated in this study, marked with bat flags, was built using the Neighbor-Joining method in Mega11.

4. Discussion

The prevalence found in this study shows that, as elsewhere on the continent, *T. cruzi* has infected vampire and non-vampire bats. The potential for transmission of the agent to other animals and humans is still under discussion and requires targeted studies. A metagenomic study of the saliva of neotropical bats revealed the presence of *T. cruzi* and suggested, together with phylogenetic results, an independent transmission cycle, where transmission through saliva is a possibility. This can occur through biting in the case of vampire bats, consumption of contaminated fruit and licking during bat socialization [17]. In addition, as these animals have contact with flies, mosquitoes and ticks capable of carrying pathogens, although the vectors of Chagas disease are triatomines, the infection of these other ectoparasites has already been proven [18,19].

There are several ways in which these animals can become infected. One of these ways is vertical transmission from mother to fetus, which has already been described in some bat species, and this is strengthened by the fact that bats have been studied as ancient reservoirs of pathogens and the source of various diseases in populations [20]. It is true that the physiology of bats, developed for flight conditions, allows a compatible and favorable blood regulation for the development and maintenance of several families of viruses, and although this characteristic cannot be applied to protozoa, considering that favorable characteristics for the maintenance of certain pathogens can be perpetuated through the generations by vertical transmission is important for the study of infections in bats [21].

Another transmission form is ingesting triatomines or fruit contaminated with triatomine feces containing *T. cruzi* forms. Our study showed a significant association between fruit bats and positivity. In Brazil, the presence of these triatomines in sugar cane plantations is a reality, and so is the presence of fruit bats in these plantations. It is believed that urban bats have a greater potential to transmit agents of diseases than other bats, as they tend to stay in the same place and experience a reduction in regular exposure to microorganisms that they used to acquire in the wild. This previous exposure helped to keep infection to a minimum. As a result, more bats, due to factors such as malnutrition, loss of habitat and other elements, end up being infected and consequently spreading a greater amount of microorganisms in urban areas [22]. There were no statistical differences in our study in relation to location; however, in this specific case, the idea runs counter to the fact that Chagas disease occurs more in rural areas, where there is a greater presence of vectors [6].

Vampire bats do not feed on fruit or insects, and the possible reason for this infection may be contact with triatomine feces in shelters and roosts or on the backs of livestock. Other wild mammals may be related to the presence of *T. cruzi* in bats since the infection has already been described in some of these species prevalent in the Brazilian Atlantic Forest [5]. Transmission through triatomine feces could be a possibility, considering that they usually contaminate sugar cane, a regional plant that is very common in the state and that bats often feed on [5]. Some bats, such as *D. rotundus*, are known to be hematophagous and transmit rabies to livestock and river-dwelling populations. Because they feed exceptionally well on blood, they are much less resistant to fasting, due to the absence of pancreatic β -cells that are characteristic of these types of bats. This absence prevents the bat's liver from producing glycogen reserves and means that the search for food has to be daily, making their contact with other animals even greater, and also increasing the chances of interaction between animals and new pathogens [23].

The flight abilities of bats influence the occurrence of new diseases not only physiologically, but also geographically, as they are able to fly long distances in search of food, depending on the relief conditions of the region, and consequently act as a migratory agent. Bats are known for their exceptional flying abilities, and their flight capacity can vary depending on the species. On average, most bat species can fly distances ranging from 20 to 50 km in a single night [21]. This ability, known in some species of the Brazilian Atlantic Forest, may influence the positivity described in this study, even though the state of São Paulo does not have many human and animal cases of infection.

N. laticaudatus, also known in Brazil as the velvety free-tailed bat, preferentially inhabits flooded forest areas and urban areas. They are social creatures, live in colonies and have seasonal migratory habits. Their migratory ability can influence the acquisition of the infection, as well as their ability to socialize with other bat species. *A. lituratus*, popularly known as the white-faced bat, inhabits forests and is also adapted to urban areas. This is a frugivorous species and travels long distances during the day, which favors the dissemination of seeds and undoubtedly makes them more susceptible to infection. *E. glaucinus* usually lives in urban areas and interacts with other bat species. It feeds on beetles and flies, which may indicate that triatomines are potential prey. *M. molossus*, or velvety free-tailed bat, is the most prevalent urban species in São Paulo. They are social and form huge colonies. They are distributed throughout the South American continent and feed preferentially on mosquitoes. The low positivity in relation to the total number of species collected may be due to the species' specific feeding habits. *D. rotundus*, the vampire bat, lives in colonies of hundreds of individuals and is known for its social habits of cleaning and self-cleaning, as well as being widely infested with ectoparasites, mainly ticks. Their low prevalence in relation to the total sampled may be due to the fact that they are found in rural areas with little or no housing, which means that there are no environments conducive to the existence of triatomines [24]. To our knowledge, this is the first study to detect DNA of *T. cruzi* in *N. laticaudatus* and *E. glaucinus*.

In our PCR results, there was no positivity in the spleen or intestine. Some strains show a preferential tropism for macrophages in the spleen, liver and bone marrow, while others are very scarce in these organs. The selection of the best tissue for investigation varies greatly [25].

The sequences detected in this study were compared with other South American sequences targeting *T. cruzi* nuclear DNA, and showed interesting positions in the tree, showing the genetic diversity found in bats. It is worth highlighting the sequences obtained from *E. glaucinus* and *M. molossus* are from two bats collected on the same day by the team. Both showed proximity and genetic similarity according to the phylogenetic analysis.

The highest concentration of bats collected was from the central–western region, and as a result, five of the positive samples were from this region. The other positive sample was detected in the northern coastal region. There are few cases of Chagas disease in São Paulo; however, the health services in the municipalities identified in this study need to be alert to new suspected cases of the disease.

One point to discuss is the COVID-19 pandemic, which occurred during the period when the samples were taken. During that period, the control of Chagas disease was neglected throughout Brazil. At the beginning of the pandemic, health resources were significantly redirected to combat COVID-19, including medical personnel, personal protective equipment and funding. This affected the capacity to diagnose and treat endemic diseases, including Chagas disease. The pandemic has temporarily interrupted health services in many areas, including routine medical consultations, diagnostic tests and epidemiological surveillance programs. In addition, the economic and social impact of the pandemic, such as unemployment and reduced resources for public health programs, may have had indirect effects on the control of Chagas disease [26]. The accuracy and reliability of health indicators are closely linked to the quality of the records, i.e., the precision of the information collected. In situations where health systems were overloaded, with a shortage of health professionals and the impossibility of carrying out laboratory tests for all cases, there may have been an overestimation of COVID-19 diagnoses, mainly related to febrile illnesses of other kinds [10].

More than any other parasitic disease, Chagas disease is closely related to social, economic, political and cultural factors, and efforts to interrupt the transmission of the agent of Chagas disease must consider improvements in housing, basic sanitation and people's quality of life [27].

5. Conclusions

Our study detected 3% of bats positive for *T. cruzi* by PCR of organs. *D. rotundus*, *N. laticaudatus*, *A. lituratus*, *E. glaucinus* and *M. molossus* species were associated with positivity. Fruit bats were statistically associated with positivity for *T. cruzi*. To our knowledge, this study detected *T. cruzi* for the first time in bats from São Paulo state and in *N. laticaudatus* and *E. glaucinus* species. Brazilian health agencies need to consider bats in their surveillance of Chagas disease.

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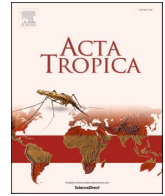
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Toxoplasma gondii in urban and rural bats from São Paulo state, Brazil

Danilo Alves de França^{*}, Benedito Donizete Menozzi, Helio Langoni

Department of Animal Production and Preventive Veterinary Medicine, School of Veterinary Medicine and Animals Science, São Paulo State University, Botucatu 18618-681, SP, Brazil

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ABSTRACT

The aim of this study was to detect *Toxoplasma gondii* in organs of urban and rural bats from 17 cities in São Paulo, Brazil. A total of 203 bats, including insectivorous, frugivorous and hematophagous species, were collected from urban and rural areas between 2020 and 2022. DNA was extracted from heart and kidney samples and analyzed using real-time PCR as a screening method, followed by conventional PCR to obtain a viable product for genetic sequencing and phylogenetic analysis. In total, 14.8 % of the samples were positive in real-time PCR and 9.4 % were sequenced. Phylogenetic analysis indicated low genetic proximity between the sequences detected in bats and those associated with domestic species, suggesting that bats may be exposed to specific sources of infection in the wild. The lack of statistical association with variables such as feeding habits and type of shelter suggests that multiple environmental factors contribute to infection. This study emphasizes the potential role of bats as intermediate hosts in the *T. gondii* transmission cycle, acting as environmental sentinels and possibly as ecological vectors. These findings contribute to understanding the distribution of *T. gondii* in bat populations and its implications for public health, especially in vulnerable communities, where poor sanitation and close contact with farm animals increase the risk of zoonotic transmission.

1. Introduction

Toxoplasmosis is a widespread zoonosis and one of the most prevalent parasitic infections in humans and animals. There is a wide variety of domestic and wild animals that can act as intermediate hosts of *Toxoplasma gondii* (Gering et al., 2021). Bats have gained increasing attention as potential reservoirs of several pathogens, including *T. gondii* (França et al. 2024a, França and Langoni, 2025). They play several ecological roles, such as pollinators, seed dispersers and insect controllers, in addition to using the blood of farm animals as a food source, which puts them in direct and indirect contact with different sources of infection, including soil, contaminated water, infected insects and domestic animals (França et al., 2024b).

The zoonotic importance of toxoplasmosis lies in its severe health impacts, including diseases in immunocompromised individuals, abortions, and congenital malformations in pregnant women (Aguirre et al., 2019). Brazil is a developing country and faces significant challenges related to the socioeconomic vulnerability of its population. Recent data indicate that approximately 24.3 % of Brazilians, corresponding to about 49 million people, live without access to basic sanitation (IBGE, 2022). Toxoplasmosis is a significant concern in vulnerable populations,

as socioeconomic factors increase the risk of exposure to the agent. In addition, these populations have less access to health services and information on preventive measures, which increases the risk of infection and its complications (Panazzolo et al., 2023; Pinto et al., 2024).

Brazil has one of the highest prevalence rates in the world, estimated at between 50 % and 80 %, with a higher incidence in rural areas and on the outskirts of large urban centers. Brazil also has a large population of bats, which, due to population growth and environmental conflicts, have come into contact with humans in both rural and urban environments (Gomez-Marin et al., 2007). The objective of this study was to detect *T. gondii* in organs of rural and urban bats from 17 cities in the state of São Paulo, Brazil, and to evaluate the genetic diversity and phylogenetic relationships of the isolates.

2. Materials and methods

2.1. Ethical statement

This study was approved by the Ethics Committee for Animal Use of São Paulo State University (protocol number 0259/2022) and obtained the appropriate environmental licenses from the Chico Mendes Institute

^{*} Corresponding author.

E-mail address: danilo.franca@unesp.br (D.A. França).

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for Biodiversity Conservation (SISBIO, protocol number 85973-1).

2.2. Study area

The bats in this study originated from 17 cities in the state of São Paulo, Brazil, a region marked by the coexistence of high ecological diversity and intense urbanization. The state is home to important remnants of the Atlantic Forest biome, recognized as one of the global biodiversity hotspots. This region is home to the majority of the Brazilian population, creating scenarios of frequent interaction between wildlife, domestic animals, and human populations (Oliveira, 2007).

To ensure that the results were representative, collection points were selected in urban and rural areas, considering the diversity of habitats and the different environmental contexts present in the studied municipalities. Geographic information, such as coordinates and environmental characteristics, were recorded for each location, allowing spatial analysis of the data. A map with the collection points (Fig. 1) was generated to illustrate the distribution of the sampled locations (França et al., 2024b).

2.3. Sample collection

Hearts and kidneys were sampled from 203 bats. One hundred and one bats were vampire bats (*Desmodus rotundus*) collected from artificial roosts in rural areas, and one hundred and two bats were urban bats, representing 12 species of insectivorous and frugivorous bats. Tables 1 and 2 present the collected bat species, along with the collection sites and their respective quantities.

Vampire bats were collected by the Agricultural Defense Office of the State of São Paulo. The collection was conducted in artificial shelters where bats live, such as abandoned houses, manholes, tunnels and water tanks. These sites were previously identified on the basis of ongoing monitoring, environmental studies and reports provided by local residents. Before the captures began, the team carried out an initial inspection to confirm the presence of bat colonies and assess the safety conditions for both the professionals and the animals. Throughout the process, team members used Personal Protective Equipment (PPE), such as waterproof overalls, leather gloves and PFF2 respirators, reducing the risk of exposure to zoonotic pathogens. The approach took place during the day, when the bats were resting. The capture nets were strategically

Table 1

Bat species and the number of individuals collected per species in São Paulo State, Brazil.

Bat Species	Eating habit	Number of Tested Bats
<i>Desmodus rotundus</i>	Hematophagous	101 (49.7 %)
<i>Molossus molossus</i>	Insectivorous	41 (20.1 %)
<i>Molossus rufus</i>	Insectivorous	2 (1 %)
<i>Myotis nigricans</i>	Insectivorous	13 (6.4 %)
<i>Eumops glaucinus</i>	Insectivorous	24 (11.8 %)
<i>Eumops auripendulus</i>	Insectivorous	4 (2 %)
<i>Eumops perotis</i>	Insectivorous	2 (1 %)
<i>Eptesicus brasiliensis</i>	Insectivorous	3 (1.5 %)
<i>Eptesicus furinalis</i>	Insectivorous	2 (1 %)
<i>Artibeus lituratus</i>	Frugivorous	4 (2 %)
<i>Lasiurus blossevillii</i>	Insectivorous	3 (1.5 %)
<i>Nyctinomops laticaudatus</i>	Insectivorous	3 (1.5 %)
<i>Histiotus velatus</i>	Insectivorous	1 (0.5 %)

positioned near the entrances, blocking the animals' possible escape routes. Controlled artificial lighting was used to locate the bats without causing major disturbances. In abandoned houses, the team carefully inspected bedrooms, attics, roofs and basements, using portable nets to catch the bats in flight.

Urban bats were collected after falling into occupied homes in urban areas. These captures were carried out by teams from the local town hall, called in by residents who reported the incidents. These actions were part of the rabies surveillance program. On receiving the call, the teams began inspecting the site to locate where the bats were sheltering. The bats were captured using specific equipment, such as boxes or hand nets, in order to minimize stress or any risk to the animal. Initial identification of the species was carried out on the spot, based on morphological characteristics such as body size, wing shape and facial details. The captured bats were kept in suitable conditions, with good ventilation and controlled temperature, before being transported to the laboratory.

The bats were taken to the laboratory in safe conditions. The identification of bat species was performed based on morphological characteristics, such as body size, wing shape, and cranial structure, following the criteria described by Reis et al. (2013). The bats were euthanized with isoflurane following ethical guidelines and submitted to necropsy in a controlled and aseptic environment. The site was sanitized with 1 % sodium hypochlorite and 70 % alcohol, and the instruments

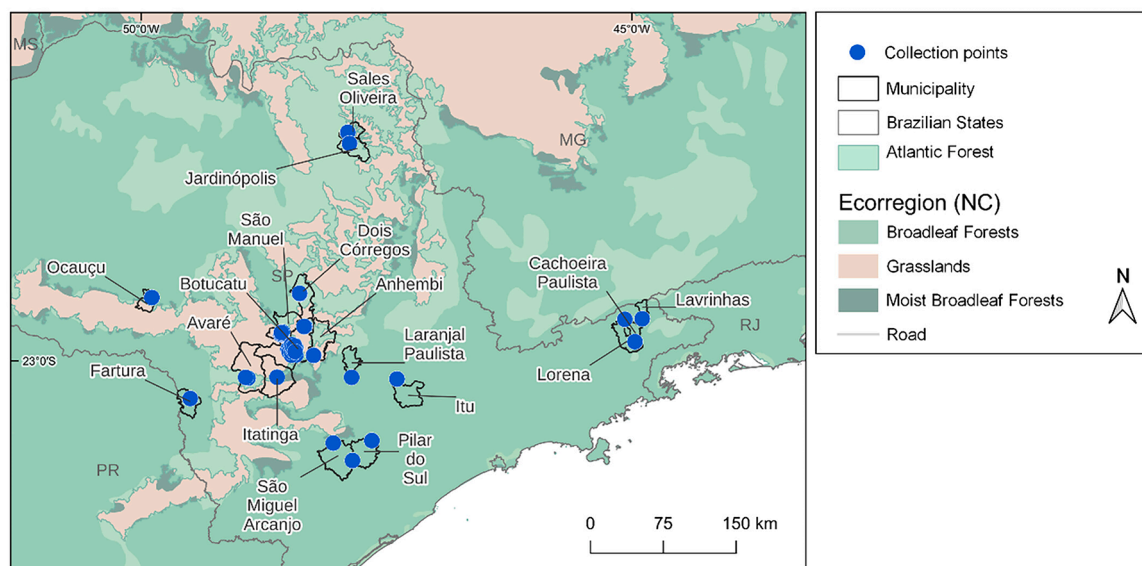


Fig. 1. Geographic representation of bat collection areas carried out between 2020 and 2022. The map shows the boundaries of the state of São Paulo and its municipalities, highlighted with lines and demarcations. The remnants of the Atlantic Forest biome are indicated in different shades of green, while the ecoregions are differentiated by a varied color palette. Bat collection sites are marked with blue icons to facilitate visualization and spatial analysis.

Table 2

Number of bat samples collected in rural and urban areas of São Paulo State, Brazil.

Location	Bat Species	Number of Tested Bats
Urban Areas		
Avaré	2, 4, 5	7 (3.4 %)
Botucatu	2, 3, 4, 5, 6, 8, 10, 11, 13	52 (25.6 %)
Dois Córregos	2, 3, 5, 7	16 (7.9 %)
Itatinga	4, 10	3 (1.5 %)
São Manuel	2, 3, 5, 6, 9, 12	24 (11.8 %)
Rural Areas		
Anhembi	1	5 (2.5 %)
Cachoeira Paulista	1	4 (2 %)
Fartura	1	16 (7.9 %)
Itu	1	3 (1.5 %)
Jardinópolis	1	4 (2 %)
Laranjal Paulista	1	2 (1 %)
Lavrinha	1	4 (2 %)
Lorena	1	11 (5.4 %)
Ocaúçu	1	17 (8.4 %)
Pilar do Sul	1	13 (6.4 %)
Sales Oliveira	1	13 (6.4 %)
São Miguel Arcanjo	1	9 (4.4 %)

Bat species: *Desmodus rotundus* (1), *Molossus molossus* (2), *Molossus rufus* (3), *Myotis nigricans* (4), *Eumops glaucinus* (5), *Eumops auripendulus* (6), *Eumops perotis* (7), *Eptesicus brasiliensis* (8), *Eptesicus furiensis* (9), *Artibeus lituratus* (10), *Lasiurus blossevillii* (11), *Nyctinomops laticaudatus* (12), *Histiotus velatus* (13).

were sterilized in an autoclave. Wearing PPE, the operators made incisions to access the internal organs, removing the heart and kidneys for *T. gondii* DNA analysis. The brain tissue was used entirely for the diagnosis of rabies. The tissues were handled carefully to avoid contamination and stored in sterile tubes, being frozen at -80°C for later processing.

2.4. DNA extraction and quality assessment

DNA was extracted using the Illustra Tissue Kit (GE Healthcare Life Sciences, Marlborough, MA, USA), following the manufacturer's protocol, with final elution in 100 μL . Sample concentrations were adjusted to 20 ng/ μL , and quality was assessed by spectrophotometry (GE Healthcare Life Sciences, Chicago, IL, USA), considering A260/280 ratios between 1.8–2.0 and A260/230 above 2.0 as indicative of high purity. Samples were stored at -80°C for later analyses.

2.5. *T. gondii* DNA detection by real-time PCR

Real-time PCR was performed as a screening method using the SYBR Green system and the StepOne Plus Real Time PCR System (Applied Biosystems®, Foster City, CA, USA) to perform the reactions. The primers selected for detection were TOX529-F (5' CAC AGA AGG GAC AGA AGT 3') and TOX529-R (5' TCG CCT TCA TCT ACA GTC 3') targeting the repeat element and amplifying an internal 94 bp fragment (Edvinsson et al., 2006). The reaction was performed in duplicate in a total volume of 25 μL , containing 1 μL of each primer (5 μM), 12.5 μL of SYBR Green qPCR Master Mix (Promega, Madison, WI, USA), 8.5 μL of ultrapure water and 2 μL of extracted DNA. The assay was conducted according to the following thermal protocol: an initial denaturation step at 95°C for 10 min; followed by 40 cycles consisting of denaturation at 95°C for 15 s, annealing at 60°C for 1 min, and extension at 95°C for 15 s; and ending with a single extension step at 72°C for 10 min. Fluorescence signals were obtained during the extension steps of the amplification cycles. We considered Ct values ≤ 36 as the threshold for positive samples. The positive control consisted of a RH strain of *T. gondii*, maintained in EMJH medium, previously centrifuged (3000 rpm for 10 min) and resuspended twice in sterile PBS solution (pH 7.6). Milli-Q water was used as a negative control.

2.6. *T. gondii* DNA detection by conventional PCR

Positive DNA samples in the real-time protocol were submitted to the conventional protocol aimed at amplification of a 529-bp fragment region and repeated 200–300 times in the *T. gondii* genome. The primers used for this amplification were TOXO4 (5' GGA ACT GCA TCC GTT CAT GAG 3') and TOXO5 (5' TCT TTA AAG CGT TCG TGG TC 3') (Homan et al., 2000). The reactions were performed in a total volume of 25 μL , containing 1 μL of each primer (5 μM), 12.5 μL of Master Mix (Promega, Madison, WI, USA), 7.5 μL of ultrapure water and 3 μL of extracted DNA. The PCR assay was performed in a thermal cycler (Applied Biosystems, Foster City, CA, USA) according to: 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min, with a final extension at 72°C for 7 mins. Reaction controls were the same as those used previously. Ten microliters of the PCR products were run on a 1.5 % agarose gel at 100 V. To establish molecular weight standards, DNA Ladder 100 bp (Invitrogen, Waltham, MA, USA) was used. The gel was visualized under ultraviolet light (Bio-Rad, Hercules, CA, USA) using Image Lab software version 4.1.

2.7. Gene sequencing

PCR products were purified using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer's guidelines and sent for Sanger sequencing at a partner laboratory. Sequences from positive samples were compared with entries in the GenBank database using the BLASTn tool. Sequence quality was checked using BioEdit Sequence Alignment Editor version 7.2, ensuring the absence of overlapping peaks, sufficient DNA quantity, smooth peak profiles and consistent spacing to confirm valid sequences.

2.8. Phylogenetic analyses

A phylogenetic tree was constructed including all sequences generated in this study, together with representative reference sequences from GenBank. *T. gondii* sequences detected in cattle, dogs and cats in Brazil were added to construct the tree. The sequence of a *Neospora caninum* isolate was used as an outgroup. Phylogenetic analysis was performed using distance methods in MEGA11 version 11.0.13. The Neighbor-Joining method was applied using Kimura's two-parameter model and the topologies of the inferred trees were evaluated by 1000 bootstrap resamplings.

2.9. Data analysis

To explore the associations between *T. gondii* positivity and the collected categorical variables, a univariate statistical analysis was performed using the chi-square test. The analysis focused on evaluating the potential correlations between *T. gondii* infection and factors such as dietary habit, sex, rurality of the area, urbanization of the area, and types of shelters. The significance level set for the tests was 0.05. All tests were run in SAS Studio (version 3.81, SAS Institute Inc., Cary, NC, USA).

3. Results

Of the 203 bats analyzed, 14.78 % [95 % CI: 10.55–20.31 %] were tested positive for *T. gondii* DNA. The cycle threshold (Ct) of positive samples ranged from 32 to 36, indicating a moderate parasite load. Ct values were consistent among the samples analyzed, indicating a relatively uniform parasite load among species and positive tissues. Ct values for heart samples were slightly lower than those for kidney, suggesting higher concentration of parasite DNA in muscle tissues. The positive samples presented melting temperature (Tm) values close to those of the positive controls, with a maximum variation of 1°C . This small variation indicates a high specificity of the amplification, suggesting that the products generated correspond to the expected target

DNA of *T. gondii*. The consistency of the Tm reinforces the reliability of the results and rules out the presence of nonspecific amplifications.

Positive bats were found in eleven cities. The prevalence was higher in rural areas (8.9 %, 18/101) compared to urban areas (5.9 %, 12/102). All positive bats from rural areas were hematophagous (*Desmodus rotundus*), whereas all positive bats from urban areas were insectivorous. Among the positive insectivorous bats, five were *Molossus molossus*, two were *Myotis nigricans*, and two were *Lasiurus blossevillii*. Additionally, one positive bat was identified for each of the following species: *Eumops glaucinus*, *Eumops auripendulus*, and *Eptesicus brasiliensis*.

Of the total positive samples, 63 % (19/30) were amplified in conventional PCR and produced sufficient DNA for sequencing. All sequenced samples showed query coverage greater than 80 % and similarity greater than 95 % with other *T. gondii* sequences available in GenBank. These results indicate that the sequences obtained in this study are of high quality and correspond specifically to the parasite's DNA. The accession numbers of the sequences generated in this study are presented in Supplementary Material 1, together with the other sample results.

The statistical association analysis between the positivity of the bats and the obtained variables are presented in Table 3. The table shows the distribution of *T. gondii* positive and negative bats according to eating habits, sex, collection points, year of collection, and area (urban vs. rural). Odds ratios (OR) with 95 % confidence intervals (CI) and p-values were calculated to assess the association. Total population for each category is also provided.

There was no statistical association between positivity and the variables evaluated. Although hematophagous and rural bats were more

positive than urban insectivorous and fruit bats, this was not statistically associated with *T. gondii* positivity, demonstrating that there is no relationship between the feeding habits of bats and the type of area they live in (rural vs urban) with infection.

Phylogenetic analysis of the *T. gondii* sequences obtained revealed the formation of distinct clades, indicating genetic diversity among the samples analyzed. The sequences from bats formed separate clusters from the *T. gondii* sequences from domestic animals, such as dogs (*Canis familiaris*), cats (*Felis catus*) and cattle (*Bos taurus*), suggesting less genetic proximity and possible diversity of infection sources. The outgroup used, *Neospora caninum*, was correctly positioned outside the main clade, reinforcing the robustness of the analysis. These results indicate that bats may be exposed to specific genotypes from the wild, different from those often found in domestic animals, which highlights their potential role in the ecological cycle of *T. gondii* (Fig. 2).

4. Discussion

The prevalence of *T. gondii* found in this study reinforces the potential role of bats as intermediate hosts in the parasite cycle, and even as possible reservoirs. As previously mentioned, other studies have investigated the presence of antibodies and DNA of *T. gondii* in bats in Brazil; however, these studies involved bats from a single city and with low sampling, in addition to not investigating bats from exclusively rural or urban areas.

Data on bat infection by *T. gondii* are still scarce and scattered, with ten studies published to date in countries such as Brazil, China, Colombia, Czech Republic, Myanmar, and the UK, reporting variable positivity rates ranging between 1 % and 29.3 % (Cabral et al., 2014; Sun et al. 2013; Jiang et al., 2014; Fournier et al., 2014; Qin et al., 2014; Dodd et al., 2014; Jesus et al., 2017; Zamora-Vélez et al., 2020; Bártová et al., 2023). Particularly in Brazil, studies in São Paulo, Natal, and Bahia have demonstrated seropositivity rates of 32.6 % (201/616), 21.6 % (11/51), and 2.1 % (2/97), respectively (Cabral et al., 2014; Fournier et al., 2014; Jesus et al., 2017). These differences may reflect regional factors, such as availability of infection sources, habitat diversity, and species-specific feeding behaviors. Furthermore, *T. gondii* genotypes found in bats from São Paulo match those reported in other domestic and wild animals, highlighting the potential role of bats not only as intermediate hosts but also as ecological vectors in the transmission dynamics of *T. gondii*. These findings underscore the need for further research to better understand the ecological and epidemiological roles of bats in the lifecycle of this parasite.

The results of this study show that bats play a role in the *T. gondii* cycle in both rural and urban environments. In rural areas, hematophagous bats (*Desmodus rotundus*) can acquire the parasite by feeding on the blood of domestic animals, such as cattle and horses, which often have direct or indirect contact with oocysts shed by felines. Although transmission of *T. gondii* via blood is generally considered unlikely under normal conditions, the higher positivity in this group raises doubts about the matter. This transmission may occur in the acute phase of the disease, when free tachyzoites are present in the bloodstream. However, there are still no experiments or evidence of this transmission in bats. A more likely hypothesis for the infection would be the use of artificial shelters, such as abandoned houses, manholes, water tanks, and tunnels, which may increase the risk of exposure due to the accumulation of contaminated organic matter, including feces from felines or other animals that shed *T. gondii* oocysts. The concentration of oocysts in these environments, combined with the proximity of bats to these sources of contamination, may significantly increase the risk of exposure to the parasite. Additionally, the lack of hygiene and the characteristic humidity of these shelters may favor the survival of oocysts in the environment, further amplifying the chances of infection. In urban environments, insectivorous and frugivorous bats can be exposed to *T. gondii* through ingesting contaminated insects, eating fruit contaminated with oocysts or direct contact with feline feces. Insectivorous bats,

Table 3

Variables associated with *Toxoplasma gondii* positivity in bats from São Paulo State, Brazil.

Variables	<i>T. gondii</i> Positive	<i>T. gondii</i> Negative	OR (95 % CI)	p Value	Total number of tested samples
Eating habit				0.3466	
Insectivorous	12 (12.2 %)	86 (87.8 %)	1.0 (ref)		98
Frugivorous	0 (0 %)	4 (100 %)	0.7 (0.31–1.50)		4
Haematophagous	18 (17.8 %)	83 (82.2 %)	1.1 (0.05–22.4)		101
Sex				0.3810	
Male	17 (17.0 %)	83 (95.0 %)	1.0 (ref)		100
Female	13 (12.6 %)	90 (98.1 %)	1.41 (0.64–3.09)		103
Collection point				0.8052	
Occupied houses	12 (11.8 %)	90 (88.2 %)	1.0 (ref)		102
Abandoned houses	7 (18.4 %)	31 (81.6 %)	0.6 (0.21–1.63)		38
Manholes	6 (17.1 %)	29 (82.9 %)	0.6 (0.22–1.87)		35
Tunnels	3 (20 %)	12 (80 %)	0.5 (0.13–2.16)		15
Water tank	2 (15.4 %)	11 (84.6 %)	0.7 (0.14–3.71)		13
Year				0.8608	
2022	5 (12.5 %)	35 (87.5 %)	1.0 (ref)		40
2021	17 (11.4 %)	132 (88.6 %)	0.9 (0.31–2.61)		149
2020	1 (7.1 %)	13 (92.9 %)	1.7 (0.20–13.6)		14
Area				0.2269	
Urban	12 (11.8 %)	90 (88.2 %)	1.0 (ref)		102
Rural	18 (17.8 %)	83 (82.2 %)	0.6 (0.27–1.35)		101

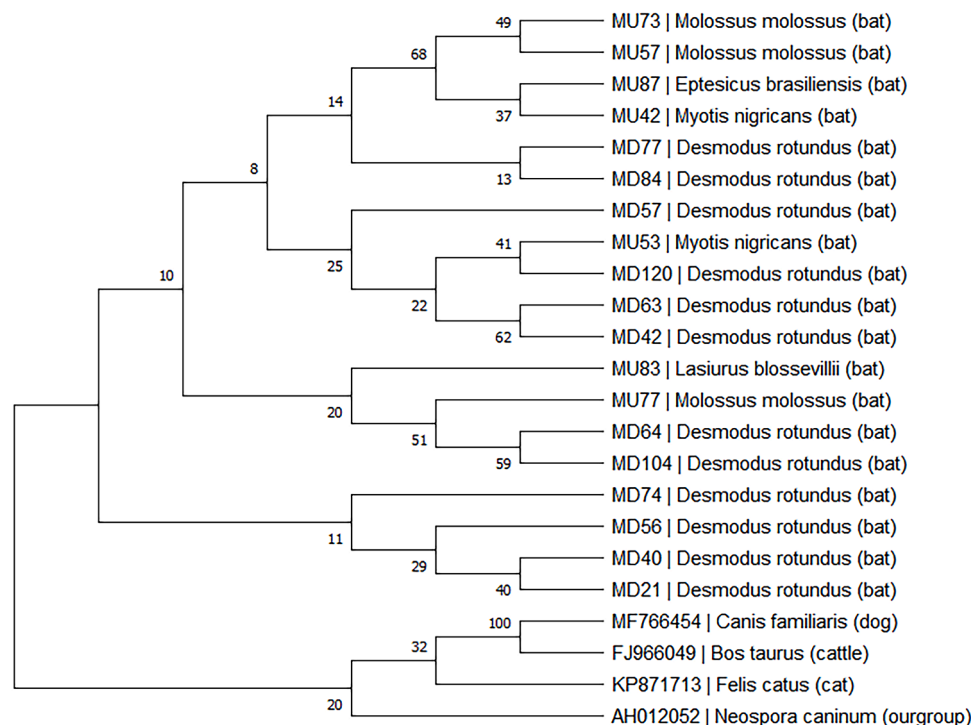


Fig. 2. Phylogenetic tree comparing *Toxoplasma gondii* DNA reference segments from isolates of other animal species in the region with the sequences generated in this study. The sequence of a *Neospora caninum* isolate was used as an outgroup. The tree was built using the Neighbor-Joining method in Mega11.

which can consume up to twice their body weight daily and require large quantities of water, are particularly at risk of ingesting oocysts from contaminated water sources (Yang et al., 2021). The detection of *T. gondii* DNA in urban and rural bats supports the hypothesis that these animals not only act as intermediate hosts, but also as ecological vectors, potentially transporting the parasite between different environments.

In the São Paulo state, *T. gondii* infection is particularly relevant, especially among pregnant women. Between 2019 and 2024, 8803 cases of gestational toxoplasmosis were reported, representing approximately 13 % of the total cases registered in Brazil during this period (Brazil, 2024). The detection of *T. gondii* in urban bats suggests that these animals may act as environmental sentinels, indicating the presence and circulation of oocysts in the environment. Although other animal species, such as cats and livestock, are widely used due to their greater availability and proximity to humans, bats offer a unique perspective by directly reflecting environmental contamination. Additionally, their presence in both urban and rural environments makes them relevant for epidemiological surveillance in peripheral areas. In these regions, where sanitation infrastructure is precarious, cases of the disease tend to be more frequent, reinforcing the importance of monitoring the circulation of the parasite and implementing preventive measures.

Toxoplasmosis is more prevalent in rural areas of the state of São Paulo. The lack of adequate sanitation, soil and food contamination, consumption of untreated water, and greater proximity to farm animals intensify the circulation of the parasite in these environments (Brazil, 2021). The serological prevalence of rural toxoplasmosis in Brazil has already exceeded 81 % (Carmo et al., 2016). The detection of *T. gondii* in vampire bats suggests that these animals share the environment with farm animals and are likely infected by common sources of contamination.

Phylogenetic analysis revealed genetically distinct clades, with sequences from bats clustering separately from isolates from domestic animals. These results indicate a lower genetic proximity between the sequences detected in bats and those often associated with domestic species, suggesting that bats may be exposed to specific sources of infection in the wild. The distance between the clades reinforces the idea

that bats from different environments may be subject to different environmental and ecosystem pressures, which influence the transmission dynamics of the parasite.

5. Conclusion

This study detected the presence of *T. gondii* in urban and rural bats of various species in the state of São Paulo, Brazil, with a prevalence of 14.78 %. The results indicate that hematophagous bats (*Desmodus rotundus*) in rural areas are more positive. These findings reinforce the potential role of bats as intermediate hosts in the *T. gondii* cycle, acting as environmental sentinels and possibly as ecological vectors. The lack of statistical association with variables such as feeding habits and type of shelter suggests that multiple environmental factors contribute to infection. In addition, phylogenetic analysis indicated low genetic proximity between the sequences detected in bats and those associated with domestic species, suggesting that bats may be exposed to specific sources of infection in the wild. This study contributes to the understanding of the epidemiology of *T. gondii* and highlights the need to strengthen zoonotic surveillance, especially in areas of high interaction between humans, domestic animals and wildlife.

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CRediT authorship contribution statement

Danilo Alves de França: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Benedito Donizete Menozzi:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation. **Helio Langoni:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition,

Conceptualization.

Declaration of competing interest

The authors have declared that no competing interests exist.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.actatropica.2025.107557](https://doi.org/10.1016/j.actatropica.2025.107557).

Data availability

Data will be made available on request.

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Bartonella spp., *Coxiella burnetii* and *Rickettsia rickettsii* in urban bats: molecular investigation of neglected zoonoses in Brazil

Danilo Alves de França¹ · Alana Vitor Barbosa da Costa² · Leandro Meneguelli Biondo^{3,4} · Ana Íris de Lima Duré² · Benedito Donizete Menozzi¹ · Felipe Fornazari¹ · Helio Langoni¹

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Abstract

In the last decade, cases of bartonellosis, Q fever and Brazilian spotted fever have been recorded in Brazil. Despite this, their occurrence is still underestimated. This study aimed to investigate *Bartonella* spp., *Coxiella burnetii* and *Rickettsia rickettsii* DNA in organs of urban bats collected from the São Paulo state, southeastern Brazil. Spleen and liver samples were collected from 102 bats from urban areas and evaluated by real-time PCR. Positive samples were submitted to conventional PCR and subsequent sequencing for species identification. In total, 3.9% of the bats were positive for *Bartonella* spp. in the real-time PCR and 2.9% were sequenced. Of the positive bats, two were *Artibeus lituratus* and two were *Myotis nigricans*. The sequences obtained were analyzed using the BLASTn, identified as *B. koehlerae* ($n=2$) and *B. clarridgeiae* ($n=1$) and deposited in Genbank. A close phylogenetic relationship was observed between the bat isolates in the study and global and Brazilian isolates from cats and humans. No sample in the study was positive for *C. burnetii* or *R. rickettsii*. The presence of *Bartonella* associated with infection of cats and humans in Brazilian bats reflects the neglected state of the disease in the country and the need for epidemiological surveillance actions. Cat scratch disease is currently a public health problem in Brazil and needs to be tracked.

Keywords Chiroptera · Cat scratch disease · Surveillance · Phylogeny · Public health

Introduction

Although some zoonoses are recognized as public health concerns in Brazil, diseases such as bartonellosis, Q fever, and Brazilian spotted fever remain largely neglected. The underdiagnosis of these diseases significantly affects public

health, leading to severe short- and long-term complications, including sepsis, endocarditis, and meningitis [1–3].

Bartonellosis is caused by different species of *Bartonella*, with different clinical forms and target hosts [4]. In southeastern Brazil, its most common zoonotic form, known as cat scratch disease, is mainly caused by *B. henselae*, followed by *B. clarridgeiae* and *B. koehlerae* [5]. The prevalence of *Bartonella* in cats can reach up to 90% in some locations, mainly in stray cats, and transmission is facilitated by *Ctenocephalides felis* fleas [4]. Among the blood samples from suspected patients analyzed by the Brazilian health system between 2011 and 2017, 38% (480/1265) were positive for anti-*Bartonella* antibodies [1]. In addition, cases of endocarditis have been diagnosed in hospitals in São Paulo [6]. Several studies have demonstrated the presence of various *Bartonella* species in bats across Latin America, indicating that these animals may serve as significant reservoirs of the disease, even in urban settings [7–11].

Q fever is caused by *Coxiella burnetii*, a highly transmissible and airborne bacterium capable of infecting a wide range of hosts, including mammals, birds, and

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✉ Danilo Alves de França
danilo.franca@unesp.br

¹ Department of Animal Production and Preventive Veterinary Medicine, School of Veterinary Medicine and Animals Science, São Paulo State University, São Paulo, Brazil

² Service of Virology and Rickettsiosis, Octavio Magalhaes Institute, Belo Horizonte, Brazil

³ National Institute of the Atlantic Forest (INMA), Brazilian Ministry of Science, Technology and Innovation, Santa Teresa, ES 29650-000, Brazil

⁴ Interdisciplinary Graduate Studies, University of British Columbia, Endowment Lands, Canada

arthropods. Despite its zoonotic potential, the disease remains underdiagnosed in Brazil, with limited epidemiological data available [2]. São Paulo stands out as the federative unit with the highest number of studies and reported cases of Q fever, including cases of infectious endocarditis, a febrile outbreak among slaughterhouse workers (21°16'00"–49°56'57"), and a seropositivity rate of 21.5% (129/604) in febrile patients initially suspected of dengue in small municipalities [6, 12]. However, the role of wildlife, particularly bats, in the epidemiology of *C. burnetii* remains poorly understood. In urban environments, bats may play a crucial role in the maintenance and transmission of *C. burnetii*, especially through their association with arthropod vectors such as ticks and fleas [13]. Although studies on *C. burnetii* in bats are scarce, evidence from other regions suggests that these animals may harbor the bacterium, particularly due to its highly transmissible nature in the airborne cycle [13–15].

Brazilian spotted fever is a severe febrile illness caused by the bacterium *Rickettsia rickettsii*, primarily transmitted by ticks of the genus *Amblyomma*. These ticks are found on a variety of hosts, including cattle, horses, dogs, birds, synanthropic animals, and capybaras, which are considered key reservoirs in the disease's transmission cycle [16]. From 2000 to 2018, following a significant effort by the Ministry of Health, 2,090 cases were confirmed, with a mortality rate of 55% [17]. Among all Brazilian states, São Paulo reported the highest number of cases (982 cases, 47%), establishing itself as an endemic area for Brazilian spotted fever [3]. Despite these numbers, the role of bats in the epidemiology of *R. rickettsii* is also poorly explored. In urban environments, bats may play an important role in the maintenance and dissemination of *R. rickettsii*, particularly through their association with *Amblyomma* ticks [18]. Although direct evidence of *R. rickettsii* in bats is limited, studies in other regions have detected *Rickettsia* species in bats and their ectoparasites, suggesting that these animals may be involved in the disease cycle [19–21].

Environmental changes, including deforestation, urbanization, and climate shifts, have intensified human-wildlife interactions, particularly with bats from the Brazilian Atlantic Forest, a global biodiversity hotspot severely impacted by human activities in recent decades [22]. While the role of bats in the cycle of endemic zoonotic diseases in Brazil has been investigated, their involvement in the transmission cycles of bartonellosis, Q fever, and Brazilian spotted fever remains poorly understood [23–25]. The aim of this study was to detect the DNA of *Bartonella* spp., *C. burnetii* and *R. rickettsii* in the organs of urban bats in the state of São Paulo, Brazil, and to evaluate the genetic diversity and phylogenetic relationships of the isolates.

Materials and methods

Study area

The bats analyzed in this study were collected from cities across the São Paulo state, Brazil, a region characterized by its remarkable ecological diversity alongside significant urban development. São Paulo harbors key remnants of the Atlantic Forest biome, which is internationally acknowledged as a critical biodiversity hotspot. This area also accommodates the largest share of Brazil's population, fostering frequent interactions between wildlife, domestic animals, and human communities [26].

The sample collections were conducted during the COVID-19 pandemic (2020–2022), a period marked by significant operational and logistical challenges. This context underscored the critical importance of strengthening zoonotic disease surveillance. In São Paulo state alone, official data reported 6 million COVID-19 cases and 181,000 deaths [27]. During this time, public health efforts were predominantly directed toward managing the pandemic, which consequently limited the monitoring of other zoonotic diseases. A map with the collection points (Fig. 1) was generated to illustrate the distribution of the sampled locations (Global Ecoregions, n.d.; World Bank Official Boundaries, n.d.; Continuous Cartographic Bases, n.d.).

Sample collection

Samples were collected from 102 bats in urban areas, representing 12 species of insectivorous and frugivorous bats. As recommended by the Brazilian Ministry of Health, passive surveillance of rabies in bats is one of the central actions of the country's environmental and epidemiological surveillance services, and is carried out by local health authorities. Bats that show atypical behavior during the day or are found inside homes are referred for rabies diagnosis. All the bats included in this study were found fallen in the homes of families and sent to our laboratory. Upon receiving the call from the families, the local teams began inspecting the area to locate where the bats were sheltering. The bats were captured using specific equipment, such as boxes or hand nets, to minimize stress or any risk to the animals.

The identification of bat species followed pre-established criteria [28]. In the laboratory, the bats were euthanized with isoflurane following ethical guidelines and submitted to necropsy in a controlled and aseptic environment. The site was sanitized with 1% sodium hypochlorite and 70% alcohol, and the instruments were sterilized in an autoclave. Using PPE, the operators made incisions to access the internal organs, removing the brain for rabies diagnosis and the liver and spleen for research into other zoonotic agents. The

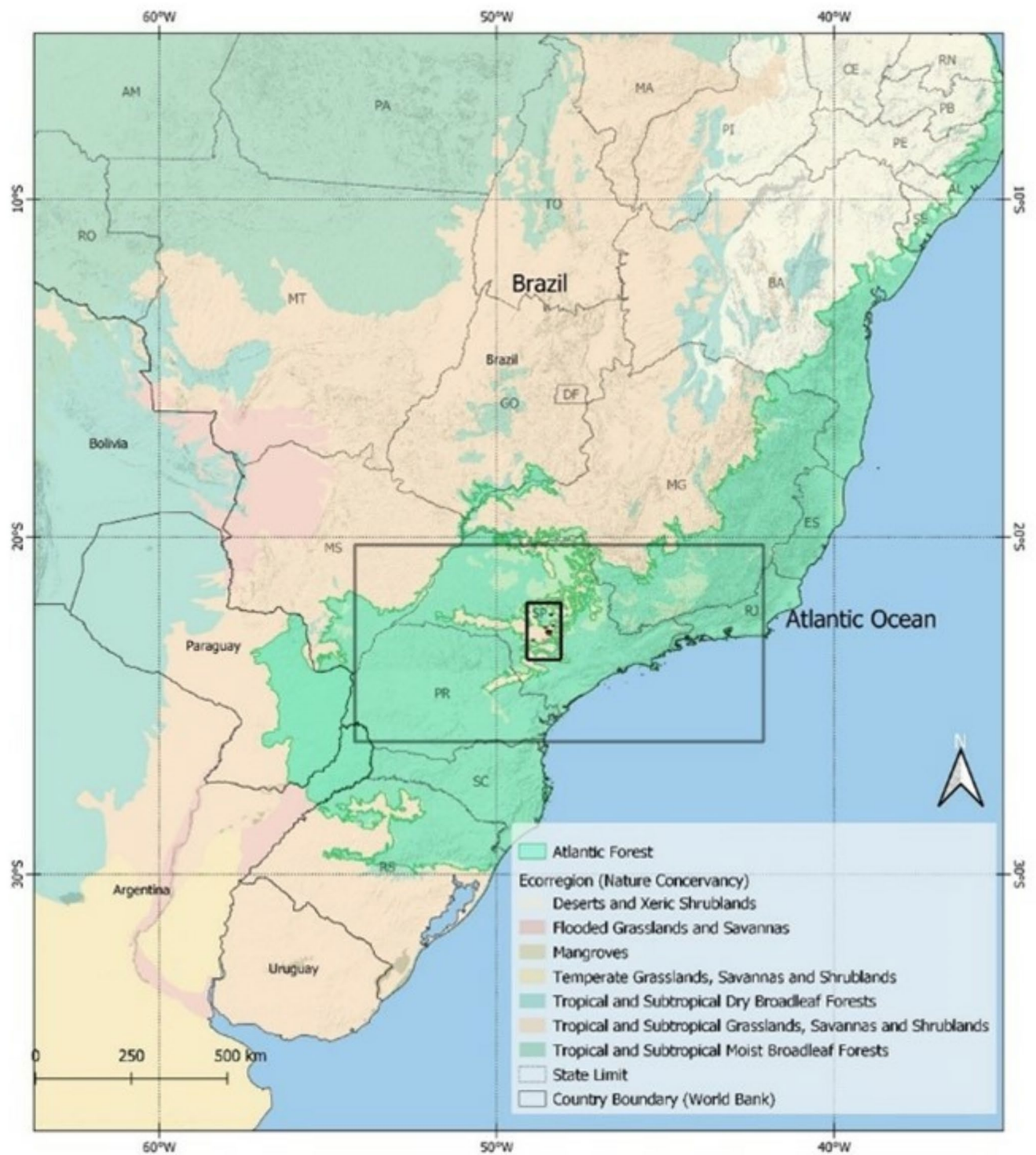


Fig. 1 Map of the study region highlighting the state of São Paulo (inside the larger square) and the collection points (inside the smaller square). The ecoregions are represented by different colors. The Atlan-

tic Forest biome, the focus of this study, is indicated in shades of dark green along the southeastern coast of Brazil. State and national boundaries are highlighted

tissues were handled carefully to avoid contamination and stored in sterile tubes, being frozen at -80 °C for further processing.

DNA extraction and quality assessment

DNA extraction was performed using the Illustra Tissue Kit (GE Healthcare Life Sciences, Marlborough, MA, USA), following the manufacturer's guidelines, with a final elution volume of 100 µL. The concentrations of the samples were standardized to 20 ng/µL, and their quality was evaluated through spectrophotometry (GE Healthcare Life Sciences, Chicago, IL, USA), with A260/280 ratios ranging from 1.8 to 2.0 and A260/230 ratios exceeding 2.0, indicating high purity.

Bartonella DNA detection

The extracted DNA was subjected to real-time PCR targeting a 338 bp fragment of the citrate synthase gene (*gltA*), using the direct primer BartGltAF (5'-TGC AGG WTC ATC RGG WGC TAA T-3') and the reverse primers BartGltAR (5'-TGA GCT GGT CCC CAA AGG-3') and BartGltAR2 (5'-TGG CTC CAC CAT GTG CGG-3' mixed 1:1). The Bartpr probe (5'-FAM-CAT TTG CAT GTA TTG CAG GTG TTG C-BQHI-3') was used to support the reaction and give greater specificity to *Bartonella* species [29]. The reaction was performed in duplicate in a total volume of 25 µL, containing 1 µL of each primer (5 µM), 0.25 µL of probe, 11.5 µL of SYBR Green qPCR Master Mix (Promega, Madison, WI, USA), 6.25 µL of ultrapure water and 5 µL of extracted DNA. The assay was conducted according to the thermal protocol: an initial denaturation step at 95 °C for 10 min; followed by 40 cycles consisting of denaturation at 95 °C for 15 s, annealing at 60 °C for 1 min, and extension at 95 °C for 15 s; and ending with a single extension step at 72 °C for 10 min. Fluorescence signals were obtained during the extension steps of the amplification cycles. The positive control was a *B. henselae* isolate provided by the Rickettsiosis and Hantavirus Service of the Central Public Health Laboratory of Minas Gerais.

To make the product viable for sequencing, the positive samples were subjected to conventional PCR targeting the 750 bp citrate synthase gene, using the primers BhCS.781p (5'-GGG GAC CAG CTC ATG GTG G-3') and BhCS.1137n (5'-AAT GCA AAA AGA ACA GTA AAC A-3') [30]. The assay was conducted in a final volume of 25 µL, containing 12.5 µL of Master Mix (Promega, Madison, WI, USA), 1 µL of each primer (10 µM), 8.5 µL of ultrapure water and 2 µL of extracted DNA. The thermal protocol included an initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for

30 s, annealing at 55 °C for 1 min and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. The amplified products were subjected to electrophoresis in a 1.5% agarose gel, stained with ethidium bromide and visualized under ultraviolet light.

C. burnetii DNA detection

The extracted DNA was subjected to real-time PCR targeting a 107 bp fragment of the IS 1111 gene, using the primers IS-202 (5'-TGT ATC CAC CGT AGC CAG TC-3') and IS-249 (5'-TGG TTC GCT CCT CCA CAC-3'). The probe (5'-TAA GGT GGG CTG CGT GGT GAT GG-BQHI-3') was used to support the reaction and give greater specificity to *C. burnetii* [31]. The reaction was performed in duplicate in a total volume of 25 µL, containing 1 µL of each primer (5 µM), 0.25 µL of probe, 11.5 µL of SYBR Green qPCR Master Mix (Promega, Madison, WI, USA), 6.25 µL of ultrapure water and 5 µL of extracted DNA. The assay was conducted according to the thermal protocol: an initial denaturation step at 95 °C for 10 min; followed by 40 cycles consisting of denaturation at 95 °C for 15 s, annealing at 60 °C for 1 min, and extension at 95 °C for 15 s; and ending with a single extension step at 72 °C for 10 min. Fluorescence signals were obtained during the extension steps of the amplification cycles. The positive control was a *C. burnetii* isolate provided by the Rickettsiosis and Hantavirus Service of the Central Public Health Laboratory of Minas Gerais.

R. rickettsii DNA detection

The extracted DNA was analyzed using a real-time PCR assay targeting a 147 bp fragment of the citrate synthase gene (*gltA*), using the primers CS-5 (5'-TGC AGG WTC ATC RGG WGC TAA T-3') and CS-6 (5'-TGA GCT GGT CCC CAA AGG-3'). The probe (5'-AAA TCA ACG GAA GAG CAA AAC CCC TCC ACT ACC TGC ATC AT-BQHI-3') was used to support the reaction and give greater specificity to *R. rickettsii* [32]. The reaction was performed in duplicate in a total volume of 25 µL, containing 1 µL of each primer (5 µM), 0.25 µL of probe, 11.5 µL of SYBR Green qPCR Master Mix (Promega, Madison, WI, USA), 6.25 µL of ultrapure water and 5 µL of extracted DNA. The assay was conducted according to the following thermal protocol: an initial denaturation step at 95 °C for 10 min; followed by 40 cycles consisting of denaturation at 95 °C for 15 s, annealing at 60 °C for 1 min, and extension at 95 °C for 15 s; and ending with a single extension step at 72 °C for 10 min. Fluorescence signals were obtained during the extension steps of the amplification cycles. We used DNA from a *R. rickettsii* isolate provided by the Rickettsiosis

and Hantavirus Service of the Minas Gerais Central Public Health Laboratory.

Gene sequencing

The PCR products were purified using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) following the protocol provided by the manufacturer. Purified products were subsequently sent to a partner laboratory for Sanger sequencing. The sequencing was carried out in both forward and reverse directions. The resulting sequences from positive samples were analyzed against the GenBank database using the BLASTn tool. Sequence quality was evaluated with BioEdit Sequence Alignment Editor version 7.2.

Phylogenetic analyses

A phylogenetic tree was constructed using all sequences obtained in this study, along with representative reference sequences retrieved from GenBank. Brazilian sequences of *Bartonella* identified in cats, dogs and fleas were included to enhance the regional relevance of the analysis. Additionally, globally representative sequences of *B. koehlerae* and *B. clarridgeiae* from humans, cats, dogs and fleas were incorporated to provide broader context. Sequences from *B. henselae*, *B. bacilliformis* and *B. ancashensis* were included as closely related species to refine the evolutionary relationships within the genus. An isolate of *Brucella abortus* was selected as the outgroup. The phylogenetic analysis was performed using Maximum Likelihood method in MEGA12 version 12.0.7. The Tamura 3-parameter model with gamma distribution (T92+G) was applied, and the inferred tree topologies were assessed using 1,000 bootstrap replicates.

Data analysis

To characterize the bat collection areas, QGIS software was used to georeference the sampling points and the places where the bats tested positive. The data was collected in the field and entered into a geographic coordinate system, ensuring the accuracy of the location of the samples. The ecoregions were integrated into the map, allowing for a more comprehensive spatial analysis. The data was processed and visualized using layers, making it possible to identify the relationships between the distribution of bats and the characteristics of the ecoregions.

To explore the associations between *Bartonella* positivity and the collected categorical variables, a univariate statistical analysis was performed using the chi-square test. The analysis focused on evaluating the potential correlations between *Bartonella* infection and factors such as dietary

habit, sex and year. The significance level set for the tests was 0.05. All tests were run in SAS Studio (version 3.81, SAS Institute Inc., Cary, NC, USA).

Results

Of the 102 bats analyzed, 3.92% [95% CI: 1.54–9.65%] tested positive for *Bartonella* DNA. The cycle threshold (Ct) of positive samples ranged from 30 to 32, indicating a moderate parasite load. Ct values were consistent among the samples analyzed, indicating a relatively uniform parasite load among species and positive tissues. Ct values for spleen samples were slightly lower than those for liver, suggesting higher concentration of parasite DNA in this tissue. The positive samples presented melting temperature (T_m) values close to those of the positive controls, with a maximum variation of 1 °C. This small variation indicates a high specificity of the amplification, suggesting that the products generated correspond to the expected target DNA of *Bartonella*. The consistency of the T_m reinforces the reliability of the results and rules out the presence of nonspecific amplifications. Positive bats were found in three cities. Among the positive bats, two were *Artibeus lituratus* (frugivorous) and two were *Myotis nigricans* (insectivorous).

Of the total number of positive samples, 2.94% (3/102) were amplified using conventional PCR and produced enough DNA for sequencing. All the sequenced samples showed query coverage of over 90% and similarity of over 95% with other *Bartonella* sequences available on GenBank. The sequences obtained were identified as *B. koehlerae* (n=2) and *B. clarridgeiae* (n=1). The high alignment coverage reflects the integrity of the amplified sequences, while the high similarity demonstrates that the isolates analyzed are genetically close to the strains previously described. The accession numbers of the sequences generated in this study are shown as PQ869012, PQ869013 and PQ869014. The Fig. 2 shows a distribution map of the samples collected in the state of São Paulo, Brazil. Positive *Bartonella* spp. bats can be seen in the red flags.

The statistical association analysis between the positivity of the bats and the obtained variables are presented in Table 1.

Odds ratios (OR) with 95% confidence intervals (CI) and p-values were calculated to assess the association. Total population for each category is also provided. The p value (0.0016) indicates that the association between eating habits and *Bartonella* positivity is statistically significant. The Odds Ratio (OR) for fruit bats was 48 (4.31–534), indicating that fruit bats are much more likely to test positive for *Bartonella* than insectivores. The confidence interval (95% CI) is wide but still significant, suggesting a strong association.

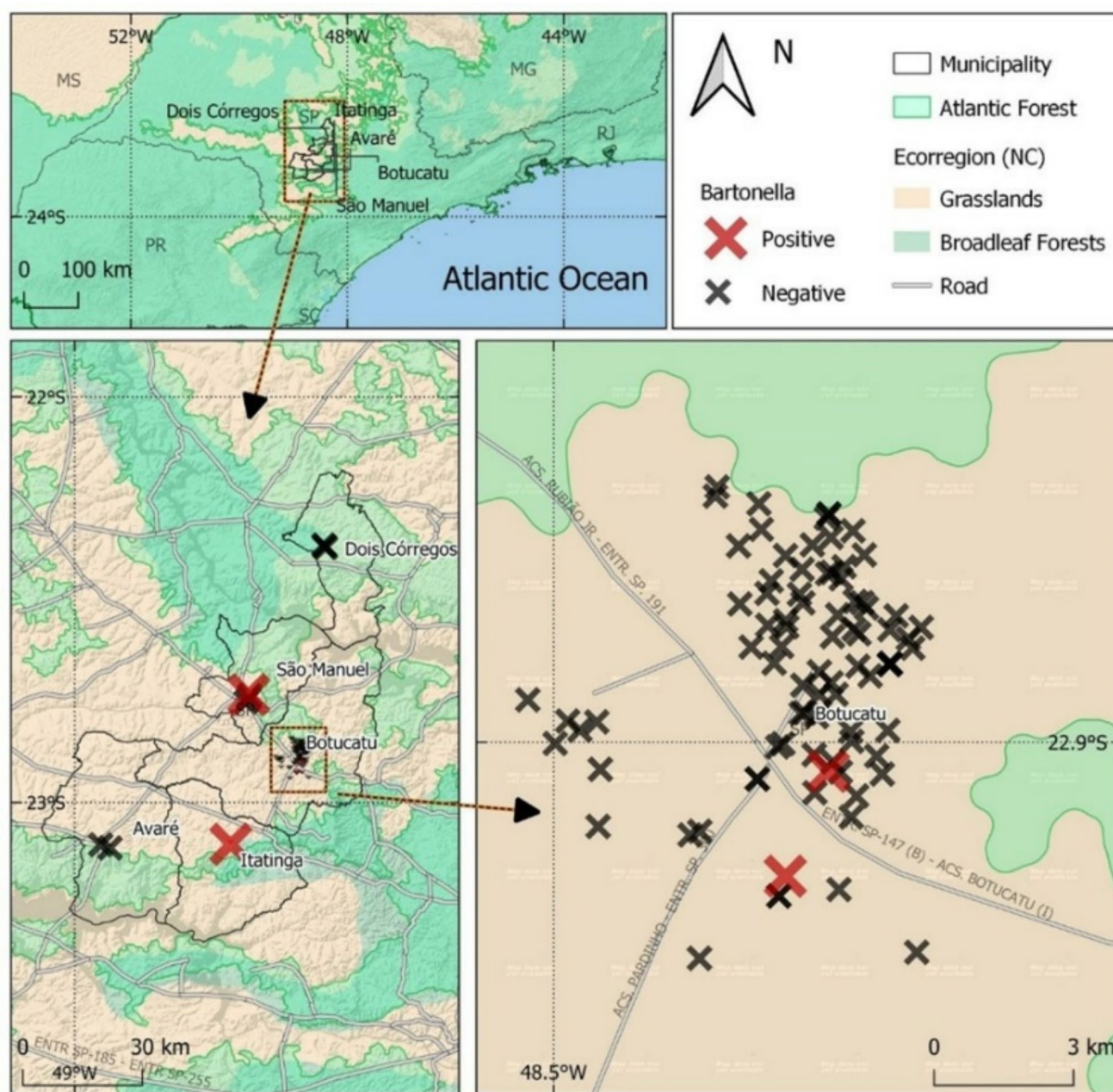


Fig. 2 Map illustrating the study areas and the distribution of positive and negative samples for *Bartonella* spp. in the state of São Paulo, Brazil (2020–2022)

The low sampling of fruit bats is a limitation to the analysis. There was no statistical association between positivity and the others variables evaluated.

Phylogenetic analysis revealed important insights into the relationships between *Bartonella* isolates obtained from bats and other sequences from different hosts (Fig. 3).

The PQ869014 sequence, identified as *B. koehlerae* in (*A. lituratus*), clustered with a global reference sequence of (*B. koehlerae* from stray cats (MK043072). This phylogenetic proximity suggests a possible ecological connection

between these hosts, highlighting the role of (*A. lituratus* as a potential reservoir of (*B. koehlerae*). Similarly, the PQ869013 sequence, identified as *B. clarridgeiae* in *M. nigricans*, clustered robustly with *B. clarridgeiae* sequences from cat fleas (MT112195) and human with endocarditis (MG385320). This alignment reinforces the hypothesis that *B. clarridgeiae* circulates among multiple host species, and may have adapted to different ecological niches. The PQ869012 sequence, identified as *B. koehlerae* in *M.*

Table 1 Relationship between categorical variables and positivity for *Bartonella* in bats in Brazil, with presentation of odds ratios (OR), confidence intervals (95% CI), p-values and population sizes

Variables	Bartonella Positive	Bartonella Negative	OR (95% CI)	<i>p</i> Value	Total Population
Eating habit				0.0016	
Frugivorous	2 (50%)	2 (50%)	1.0 (ref)		4
Insectivorous	2 (2%)	96 (98%)	48 (4.31–534)		98
Sex				0.3140	
Male	1 (2%)	51 (98%)	1.0 (ref)		52
Female	3 (6%)	47 (94%)	3.25 (0.32–32.4)		50
Year				0.6852	
2022	2 (6%)	33 (94%)	1.0 (ref)		35
2021	2 (4%)	50 (96%)	1.5 (0.20–11.2)		52
2020	0 (0%)	15 (100%)	2.3 (0.10–51.1)		15

nigricans, exhibited no close phylogenetic association with the reference sequences, as indicated by low bootstrap support.

DNA from *C. burnetii* and *R. rickettsii* was not detected in any of the samples.

Discussion

The positivity found for *Bartonella* shows that urban bats can be infected and can act in the transmission cycle of the disease, either through direct contact with other animals or through contact with fleas and ticks that parasitize these animals. The species identified in this study, *B. koehlerae* and *B. clarridgeiae*, cause cat scratch disease, a zoonosis that occurs mainly after scratches or bites from infected cats, or through contact with their fleas (*Ctenocephalides felis*). Clinically, cat scratch disease is characterized by regional lymphadenopathy, fever and fatigue, but in more severe cases it can develop into complications such as endocarditis, which has already been described in patients in southeastern Brazil and has been associated with these *Bartonella* species [4, 5]. This is the first identification of *B. koehlerae* in bats in southeastern Brazil and of *B. clarridgeiae* in the whole country.

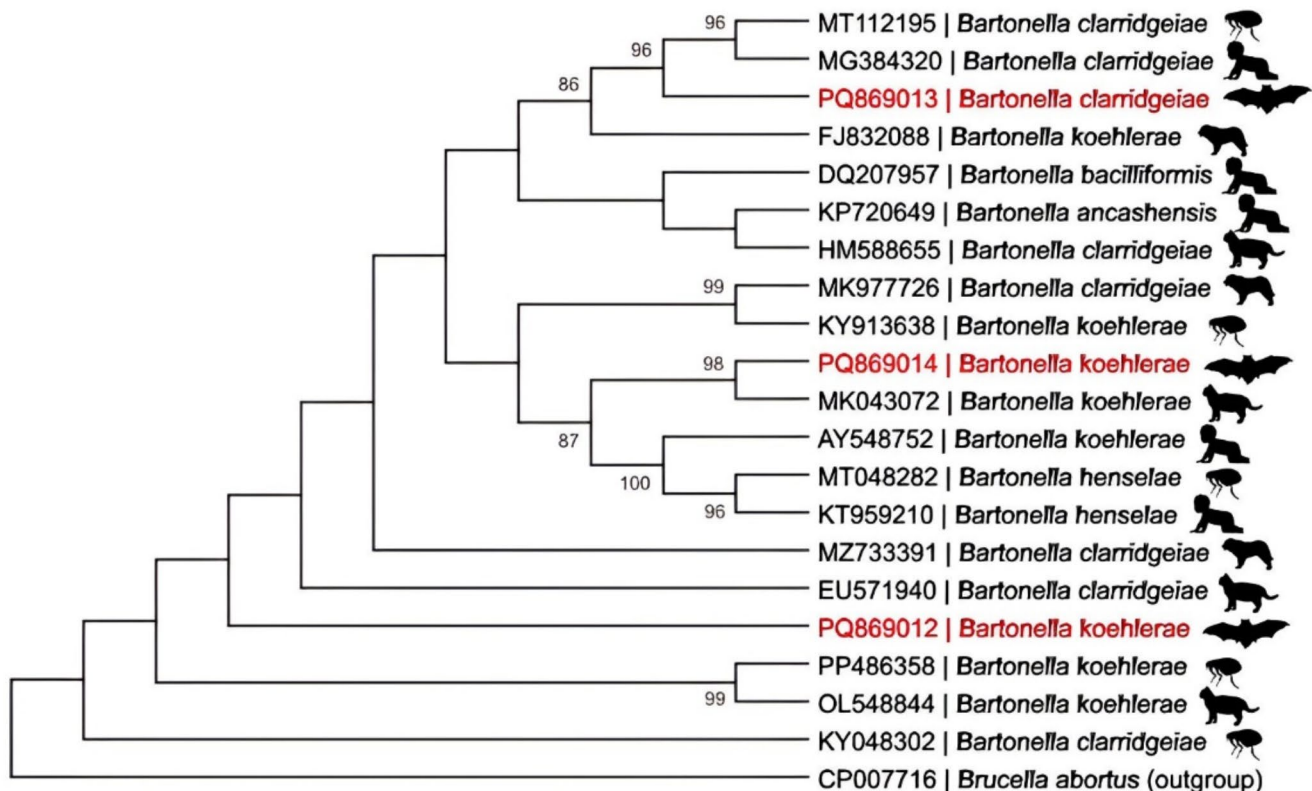


Fig. 3 Phylogenetic tree comparing *Bartonella* DNA reference segments from global and Brazilian isolates with the sequences generated in this study. The sequence of a *Brucella abortus* isolate was used as an outgroup. The tree was built using the Maximum Likelihood method in MEGA12

There are few reports of *Bartonella* in bats in Brazil. A study of bat samples from five states in the north and south detected 5.3% (17/322) positivity for *B. taylorii* and *B. koehlerae* in liver and spleen samples [33]. In another study involving three states, *Bartonella* was detected in 18.5% (22/119) of spleen samples, but only at genus level [15]. The only study involving exclusively hematophagous bats from rural areas, collected from states in the northern region, detected 24.5% (51/208) positivity for *B. bacilliformis* and *B. ancachensis* in liver samples [34]. These species are related to trench typhus, a common disease in humans in Peru, and have already been detected in phlebotomine vectors [35]. Our study was the first to exclusively evaluate bats collected from urban areas and the first to detect species related to cat scratch disease in the southeast Brazil, the region with the most reported human cases. We don't know if bats act in the disease cycle or if they are a reservoir, but we believe that infected fleas and ticks may be the cause of this infection, and although they may be potential vectors for cats and humans, more studies are needed to understand their vectorial function for bats.

In addition to fleas, the main vectors of this zoonosis, we can consider the influence of ticks and flies in the transmission cycle. Two Brazilian studies evaluated the presence of *Bartonella* in bat flies, finding 19.8% (40/202) and 20% (3/15) of positive flies, respectively [36, 37].

This study revealed a significant difference in fruit bats, indicating a higher risk of infection for this category. A previous study showed that fleas digest the blood of fruit bats significantly faster than the blood of insectivorous bats, suggesting physiological differences that may influence the transmission of pathogens [38]. In addition, urban bats are considered potential disease transmitters on a larger scale than other bats. This is because, by staying in urban environments, these animals experience a reduction in natural exposure to microorganisms present in wild habitats, exposure that previously helped control infections. However, factors such as malnutrition, habitat loss and environmental stress have increased the susceptibility of urban bats to infections. Consequently, infected bats can spread a greater load of microorganisms in urban areas, increasing the risks to public health [39].

The negative results for *C. burnetii* in bats are interesting, since Q fever has been widely reported in southeastern Brazil, and the airborne route is considered its main mode of transmission [40]. The only previous study published in Brazil detected 3.4% (4/119) of positivity in bat spleens [15]. In our neighboring country, Colombia, a group investigated the presence of the bacteria in the blood of bats captured in caves, detecting 6.3% (8/126) of positivity [41]. The absence of positivity in bats from an area with described

human and animal cases suggests that these species are not affected by the bacteria.

The negative results for *R. rickettsii* in bats are similar to another Brazilian study which investigated bat tissues and found no positives [42]. Although the presence of DNA has not been described in the tissues, seropositivity and the presence of DNA in ectoparasites of Brazilian bats has already been evidenced [43]. The possibility of infection exists, as was demonstrated in a study from Sweden that detected *Rickettsia* pathogenic to humans in bats and their ticks [44], however, molecular evidence of *Rickettsia* causing Brazilian spotted fever has not yet been described. This is an interesting result because the bat collection points are endemic areas for the disease.

The phylogenetic results of this study reinforce the potential role of bats as reservoirs for *Bartonella* species associated with cat scratch disease. The detection of *B. koehlerae* and *B. clarridgeiae* in urban bats suggests that these animals may play an underestimated role in the transmission cycle of bartonellosis, connecting wild and urban habitats. These observations have significant implications for public health, especially in densely populated areas where interactions between bats, domestic animals and humans are frequent. The presence of *Bartonella* in bats also indicates that these animals may be part of wider transmission cycles, potentially amplifying the spread of pathogens through vectors such as fleas and ticks. Urban factors, such as loss of natural habitat, cat population density and adaptation of bats to anthropized environments, may be intensifying these interactions and increasing zoonotic risks. These findings reinforce the need for integrated surveillance that includes bats as part of zoonosis control strategies. Future studies should explore transmission mechanisms involving bats and their vectors, as well as assessing the ecological and epidemiological impact of these interactions in urban environments.

A potential limitation of this study is related to the choice of the *gltA* gene for the detection of *Bartonella*. Although *gltA* is widely used for the identification and characterization of *Bartonella* species, it is less sensitive compared to other molecular markers, such as ITS. This lower sensitivity may have resulted in false negatives, underestimating the true prevalence of *Bartonella* infection in the studied population. Therefore, future studies could consider using more sensitive markers or a combination of multiple genes to enhance the accuracy of detecting these pathogens [45, 46].

The COVID-19 pandemic coincided with the sample collection period. During this interval, attention to other infectious diseases was being neglected throughout Brazil. At the beginning of the pandemic, there was a significant reallocation of health resources to tackle COVID-19, including medical personnel, personal protective equipment and funding. This impacted the ability to diagnose and treat diseases,

especially those that were already underdiagnosed. The pandemic resulted in the temporary interruption of health services in several regions, covering routine medical consultations, diagnostic tests and epidemiological surveillance programs [27]. Our result is a warning to Brazilian health authorities and the importance of not interrupting basic services to combat bartonellosis.

Conclusions

This study is a pioneer in the molecular investigation of *Bartonella* spp., *Coxiella burnetii* and *Rickettsia rickettsii* in urban bats in the São Paulo state, Brazil. *B. koehlerae* and *B. clarridgeiae*, both associated with cat scratch disease, were identified. This is the first identification of *B. koehlerae* in bats in southeastern Brazil and of *B. clarridgeiae* in the whole country. A close phylogenetic relationship was also observed between the bat isolates from study and global and Brazilian isolates from cats and humans. In addition, positivity was significantly higher in fruit bats, highlighting a possible increased risk of infection in this category. Although *Coxiella* and *Rickettsia* were not detected in the samples analyzed, the results reinforce the importance of urban bats as possible reservoirs of zoonoses and their role in the transmission dynamics of these diseases. This work contributes to the understanding of the ecology of these zoonoses in urban environments and highlights the need for further studies to investigate the role of bats in the transmission cycle and the potential vectors involved.

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Author contributions Conceptualization: [Danilo Alves de França]; Methodology: [Danilo Alves de França, Alana Vitor Barbosa da Costa, Leandro Meneguelli Biondo, Benedito Donizete Menozzi]; Formal analysis and research: [Danilo Alves de França, Alana Vitor Barbosa da Costa, Leandro Meneguelli Biondo]; Writing - preparation of original draft: [Danilo Alves de França]; Writing - proofreading and editing: [Ana Íris de Lima Duré, Benedito Donizete Menozzi, Felipe Fornazari, Helio Langoni]; Obtaining funding: [Ana Íris de Lima Duré, Helio Langoni]; Resources: [Helio Langoni]; Supervision: [Felipe Fornazari, Helio Langoni].

Data availability All relevant data are within the manuscript.

Declarations

Ethical approval Ethics Committee for the Use of Animals (number 0259/2022), Faculty of Veterinary Medicine and Zootechny, UNESP, Brazil. Environmental authorization (number 85973-1).

Competing Interests The authors have declared that no competing interests exist.

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PLOS Neglected Tropical Diseases

Vampire bats (*Desmodus rotundus*) as potential reservoirs of *Leptospira* in rural areas

--Manuscript Draft--

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Short Title:	<i>Leptospira</i> in vampire bats from Brazil
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Corresponding Author:	Danilo Alves de França, M.D. UNESP: Universidade Estadual Paulista Julio de Mesquita Filho Botucatu, SP BRAZIL
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	UNESP: Universidade Estadual Paulista Julio de Mesquita Filho
Corresponding Author's Secondary Institution:	
First Author:	Danilo Alves de França
First Author Secondary Information:	
Order of Authors:	Danilo Alves de França
	Rafael H. Mateus-Vargas
	Ina Fischer
	Benedito Donizete Menozzi
	Felipe Fornazari
	Helio Langoni
Order of Authors Secondary Information:	
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Suggested Reviewers:	Laurindo Guasselli UFRGS: Universidade Federal do Rio Grande do Sul laurindo.guasselli@ufrgs.br

Vampire bats (*Desmodus rotundus*) as potential reservoirs of *Leptospira* in rural areas

Danilo Alves de França^{1*}, Rafael H. Mateus-Vargas², Ina Fischer³, Benedito Donizete Menozzi¹, Felipe Fornazari¹, Helio Langoni¹

¹ São Paulo State University, Faculty of Veterinary Medicine and Animals Science, Department of Animal Production and Preventive Veterinary Medicine, Botucatu, SP, Brazil.

² University of Göttingen, Faculty of Agricultural Sciences, Department of Animal Sciences, Göttingen, Germany.

³ University of Göttingen, Faculty of Geoscience and Geography, Department Cartography, GIS and Remote Sensing, Göttingen, Germany.

* Author to whom correspondence should be addressed: danilo.franca@unesp.br

Abstract: This study explores the role of vampire bats in the epidemiology of leptospirosis in rural Brazil. The aim was to detect *Leptospira* spp. in *Desmodus rotundus* bats using different molecular methods, compare the accuracy of the techniques for epidemiological purposes, phylogenetically evaluate the sequences obtained and spatially evaluate the relationship between bat infection rates and regional land use characteristics. Samples were collected from 101 bats in 12 municipalities in the state of São Paulo, Brazil. Molecular techniques, including conventional PCR and real-time PCR, were used to detect *Leptospira* DNA. Spatial analysis evaluated positivity and land use factors, such as livestock density, local settlement extent, pastures, agriculture, forest cover, and water bodies. *Leptospira* DNA was detected in 43% of bat kidneys by real-time PCR and 31% by conventional PCR. Although less sensitive, conventional PCR had a good kappa coefficient and was 100% specific. Genetic sequencing identified two species, *Leptospira borgpetersenii* (30%) and *Leptospira interrogans* (37%). The phylogenetic evaluation showed similarity between the bat sequences and those of cattle from the region. However, the spatial analyses revealed no correlation between the detection rate of *Leptospira* DNA and cattle

density or any type of land use. Vampire bats may play a relevant role in the maintenance of *Leptospira* spp. in rural areas, potentially contributing to the exposure of livestock and posing a zoonotic risk due to their close interactions with farm animals. The findings underscore the importance of targeted surveillance and control measures in rural settings.

Keywords: pathogenic *Leptospira*, hematophagous bats, real-time PCR, phylogenetic analysis, spatial analysis.

Author Summary: This study investigates the interaction between blood-feeding bats (vampire bats) and the bacterium responsible for leptospirosis (*Leptospira*) in rural areas of Brazil. Leptospirosis is a significant zoonotic disease that can affect both humans and animals, often with serious health and economic consequences. The research highlights the importance of these bats as potential reservoirs of the pathogen, which could contribute to the spread of leptospirosis in regions where wildlife, livestock, and humans coexist. By using advanced molecular and phylogenetic analyses, the study reveals a possible link between bats and the transmission of *Leptospira* to cattle, particularly in areas with frequent and close interactions between these species. These findings underscore the critical need for improved disease surveillance and control strategies in rural settings. They also contribute to the global understanding of how infectious diseases spread in ecosystems shared by humans, domestic animals, and wildlife, offering valuable insights into the prevention of zoonotic diseases.

1. Introduction

Leptospirosis is a zoonosis caused by spirochetes of the genus *Leptospira*. Its main urban reservoirs are rodents, which eliminate the bacteria in the urine throughout their lives. Infection occurs mainly through direct contact with the urine of infected animals or indirectly through contaminated water and soil [1]. Humans and other animals can be accidental hosts, especially when they come into contact with these reservoirs. There are several species and serovars of *Leptospira*, and their occurrence and pathogenicity will depend on the geographical region being studied [2]. Leptospirosis is of great importance for both public and animal health. In humans, it

can manifest as an acute febrile illness with possible severe complications such as kidney and liver failure. In animals, it can result in substantial economic losses due to reduced production and increase of treatment costs [3].

In urban areas in Brazil, the main species associated with human cases of the disease is *Leptospira interrogans*, serovars Copenhageni, Icterohaemorrhagiae and Canicola [4]. It is also associated with cases of the disease in dogs, including serovars Canicola and Icterohaemorrhagiae [5]. In rural areas of Brazil, leptospirosis mainly affects cattle, causing abortions and other reproductive problems. The main species associated with these infections is *Leptospira borgpetersenii*, serovars Hardjo and Wolffi [6].

In Brazil, urban areas are the most affected, especially those located in the Southeast region, where the state of São Paulo is located. According to the latest Brazilian censuses, home environments are most associated with confirmed cases and the presence of rats is the greatest related risk factor [7]. Brazil is endemic for leptospirosis, however what usually accentuates the number of cases in municipalities are the epidemic outbreaks that occur in times of rain and flooding [7]. This disease is intrinsically linked to the infrastructural conditions of cities, basic sanitation and the education of the population, affecting communities with the most precarious sanitary conditions [8]. On the other hand, rural areas remain little studied, and there is still no consensus on whether rodents are also the main reservoirs in these environments, or whether wild animals act as reservoirs in a more significant and predominant way [3].

Wildlife has been investigated for its role in maintaining leptospirosis. Few studies on bats have been carried out in Brazil, but what we have is already an indication that they may be important reservoirs. A study carried out in the city of São Paulo evaluated the presence of the bacteria in bat kidneys, finding 1.7% (6/343) of pathogenic leptospires [9]. In Rio Grande do Sul, two studies found high positivity rates: one of the studies evaluated kidney samples by real-time PCR and obtained 39% (36/92) of pathogenic leptospires [10], while the second obtained, by conventional PCR, 22.5% (23/102) of positive leptospires of the *L. interrogans* group [11]. In none of the

studies was there any detection of the bacteria in vampire bats or bats from rural areas, so there is a gap here.

Animal reservoirs are undoubtedly essential for the occurrence of leptospirosis. As in the case of urban rats, bats carry numerous infectious agents and are potential suspects for the occurrence of rural leptospirosis [12]. The aim of this study was to detect *Leptospira* species by different molecular methods in kidneys of vampire bats in Brazil, to compare the accuracy of the techniques for epidemiological purposes, and to test possible relationships between the prevalence obtained and the regional land use characteristics of the areas where the vampires were captured. The spatial analyses considered factors such as the density of livestock as well as the local extent of the settlement, grassland, agriculture, forest cover and water bodies.

2. Materials and methods

Ethical statement

The Ethics Committee for Animal Use of the School of Veterinary Medicine and Zootechnics, São Paulo State University, Brazil (protocol number 0259/2022) approved this study. We obtained environmental authorization through the Biodiversity Authorization and Information System (SISBIO) from the Chico Mendes Institute for Biodiversity Conservation (ICMBio), protocol number 85973-1.

Study area and sample collection

The bats were collected between 2020 and 2022. In total, 101 bats were collected from 12 cities in the São Paulo state, located in a part of the Brazilian Atlantic Forest that has a rich wildlife fauna (Figure 1). One of the greatest biodiversities in the world is found in the Atlantic Rainforest, which corresponds to the area where the animals were collected. Around 70% of the Brazilian population lives in this region, and added to the migration of wildlife to urban environments, the interaction between species becomes greater [13].

The vampire bats were collected by the Agricultural Defense Office for rabies epidemiological surveillance in São Paulo. The bats were collected at their roosting sites, in artificial shelters that included culverts, tunnels, water tanks and abandoned houses. They arrived alive at the laboratory, respecting all ethical procedures for handling and transportation, and were later euthanized with an overdose of isoflurane. To diagnose rabies, the brain was extracted, followed by aseptic extraction of kidneys to investigate the presence of *Leptospira* spp. The collected material was kept at -80 °C until further examination.

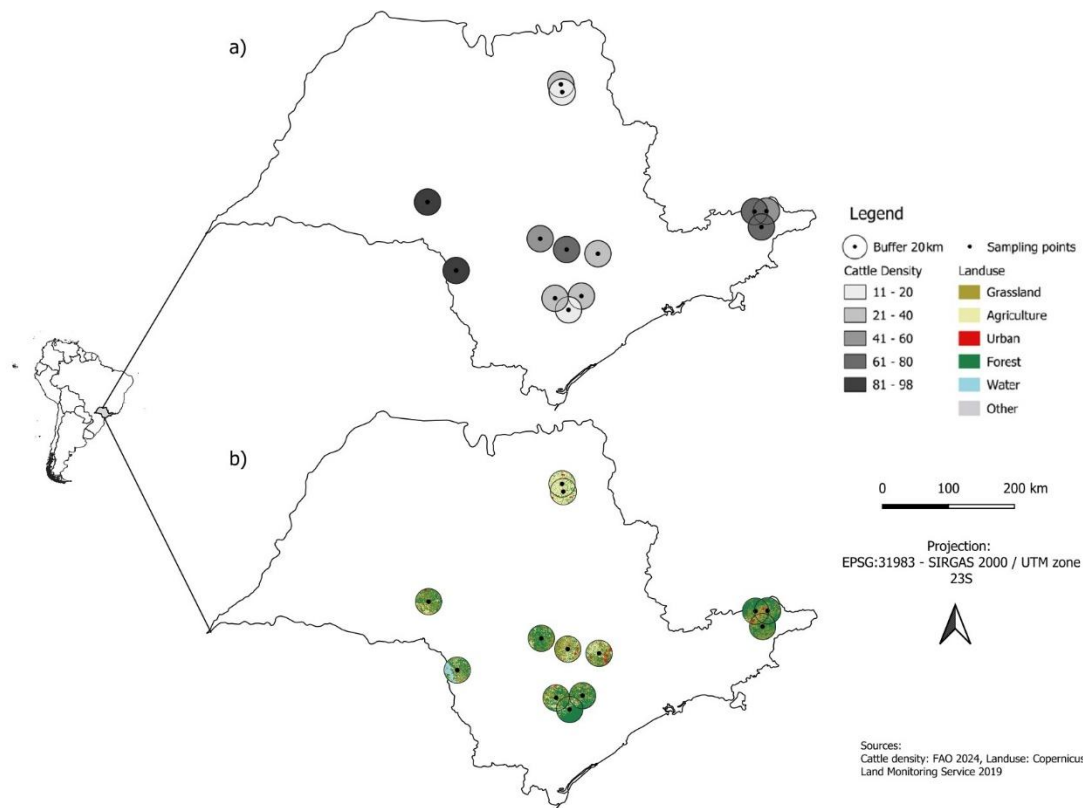


Figure 1. Maps of livestock density and land use in sampled regions in the São Paulo state, Brazil. (a) Distribution of livestock density within 20 km buffers around the collection points, indicating areas with densities ranging from 11 to 98 animals per km². (b) Land use in the sampled areas, categorized into six main classes: pasture, agriculture, urban, forest, water, and other. The outline of South America was obtained from GADM (Global Administrative Areas, University of California, Berkeley, 2025), available at: https://gadm.org/download_world.html, under license: <https://gadm.org/license.html>. The administrative boundaries of São Paulo state were obtained from SimpleMaps (<https://simplemaps.com/gis/country/br#admin1>), and are licensed under

Creative Commons BY 4.0 (<https://creativecommons.org/licenses/by/4.0/>). Layers were clipped and reprojected to UTM Zone 23S. .

The sampled regions are characterized by predominantly rural land use, with livestock farming as the main economic activity. Several municipalities also include small-scale agriculture and human settlements. These land characteristics suggest the possible presence of other domestic and wild animals that could act as reservoirs of *Leptospira*. According to the Notifiable Diseases Information System (SINAN) of the Brazilian Ministry of Health, the São Paulo state recorded 9,388 confirmed cases of leptospirosis between 2007 and 2023 [14]. Specific data by city is not available at this time. Additionally, bovine leptospirosis is considered endemic in the state, with the Hardjo serovar of *Leptospira borgpetersenii* frequently associated with reproductive disorders in cattle herds. Veterinary health authorities, such as the Instituto Biológico, report ongoing serological and molecular diagnoses in cattle, reinforcing the endemic nature of the disease in São Paulo's livestock sector [15].

DNA extraction and quality assessment

DNA was extracted from bat kidneys using the Illustra Tissue Kit (GE Healthcare Life Sciences, Marlborough, MA, USA), following the manufacturer's protocol, with final elution in 100 µL. Sample concentrations were adjusted to 20 ng/µL, and quality was assessed by spectrophotometry (GE Healthcare Life Sciences, Chicago, IL, USA), considering A260/280 ratios between 1.8–2.0 and A260/230 above 2.0 as indicative of high purity. Samples were stored at -80 °C for later analyses.

***Leptospira* DNA detection by Conventional PCR**

The samples were subjected to a first cPCR reaction targeting the primary structure of the 16S rRNA gene of 331 bp, using primers Lep-1 (5' GGC GCG TCT TAA ACA TG 3') and Lep-2 (5' TTC CCC CCA TTG AGC AAG ATT 3) [16]. The 16S rRNA gene is highly conserved for the detection of all *Leptospira* species. The samples were subjected to a second cPCR reaction targeting the 242 bp Lip132 gene, using the primers Lip132- 45F (5' AAG CAT TAC CGC TTG

TGG TG 3') and Lip132-286R (5' GAA CTC CCA TTT CAG CGA TT 3'). The LipL32 gene encodes an outer membrane lipoprotein that is present exclusively in pathogenic species [17]. The reactions were performed in a total volume of 25 µL, containing 5 pMol of each forward and reverse primer, 12.5 µL Master Mix (Promega, Madison, WI, USA), 7.5 µL of ultrapure water and 3 µL of extracted DNA. The PCR assay was performed in a thermal cycler (Applied Biosystems, Foster City, CA, USA), according to the following thermal protocol: an initial denaturation at 94°C for 3 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 63°C for 1 minute, and extension at 72°C for 2 minutes [16]. The positive control consisted of a *L. interrogans* serovar Hardjo strain, kept in EMJH media, previously centrifuged (3000 rpm for 10 min) and resuspended twice in sterile PBS (pH 7.6) solution. Milli-Q water was used as negative control. Ten microliters of the PCR product was run in 1.5% agarose gel at 100 V. To establish molecular weight standards, the DNA Ladder 100 bp (Invitrogen, Waltham, MA, USA) was employed to visually assess the amplified fragment sizes. Gel visualization was performed under ultraviolet light (Bio-Rad, Hercules, CA, USA).

***Leptospira* DNA detection by Real-Time PCR**

The real time PCR was performed using the SYBR Green system [16], by means of StepOne Plus Real Time PCR (Applied Biosystems, Foster City, California, USA), with Lip132- 45F and Lip132-286R primers described for conventional PCR. The reaction was performed in duplicate in a total volume of 25 µL, containing 1 µL of each primer (5 µM), 12.5 µL SYBR Green qPCR Master Mix (Promega, Madison, WI, USA), 8.5 µL of ultrapure water and 2 µL of extracted DNA. The real-time PCR assay was performed according to the following thermal protocol: an initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 1 minute, and denaturation at 95°C for 15 seconds, and a final extension at 72°C for 10 minutes [16]. The fluorescence signals were obtained along the extension steps of the amplification cycles. The final values were determined by the equipment software.

Gene sequencing

The amplified products from both cPCR and qPCR were purified using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) following the manufacturer's instructions. Sequencing was performed with 10 µL of the purified sample combined with a solution containing 5 µL of the primer (3.2 pmol). Sequencing was carried out bidirectionally using the respective PCR primers, utilizing the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequences from positive samples were analyzed using the BLASTn program to compare nucleotide sequences with those in the international database (GenBank). Sequence quality was assessed using BioEdit Sequence Alignment Editor version 7.2, evaluating criteria such as absence of overlapping peaks, adequate DNA quantity, peak smoothness, and uniform spacing to select valid sequences.

Phylogenetic analyses

A phylogenetic tree was constructed including all the sequences generated in this study, together with representative reference sequences from GenBank. *Leptospira interrogans* and *Leptospira borgpetersenii* sequences detected in cattle, dog, cat, human and rodents in Brazil were added to build the phylogenetic tree. The sequence of a *Leptonema illini* isolate was used as an outgroup. Phylogenetic analysis was performed using distance methods in MEGA11 version 11.0.13. The Neighbor-Joining method was applied using Kimura's two-parameter model. The topologies of the inferred trees were evaluated by 1000 bootstrap resamplings.

Comparing tests accuracy

The cPCR results were compared with the real-time PCR results. Qualitative agreement was assessed by calculating the Kappa coefficient. Kappa values were interpreted according to the usual scale [18]. Associations between *Leptospira* infection and PCR results were explored by calculating sensitivity, specificity, positive and negative predictive values, accuracy, positive test likelihood ratio and negative test likelihood ratio [19]. From a 2×2 table, the quantitative agreement parameters of the techniques were calculated. The formulas $(a/a+c)$ and $(d/b+d)$ were

used to calculate sensitivity and specificity, respectively. To calculate the positive and negative predictive values, the formulas $(a/a+b)$ and $(d/c+d)$ were used, respectively.

Land use categorization of localities

Buffers of radii 2.5, 5, 10, 15 and 20 km were generated around the bat collection points using the Buffer-Tool in QGIS Geographic Information System. The size of the radii was chosen based on published literature regarding the home range size and reported flying distances for *D. rotundus* in Latin-America [20-22].

A FAO raster layer of global cattle densities was downloaded. The spatial resolution is 10 km at the equator and the unit is animals per km² [23]. First the dataset was projected to the coordinate system EPSG:31983 - SIRGAS 2000 / UTM zone 23S. Then the cattle density layer was clipped to the buffer zones by using the tool Clip raster by mask layer. The output resolution was set to 100 by 100 m to match the buffers shape. Afterwards the cattle densities inside the buffers could be retrieved by converting the raster to a vector layer (Raster pixels to polygons). This new layer was then intersected with the original buffer layer that contains the location coordinates and sample data. Later, the area (m²) of each pixel inside the buffers was determined using the Field calculator (\$area). Lastly, a weighted cattle density for each of the buffers was calculated using a python script [24]. This was done by deriving weights based on the different proportions of densities inside the buffers and multiplying these by cattle density.

Additionally, the spatial analysis was based on classified land use data published by the European Union's Copernicus Land Monitoring Service [25]. The dataset was reprojected to the specified coordinate system. For simplicity, the land use classes were consolidated into broader categories, resulting in six types: grassland, agriculture, urban, forest, water, and other. The workflow followed a similar approach to that of the cattle density data, with the main difference being that the goal was to calculate the proportion of each land use class within the buffer, rather than a weighted average.

Statistical Analysis

Initially, descriptive statistics were determined regarding the prevalence by collection site 95% confidence intervals (95% CI). Using the characterization made with geographic data, Spearman's rank correlation coefficient was calculated to statistically evaluate the degree of correlation of the proportional extent of land covered by settlements, agricultural land, grassland, forest, or surface water (%), as well as the cattle density (animals/km²), and the detection rate of *Leptospira* in *D. rotundus* (%) within each of the buffers. The significance level set for the tests was 0.05.

3. Results

Of the 101 bats analyzed by real-time PCR, 42.57% [CI (95%) = 33.38-52.31%] tested positive for *Leptospira* DNA. The cycle threshold (Ct) of the positive samples ranged from 18 to 38, with differences in the initial amount of detectable DNA between the samples. The positive samples showed melting temperature (T_m) values close to those of the positive controls, with a maximum variation of 2 °C. Positive bats were found in all twelve cities studied. By conventional PCR, 31 samples were amplified with products between 242-330 bp, representing 30.7% of the total number of samples. All the samples tested by conventional PCR amplified both target genes. Genetic sequencing was possible in 29 (28.7%, 29/101) of the samples and allowed the identification of two *Leptospira* species, including *L. borgpetersenii* (30.2%, 13/43) and *L. interrogans* (37.2%, 16/43). All the sequenced samples showed query coverage of over 90% and similarity of over 92% with other *Leptospira* sequences available on GenBank. The access numbers (Genbank) of the sequences generated in this study, as well as all the other results described so far, are presented in Supplementary Material (S1).

Phylogenetic analyses

Phylogenetic analysis of positive *Leptospira* samples obtained from vampire bats shows a particularly strong evolutionary proximity to reference strains isolated from *Bos taurus* (cows), compared to other species such as humans and dogs, as shown in Figure 2.

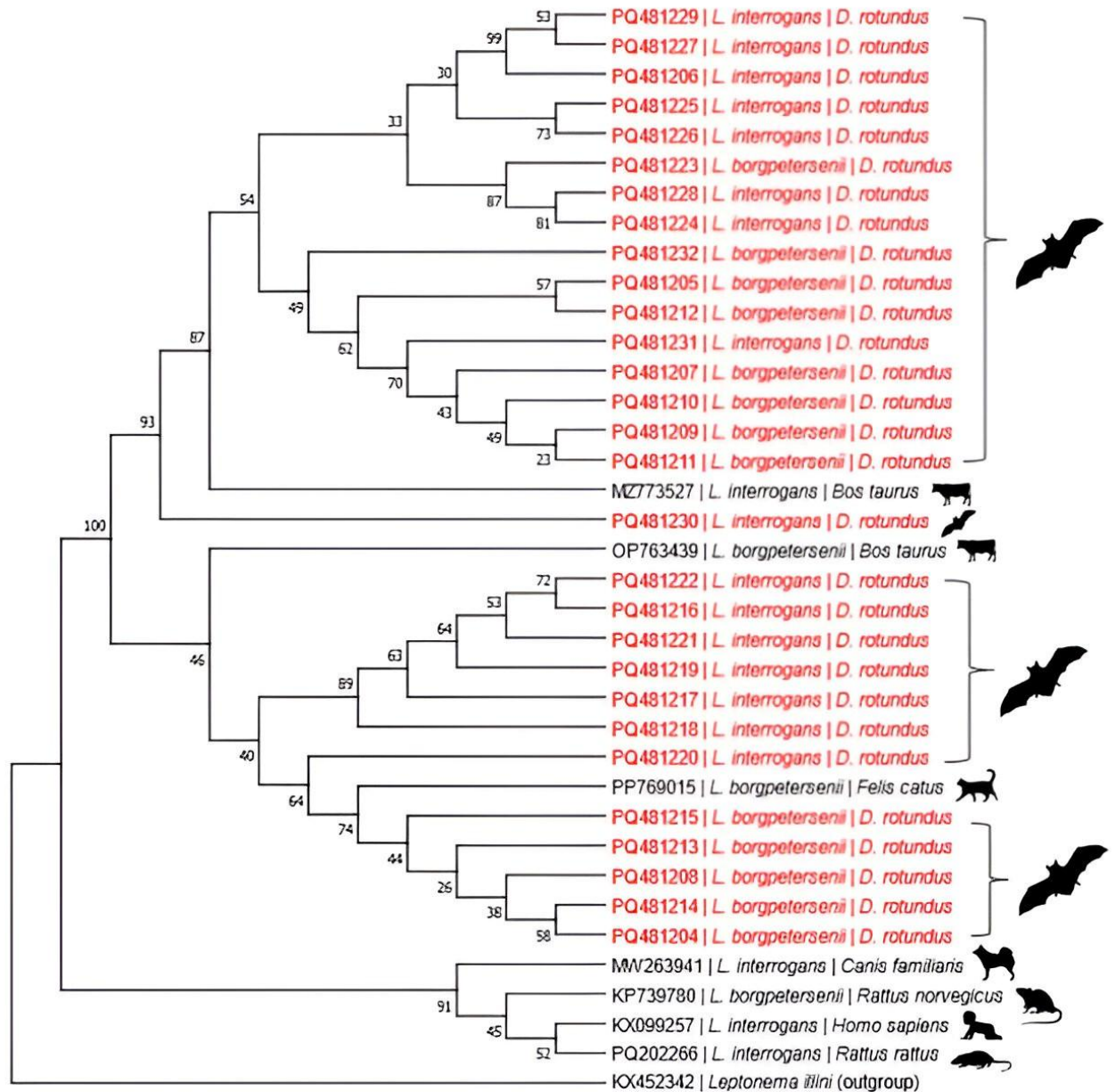


Figure 2. Phylogenetic tree comparing *L. interrogans* and *L. borgpetersenii* DNA reference segments from isolates of other animal species in the region with the sequences generated in this study (highlighted in red). The tree was built using the Neighbor-Joining method in MEGA11. Silhouettes obtained from [PhyloPic.org](https://www.phylopic.org/) (available under CC BY 3.0 license).

The distances on the phylogenetic tree represent nucleotide substitutions per position, with a degree of genetic divergence between the sequences analyzed. Bat sequences clustered into well-defined clades with bootstrap values above 70% in most cases, with robust support for the observed phylogenetic relationships.

Comparing tests accuracy

According to real-time PCR, 42.6% of the samples were positive for *Leptospira*. In comparison, conventional PCR detected 30.7% of the positives. The association between the diagnostic tests can be seen in Table 1.

Table 1. Comparison between real-time PCR (gold standard) and conventional PCR to *Leptospira* diagnosis

		Real-time PCR		Total
		(+)	(-)	
Conventional PCR	(+)	31	0	31
	(-)	12	58	70
Total		43	58	101

Conventional PCR showed strong agreement with real-time PCR, with a Cohen's Kappa index of 0.7479. Compared to real-time PCR, 72.1% of positive samples were also detected by conventional PCR, while no sample was identified as positive exclusively by conventional PCR. The data obtained from the analysis of the diagnostic accuracy of the tests is shown in Table 2.

Table 2. Results of the comparative analysis between real-time PCR and the conventional PCR to *Leptospira* diagnosis

Parameter	Calculation	CI 95% Inferior–Superior	Method
Sensitivity	72.09%	(57.31–83.25)	Wilson's Points
Specificity	100%	(93.79–100)	Wilson's Points
Positive Predictive Value	100%	(88.97–100)	Wilson's Points
Negative Predictive Value	82.86%	(72.38–89.91)	Wilson's Points
Accuracy	88.82%	(80.37–93.07)	Wilson's Points

Likelihood Ratio of Positive Test	Undefined	Undefined	-
Likelihood Ratio of Negative Test	0.2791	(0.2370–0.3286)	-
Cohen's Kappa	0.7479	(0.5592–0.9366)	-

Confidence interval: CI

Although less sensitive than real-time PCR, conventional PCR proved to be specific (100%). The result of the negative predictive value (100%) complements this observation. The likelihood ratio of negative test showed that negative conventional PCR results have only a 0.27 chance of do not correspond to reality.

Spatial analysis based on land use characteristics

Based on the geographic data, the sampled localities exhibited considerable diversity in terms of their land use composition. This variability was similarly reflected in the varying levels of cattle density observed across the different localities. For instance, surface water bodies were largely underrepresented, accounting for less than 1% within a 2.5 km radius, and this trend persisted up to the 20 km radius, with the exception of one point (Locality 3, Fartura city), where it increased to 15.3%. In terms of cattle density, it ranged from 7 to 98 animals per km² within the 2.5 km radius, with most sampling points showing stable values across all buffers. However, in Locality 5 (Jardinopolis city), the density doubled, rising from 7 to 14 animals per km² as the radius expanded to 20 km. Supplementary Material (S2) provides details on the sampled localities, specifically in terms of the average build derived from five buffer areas. Despite these variations in land use and cattle density, neither the proportional extent of land use features nor the surrounding cattle density showed any statistical association with the detection rate of *Leptospira* in *D. rotundus*, as determined by PCR in the study areas.

4. Discussion

Could vampire bats (*Desmodus rotundus*) play a role in the epidemiology of leptospirosis in rural Brazil? With the expansion of cattle production in Brazil, vampire bats began to migrate from the forest to rural areas in search of food. These bats played an important role in population control

in wild environments, and now have abundant food from cattle farms [26]. Recent research in São Paulo has shown that this bat is capable of harboring various zoonotic agents [27-29]. Leptospirosis in urban environments has previously been associated with urban rodents and flooding, however, in rural environments, the origin of leptospirosis remains uncertain [30]. The role of wild animals in the occurrence of leptospirosis on livestock farms has been questioned, however, the role of bats has not been much debated [31,32]. In Brazil, these bats feed on the blood of cattle, a route through which pathogens other than the rabies virus can be transmitted. However, pathogens can also be transmitted by bats through urine, which is probably the way it occurs, through environmental contamination of pasture, troughs and drinking water [33].

Bovine abortion is one of the biggest problems in cattle farming, negatively impacting the national economy and family farmers. *Leptospira* miscarriage has been recorded in several regions of South America, but it is still highly underreported [34-36]. Control is difficult, since there are many possible causes of miscarriage and most animals in the herd are usually infected [36].

The search for reservoirs responsible for introducing the disease into herds would be essential for effective control of the disease in rural environments. The role of wildlife in the occurrence of infectious diseases is a big mystery and deserves much more attention than it is currently getting [31]. The vampire bats collected in this study have direct contact with farm animals in several localities in São Paulo state, and the high positivity observed suggests that these bats may become infected in environments where *Leptospira* circulates, and raises the hypothesis that they could contribute to the maintenance or introduction of the bacteria into domestic herds.

Overall, the molecular detection was 42.57% (43/101), with the majority of positive samples sequenced. *Leptospira borgpetersenii* and *Leptospira interrogans* were the species identified by sequencing, 30% and 37% of the positive samples, respectively, and have distinct epidemiological characteristics.

When comparing molecular diagnostic techniques, we found that conventional PCR is an effective tool for epidemiological studies, such as this one, and for providing an overview of the presence of the disease in a given area. However, it is not recommended for the clinical diagnosis

of animals or humans in contact with bats due to its lower sensitivity. We strongly recommend the use of real-time PCR, which has proven to be highly sensitive for the analysis of bat tissues. These tissues often present interferents that can compromise the effectiveness of conventional PCR reactions. Although real-time PCR has a higher initial cost, it may be more cost-effective over time due to its higher reliability and reduced need for repeat testing caused by inconclusive or false-negative results.

In addition to its increased sensitivity, the real-time PCR assay demonstrated high diagnostic reliability, as all qPCR-positive samples selected for sequencing showed 100% identity with pathogenic *Leptospira* species. This molecular confirmation supports a high positive predictive value (PPV) for the assay under the conditions used in this study. While a formal calculation of diagnostic accuracy indicators such as sensitivity, specificity, PPV, and NPV was not feasible in the absence of a gold standard or known true status of all samples, the absence of amplification in negative controls and the reproducibility of the results across different runs reinforce the analytical specificity of the test. These findings underscore the potential of qPCR not only as a sensitive but also as a reliable tool for epidemiological surveillance in wild reservoirs.

L. borgpetersenii is the most common species in cattle herds and its life cycle involves wild animals. Its presence in rural areas is frequent [37,38]. Transmission to humans occurs through the urine and milk of infected cattle and can cause complications such as fever, kidney failure and meningitis [39,40]. The effect of *L. borgpetersenii* serovar Hardjo on fertilized bovine embryos has already been proven, causing damage to the membrane and cytoplasm of the embryos [41]. The presence of this pathogen in vampire bats may be associated with ecological interactions between bats and cattle in the region. Although previous studies have discussed the role of wildlife in introducing this pathogen into cattle farms [42], the possible involvement of *D. rotundus* in this dynamic is suggested here based on molecular detection and phylogenetic similarity, and warrants further investigation.

L. interrogans is the most pathogenic species of the genus and is also associated with bovine infections and abortions, although it is less frequent in rural areas. Because it is more pathogenic,

non-specific symptoms and acute complications are more prevalent [1]. Wild reservoirs have not previously been associated as a cause of their presence in rural areas, unlike *L. borgpetersenii*. However, its detection in bat kidneys raises the possibility of an ecological relationship that warrants further investigation. In Italy, an outbreak of *L. interrogans* serogroup Pomona in cattle was reported, associating this infection with habitat sharing with wild animals [43]. Two outbreaks of bovine abortion caused by *L. interrogans* serogroup Sejroe serovar Hardjo were also reported [44]. In Ukraine, 24.3% (139/573) of the cows studied were seropositive for the pathogen [45].

The evolutionary proximity to the bovine strains suggests a possible epidemiological interaction between bats and herds, especially in areas where these animals share the same environment. This hypothesis includes the possibility of bidirectional exposure, either from bats to cattle or vice versa, depending on environmental and ecological conditions. In addition, the samples showed low proximity to isolates from dogs and humans, suggesting a closer genetic relationship with bovine strains under the conditions analyzed. The evolutionary proximity to the cat sequence suggests that these bats may share the same water source with cats. Although our discussion focused primarily on the relationship with cattle due to the known feeding habits of *Desmodus rotundus*, the observed genetic similarity with *Felis catus* sequences may indicate another relevant epidemiological link. This proximity raises the hypothesis of shared environmental exposure, which should be further investigated in future studies.

These phylogenetic observations highlight the importance of integrating spatial analyses in the study of zoonoses. The genetic diversity of *Leptospira* found in bats sharing the same roost suggests multiple sources of infection or the movement of these animals between different geographical areas. Furthermore, roosts may serve as important points of interaction between wild and domestic animals, potentially facilitating the circulation of *Leptospira*. The phylogenetic proximity observed between some samples from different roosts indicates regional connectivity between shelters, suggesting that bats may contribute to the dispersal of bacterial strains across ecosystems. These findings highlight the complexity of *Leptospira* transmission dynamics and

the need for approaches that combine phylogenetics and spatial analysis to understand the factors that influence the ecology of the bacterium and its dispersal.

A relevant finding was the phylogenetic proximity between some *L. borgpetersenii* sequences from *D. rotundus* and those from *Felis catus*. This relationship, closer than that observed with *Bos taurus*, raises questions about possible epidemiological interactions between bats and cats, probably related to access to the same source of contamination.

Phylogenetic analysis also revealed a significant genetic diversity of *Leptospira* in bats caught in the same roosts, highlighting the coexistence of distinct lineages within the same environment. In Sales de Oliveira, for example, samples of *L. borgpetersenii* collected from the water tank were grouped into two main clades, with samples PQ481207 and PQ481209 closely related, while samples PQ481206 and PQ481208 showed greater genetic divergence, the latter being grouped into a completely distinct clade. Similarly, in the abandoned house in Ocaçu, samples of *L. interrogans* formed two well-defined clusters, with samples PQ481228, PQ481227, PQ481224 and PQ481229 showing high genetic proximity, while samples PQ481221 and PQ481217 were grouped in a separate clade.

It is important to note that the phylogenetic inferences in this study are based on the LipL32 gene, which is highly conserved among pathogenic *Leptospira* species and may not allow for high-resolution discrimination between closely related strains. Although the LipL32 gene is a conserved marker, the phylogenetic analysis revealed that sequences from bats were consistently grouped with bovine isolates, and not with sequences from other species such as dogs, rodents, or humans. This proximity may reflect a shared environmental source or epidemiological link between these hosts. However, due to the limited resolution of this gene, further analyses using additional targets are needed to confirm these associations. Additionally, we acknowledge that using only regional reference sequences may introduce a geographic bias. However, our goal was to explore phylogenetic proximity within the local epidemiological context. Future studies should include sequences from a wider range of hosts and regions to enhance comparative analyses.

The only study that published a similar result, with bats from rural areas, was a study from Xianning, Hubei province in China, which detected 57% (34/60) of bats positive for *Leptospira* in their kidneys [46]. In Hubei, poultry and pig farming is larger than cattle farming, and there are no studies showing the occurrence on these farms. Another study in Hubei province investigated infection in rodents, obtaining 4.8% (46/964) of positives, mainly in *Rattus norvegicus* [46]. This result is noteworthy because rodents, considered to be the possible reservoirs of rural leptospirosis, were much less positive for *Leptospira* than bats in Hubei. In addition, this study found that the presence of rivers near human settlements increases the risk of *Leptospira* infection [47]. In both Chinese studies, *Leptospira interrogans* and *Leptospira borgpetersenii* were the species detected. These findings support the hypothesis that rural bats may have higher infection rates and could potentially contribute to the risk of transmission to animals and humans in these environments. The lower positivity in rodents compared to bats raises interesting questions about the potential role of bats in local transmission cycles.

These hypotheses should be tested in the future by systematically sampling individuals from both orders (Rodentia and Chiroptera) at the same collection points. By contrasting the level of urbanization of the sampled localities, it may be possible to better understand the transmission patterns between these two groups and to test whether they participate in distinct cycles for *Leptospira* and how their interactions influence the epidemiology of the infection for local human and animal populations.

The ingestion of blood may represent a potential, yet unconfirmed, route of infection for bats. Vampire bats feed directly on the blood of cattle, which raises the hypothesis of pathogen exchange during periods of bovine bacteremia, particularly in the acute phase of infection [48]. Although there is currently no direct evidence of *Leptospira* transmission through this route, the close ecological interaction between bats and cattle, combined with the phylogenetic proximity of the sequences identified, suggests that this hypothesis deserves further investigation.

In the present study, spatial analyses were conducted on the localities where the vampire bats were collected, using data on land use and cattle density. The analysis approach involved

summarizing and extracting data from different buffers, then statistically assessing correlations between the extracted data and the prevalence observed in the individuals. An important challenge was that *D. rotundus* exhibits highly opportunistic behavior in terms of its home range, roosting sites, and prey spectrum [20,49,50]. Due to uncertainty about the most probable movements of the collected vampire bats, various radii were used to improve comparability with other studies and to account for the variable ranges covered by *D. rotundus*, as reported by other researchers [51]. A similar approach was previously used to investigate the occurrence of Staphylococcaceae in wild ruminants in Germany [52], and was also applied by Gomes et al. [51] to study landscape factors influencing vampire bat attacks on cattle in São Paulo, Brazil.

According to Spearman's correlation test, no significant correlation was found between the prevalence of *Leptospira* DNA in the kidneys of *D. rotundus* and land use characteristics or cattle density. At first glance, these results suggest that the dynamics of infection in these bats are not directly linked to human activities or the presence of cattle within a defined radius around the collection point. This implies that other ecological factors, such as microenvironmental conditions in the bats' roosts or interactions with other wild reservoirs, may play a more important role in maintaining the bacterium in bat populations. It is important to note that these observations do not exclude the possibility of a relationship between vampire bat positivity and cattle, as we do not know the infection status of the cattle in the herds or the level of environmental contamination. The presence of distant *Leptospira* strains in the same collection points, as indicated by the phylogenetic tree based on DNA sequencing, complicate drawing definitive conclusions from our observations. Additionally, the plasticity of *D. rotundus* regarding prey spectrum adds further complexity to the analyses [20,49,50].

From another perspective, these findings suggest that cattle density alone may not be directly associated with bat positivity, providing an important initial answer to our research question. This outcome highlights the complexity of the epidemiological cycle of *Leptospira* in rural environments and underscores the need for a broader investigation of the environment and the ecology of bats to understand their role as potential zoonotic reservoirs. For future studies, it

would be beneficial to collect additional vampire bats from a variety of sites within similar localities to gain a more nuanced understanding of how collection sites, land use, and cattle density or holding types (e.g. extensive vs. intensive animal holdings) influence *Leptospira* prevalence. To establish a clearer connection between *Leptospira* in cattle and bats, it is recommended to study both populations within various radii.

It is important to emphasize that, although this study focused specifically on vampire bats due to their ecological interactions with livestock, other wildlife species, especially wild rodents, are known to play significant roles in the environmental contamination and epidemiology of leptospirosis. Future studies should simultaneously evaluate rodents and bats to clearly elucidate their comparative contributions to the epidemiology of *Leptospira* in rural environments.

We still don't know how bats become infected by bacteria or how they function in the epidemiology of leptospirosis, and for this more studies are needed to allow robust comparisons with human data and to assess bat habits. It is a fact that the infection of bats attracted to rural environments and cattle farms is a novelty in the study of leptospirosis, and allows for new possibilities, in addition to rodents, as reservoirs of the disease in these environments.

5. Conclusion

The results of this study showed that 42.6% of the vampire bats (*Desmodus rotundus*) kidneys analyzed were positive for *Leptospira*, with the identification of *Leptospira borgpetersenii* and *Leptospira interrogans*. Phylogenetic analysis revealed a close relationship between the strains detected in bats and those previously reported in cattle, suggesting a possible epidemiological interaction between these species, potentially mediated by feeding habits and environmental sharing. Although no direct correlation with cattle density or land use characteristics was observed, the results raise the hypothesis that vampire bats may play a relevant role in the maintenance of *Leptospira* in rural areas, potentially increasing the risk of infection in livestock and contributing to zoonotic exposure in humans. This study highlights the importance of ecological interactions between bats and domestic animals in the epidemiology of leptospirosis

in rural environments, proposes alternative pathways for bacterial circulation, and reinforces the need for integrated surveillance and control strategies within the One Health framework.

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7. Legends for Supporting Information

Supplementary Material S1 – Molecular and epidemiological data from analyzed bat samples. This table contains detailed information on each sampled bat, including sample ID, sex, collection point, city, qPCR and conventional PCR results, cycle threshold (Ct) values, melting temperature (Melt), detected species, GenBank accession numbers of the sequences generated in this study, and their closest GenBank matches. M: male. F: Female. +: positive. NA: not amplified. SNI: species not identified. NS: not sequenced. Ct: cycle threshold. Melt: melting temperature.

Supplementary Material S2 – This table provides details on each locality sampled in the study, including the type of collection point, number of positive and negative samples, prevalence of infection, average cattle density (animals/km²), and the percentage coverage of different land use types (grassland, agriculture, urban area, forest, water, and other) calculated from five buffer areas surrounding each locality.

Sample ID	Sex	Collection point	City	qPCR	Ct	Melt	cPCR
D18	M	Abandoned house	Pilar do Sul	+	24	79.19	+
D19	M	Abandoned house	Pilar do Sul	+	32	78.29	+
D21	M	Abandoned house	Pilar do Sul	+	33	78.74	NA
D22	M	Abandoned house	Pilar do Sul	+	24	78.74	+
D24	M	Abandoned house	Itu	+	28	79.93	+
D25	M	Abandoned house	Itu	+	28	79.62	+
D27	M	Tunnel	São Miguel Arcanjo	+	18	78.59	+
D28	M	Tunnel	São Miguel Arcanjo	+	32	79.70	+
D29	F	Tunnel	São Miguel Arcanjo	+	26	78.77	+
D32	F	Tunnel	São Miguel Arcanjo	+	38	79.49	NA
D33	F	Tunnel	São Miguel Arcanjo	+	28	78.81	+
D38	M	Manhole	Anhembi	+	37	79.94	NA
D41	F	Cave	Laranjal Paulista	+	28	78.10	+
D42	M	Water tank	Sales Oliveira	+	34	79.93	NA
D43	F	Water tank	Sales Oliveira	+	32	78.96	+
D44	M	Water tank	Sales Oliveira	+	36	78.74	NA
D46	F	Water tank	Sales Oliveira	+	30	79.34	+
D47	F	Water tank	Sales Oliveira	+	28	79.48	+
D48	F	Water tank	Sales Oliveira	+	28	78.59	+
D50	F	Water tank	Sales Oliveira	+	30	79.64	+
D53	M	Water tank	Sales Oliveira	+	30	78.99	+
D57	M	Tunnel	Lavrinha	+	38	79.79	NA
D58	M	Tunnel	Lavrinha	+	28	79.63	+
D60	M	Manhole	Cachoeira Paulista	+	32	79.34	+
D61	M	Manhole	Cachoeira Paulista	+	28	79.38	+
D65	F	Abandoned house	Lorena	+	28	78.97	+
D66	M	Abandoned house	Lorena	+	35	79.93	NA
D67	F	Abandoned house	Lorena	+	37	79.79	+
D68	F	Abandoned house	Lorena	+	35	79.69	NA

D69	F	Abandoned house	Lorena	+	28	78.89	+
D77	F	Manhole	Fartura	+	34	78.49	+
D80	F	Manhole	Fartura	+	30	79.18	+
D86	F	Manhole	Fartura	+	28	78.30	+
D90	M	Abandoned house	Ocaçu	+	30	75.96	+
D91	M	Abandoned house	Ocaçu	+	30	78.97	+
D92	M	Abandoned house	Ocaçu	+	38	79.64	NA
D93	M	Abandoned house	Ocaçu	+	24	78.29	+
D94	M	Abandoned house	Ocaçu	+	26	78.30	+
D95	M	Abandoned house	Ocaçu	+	36	78.74	NA
D96	M	Abandoned house	Ocaçu	+	30	79.93	+
D99	F	Abandoned house	Ocaçu	+	26	79.49	+
D106	F	Abandoned house	Ocaçu	+	38	79.79	NA
D120	F	Manhole	Jardinópolis	+	32	79.78	NA

M: male. F: Female. +: positive. NA: not amplified. SNI: species not identified. NS: not sequenced. Ct

Species	Sequence (Access Number)	Closest GenBank Match
<i>L. borgpetersenii</i>	PQ481204	98.94% (CP047516.1)
<i>L. interrogans</i>	PQ481223	92.77% (MN906895.1)
SNI	NS	-
SNI	NS	-
<i>L. interrogans</i>	PQ481220	96.00% (MN906895.1)
<i>L. borgpetersenii</i>	PQ481205	97.79% (CP047516.1)
<i>L. interrogans</i>	PQ481218	96.93% (CP048830.1)
SNI	NS	-
SNI	NS	-
<i>L. interrogans</i>	PQ481231	96.52% (MN906895.1)
SNI	NS	-
<i>L. interrogans</i>	PQ481225	97.54% (MN906895.1)
SNI	NS	-
<i>L. borgpetersenii</i>	PQ481206	98.00% (CP047516.1)
SNI	NS	-
SNI	NS	-
<i>L. borgpetersenii</i>	PQ481207	99.00% (CP047516.1)
<i>L. borgpetersenii</i>	PQ481208	98.54% (MK328837.1)
<i>L. interrogans</i>	PQ481222	99.10% (OR269719.1)
<i>L. borgpetersenii</i>	PQ481209	98.01% (CP047516.1)
SNI	NS	-
<i>L. interrogans</i>	PQ481226	94.58% (MN906895.1)
<i>L. borgpetersenii</i>	PQ481210	99.00% (MK568984.1)
<i>L. borgpetersenii</i>	PQ481211	99.00% (CP047516.1)
SNI	NS	-
SNI	NS	-
<i>L. interrogans</i>	PQ481230	92.18% (MN906895.1)
<i>L. borgpetersenii</i>	PQ481212	92.67% (MK568984.1)
SNI	NS	-

SNI	NS	-
<i>L. interrogans</i>	PQ481216	99.95% (CP048830.1)
<i>L. borgpetersenii</i>	PQ481213	98.02% (CP047516.1)
<i>L. interrogans</i>	PQ481219	99.62% (MN906895.1)
SNI	NS	-
<i>L. interrogans</i>	PQ481228	90.82% (MN906895.1)
<i>L. interrogans</i>	PQ481227	99.02% (ON619829.1)
<i>L. interrogans</i>	PQ481221	99.95% (CP048830.1)
<i>L. interrogans</i>	PQ481217	95.93% (MN906895.1)
<i>L. interrogans</i>	PQ481224	90.95% (ON209446.1)
<i>L. borgpetersenii</i>	PQ481214	97.56% (MK328837.1)
<i>L. borgpetersenii</i>	PQ481215	97.93% (MK328837.1)
<i>L. interrogans</i>	PQ481229	99.01% (MN906895.1)
<i>L. borgpetersenii</i>	PQ481232	92.50% (MK568984.1)

: cycle threshold. Melt: melting temperature



Rabies virus, *Bartonella koehlerae* and *Leishmania infantum* coinfection in a black *Myotis* (*Myotis nigricans*) from southeastern Brazil

Danilo Alves de França¹ · Bruna Letícia Devidé Ribeiro¹ · Benedito Donizete Menozzi¹ · Helio Langoni¹

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Abstract

The aim of this study was to investigate the presence of important zoonotic agents in Brazil in urban bats and to evaluate the existence of coinfections in bats diagnosed with rabies. In 2021, organ samples from 52 bats from urban areas in southeastern Brazil were used to diagnose rabies and other zoonoses occurring in the region. The positive samples were sequenced, characterized and included in GenBank. A co-infection involving the rabies virus, *Bartonella koehlerae*, and *Leishmania infantum* was identified in a Black *Myotis* bat collected from a household in São Manuel, a city endemic for visceral leishmaniasis. Phylogenetic analysis showed similarity between the RABV sequence obtained and reference sequences from humans and wild canids, as well as confirming the identity of the *Bartonella* and *Leishmania* species detected. To the best of our knowledge, this is the first report of a bat co-infected with rabies virus, *B. koehlerae*, and *L. infantum*. The co-infection of three important pathogens in a Black *Myotis* highlights the multifaceted role of neotropical bats as reservoirs of zoonotic agents. This unprecedented finding reinforces the potential of these animals to contribute to the transmission dynamics of viral, bacterial and protozoan pathogens, and the importance of molecular surveillance in bats.

Keywords RABV · Bartonellosis · Leishmaniasis · Chiroptera · Reservoir · Molecular surveillance

Introduction

Zoonoses pose a significant challenge to global public health, particularly in biodiverse and tropical regions (Allen et al. 2017). The increasing interaction between humans and wildlife, driven by urbanization, deforestation, and environmental imbalances, is one of the primary factors contributing to the emergence and spread of zoonotic diseases (Jones et al. 2008). Bats, owing to their extensive genetic and ecological diversity, play a crucial role as reservoirs for various pathogens, including viruses, bacteria, and protozoa (Alexander et al. 2018; França et al. 2024).

Rabies, bartonellosis and visceral leishmaniasis are zoonoses that occur in Brazil. Rabies is endemic in some regions, especially in rural and forest areas, and bats are currently

recognized as the main source of infection, transmitting the virus to humans and animals (Mayen 2003). According to the Brazilian Ministry of Health, between 2010 and 2024, there were approximately 48 human cases of rabies reported in Brazil. Of these, 24 were caused by bats (half of all cases), nine by dog bites, six by non-human primates, two by foxes, four by felines, and one by a bovine. In two cases, it was not possible to identify the animal responsible for the aggression (Brasil 2024). Bartonellosis is an underdiagnosed zoonosis in the country, mainly associated with fleas on pets, which has shown high prevalence in recent epidemiological studies (Ikeda et al. 2017; Gonçalves-Oliveira et al. 2020; Raimundo et al. 2022; Faccini-Martínez et al. 2023; França et al. 2025a). Between 2011 and 2017, 37.94% (480/1265) of suspected cases were confirmed, with the urban population more affected than the rural population (Silva et al. 2019). Visceral leishmaniasis is endemic in several regions of the country, and Brazil accounts for 93% of the world's cases (Desjeux 2004). More than 500,000 cases occur annually (Desjeux 2004). It is transmitted by phlebotomines that frequently interact with humans, domestic and wild animals

✉ Danilo Alves de França
danilo.franca@unesp.br

¹ Department of Animal Production and Preventive Veterinary Medicine, São Paulo State University, Botucatu, São Paulo, Brazil

(Gómez-Hernández et al. 2017; Castro et al. 2020; Riva et al. 2021; França et al. 2025b).

Bats are recognized as important reservoirs for numerous zoonotic pathogens (Han et al. 2015; Gupta et al. 2021). Although bats are recognized as reservoirs of various pathogens, little is known about the occurrence of co-infections in these animals, especially with pathogens of zoonotic importance. Understanding the co-circulation of zoonotic pathogens in bats may provide insights into potential risks for spillover events (Brook and Dobson 2015). The aim of this study was to investigate the presence of important zoonotic agents in Brazil in urban bats and to evaluate the existence of coinfections in bats diagnosed with rabies.

Materials and methods

In 2021, 52 bats from urban areas were submitted to rabies surveillance and subsequently investigated for other neglected zoonotic agents occurring in the region (*Bartonella* and *Leishmania*). As recommended by the Brazilian Ministry of Health, passive surveillance of rabies in bats is a key component of the country's environmental and epidemiological surveillance services, conducted by local health authorities (Brasil 2022). Bats that show atypical behavior, such as aggression or flying during the day, or are found inside homes are collected by the municipal zoonoses service and sent for rabies diagnosis. The population is instructed to contact these authorities when a bat is sighted in their homes. The identification of bat species was based on morphological characteristics, such as body size, wing shape and cranial structure, following the criteria described by Reis et al. (2013). The bat species collected were *Molossus molossus* (18), *Eumops glaucinus* (15), *Myotis nigricans* (9), *Eumops auripendulus* (3), *Eptesicus furinalis* (2), *Eumops perotis* (1), *Molossus rufus* (2), *Eptesicus brasiliensis* (1), and *Lasiurus blossevillii* (1).

The animals were euthanized by exposing them to a concentration of 5% isoflurane in a closed chamber, followed by continuous exposure until cardiorespiratory arrest. Subsequently, the animals were subjected to necropsy in a controlled aseptic environment. Initially, the skull was opened to remove the brain, followed by the extraction of the spleen and liver. During necropsy, the bats were thoroughly inspected to identify possible anomalies or signs associated with infection by zoonotic agents. The inspection focused on macroscopic pathological signs suggestive of zoonotic infections, including organ discoloration, necrosis, granulomas, or atypical fluid accumulation. All examined tissues appeared within normal parameters. The brain tissue was used entirely for the immediate diagnosis of rabies, while

the other tissues were stored in sterile tubes and frozen at -80°C for subsequent analysis of other zoonotic agents.

For rabies virus detection, fragments of the brain were used for both the direct fluorescent antibody test (DFAT) and the mouse inoculation test (MIT). The DFAT involves applying fluorescently labeled antibodies to brain tissue imprints, enabling the direct detection of RABV antigens in the brain tissue through fluorescence microscopy (Rupprecht et al. 2018). The MIT involves injecting macerated brain material into healthy mice and monitoring them for clinical signs over a seven-day period to confirm the presence of RABV (Rupprecht et al. 2018). Viral RNA was extracted from brain fragments using the InviSorb® Spin Universal Kit (Invitex Molecular, Berlin, Germany) following the manufacturer's protocol. The extracted RNA served as a template for cDNA synthesis through reverse transcription, using primer 21 g (5'-ATG TAA CAC CTC TAC AAT G-3') (Menozi et al. 2020). The cDNA was amplified using primers 21 g and 304 (5'-TTG ACG AAG ATC TTG CTC AT-3'), targeting a 1,478 bp fragment of the N gene, as described elsewhere (Menozi et al. 2020).

For *Bartonella* detection, DNA was extracted from spleen samples using the same kit employed for RNA extraction. Real-time PCR was conducted as a screening method, targeting a 338 bp fragment of the citrate synthase encoding gene (*gltA*), as described elsewhere (Buss et al. 2012). The forward primer BartGltAF (5'-TGC AGG WTC ATC RGG WGC TAA T-3') and the reverse primers BartGltAR (5'-TGA GCT GGT CCC CAA AGG-3') and BartGltAR2 (5'-TGG CTC CAC CAT GTG CGG-3', mixed at a 1:1 ratio) were used. To enhance the reaction and improve specificity for *Bartonella* species, the Bartpr probe (5'-FAM-CAT TTG CAT GTA TTG CAG CAG GTG TTG C-BQHI-3') was utilized. DNA from a *B. henselae* isolate, provided by the Rickettsiosis and Hantavirus Service of the Minas Gerais Central Public Health Laboratory, was used as a positive control. After confirmation of positivity, the sample was amplified by conventional PCR using primers BhCS.781p (5'-GGG GAC CAG CTC ATG GTG G-3') and BhCS.1137n (5'-AAT GCAAAAAGACACA GTAAAC A-3') targeting the 750 bp citrate synthase encoding gene, as described elsewhere (Norman et al. 1995).

For *Leishmania* detection, DNA extracted from spleen samples was used. Real-time PCR was conducted as a screening method, targeting a 204 bp fragment of the putative spermidine synthase 1 gene, as described elsewhere (Wu et al. 2022). The forward primer CL-SPDF (5'-AGA TCA TTG CGT ACT TGA C-3') and the reverse primer CL-SPDR (5'-TCA TCG ACA CAA CAG ACC-3') were used. DNA from the MHOM/BR/IOC/L2906 strain of *L. infantum*, provided by the Zoonoses Laboratory of São Paulo State University, was used as a positive control. After

confirmation of positivity, the sample was amplified by conventional PCR using primers Leish_18S/ITS1 (5' CTG GAT CAT TTT CCG ATG ATT A 3') and Leish_18S/ITS1 (5' AAC ACA AAC ACA CGC CTC CT 3') targeting a 530 bp fragment of the 18 S gene and the beginning of the ITS1 ribosomal RNA gene, as described in the referenced method (França et al. 2025b).

Ten microliters of the PCR product was then subjected to agarose gel electrophoresis.

Positive PCR products were purified using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer's guidelines and sent for Sanger sequencing. Sequences from positive samples were compared with previously deposited sequences in the GenBank database using the BLASTn tool (Altschul et al. 1990). Sequence quality was checked using BioEdit Sequence Alignment Editor version 7.2 (Ibis Therapeutics, Carlsbad, CA, USA).

Phylogenetic analysis was conducted using Maximum Likelihood methods in MEGA12 version 12.0.7 (Tamura et al. 2021). The Tamura 3-parameter model with gamma distribution (T92 + G) was applied, and the topologies of the inferred trees were assessed using 1000 bootstrap replications (Tamura 1993). The sequence obtained from the RABV isolate was aligned with a dataset composed of 20 Brazilian N gene sequences, representing various hosts, including dogs, cats, horses, cows, wild canids, humans, non-human primates, and different bat species. The sequences obtained from *Bartonella* and *Leishmania* were compared with worldwide reference sequences. Outgroups were selected to root the trees: RABV from a European bat,

Brucella abortus for the *Bartonella* analysis, and *Trypanosoma cruzi* for the *Leishmania* analysis.

Results

Among the bats analyzed, 1.9% (1/52) were positive for RABV, 3.9% (2/52) were positive for *Bartonella* spp. and 5.8% (3/52) were positive for *Leishmania* spp. Co-infection of rabies virus, *Bartonella* and *Leishmania* was observed in one individual of the bat species Black *Myotis*. This bat was an adult female.

The cycle threshold (Ct) values of the positive samples in real-time PCR ranged from 30 to 32, indicating a moderate microbial load. The positive samples exhibited melting temperature (Tm) values consistent with those of the positive controls, with a maximum variation of 1 °C.

All samples were successfully amplified by conventional PCR, yielding sufficient DNA for sequencing. The sequenced samples exhibited query coverage greater than 80% and sequence similarity greater than 97% with other sequences available in GenBank. The accession numbers for the sequences obtained from the co-infected Black *Myotis* are PQ812415, PQ812416, and PP939070, showing a high degree of similarity to rabies virus, *Bartonella koehlerae*, and *Leishmania infantum*, respectively.

The phylogenetic analysis, shown in Fig. 1, revealed that the N gene sequence of the rabies virus (RABV) obtained from the bat Black *Myotis* (PQ812415) is grouped in a clade close to that of humans (*Homo sapiens*) and wild canids (*Lycalopex vetulus*). Figure 2 shows the genetic

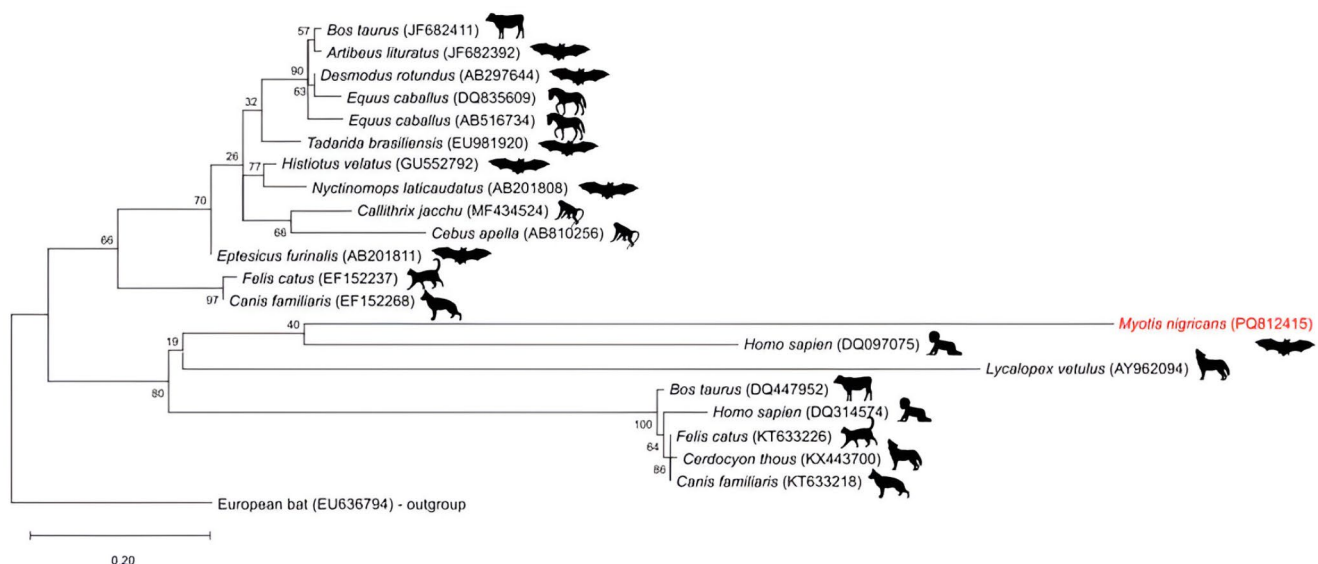


Fig. 1 Phylogenetic analysis of the N gene of rabies virus (RABV) obtained from a Black *Myotis* bat, compared to reference sequences of RABV from different hosts in Brazil. The tree was constructed using the Maximum Likelihood method in MEGA12 software, with Tamura

3-parameter model and 1000 bootstrap replicates to assess branch support. The RABV sequence from a European bat was used as the outgroup

similarity between our sequence (PQ812416) and the reference sequence of *B. koehlerae* (MT095046), confirming the identity of the detected *Bartonella*. Figure 3 shows the genetic similarity between our sequence (PP939070) and the reference sequence of *L. infantum* (PP976612), confirming the identity of the detected *Leishmania*.

Discussion

The co-infection with rabies virus, *B. koehlerae* and *L. infantum* detected in one of the bats represents an unprecedented finding. This result underscores the capacity of these animals to simultaneously harbor multiple zoonotic agents of significant public health importance. The positive bat in this study belongs to the São Manuel city (48°27'4.91"W, 22°55'41.45"S), located in the southeastern of Brazil, in the interior of the São Paulo state. This region is covered by the Brazilian Atlantic Forest, an important ecological hotspot, characterized by the coexistence of high biological diversity and intense urbanization (Oliveira 2007). This bat is popularly known as the Black *Myotis*, an insectivorous bat widely distributed throughout Latin America and found in various habitats, from forest areas to urban environments, characterized by its small size and dark fur (Wilson 1971).

In 2017, a database containing over 2,800 viral infections in mammals was analyzed, revealing that bats are the

most affected species by such infections (Olival et al. 2017). The rabies virus has been reported in 41 species of Brazilian bats, and they are now considered the primary vectors responsible for rabies occurrences in urban areas (Menozzi et al. 2020). The Black *Myotis* is capable of traveling long distances in search of food and inhabiting various shelters, often in close proximity to humans and domestic animals. This behavior increases the likelihood of exposure to and transmission of zoonotic agents (Wilson 1971; Ferreira et al. 2018). The infection of this animal by not only RABV but also other pathogens highlights its ability to survive infections and underscores its potential role as a reservoir for other significant diseases. Given that Black *Myotis* frequently roosts in anthropogenic areas, this co-infection raises concerns about overlapping transmission routes (e.g., vector-borne vs. direct contact) and underscores the need for integrated surveillance in fragmented habitats where humans, domestic animals, and bats converge (Ferreira et al. 2018).

The Brazilian Atlantic Forest is a neotropical region characterized by high humidity and elevated temperatures, conditions that favor not only a rich diversity of animal species but also the proliferation of phlebotomine sandflies (Pinto et al. 2010). These insects interact with bats in both natural and artificial shelters, feeding on their blood and acting as vectors for pathogen transmission (Lampo et al. 2000). In Brazil, *Leishmania* species are transmitted by these insects,

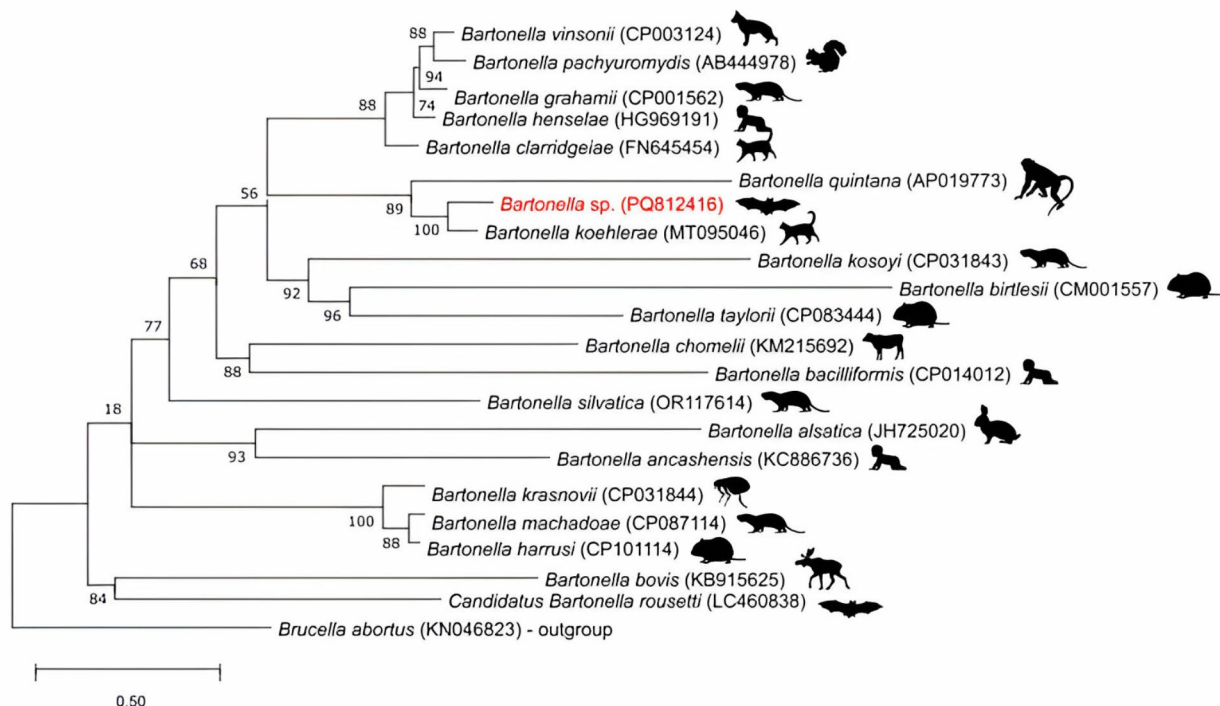


Fig. 2 Phylogenetic analysis of the citrate synthase encoding gene (*gltA*) of *Bartonella* obtained from a Black *Myotis* bat, compared to reference sequences. The tree was constructed using the Maximum

Likelihood method in MEGA12 software, with Tamura 3-parameter model and 1000 bootstrap replicates to assess branch support. *Brucella abortus* was used as the outgroup

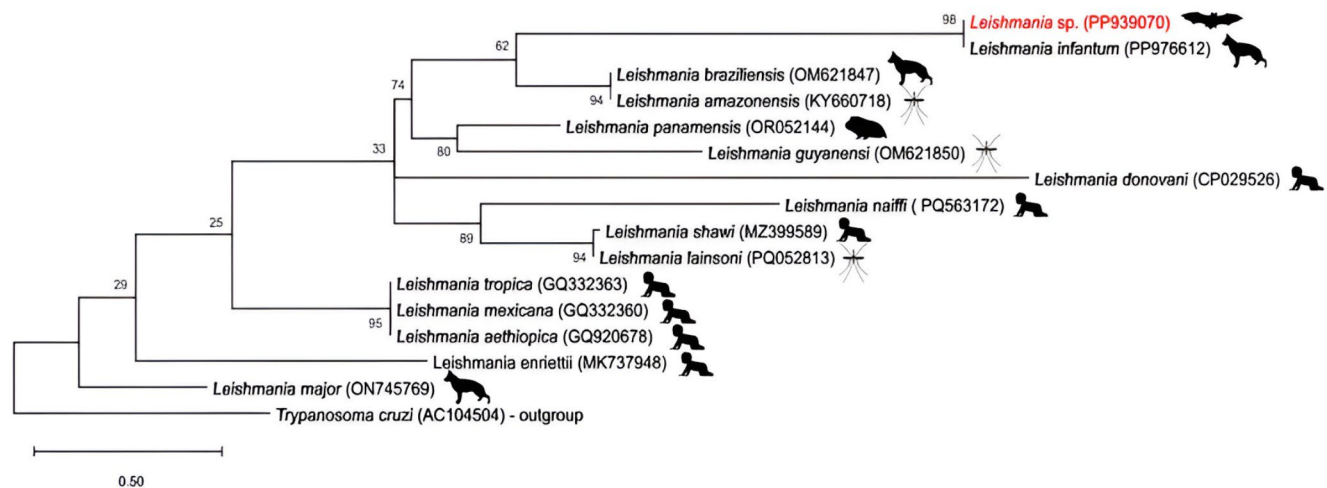


Fig. 3 Phylogenetic analysis of the ITS1 region of *Leishmania* obtained from a Black *Myotis* bat, compared to reference sequences. The tree was built constructed the Maximum Likelihood method in

MEGA12 software, with Tamura 3-parameter model and 1000 bootstrap replicates to assess branch support. *Trypanosoma cruzi* was used as the outgroup

and recent studies have shown that *Bartonella* species can also be transmitted by them (Lee et al. sh2024). Additionally, a study from the United States evaluated the consumption of *Culex* mosquitoes by insectivorous bats, suggesting another possible transmission route to bats (Hughes et al. 2022). These findings suggest that phlebotomine sandflies may contribute to co-infections in bats.

The city where the bat was collected is endemic for visceral leishmaniasis. *Leishmania* species have previously been detected in bats from endemic areas in Brazil; however, co-infection with other pathogens has not yet been reported (Ratzlaff et al. 2023). Although cases of bartonelosis have not been reported in the city, the disease remains widely underestimated in Brazil despite its documented occurrence. Recent studies in the Southeast have indicated a high prevalence of *Bartonella* in shelter cats, homeless individuals, and blood donors (Pitassi et al. 2015; Raimundo et al. 2022; Faccini-Martínez et al. 2023). Domestic cats, potential transmitters of *B. koehlerae* to humans, typically spread the bacterium through scratching after being infected by flea vectors. While clinical signs in cats are generally mild, in humans, the infection can lead to severe conditions such as endocarditis (Avidor et al. 2004). The presence of *B. koehlerae* has been previously reported in Brazilian neotropical bats; however, co-infection with other pathogens has not (Ikeda et al. 2017).

In addition to interactions with phlebotomines and fleas, the bat's association with other ectoparasites may explain this multi-agent infection. In Brazil, *Bartonella* has been detected in 19.8% (40/202) of Streblidae flies (Amaral et al. 2018).

The position of our RABV sequence in the phylogenetic tree highlights the relevance of bats in the RABV transmission chain, especially in areas where different mammal species share habitats. The phylogenetic proximity between the bat RABV sequence and the sequences from humans and wild canids suggests a possible epidemiological connection, reinforcing the wide variety of potential hosts and reservoirs of the rabies virus.

An important limitation of this study is the fact that only one bat showed co-infection, which may limit the generalizability of the results. At the same time, this finding is relevant, as it highlights the complexity of interactions between pathogens in bats and their potential ability to act as multiple reservoirs. Furthermore, it is important to contextualize this finding within the Brazilian surveillance system, which prioritizes the collection and analysis of bats found fallen or in atypical situations, such as inside homes. This system, although effective for detecting rabies cases, may underestimate the occurrence of other zoonoses, since active surveillance for other pathogens in bats is still limited.

Conclusion

This study reports, for the first time, the co-infection of rabies virus, *Bartonella koehlerae*, and *Leishmania infantum* in a Black *Myotis* bat in southeastern Brazil. The results highlight the potential role of these animals as multifaceted reservoirs of zoonotic agents with different transmission cycles, reinforcing the need for integrated surveillance in regions where humans, domestic animals, and wildlife coexist. The detection of these pathogens in a single host

underscores the complexity of pathogen-host interactions in anthropized environments and the importance of further studies to assess the ecological and epidemiological impact of these co-infections.

Author contributions All the authors contributed to the conception and design of the study. Material preparation and data collection were carried out by Danilo Alves de França, Benedito D. Menozzi and Helio Langoni. The methodology and analysis of the results were carried out by Danilo Alves de França and Bruna L. D. Ribeiro. The first draft of the manuscript was written by Danilo Alves de França. All the authors read it, contributed to the writing and approved the final manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval Ethics Committee for the Use of Animals (number 0259/2022), Faculty of Veterinary Medicine and Zootechny, UNESP Botucatu, Brazil. Environmental authorization (number 85973 -1).

Competing interests The authors declare no competing interests.

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Prevalent zoonoses in Sao Paulo State, Brazil: the role of bats and molecular diagnosis

Danilo Alves de França ¹, Helio Langoni ¹

ABSTRACT

This review explores the landscape of prevalent zoonotic diseases in Sao Paulo State, Brazil, focusing on the role of bats as reservoirs and the application of molecular biology in the diagnosis. The zoonoses covered include visceral and cutaneous leishmaniasis, Chagas disease, toxoplasmosis, bartonellosis, Q fever, Brazilian spotted fever, and leptospirosis. Molecular techniques can improve public health responses by accurately identifying pathogens and tracking their transmission dynamics in populations, thus enhancing early detection, characterization of strains, and monitoring of disease outbreaks. By elucidating the epidemiology and molecular aspects of zoonoses associated with bats in Sao Paulo State, we highlight the importance of integrated surveillance systems and multidisciplinary approaches to effectively manage and prevent these diseases.

KEYWORDS: Surveillance. Reservoir. Infectious agents. Zoonoses. Literature review.

INTRODUCTION

Brazil faces significant challenges related to zoonoses, which are infectious diseases transmitted between animals and humans. Bats are important reservoirs of zoonotic agents, being vital to better understand and mitigate these diseases. With advances in molecular biology, new diagnostic techniques have revolutionized the identification and study of these zoonoses, offering new perspectives to comprehend epidemiology and implement control measures¹.

Many zoonoses are classified as public health issues in Sao Paulo State. Regarding prevalence, severity and lethality, leishmaniasis and leptospirosis are among the most significant zoonoses. Chagas disease and toxoplasmosis are also prevalent and, in specific cases, can also lead to death. Some other zoonoses also stand out in terms of severity and lethality, but are highly underdiagnosed, and recent studies have shown that they circulate in animals and humans in the statewide, such as Brazilian spotted fever, bartonellosis and Q fever².

These diseases share common reservoirs, which participate in transmission cycles and their occurrence in humans. This is the case with dogs, which are very close to humans in urban and rural environments, being directly linked to the occurrence of visceral and cutaneous leishmaniasis in various municipalities in Sao Paulo State³. Dogs are also directly linked to urban leptospirosis, Chagas disease in rural environments, and toxoplasmosis⁴⁻⁶. Bovines are also a common source of infection and an important reservoir for diseases such as rural leptospirosis and Q fever, especially during the reproductive period, when the bacteria are eliminated by calving^{2,6}. Cats are also important reservoirs, acting in the cycle of bartonellosis and toxoplasmosis^{5,7}. Bats have adapted to both urban and rural environments,

¹Universidade Estadual Paulista, Departamento de Produção Animal e Medicina Veterinária Preventiva, Botucatu, São Paulo, Brazil

Correspondence to: Helio Langoni
Universidade Estadual Paulista,
Departamento de Produção Animal e
Medicina Veterinária Preventiva, Prof.
Doutor Walter Mauricio Correa, S/N, CEP
01049-010, Botucatu, SP, Brazil

E-mail: helio.langoni@unesp.br

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and their proximity to these reservoirs is common in Sao Paulo State. Vampire bats, for example, mainly feed on cattle herds, while insectivorous and frugivorous bats have a lot of contact with dogs and cats, since their main shelters are located in urban areas. The possibility of these bats acting as reservoirs and players in the cycle of these zoonoses is something to be investigated and debated among researchers¹.

This review aims to explore the prevalence of zoonoses in Sao Paulo State, Brazil, focusing on the role of bats and the contribution of molecular biology and techniques such as polymerase chain reaction (PCR) to the diagnosis.

Visceral leishmaniasis and cutaneous leishmaniasis

Leishmaniasis are caused by the flagellated protozoan *Leishmania* spp. There are approximately 1.5 million cases of cutaneous leishmaniasis (CL) and 500,000 cases of visceral leishmaniasis (VL) per year. A population of 350 million is at risk, mainly distributed in tropical and developing countries. Ninety percent of global cases of VL occur in five countries, including Brazil. The same applies to CL, with seven countries, including Brazil, accounting for 90% of global cases. There are currently 20 species of *Leishmania* and 30 species of phlebotomine vectors. The main vectors that transmit the disease are female sandflies of *Lutzomyia*, popularly known as *mosquito-palha*. In Brazil, deforestation, encroachment into primary forests, mass rural-to-urban migration and the construction of dams have increased the incidence of the disease in recent years. These factors enabled a greater development of mosquito vectors, which is undoubtedly associated with the country's economic inequality^{3,8}.

The main causative species of VL in Brazil is *L. infantum* (syn. *chagasi*), while for CL, they are *L. amazonensis* and *L. braziliensis*, distributed nationwide, and *L. guyanensis*, concentrated in the Northern region. In humans, VL symptoms range from fever, weight loss and anemia to more serious complications, such as splenosis and hepatomegaly. In turn, CL manifests ulcerative lesions on the skin and mucous membranes, coryza, nosebleeds and sore throats^{9,10}.

In Sao Paulo State, 8,553 cases were reported from 1999 to 2019. Among these cases, 3,046 (35.6%) were confirmed as autochthonous, with 266 resulting in death; an 8.7% overall mortality rate. Among the 645 municipalities, 107 (16.6%) reported autochthonous human transmission during the period. A total of 25.7% of the municipalities confirmed canine transmission and the presence of *Lutzomyia longipalpis*¹¹.

Bats are a natural food source for Phlebotominae, as they share the same habitats in caves and shelters, as well as being reservoirs for various species of Trypanosomatidae. In Brazil, there are environmental factors that favor the development of these vectors, as Brazil is a tropical country with high temperatures and excessive organic matter¹².

A recent Brazilian study evaluated the detection of *Leishmania* species in bat species from their first detection to the present day. Among all bats evaluated, 11.6% (251/2,167) were infected. In total, 63 different bat species were analyzed, and *Leishmania* spp. were found mainly in *Molossus rufus* (77.3%, 34/44), *Desmodus rotundus* (22.7%, 15/66), *Artibeus planirostris* (18.7%, 12/64), *Carollia perspicillata* (16.9%, 12/71), *Artibeus lituratus* (16.7%, 20/120), *Molossus* (14.9%, 53/356) and *Glossophaga soricina* (8.6%, 31/361)¹³.

L. amazonensis has already been identified in several bat species, as have *L. infantum* and *L. braziliensis*. The findings of protozoans in bats from endemic areas suggest a direct relationship with the disease cycle in these places, as is the case for studies in Mato Grosso, with 28.9% positive cases (70/242); Belo Horizonte, with 1.67% (2/120); Montes Claros, with 4.4% (11/247); Espirito Santo, with 4.8% (5/105); Campo Grande, with 22.5% (18/80); and Sao Paulo, with 3.1% (21/683). The most commonly used molecular methods involve the detection of specific genes via conventional and nested PCR¹⁴⁻¹⁸. Interestingly, in non-endemic areas, positive results were also found, such as in Rio Grande do Sul with 1.2% (5/402) and in the Triangulo Mineiro and Alto da Paranaíba areas, with 8% (36/448)^{19,20}.

Chagas disease

Chagas disease (CD) results from infection caused by the protozoan *Trypanosoma cruzi*, being characteristic of the American continent. There are approximately 10 million people infected, 25 million at risk of contracting the infection and approximately 20,000 deaths from the disease per year⁴. *T. cruzi* vectors are hematophagous bedbugs, popularly known as kissing-bugs and technically called triatomines. Three important species are currently described: *Triatoma infestans*, *Rhodnius prolixus* and *Triatoma dimidiata*, which are widely distributed throughout South America²¹. Wild species of triatomines have an even wider distribution, reaching North America, and are important for the wild cycle of the disease. These vectors are nocturnal and transmit *T. cruzi* via feces during hematophagy²¹.

In Brazil, an overall 4.2% prevalence of *T. cruzi* infection has been estimated in rural populations, with highest rates in Minas Gerais and Rio Grande do Sul (8.8%),

Goiás (7.4%), the Federal District (6.1%), Sergipe (6.0%) and Bahia (5.4%). From 2000 to 2013, 1,570 cases of acute or chronic CD were recorded by municipal health agencies, whereas only four cases were confirmed in Sao Paulo State during the entire period²².

T. cruzi vectors are widely present in homes and peridomestic areas, as well as caves and shelters, and can have close contact with bats, either via feeding in the case of insectivorous bats or via contact with the feces of bedbugs. A Brazilian study in Acre State, in the Amazon region, analyzed 367 bats of different species and detected *Trypanosoma* in 81 samples. Among these strains, 16.5% (60/367) were *T. cruzi*^{23,24}. Acre, like Sao Paulo, has few recorded human cases of CD in the last decade. Another study in the same region detected 20.3% (15/74) positive cases²⁵. A research group assessed the presence of *T. cruzi* in bats from different regions, including Para, Rio de Janeiro, Mato Grosso do Sul and Piaui, targeting species from all biomes, and the agent was detected in bats from all locations, resulting in 14% (13/93) positivity²⁶. In Sao Paulo, a recent study detected 2.97% (6/20) positive cases in the hearts and kidneys of urban bats and vampire bats²⁷. In addition to other parasitic diseases, CD is closely related to social, economic, political and cultural factors^{28,29}.

Toxoplasmosis

Toxoplasmosis is a parasitic disease caused by the protozoan *Toxoplasma gondii*. This protozoan is spread worldwide, infecting approximately one-third of the population³⁰. Infection occurs mainly by consuming food or water contaminated by oocysts released in the feces of animal hosts. There has been much discussion about the complications that occur when pregnant women are infected during pregnancy and the risk factors associated with it³¹. Toxoplasmosis control is extremely complex and requires attention to all variables, including the role of wildlife in maintaining endemicity³⁰.

The burden of toxoplasmosis in children born with congenital infection is very high³². Among congenitally infected children, several do not survive long after birth; 35% of them face neurological problems such as hydrocephalus, microcephaly and intellectual disabilities; 80% have eye damage; and 40% suffer hearing loss. The severity of clinical toxoplasmosis in Brazilian children may be related to the genetic characteristics of *T. gondii* strains prevalent in animals and humans in Brazil⁵.

The number of wild species infected by *T. gondii* is extensive. The routes of infection are associated with predation on intermediate hosts and consumption of contaminated vegetables or water⁵. In relation to bats,

some suspicions have been raised, such as the consumption of fruit or water contaminated with oocysts or by vertical transmission, although few studies have proposed a real possibility according to the pathogenesis of the protozoan. The parasite has tissue tropism to the brain, heart and pectoral muscle of bats³³. A study of bats in Sao Paulo city found that 32.6% (201/616) were seropositive for *T. gondii*, demonstrating exposure of local bats³⁴. Studies have highlighted the potential role of bats in the toxoplasmosis cycle in Brazil. Research conducted in Natal city found that 21.6% (11/51) of bat tissue samples tested positive for *Toxoplasma gondii* using conventional PCR across different organs. Similarly, a study in Bahia reported 2.1% (2/97) positive results in bat brain samples using the same method^{35,36}. *T. gondii* has already been genotyped in bat species from Sao Paulo city in non-hematophagous and hematophagous bats, pointing to the previous presence of these genotypes in other animal species, both domestic and wild, suggesting that this genotype is circulating in other Brazilian regions³⁴.

Bartonellosis

Bartonellosis is a bacterial disease caused by species of *Bartonella*, with different clinical forms, target hosts and pathogenic species. In Brazil, its most common zoonotic form, known as cat scratch disease (CSD), is caused by the species *B. henselae*. In cats, the infection prevalence is very high, reaching 90% in certain locations, making it difficult to differentiate infection from disease in some cases³⁷. This high prevalence can be explained by persistent bacteremia in asymptomatic reservoir hosts, which is common and can facilitate the agent's transmission process³⁸. Bacterial transmission between cats occurs via fleas of the species *Ctenocephalides felis*, via inoculation, while transmission between cats and humans occurs via scratches or bites³⁷. Ticks have also been studied as potential transmitters of *B. henselae* to humans³⁷.

In cats, the disease is generally asymptomatic³⁹. In humans, CSD is also asymptomatic, but when it persists in the body, it can develop into recurrent fever, endocarditis, encephalopathy in children, and osteomyelitis³⁷.

In the United States, where the disease is frequently investigated, more than 40,000 cases of CSD and around 2,000 hospitalizations are reported per year⁴⁰. In Brazil, there are few data available on the occurrence of cases at the population level. The distribution of samples related to bartonellosis in humans in Brazil from 2011 to 2017 indicates that 37.94% (480/1265) of samples were positive for anti-*B. henselae* antibodies. In terms of region, Southeast China was the most reactive, accounting for 85.42%

(410/480) of the cases, with 31.04% (149/480) in Rio de Janeiro and 29.38% (141/480) in Minas Gerais. Compared with the rural population, the urban population was the most affected⁴¹.

A study of bat samples from five Brazilian states (Mato Grosso, Sao Paulo, Parana, Para and Tocantins), aimed at detecting the *nuoG* gene by real-time PCR, revealed *Bartonella* positivity in 5.3% (17/322) of liver, spleen and blood samples. None of the samples sequenced were *B. henselae*, but rather *B. taylorii* and *B. koehlerae*⁷. Real-time PCR was also used to investigate the bacteria in bats from Maranhao, and of the 29 samples tested, none were positive⁴². Another study, in the states of Rio de Janeiro, Santa Catarina and Bahia, evaluated the presence of *Bartonella* in 18.5% (22/119) of the spleen samples via conventional PCR (*gltA* gene), but only at the genus level⁴³. In another study with samples from Rio de Janeiro, 3.6% (4/110) of liver and spleen samples were positive by conventional PCR (*gltA* gene)⁴⁴. When the presence of *Bartonella* in bat ectoparasites was assessed, 19.8% (40/202) of the flies were positive, whereas no mites out of 100 were positive according to qPCR (*nuoG* gene)⁴⁵. A large study with samples from fifteen Brazilian states investigated the presence of the bacterium in the liver of hematophagous bats via real-time PCR combined with conventional *gltA* PCR. They reported 24.5% (51/208) positive samples, and after sequencing, the only species found were *B. bacilliformis* and *B. ancachensis*⁴⁶.

Q fever

Q fever is a febrile human disease caused by the bacterium *Coxiella burnetii*. This pathogen can infect vertebrate and invertebrate animals and cause a disease called coxiellosis, characterized by reproductive disorders⁴⁷. This is a highly transmissible and infectious bacterium, and its main and most important route of transmission is airborne, in which the bacterium is dispersed in the environment after calving or animal abortion, contaminating it and making animals and humans close to the area susceptible⁴⁸. From its aerosolization site, the bacterium can be carried by the wind for up to 30km, reaching populations that are not necessarily in direct contact with infected animals⁴⁸.

In animals, reproductive complications such as abortions, stillbirths and premature births can occur⁴⁷. In humans, most cases are asymptomatic; however, approximately 35 to 39% of patients can develop fever, body aches and headaches, and approximately 1 to 5% of patients usually develop more serious complications as a result of bacterial persistence in the body, such

as pneumonia, hepatitis and endocarditis⁴⁷. Although the percentage of persistent infections is minimal, the prevalence of the disease in Brazil does not seem to be, and cases of *C. burnetii* endocarditis have already been diagnosed in Brazilian hospitals, which suggests a much larger number of chronic diseases that have not yet been associated with the etiological agent⁴⁷.

Sao Paulo is the largest Brazilian state with studies and reports of the disease, including an outbreak in slaughterhouse workers in the municipality of Barbosa (21°16'00"49°56'57"), and a seropositivity of 21.5% (129/604) in febrile patients suspected of having dengue in municipalities in the countryside⁴⁷.

Bats are considered potential reservoirs of the disease since they are mammals with an aerial cycle and often have rural environments as natural habitats. The only study involving Q fever in bats in Brazil was carried out with samples from the states of Rio de Janeiro, Santa Catarina and Bahia. A total of 119 spleen samples were evaluated via conventional PCR (*IS1111* gene), and 3.4% (4/119) were positive⁴³. In Colombia, a group studied the presence of the bacterium in the blood of bats captured from caves and detected 6.3% (8/126) of positive bacteria by qPCR (*IS1111* gene). The absence of human cases in the country also makes possible associations difficult⁴⁹.

Brazilian spotted fever

Brazilian spotted fever (BSF) is a serious febrile disease caused by the bacterium *Rickettsia rickettsii* and transmitted by *Amblyomma* ticks. This tick can be present on cattle, horses, dogs, poultry and some synanthropic animals; however, the main reservoirs of *Rickettsia* are capybaras⁵⁰. In humans, the disease can cause fever, reddish patches on the body, muscle and joint pain, severe headache, nausea and neurological disorders. In animals, the disease is accompanied by fever, severe weight loss, hemorrhages, difficulty breathing and convulsions. Capybaras, in particular, are resistant to the disease, and thus are the preferred targets for ticks, being an epidemiological danger to humans⁵⁰.

In Brazil, sporadic cases of BSF have been reported since it was first described, in 1929, until 1980. From 2000 to 2018, after a major effort by the Ministry of Health, 2090 cases were confirmed, 55% of which resulted in death. Sao Paulo ranked first among all Brazilian states, with 982 cases (47%). The Central-eastern region of Sao Paulo is considered an endemic area for BSF⁵¹.

Many tick infestations have already been described in bats, including the presence of mutual infection of zoonotic agents in bats and their parasites, as in the case

of rickettsiae pathogenic to humans found in bats and their ticks in Sweden⁵². A study of bats in Sao Paulo city evaluated *R. rickettsii* seropositivity, showing that 8.6% (39/451) of them were seropositive⁵³. In the states of Rio de Janeiro, Santa Catarina and Bahia, a study of 119 total spleen samples revealed no positives⁴³. Also in Sao Paulo city, an interesting report of infection of ticks that feed on bats and humans has been published; however, it is a *Candidatus R. paranaensis* species⁵⁴. The same occurred in Rio de Janeiro, with the sequencing of a positive sample for *Rickettsia* spp. in bat flies, in which similarity was found with *Candidatus R. andaenae*⁴⁴. Molecular evidence of *Rickettsia* spp. species in bat tissues has not yet been described in Brazil, but few studies on this topic exist. There is a need for more studies with samples from other locations, including endemic areas in the Sao Paulo countryside, to draw more conclusions about the importance of bats in the BSF cycle.

Leptospirosis

Leptospirosis caused by the bacteria *Leptospira* is widely distributed worldwide. The main species associated with clinical cases in Brazil is *L. interrogans* and its serovars. Leptospirosis is considered a disease of global importance, affecting overcrowded urban areas to a greater extent; however, rural populations that deal directly with farm animals are also affected. Mortality from this disease is significant, being associated with pathogenic strains and lack of early clinical diagnosis⁵⁵.

Dogs are the most affected by the disease and have the highest mortality rate. Acute renal failure is the most serious consequence, and is usually the cause of death. In farm animals, especially cattle, leptospirosis is more common in nature, leading to abortions and low milk production. In humans, leptospirosis acts as an acute disease, leading not only to nonspecific signs, such as fever and body aches, but also to rapid kidney damage, causing acute renal failure, which is the most common cause of death. In severe cases, respiratory arrest can occur due to lung involvement, caused by intense breathing difficulties⁶.

Animal reservoirs are undoubtedly essential for the occurrence of leptospirosis. As is the case of urban rats, bats carry numerous infectious agents. The role of rats in the epidemiology of the disease has been well defined, but the role of bats in maintaining and/or transmitting *Leptospira* spp. to other animals or to humans has not been defined. Some studies have investigated the influence of wildlife in this chain, but few studies involving bats exist. One study assessed the presence of the bacterium via conventional PCR (LipL32 gene) in the kidneys of bats in Sao Paulo city, finding 1.7% (6/343) pathogenic leptospires⁵⁶. In Rio

Grande do Sul, one study obtained higher positivity, with 39% (36/92) of pathogenic leptospires⁵⁷.

In Table 1, we organized the data resulting from the molecular investigations carried out in Brazil to date to detect the zoonotic agents discussed in this article in urban and rural bats.

Application of molecular biology in the diagnosis and determination of zoonoses in populations

Molecular biology is crucial for the diagnosis, monitoring and prevention of zoonoses in populations. The ability to rapidly identify pathogens, trace their genetic evolution and understand transmission dynamics is essential to protect public health and minimize zoonotic impact⁵⁸.

Compared with conventional diagnostic tools, molecular tools enable the etiological agent within a population to be diagnosed with greater efficiency and precision. These tools have increased the understanding of the relationship with the host, pathogenicity and virulence of etiological agents, provided information on their genetic structure and taxonomy, and enabled the determination of the zoonotic potential of previously unidentified agents. Molecular tools have also provided a dimension to disease epidemiology that would be unattainable via conventional diagnostic tools. Molecular epidemiology was defined as a science that focuses on the contribution of potential genetic and environmental risk factors, identified at the molecular level, to the etiology, distribution and prevention of diseases within families and between populations⁵⁹.

Molecular biology enables sensitive and specific detection of pathogens. This is accomplished via techniques such as PCR, which amplifies the genetic material of a pathogen, making it easier to identify. The development of nucleic acid amplification techniques led to further improvements in the identification and taxonomy of microorganisms. The main advantages of these techniques are speed, increased discriminatory power and the ability to analyze small amounts of sample⁵⁹. An important advantage of PCR-based procedures is their ability to detect and characterize the genetic variability of infectious agents, particularly at the intraspecific level. In addition, PCR tests, if properly designed, can enable the direct detection of parasite DNA from mixed templates, such as clinical or environmental samples, quickly and with high levels of sensitivity and specificity⁵⁸.

Some specific methods are commonly applied in the diagnosis of the zoonoses discussed in this review, and have shown good results in direct diagnosis in bat tissues. Table 2 shows the main methods, gene targets and oligonucleotides used for molecular diagnosis in bat tissues.

Table 1 - Molecular investigations carried out on Brazilian bats to detect zoonotic agents.

Type of bat	Molecular prevalence	Brazilian states	Article
<i>Leishmania</i>			
Urban bats	11.6% (251/2167)	MG, SP, RJ, ES, MS, MA	Ratzlaff <i>et al.</i> ¹³
Urban and rural bats	28.9% (70/242)	MT	Castro <i>et al.</i> ¹⁴
Urban bats	1.67% (2/120)	MG	Araújo <i>et al.</i> ¹⁵
Urban bats	4.4% (11/247)	MG	Araújo <i>et al.</i> ¹⁵
Urban bats	4.8% (5/105)	ES	Riva <i>et al.</i> ¹⁶
Urban bats	22.5% (18/80)	MT	Rezende <i>et al.</i> ¹⁷
Urban and rural bats	3.1% (21/683)	SP	Savani <i>et al.</i> ¹⁸
Urban bats	1.2% (5/402)	RS	Ratzlaff <i>et al.</i> ¹⁹
Urban bats	8% (36/448)	MG	Gómez-Hernández <i>et al.</i> ²⁰
<i>Trypanosoma cruzi</i>			
Urban bats	16.5% (60/367)	AC	Santos <i>et al.</i> ²⁴
Urban bats	20.3% (15/74)	AM	Coura <i>et al.</i> ²⁵
Urban and rural bats	14% (13/93)	PA, RJ, MS, PI	Lisboa <i>et al.</i> ²⁶
Urban and rural bats	2.97% (6/20)	SP	França <i>et al.</i> ²⁷
<i>Toxoplasma gondii</i>			
Urban and rural bats	32.6% (201/616)	SP	Cabral <i>et al.</i> ³⁴
Urban bats	21.6% (11/51)	RN	Fournier <i>et al.</i> ³⁵
Urban bats	2.1% (2/97)	BA	Jesus <i>et al.</i> ³⁶
<i>Bartonella</i>			
Urban bats	5.3% (17/322)	MT, SP, PR, PA, TO	Ikeda <i>et al.</i> ⁴¹
Urban bats	0% (0/29)	MA	Braga <i>et al.</i> ⁴²
Urban bats	18.5% (22/119)	RJ, SC, BA	Ferreira <i>et al.</i> ⁴³
Urban bats	3.6% (4/110)	RJ	Gonçalves-Oliveira <i>et al.</i> ⁴⁴
Bat ectoparasites	19.8% (40/202)	RJ	Amaral <i>et al.</i> ⁴⁵
Rural bats	24.5% (51/208)	All biomes	André <i>et al.</i> ⁴⁶
<i>Coxiella burnetii</i>			
Urban bats	3.4% (4/119)	RJ, SC, BA	Ferreira <i>et al.</i> ⁴³
<i>Rickettsia rickettsii</i>			
Urban bats	8.6% (39/451)	SP	Tompa <i>et al.</i> ⁵²
Urban bats	0% (0/119)	RJ, SC, BA	Ferreira <i>et al.</i> ⁴³
<i>Leptospira</i>			
Urban bats	1.7% (6/343)	SP	Bessa <i>et al.</i> ⁵⁶
Urban bats	39% (36/92)	RS	Mayer <i>et al.</i> ⁵⁷

In addition to PCR, nucleic acid sequencing makes it possible not only to confirm the presence of the agent, but also to identify mutations and genetic variations in pathogens that can affect transmission and virulence. It rapidly provides large amounts of genetic information and tends to become one of the most important pillars in the routine of public health laboratories⁶⁰. Sequencing was initially expensive and time-consuming, especially when it was performed manually, but with the development of cycle sequencing and automated systems, cost and time have greatly decreased, making

large-scale sequencing faster and more accessible⁶⁰. Direct sequencing of a PCR product is commonly used to detect pathogens; however, if there are multiple related sequences in an amplification product, either due to mixed infection with similar genotypes or due to polymorphic alleles, deciphering the sequence may be impossible, or the mixed template may be completely lost by preferential amplification. Cloning the template can overcome this problem, but this is a laborious process, and variant molecules can still be lost if they exist at low frequencies⁵⁸.

Table 2 - Main methods, gene targets and oligonucleotides used for the molecular diagnosis of zoonotic agents prevalent in Sao Paulo State in bat tissues.

Zoonotic agent	Method	Target gene	Oligonucleotide	Article
<i>Leishmania</i>	cPCR	IRBP	IRBPfw (TCC AAC ACC ACT GAG ATC TGG AC); IRBPrev (GTG AGG AAG AAA TCG GAC TGG CC)	Castro <i>et al.</i> ¹⁴
	cPCR	ITS-1	LITSR (CTG GAT CAT TTT CCG ATG); L58S (TGA TAC CAC TTA TCG CAC TT).	Araújo <i>et al.</i> ¹⁵
	cPCR	kDNA	D1 (GGG GAG GGG CGT TCT GCG AA); D2 (CCG CCC CTA TTT TAC ACC AAC CCC)	Riva <i>et al.</i> ¹⁶
	nPCR	SSU rDNA	S4 (GAT CCA GCT GCA GGT TCA CC); S12 (GGT TGA TTC CGT CAA CGG AC); S17 (CCA AGC TGC CCA GTA GAA T); S18 (TCG GGC GGA TAA AAC CC)	Ratzlaff <i>et al.</i> ¹⁷
<i>Trypanosoma cruzi</i>	cPCR	mini-exon	TCZ1 (CGA GCT CTT GCC CAC ACG GGT GCT); TCZ2 (CCT CCA AGC GGA TAG TTC AGG)	Lisboa <i>et al.</i> ²⁶
	cPCR	kDNA	121 (AAA TAA TGT ACG GGT GAG ATG CAT GA); 122 (GGG TTC GAT TGG GGT TGG TGT)	França <i>et al.</i> ²⁷
<i>Toxoplasma gondii</i>	cPCR	B1	T1 (AGC GTC TCT CTT CAA GCA GCG TA); T2 (TCC GCA GCG ACT TCT ATC TCT GT)	Fournier <i>et al.</i> ³⁵
	cPCR	B1	TOX4 (CGC TGC AGG GAG GAA GAC GAA AGT TG); TOX5 (CGC TGC AGA CAC AGT GCA TCT GGA TT)	Jesus <i>et al.</i> ³⁶
<i>Bartonella</i>	qPCR	nuoG	F-Bart (CAA TCT TTT GCT TCA CC); R-Bart (TCA GGG CTT TAT GTG AAT AC)	Ikeda <i>et al.</i> ⁴¹
	cPCR	gltA	F (GCT ATG TCG CVT TCT ATC AYG A); R (AGA ACA GTA AAC ATT TCN GTH GG)	Ferreira <i>et al.</i> ⁴³
<i>Coxiella burnetii</i>	qPCR	IS1111	F (TAT GTA TCC ACC GTA GCC AGC); R (CCC AAC ACC TCC TTA TTC)	Ferreira <i>et al.</i> ⁴³
<i>Rickettsia rickettsii</i>	cPCR	gltA	F (AAA TGA GTA TTT TTA GTA GG); R (AAG GTT TAT CCG TTT AGG CT)	Tompa <i>et al.</i> ⁵²
<i>Leptospira</i>	qPCR	LipI32	45F (AAG CAT TAC CGC TTG TGG TG); 286R (GAA CTC CCA TTT CAG CGA TT)	Mayer <i>et al.</i> ⁵⁷

Small amounts of information from sequencing can be used to identify pathogens at the species level, even if primers are used at the genus or family level. On the other hand, large amounts of information are essential to perform a phylogenetic analysis⁵⁸. This analysis is crucial for the study of molecular epidemiology, as it provides a solid understanding of the taxonomy and population structure of the microorganism, which is essential to study aspects of the pathogen's biology and epidemiology, including its host specificity, genetic diversity and zoonotic potential⁶⁰. PCR sequencing in combination with phylogenetic analysis has been used to investigate the biology of a variety of agents, as well as to identify the relationship of agents detected in different geographical areas and the genetic proximity between them⁶⁰.

Another advance in the application of molecular techniques to epidemiology has been the development of technology to measure nucleic acid amplification in real time, such as qPCR, to quantify the number of parasites in a given sample or measure gene expression levels⁵⁸.

A variety of chemicals can be used to detect nucleic acid amplification in real time, including SYBR Green dyes and Taqman probes. Real-time amplification has several advantages over conventional amplification, including simple DNA quantification, high-speed analysis and high-throughput analysis. Real-time PCR is much more sensitive than conventional PCR, detecting fragments that cannot be detected by conventional equipment⁵⁸. The ability of qPCR to perform analysis in closed tubes eliminates the need for post-PCR handling, such as agarose gel electrophoresis, reducing the risk of contamination and reducing sample handling, while also reducing costs⁵⁹. In the case of probe-based systems, it is possible to detect single nucleotide polymorphisms, which greatly increases sensitivity and confirms the agent at species level without the need for genetic sequencing. With real-time molecular approaches, it is possible to obtain more information in genotyping, as it can theoretically detect and differentiate all mutants at different base positions within the probe's binding region, which enables tracing the origin and spread of zoonoses in populations⁵⁹.

PCR-based techniques may be the only means of diagnosis to uncover the role of animals as mechanical or zoonotic transmitters of diseases⁶⁰. It is imperative that, in situations such as these, simultaneous surveys are carried out in human and animal populations to correlate data at the epidemiological and molecular levels and to rule out the possibility of geographical variation. A good choice of genetic markers according to the epidemiological issue being investigated is essential for greater sensitivity in detecting the agent present in the study area, making the technique better suited to the epidemiological context. A robust and well-designed PCR is an ideal diagnostic tool for epidemiological studies aimed at uncovering zoonotic relationships⁵⁸.

A better accuracy of diagnostic tests invariably results in a greater degree of confidence for epidemiological statistics and provides another dimension to the study of microbial epidemiology⁵⁸⁻⁶⁰.

CONCLUSION

This study summarizes the importance of understanding the complex dynamics of the zoonoses discussed and the possible role of bats in maintaining these diseases in Brazil. The prevalence and epidemiology of these diseases highlight the urgent need for effective control and prevention measures, especially in São Paulo State, where interactions between humans and bats are significant. Studies focusing on bats as natural reservoirs reveal the relevance of these animals in the chain of transmission of various pathogens. In addition, the application of molecular biology techniques in the diagnosis of these zoonoses is essential for early and accurate detection, enabling rapid and targeted interventions. The integration of ecological and molecular approaches is, therefore, fundamental to mitigate these diseases and promote public health.

AUTHORS' CONTRIBUTIONS

DAF: conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing—original draft, writing—review and editing; HL: conceptualization, supervision, validation, visualization, funding acquisition, project administration, resources, writing—review and editing.

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CAPÍTULO 9: CONSIDERAÇÕES FINAIS

9.1. DISCUSSÃO GERAL

O presente estudo investigou a presença de importantes agentes zoonóticos em diferentes espécies de morcegos do Estado de São Paulo, utilizando técnicas moleculares para detecção e caracterização desses patógenos. A pesquisa trouxe novos dados sobre o papel desses animais na manutenção e amplificação de patógenos como *Leishmania* spp., *Trypanosoma cruzi*, *Toxoplasma gondii*, *Bartonella* spp., *Coxiella burnetii*, *Rickettsia rickettsii* e *Leptospira* spp., oferecendo subsídios valiosos para a vigilância epidemiológica e controle dessas zoonoses em áreas urbanas e rurais.

Ao longo dos diferentes capítulos, foram discutidos os achados relacionados a cada patógeno, com ênfase na associação entre a biologia dos morcegos, seus hábitos alimentares, e a sua capacidade de atuar como reservatórios de agentes zoonóticos. Em particular, os morcegos hematófagos, *Desmodus rotundus*, destacaram-se pela alta positividade para *Leptospira* spp., sugerindo seu papel na manutenção da leptospirose em áreas rurais, especialmente em regiões com grande concentração de gado bovino. Este achado é particularmente relevante, uma vez que a leptospirose continua a ser uma zoonose negligenciada no Brasil, com impacto direto na saúde pública e na economia devido às perdas na produção animal.

Além disso, a detecção de *Leishmania amazonensis* e *Leishmania infantum* em morcegos urbanos nos trouxe uma nova perspectiva sobre a epidemiologia da leishmaniose em áreas não endêmicas, indicando que esses animais podem estar envolvidos na manutenção silenciosa do ciclo de transmissão da doença. A correlação entre a positividade para *Leishmania* spp. e os hábitos alimentares dos morcegos insetívoros ressalta a importância de estudos futuros para explorar mais profundamente a interação entre esses vetores e a fauna silvestre.

Outro ponto de destaque foi a detecção de *Trypanosoma cruzi* em diferentes espécies de morcegos, incluindo *Nyctinomops laticaudatus* e *Eumops glaucinus*. Esta descoberta amplia o conhecimento sobre os hospedeiros potenciais da doença de Chagas no Brasil, evidenciando a necessidade de um monitoramento contínuo desses reservatórios naturais, especialmente em áreas

de contato entre humanos e fauna silvestre, como as regiões de Mata Atlântica. A presença de *T. cruzi* em morcegos frugívoros sugere que a transmissão desse protozoário pode estar associada a hábitos alimentares e à interação com triatomíneos em áreas peridomiciliares.

A pesquisa também trouxe contribuições importantes para a compreensão da ecologia de patógenos como *Bartonella* spp., *Coxiella burnetii* e *Rickettsia rickettsii* em morcegos urbanos. Embora a prevalência de *Bartonella* spp. tenha sido baixa, a detecção em morcegos sugere que esses animais podem servir como reservatórios da bartonelose, que vem sendo subdiagnosticada no Brasil. Não foi possível detectar *C. burnetii* e *R. rickettsii* nos morcegos estudados, o que reforça a necessidade de mais investigações sobre a epidemiologia dessas bactérias em populações de morcegos e suas possíveis interações com vetores como carrapatos e pulgas.

Além dos agentes já mencionados, a detecção de *Toxoplasma gondii* nos morcegos representa outro achado significativo deste estudo. A presença do protozoário foi confirmada em morcegos urbanos insetívoros e frugívoros, bem como em morcegos hematófagos de áreas rurais, o que sugere uma ampla disseminação desse agente em diferentes nichos ecológicos. A detecção de *T. gondii* em placenta de uma fêmea de morcego e a não detecção em seu filhote recém-nascido também são dignas de nota, levantando questões sobre a possível transmissão vertical do parasita e respondendo inicialmente a essa questão.

No conjunto, os resultados desta tese fornecem uma visão abrangente sobre a diversidade de agentes zoonóticos presentes em morcegos no Estado de São Paulo e destacam a importância desses animais como sentinelas na vigilância de zoonoses. A utilização de métodos moleculares como a PCR em tempo real permitiu não apenas a detecção sensível e específica desses patógenos, mas também a caracterização genética, o que contribui para a compreensão da dinâmica de transmissão e para a implementação de estratégias de controle mais eficazes.

As descobertas aqui apresentadas também reforçam a relevância da abordagem de One Health, integrando saúde humana, animal e ambiental para lidar com os desafios das zoonoses emergentes. A urbanização crescente, a

fragmentação de habitats e as mudanças climáticas continuam a promover o contato entre humanos e a vida selvagem, o que aumenta o risco de transmissão de patógenos. A partir dos dados coletados, torna-se claro que o monitoramento contínuo de morcegos e seus patógenos deve ser uma prioridade nas ações de saúde pública, visando prevenir surtos de doenças e mitigar os impactos na saúde das populações humanas e animais.

Em suma, esta tese contribuiu significativamente para o conhecimento científico sobre a relação entre morcegos e zoonoses no Brasil, trazendo novos insights sobre a ecologia de patógenos e o papel desses mamíferos na manutenção de doenças infecciosas. Além disso, as técnicas moleculares empregadas abriram novas possibilidades para o monitoramento epidemiológico em tempo real, com aplicações práticas no controle de zoonoses. O avanço no conhecimento científico gerado por esta pesquisa oferece bases sólidas para a formulação de políticas públicas que integram as necessidades de conservação da biodiversidade e a proteção da saúde pública.

9.2. CONCLUSÕES GERAIS

Este estudo trouxe uma contribuição significativa para o entendimento do papel dos morcegos como reservatórios e amplificadores de agentes zoonóticos no Estado de São Paulo. Os resultados obtidos reforçam a importância desses animais na manutenção de agentes como *Leishmania* spp., *Trypanosoma cruzi*, *Toxoplasma gondii*, *Bartonella* spp. e *Leptospira* spp., e também traz resultados significativos sobre a não ocorrência da *Coxiella burnetii* e da *Rickettsia rickettsii*.

A alta prevalência de *Leptospira* spp. em morcegos hematófagos destaca a possível participação desses animais na cadeia de transmissão da leptospirose em áreas rurais. Já a presença de *Leishmania* spp. em morcegos urbanos sugere que esses animais podem participar da manutenção da leishmaniose, sendo relevante investigar esse papel mesmo em áreas não endêmicas. A detecção de *Trypanosoma cruzi* e *Toxoplasma gondii* em várias espécies de morcegos contribui para o entendimento do possível papel desses animais na manutenção dessas zoonoses, com a detecção de *Toxoplasma gondii* até mesmo em placenta, levantando questões sobre a sua transmissão vertical. *Bartonella* spp. foi detectada em morcegos urbanos, indicando uma possível participação desses

animais na ecologia da bartonelose em ambientes urbanos. Por outro lado, não foram detectadas *Coxiella burnetii* e *Rickettsia rickettsii*, sugerindo que, pelo menos nessas amostras, esses agentes não parecem estar presentes em morcegos da região estudada.

Finalmente, este trabalho reforça a necessidade de monitoramento contínuo de morcegos e seus patógenos, especialmente em um contexto de crescente urbanização e fragmentação de habitats. O uso de ferramentas moleculares no diagnóstico e na vigilância de zoonoses provou ser uma estratégia eficaz para detectar e caracterizar agentes patogênicos, contribuindo para o desenvolvimento de políticas públicas mais eficazes no controle dessas doenças e na preservação da saúde pública.

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