UNIVERSIDADE FEDERAL DE MINAS GERAIS
Escola de Veterinária
Programa de Pós-graduação em Ciência Animal

Daniel Oliveira dos Santos

INVESTIGATION OF ZOONOTIC DISEASES IN FREE-RANGING BLACK-TUFFED MARMOSETS (*Callithrix penicillata*) AND SOUTH AMERICAN COATIS (*Nasua nasua*)

Investigação de doenças zoonóticas em micos estrela (*Callithrix penicillata*) e quatis (*Nasua nasua*) de vida livre

Belo Horizonte
2024

Daniel Oliveira dos Santos

INVESTIGATION OF ZOONOTIC DISEASES IN FREE-RANGING BLACK-TUFFED MARMOSETS (*Callithrix penicillata*) AND SOUTH AMERICAN COATIS (*Nasua nasua*)

Investigação de doenças zoonóticas em micos estrela (*Callithrix penicillata*) e quatis (*Nasua nasua*) de vida livre

Tese apresentada ao Programa de Pós-graduação em Ciência Animal da Universidade Federal de Minas Gerais como requisito parcial para obtenção do título de Doutor em Ciência Animal.

Área de concentração: Patologia Animal

Orientador: Renato de Lima Santos

Coorientadora: Ayisa Rodrigues de Oliveira

Coorientador: Marcelo Pires Nogueira de Carvalho

Belo Horizonte
2024

Santos, Daniel Oliveira dos, 1993 -
S237i Investigation of zoonotic diseases in free-ranging Black-Tuffed Marmosets (Callithrix Penicillata) and south American Coatis (Nasua Nasua) / Daniel Oliveira dos Santos. – 2024.

114f: il

Orientador: Renato de Lima Santos
Coorientadores: Ayisa Rodrigues de Oliveira.
Marcelo Pires Nogueira de Carvalho.
Tese apresentada ao Programa de Pós-Graduação em Ciência Animal da Escola de Veterinária da Universidade Federal de Minas Gerais, como requisito para obtenção do título de Doutor em Ciência Animal.
Á**rea de concentração:** Patologia animal.
Contém Bibliografia.

1. Patologia animal - Teses - 2. Leishmaniose - Teses – 3. Arbovirus – Teses - I. Santos, Renato de Lima. – II. Oliveira, Ayisa Rodrigues de. - III. Carvalho, Marcelo Pires Nogueira de. IV. -Universidade Federal de Minas Gerais, Escola de Veterinária – V. Título.

CDD – 636.089 607

Bibliotecário responsável Marcio Alves dos Santos CRB 3589
Biblioteca da Escola de Veterinária, UFMG.

UNIVERSIDADE FEDERAL DE MINAS GERAIS
ESCOLA DE VETERINÁRIA
COLEGIADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA ANIMAL

FOLHA DE APROVAÇÃO

DANIEL OLIVEIRA DOS SANTOS

Tese submetida à banca examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIA ANIMAL, como requisito para obtenção do grau de DOUTOR em CIÊNCIA ANIMAL, área de concentração PATOLOGIA ANIMAL.

Aprovado(a) em 27 de maio de 2024, pela banca constituída pelos membros:

Dr.(a). Renato de Lima Santos - Orientador(a)

Dr.(a). JEANN LEAL

Dr.(a). Juliana Mariotti Guerra

Dr.(a). Josué Diaz Delgado

Dr.(a). Tatiane Alves da Paixão

Documento assinado eletronicamente por **Renato de Lima Santos**, **Professor do Magistério Superior**, em 13/06/2024, às 20:39, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Tatiane Alves da Paixao**, **Professora do Magistério Superior**, em 14/06/2024, às 02:42, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Jeann Leal de Araújo**, **Usuário Externo**, em 15/06/2024, às 09:40, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Juliana Mariotti Guerra**, **Usuário Externo**, em 16/06/2024, às 17:22, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Josue Diaz Delgado**, **Usuário Externo**, em 18/06/2024, às 15:31, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

A autenticidade deste documento pode ser conferida no site https://sei.ufmg.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **3302813** e o código CRC **8F544913**.

A todos os animais que se permitiram ser capturados.

AGRADECIMENTOS

Mais uma vez eu agradeço ao universo por ter colocado as pessoas certas no meu caminho.

As agências de fomento que tornaram esse projeto viável, CNPq, CAPES e FAPEMIG.

A Fundação de Parques Municipais e Zoobotânica de Belo Horizonte pelo apoio na realização das atividades campo.

A toda equipe de campo pelas infinitas horas trabalhadas inclusive nos finais de semana e feriados, especialmente a Bruna, Nadja, André, Caio, Janaína, Letícia e Vinícius.

Um agradecimento especial a Bruna por ter embarcado nessa "aventura" comigo, o universo não sabia o que tava fazendo quando juntou nós dois nesse projeto.

Aos laboratórios parceiros que foram suporte para esse projeto. Ao Professor Ricardo Fujiwara e sua equipe, especialmente Ana Laura, Bryan e Ramayana. Ao Professor Nelder Gontijo e sua equipe, especialmente ao Luccas. A Fundação Ezequiel Dias, especialmente Felipe, Talita, Alana, Maira e Marília. Ao Professor Rodrigo Silva e sua equipe, especialmente a Yasmin.

Aos muitos colegas de laboratório pela convivência durante todos esses anos, especialmente Lucas, Nayara, Thaynara e Monique.

Ao meu orientador, Professor Renato, pela confiança e por me ensinar que o que temos de mais valioso é o nosso tempo.

A Professora Tatiane pelo apoio e confiança durante todos esses anos.

A Professora Ayisa pelos muitos anos de convivência e por ser inspiração, apoio e orientação.

A~~o hospício~~ casa do Seu João, que foi o lugar que eu chamei de casa por muitos anos, e as muitas pessoas que passaram por lá. Ao Senhor João pelas palavras de sabedoria e apoio durante todos esses anos.

Por fim, como diria meu amigo Andrés: casa não é um lugar, casa são as pessoas. Então eu agradeço as pessoas que foram "casa": Ágna, Andrés, Ayisa, Beatriz, Carol Valente, Carol Vital, Fagner, Filipe, Karina, Lucas, Nayara, Sara, Sérgio, Sidmar, Thaynanne, Thaynara e Victor.

“Though you can see when you’re wrong.

You know, you can’t always see when you’re right.”

(Billy Joel, 1977)

RESUMO

A maioria das doenças infecciosas emergentes são zoonóticas originárias da vida selvagem. A urbanização impacta áreas florestais que ficam isoladas ou fragmentadas. Alguns animais selvagens podem adaptar-se e proliferar em áreas urbanizadas, e a monitorização de doenças zoonóticas na população de animais selvagens pode levar à identificação precoce de agentes patogênicos importantes. Saguis (*Callithrix* sp.) e quatis (*Nasua nasua*) são mamíferos silvestres altamente adaptados a ambientes urbanizados e podem hospedar múltiplos patógenos zoonóticos. Leishmaniose visceral é uma doença zoonótica importante causada por *Leishmania infantum* que afeta diversos mamíferos, incluindo humanos, animais domésticos e selvagens. Arbovírus são patógenos importantes que afetam a saúde humana e podem ter animais silvestres como amplificadores. O vírus da febre amarela, um *Flavivirus*, é um importante causador doença e óbito em primatas neotropicais. Há evidências de infecção por outros arbovírus, como zika, dengue e chikungunya, em primatas neotropicais. *Staphylococcus* spp. fazem parte da microbiota de muitos hospedeiros e levam a infecções oportunistas graves. Isolados de animais silvestres podem ser resistentes a antimicrobianos, porém poucos estudos avaliaram *Staphylococcus* spp. em primatas neotropicais. O objetivo deste estudo foi investigar doenças zoonóticas em micos estrela (*Callithrix penicillata*) e quatis (*N. nasua*) de vida livre capturados em parques urbanos de Belo Horizonte (Minas Gerais, Brasil). A frequência de *L. infantum* através de testes sorológicos em quatis foi de 29,72% (44/148). Cinco quatis capturados múltiplas vezes apresentaram soroconversão e outros cinco quatis reverteram seu status sorológico, tornando-se não reativos após um primeiro resultado reagente. Quatis também foram capazes de transmitir *L. infantum* para *Lutzomyia longipalpis*. A frequência de sororreatividade para *L. infantum* em micos foi de 3,79% (3/79) e 12,5% (4/32) dos animais foram capazes de infectar *Lu. longipalpis*, todos não reativos a *L. infantum*. A frequência de sororreatividade contra arbovírus em micos foi baixa, com 2,94% (2/68) reativos para chikungunya (IgM) e 1,47% (1/68) reativos para zika (IgM). Um animal foi sororreativo a ambos os vírus. Mais de 30% dos micos capturados foram positivos para *Staphylococcus* spp., sendo *S. aureus* a espécie mais isolada seguida de *S. sciuri*. A maioria dos isolados foi suscetível a antimicrobianos, porém um isolado de *S. epidermidis* foi resistente a múltiplos. *S. aureus* foi considerado o principal estafilococo a colonizar micos.

Palavras-chave: Callithrichidae, Procyonidae, leishmaniose, arbovírus, *Staphylococcus*.

ABSTRACT

Most infectious emergent diseases are zoonotic originating from wildlife. Urbanization impact forested areas that become isolated or fragmented. Some wild animals can adapt and proliferate in urbanized areas, so monitoring zoonotic diseases in wildlife populations can lead to early identification of important pathogens. Marmosets (*Callithrix* sp.) and South American coatis (*Nasua nasua*) are wild mammals highly adapted to urbanized environments that can host multiple zoonotic pathogens. Visceral leishmaniosis is an important zoonotic disease caused by *Leishmania infantum* that affects several mammals, including humans, domestic and wild animals. Arboviruses are important pathogens that affect human health and can have wild animals as amplifiers. Yellow fever virus, a *Flavivirus*, is an important cause of disease and death in neotropical primates. There is evidence of infection by other arboviruses, such as zika, dengue and chikungunya, in neotropical primates. *Staphylococcus* spp. are part of the microbiota of many different hosts and lead to opportunistic severe infection. Isolates from wild animals can be resistant to antimicrobial drugs, however few studies evaluated *Staphylococcus* spp. in neotropical primates. The goal of this study was to investigate zoonotic diseases in free-ranging black-tuffed marmosets (*Callithrix penicillata*) and coatis (*N. nasua*) captured in urban parks of Belo Horizonte (Minas Gerais, Brazil). Frequency of *L. infantum* by serological tests in coatis was 29.72% (44/148). Five coatis captured multiple times, showed seroconversion and other five coatis reversed their serological status, becoming non-reactive after a first reactive result. Coatis were also able to transmit *L. infantum* to *Lutzomyia longipalpis*. Frequency of seroreactivity to *L. infantum* in *C. penicillata* was 3.79% (3/79) and 12.5% (4/32) of the animals were able to infect *Lu. longipalpis*, all of them non-reactive to *L. infantum*. Frequency of seroreactivity against arboviruses in black-tuffed marmosets was low with 2.94% (2/68) reactive to chikungunya (IgM) and 1.47% (1/68) reactive to zika (IgM). One animal was seroreactive to both viruses. Over 30% of captured marmosets were positive for *Staphylococcus* spp., and *S. aureus* was the most isolated species followed by *S. sciuri*. Most isolates were susceptible to antimicrobials, however one *S. epidermidis* isolate was resistant to multiples antimicrobials. *S. aureus* was considered the main staphylococci to colonize black-tuffed marmosets.

Keywords: Callithrichidae, Procyonidae, leishmaniosis, arbovirus, *Staphylococcus*.

LIST OF FIGURES

LIST OF TABLES

SUMMARY

1. INTRODUCTION

Zoonotic diseases correspond to more than 60% of all emerging human infectious diseases, and more than 70% of those diseases are originated from wildlife (Jones et al., 2008). Even though these diseases are primarily identified in humans, their main reservoirs are other species (Frank, 2008). Forested areas have been intensely impacted by urbanization becoming isolated and fragmented. These areas are inhabited by a variety of wild animals and some of them can adapt and survive under those conditions (Uttara et al., 2012). This process leads to more contact between humans and wild animals, and consequently it facilitates transmission of zoonotic diseases. Therefore, monitoring of zoonotic diseases should focus on investigating both human and animal populations.

Monitoring zoonotic diseases in wildlife can lead to early identification of important pathogens, assisting directly in public health strategies. For instance, in early 2000s in New York City, West Nile virus was first detected in dead wild birds while human cases of encephalitis with unknown cause were occurring, which was later identified as West Nile virus infections (Edison et al., 2001; Ludwig et al., 2002). Also, in Brazil the Yellow Fever vigilance program is based on monitoring and investigation of deaths among neotropical primates. Confirmed cases of the disease in primates has lead to implementation of prevention and control measures for the human population (Brazil, 2017).

Some wildlife species are able to proliferate more in urban environments than in their natural habitats, which is the case of marmosets (*Callithrix* sp.) and South American coatis (*Nasua nasua*) (Teixeira et al., 2015; Hemetrio, 2007). Marmosets (*Callithrix* spp.) are neotropical primates belonging to the Callithrichidae family that includes black-tuffed marmosets (*C. penicillata*), a small primate that lives in groups of 2 to 19 individuals and have a diet consisting of fruits, flowers, insects, small vertebrates, and tree gums (Miranda e Faria 2001; Silva, 2008; Malukiewics et al., 2020). Conditions associated with urban environment, as introduction of exotic plants and reduction of predators, are factors that influence the expansion of *C. penicillata* population in urban areas (Malukiewicz et al., 2020). South American coatis (*Nasua nasua*) are procyonids native to South America that feed of invertebrates, fruits, and small vertebrates, however in anthropized environments can also feed on discarded human food (Alves-Costa et al., 2004; Emmons & Helgens, 2016; Rodrigues et al., 2021). Coatis mainly inhabits forested areas, however in urban areas the population may grow larger than in preserved areas (Hemetrio, 2007; Hemetrio, 2011; Barreto et al., 2021).

Marmosets and coatis can host important zoonotic pathogens, including viral (Alphaherpesvirus, Yellow Fever virus, Rabies virus, poxviruses, coronaviruses) (Costa et al., 2011; Santos et al., 2020; Abreu et al., 2022; Benavides et al., 2022; Wilson et al., 2022; Stoffella-Dutra et al., 2023), bacterial (*Staphylococcus* sp., *Salmonella* sp., *Clostridioides difficile*, *Leptospira* sp., *Mycobacterium* sp.) (Langoni et al., 2009; Murakami et al., 2012; Silva et al., 2014; De-La-Rosa-Arana et al., 2016; Armstrong et al., 2019; Albuquerque et al., 2020; Wilson et al., 2021; Carvalho et al., 2022; Santana et al., 2023) or protozoan pathogens (*Toxoplasma gondii*, *Leishmania* sp., *Trypanossoma* sp.) (Lisboa et al., 2004; Herrera et al., 2008; Malta et al., 2010; Maia et al., 2016; Paiz et al., 2018; Coimbra et al., 2020; Paula et al., 2020; Oliveira et al., 2022; Macedo et al., 2023). Considering that both black-tuffed marmosets (*C. penicillata*) and coatis (*N. nasua*) inhabit urban areas and can host several important zoonotic pathogens, both species can be potential sentinels for surveillance.

The city of Belo Horizonte (Minas Gerais, Brazil) has around 82 parks with forested areas that are inhabited by several wild mammals, including primates (*C. penicillata*), carnivores (*N. nasua*, *Cerdocyon thous*, *Eira barbara*, and *Puma concolor*) and marsupials (*Didelphis albiventris* and *D. aurita*). Considering the high adaptability of some of these species to urban environments and their potential to host several zoonotic pathogens, the goal of this study was to investigate zoonotic diseases in free-ranging black-tuffed marmosets and coatis living in urban parks.

2. LITERATURE REVIEW

2.1. Zoonotic pathogens in *Callithrix* sp. and *Nasua nasua*

In urban areas, some wild animals can be more prolific and closer to humans, which favors transmission of diseases. Both marmosets (*Callithrix* sp.) and coatis (*N. nasua*) are adapted to urban environments and can host multiple zoonotic pathogens. This section explores the occurrence of viral, bacterial, and protozoan pathogens in both species.

2.1.1. Virus

2.1.1.1. Human herpesviruses

Marmosets can be infected with human herpesviruses, mainly *Simplexvirus humanalpha1*, by direct contact with infected humans or through infected food. Herpesvirus-

elicited disease in these animals is acute and severe, presenting oral and mucocutaneous ulcers and encephalitis (Costa et al., 2011; Wilson et al., 2022). Disease is usually associated with outbreaks affecting multiple animals from the group with high mortality, however animals that survive infection can develop antibodies for at least four years (Hatt et al., 2004). Human herpesviruses were not reported in coatis.

2.1.1.2. Yellow Fever virus (*Orthoflavivirus flavi*)

Yellow fever (*Orthoflavivirus flavi*) is an arbovirus (Arthropod Borne Virus) transmitted by *Sabethes* sp. and *Haemagogus* sp. in the sylvatic cycle or by *Aedes* sp in the urban cycle (Litvoc et al., 2018). Neotropical primates are sentinels for Yellow fever surveillance, and in Brazil, prevention and control measures for human population are implemented after the initial diagnosis in primates (Brasil, 2017). Susceptibility to infection is different among the different species of neotropical primates. Howler monkeys (*Alouatta* sp.) are highly susceptible, with higher frequency of positive animals and developing severe hepatic lesions. However, frequency of positive marmosets (*Callithrix* sp.) is lower and positive animals usually do not present hepatic lesions (Santos et al., 2020).

2.1.1.3. Other arboviruses

Marmosets are frequently used as experimental model in biomedical research, including studies with Dengue virus (*Orthoflavivirus denguei*), Zika virus (*Orthoflavivirus zikaense*) and West Nile virus (*Orthoflavivirus nilense*) (Omatsu et al., 2011; Ferreira et al., 2014; Moi et al., 2014; Verstrepen et al., 2014; Lum et al., 2018). Serological and molecular signs of natural infection by Zika virus was reported in free-ranging marmosets (Moreira-Soto et al., 2018; Terzian et al., 2018; Favoretto et al., 2019). However, histopathological lesions observed in zika positive individuals were not associated with the virus (Terzian et al., 2018).

There is serological evidence of infection in free-ranging coatis (*N. nasua*), with animals presenting antibodies against several arboviruses including yellow fever (*O. flavi*), Ilheus (*Orthoflavivirus ilheusense*), Saint Louis (*Orthoflavivirus louisense*), West Nile and Dengue (*O. denguei*) (types 1, 2, 3 and 4) viruses (Rodrigues et al., 2022).

2.1.1.4. Rabies

Rabies is an important zoonotic disease caused by a Lyssavirus with lethality of 100%. Cases of rabies in marmosets, mainly in common marmosets (*Callithrix jacchus*) from the northeastern region of Brazil, are associated with transmission to humans (Favoreto, et al., 2001; Benavides et al., 2022). More recently, an infected free-ranging marmoset was diagnosed with rabies in the urban area of Niterói (Rio de Janeiro, Brazil), raising concerns that marmosets may act as potential hosts for rabies in other regions of Brazil (Moutinho et al., 2020). Importantly, there are no reports of rabies in South American coatis (*N. nasua*), however the white-nosed coati (*Nasua narica*) can be naturally infected with the rabies virus (Krebs et al., 2003; Aréchiga-Ceballos et al., 2010).

2.1.1.5. Respiratory coronavirus (SARS-CoV-2) (*Betacoronavirus pandemicum-2*)

In 2019, a new respiratory coronavirus (SARS-CoV-2) emerged quickly spreading across the globe by March of 2020. Studies investigated free-ranging neotropical primates during the pandemic, however there were no molecular or serological evidences of SARS-CoV-2 circulation in marmosets (Abreu et al., 2021; Sachetto et al., 2021). However, a free-ranging black-tailed marmoset (*Mico melanurus*), a species belonging to the Callithichidae family, was positive to SARS-CoV-2 by PCR and immunohistochemistry (Pereira et al., 2022) and the Zeta variant of SARS-CoV-2 was identified in free-ranging coatis in Belo Horizonte (Brazil) (Stoffella-Dutra et al., 2023).

2.1.2. Bacteria

2.1.2.1. *Staphylococcus* sp.

Staphylococcus sp. are Gram-positive bacteria, part of the microbiota of most mammals, opportunistic and have zoonotic potential. *S. aureus* is the most important species associated with diseases in primates and was previously isolated from free-ranging callithrichids (Albuquerque et al., 2020). Coatis can also host *Staphylococcus* sp., nine different species were isolated from free-ranging animals and only one strain was resistant to multiple antibiotics. *S. intermedius* was the most isolated species in this study (Santana et al., 2023).

2.1.2.2. *Salmonella* sp.

Some bacteria are important intestinal pathogens for humans and animals. *Salmonella* sp. can colonize the intestines of non-human primates, but the animals are usually asymptomatic, although disease outbreaks may happen (Verona & Pissinati, 2014). Yoruba serotype was isolated from common marmosets presenting diarrhea (Knobl et al., 2011). No *Salmonella* isolation from coatis have been reported. However, a free-ranging white-nosed coati from Mexico presented antibodies against *Salmonella* Typhimurium (De-La-Rosa-Arana et al., 2016).

2.1.2.3. *Clostridioides difficile*

Clostridioides difficile, previously named *Clostridium difficile*, can be part of the intestinal microbiota and usually causes diarrhea associated with dysbiosis. *C. difficile* was isolated from common marmoset and a buffy-tuffed-ear marmoset (*C. aurita*) kept under human care. In both cases the animals presented diarrhea (Armstrong et al., 2019; Carvalho et al., 2022). Toxigenic strains of *C. difficile* were isolated from fecal samples of free-ranging coatis (Silva et al., 2014).

2.1.2.4. *Leptospira* sp.

Leptospirosis is a zoonotic disease caused by spirochetes of the genus *Leptospira*. In a serological study with 48 black-tuffed marmosets, no animal presented antibodies against *Leptospira* sp. (Molina et al., 2014), however a free-ranging black-tuffed marmosets was positive for *Leptospira interrogans*, presenting dissociation and necrosis of hepatocytes, interstitial nephritis with tubular degeneration and jaundice. In this case, renal tubules had multiple spirochetes, suggesting that *L. interrogans* can be eliminated through urine (Wilson et al., 2021). Serological evidence of infection by *Leptospira* sp. was reported in both free-ranging coatis and animals kept under human care (Langoni et al., 2009; Vieira et al., 2016). In the study with free-ranging animals, *Leptospira* sp. DNA was found in urine samples from one individual, suggesting that elimination can also happen through urine (Vieira et al., 2016).

2.1.2.5. *Mycobacterium* sp.

Tuberculosis, mainly associated with *Mycobacterium tuberculosis* or *M. bovis*, is a zoonotic disease that causes granulomatous lesions that can be restricted to the pulmonary tract or disseminated. Disease in non-human primates is mainly caused by *M. tuberculosis* and more frequent in animals kept under human care (Matz-Rensing & Lowenstine, 2018). Marmosets are used as models for experimental infection of multiple species of *Mycobacterium* sp. (Via et al., 2013; Mangat et al., 2017) however natural infection was not reported. *M. bovis*-associated tuberculosis was reported in coatis kept under human care at a triage center that presented pulmonary granulomas (Murakami et al., 2012). Another report described coatis kept under human care presenting granulomas in multiple organs (lungs, liver and lymph nodes). *M. kansaii* was identified in these animals, an opportunistic pathogen that can cause disease in immunosuppressed humans (Rocha et al., 2013).

2.1.2.6. *Brucella* sp.

Brucellosis is a disease caused by *Brucella* sp. Gram-negative bacteria, including several species and various hosts (Olsen & Palmer, 2014). *Brucella papionis* is a species isolated from baboons (*Papio* sp.), which raises concerns its zoonotic potential (Schlabritz-Loutsevitch et al., 2009; Whatmore et al., 2014). No antibodies were previously found in serum samples from marmosets (Ricciardi et al., 1976; Molina et al., 2014), however frequency of seroreactive coatis ranged from 8.8% for free-ranging animals to 76% in animals kept under human care (Dorneles et al., 2014; Oliveira-Filho et al., 2012).

2.1.3. Protozoan

2.1.3.1. *Toxoplasma gondii*

Toxoplasmosis, caused *Toxoplasma gondii*, is transmitted by ingestion of water and food contaminated with oocysts that are eliminated by infected cats through their feces. In humans, infection by *T. gondii* is more important in pregnant and immunosuppressed individuals, however for most neotropical primates the disease is systemic and severe leading quickly to death (Paula et al., 2020; Santana et al., 2021). Reported frequency of toxoplasmosis in free-ranging marmosets was 1.6% and animals presented necrotizing lesions in multiple organs associated with *T. gondii* zoites (Oliveira et al., 2022). Serological assays for diagnosis of

toxoplamosis in neotropical primates are usually ineffective, as animals develop hyperacute disease upon infection with *T. gondii* leading to death serological response is not detectable (Paula et al., 2020). However, serological response was reported in 16.6% of free-ranging black-tuffed marmosets (Molina et al., 2014), which suggest that this specie can survive *T. gondii* infection and develop antibodies. Disease associated with *T. gondii* was not described in coatis, however there is serological evidence of exposure in free-ranging animals from urban areas. Frequency of reactivity was 70.7% (Maia et al., 2016), indicating that those animals were exposed to *T. gondii*.

2.1.3.2. *Leishmania* sp.

Leishmania sp. is a protozoan that causes leishmaniosis, a zoonotic disease transmitted by sand flies. In Brazil, visceral leishmaniosis is the most important form of the disease, caused by *L. infantum* and transmitted by *Lutzomyia longipalpis* (Lainson & Rangel, 2005; WHO, 2010). Both humans and animals can host *L. infantum*, including several species of wild mammals. Non-human primates not only host *L. infantum* but also develop clinical disease (Malta et al., 2010; Tinoco et al., 2018). There is serological and molecular evidence of infection by *L. infantum* in marmosets and coatis (Paiz et a, 2015; Paiz et al., 2018; Porfirio et al., 2018; Trüeb et al., 2018; Venial et al., 2022; Macedo et al., 2023). For these species there is no report of clinical disease and their ability to transmit *Leishmania* sp. to the vector has not been accessed.

2.1.3.3. *Trypanossoma* sp.

Trypanossoma cruzi is a parasite that causes chagas disease, an endemic disease in Latin America transmitted by triatomine bugs (Minuzzi-Souza et al., 2016). *T. cruzi* was reported in neotropical primates (Lisboa et al., 2004; Monteiro et al., 2006; Monteiro et al., 2010) and another species, *T. minanense*, was identified in free-ranging marmosets. (Coimbra et al., 2020). Coatis can be infected with *T. cruzi* and develop antibodies (Herrera et al., 2008) and experimental infection with *T. evansi* resulted in persistent anemia and myocarditis (Herrera et al., 2002).

2.2. Leishmaniosis in wild carnivores

The Carnivora order is composed of 286 animal species divided in two suborder: Caniformia and Feliformia. Feliformia suborder contains four families: Felidae, Hyaenidae, Herpestidae and Viverridae. The Carnivora suborder has seven families: Canidae, Mustelidae, Ursidae, Procyonidae, Otariidae, Phocidae and Odobenidae.

2.2.1. Canidae

The Canidae family is made up of approximately 35 species of carnivores, 6 species of which occur in Brazil. They are *Cerdocyon thous*, *Speothos venaticus*, *Chrysocyon brachyurus*, *Lycalopex gymnocercus*, *Lycalopex vetulus*, and *Atelocynus microtis* (Keel et al., 2018; Jorge & Jorge, 2014).

2.2.1.1. Crab-eating foxes (*Cerdocyon thous*)

Among the species of the Canidae family, most information about leishmaniosis is reported in crab-eating foxes (*Cerdocyon thous*), a medium-sized canid with a wide distribution in Brazilian territory and a low risk of extinction (Jorge & Jorge, 2014; Lucherini, 2015). Frequency of seroreactive crab-eating foxes is variable, ranging from 3.8% (Brandão et al., 2020) to 78.4% (Courtenay et al., 2002) for free-ranging animals. From a group of 11 free-living individuals, all serologically non-reactive, one animal had amastigotes in bone marrow cytology and the same sample was positive in PCR (Gomes et al., 2007). Several authors have used molecular methods to identify the genetic material of *Leishmania* sp. successfully in organ samples collected during necropsy procedures (Silva et al., 2000; Gomes et al., 2007; Souza et al., 2010; Jusi et al., 2011; Tenório et al., 2011; Richini-Pereira et al., 2014;) and biological samples collected from still-living animals such as bone marrow and blood (Courtenay et al., 2002; Porfirio et al., 2018; Trüeb et al., 2018; Reis et al., 2020; Lima et al., 2021).

The clinical signs observed in naturally infected crab-eating foxes are ulcerative skin lesions, lymphadenopathy, onychogryphosis, uveitis, weight loss, dehydration, vomiting, diarrhea, oliguria, anuria, and anemia (Silva et al., 2000; Courtenay et al., 2002; Gomes et al., 2007; Souza et al., 2010; Tenório et al., 2011). Some authors have observed serologically reactive animals without clinical signs compatible with leishmaniosis (Curi et al., 2006; Luppi

et al., 2008) and Trüeb et al. (2018) described an asymptomatic individual with a positive blood sample through PCR and isolation.

Anatomopathological findings were described by Silva et al. (2000) and Tenório et al. (2011). Silva et al. (2000) reported a free-ranging individual with histiocytic dermatitis with intracytoplasmic amastigotes in the snout region. Tenório et al. (2011), an animal kept under human care that presented hepatomegaly, splenomegaly with white pulp hyperplasia, enlargement of lymph nodes in the superficial chain. In this case, amastigote forms of *Leishmania* sp. in various organs through cytology and histology, in addition to immunohistochemistry. To evaluate the transmission capacity of *Leishmania* sp. from crab-eating foxes to the vector, Courtenay et al. (2002) subjected 26 free-ranging individuals to xenodiagnosis using *Lu. longipalpis* raised in laboratory conditions and none of the sandflies evaluated after feeding were infected.

2.2.1.2. Bush dog (*Speothos venaticus*)

The bush dog (*Speothos venaticus*) is a canid with wide distribution in Brazilian territory and in most Latin American countries, being considered by the IUCN as a near-threatened species on the red list (DeMatteo et al., 2011; Jorge & Jorge, 2014). Unlike other canid species, bush dogs have less data available in the literature. The Belo Horizonte Zoo had a group of individuals of this species that allowed some information regarding this species to be obtained. In 2004, an adult female kept at the institution presented progressive weight loss, vomiting, diarrhea, anemia, polyuria, polydipsia and an increase in urea and creatinine (Luppi et al., 2008). The animal died and presented lesions compatible with infection by *Leishmania* sp. such as splenomegaly associated with lymphoid hyperplasia and renal lesions (lymphohistioplasmacytic interstitial nephritis and membranous glomerolopathy), in addition to the presence of intrahistiocytic amastigotes in several organs confirmed as *L. infantum* through PCR. Years later, Mol et al. (2015) subjected some species of canids, including bush dogs, to xenodiagnosis to evaluate the transmissibility potential of these individuals for *Lu. longipalpis*. On this occasion, of the five individuals evaluated, three were seroreactive and all were capable of transmitting *L. intantum* to the vector with low parasite loads.

Clinical signs described by other authors include foot dermatitis, alopecia, weight loss, onychogryphosis, vomiting and conjunctivitis (Lima et al., 2009; Souza et al., 2010). Other reports involving bush dogs described an asymptomatic animal kept under human care from

which *Leishmania* sp. was isolated from the skin (Figueiredo et al., 2008) and a seropositive individual who died and was negative in organ samples collected during necropsy (Jusi et al., 2011).

2.2.1.3. Maned wolf (*Chrysocyon brachyurus*)

The maned wolf (*Chrysocyon brachyurus*) is the largest Brazilian canid also present in some Latin American countries. Its classification on the IUCN red list is as a near-threatened species (Jorge & Jorge, 2014; Paula & DeMatteo, 2015). Few studies have investigated the relationship between maned wolves and *Leishmania* sp. Serological assessments observed frequencies ranging from 0% (0/10 and 0/11) (Paiz et al., 2015; Brandão et al., 2020) to 28.57% (2/7) (Curi et al., 2006) in samples of free-ranging animals, and 14.28% (1/7) (Luppi et al., 2008) to 75% (3/4) (Mol et al., 2015) in animals kept under human care. Both serological investigations of animals kept in human care were carried out at the same institution at different periods. Additionally, Reis et al. (2020) identified three animals kept in human care with positive blood samples using PCR.

Clinical signs associated with infection by Leishmania sp. in maned wolves were onychogryphosis and ulcerative dermatitis (Luppi et al., 2008; Jusi et al., 2011). In an animal with ulcerative dermatitis on the pinna described by Luppi et al. (2008), intrahistiocytic amastigotes were also observed in the cytological evaluation of the lesion. In some situations, serologically reactive animals were asymptomatic (Curi et al., 2006; Mol et al., 2015). However, even asymptomatic, serologically positive individuals were able to infect the vector with low parasite loads when subjected to xenodiagnosis (Mol et al., 2015). Furthermore, two animals kept under human care underwent necropsy and both animals presented lymphohistioplasmacytic adrenalitis with intracytoplasmic amastigotes without evidence of *Leishmania* sp. in other organs (Carvalho et al., 2015; unpublished data).

2.2.1.4. Hoary Fox (*Lycalopex vetulus*)

The hoary fox, *Lycalopex vetulus*, is the smallest of the Brazilian canids, found mainly in the central region of the country in the cerrado biome and is one of the canid species considered near threatened by the IUCN (Jorge & Jorge, 2014; Lemos et al., 2020).

Few authors described clinical and pathological changes in hoary foxes infected with *Leishmania* sp. Luppi et al. (2008) described an individual kept under human care at the Belo Horizonte Zoo who presented ulcerative dermatitis, prostration, weight loss and lymphadenopathy, from which intrahistiocytic amastigotes were observed on cytology. At necropsy, anemia, enlarged cervical lymph node, emaciation and hepatoeplenomegaly were observed. In this animal, amastigotes were observed in the cytoplasm of macrophages in several organs (kidneys, lymph nodes, spleen, lung, liver, and intestines), in addition, the animal presented membranoproloferative glomerulopathy.

A previous serological evaluation was carried out on this group of hoary foxes that resided at the Belo Horizonte Zoo, two of the six animals were reactive (33%) and both had bone marrow samples positive in PCR for *Leishmania* sp. Other investigations used serological methods with varying amounts of samples resulting in frequencies ranging from 0% (0/2, 0/8) (Curi et al., 2006; Paiz et al., 2015), 4.7% (2/42) (Brandão et al., 2020) to 100% (2/2) (Voltarelli et al., 2009) in free-ranging animals. Animals kept under human care were less described, one animal evaluated by Jusi et al. (2011) was negative in the tests carried out (serology and PCR) and one of the two individuals evaluated by Reis et al. (2020) was positive in the PCR using blood samples.

2.2.1.5. Pampas fox (*Lycalopex gymnocercus*)

The pampas fox (*Lycalopex gymnocercus*) is considered of least concern on the IUCN list of threatened animals and is found in the southern region of Brazil, mainly Rio Grande do Sul and Santa Catarina, as well as in other Latin American countries (Jorge & Jorge, 2014; Lucherini, 2016a). Only one study investigated the presence of anti-Leishmania antibodies in free-ranging pampas foxes and none of the 22 samples submitted to the ELISA test was reactive (Padilha et al., 2021).

2.2.2. Felidae

Wild felids have a wide geographic distribution. In Brazil, neotropical felids are divided into four main genera: *Panthera*, represented by *P. onca*; *Puma*, which has *P. concolor* as its main representative; *Leopardus*, formed by *L. pardalis*, *L. tigrinus*, *L. colocolo*, *L. guttulus*, *L. wiedii*, and *L. geoffroyi*; and *Herpailurus*, whose representative is *H. yagouaroundi* previously

belonging to the genus *Puma* (Adania et al., 2014). Furthermore, exotic felids are frequently found in zoos in Brazil, therefore *Panthera leo* and *Panthera tigris* were included in this review.

2.2.2.1. Ocelot (*Leopardus pardalis*)

Ocelots (*Leopardus pardalis*) are medium-sized felids that inhabit almost all of the Brazilian territory, with the exception of Rio Grande do Sul, and countries in Central and South America. Ocelots are considered as least concern regarding their risk of extinction (Adania et al., 2014; Paviolo et al., 2015).

Clinical disease or anatomopathological findings associated with *Leishmania* sp. infection. in ocelots have not been reported, however Reis et al. (2020) and Lima et al. (2021) found genetic material from *Leishmania* sp. in blood samples from an individual kept under human care and another sent to a rescuing and triage center after animal trafficking apprehension. Furthermore, Dahroug et al. (2010) evaluated blood and lymph node aspirate samples from four animals kept under human care, all of which were negative in molecular tests. A serum sample from a free-ranging animal was submitted to the direct agglutination test as part of a study involving several free-living species and did not react in the test (Paiz et al., 2015), however Tolentino et al. (2019) observed two seroreactive animals kept under human care.

2.2.2.2.Northern and southern tiger cats (*Leopardus tigrinus* and *L. guttulus)*

The northern tiger cat (*Leopardus tigrinus*) is the smallest feline species found in Brazil and has a poorly defined geographic distribution, being recorded in some countries in South America and is considered vulnerable on the list of endangered species (Adania et al., 2014; Payan & Oliveira, 2016). No evidence of infection by *Leishmania* sp. in northern tiger cats. Organ samples from a found dead individual were negative in PCR (Richini-Pereira et al., 2014) and a serum sample from a free-ranging animal was negative in the direct agglutination test (Paiz et al., 2015).

The southern tiger cat (*Leopardus guttulus*) is a recently described species previously considered *L. tigrinus* and occurs in Brazil in the central and southern areas, as well as Argentina and Paraguay. It is considered vulnerable by the IUCN (Trigo et al., 2013; Oliveira et al., 2016). In a study carried out by Reis et al. (2020), a blood sample from an individual kept under human

care was positive in the PCR. There is no other information about infection by *Leishmania* sp. in this species.

2.2.2.3. Pampas cat (*Leopardus colocolo*)

The pampas cat (*Leopardus colocolo*) is a small feline with little information available, found in several countries in South America and considered near threatened by the IUCN (Adenia et al., 2014; Lucherini et al., 2016b). Reis et al. (2020) tested blood samples from two individuals kept in human care that were positive by PCR. However, there is no description of clinical signs, anatomopathological changes or serological evidence of infection by *Leishmania* sp. in this species.

2.2.2.4. Margay (*Leopardus wiedii*)

The margay (*Leopardus wiedii*) occurs in several countries in Central and South America, including Brazil, and is considered near threatened by the IUCN (Adania, 2014; Oliveira et al., 2015). No information about the relationship of this species with *Leishmania* sp. was reported.

2.2.2.5. Geoffroy' cat (*Leopardus geoffroyi*)

The Geoffroy' cat (*Leopardus geoffroyi*) is a medium-sized feline found in Brazil, inhabiting the state of Rio Grande do Sul as well as other countries in South America. It is classified as being of least concern on the list of endangered species (Adania et al., 2014; Pereira et al., 2015). No information was described regarding the interaction between this species and *Leishmania* sp.

2.2.2.6.Puma (*Puma concolor*)

The puma (*Puma concolor*) is the second largest feline species native to Brazil, originally distributed throughout the American continent, but today it is already considered extinct in some regions, however it is still considered to be of leats concern regarding the risk of extinction (Adania et al., 2014; Nielsen et al., 2015). Little information about the interaction of these

animals with *Leishmania* sp. are available. Dahroug et al. (2010) evaluated seven animals kept under human care and five of them were positive in PCR from lymph node and blood samples and Reis et al. (2020) described an individual kept under human care that was also positive by PCR from a blood sample. In relation to free-ranging animals, organ samples from two roadkilled animals were negative in PCR (Richini-Pereira et al., 2014) and a serum sample was negative in the direct agglutination test (Paiz et al., 2015). Tolentino et al. (2019) evaluated three animals kept in human care by various serological methods and all results were negative.

2.2.2.7. Jaguar (*Panthera onca*)

The jaguar (*Panthera onca*) is the largest felid in the Americas and the only representative of its genus among New World felids occurring in Central and South America and considered near threatened (Adania et al., 2014; Quigley et al., 2018). Information on this species is scarce. One animal kept under human care of the five animals evaluated had positive blood and lymph node samples in PCR (Dahroug et al., 2010). Reis et al. (2020) evaluated a blood sample from an individual that was negative in the PCR.

2.2.2.8. Jaguarundi (*Herpailurus yagouaroundi*)

The jaguarundi (*Herpailurus yagouarundi*), previously included in the genus *Puma*, is a small felid distributed throughout Central and South America with a wide distribution in Brazil and considered by the IUCN as least concern regarding the risk of extinction (Adania et al., 2014; Caso et al., 2015). No evidence of the circulation of *Leishmania* sp. was registered. In a serological study with serum samples from several species of free-living mammals, four animals were evaluated using the direct agglutination test, all of which were non-reactive (Paiz et al., 2015). A blood sample from an individual held in human care was PCR negative (Reis et al., 2020).

2.2.2.9. Lion (*Panthera leo*)

Lions (*Panthera leo*) are large felids that were originally distributed throughout almost the entire African continent and parts of Europe and Asia and today their habitat have been severely reduced. In Brazil, they are often part of zoos and are considered vulnerable by the

IUCN in terms of their risk of extinction (Adania et al., 2014; Nicholson et al., 2023). Little information about the susceptibility of this species has been described, however Libert et al. (2012) evaluated a group of *P. leo leo* kept under human care in which one of the five animals presented cachexia, anemia, leukocytosis, hypercholesterolemia and hypoalbuminemia. Blood and serum samples from the five animals were subjected to PCR and indirect immunofluorescence testing, two of which were positive in PCR and three were positive in serology. Furthermore, in a study carried out by Dahroug et al. (2011), a blood sample from a lion kept under human care was positive in PCR and a serum sample from a female kept under human care was a reagent in serological tests carried out by Tolentino et al. (2019).

2.2.2.10. Tiger (*Panthera tigris*)

Tigers (*Panthera tigris*) are large felids native to the Asian continent, an endangered species that has had its habitat severely reduced (Goodrich et al., 2022). Information on leishmaniosis in this species is scarce, however Cavalera et al. (2020) evaluated a group of 20 individuals under human care who did not show clinical signs, but who showed seroreactivity in the indirect immunofluorescence test in 45% of the animals (9/20) and 23% (4/17) of the animals were positive in PCR using lymph node aspirates. Furthermore, Tolentino et al. (2019) evaluated serum samples from two individuals of the species *P. tigris altaica*, one of which was positive in serological tests. One of these tigers presented a mandibular squamous cell carcinoma (Oliveira et al., 2018). *L. infantum* DNA was detected in spleen and lymph node samples.

2.2.3. Mustelidae

Mustelids are mammals with a wide distribution around the world, covering 22 genera and 58 species of animals. In Brazil there are 6 species of native mustelids: *Eira barbara*, *Galictis vittata*, *Galictis cuja*, *Pteronura brasiliensis*, *Lontra longicaudis* and *Mustela africana* (Javorouski & Passerino, 2014).

2.2.3.1. Tayra (*Eira barbara*)

The tayra (*Eira barbara*), only species of the genus, is a mustelid that occurs from Mexico to Brazil and is classified as least concern by the IUCN (Javorouski & Passerino, 2014; Cuarón et al., 2016b). The only evidence of contact of this species with *Leishmania* sp. was observed by Paiz et al. (2015) who submitted two tayras serum samples to the direct agglutination test and one of them was reactive.

2.2.3.2. Lesser and greater grisson (*Galictis cuja* and *Galictis vittata*)

Grissons are mustelids of the genus *Galictis*, which includes two species: *G. vittata* (greater grisson) and *G. cuja* (lesser grisson). *G. vittata* occurs from Mexico to part of the Brazilian territory, encompassing the north, northeast and part of the central-west region and Lesser grissons occurs in Brazil, extending from the northeast to the south, and in other countries in South America. Both are considered of least concern by the IUCN in terms of risk of extinction (Javorouski & Passerino, 2014; Cuarón et al., 2016a; Helgen & Schiaffini, 2016).

Serological evidence of infection by *Leishmania* sp. in free-ranging lesses grissons were observed in three animals evaluated by Paiz et al. (2015). Furthermore, an elderly lesses grisson kept under human care at the Belo Horizonte Zoo, presented an increase in lymph nodes in the superficial chain and, in the cytological evaluation, there was a large number of intra-histiocytic amastigotes (unpublished data). The animal was euthanized, and amastigotes were observed in several organs, in addition the animal presented chronic interstitial nephritis with membranous glomerulopathy. Tissue samples from two greater grissons were subjected to PCR by Richini-Pereira et al. (2014), all considered negative.

2.2.3.3. Neotropical otter (*Lontra longicaldis*)

Otters are mustelids with aquatic habits, with *Lontra longicaudis* being the species that occurs in Brazil and is considered near threatened by the IUCN (Javorouski & Passerino, 2014; Rheingantz et al., 2022). The only evidence of *Leishmania* sp. in this species was reported by Reis et al. (2020) who performed PCR on two blood samples from animals kept under human care and one of them was positive.

2.2.4. Procyonidae

The procyonid family is made of carnivores of six genera, of which four genera are present in South America, they are *Procyon*, *Nasua*, *Potos*, and *Bassaricyon* (Teixeira & Ambrosio, 2014). Most of the available information discusses the species *Procyon cancrivorus* and *Nasua nasua*. No information was found on the species of the genera *Potos* and *Bassaricyon*.

2.2.4.1. Crab-eating racoon (*Procyon cancrivorus*)

The crab-eating raccoon (*Procyon cancrivorus*) is a medium-sized animal similar to the raccoons found in North America (*Procyon lotor*) and its distribution includes countries in Central and South America. It has a low risk of extinction (Teixeira & Ambrosio, 2014; Reid et al., 2016). There is little evidence of *Leishmania* sp. infection in crab-eating raccoons. Voltarelli et al. (2009) identified a free-ranging seropositive individual through the direct agglutination test. While Jusi et al. (2011), evaluating six animals kept under human care using serological and molecular methods did not identify positive animals. Infection by *Leishmania* sp. was observed in organ samples collected from a free-living road-killed animal by PCR (Richini-Pereira et al., 2014). However, none of these studies described clinical or anatomopathological changes associated with leishmaniosis.

2.2.4.2.South American coati (*Nasua nasua*)

South American coatis (*Nasua nasua*) are procyonids with diurnal, terrestrial and arboreal habits that live in groups. They are animals classified as being of least concern in terms of risk of extinction and inhabit a large part of South America and Brazil (Texeira & Ambrosio, 2014; Emmons & Helgen, 2016).

There is serological evidence of infection in free-ranging animals, with frequencies ranging from 4.6% (Paiz et al., 2015) to 44% (Porfirio et al., 2018). In a recent study, Macedo et al. (2023) evaluated 110 free-ranging animals in the city of Campo Grande and the animals, in addition to showing reactive serology, were also positive in the PCR of bone marrow samples (33/85, 41.2%). Another study with a substantial number of animals evaluated was carried out by Estevam et al. (2020), who used blood samples collected from free-ranging animals over a period of 7 years and investigated several hemopathogens, including *Leishmania infantum*,

however, none of the samples evaluated was positive. This contrasts with the results obtained by Reis et al. (2020) who evaluated captive animals and 3 of the six animals collected had a positive blood sample in the PCR. Clinical and pathological aspects have not been described in this species.

2.3. Leishmaniosis in neotropical primates

Neotropical primates are classified into five families: Callithrichidae – comprising species of the genera *Callithrix*, *Cebuella*, *Callibella*, *Mico*, *Saguinus*, *Leontopithecus*, and *Callimico* –, Cebidae – which encompasses the genera *Cebus*, *Sapajus* and *Saimiri* –, Aotide – represented by a single genus, *Aotus* –, Atelidae – with the genera *Ateles*, *Brachyteles*, *Alouatta* and *Lagothrix* – and Pithecidae – which includes the genera *Cacajao*, *Callicebus*, *Chiropotes* and *Pithecia* (Rylands et al., 2012). The clinical and pathological aspects of leishmaniosis in non-human primates, including animals from the old and new world in cases of natural or experimental infection, were explored in a recent review (Santos & Oliveira, 2020).

2.3.1. Callithrichidae

The Callithrichidae family is made up of primates from seven genera: *Callithrix*, *Cebuella*, *Callibella*, *Mico*, *Saguinus*, *Leontopithecus*, and *Callimico*. These animals are small and are often used as experimental animals (Verona & Pissinatti, 2014; Whitney, 1995). In these genera, vulnerability is quite variable, with species classified as being of least concern to endangered species such as the buffy-headed marmosets (*Callithrix flaviceps*) and golden lion tamarins (*Leontopithecus rosalia*) (Melo et al., 2021; Ruiz- Miranda et al., 2021).

For primates of the genus *Callithrix*, serological evidence was observed in free-ranging animals. Paiz et al. (2018) evaluated serum samples of common marmosets and black-tuffed marmosets and found 25% (2/8) and 27.7% (5/18) of seroreactive animals respectively. Blood, lymph node and skin samples from free-ranging animals were used in some studies for molecular tests and frequencies of 40% (2/5), 23.07% (3/13) and 12.5% (1/ 8) respectively (Paiz et al., 2018; Trüeb et al., 2018; Venial et al., 2022). In these species, no clinical signs or pathological changes associated with infection by *Leishmania* sp.

Leishmaniosis in primates of the genus *Saguinus* was investigated on two occasions in primates kept under human care at the Belo Horizonte Zoo. Malta et al. (2010) used PCR on

blood samples from emperor tamarins (*Saguinus imperator*) and the three animals evaluated were positive. Oliveira et al. (2019) evaluated four individuals of the same species, all negative serologically and through xenodiagnosis. Blood samples from two other species, pied tamarin (*S. bicolor*) and black-handed tamarin (*S. niger*), were included in the study by Reis et al. (2020) and were negative by PCR.

In primates of the genus *Leontopithecus*, anti-*Leishmania* antibodies were found in two golden lion tamarins (*L. rosalia*) (Oliveira et al., 2019). In that same study, fifteen golden lion tamarins were subjected to xenodiagnosis and three animals were able to transmit *L. infatum* to the vector. Furthermore, one gonden-headed lion tamarin (*L. chrysomelas*) and two black lion tamarins (*L. chrysopygus*) were subjected to the same tests and were negative in serology and PCR. Some studies used golden-headed lion tamarins blood samples for PCR, with one positive animal out of 5 and 6 animals evaluated by Malta et al. (2010) and Reis et al. (2020) respectively.

2.3.2. Cebidae

Primates of the Cebidae family are composed of medium-sized primates of the genera *Cebus* and *Sapajus* (capuchin monkeys) and from the genera *Saimiri* (squirrel monkeys) (Verona & Pissinatti, 2014). The vulnerability of these species is variable, with some being considered critically endangered such as the buff-headed capuchin (*Sapajus xanthosternus*) (Canale et al., 2021).

For the Cebidae family, frequencies of serologically positive animals were 100% (4/4) for free-ranging black-capped capuchin (*Sapajus apella*) (Voltarelli et al., 2009), 16.6% (1/6) for black-capped capuchins kept under human care (Oliveira et al., 2019) and 53.3% (8/15) for free-ranging black-horned capuchins (*S. nigritus*) (Lopes et al., 2022). Parasitemia, assessed through PCR, was observed in 56% (5/9) of free-ranging Azara's capuchins (*S. cay*) (Porfirio et al., 2018), 26.66% (4/15) of free-ranging black-horned capuchins (Lopes et al., 2022) and in 60% of yellow-breasted capuchin (*S. xanthosternos*) kept in human care (Malta et al. 2010). Of the six animals subjected to xenodiagnosis by Oliveira et al. (2019), only one black-capped capuchin was capable of transmitting *L. infatum* to *Lu. longipalpis*.

2.3.3. Aotidae

Aotidae family is made up of just one genus, with owl monkeys (*Aotus nigriceps*) being the main representative, a nocturnal primate that inhabits states in the northern region of Brazil and Mato Grosso. Its risk of extinction is considered of least concern by the IUCN (Shanee et al., 2021).

No serological evidence of infection by *Leishmania* sp. in primates of the genus *Aotus*, however some authors report positive blood samples in PCR in owl monkeys kept under human care (Malta et al., 2010; Reis, et al., 2020) and free-living Azara's night monkey (*A. azarai*) (Acardi et al., 2013). Two animals included in the study by Oliveira et al. (2019) were serologically negative and were not capable of infecting the vector.

2.3.4. Pithecidae

Although no serologically positive animals have been reported within the species that make up the Pithecidae family, a black-fronted titi monkey (*Callicebus nigrifrons*) kept under human care at the Belo Horizonte Zoo died and presented emaciation, pulmonary edema, splenomegaly, and lymphadenopathy at necropsy (Malta et al., 2010). Furthermore, histologically, amastigotes were observed in the cytoplasm of macrophages in various organs, including the liver, spleen, and lymph nodes, and were confirmed as *L. infantum* using PCR and immunohistochemistry. After confirmation of leishmaniosis in this individual, 8 other animals of the same species were tested using PCR from blood samples, 6 of which were positive. Positive blood samples have also been reported in black-bearded sakis (*Chiropotes Satanas*) (Reis et al., 2020), Gray's bald-faced saki (*Pithecia irrorate*) (Malta et al., 2010) and Vieira's titi monkey *Plecturocebus vieirai* (Guiraldi et al., 2022).

2.3.5. Atelidae

No anti-*Leishmania* antibodies were detected in samples of brown howler monkeys (*Alouatta guariba*) (Paiz et al., 2015; Paiz et al., 2015; Oliveira et al., 2019), black-and-gold howler monkeys (*Alouatta caraya*) (Oliveira et al., 2019), southern muriqui (*Brachyteles arachnoides*) (Paiz et al., 2015) and common woolly monkeys (*Lagothrix cana*) (Oliveira et al., 2019). However, some authors describe positive blood samples using PCR in brown howler monkeys (Malta et al., 2010), black-and-gold howler monkeys (Guiraldi et al., 2022), black-

faced black spider monkey (*Ateles chamek*) (Guiraldi et al., 2022), Guiana spider monkey (*Ateles paniscus*) (Lima et al., 2012) and common woolly monkeys (Reis et al., 2020). A Guiana spider monkey with a positive blood sample reported by Lima et al., (2012) presented weight loss, pallor of the mucous membranes, leukocytosis with neutrophilia and increased alkaline phosphatase. None of the animals subjected to xenodiagnosis were capable of transmitting *L. infantum* to the vector in the study carried out by Oliveira et al., 2019, which included howler monkeys and wooly monkeys.

2.4. Xenodiagnosis

Xenodiagnosis, from the Greek *xenos* (foreign) and *diagnosi* (diagnosis), is an indirect method of diagnosis that uses a vector where the infectious agent to be identified can multiply more and be easily identified (Meiser & Schaub, 2011). This technique was initially used for the diagnosis of *Trypanosoma cruzi* in human patients with low parasite load, however with the advancement of diagnostic methods it became less relevant (Schenome, 1999). Furthermore, it still is an important method for evaluating the interaction between parasite, vector and host, being able to evaluate the ability of a host to transmit a certain infectious agent to the vector (Sadlova et al., 2015).

2.4.1. Investigation of leishmaniosis in wild animals through xenodiagnosis

Xenodiagnosis is a method used to evaluate interactions between infectious agents, vectors and hosts, useful for determining the ability of a vertebrate host to transmit *Leishmania* sp. for vectors and consequently their role as a potential reservoir. This method has already been used in domestic animals to demonstrate the ability of *Lutzomyia longipalpis* raised in laboratory conditions to become infected after feeding on dogs and cats (Costa-Val et al., 2007; Silva et al., 2010).

The ability of wild animals to transmit *Leishmania* sp. for *Lu. longipalpis* has been evaluated in some studies. Canids were evaluated by Courtenay et al. (2002) and Mol et al. (2015). Free-ranging crab-eating foxes (*C. thous*) were not able to transmit *Leishmania* sp. (Courtenay et al., 2002), while maned wolves (*C. brachyurus*) and bush dogs (*S. venaticus*) kept under human care transmitted *L. infantum* to the vectors in low quantities (Mol et al., 2015).

Travi et al. (1998) carried out experimental infection of *L. infantum* in common opossums (*Didelphis marsupialis*), demonstrating not only the development of clinical disease but also the ability of some animals to transmit the protozoan to the vector. Free-ranging lagomorphs (*Lepus granatensis* and *Oryctolagus cuniculus*) were able to transmit *Leishmania* sp. to vectors in studies carried out in endemic areas of Spain (Molina et al., 2012; Jiménez et al., 2014).

Clinical disease associated with *L. infantum* infection has been reported in New and Old-World primates maintained under human care (Malta et al., 2010; Tinoco et, 2018). Non-human primates (*Leontopithecus rosalia*, *Sapajus apella*, *Pan troglodytes*, and *Myopithecus talapoin*) kept under human care demonstrated the ability to transmit low amounts of *L. infantum* to *Lu. longipalpis* (Oliveira et al., 2019). Free-ranging Neotropical primates presented serological and molecular evidence of infection by *Leishmania* sp. (Paiz et al., 2018; Porfirio et al., 2018; Trüeb et al., 2018; Martínez et al., 2020; Lopes et al., 2022) however, the ability of these free-ranging individuals to transmit *Leishmania* sp. to vectors has not yet been explored.

2.4.2. *Leishmania* sp. development in *Lutzomyia longipalpis*

The life cycle of *Leishmania* sp. is digenetic, alternating between mammalian hosts and invertebrate vectors, sandflies of the genus *Phlebotomus* and *Lutzomyia* (Killick-Kendrick, 1999). Stages of *Leishmania* sp. development inside the vector are critical for the establishment of infection in the vector. In sandflies, subgenera of *Leishmania* sp. develop in different segments of the digestive tract. Parasites of the subgenus *Leishmania* are the most studied in terms of interactions with the vector and develop exclusively in the midgut, while those of the subgenus *Viannia* pass through the hindgut before migrating to the midgut (Lainson et al., 1977).

Female sandflies become infected when they feed on infected animals. When eating, sandflies insert their mouthparts forming a “pool” containing blood and cells present in the skin, in this case macrophages containing amastigotes. Amastigote forms of *Leishmania* sp., round and non-motile with approximately 3-5 µm, are then ingested by the sandfly. Changes in the host environment for the vector's midgut lead to the transformation of amastigotes into procyclic promastigotes, poorly mobile forms measuring 6-8 µm and with a short flagellum located in the anterior portion of the cell. This form replicates in the blood ingested during the meal and around 48 to 72 hours later the replication rate decreases and the change to

nectomonad promastigote occurs, long forms with greater motility that measure 12-20 μm. The nectomonads migrate to the anterior portion of the midgut, undergo yet another transformation, becoming shorter (6-8 μm), called leptomonads, and begin another phase of proliferation. Leptomonads can adhere to the intestinal epithelium and produce a gelatinous substance (Promastigote Secretory Gel – PSG) that forms a plug that contains *Leishmania* sp. cells. At this stage, the final transformation of promastigotes occurs, which become highly mobile and infective to mammalian hosts and are called metacyclic promastigotes. This form has no ability to divide or adhere to the surface of the epithelium and is positioned in the lumen of the mid or anterior intestine, facilitating transmission in the next meal (Bates & Rogers, 2004; Bates, 2007; Dostálová & Volf, 2012).

A female sand fly capable of transmitting *Leishmania* sp. to a vertebrate host must have metacyclic promastigote forms in the proboscis and midgut. There are two main theories about how these promastigotes are inoculated into the host: regurgitation or inoculation. The regurgitation theory is based on the hypothesis that promastigotes located in the intestine form a physical obstruction that is expelled during eating. The inoculation theory states that only the promastigotes present in the proboscis are inoculated at the time of feeding (Bates & Rogers, 2004; Bates, 2007).

The regurgitation theory is the most accepted and is supported by the formation of the plug associated with the PSG that facilitates physical obstruction by forming a structure that fills and distends the midgut and projects into the foregut. This plug is composed of PSG and metacyclic promastigote forms. It is still unclear whether plug formation is essential for transmission to the host. In addition to the plug containing promastigotes, the regurgitation process releases sandfly saliva, which has vasodilatory and anti-hemostatic properties, which are necessary for blood meal (Ribeiro, 1987; Bates & Rogers, 2004; Rogers et al., 2004; Bates, 2007).

3. REFERENCES

ABREU, F.V.S.; MACEDO, M.V.; SILVA, A.J.J. et al. No Evidence of SARS-CoV-2 infection in neotropical primates sampled during COVID-19 pandemic in Minas Gerais and Rio Grande do Sul, Brazil. **EcoHealth**, v. 18, p. 414-420, 2021.

ABREU, F.V.S.; ROCHA, K.L.S.; SILVA-OLIVEIRA, R. et al. Serological Evidence of Orthopoxvirus Infection in Neotropical Primates in Brazil. **Pathogens**, v. 11, n. 10, p. 1167, 2022.

ACARDI, S.A.; RAGO, M.V; LIOTTA, D.J. et al. *Leishmania* (*Viannia*) DNA detection by PCR-RFLP and sequencing in free-ranging owl monkeys (*Aotus azarai azarai*) from Formosa, Argentina. **Veterinary Parasitology**, v. 193, n. 1-3, p. 256-259, 2013.

ADANIA, C.H.; SILVA, J.C.R.; FELIPPE, P.A.N. **Carnivora – Felidae (Onça, Suçuarana, Jaguatirica e Gato-do-mato)**. In: CUBAS, Z.S.; SILVA, J.C.R.; CATÃO-DIAS, J.L.; Tratado de Animais Selvagens. 2 ed. São Paulo : Roca, 2014.

ALBUQUERQUE, D.D.A.; FIGUEIREDO, F.B.; BRANDÃO, M.L. et al. Oral and rectal microbiota of callitrichids (*Callithrix* sp.) in an anthropized area of the Atlantic Forest in Rio de Janeiro, Brazil. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 72, n. 4, p. 1113-1121, 2020.

ALVES-COSTA, C.P.; FONSECA, G.A.B.; CHRISTÓFARO, C. Variation in the diet of the brown-nosed coati (*Nasua nasua*) in southeastern Brazil. **Journal of Mammalogy**, v. 85, n. 3, p. 478-482, 2004.

ARÉCHIGA-CEBALLOS, N.; VELASCO-VILLA, A.; SHI.M. et al. New rabies virus variant found during an epizootic in white-nosed coatis from the Yucatan Peninsula. **Epidemiology & Infection**, v. 138, n. 11, p. 1586-1589, 2010.

ARMSTRONG, A.R.; WUNSCHMANN, A.; RIGATTI, L.H. et al. *Clostridium difficile* enterocolitis in a captive Geoffroy's spider monkey (*Ateles geoffroyi*) and common marmosets (*Callithrix jacchus*). **Veterinary Pathology**, v. 56, n. 6, p. 959-963, 2019.

BARRETO, W.T.G.; HERRERA, H.M.; MACEDO, G.C. et al. Density and survivorship of the South American coati (*Nasua nasua*) in urban areas in Central–Western Brazil. **Hystrix**, v. 32, n. 1, p. 82, 2021.

BATES, P.A. Transmission of *Leishmania* metacyclic promastigotes by phlebotomine sand flies. **International Journal for Parasitology**, v. 37, n. 10, p. 1097-1106, 2007.

BATES, P.A.; ROGERS, M.E. New insights into the developmental biology and transmission mechanisms of *Leishmania*. **Current Molecular Medicine**, v. 4, n. 6, p. 601-609, 2004.

BENAVIDES, J.A.; RAGHAVAN, R.K.; BOERE, V. et al. Spatio-temporal dynamics of rabies and habitat suitability of the common marmoset *Callithrix jacchus* in Brazil. **PLoS Neglected Tropical Diseases**, v. 16, n. 3, p. e0010254, 2022.

BRANDÃO, E.M.V.; XAVIER, S.C.C.; ROCHA, F.L. et al. Wild and domestic canids and their interactions in the transmission cycles of *Trypanosoma cruzi* and *Leishmania* spp. in an area of the Brazilian Cerrado. **Pathogens**, v. 9, n. 10, p. 818, 2020.

BRASIL. Guia de vigilância de epizootias em primatas não humanos e entomologia aplicada à vigilância da febre amarela. 2 ed. Brasília : Ministério da Saúde, p. 50-60, 2017.

CANALE, G.R.; ALONSO, A.C.; MARTINS, W.P. et al. *Sapajus xanthosternos* (amended version of 2020 assessment). **The IUCN Red List of Threatened Species 2021** : e.T4074A192592138.

CARVALHO, T.F.; NEVES, G.G.; GOMES, P.P.R. et al. Adrenalitis associated with *Leishmania infantum* infection in a maned wolf (*Chrysocyon brachyurus*). **Brazilian Journal of Veterinary Pathology**, v. 8, n. 3, p. 88-94, 2015.

CARVALHO, T.P.; SANTOS, D.O.; OLIVEIRA, A.R. et al. Lethal acute diarrhea associated with *Clostridioides difficile* toxin A and B in a buffy-tufted-ear marmoset (*Callithrix aurita*). **Journal of Medical Primatology**, v. 51, n. 6, p. 400-403, 2022.

CASO, A.; OLIVEIRA, T. CARVAJAL, S.V. *Herpailurus yagouaroundi*. **The IUCN Red List of Threatened Species 2015** : e.T9948A50653167.

CAVALERA, M.A.; IATTA, R.; LARICCHIUTA, P. et al. Clinical, haematological and biochemical findings in tigers infected by *Leishmania infantum*. **BMC Veterinary Research**, v. 16, p. 1-7, 2020.

COIMBRA, D.P.; PENEDO, D.M.; SILVA, M.O. et al. Molecular and morphometric identification of *Trypanosoma* (*Megatrypanum*) *minasense* in blood samples of marmosets (*Callithrix*: Callithrichidae) from the city of Rio de Janeiro, Brazil. **Parasitology International**, v. 75, p. 101999, 2020.

COSTA, E.A.; LUPPI, M.M.; MALTA, M.C.C. et al. Outbreak of human herpesvirus type 1 infection in nonhuman primates (*Callithrix penincillata*). **Journal of Wildlife Diseases**, v. 47, n. 3, p. 690-693, 2011.

COSTA-VAL, A.P.; CAVALCANTI, R.R.; GONTIJO, N.F. et al. Canine visceral leishmaniasis: relationships between clinical status, humoral immune response, haematology and *Lutzomyia* (*Lutzomyia*) *longipalpis* infectivity. **The Veterinary Journal**, v. 174, n. 3, p. 636-643, 2007.

COURTENAY, O.; QUINNELL, R.J.; GARCEZ, L.M. et al. Low infectiousness of a wildlife host of *Leishmania infantum*: the crab-eating fox is not important for transmission. **Parasitology**, v. 125, n. 5, p. 407-414, 2002.

CUARÓN, A.D.; REID, F. GONZÁLEZ-MAYA, J.F. et al. *Galictis vittata*. **The IUCN Red List of Threatened Species 2016a** : e.T41640A45211961.

CUARÓN, A.D.; REID, F.; HELGEN, K. et al. *Eira barbara*. **The IUCN Red List of Threatened Species 2016b** : e.T41644A45212151.

CURI, N.H.A.; MIRANDA, I.; TALAMONI, S.A. Serologic evidence of *Leishmania* infection in free-ranging wild and domestic canids around a Brazilian National Park. **Memórias do Instituto Oswaldo Cruz**, v. 101, p. 99-101, 2006.

DAHROUG, M.A.A.; ALMEIDA, A.B.P.F.; SOUZA, V.R.F. et al. *Leishmania* (*Leishmania*) *chagasi* in captive wild felids in Brazil. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, v. 104, n. 1, p. 73-74, 2010.

DAHROUG, M.A.A.; ALMEIDA, A.B.P.F.; SOUZA, V.R.F. et al. The first case report of *Leishmania* (*leishmania*) *chagasi* in Panthera leo in Brazil. **Asian Pacific Journal of Tropical Biomedicine**, v. 1, n. 3, p. 249-250, 2011.

DE-LA-ROSA-ARANA, J.L.; MUÑOZ-GARCÍA, C.I.; GODÍNEZ-GARCÍA, V.H. et al. Serological survey of anti-*Salmonella* antibodies in coatis (*Nasua narica*) and raccoons (*Procyon lotor*) in southeast Mexico. **Austral Journal of Veterinary Sciences**, v. 48, n. 3, p. 283-288, 2016.

DEMATTEO, K.; MICHALSKI F.; LEITE-PITMAN, M.R.P. *Speothos venaticus*. **The IUCN Red List of Threatened Species 2011** : e.T20468A9203243.

DORNELES, E.M.S.; PELLEGRIN, A.O.; PÉRES, I.A.H.F.S. et al. Serology for brucellosis in free-ranging crab-eating foxes (*Cerdocyon thous*) and brown-nosed coatis (*Nasua nasua*) from Brazilian Pantanal. **Ciência Rural**, v. 44, p. 2193-2196, 2014.

DOSTÁLOVÁ, A.; VOLF, P. *Leishmania* development in sand flies: parasite-vector interactions overview. **Parasites & Vectors**, v. 5, p. 1-12, 2012.

EIDSON, M.; MILLER, J.; KRAMER, L. et al. Dead crow densities and human cases of West Nile virus, New York State, 2000. **Emerging Infectious Diseases**, v. 7, n. 4, p. 662, 2001.

EMMONS, L.; HELGEN, K. *Nasua nasua*. **The IUCN Red List of Threatened Species 2016:** e.T41684A45216227.

ESTEVAM, L.G.T.M.; FONSECA JUNIOR, A.A.; SILVESTRE, B.T. et al. Seven years of evaluation of ectoparasites and vector-borne pathogens among ring-tailed coatis in an urban park in southeastern Brazil. **Veterinary Parasitology: Regional Studies and Reports**, v. 21, p. 100442, 2020.

FAVORETTO, S.R.; ARAÚJO, D.B.; DUARTE, N.F. et al. Zika Virus in peridomestic neotropical primates, Northeast Brazil. **Ecohealth**, v. 16, p. 61-69, 2019.

FAVORETTO, S.R.; MATTOS, C.C.; MORAIS, N.B. et al. Rabies in marmosets (*Callithrix jacchus*), Ceará, Brazil. **Emerging Infectious Diseases**, v. 7, n. 6, p. 1062, 2001.

FIGUEIREDO, F.B.; GREMIÃO, I.D.F.; PEREIRA, S.A. et al. First report of natural infection of a bush dog (*Speothos venaticus*) with *Leishmania* (*Leishmania*) *chagasi* in Brazil. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, v. 102, n. 2, p. 200-201, 2008.

FRANK, D. One world, one health, one medicine. **The Canadian Veterinary Journal**, v. 49, n. 11, p. 1063, 2008.

GOMES, R.B.; MENDONÇA, I.L.; SILVA, V.C. et al. Antibodies against *Lutzomyia longipalpis* saliva in the fox *Cerdocyon thous* and the sylvatic cycle of Leishmania chagasi. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, v. 101, n. 2, p. 127-133, 2007.

GOODRICH, J.; WIBISONO, H.; MIQUELLE, D. et al.. *Panthera tigris*. **The IUCN Red List of Threatened Species 2022** : e.T15955A214862019.

GUIRALDI, L.M.; SANTOS, W.J.; MANZINI, S. et al. Identification of *Leishmania infantum* and *Leishmania braziliensis* in captive primates from a zoo in Brazil. **American Journal of Primatology**, v. 84, n. 4-5, p. e23376, 2022.

HATT, J.M.; GREST, P.; POSTHAUS, H. et al. Serologic survey in a colony of captive common marmosets (*Callithrix jacchus*) after infection with herpes simplex type 1–like virus. **Journal of Zoo and Wildlife Medicine**, v. 35, n. 3, p. 387-390, 2004.

HELGEN, K.; SCHIAFFINI, M. *Galictis cuja*. **The IUCN Red List of Threatened Species 2016** : e.T41639A45211832.

HEMETRIO, N.S. Levantamento populacional de quatis (PROCYONIDAE: *Nasua nasua*) no Parque das Mangabeiras, Belo Horizonte, MG. **Monografia. Universidade Federal de Minas Gerais**. 30p, 2007.

HEMETRIO, N.S. Levantamento populacional e manejo de Quatis (PROCYONIDAE: *Nasua nasua*) no Parque das Mangabeiras, Belo Horizonte, MG. **Dissertação. Universidade de Minas Gerais**. 106p, 2011

HERRERA, H.M.; ALESSI, A.C.; MARQUES, L.C. et al. Experimental *Trypanosoma evansi* infection in South American coati (*Nasua nasua*): hematological, biochemical and histopathological changes. **Acta Tropica**, v. 81, n. 3, p. 203-210, 2002.

HERRERA, H.M.; LISBOA, C.V.; PINHO, A.P. et al. The coati (*Nasua nasua*, Carnivora, Procyonidae) as a reservoir host for the main lineages of *Trypanosoma cruzi* in the Pantanal region, Brazil. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, v. 102, n. 11, p. 1133-1139, 2008.

JAVOROUSKI, M.L.; PASSERINO, A.S.M. **Carnivora – Mustelidae (Ariranha, Lontra e Irara)**. In: CUBAS, Z.S.; SILVA, J.C.R.; CATÃO-DIAS, J.L.; Tratado de Animais Selvagens. 2 ed. São Paulo : Roca, 2014.

JIMÉNEZ, M.; GONZÁLEZ, E.; MARTÍN-MARTÍN, I et al. Could wild rabbits (*Oryctolagus cuniculus*) be reservoirs for *Leishmania infantum* in the focus of Madrid, Spain?. **Veterinary Parasitology**, v. 202, n. 3-4, p. 296-300, 2014.

JONES, K.E.; PATEL, N.G.; LEVY, M.A. et al.. Global trends in emerging infectious diseases. **Nature**, v. 451, n. 7181, p. 990-993, 2008.

JORGE, R.S.P.; JORGE, M.L.S.P. **Carnivora – Canidae (Cachorro-do-mato, Cachorro-vinagre, Lobo-guará e Raposa-do-campo)**. In: CUBAS, Z.S.; SILVA, J.C.R.; CATÃO-DIAS, J.L.; Tratado de Animais Selvagens. 2 ed. São Paulo : Roca, 2014.

JUSI, M.M.G.; STARKE-BUZETTI, W.A.; OLIVEIRA, T.M.F.S. et al. Molecular and serological detection of *Leishmania* spp. in captive wild animals from Ilha Solteira, SP, Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 20, p. 219-222, 2011.

KEEL, M.K.; TERIO, K.A.; MCALOOSE, D. Canidae, Ursidae, and Ailuridae. In: TERIO, K.A.; MCALOOSE, D.; LEGER, J.St. **Pathology of Wildlife and Zoo Animals**. Elsevier, 2018. p. 229-261.

KILLICK-KENDRICK, R. The biology and control of phlebotomine sand flies. **Clinics in Dermatology**, v. 17, n. 3, p. 279-289, 1999.

KREBS, J.W.; WILLIAMS, S.M.; SMITH, J.S. et al. Rabies among infrequently reported mammalian carnivores in the United States, 1960–2000. **Journal of Wildlife Diseases**, v. 39, n. 2, p. 253-261, 2003.

LAINSON, R.; RANGEL, E.F. *Lutzomyia longipalpis* and the eco-epidemiology of American visceral leishmaniasis, with particular reference to Brazil: a review. **Memórias do Instituto Oswaldo Cruz**, v. 100, p. 811-827, 2005.

LAINSON, R.; WARD, R.D.; SHAW, J. *Leishmania* in phlebotomid sandflies: VI. Importance of hindgut development in distinguishing between parasites of the *Leishmania mexicana* and *L. braziliensis* complexes. **Proceedings of the Royal society of London. Series B. Biological Sciences**, v. 199, n. 1135, p. 309-320, 1977.

LANE, R. P. **Sandflies (Phlebotominae)**. In: Medical insects and arachnids. Dordrecht: Springer Netherlands, 1993. p. 78-119.

LANGONI, H.; KAWAGUCHI, M.F.; OSHIKA, J.C. et al.. *Leptospira* spp. antibodies in captive coatis (*Nasua nasua* Storr, 1780) (Carnivora: Procyonidae). **Journal of Venomous Animals and Toxins including Tropical Diseases**, v. 15, p. 762-767, 2009.

LEMOS, F.G.; AZEVEDO, F.C.; PAULA, R.C. et al. *Lycalopex vetulus*. **The IUCN Red List of Threatened Species** 2020 : e.T6926A87695615.

LIBERT, C.; RAVEL, C.; PRATLONG, F. et al.. *Leishmania infantum* infection in two captive barbary lions (*Panthera leo leo*). **Journal of Zoo and Wildlife Medicine**, v. 43, n. 3, p. 685-688, 2012.

LIMA, V.F.S.; RAMOS, R.A.N.; GIANELLI, A. et al. Zoonotic parasites in wild animals such as carnivores and primates that are traded illegally in Brazil. **Brazilian Journal of Veterinary Medicine**, v. 43, 2021.

LIMA, V.M.F.; FATTORI, K.R.; MICHELIN, A.F. et al. Evidence of *Leishmania* spp. antibodies and DNA in bush dogs (*Speothos venaticus*) in Brazil. **Journal of Zoo and Wildlife Medicine**, v. 40, n. 1, p. 91-94, 2009.

LIMA, V.M.F.; SANTIAGO, M.E.B.; SANCHES, L.C. et al. Molecular diagnosis of *Leishmania amazonensis* in a captive spider monkey in Bauru, São Paulo, Brazil. **Journal of Zoo and Wildlife Medicine**, v. 43, n. 4, p. 943-945, 2012.

LISBOA, C.V.; MANGIA, R.H.; LIMA, N.R.C. et al. Distinct patterns of *Trypanosoma cruzi* infection in *Leontopithecus rosalia* in distinct Atlantic coastal rainforest fragments in Rio de Janeiro–Brazil. **Parasitology**, v. 129, n. 6, p. 703-711, 2004.

LITVOC, M.N.; NOVAES, C.T.G.; LOPES, M.I.B.F. Yellow fever. **Revista da Associação Médica Brasileira**, v. 64, p. 106-113, 2018.

LOPES, K.F.C.; DELAI, R.M.; ZANIOLO, M.M. et al.. Urban capuchin monkeys *Sapajus nigritus* (Goldfuss, 1809) (Primates, Cebidae) as environmental bioindicators of leishmaniasis. **Transboundary and Emerging Diseases**, v. 69, n. 4, p. 2320-2325, 2022.

LUCHERINI, M. *Lycalopex gymnocercus*. **The IUCN Red List of Threatened Species 2016a** : e.T6928A85371194.

LUCHERINI, M.; EIZIRIK, E.; OLIVEIRA, T. et al. *Leopardus colocolo*. **The IUCN Red List of Threatened Species 2016b** : e.T15309A97204446.

LUDWIG, G.V.; CALLE, P.P.; MANGIAFICO, J.A. et al. An outbreak of West Nile virus in a New York City captive wildlife population. **The American Journal of Tropical Medicine and Hygiene**, v. 67, n. 1, p. 67-75, 2002.

LUM, F.M.; ZHANG, W.; LIM, K.C. et al. Multimodal assessments of Zika virus immune pathophysiological responses in marmosets. **Scientific Reports**, v. 8, n. 1, p. 17125, 2018.

LUPPI, M.M.; MALTA, M.C.C.; SILVA, T.M.A. et al.. Visceral leishmaniasis in captive wild canids in Brazil. **Veterinary Parasitology**, v. 155, n. 1-2, p. 146-151, 2008.

MACEDO, G.C.; BARRETO, W.T.G.; OLIVEIRA, C.E. et al.. *Leishmania infantum* infecting the carnivore *Nasua nasua* from urban forest fragments in an endemic area of visceral leishmaniasis in Brazilian Midwest. **Frontiers in Cellular and Infection Microbiology**, v. 12, p. 1050339, 2023.

MAIA, J.F.; GENNARI, S.M.; MILANELO, L. et al. Serological survey of Toxoplamosis in South American coatis (*Nasua nasua*) in Tietê Ecological Park, São Paulo, SP, Brazil. **Brazilian Journal of Veterinary Research and Animal Science**, v. 53, n. 3, p. 1-6, 2016.

MALTA, M.C.C.; TINOCO, H.P.; XAVIER, M.N. et al. Naturally acquired visceral leishmaniasis in non-human primates in Brazil. **Veterinary Parasitology**, v. 169, n. 1-2, p. 193-197, 2010.

MALUKIEWICZ, J.**;** BOERE, V.; OLIVEIRA, M.A.B. et al. An introduction to the *Callithrix* genus and overview of recent advances in marmoset research. **ILAR journal**, v. 61, n. 2-3, p. 110-138, 2020.

MANGAT, M.; PETERS, J.; GRIFFITH, D.E. C25 NON-TUBERCULOUS MYCOBACTERIA: FROM BENCH TO CLINIC: *Callithrix Jacchus*: A Non-Human Primate Model of *Mycobacterium Avium* Complex Pulmonary Infection? **American Journal of Respiratory and Critical Care Medicine**, v. 195, 2017.

MARQUES, A.; TELFORD III, S.R.; TURK, S.P. et al. Xenodiagnosis to detect *Borrelia burgdorferi* infection: a first-in-human study. **Clinical Infectious Diseases**, v. 58, n. 7, p. 937-945, 2014.

MARTÍNEZ, M.F.; KOWALEWSKI, M.M.; GIULIANI, M.G. et al. Molecular identification of *Leishmania* in free-ranging black and gold howler monkeys (*Alouatta caraya*) in northeastern Argentina. **Acta Tropica**, v. 210, p. 105534, 2020.

MÄTZ-RENSING, K.; LOWENSTINE, L.J. **New World and Old World Monkeys**. In: TERIO, K.A.; MCALOOSE, D.; LEGER, J.S. Pathology of Wildlife and Zoo Animals. Elsevier Inc : Cambridge. 2018.

MEISER, C.K.; SCHAUB, G.A. **Xenodiagnosis**. In: Nature Helps... How Plants and Other Organisms Contribute to Solve Health Problems. Berlin, Heidelberg: Springer Berlin Heidelberg, 2011. p. 273-299.

MELO F.R.; HILÁRIO, R.R.; FERRAZ, D.S. et al. *Callithrix flaviceps* (amended version of 2020 assessment). **The IUCN Red List of Threatened Species 2021** : e.T3571A191700879.

MIRANDA, G.H.B.; FARIA, D.S. Ecological aspects of black-pincelled marmoset (*Callithrix penicillata*) in the cerradão and dense cerrado of the Brazilian Central Plateau. **Brazilian Journal of Biology**, v. 61, p. 397-404, 2001.

MOL, J.P.S.; SOAVE, S.A.; TURCHETTI, A.P. et al. Transmissibility of *Leishmania infantum* from maned wolves (*Chrysocyon brachyurus*) and bush dogs (*Speothos venaticus*) to *Lutzomyia longipalpis*. **Veterinary Parasitology**, v. 212, n. 3-4, p. 86-91, 2015.

MOLINA, C.V.; CATÃO-DIAS, J.L.; FERREIRA NETO, J.S. et al. Sero-epidemiological survey for brucellosis, leptospirosis, and toxoplasmosis in free-ranging *Alouatta caraya* and *Callithrix penicillata* from São Paulo State, Brazil. **Journal of Medical Primatology**, v. 43, n. 3, p. 197-201, 2014.

MOLINA, R.; JIMÉNEZ, M.I.; CRUZ, I. et al. The hare (*Lepus granatensis*) as potential sylvatic reservoir of *Leishmania infantum* in Spain. **Veterinary Parasitology**, v. 190, n. 1-2, p. 268-271, 2012.

MOREIRA-SOTO, A.; CARNEIRO, I.D.O.; FISCHER, C. et al. Limited evidence for infection of urban and peri-urban nonhuman primates with Zika and chikungunya viruses in Brazil. **Msphere**, v. 3, n. 1, p. 10.1128/msphere. 00523-17, 2018.

MOURYA, D.T.; GOKHALE, M.D.; KUMAR, R. Xenodiagnosis: use of mosquitoes for the diagnosis of arboviral infections. **Journal of Vector Borne Diseases**, v. 44, n. 4, p. 233, 2007.

MOUTINHO, F.F.B.; ANDRADE, M.G.A.D.; NUNES, V.M.A. et al. Rabies in *Callithrix* sp. in the urban area of Niterói, Rio de Janeiro, Brazil. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 53, 2020.

MURAKAMI, P.S.; MONEGO, F.; HO, J.L. et al. An outbreak of tuberculosis by *Mycobacterium bovis* in coatis (*Nasua nasua*). **Journal of Zoo and Wildlife Medicine**, v. 43, n. 2, p. 338-341, 2012.

NICHOLSON, S.; BAUER. H.; STRAMPELLI, P. et al. *Panthera leo*. **The IUCN Red List of Threatened Species 2023** : e.T15951A231696234.

NIELSEN, C.; THOMPSON, D.; KELLY, M. et al. *Puma concolor*. **The IUCN Red List of Threatened Species 2015** : e.T18868A97216466.

OLIVEIRA, A.R.; CARVALHO, T.D.; ARENALES, A. et al. Mandibular squamous cell carcinoma in a captive Siberian tiger (*Panthera tigris altaica*). 2018. **Brazilian Journal of Veterinary Pathology,** v. 11, n. 3, p. 97-101, 2018.

OLIVEIRA, A.R.; PINHEIRO, G.R.G.; TINOCO, H.P. et al. Competence of non-human primates to transmit *Leishmania infantum* to the invertebrate vector *Lutzomyia longipalpis*. **PLoS Neglected Tropical Diseases**, v. 13, n. 4, p. e0007313, 2019.

OLIVEIRA, A.R.; RITTER, J.M.; SANTOS, D.O. et al. Pathology and epidemiology of fatal toxoplasmosis in free-ranging marmosets (*Callithrix* spp.) from the Brazilian atlantic forest. **PLOS Neglected Tropical Diseases**, v. 16, n. 9, p. e0010782, 2022.

OLIVEIRA, T.; PAVIOLO, A.; SCHIPPER, J. et al. *Leopardus wiedii*. **The IUCN Red List of Threatened Species 2015** : e.T11511A50654216.

OLIVEIRA, T.; TRIGO, T.; TORTATO, M. et al.. *Leopardus guttulus*. **The IUCN Red List of Threatened Species 2016** : e.T54010476A54010576.

OLIVEIRA-FILHO, E.F.; JÚNIOR, J.W.P.; SOUZA, M.M. et al. Serologic survey of brucellosis in captive neotropical wild carnivores in northeast Brazil. **Journal of Zoo and Wildlife Medicine**, v. 43, n. 2, p. 384-387, 2012.

OLSEN, S.C.; PALMER, M.V. Advancement of knowledge pf *Brucella* over the past 50 years. **Veterinary Pathology**, n. 51, v. 6, p. 1076-1089, 2014.

PADILHA, T.C.; ZITELLI, L.C.; WEBSTER, A. et al. Serosurvey of antibodies against zoonotic pathogens in free-ranging wild canids (*Cerdocyon thous* and *Lycalopex gymnocercus*) from Southern Brazil. **Comparative Immunology, Microbiology and Infectious Diseases**, v. 79, p. 101716, 2021.

PAIZ, L.M.; FORNAZARI, F.; MENOZZI, B.D. et al. Serological evidence of infection by *Leishmania* (*Leishmania*) *infantum* (Synonym: *Leishmania* (*Leishmania*) *chagasi*) in free-ranging wild mammals in a nonendemic region of the State of São Paulo, Brazil. **Vector-Borne and Zoonotic Diseases**, v. 15, n. 11, p. 667-673, 2015.

PAIZ, L.M.; MOTOIE, G.; RICHINI-PEREIRA, V.B. et al. Antibodies and molecular detection of *Leishmania* (*Leishmania*) *infantum* in samples of free-ranging marmosets (Primates: Callitrichidae: *Callithrix* spp.) in an area of canine visceral leishmaniasis in Southeastern Brazil. **Vector-Borne and Zoonotic Diseases**, v. 19, n. 4, p. 249-254, 2018.

PAIZ, L.M.; MOTOIE, G.; RICHINI-PEREIRA, V.B. et al. Antibodies and molecular detection of *Leishmania* (*Leishmania*) *infantum* in samples of free-ranging marmosets (Primates: Callitrichidae: *Callithrix* spp.) in an area of canine visceral leishmaniasis in Southeastern Brazil. **Vector-Borne and Zoonotic Diseases**, v. 19, n. 4, p. 249-254, 2018.

PAULA, N.F.; DUTRA, K.S.; OLIVEIRA, A.R. et al. Host range and susceptibility to *Toxoplasma gondii* infection in captive neotropical and Old-world primates. **Journal of Medical Primatology**, v. 49, n. 4, p. 202-210, 2020.

PAULA, R.C.; DEMATTEO, K. *Chrysocyon brachyurus*. **The IUCN Red List of Threatened Species 2015** : e.T4819A88135664.

PAVIOLO, A.; CRAWSHAW, P.; CASO, A. et al. *Leopardus pardalis*. **The IUCN Red List of Threatened Species 2015** : e.T11509A97212355.

PAYAN, E.; OLIVEIRA, T. *Leopardus tigrinus*. **The IUCN Red List of Threatened Species 2016** : e.T54012637A50653881.

PEREIRA, A.H.B.; VASCONCELOS, A.L.; SILVA, V.L. et al. Natural SARS-CoV-2 infection in a free-ranging black-tailed marmoset (*Mico melanurus*) from an urban area in mid-west Brazil. **Journal of Comparative Pathology**, v. 194, p. 22-27, 2022.

PEREIRA, J.; LUCHERINI, M; TRIGO, T. *Leopardus geoffroyi*. **The IUCN Red List of Threatened Species 2015** : e.T15310A50657011.

PORFIRIO, G.E.O.; SANTOS, F.M.; MACEDO, G.C. et al. Maintenance of *Trypanosoma cruzi*, *T. evansi* and *Leishmania* spp. by domestic dogs and wild mammals in a rural settlement in Brazil-Bolivian border. **International Journal for Parasitology: Parasites and Wildlife**, v. 7, n. 3, p. 398-404, 2018.

PORFIRIO, G.E.O.; SANTOS, F.M.; MACEDO, G.C. et al. Maintenance of *Trypanosoma cruzi*, *T. evansi* and *Leishmania* spp. by domestic dogs and wild mammals in a rural settlement in Brazil-Bolivian border. **International Journal for Parasitology: Parasites and Wildlife**, v. 7, n. 3, p. 398-404, 2018.

QUIGLEY, H.; FOSTER, R.; PETRACCA, L. et al.. *Panthera onca* (errata version published in 2018). **The IUCN Red List of Threatened Species 2018** : e.T15953A123791436.Reid et al., 2016

REID, F.; HELGEN, K. GONZÁLEZ-MAYA, J.F. *Procyon cancrivorus*. **The IUCN Red List of Threatened Species 2016** : e.T41685A45216426.

REIS, F.C.; MINUZZI-SOUZA, T.T.C.; NEIVA, M. et al. Trypanosomatid infections in captive wild mammals and potential vectors at the Brasilia Zoo, Federal District, Brazil. **Veterinary Medicine and Science**, v. 6, n. 2, p. 248-256, 2020.

RHEINGANTZ, M.L.; ROSAS-RIBEIRO, P.; GALLO-REYNOSO, J. et al. *Lontra longicaudis* (amended version of 2021 assessment). **The IUCN Red List of Threatened Species 2022** : e.T12304A219373698.

RIBEIRO, J.M.C. Role of saliva in blood-feeding by arthropods. **Annual Review of Entomology**, v. 32, n. 1, p. 463-478, 1987.

RICHINI-PEREIRA, V.B.; MARSON, P.M.; HAYASAKA, E.Y. et al. Molecular detection of *Leishmania* spp. in road-killed wild mammals in the Central Western area of the State of São Paulo, Brazil. **Journal of Venomous Animals and Toxins including Tropical Diseases**, v. 20, p. 01-07, 2014.

ROCHA, V.C.M.; CORRÊA, S.H.R.; SETZER, A.P. et al. *Mycobacterium kansasii* isolation from captive South American coati (*Nasua nasua*). **Journal of Zoo and Wildlife Medicine**, v. 44, n. 1, p. 167-168, 2013.

RODRIGUES, J.C.P.; PARRY, I.D.S.; LOPES, T.D.C.M. et al. Seroprevalence of arboviruses in *Nasua nasua* (Mammalia, Carnivora, Procyonidae) of synanthropic habitats in the Iguaçu National Park, Brazilian Atlantic Forest. **Ciência Rural**, v. 53, p. e20210713, 2022.

ROGERS, M. E.; ILG, T.; NIKOLAEV, A.V. et al. Transmission of cutaneous leishmaniasis by sand flies is enhanced by regurgitation of fPPG. **Nature**, v. 430, n. 6998, p. 463-467, 2004.

RUIZ-MIRANDA, C.R.; PISSINATTI, A.; KIERULFF, M.C.M. et al. *Leontopithecus rosalia* (amended version of 2019 assessment). **The IUCN Red List of Threatened Species 2021** : e.T11506A192327291.

RYLANDS, A. B.; MITTERMEIER, R. A.; SILVA JR, J. S. Neotropical primates: taxonomy and recently described species and subspecies. **International Zoo Yearbook**, v. 46, n. 1, p. 11-24, 2012.

SACCHETTO, L.; CHAVES, B.A.; COSTA, E.R. et al. Lack of Evidence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Spillover in Free-Living Neotropical Non-Human Primates, Brazil. **Viruses 2021**, v. 13, p. 1933, 2021.

SADLOVA, J.; SEBLOVA, V.; VOTYPKA, J. et al.. Xenodiagnosis of *Leishmania donovani* in BALB/c mice using *Phlebotomus orientalis*: a new laboratory model**. Parasites & Vectors**, v. 8, p. 1-8, 2015.

SANTANA, C.H.; OLIVEIRA, A.R.; SANTOS, D.O. et al. Genotyping of *Toxoplasma gondii* in a lethal toxoplasmosis outbreak affecting captive howler monkeys (*Alouatta* sp.). **Journal of Medical Primatology**, v. 50, n. 2, p. 99-107, 2021.

SANTANA, J.A.; ZANON, I.P.; FREITAS, R.J.S.R. et al. Distribution and antimicrobial resistance of staphylococci isolated from free-living south american coati (*Nasua nasua*) in an urban park in Minas Gerais, Brazil. **Journal of Zoo and Wildlife Medicine**, v. 54, n. 3, p. 578-583, 2023.

SANTOS, D.O.; OLIVEIRA, A.R.; LUCENA, F.P. et al. Histopathologic patterns and susceptibility of neotropical primates naturally infected with yellow fever virus. **Veterinary Pathology**, v. 57, n. 5, p. 681-686, 2020.

SANTOS, R.L.; OLIVEIRA, A.R. Leishmaniasis in non-human primates: Clinical and pathological manifestations and potential as reservoirs. **Journal of Medical Primatology**, v. 49, n. 1, p. 34-39, 2020.

SCHENONE, H. Xenodiagnosis. **Memórias do Instituto Oswaldo Cruz**, v. 94, p. 289-294, 1999.

SCHLABRITZ-LOUTSEVITCH, N.E.; WHATMORE, A.M.; QUANCE, C.R. et al. A novel *Brucella* isolate in association with two cases of stillbirth in non-human primates–first report. **Journal of Medical Primatology**, v. 38, n. 1, p. 70-73, 2009.

SHANEE, S.; ALVES, S.L.; CALOURO, A.M. et al. *Aotus nigriceps* (amended version of 2018 assesment). **The IUCN Red List of Threatened Species 2021** : e.T41542A190455194.

SILVA, E.S.; PIRMEZ, C.; GONTIJO, C.M.F. et al. Visceral leishmaniasis in the crab-eating fox. **Veterinary Record**, v. 147, p. 422, 2000.

SILVA, I.O. Socialidade e acesso a recursos alimentares por fêmeas de saguis (*Callithrix penicillata*) em grupos em ambiente natural. **Tese, Universidade de Brasília**, 2008.

SILVA, R.O.S.; ALMEIDA, L.R.; JUNIOR, C.A.O. et al. Carriage of *Clostridium difficile* in free-living South American coati (*Nasua nasua*) in Brazil. **Anaerobe**, v. 30, p. 99-101, 2014.

SILVA, S.M.; RABELO, F.P.B.; GONTIJO, N.F. et al. First report of infection of *Lutzomyia longipalpis* by *Leishmania* (*Leishmania*) *infantum* from a naturally infected cat of Brazil. **Veterinary Parasitology**, v. 174, n. 1-2, p. 150-154, 2010.

SOUZA, N.P.; ALMEIDA, A.B.P.F.; FREITAS, T.P.T. et al. *Leishmania* (*Leishmania*) *infantum chagasi* em canídeos silvestres mantidos em cativeiro, no Estado de Mato Grosso. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 43, p. 333-335, 2010.

STOFFELLA-DUTRA, A.G.; CAMPOS, B.H.; SILVA, P.B.H. et al. SARS-CoV-2 Spillback to Wild Coatis in Sylvatic–Urban Hotspot, Brazil. **Emerging Infectious Diseases**, v. 29, n. 3, p. 664, 2023.

TEIXEIRA, B**.;** HIRSCH, A.; GOULART, V.D.L.R. et al. Good neighbours: distribution of black-tufted marmoset (*Callithrix penicillata*) in an urban environment. **Wildlife Research**, v. 42, n. 7, p. 579-589, 2015.

TEIXEIRA, R.H.F.; AMBROSIO, S.R. **Carnivora – Procyonidae (Quati, Mão-pelada e Jupará)**. In: CUBAS, Z.S.; SILVA, J.C.R.; CATÃO-DIAS, J.L.; Tratado de Animais Selvagens. 2 ed. São Paulo : Roca, 2014.

TENÓRIO, M.S.; SOUSA, L.O.; PAIXÃO, M.S. et al. VISCERAL LEISHMANIASIS IN A CAPTIVE CRAB-EATING FOX (*Cerdocyon thous*). **Journal of Zoo and Wildlife Medicine**, v. 42, n. 4, p. 608-616, 2011.

TERZIAN, A.C.B.; ZINI, N.; SACCHETTO, L. et al. Evidence of natural Zika virus infection in neotropical non-human primates in Brazil. **Scientific Reports**, v. 8, n. 1, p. 1-15, 2018.

TINOCO, H.P.; COSTA, M.E.L.T.; PESSANHA, A.T. et al. Visceral leishmaniasis in an infant gorila (*Gorilla gorila gorilla*): clinical signs, diagnosis, and successful treatment with single-dose liposomal amphotericin B. **Journal of Medical Primatology**, v.47, n.6, p.1-3, 2018.

TOLENTINO, N.; PINHEIRO, G.R.G.; OTTINO, J. et al. Serological evidence of *Leishmania* infection by employing ELISA and rapid tests in captive felids and canids in Brazil. **Veterinary Parasitology: Regional Studies and Reports**, v. 17, p. 100308, 2019.

TRAVI, B.L.; OSORIO, Y.; GUARIN, N. et al. *Leishmania* (*Leishmania*) *chagasi*: clinical and parasitological observations in experimentally infected *Didelphis marsupialis*, reservoir of New World visceral leishmaniasis. **Experimental Parasitology**, v. 88, n. 1, p. 73-75, 1998.

TRIGO, T.C.; SCHNEIDER, A.; DE OLIVEIRA,T.G. et al. Molecular data reveal complex hybridization and a cryptic species of Neotropical wild cat. **Current Biology**, v. 23, n. 24, p. 2528–2533, 2013.

TRÜEB, I.; PORTELA, R.D.; FRANKE, C.R. et al. *Trypanosoma cruzi* and *Leishmania* sp. infection in wildlife from urban rainforest fragments in northeast Brazil. **Journal of Wildlife Diseases**, v. 54, n. 1, p. 76-84, 2018.

UTTARA, S.; BHUVANDAS, N.; AGGARWAL, V. Impacts of urbanization on environment. **International Journal of Research in Engineering and Applied Sciences**, v. 2, n. 2, p. 1637-1645, 2012.

VENIAL, H.J.; MONTOYA, A.; CHECA, R. et al. Investigation of *Leishmania* (*Viannia*) *braziliensis* infection in wild mammals in Brazil. **Acta Parasitologica**, p. 1-10, 2022.

VERONA, C.E.; PISSINATTI, A. **Primates – Primatas do Novo Mundo (Sagui, Macaco-prego, Macaco-aranha, Bugio e Muriqui)**. In: CUBAS, Z.S.; SILVA, J.C.R.; CATÃO-DIAS, J.L.; Tratado de Animais Selvagens. 2 ed. São Paulo : Roca, 2014.

VIA, L.E.; WEINER, D.M.; SCHIMEL, D. et al. Differential virulence and disease progression following *Mycobacterium tuberculosis* complex infection of the common marmoset (*Callithrix jacchus*). **Infection and Immunity**, v. 81, n. 8, p. 2909-2919, 2013.

VIEIRA, A.S.; NARDUCHE, L.; MARTINS, G. et al. Detection of wild animals as carriers of Leptospira by PCR in the Pantanal biome, Brazil. **Acta Tropica**, v. 163, p. 87-89, 2016.

VOLTARELLI, E.M.; ARRAES, S.M.A.A.; PERLES, T.F. et al. Serological survey for *Leishmania* sp. infection in wild animals from the municipality of Maringá, Paraná State, Brazil. **Journal of Venomous Animals and Toxins including Tropical Diseases**, v. 15, p. 732-744, 2009.

WHATMORE, A.M.; DAVISON, N.; CLOECKAERT, A. et al. *Brucella papionis* sp. nov., isolated from baboons (*Papio* spp.). **International Journal of Systematic and Evolutionary Microbiology**, v. 64, n. Pt_12, p. 4120-4128, 2014.

WHITNEY, R.A. Taxonomy. In: BENNETT, B.; ABEE, C.; HENRICKSON, R. Nonhuman primates in biomedical research. **Biology and Management**. Elsevier: Missouri, 1995.

WHO Expert Committee on the control of leishmaniasis. **WHO Technical Report Series**, no. 949; 2010.

WILSON, T.M.; RITTER, J.M.; MARTINES, R.B. et al. Fatal Human alphaherpesvirus 1 infection in free-ranging black-tufted marmosets in anthropized environments, Brazil, 2012–2019. **Emerging Infectious Diseases**, v. 28, n. 4, p. 802, 2022.

WILSON, T.M.; RITTER, J.M.; MARTINES, R.B. et al. Pathology and One Health implications of fatal *Leptospira interrogans* infection in an urbanized, free-ranging, black-tufted marmoset (*Callithrix penicillata*) in Brazil. **Transboundary and Emerging Diseases**, v. 68, n. 6, p. 3207-3216, 2021.

CHAPTER 1

Leishmaniosis in free-ranging South American coatis (*Nasua nasua*) in an endemic urban area: serological response to *Leishmania infantum* and host-vector interactions.

Abstract

Visceral leishmaniosis is an important zoonotic disease caused by *Leishmania infantum* that affects several mammals, including humans and domestic dogs. Wild animals can act as hosts to *L. infantum*. A population of free-ranging South American coatis (*Nasua nasua*) inhabiting an urban endemic area was evaluated in this study. Serum samples were tested by two serological methods (immunochromoatographic rapid test and ELISA). Animals were considered seroreactive if at least one serological test was reactive. Frequency of serorectivity to *L. infantum* was 29.72% (44/148). Five coatis captured multiple times became non-reactive after having a first reactive serological results. Both *L. infantum* reactive and non-reactive individuals shown the same exposure to *Lutzomyia longipalpis* saliva and were both able to transmit *L. infantum* to sand flies.

Keywords: xenodiagnosis, seroreversion, procyonid, *Lutzomyia longipalpis*.

1. INTRODUCTION

Leishmaniosis is a zoonotic disease caused by protozoa of the genus *Leishmania*. (WHO, 2010). Transmission occurs via sand flies belonging to the genera *Phlebotomus* and *Lutzomyia*. (Lainson & Rangel, 2005). In the New World, visceral leishmaniosis affects humans and domestic dogs and is associated with *Leishmania infantum*, which is mainly transmitted by *Lutzomyia longipalpis* (Brindha et al., 2021; Morales-Yuste et al., 2022).

One of the criteria to consider an animal as a reservoir is that the suspected host species must maintain enough parasites in the skin to transmit to sand flies, and domestic dogs are the most important reservoirs in urban environments (Giunchetti et al., 2006; Diniz et al., 2008; WHO, 2010). Wild animals may also be potential reservoirs, including marsupials, rodents, canids, primates and bats (Roque & Jansen, 2014).

South American coatis (*Nasua nasua*) are wild carnivores highly adapted to the peri-urban environment (Barreto et al., 2021). Infection by zoonotic pathogens have been reported in coatis, including *Clostridoides difficile*, coronavirus (SARS-CoV-2) and leishmaniosis (Silva et al., 2014; Paiz et a, 2015; Porfirio et al., 2018; Macedo et al., 2023; Stoffella-Dutra et al., 2023). Coatis have been shown to develop antibodies against *L. infantum* and DNA of *L. infantum* was found in blood and bone marrow samples, however there is no information regarding their potential as reservoirs (Paiz et al., 2015; Porfirio et al., 2018; Macedo et al., 2023). The goal in this chapter was to assess anti-*Leishmania* sp. antibodies in free-ranging coatis from peri-urban areas of Belo Horizonte (an endemic area for visceral leishmaniosis) as well as to evaluate through xenodiagnosis the capacity of these animals to transmit *Leishmania* to sand flies.

2. MATERIAL AND METHODS

2.1. Ethics

All procedures were approved by the Ethics Committee on the Use of Animals of the Universidade Federal de Minas Gerais (CEUA/UFMG) under protocol numbers 100/2021 and 80/2022, by the Instituto Chico Mendes de Conservação de Biodiversidade under protocol numbers 75831 and 81107, by Fundação de Parques Municipais e Zoobotânica de Belo Horizonte (FPMZ-BH) under protocol numbers FU002/2021 and FU004/2022, and Sisgen under protocols ABB00DB and ACDCB02.

2.2. Animal procedures

Coatis were captured in three parks in the city of Belo Horizonte, Brazil (PM – *Parque das Mangabeiras*, SC – *Parque da Serra do Curral*, and AP – *Parque Aggeo Pio*) (Figure 1.1), using Tomahawk traps distributed in areas where the animals were known to circulate. Captures happened in PM from 2021 to 2023, two times per year except for 2021, when captures happened only on the second semester. In SC captures happened from the second semester of 2022 to 2023. AP was only included as place of capture on the second semester of 2023.

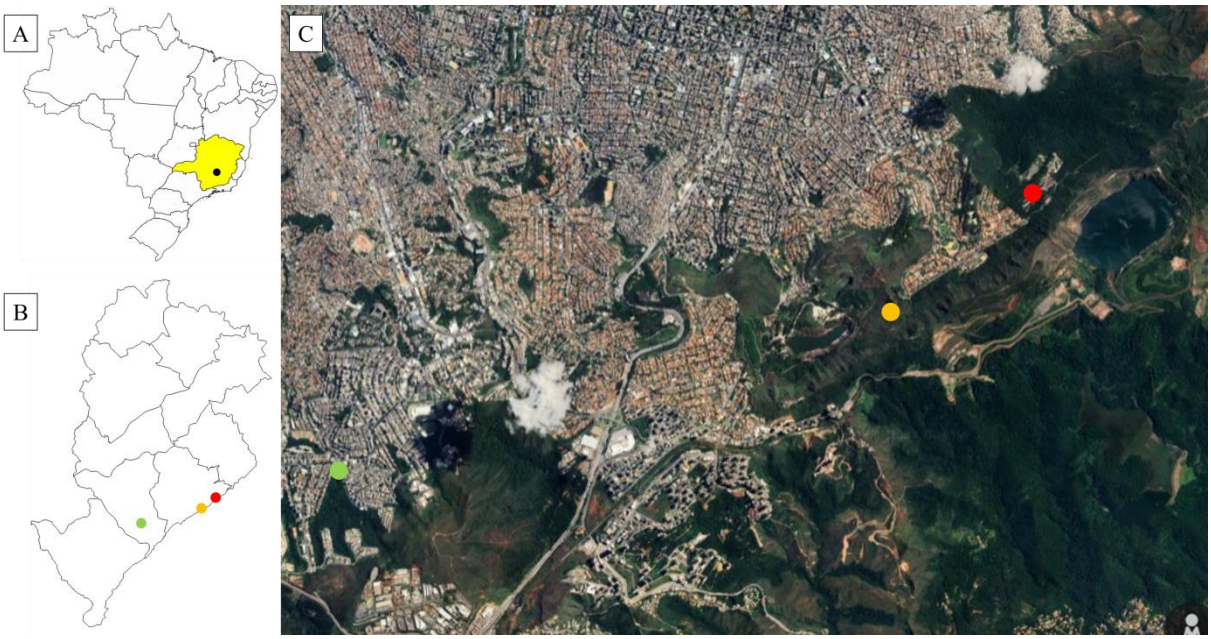

Figure 1.1. Geographic location of the areas of capture in Belo Horizonte (Minas Gerais, Brazil). A. Brazil map highlighting Minas Gerais state (yellow) and the city of Belo Horizonte (black dot). B. Belo Horizonte map with different colored dots marking the location of the parks. C. Green: *Parque Aggeo Pio* (AP); Orange: *Parque da Serra do Curral* (SC); Red: *Parque das Mangabeiras* (PM);

Traps were baited with bananas and checked twice a day during the capture period. Captured animals were sedated with ketamine (IM, 8 mg/kg) and midazolam (IM, 1 mg/kg) or tiletamine with zolazepam (Zoletil, IM, 7 mg/kg). After sedation, animals were clinically evaluated, and sex and age were recorded. Clinical evaluation consisted of external exam, body condition, superficial lymph nodes palpation, abdominal palpation, respiratory frequency, heartbeats frequency and rectal temperature. Age was estimated based on weight, teeth condition, and reproductive condition (Gompper, 1995; Conforti et al., 2017). Infants, estimate

to be up to six months old, have medium weight of 1.5 Kg and still have deciduous teeth. Young individuals have estimated age between six and 12 months, medium weight of 2 Kg and permanent tooth started to erupt. Subadults were estimated to be 13 to 24 months-old, have medium weight of 2 to 3 Kg. Adults, over 24 months of age, weight more than 3 kg and had permanent dentition. Males were considered adults if testicles were inside the scrotum, and females based on mammary gland development and/or if they show signs of gestation (current or previous).

Blood was sampled from the jugular vein and placed in tubes containing clot activators for serum separation. Serum was stored at -20°C until serological evaluation. All animals were tagged with subcutaneous microchip and numbered ear tag for later identification. After total recovery from sedation, animals were released near the same place of capture inside the parks.

2.3. Serologic tests

rKDDR Immunochromatographic rapid test assay: serum samples were used for the rKDDR Immunochromatographic assay (Rapid test, Safetest Diagnósticos, Brazil) according to manufacturer's instructions. Briefly, 20 µL of serum was added, followed by 40 µL of PBS buffer. Results were read after 20 minutes of incubation at room temperature, being considered positive if two red marks were visualized (Figure 1.2).

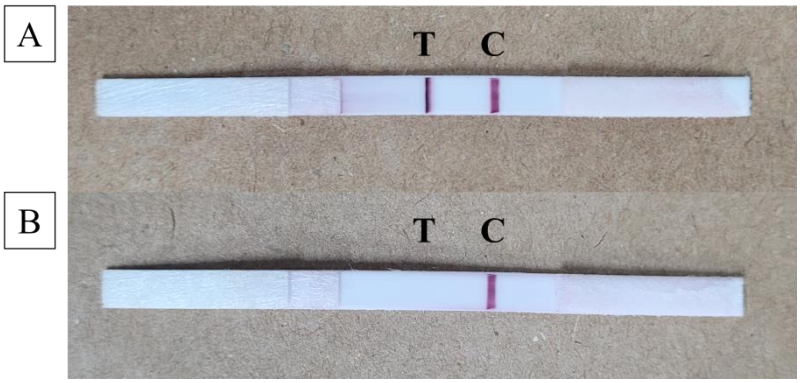

Figure 1.2. Reactive (A) and non-reacitve (B) results of rKDDR immunochromatographic rapid test assay. T indicates sample results and C indicates test internal control. A. Reactive test showing two red marks. B. Non-reactive test showing only internal control reaction.

ELISA anti-*Leishmania* (rKDDR): ELISA was performed as described in Oliveira et al. (2019) with adaptations. 96-well-plates (Costar, Cornig, USA) coated with 50 ng/well of

rKDDR antigen diluted in carbonate buffer overnight at 4°C. Then, wells were blocked with a solution of PBS with 5% BSA for 2 hours at 37°C. Serum samples were diluted in 2.5% PBS-BSA at 1:100 proportion, added to the wells and incubated for 12 hours at 4°C. Racoon anti-IgG (Bethyl Laboratories, USA) was diluted in 2.5% PBS-BSA at 1:70000 (Macedo et al., 2023). Added to each well for 1 hour and 30 minutes at 37°C. Reaction was revealed with a solution of 0.05% o-phenylenediamine (OPD), 0.1% hydrogen peroxide in citrate buffer. Reaction was stopped after 10 minutes with 4 M sulfuric acid and optical densities (O.D.) were measured at 490 nm with an ELISA reader (BioRad 550, Brazil). Between each step, plates were washed five times with 0.05% PBS-Tween. Pools of coati serum samples previously tested with rKDDR rapid test were used as positive and negative controls. Also, eight individual samples of coatis with at least two negative rapid test results in different times of capture were used to calculate the cut off which was stablished at three standard deviations above average O.D. of the eight samples. All serum samples were tested at the same moment in duplicates.

ELISA anti-*Lutzomyia longipalpis* saliva: salivary glands were dissected from *Lu. longipalpis* raised at laboratory conditions and stored in PBS at -80°C. For saliva collection, salivary glands were disrupted by ultrasonication (10 rounds of 1 min with amplitude of 20) and centrifuged at 3200 xg for 2 min. Supernatant was used as antigen at a proportion of five pairs of gland/mL as described previously by Barral et al. (2000). Protein concentration of the antigen was determined using Pierce BCA Protein Assay Kit (Thermo Scientific, USA) resulting in a final concentration of 470 ng/well. 96-well-plates were coated with the salivary gland preparation overnight at 4°C, followed by blocking with 5% PBS-BSA for 1 hour at 37°C. Serum samples were diluted in 2.5% PBS-BSA at 1:100 proportion, added to the wells and incubated for 1 hour at 37°C. Racoon anti-IgG (Bethyl Laboratories, USA) was diluted in 2.5% PBS-BSA at 1:70000 and added to each well for 1 hour at 37°C. Reaction was revealed with a solution of 0.05% o-phenylenediamine (OPD), 0.1% hydrogen peroxide in citrate buffer. Reaction was stopped after 10 minutes with 4 M sulfuric acid and optical densities (O.D.) were measured at 490 nm with an ELISA reader (BioRad 550, Brazil). Between each step, plates were washed five times with 0.05% PBS-Tween. No positive or negative controls were used for this experiment, so no cut off was established. All serum samples were tested at the same moment in duplicates.

2.4. Xenodiagnosis

Xenodiagnosis was performed using four-day-old female *Lu. longipalpis* sand flies raised in laboratory conditions and free of *Leishmania* sp. Fifty sand flies were placed in a

FleboContainer as previously described (Mol et al., 2015; Oliveira et al., 2019). Sedated coatis were exposed to sand flies on the axillary skin (Figure 1.3) for 30 minutes as described by Scorza et al. (2021). After feeding, the sand flies were kept for two days and fed with 15% sucrose solution. Sand flies were euthanized by freezing and ingurgitated females were separated in induvial microtubes and stored at -20°C until analysis.

Figure 1.3. Xenodiagnosis in free-ranging South American coatis (*Nasua nasua*). Adult female coati, sedated and exposed to *Lutzomyia longipalpis* on the skin of the axillary region.

DNA extraction: DNA extraction was performed as previously described (Oliveira et al.., 2019). Ten ingurgitated female sand flies were selected from each individual *N. nasua* for analysis. Sand flies were macerated in a 1.5 mL microtube with 50 µL of lysis buffer (0.08 M sodium chloride, 0.16 M sucrose, 0.06 M EDTA, 0.5% SDS, 0.1 M Tris-Cl, pH 8.6) followed by incubation at 65°C for 30 minutes. Then, 7.1 µL of 8 M potassium acetate was added and vortex homogenized followed by incubation for 30 minutes at 4°C. Homogenate was then centrifuged at 13000 xg for 10 minutes and supernatant was transferred to another microtube, added 100 µL of 95% ethanol and centrifuged at 13000 xg for 10 minutes. Supernatant was discarded and the pellet was washed with 100 µL of 70% ethanol and centrifuged at 13000xg for 10 minutes. Supernatant was discarded and after complete drying of the residual ethanol the pellet was resuspended in 20 µL of ultrapure water. DNA concentration was determined with

NanoDropTM One/OneC Microvolume UV-Vis Spectrophotometer (ThermoFischer, USA) and extracted samples were stored at -20°C until analysis.

Real Time PCR (qPCR): identification of *L. infantum* in sand flies was performed through Real Tima PCR (qPCR) as described by Oliveira et al. (2019). Two sets of primers were used: one targeting *Leishmania* sp. minicircle kinetoplast DNA (kDNA) specific for the *donovani* complex – forward 5'-CTTTTCTGGTCCCGCGGGTAGG-3' and reverse 5'-CCACCTGGCCTATTTTACACCA-3'. qPCR reaction was performed in a final volume of 10 µL – 5 µL of 1x SYBR Green PCR master mix (Thermo Fisher Scientific, Applied Biosystems, USA), 0.2 µM od each primer and 10 ng of DNA. Reaction was performed in StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, USA) with the following parameters: initial denaturation as 95°C for 10 minutes, 40 cycles of denaturation as 95°C for 15 seconds, annealing and extension at 60°C for 1 minute. DNA extracted from *L. infantum* culture was used as positive control and for negative control DNA template was substituted by water.

Sand flies were evaluated as pools. From each coati two pools were tested, each one containing DNA from 5 individual sand fly. Animals were considered positive if at least one of the pools had amplification.

2.5. Statistical analysis

GraphPad Prism software (version 8.0.1) was used to analyze data. Frequencies were evaluated by Chi-square test. Kappa Coefficient was used to evaluate concordance between serological tests. Mann-Whitney test was used to compare O.D. values.

3. RESULTS

A total of 148 coatis were captured in three parks, with 32 of them being recaptured in at least one other period of capture, resulting in 192 sampling procedures. Table 1.1. details data from the captures by period and by location.

Table 1.1. Data from free-ranging coatis (*Nasua nasua*) captured in urban parks of Belo Horizonte (MG, Brazil).

		Sex			Age					
Period	Location	Male	Female	Total	Infant	Young	Subadult	Adult	Total	Recap.
2021.1	PM	10	15	25	11	3	4	7	25	0
2021.2	PM	14	22	36	0	7	7	22	36	11
2022.2	PM	21	36	57	0	27	0	30	57	9
	SC	1	0	1	0	1	0	0	1	0
	Total	22	37	58	0	28	0	30	58	9
2023.1	PM	10	15	25	8	3	5	9	25	8
	SC	2	8	10	0	0	5	5	10	1
	Total	12	23	35	8	3	10	14	35	9
2023.2	PM	12	10	22	0	7	1	14	22	10
	SC	3	0	3	1	0	0	2	3	1
	AP	5	8	13	0	2	4	7	13	1
	Total	20	18	38	1	9	5	23	38	12

PM: *Parque das Mangabeiras*; SC: *Parque da Serra do Curral*; AP: *Parque Aggeo Pio*; Recap.: number of recaptures;

3.1. Anti-*Leishmania infantum* serology

To evaluate exposure to *L. infantum*, a total of 192 serum samples from 148 coatis were submitted to serological tests. All samples were submitted to rKDDR immunochromatographic rapid test (Safetest Diagnóstico, Brazil) and 20.31% (39/192) were considered seroreactive. Since six serum samples were insufficient for further analysis, for ELISA assays 186 samples were tested with 25.26% (47/186) of seroreactive samples for anti-*L. infantum* IgG antibodies. Kappa coefficient test resulted in a value of 0.786, a strong agreement between tests. Table 1.2 shows the number of serum samples that were reactive and non-reactive in both serological tests.

Table 1.2. Serological anti-*Leishmania infantum* assays using free-ranging South American coatis (*Nasua nasua*).

		ELISA		
		Reactive	Non-reactive	Total
Immunochromatographic rapid test	Reactive	35	2	37
	Non-reactive	12	137	149
	Total	47	139	186

Individuals were considered seroreactive to *L. infantum* if reactive in at least one of the serological tests employed. Since no animal captured in AP had seroreactvity for *L. infantum* (0/13), both PM and SC frequencies were higher, 27.3% (45/165) and 45.9% (6/14) (Chi square test, p = 0.0294 and 0.0074 respectively) (Figure 1.4A). No difference between PM and SC frequencies was found (Chi square test, p = 0.3779). To compare the frequencies between different moments of capture we used the results from animals captured at PM and SC. Animals captured in AP were excluded due to lack of seroreactivity. Frequency of seroreactivity was lower in the last period of captures (2023.2) with 12% (3/25) of seroreactive animals than the frequency of 2022.2 (39.7%, 23/58) (Figure 1.4B).

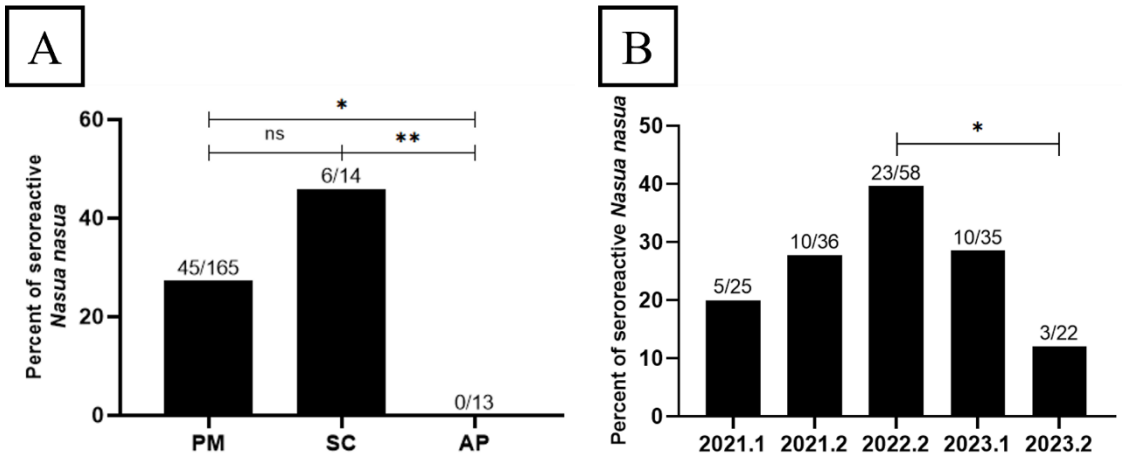

Figure 1.4. Frequency of anti-*Leishmania infantum* seroreactive free-ranging *Nasua nasua* by site (PM – *Parque das Mangabeiras*, SC – *Parque da Serra do Curral*, and AP – *Parque Aggeo Pio*) and period of sampling (2021.1, 2021.2, 2022.2, 2023.1, 2023.2). A and B. Seroreactivity was based on rKDDR immunochromatographic rapid test (Safetest Diagnóstico, Brazil) and rKDDR ELISA. Statistical difference was based on Chi-square test. B. For this analysis samples obtained from *N. nasua* captured in AP were excluded.

Overall frequencies of seroreactivity were analyzed considering as reactive an individual that presented reactivity in one of the serological tests at any moment. Frequency of seroreactivity to *L. infantum* in coatis was 29.72% (44/148), with 34.09% (15/44) of males and 65.91% (29/44) of females (Figure 1.5A). No difference was observed between male and female seroreactive samples (Mann-Whitnney test, p = 0.1141) (Figure 1.5A). Since recaptured coatis presented different age categories over 3 years, for age comparison we included all samples for

frequency analysis, including recaptured animals. Adult coatis had higher frequency of *L. infantum* seroreactivity than young and infants (Figure 1.5B).

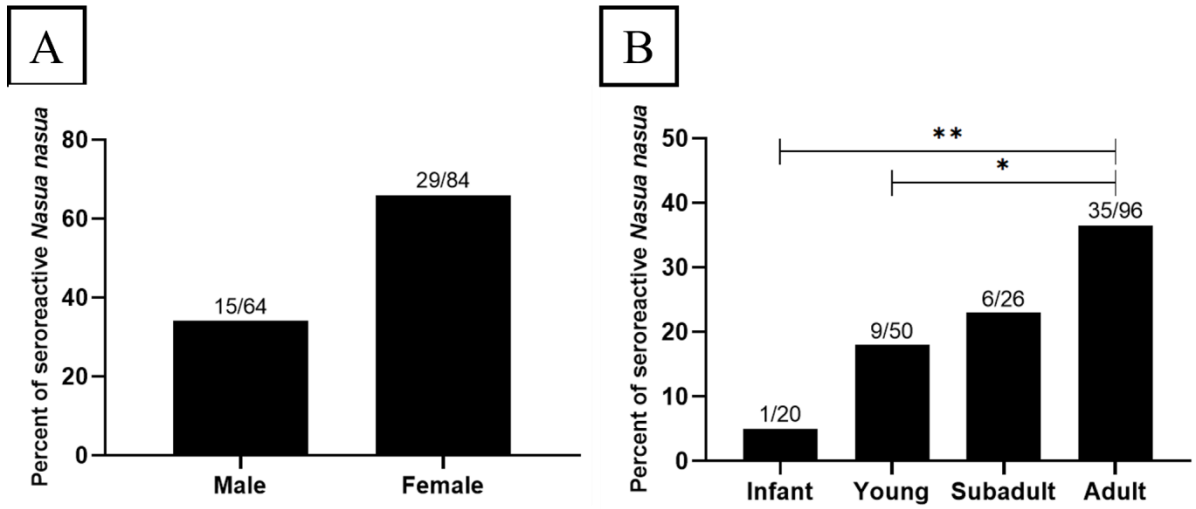

Figure 1.5. Frequency of *Leishmania infantum* seroreactive free-ranging *Nasua nasua* by sex (A) and estimated age (infant, young, subadult, adult) (B). Seroreactivity was based on rKDDR immunochromatographic rapid test (Safetest Diagnóstico, Brazil) and rKDDR ELISA. A. No statitistical diferrence was found based on Chi-square test (p = 0.1438). B. Statitistical diferrence was based on Chi-square test.

Thirty-four coatis were captured more the one time, allowing the evaluation of antibody response persistence over the time course of this study. Seven animals were captured three times, and the others were captured twice. Four animals (11.8%) were captured twice and were seroreactive both times. Five animals (14.7%) had a first serum sample non-reactive and then in following captures were seroreactive (Figure 6A). One of them maintained seroreactivity for at least one year. Interestingly, five individuals (14.7%) presented seroreactivity for the first sample, however for the next captures serum samples were non-reactive (Figure 1.6B). For two of these animals, negative serological response persisted at least for one year.

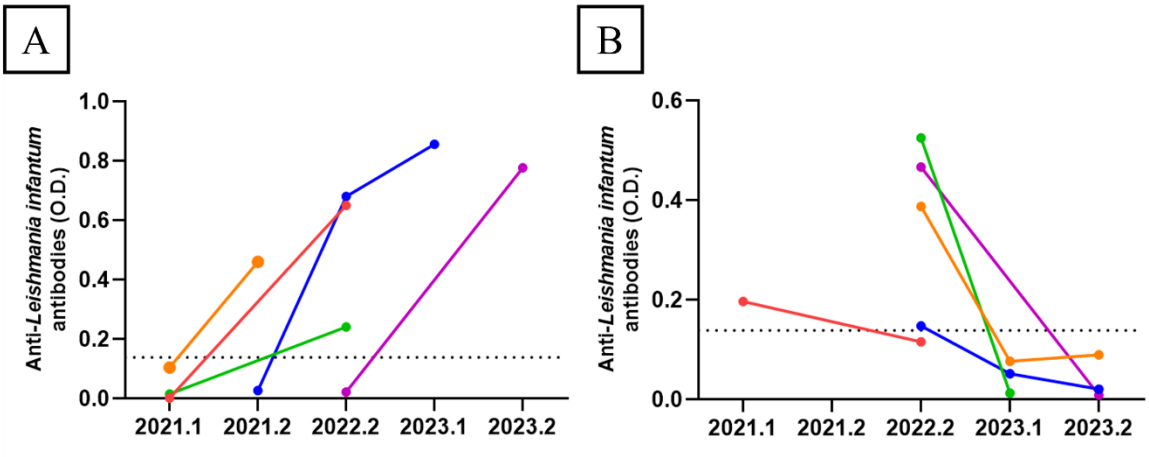

Figure 1.6. Anti-*Leishmania infantum* antibodies in free-ranging *Nasua nasua* captured multiple times with seroconvertion (A) or seroreversion (B). Each point represents optic density (O.D.) of a serum sample submitted to rKDDR ELISA. Different animals are identified with a different symbol/color and their samples are connected by lines. Dashed line represents the cut-off value (0.1385).

3.2. Anti-*Lutzomyia longipalpis* saliva serology

To evaluate exposure to *Lu. longipalpis* saliva, 187 serum samples were tested by ELISA using *Lu. longipalpis* saliva extract as antigen. No difference was found between males and females, however infants showed lower O.D. values than young ($p = 0.0046$), subadults ($p = 0.0008$) and adults ($p = 0.0007$) and the young ones were lower than subadults ($p = 0.035$) (Figure 1.7A). These results indicate that exposure increases with age. No difference of O.D. was found between *L. infantum* reactive or non-reactive samples (Figure 1.7B).

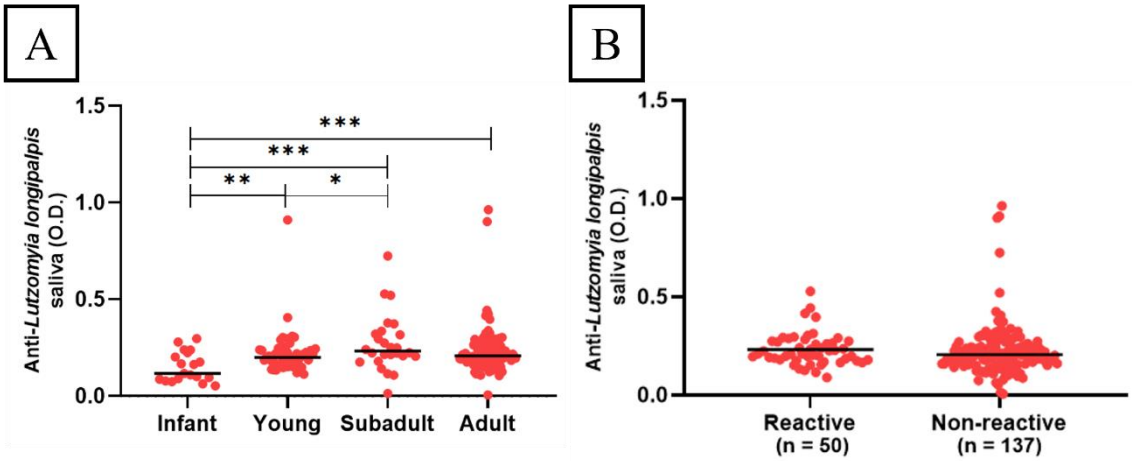

Figure 1.7. Anti-*Lutzomyia longipalpis* saliva IgG antibodies in free-ranging *Nasua nasua* evaluated by estimated age (A) and by anti-*Leishmania infantum* serological status (B). A and B. Each dot represents

a different serum sample submitted to ELISA. Optical densities (O.D.) values were compared with Mann-Whitney test.

3.3.Xenodiagnosis

Twenty-four coatis were exposed to *Lu. longipalpis* to evaluate their ability to transmit *L. infantum* to the vector (Table 1.3), 41.66% (10/24) of them were considered seroreactive by the serological tests employed. Seven (29.16%) coatis were capable of transmit *L. infantum* to the vector, interestingly two of them were non-reactive on serological tests. From the ten seroreactive individuals, five were not capable of transmission. However, there was no difference in the frequency of xenodiagnoses positivity between reactive or non-reactive animals (Chi square test, p = 0.0577). There was no difference between sex or estimated age.

Table 1.3. Estimated age, sex, serological status and xenodiagnosis results from free-ranging *Nasua nasua* exposed to *Lutzomyia longipalpis*.

ID	Estimated age	Sex	Serology	Xenodiagnosis	Ct
N015	Adult	F	R	Negative	-
N022	Adult	F	NR	Negative	-
N045	Adult	M	NR	Negative	-
N058	Adult	F	NR	Negative	-
N061	Adult	F	R	Negative	-
N067	Young	F	NR	Negative	-
N071	Adult	F	R	**Positive**	**32,64**
N072	Young	F	NR	Negative	-
N074	Young	F	NR	Negative	-
N076	Young	F	R	**Positive**	**30,2**
N077	Adult	M	R	**Positive**	**31,65**
N083	Young	F	NR	Negative	-
N085	Adult	M	R	Negative	-
N087	Adult	M	R	**Positive**	**27,87**
N090	Young	F	NR	Negative	-
N091	Young	M	NR	Negative	-
N093	Adult	F	R	Negative	-
N094	Young	F	NR	**Positive**	**32,41**
N095	Young	M	NR	**Positive**	**32,04**
N100	Young	F	NR	Negative	-
N103	Subadult	M	NR	Negative	-

N104	Adult	M	NR	Negative	-
N125	Subadult	M	R	**Positive**	**27,31**
N126	Subadult	F	R	Negative	-

F: female; M: male; R: reactive; NR: non-reactive;

4. Discussion

This study described the sero-epidemiology of *Leishmania infantum* in a free-ranging population of coatis (*Nasua nasua*) in an urban area demonstrating the seroreversion phenomenon and providing insights on their interaction with the vector, *Lutzomyia longipalpis.* Coatis showed antibodies against *L. infantum* by two different methods, rKDDR rapid test and ELISA, with strong concordance according to Kappa coefficient.

Frequency of coatis seroreactive to *L. infantum* was 29.7%, similar to previous results in free-ranging coatis that reported 30% to 44% of seroreactive individuals in endemic areas (Porfirio et al.., 2018; Macedo et al.., 2023). However, other authors found lower frequencies of seroreactive animals (4.6%) also evaluating samples from free-ranging coatis (Paiz et al.., 2015). Serological studies with other procyonids are rare. Studies evaluating crab-eating racoons (*P. cancrivorus*) used few serum samples (Voltarelli et al.., 2009; Jusi et al., 2011; Paiz et al.., 2015) and only Voltarelli et al. (2009) reported one seroreactive individual.

More serological studies evaluated other free-ranging carnivore species, mainly canids (Courteney et al.., 2002; Curi et al.., 2006; Lima et al., 2009; Voltarelli et al., 2009; Paiz et al., 2015; Almeida et al., 2018; Porfirio et al., 2018; Brandão et al., 2020; Padilha et al., 2021). Studies that evaluated higher numbers of individuals reported 3.8% to 78.4% of seroreactive free-ranging crab-eating foxes (*C. thous*) and 4.7% of hoary foxes (*L. vetulus*) (Courteneay et al., 2002; Brandão et al., 2020). Other canids, such as bush dogs (*S. venaticus*) and maned wolves (*C. brachyurus*), have been shown to develop antibodies against *L. infantum* (Curi et al., 2006; Lima et al., 2009; Brandão et al., 2020).

Frequency of visceral leishmaniosis in domestic dogs from endemic regions varies from 16.08 to 64.6% (Silva et al., 2001; Evaristo et al., 2020; Rodrigues et al., 2020), therefore, the frequency of seroreactive coatis observed in this study is within the range reported for domestic dogs. There was no statistical difference in frequency between males and females in this study, which was also reported in domestic dogs (Brito et al., 2016). However other study in domestic dogs shows higher frequency for males (Dantas-Torres et al., 2006). Here, younger coatis,

classified as infant and young, showed lower frequencies of serologic response than adults. Contrasting with reports in domestic dogs that show higher frequencies for younger dogs (Dantas-Torres et al., 2006). Previous studies in coatis did not access the differences between sex and age of the evaluated animals (Paiz et al., 2015; Porfirio et al., 2018; Macedo et al., 2023).

Serological analysis from recaptured animals allowed us to evaluated persistence of antibodies throughout time. Seroconversion was observed in five animals, indicating that these individuals were exposed to *L. infantum* between sampling procedures. One of them were able to sustain antibody response for approximately six months (Figure 1.6A, blue circle). Seroreversion occurred in five different individuals, whom were all reactive in the first capture but in the following sampling events were all non-reactive in rKDDR ELISA assay. This phenomenon was previously described in humans, domestic dogs, and in one coati (Vulpiani et al., 2009; Ostyn et al., 2011; Macedo et al., 2023). For domestic dogs, seroreversion can happen after leishmaniosis treatment (Vulpiani et al., 2009). The coatis evaluated here probably had a self-limiting infection that resulted in transient antibody production.

Domestic dogs and non-human primates that are seroreactive to *Leishmania* sp. have more antibodies against *Lu. longipalpis* saliva, indicating that these individuals were more exposed to sand flies (Quinnel et al., 2018; Oliveira et al., 2019). For free-ranging coatis, both *L. infantum* seroreactive and non-reactive animal showed similar *Lu. longipalpis* saliva antibody response, indicating that more exposition to sand flies is not associated with *L. infantum* exposure. However, when comparing different ages, O.D. values of *Lu. longipalpis* saliva antibodies were lower in younger individuals. That is probably due to continuous exposure over the years.

Xenodiagnosis is an important tool to better understand parasite-vector-host interaction (Sadlova et al., 2015). Here we used female *Lu. longipalpis* raised in laboratory condition free of *Leishmania* sp. to feed on free-ranging coatis. Two days after feeding, sand flies were evaluated by qPCR to identify *L. infantum* DNA. At this time point any parasite that was ingested from the tested host is trapped within the ingested blood by a chitin and protein mesh that is secreted by the sand fly midgut (Secundino et al., 2005; Bates, 2007). Xenodiagnosis in these conditions were used to determine if *Lu. longipalpis* were able to acquire *L. infantum* available on the skin of free-ranging coatis. This was the first time coatis were submitted to this xenodiagnosis, and 29.16% of them were positive. Other wild carnivores were evaluated through xenodiagnosis. Free-ranging crab-eating foxes were unable to transmit *L. infantum* to

sand flies, however bush dogs and maned wolves kept under human care transmitted (Courteney et al., 2002; Mol et al., 2015). Xenodiagnosis positive individuals included *L. infantum* seroreactive and non-reactive coatis. Two coatis were able to transmit *L. infantum* to sand flies and were non-reactive to *L. infantum*, they were probably in early stages of infection and did not develop an antibody response yet.

This study demonstrated that free-ranging coatis in a visceral leishmaniosis endemic region develop anti-*L. infantum* antibodies with no sex predisposition but occurring more frequently in adult animals. Seroreversion phenomena observed in five individuals supports the idea that *L. infantum* infection in this species can be self-limited and antibody response transient. We also provided insights in parasite-host-vector interaction. *Lu. longipalpis* saliva exposure is not associated with *L. infantum* IgG antibody production and sand flies can ingest *L. infantum* from coatis skin.

5. REFERENCES

ALMEIDA, J.C.; MELO, R.P.B.; KIM, P.C.P. et al. Molecular and serological investigation of infectious diseases in captive and free-range crab-eating fox (*Cerdocyon thous*–Linnaeus, 1776) from northeastern Brazil. **Acta Parasitologica**, v. 63, n. 1, p. 184-189, 2018.

BARRAL, A.M.P.; HONDA, E.; CALDAS, A.D.J.M. et al. Human immune response to sand fly salivary gland antigens: a useful epidemiological marker?. **American Journal of Tropical Medicina and Hygiene**, v. 62, n. 6, p. 740-745, 2000.

BARRETO, W.T.G.; HERRERA, H.M.; MACEDO, G.C. et al. Density and survivorship of the South American coati (*Nasua nasua*) in urban areas in Central–Western Brazil. **Hystrix**, v. 32, n. 1, p. 82, 2021.

BATES, P.A. Transmission of *Leishmania* metacyclic promastigotes by phlebotomine sand flies. **International journal for parasitology**, v. 37, n. 10, p. 1097-1106, 2007.

BRANDÃO, E.M.V.; XAVIER, S.C.C.; ROCHA, F.L. et al. Wild and domestic canids and their interactions in the transmission cycles of *Trypanosoma cruzi* and *Leishmania* spp. in an area of the Brazilian Cerrado. **Pathogens**, v. 9, n. 10, p. 818, 2020.

BRINDHA, J.; BALAMURALI, M.M.; CHANDA, K. An overview on the therapeutics of neglected infectious diseases—Leishmaniasis and Chagas diseases. **Frontiers in Chemistry**, v. 9, p. 622286, 2021.

BRITO, F.G.; LANGONI, H.; SILVA, R.C.D. et al. Canine visceral leishmaniasis in the Northeast Region of Brazil. **Journal of Venomous Animals and Toxins including Tropical Diseases**, v. 22, 2016.

CONFORTI, V.A.; AZEVEDO, F.C.C.; PAULO, O.L.O.H. et al. Chemical restraint of free-ranging South American coatis (*Nasua nasua*) with a combination of tiletamine and zolazepam. **Journal of wildlife diseases**, v. 53, n. 1, p. 140-143, 2017.

COURTENAY, O.; QUINNELL, R.J.; GARCEZ, L.M. et al. Low infectiousness of a wildlife host of *Leishmania infantum*: the crab-eating fox is not important for transmission. **Parasitology**, v. 125, n. 5, p. 407-414, 2002.

CURI, N.H.A.; MIRANDA, I.; TALAMONI, S.A. Serologic evidence of *Leishmania* infection in free-ranging wild and domestic canids around a Brazilian National Park. **Memórias do Instituto Oswaldo Cruz**, v. 101, p. 99-101, 2006.

DANTAS-TORRES, F.; BRITO, M.E.F.; BRANDÃO-FILHO, S.P. Seroepidemiological survey on canine leishmaniasis among dogs from an urban area of Brazil. **Veterinary parasitology**, v. 140, n. 1-2, p. 54-60, 2006.

DINIZ, S.A.; SILVA, F.L.; NETA, A.C.C. et al. Animal reservoirs for visceral leishmaniasis in densely populated urban areas. The Journal of Infection in Developing Countries, v. 2, n. 01, p. 024-033, 2008.

EVARISTO, A.M.C.F.; SEVÁ, A.D.P.; OLIVEIRA, G.M.B.D. et al. Canine leishmaniasis in the semi-arid region of Pernambuco, northeastern Brazil: epidemiology, factors associated with seropositivity and spatial analysis. **Revista Brasileira de Parasitologia Veterinária**, v. 29, p. e001120, 2020.

GIUNCHETTI, R.C.; MAYRINK, W.; GENARO, O. et al. Relationship between canine visceral leishmaniosis and the *Leishmania* (*Leishmania*) *chagasi* burden in dermal inflammatory foci. **Journal of comparative pathology**, v. 135, n. 2-3, p. 100-107, 2006.

GOMPPER, M.E. *Nasua narica*. **Mammalian species**, n. 487, p. 1-10, 1995.

JUSI, M.M.G.; STARKE-BUZETTI, W.A.; OLIVEIRA, T.M.F.S. et al. Molecular and serological detection of *Leishmania* spp. in captive wild animals from Ilha Solteira, SP, Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 20, p. 219-222, 2011.

LAINSON, R.; RANGEL, E.F. *Lutzomyia longipalpis* and the eco-epidemiology of American visceral leishmaniasis, with particular reference to Brazil: a review. **Memórias do Instituto Oswaldo Cruz**, v. 100, p. 811-827, 2005.

LIMA, V.M.F.; FATTORI, K.R.; MICHELIN, A.F. et al. Evidence of *Leishmania* spp. antibodies and DNA in bush dogs (*Speothos venaticus*) in Brazil. **Journal of Zoo and Wildlife Medicine**, v. 40, n. 1, p. 91-94, 2009.

MACEDO, G.C.; BARRETO, W.T.G.; OLIVEIRA, C.E. et al. *Leishmania infantum* infecting the carnivore *Nasua nasua* from urban forest fragments in an endemic area of visceral leishmaniasis in Brazilian Midwest. **Frontiers in Cellular and Infection Microbiology**, v. 12, p. 1993, 2023.

MOL, J.P.S.; SEMÍRAMIS, A.S.; TURCHETTI, A.P.; et al. Transmissibility of *Leishmania infantum* from maned wolves (*Chrysocyon brachyurus*) and bush dogs (*Speothos venaticus*) to *Lutzomyia longipalpis*. **Veterinary Parasitology**, v. 212, n. 3-4, p. 86-91, 2015.

MORALES-YUSTE, M.; MARTÍN-SÁNCHEZ, J.; CORPAS-LOPEZ, V. Canine leishmaniasis: Update on epidemiology, diagnosis, treatment, and prevention. **Veterinary Sciences**, v. 9, n. 8, p. 387, 2022.

OLIVEIRA, A.R.; PINHEIRO, G.R.G.; TINOCO, H.P. et al. Competence of non-human primates to transmit *Leishmania infantum* to the invertebrate vector *Lutzomyia longipalpis*. **PLoS neglected tropical diseases**, v. 13, n. 4, p. 1-14, 2019.

OSTYN, B.; GIDWANI, K.; KHANAL, B. et al. Incidence of symptomatic and asymptomatic *Leishmania donovani* infections in high-endemic foci in India and Nepal: a prospective study. **PLoS Neglected Tropical Diseases**, v. 5, n. 10, p. e1284, 2011.

PADILHA, T.C.; ZITELLI, L.C.; WEBSTER, A. et al. Serosurvey of antibodies against zoonotic pathogens in free-ranging wild canids (*Cerdocyon thous* and *Lycalopex gymnocercus*) from Southern Brazil. **Comparative Immunology, Microbiology and Infectious Diseases**, v. 79, p. 101716, 2021.

PAIZ, L.M.; FORNAZARI, F.; MENOZZI, B.D. et al. Serological evidence of infection by *Leishmania* (*Leishmania*) *infantum* (Synonym: *Leishmania* (*Leishmania*) *chagasi*) in free-ranging wild mammals in a nonendemic region of the State of São Paulo, Brazil. Vector-Borne and Zoonotic Diseases, v. 15, n. 11, p. 667-673, 2015.

PORFIRIO, G.E.O.; SANTOS, F.M.; MACEDO, G.C. et al. Maintenance of *Trypanosoma cruzi*, *T. evansi* and *Leishmania* spp. by domestic dogs and wild mammals in a rural settlement in Brazil-Bolivian border. **International Journal for Parasitology: Parasites and Wildlife**, v. 7, n. 3, p. 398-404, 2018.

QUINNELL, R.J.; SOREMEKUN, S.; BATES, P.A. et al. Antibody response to sand fly saliva is a marker of transmission intensity but not disease progression in dogs naturally infected with *Leishmania infantum*. **Parasites & vectors**, v. 11, p. 1-12, 2018.

RODRIGUES, T.F.; BENITEZ, A.D.N.; SEVÁ, A.D.P. et al. Spatial and seroepidemiology of canine visceral leishmaniasis in an endemic Southeast Brazilian area. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 53, 2020.

ROQUE, A.L.R.; JANSEN, A.M. Wild and synanthropic reservoirs of *Leishmania* species in the Americas. **International Journal for Parasitology: Parasites and Wildlife**, v. 3, n. 3, p. 251-262, 2014.

SADLOVA, J.; SEBLOVA, V.; VOTYPKA, J. et al. Xenodiagnosis of *Leishmania donovani* in BALB/c mice using *Phlebotomus orientalis*: a new laboratory model**. Parasites & vectors**, v. 8, p. 1-8, 2015.

SCORZA, B.M.; MAHACHI, K.G.; COX, K.G. et al. *Leishmania infantum* xenodiagnosis from vertically infected dogs reveals significant skin tropism. **PLoS neglected tropical diseases**, v. 15, n. 10, p. e0009366, 2021.

SECUNDINO, N.F.C.; EGER-MANGRICH, I.; BRAGA, E.M. et al. *Lutzomyia longipalpis* peritrophic matrix: formation, structure, and chemical composition. **Journal of medical entomology**, v. 42, n. 6, p. 928-938, 2005.

SILVA, E.S.; GONTIJO, C.M.; PACHECO, R.S. et al. Visceral leishmaniasis in the metropolitan region of Belo Horizonte, state of Minas Gerais, Brazil. **Memórias do Instituto Oswaldo Cruz**, v. 96, p. 285-291, 2001.

SILVA, R.O.S.; ALMEIDA, L.R.; OLIVEIRA JUNIOR, C.A. et al. Carriage of *Clostridium difficile* in free-living South American coati (*Nasua nasua*) in Brazil. **Anaerobe**, v. 30, p. 99-101, 2014.

STOFFELLA-DUTRA, A.G.; CAMPOS, B.H.; SILVA, P.B.H. et al. SARS-CoV-2 Spillback to Wild Coatis in Sylvatic–Urban Hotspot, Brazil. **Emerging Infectious Diseases**, v. 29, n. 3, p. 664, 2023.

VULPIANI, M.P.; IANETTI, L.; DI MATTIA, T. et al. Leishmania infantum in a Central Italy dog shelter: retrospective study of serologic reactivity during a 4-year period in a confined dog population subjected to preventive and therapeutic treatment. **Veterinary parasitology**, v. 160, n. 3-4, p. 190-197, 2009.

WHO Expert Committee on the control of leishmaniosis. WHO technical report series, no. 949; 2010.

CHAPTER 2

Transmission of *Leishmania* infantum from free-ranging black-tuffed marmosets (*Callithrix penicillata*) to the invertebrate vector *Lutzomyia longipalpis*.

Abstract

Non-human primates can host *Leishmania infantum*, a protozoan that causes visceral leishmaniosis. Most reports of *L. infantum* infection are based on serological and molecular tests, with most primates being asymptomatic. However, clinical disease has been reported in both neotropical and Old-world primates. Some species of non-human primates kept under human care were able to transmit *L. infantum* to its vector *Lutzomyia longipalis*, however there are no previous studies on free-ranging neotropical primates. This study evaluated free-ranging black-tuffed marmosets (*Callithrix penicillata*) in an endemic urban area. Serum samples from free-ranging marmosets were submitted to two serological tests (immunochromatographic rapid test and ELISA). Xenodiagnosis was performed with *Lu. longipalpis* to evaluated *L. infantum* transmission. Frequency of seroreactivity to *L. infantum* was 3.79% (3/79) and 12.5% (4/32) of the animals were able to transmit *L. infantum* to *Lu. longipalpis*. All xenodiagnosis positive marmosets were non-reactive in serological tests.

Keywords: neotropical primates, leishmaniosis, xenodiagnosis, serology.

1. INTRODUCTION

Non-human primates can be hosts to *Leishmania infantum*, a zoonotic protozoan that causes visceral leishmaniosis (Santos & Oliveira, 2020). Both neotropical and Old-World primates have been shown to develop significant disease associated with *L. infantum* infection (Malta et al., 2010; Miró et al., 2018; Tinoco et al., 2018). There is evidence of natural infection in several species of neotropical primates based on serological assays and molecular tests, however in most of these reports animals are asymptomatic (Porfirio et al., 2018; Trüeb et al., 2018; Oliveira et al., 2019; Guiraldi et al., 2022; Lopes et al., 2022).

Several species of non-human primates, including New and Old-World primates, kept under human care have been evaluated as potential reservoirs through xenodiagnosis (Oliveira et al., 2019). Asymptomatic golden lion tamarins (*Leontopithecus rosalia*), black-capped capuchins (*Sapajus apella*), chimpanzees (*Pan troglodytes*), and talapoin (*Miopithecus talapoin*) were able to infect *Lutzomyia longipalpis*, demonstrating that non-human primates can host *L. infantum* in endemic areas and transmit it to invertebrarte hosts.

Among neotropical primates, the Callithrichidae family include the genus *Callithrix*, with six species that are endemic in Brazil and some of them are highly adapted to urban areas (Malukiewics et al., 2020). Information about visceral leishmaniosis in this species are limited, however serological and molecular studies indicate that black-tuffed marmosets (*C. penicillata*), common marmosets (*C. jacchus*), and Geoffroy's tufted-ear marmoset (*C. geoffroyi*) can be infected by *L. infantum* (Paiz et al., 2015; Paiz et al., 2018; Venial et al., 2022). The goal of this study was to determine the frequency of *L. infantum* infection in free-ranging black-tuffed marmosets (*C. penicillata*) from an endemic urban area trough serological assays and xenodiagnosis.

2. MATERIAL AND METHODS

2.1.Ethics

All procedures were authorized by the Ethics Committee on the Use of Animals of the Universidade Federal de Minas Gerais (CEUA/UFMG) under protocol number 78/2022, by Instituto Chico Mendes de Conservação de Biodiversiade under protocol number 81392, and by Fundação de Parques Municipais e Zoobotânica de Belo Horizonte (FPMZ-BH) under protocol numbers FU002/2022, and Sisgen under protocols ABB00DB and ACDCB02.

2.2.Animal procedures

Black-tuffed marmosets (*C. penicillata*) were captured in six parks in the city of Belo Horizonte, Brazil (Figure 2.1) (*Parque Aggeo Pio* – AP, *Parque Fazenda Lagoa do Nado* – LN, *Parque das Mangabeiras* – PM, *Parque da Serra do Curral* – SC, *Parque Ursulina de Andrade Mello* – U and Belo Horizonte Zoo – Z) from 2022 and 2023 with two capture periods per year, from January to June and from July to December. The first period of capture (2022.1) was a pilot that took place only in PM.

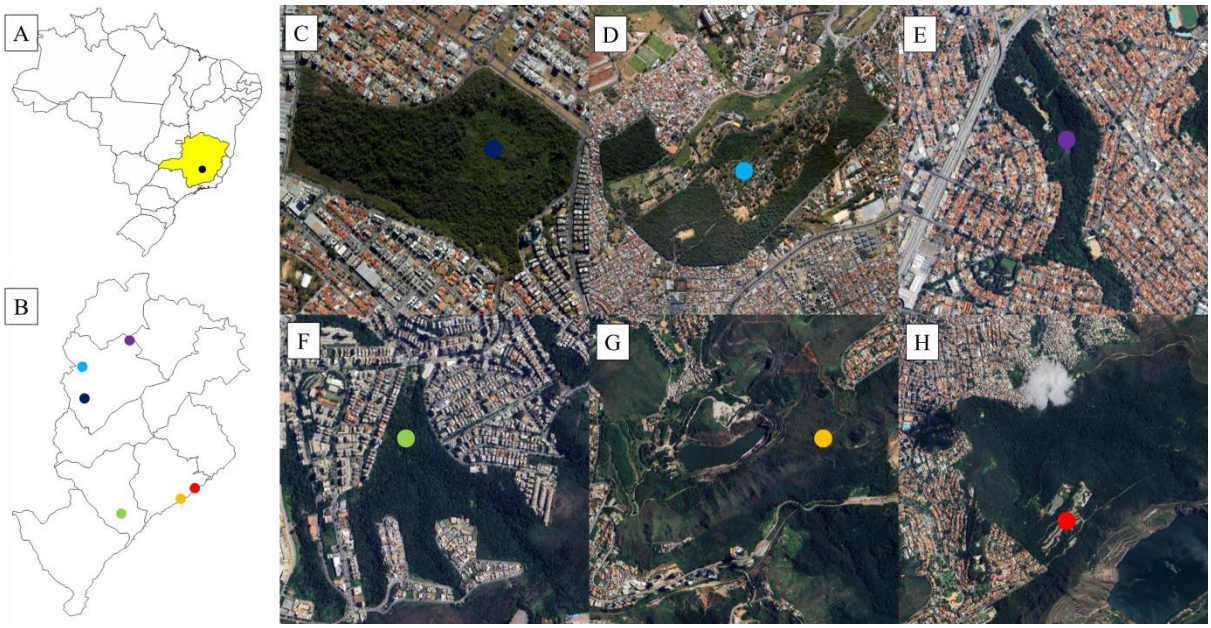

Figure 2.1. Geographic location of the areas of capture in Belo Horizonte (Minas Gerais, Brazil). A. Brazil map highlighting Minas Gerais state (yellow) and the city of Belo Horizonte (black dot). B. Belo Horizonte map with different colored dots marking the location of the parks. C – H: Dark blue: *Parque Ursulina de Andrade Mello* (U); light blue: Belo Horizonte Zoo (Z); Purple: *Parque Fazenda Lagoa do Nado* (LN); Green: *Parque Aggeo Pio* (AP); Orange: *Parque da Serra do Curral* (SC); Red: *Parque das Mangabeiras* (PM);

Traps were baited with bananas, distributed in multiple points and checked twice a day. Captured animals were weighted and sedated with ketamine (IM, 20 mg/kg) and midazolam (IM, 1 mg/kg). During sedation, animals were clinically evaluated, sex determined and age estimated. Clinical evaluation consisted of external exam, body condition, superficial lymph nodes palpation, abdominal palpation, respiratory frequency, heartbeats frequency and rectal temperature. Age was estimated based on weight, physical characteristics, and reproductive

condition. Infants weight less than 100 g, fur details are not fully defined, and tuffs are shorter. Young marmosets weighted between 100 and 250 g, fur details are also not fully defined, and tuffs are shorter. Adults weight more than 250 g, fur details are fully defined, and tuffs are longer. Pregnant and lactating females were also considered adults. Blood was sampled from the femoral vein and placed in tubes containing clot activators. Volume of sampled blood was under 1% of the marmoset weight. After separation, serum samples were stored at -20°C until serological evaluation. All animals were tagged with subcutaneous microchips for later identification. After total recovery from sedation, animals were released near the same place of capture inside the parks.

2.3.Serologic tests

rKDDR Immunochromatographic assay: rKDDR Immunochromatographic assay (Rapid test, Safetest Diagnósticos, Brazil) was used according to manufacturer's instructions. Briefly, 20 µL of serum was added, followed by 40 µL of PBS buffer. After 20 minutes of incubation at room temperature, samples were considered positive if two red marks were visualized (Figure 1.2).

ELISA anti-*Leishmania* (rKDDR): ELISA was performed as described in Oliveira et al. (2019) with adaptations. 96-well-plates (Costar, Cornig, USA) coated with 50 ng/well of rKDDR antigen diluted in carbonate buffer overnight at 4°C. Then, wells were blocked with a solution of PBS with 5% BSA for 2 hours at 37°C. Serum samples were diluted in PBS-BSA 2.5% at 1:100 proportion, added to the wells and incubated for 12 hours at 4°C. Human anti-IgG (Rhea Biotech, Brazil) was diluted in 2.5% PBS-BSA at 1:2500 and added to each well for 1 hour and 30 minutes at 37°C. Reaction was revealed with a solution of 0.05% o-phenylenediamine (OPD), 0.1% hydrogen peroxide in citrate buffer. Reaction was stopped after 10 minutes with 4 M sulfuric acid and optical densities (O.D.) were measured at 490 nm with an ELISA reader (BioRad 550, Brazil). Between each step, plates were washed five times with 0.05% PBS-Tween. Primate serum samples previously tested (Oliveira et al., 2019) were used as positive and negative controls. Cut-off was stablished at three standard deviations above average O.D. of the eight samples of primate serum samples previously tested. All serum samples were tested at the same moment in duplicates.

ELISA anti-*Lutzomyia longipalpis* saliva: salivary glands were dissected from *Lutzomyia longipalpis* raised at laboratory conditions and stored in PBS at -80°C. For saliva collection, salivary glands were disrupted by ultrasonication (10 rounds of 1 min with amplitude of 20) and centrifuged at 3200 xg for 2 min. Supernatant was used as antigen at a proportion of 5 pairs of gland/mL as described previously by Barral et al. (2000). Protein concentration was determined using Pierce BCA Protein Assay Kit (Thermo Scientific, USA) resulting in a final concentration of 470 ng/well. 96-well-plates were coated with the salivary gland preparation overnight at 4°C, followed by blocking with 5% PBS-BSA for 1 hour at 37°C. Serum samples were diluted in PBS-BSA 2.5% at 1:100 proportion, added to the wells and incubated for 1 hour at 37°C. Human anti-IgG (Rhea Biotech, Brazil) was diluted in 2.5% PBS-BSA at 1:2500 and added to each well for 1 hour at 37°C. Reaction was revealed with a solution of 0.05% o-phenylenediamine (OPD), 0.1% hydrogen peroxide in citrate buffer. Reaction was stopped after 10 minutes with 4 M sulfuric acid and optical densities (O.D.) were measured at 490 nm with an ELISA reader (BioRad 550, Brazil). Between each step, plates were washed five times with 0.05% PBS-Tween. No positive or negative controls were used for this experiment, so no cut off was established. All serum samples were tested at the same moment in duplicates.

2.4.Xenodiagnosis

Xenodiagnosis was performed using four-day-old female sand flies (*Lu. longipalpis*) raised under laboratory conditions and free of *Leishmania* sp. Fifty sand flies were placed in a FleboContainer as previously described (Mol et al., 2015; Oliveira et al., 2019). Sedated marmosets were exposed to sand flies on the ear for 30 minutes (Figure 2.2). After feeding, the sand flies were kept for 2 days and fed with 50% sucrose solution. Sand flies were euthanized by freezing and ingurgitated females were separated in individual microtubes and stored at -20°C until analysis.

Figure 2.2. Xenodiagnosis in free-ranging black-tuffed marmosets (*Callithrix penicillata*). Adult male black-tuffed marmoset, sedated and exposed to *Lutzomyia longipalpis* on the ear.

DNA extraction: DNA extraction was performed as previously described (Oliveira et al., 2019). Ten ingurgitated female sand flies were randomly selected from each individual marmoset exposed to them for analysis. Sand flies were macerated in a 1.5 mL microtube with 50 µL of lysis buffer (0.08 M sodium chloride, 0.16 M sucrose, 0.06 M EDTA, 0.5% SDS, 0.1 M Tris-Cl, pH 8.6) followed by incubation at 65°C for 30 minutes. Then, 7.1 µL of 8 M potassium acetate was added and vortex homogenized followed by incubation for 30 minutes at 4°C. Homogenate was then centrifuged at 13000 x g for 10 minutes and supernatant was transferred to another microtube, added 100 µL of 95% ethanol and centrifuged at 13000 x g for 10 minutes. Supernatant was discarded and the pellet was washed with 100 µL of 70% ethanol and centrifuged at 13000xg for 10 minutes. Supernatant was discarded and after complete drying of the residual ethanol the pellet was resuspended in 20 µL of ultrapure water. DNA concentration was determined with NanoDrop One/OneC Microvolume UV-Vis Spectrophotometer (ThermoFischer, USA) and extracted samples stored at -20°C until analysis.

Real Time PCR: identification of *L. infantum* in sand flies was made through quantitative PCR as described by Oliveira et al. (2019). Two sets of primers were used: one targeting *Leishmania* sp. minicircle kinetoplast DNA (kDNA) specific for the *donovani* complex – forward 5'-CTTTTCTGGTCCCGCGGGTAGG-3' and reverse 5'-CCACCTGGCCTATTTTACACCA-3'. qPCR reaction was performed in a final volume of 10 µL – 5 µL of 1x SYBR Green PCR master mix (Thermo Fisher Scientific, Applied Biosystems, USA), 0.2 µM od each primer and 10 ng of DNA. Reaction was performed in StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, USA) with the following parameters: initial

denaturation as 95°C for 10 minutes, 40 cycles of denaturation as 95°C for 15 seconds, annealing and extension at 60°C for 1 minute. DNA extracted from *L. infantum* culture was used as positive control and for negative control DNA template was substituted by water.

Sand flies were first evaluated as pools. From each marmoset two pools were tested, each one containing DNA from 5 individual sand fly. Xenodiagnosis was considered positive if at least one of the pools were positive.

2.5.Statistical analysis

GraphPad Prism software (version 8.0.1) was used to analyze data. Frequencies were evaluated by Chi-square test. Mann-Whitney test was used to compare O.D. values. Kappa Coefficient was used to evaluate concordance between serological tests.

3. RESULTS

A total of 79 black-tuffed marmosets were captured, with five of them being recaptured one more time. Information about the animals captured (site of capture, sex and estimated age) are detailed in Table 2.1. A pilot capture took place in 2021.1 only in PM, that resulted in one marmoset captured.

Table 2.1. Free-ranging *Callithrix penicillata* captured in urban parks of Belo Horizonte (Minas Gerais, Brazil).

		Sex			**Estimated age**			
Period	Location	Male	Female	Total	Infant	Young	Adult	Total
2022.1	PM	1	0	1	0	0	1	1
2022.2	LN	4	3	7	0	2	5	7
	PM	7	10	17	0	0	17	17
	SC	1	0	1	0	0	1	1
	U	2	2	4	0	0	4	4
	Z	4	2	6	1	0	5	6
	Total	18	17	35	1	2	32	35
2023.1	LN	3	2	5	0	1	4	5
	PM	5	3	8	0	2	6	8
	U	1	0	1	0	0	1	1
	Z	2	1	3	0	1	2	3
	Total	11	6	17	0	4	13	17
2023.2	AP	1	1	2	0	0	2	2
	LN	4	1	5	0	1	4	5

	PM	4	8	12	0	3	9	12
	SC	1	2	3	0	1	2	3
	U	0	2	2	0	0	2	2
	Z	4	3	7	0	2	5	7
	Total	14	17	31	0	7	24	31

PM: *Parque das Mangabeiras*; LN: *Parque Fazenda Lago*; SC: *Parque da Serra do Curral*; U: *Parque Ursulina de Andrade Mello*; Z: Belo Horizonte Zoo; AP: *Parque Aggeo Pio*;

3.1.Anti-*Leishmania infantum* serology

Exposure to *L. infantum* was evaluated by two serological tests, rKDDR immunochromatographic rapid test (Safetest Diagnóstico, Brazil) and ELISA using rKDDR. Eighty-four serum samples from 79 marmosets were evaluated. No samples were reactive in the rapid test and 3 (3.57%) were reactive in the ELISA, therefore, there was no agreement between these tests (Kappa coefficient = 0.0).

Since the rapid test was not sensitive enough to detect seroreactive animals, only ELISA results were criteria for seroreaction. To evaluate individual serological status, we considered an individual as seroreactive if one of the serum samples collected at any point in time was reactive. Overall frequency was 3.79% (3/79) of marmosets seroreactive to *L. infantum*. There was no difference in the frequency when comparing age, sex or time of capture (Figure 2.3A, B and C). All seroreactive marmosets were male, two adults and one young. Two of them were captured at PM and the other at SC (Figure 2.3D). All recaptured animals were seronegative in both periods of capture.

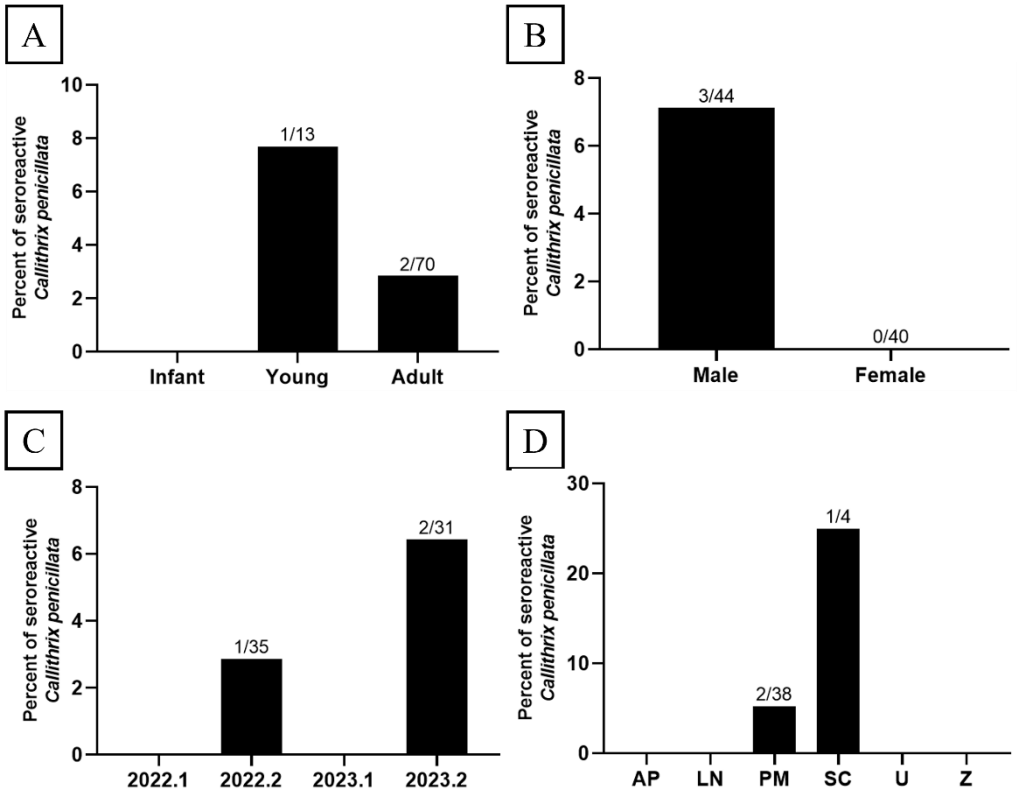

Figure 2.3. Frequency of *Leishmania infantum* seroreactive free-ranging *Callithrix penicillata* by estimated age, sex, period, and site of sampling. A-D. Seroreactivity was based on rKDDR ELISA. Statistical difference was based on Chi-square test. Capture locations: AP: *Parque Aggeo Pio*; LN: *Parque Fazenda Lagoa do Nado*; PM: *Parque das Mangabeiras*; SC: *Parque da Serra do Curral*; U: *Parque Ursulina Andrade de Mello*; Z: Belo Horizonte Zoo.

3.2.Anti-*Lutzomyia longipalpis* saliva serology

Seroreaction to *Lu. longipalpis* saliva was evaluated through ELISA. There was no difference between males and females (Mann-Whitney, p = 0.9693) or between *L. infantum* reactive and non-reactive individuals (Mann-Whitney, p = 0.7553) (Figure 2.4A). When comparing estimated ages, adults showed higher O.D. values than young ones (Mann-Whitney, p = 0.0052) (Figure 2.4B).

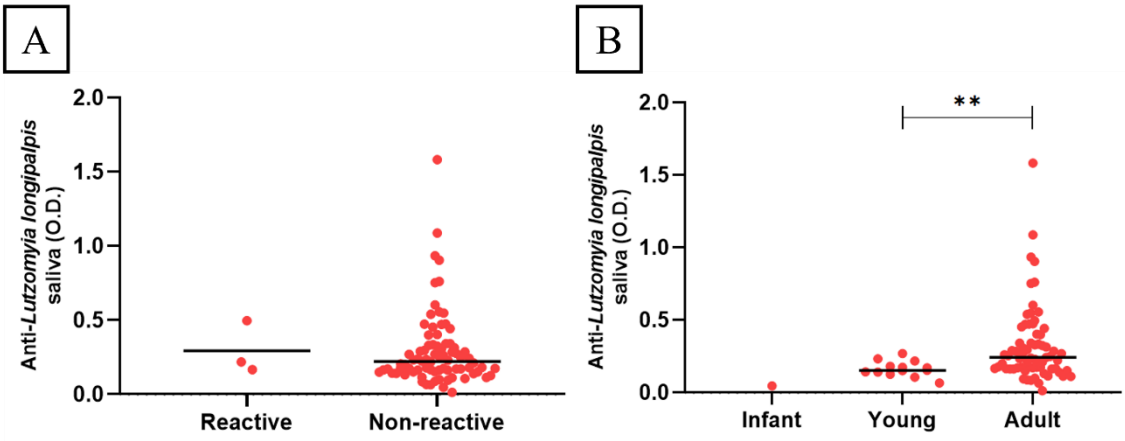

Figure 2.4. Anti-*Lutzomyia longipalpis* saliva IgG antibodies in free-ranging *Callithrix penicillata* by anti-*Leishmania infantum* serological status (A) and estimated age (B). Each dot represents a different serum sample submitted to ELISA. Optical densities (O.D.) values were compared with Mann-Whitney test. A. Number of samples evaluated: 3 (reactive) and 80 (non-reactive). B. Number of samples evaluated: 1 (infant), 13 (young) and 69 (adult).

3.3.Xenodiagnosis

Thirty-two marmosets were submitted to xenodiagnosis with *Lu. longipalpis*, Table 2.2 contains data of all individuals evaluated. Four animals (12.5%) were able to transmit *L. infantum* to sand flies. Interestingly all four individuals were non-reactive on serological tests. Only one seroreactive marmoset was included in the analysis by xenodiagnosis, and no *L. infantum* DNA was detected. The four xenodiagnosis positive individuals included two males and two females. Both females and one of the males were adults, the other male was young. There was no difference between *L. infantum* serological status, sex or age category.

Table 2.2. Estimated age, sex, serological status and xenodiagnoses results from free-ranging *Callithrix penicillata* exposed to *Lutzomyia longipalpis*.

ID	Estimated age	Sex	Serology	Xenodiagnosis	Ct
C001	Adult	Male	NR	Negative	-
C002	Adult	Male	NR	Negative	-
C004	Adult	Female	NR	Negative	-
C006	Adult	Female	NR	Negative	-
C007	Adult	Male	NR	Negative	-
C008	Adult	Female	NR	Negative	-
C009	Adult	Male	NR	Negative	-
C011	Adult	Female	NR	Negative	-
C015	Young	Male	NR	**Positive**	**30,48**
C016	Adult	Male	NR	Negative	-
C017	Adult	Female	NR	Negative	-

C019	Adult	Female	NR	Negative	-
C020	Adult	Female	NR	**Positive**	**30,43**
C021	Adult	Female	NR	Negative	-
C022	Adult	Female	NR	Negative	-
C023	Adult	Male	NR	Negative	-
C024	Adult	Male	NR	Negative	-
C025	Adult	Female	NR	Negative	-
C026	Adult	Male	NR	Negative	-
C027	Adult	Male	NR	Negative	-
C028	Adult	Female	NR	Negative	-
C029	Adult	Male	NR	**Positive**	**26,17**
C030	Adult	Female	NR	Negative	-
C031	Adult	Male	NR	Negative	-
C032	Adult	Male	R	Negative	-
C033	Adult	Female	NR	Negative	-
C034	Adult	Female	NR	Negative	-
C038	Adult	Female	NR	**Positive**	**33,1**
C039	Adult	Male	NR	Negative	-
C048	Adult	Female	NR	Negative	-
C049	Adult	Male	NR	Negative	-
C051	Adult	Male	NR	Negative	-

F: female; M: male; R: reactive; NR: non-reactive;

4. DISCUSSION

This study investigated *L. infantum* infection in free-ranging black-tuffed marmosets (*C. penicillata*) through serological assays and xenodiagnosis. Only 3.79% of the evaluated animals were seroreactive to *L. infantum*. Another serological investigation evaluated fewer marmosets from Campinas (São Paulo, Brazil), identifying 25% and 27.7% of seroreactive free-ranging common marmosets (*C. jacchus*) and black-tuffed marmosets (*C. penicillata*) (Paiz et al., 2018). Other species of neotropical primates have been evaluated through serological tests. For other callitrichids, antibodies against *L. infantum* were reported in 13% of golden lion tamarins (*L. rosalia*) kept under human care (Oliveira et al., 2019). There is also serological evidence for other species such as black-capped capuchins (*S. apella*) (Voltarelli et al., 2009; Oliveira et al., 2019) and black-horned capuchins (*S. nigritus*) (Lopes et al., 2022). Frequency of *L. infantum* seroreative in black-tuffed marmosets observed here was lower than other reports, indicating that free-ranging marmosets evaluated here might have been less exposed to *L. infantum*.

In previous investigation of leishmaniosis in non-human primates kept under human care, seroreactive animals developed more antibodies against *Lu. longipalpis* saliva, indicating that those individuals were more exposed to sand flies bite (Oliveira et al., 2019). In this study, there was no significant difference in O.D values of *Lu longipalpis* saliva antibodies between

serologically reactive and non-reactive marmosets, even with similar exposure to sand flies bites most marmosets evaluated here did not develop *L. infantum* antibodies.

In this study, xenodiagnosis was used for the first time as a diagnostic tool in black-tuffed marmosets to better understand their role in visceral leishmaniosis epidemiology. Thirty-two individuals were evaluated with four of them being able to transfer *L. intantum* to *Lu. longipalpis*. Interestingly, all the xenodiagnosis positive marmosets were non-reactive to *L. infantum* and the only seroreactive animal was xenodignosis negative. This was also reported in one golden lion tamarin kept under human care that was able to infect sand flies even with no serological response (Oliveira et al., 2019). Development of *L. infantum* inside the sand fly is important to understand xenodiagnosis results. At two days after feeding, as evaluated in this study, *L. infantum* acquired from the skin and blood of tested animals is still developing in the ingested blood and not yet fully infecting the sand fly (Secundino et al., 2005; Bates, 2007). Our interpretation here is that the four positive marmosets had enough parasites available on their skin to be acquired by the sand fly. Further studies are needed to better understand if infection of sand flies with *L. infantum* under these conditions could render the sand flies infective to other vertebrate hosts.

Black-tuffed marmosets are small neotropical primates highly adapted to urban environment and shown a low frequency of *L. infantum* antibodies even with exposure to *Lu. longipalpis* bites. However, even without serological response some animals were able to transfer *L. infantum* to sand flies. These results support the hypothesis that black-tuffed marmosets might play a minor role as a reservoir of *L. infantum* in urban or periurban areas.

5. REFERENCES

BARRAL, A.M.P.; HONDA, E.; CALDAS, A.D.J.M. et al. Human immune response to sand fly salivary gland antigens: a useful epidemiological marker?. **American Journal of Tropical Medicina and Hygiene**, v. 62, n. 6, p. 740-745, 2000.

BATES, P.A. Transmission of *Leishmania* metacyclic promastigotes by phlebotomine sand flies. **International Journal for Parasitology**, v. 37, n. 10, p. 1097-1106, 2007.

GUIRALDI, L.M.; SANTOS, W.J.; MANZINI, S. et al. Identification of *Leishmania infantum* and *Leishmania braziliensis* in captive primates from a zoo in Brazil. **American Journal of Primatology**, v. 84, n. 4-5, p. e23376, 2022.

LOPES, K.F.C.; DELAI, R.M.; ZANIOLO, M.M. et al. Urban capuchin monkeys *Sapajus nigritus* (Goldfuss, 1809) (Primates, Cebidae) as environmental bioindicators of leishmaniasis. **Transboundary and Emerging Diseases**, v. 69, n. 4, p. 2320-2325, 2022.

MALTA, M.C.C.; TINOCO, H.P.; XAVIER, M.N. et al. Naturally acquired visceral leishmaniasis in non-human primates in Brazil. **Veterinary Parasitology**, v. 169, n. 1-2, p. 193-197, 2010.

MALUKIEWICZ, J.**;** BOERE, V.; OLIVEIRA, M.A.B. et al. An introduction to the *Callithrix* genus and overview of recent advances in marmoset research. **ILAR journal**, v. 61, n. 2-3, p. 110-138, 2020.

MIRÓ, G.; TROYANO, A.; MONTOYA, A. et al. First report of *Leishmania infantum* infection in the endangered orangutan (*Pongo pygmaeus pygmaeus*) in Madrid, Spain. **Parasites & Vectors**, v. 11, p. 1-7, 2018.

MOL, J.P.S.; SEMÍRAMIS, A.S.; TURCHETTI, A.P.; et al. Transmissibility of *Leishmania infantum* from maned wolves (*Chrysocyon brachyurus*) and bush dogs (*Speothos venaticus*) to *Lutzomyia longipalpis*. **Veterinary Parasitology**, v. 212, n. 3-4, p. 86-91, 2015.

OLIVEIRA, A.R.; PINHEIRO, G.R.G.; TINOCO, H.P. et al. Competence of non-human primates to transmit *Leishmania infantum* to the invertebrate vector *Lutzomyia longipalpis*. **PLoS Neglected Tropical Diseases**, v. 13, n. 4, p. 1-14, 2019.

PAIZ, L.M.; FORNAZARI, F.; MENOZZI, B.D. et al. Serological evidence of infection by *Leishmania* (*Leishmania*) *infantum* (Synonym: *Leishmania* (*Leishmania*) *chagasi*) in free-

ranging wild mammals in a nonendemic region of the State of São Paulo, Brazil. **Vector-Borne and Zoonotic Diseases**, v. 15, n. 11, p. 667-673, 2015.

PAIZ, L.M.; MOTOIE, G.; RICHINI-PEREIRA, V.B. et al. Antibodies and molecular detection of *Leishmania* (*Leishmania*) *infantum* in samples of free-ranging marmosets (Primates: Callitrichidae: *Callithrix* spp.) in an area of canine visceral leishmaniasis in Southeastern Brazil. **Vector-Borne and Zoonotic Diseases**, v. 19, n. 4, p. 249-254, 2018.

PORFIRIO, G.E.O.; SANTOS, F.M.; MACEDO, G.C. et al. Maintenance of *Trypanosoma cruzi*, *T. evansi* and *Leishmania* spp. by domestic dogs and wild mammals in a rural settlement in Brazil-Bolivian border. **International Journal for Parasitology: Parasites and Wildlife**, v. 7, n. 3, p. 398-404, 2018.

SANTOS, R.L.; OLIVEIRA, A.R. Leishmaniasis in non-human primates: Clinical and pathological manifestations and potential as reservoirs. **Journal of Medical Primatology**, v. 49, n. 1, p. 34-39, 2020.

SCORZA, B.M.; MAHACHI, K.G.; COX, K.G. et al. *Leishmania infantum* xenodiagnosis from vertically infected dogs reveals significant skin tropism. **PLoS Neglected Tropical Diseases**, v. 15, n. 10, p. e0009366, 2021.

SECUNDINO, N.F.C.; EGER-MANGRICH, I.; BRAGA, E.M. et al. *Lutzomyia longipalpis* peritrophic matrix: formation, structure, and chemical composition. **Journal of Medical Entomology**, v. 42, n. 6, p. 928-938, 2005.

TINOCO, H.P.; COSTA, M.E.L.T.; PESSANHA, A.T. et al. Visceral leishmaniasis in an infant gorilla (*Gorilla gorilla gorilla*): Clinical signs, diagnosis, and successful treatment with single-dose liposomal amphotericin B. **Journal of Medical Primatology**, v. 47, n. 6, p. 416-418, 2018.

TRÜEB, I.; PORTELA, R.D.; FRANKE, C.R. et al. *Trypanosoma cruzi* and *Leishmania* sp. infection in wildlife from urban rainforest fragments in northeast Brazil. **Journal of Wildlife Diseases**, v. 54, n. 1, p. 76-84, 2018.

VENIAL, H.J.; MONTOYA, A.; CHECA, R. et al. Investigation of *Leishmania* (*Viannia*) *braziliensis* infection in wild mammals in Brazil. **Acta Parasitologica**, p. 1-10, 2022.

VOLTARELLI, E.M.; ARRAES, S.M.A.A.; PERLES, T.F. et al. Serological survey for *Leishmania* sp. infection in wild animals from the municipality of Maringá, Paraná State,

Brazil. **Journal of Venomous Animals and Toxins including Tropical Diseases**, v. 15, p. 732-744, 2009.

CHAPTER 3

Serological evidence of arboviruses exposure in free-ranging black-tuffed marmosets (*Callithrix penicillata*)

Abstract

Arboviruses are important causes of disease to humans and can have wild animals as amplifiers. Yellow fever virus (*Orthoflavivirus flavi*), a flavivirus, is an important cause of disease and death in neotropical primates. Other arboviruses have been reported in neotropical primates. Zika virus (*Orthoflavivirus zikaense*) can infect free-ranging marmosets. and capuchins. Dengue virus (*Orthoflavivirus denguei*) antibodies were detected in *Alouatta* sp. and *Ateles* sp. *Sapajus* sp., *Ateles marginatus*, and *Callithrix jacchus* may develop neutralizing antibodies against chikungunya (*Alphavirus chikungunya*). In this study we investigated arboviruses (*A. chikungunya*, *O. zikaense*, *O. denguei*, and *O. flavi*) exposure in free-ranging black-tuffed marmosets (*Callithrix penicillata*) captured in urban parks through serological assays. Frequency of seroreactivity was low with 2.94% (2/68) seroreactive to *A. chikungunya* (IgM) and 1.47% (1/68) seroreactive to *O. zikaense* (IgM). One marmoset was seroreactive to both viruses. Frequency of seroreactive marmosets was low as was also reported in other studies with neotropical primates, and could be a result of low exposure to arboviruses.

Keywords: Chikungunya, dengue, yellow fever, zika, serology.

1. INTRODUCTION

Arboviruses are transmitted by arthropods and cause great impact in human health. Several viruses belonging to the *Flavivirus* genus are included in this group such as Yellow Fever virus (*Orthoflavivirus flavi*), Dengue virus (*Orthoflavivirus denguei*), Saint Louis virus (*Orthoflavivirus louisense*), West Nile virus (*Orthoflavivirus nilense*), and Zika virus (*Orthoflavivirus ziakense*). Relevant arboviruses also include Chikungunya virus (*Alphavirus chikungunya*), an *Alphavirus* (Gould and Solomon, 2008; Gould et al., 2017). These viruses can be transmitted from human to human through an invertebrate vector or transmission to the invertebrate vector may come from wild or domestic animals, which may act as amplifiers resulting in human infections (Weaver et al., 2018).

Yellow fever is an important disease associated with outbreaks in human and non-human primate populations (Litvoc et al., 2018). More than 2,000 human cases occurred during the last outbreak in Brazil from 2016 to 2019 (Giovanetti et al., 2019). Neotropical primates were greatly impacted during this outbreak with reduction of free-ranging populations of threatened species such as golden lion tamarins (*Leontopithecus rosalia*) and northern muriquis (*Brachyteles hypoxanthus*) (Dietz et al., 2019; Strier et al., 2019). Severity of disease for neotropical primates varies according to the affected species that may develop severe necrotic hepatitis, whereas other species may develop no histopathological lesions upon infection with *O. flavi* (Santos et al., 2020; Fernandes et al., 2021).

Other arboviruses have been investigated in neotropical primates. squirrel monkeys (*Saimiri* sp.), owl monkeys (*Aotus* sp.), and common marmosets (*Callithrix jacchus*) develop viremia and antibodies after experimental infection with *O. zikaense* (Chiu et al., 2017; Vanchiere et al., 2018), and natural infections with *O. zikaense* has been reported in free-ranging marmosets and capuchins as diagnosed by molecular and serological methods (Terzian et al., 2018; Favoretto et al., 2019). *O. denguei* seroreactivity was reported in howler monkeys (*Alouatta* sp.) and spider monkeys (*Ateles* sp.) (Morales et al., 2017; Chaves et al., 2021). Neutralizing antibodies against *A. chikungunya* have been detected in capuchins (*Sapajus* sp.), white-cheeked spider monkeys (*Ateles marginatus*), and common marmosets (*Callithrix jacchus*) (Moreira-Soto et al., 2018).

Black-tuffed marmosets (*Callithrix penicillata*) are small neotropical primates highly adapted to urban environments (Malukiewics et al., 2020). Marmosets can host *O. flavi* and *O. zikaense* and there is serological evidence of natural infection with *A. chikungunya* in this

species (Moreira-soto et al., 2018; Terzian et al., 2018; Santos et al., 2020). Since neotropical primates can act as sentinels for Yelow Fever, mainly howler monkeys (*Alouatta* sp.), and other arboviruses can infect neotropical primates, the goal of this study was to investigate arboviruses exposure in free-ranging black-tuffed marmosets (*C. penicillata*), through serological assays.

2. MATERIAL AND METHODS

2.1. Ethics

All procedures were authorized by the Ethics Committee on the Use of Animals of the Universidade Federal de Minas Gerais (CEUA/UFMG) under protocol number 78/2022, by Instituto Chico Mendes de Conservação de Biodiversiade under protocol number 81392, and by Fundação de Parques Municipais e Zoobotânica de Belo Horizonte (FPMZ-BH) under protocol numbers FU002/2022, and Sisgen under protocols ABB00DB and ACDCB02.

2.2. Animal procedures

Black-tuffed marmosets (*C. penicillata*) were captured in six parks in the city of Belo Horizonte, Brazil (Figure 2.1) (*Parque Aggeo Pio* – AP, *Parque Fazenda Lagoa do Nado* – LN, *Parque das Mangabeiras* – PM, *Parque da Serra do Curral* – SC, *Parque Ursulina de Andrade Mello* – U and Belo Horizonte Zoo – Z) from 2022 and 2023 with two capture periods per year, from January to June and from July to December. The first period of capture (2022.1) was a pilot that took place only in PM.

Traps were baited with bananas, distributed in multiple points and checked twice a day. Captured animals were weighted and sedated with ketamine (IM, 20 mg/kg) and midazolam (IM, 1 mg/kg). During sedation, animals were clinically evaluated, sex determined and age estimated. Clinical evaluation consisted of external exam, body condition, superficial lymph nodes palpation, abdominal palpation, respiratory frequency, heartbeats frequency and rectal temperature. Age was estimated based on weight, physical characteristics and reproductive condition. Infants weight less than 100 g, fur details are not fully defined, and tuffs are shorter. Young marmosets weighted between 100 and 250 g, fur details are also not fully defined, and tuffs are shorter. Adults weight more than 250 g, fur details are fully defined, and tuffs are longer. Pregnant and lactating females were also considered adults. Blood was sampled from the femoral vein and placed in tubes containing clot activators. Volume of sampled blood was

under 1% of the marmoset weight. After separation, serum samples were stored at -20°C until serological evaluation. All animals were tagged with subcutaneous microchips for later identification. After total recovery from sedation, animals were released near the same place of capture inside the parks.

2.3. Serological tests

Chikungunya (*A. chikungunya*) IgG and IgM ELISA assays: anti-*A. chikungunya* IgG or IgM antibodies were detected with two commercial kits (EUROIMMUN, Germany) following manufacturer's instructions. Sensitized plates were incubated with positive and negative controls, calibrators and serum samples for one hour at 37°C. Secondary antibody (anti-human IgG or IgM) was added to each well and incubated for 30 minutes at room temperature. Chromogenic solution was added to each well and incubated for 15 minutes at room temperature, followed by the stop solution. Optic density was read at 450 nm. Plates were washed 3 times between each step. All reagents, including positive and negative controls are provided by the kit. The following formula was used to evaluate the results: ratio = sample O.D. / calibrator O.D. Samples were considered reactive if ratio was above 1.1.

Zika virus (*O. zikaense*) IgG and IgM ELISA assays: detection of anti-*O. zikaense* virus antibodies was by two commercial kits (Vircell, Spain) following manufacturer's instructions. Sensitized plates were incubated with positive and negative controls, calibrators and serum samples for 45 minutes at 37°C. Secondary antibody (anti-human IgG or IgM) was added to each well and incubated for 30 minutes at 37°C, followed by chromogenic solution for 20 minutes at room temperature. Stop solution was added and O.D. read at 450 nm. Plates were washed 5 times between incubation steps. All reagents, including positive and negative controls are included in the kit. The following formula was used to evaluate the results: ratio = (sample O.D. / mean calibrators O.D.) x 10. Samples with ratio value above 11 were considered reactive.

Dengue virus (*O. denguei*) IgM ELISA assay: anti-*O. denguei* IgM antibodies were detected by a commercial kit (Panbio Dengue IgM Capture ELISA, Abbot laboratories, USA) following manufacturer's instructions. Positive and negative controls, calibrators and serum samples were incubated for 1 hour at 37°C. Then a combination of antigen and antibody detector was added and incubated for 1 hour at 37°C. Chromogenic solution was added and

incubated for 10 minutes at room temperature and then added the stop solution. Optic density (O.D.) was read at 450nm. Wells were washed 6 times between each step.

Yellow fever (*O. flavi*) ELISA assay: 96-well plates were incubated with anti-human IgM antibodies, diluted in carbonate buffer at 1:500 dilution, at 4°C overnight. Then, a PBS solution with 5% Difco and 0.5 Tween20 was added for 30 minutes at room temperature. Serum samples and controls (positive and negative) at 1:400 dilution were incubated for 60 minutes at 37°C. *O. flavi* antigen was added and incubated overnight at 4°C. Anti-flavivirus conjugate (6B6C-1) at 1:3000 dilution was incubated at 37°C for 60 minutes. Then chromogenic TBM solution was added and incubated for 15 minutes, followed by stop solution. Optic density (O.D.) was read at 450nm. Samples were considered reactive if O.D. was higher than 0.3.

2.4. Statistical analysis

GraphPad Prism software (version 8.0.1) was used to analyze data. Frequencies were evaluated by Fisher's exact test.

3. RESULTS

To evaluate arbovirus circulation, 72 serum samples from 68 free-ranging black-tuffed marmosets were submitted to serological evaluation. Two animals (2/68, 2.94%) were seroreactive by the *A. chikungunya* anti-IgM assay. One of them was evaluated in two different periods of capture, with three months of interval between samplings. Both samples from the recaptured *A. chikungunya*-reactive animal were also seroreactive by the *O. zikaense* anti-IgM assay. Frequency of seroreactivity for *O. zikaense* (IgM) was 1.47% (1/68). The *A. chikungunya* and *O. zikaense*-reactive animal was an adult male, and the *A. chikungunya*-reactive was an adult female. Both animals were captured in *Parque das Mangabeiras*, both samplings of the male were in 2022 (April and July), and the female was captured in 2023 (September).

All serum samples were non-reactive for *A. chikungunya* anti-IgG, *O. denguei* anti-IgM, *O. zikaense* anti-IgG and *O. flavi* anti-IgG. No difference between sex or estimated age was found (for all analysis $p > 0.9999$). Table 3.1 details information about the seroreactive animals.

Table 3.1. Free-ranging *Callithrix penicillata* seroreactive to arboviruses.

ID	PC	Age	Sex	CHIK-IgM	CHIK-IgG	DNV-IgM	ZK-IgM	ZK-IgG	YF-IgG
C001	22.1	A	M	R	NR	NR	R	NR	NR
C001	22.2	A	M	R	NR	NR	R	NR	NR
C072	23.2	A	F	R	NR	NR	NR	NR	NR

Abbreviations: ID: animal identification; PC: period of capture; A: adult; M: male; F: Female; CHIK-IgM: *A. chikungunya* anti-IgM assay; CHIK-IgG: *A. chikungunya* anti-IgG assay; DNV-IgM: *O. denguei* anti-IgM assay; ZK-IgM: *A. zikaense* anti-IgM assay; ZK-IgG: *A. zikaense* anti-IgG assay; YF-IgG: *O. flavi* fever anti-IgG assay; R: reactive; NR: not reactive.

4. DISCUSSION

This study investigated serological evidence of circulation of arboviruses in free-ranging black-tuffed marmosets (*C. penicillata*) from urban or periurban areas. Serological response was low with only two animals (2.94%) seroreactive to *A. chikungunya* and one (1.47%) reactive to *O. zikaense*. Both seroreactive animals were from *Parque das Mangabeiras* (PM), the biggest park in the city of Belo Horizonte with 236 hectares, and delimited by residential areas and by a mining area. Other studies also evaluated neotropical primates through serological tests. *A. chikungunya* and *O. zikaense* neutralizing antibodies were found in 2.9% of callithrichids from northeastern region of Brazil, one common marmoset (*C. jacchus*) was seroreactive to *A. chikungunya* and one black-tuffed marmoset reactive to *O. zikaense* (Moreira-Soto et al., 2018). Favoretto et al. (2019) investigated *O. zikaense* infection in peridomestic common marmosets in northeastern Brazil (Ceará) and one animal (1.8%, 1/55) had neutralizing antibodies against *O. zikaense*.

In 2022, Minas Gerais had 70,421 confirmed human cases of dengue infection, 6,382 of *A. chikungunya* infection and 18 of *O. zikaense* infection (SES-MG, 2022). *A. chikungunya* cases occurred mostly during two periods of the year, between March and April, and in December. *O. zikaense* infection in humans was less frequent than other arboviruses in this year and was distributed throughout the year. One animal was captured and evaluated two times in 2022, both times it was seroreactive to *A. chikungunya* and *O. zikaense* with IgM response in this animal persisting at least until July for both viruses. The first capture was in April, at the same time *A. chikungunya* was happening in human population. The other animal was captured in September of 2023 and was seroreactive to *A. chikungunya*. In this year, human *A.*

chikungunya cases were concentrated between February and May (SES-MG, 2023), which was before diagnosis in the marmoset.

Arboviruses can cross-react with each other in serological tests, which is more commonly observed between *O. zikaense* and *O. denguei* viruses. However, other flaviviruses can cross-react with each other and with *A. chikungunya* (Stettler et al., 2016; Endale et al., 2021; Kasbergen et al., 2023). Serologic studies with neotropical primates demonstrated cross-reactivity between *O. denguei* and other flaviviruses (*O. zikaense*, *O. nilense* and *O. louisense*) (Moreira-Soto et al., 2018; Chaves et al., 2021). Based on known cross-reactivity between these viruses, serological assays have limited specificity to determine which virus induced that serological response. One of the marmosets evaluated in this study had IgM against both *A. chikungunya* and *O. zikaense*, but it is impossible, based on serology, to determine which of these viruses triggered this antibody response, and exposure to both viruses cannot be ruled out as well.

Yellow fever is an important cause of death to neotropical primates (Santos et al., 2020; Fernandes et al., 2021). Frequency of death in marmosets associated with *O. flavi* infection is lower than other species. In this study all marmosets evaluated were non-reactive in the *O. flavi* serological assay, indicating that no recent exposure to the virus. Assays to determine the presence of neutralizing antibodies would be useful to access their susceptibility in a new outbreak.

Frequency of seroreactivity to arboviruses was low (2.94%) as reported by other studies with neotropical primates (Morales et al., 2017; Moreira-Soto et al., 2018; Chaves et al., 2021). Also, all seroreactive animals presented immunoglobulins M, and acute immunoglobulin, supporting the hypothesis that arboviral exposure in black-tuffed marmosets is low and may not result in persistent antibody production.

5. REFERENCES

CHIU, C.Y.; SÁNCHEZ-SAN MARTÍN, C.; BOUQUET, J. et al. Experimental Zika virus inoculation in a New World monkey model reproduces key features of the human infection. **Scientific Reports**, v. 7, n. 1, p. 17126, 2017.

ENDALE, A.; MEDHIN, G.; DARFIRO, K. et al. Magnitude of antibody cross-reactivity in medically important mosquito-borne flaviviruses: a systematic review. **Infection and Drug Resistance**, p. 4291-4299, 2021.

FAVORETTO, S.R.; ARAÚJO, D.B., DUARTE, N.F. et al. Zika Virus in peridomestic neotropical primates, Northeast Brazil. **Ecohealth**, v. 16, p. 61-69, 2019.

FERNANDES, N.C.C.A.; GUERRA, J.M.; DÍAZ-DELGADO, J. et al. Differential yellow fever susceptibility in new world nonhuman primates, comparison with humans, and implications for surveillance. **Emerging Infectious Diseases**, v. 27, n. 1, p. 47, 2021.

FERREIRA, M.S.; CASTRO, P.H.G.; SILVA, G.A. et al. *Callithrix penicillata*: a feasible experimental model for dengue virus infection. **Immunology Letters**, v. 158, n. 1-2, p. 126-133, 2014.

GIOVANETTI, M.; MENDONÇA, M.C.L.; FONSECA, V. et al. Yellow fever virus reemergence and spread in Southeast Brazil, 2016–2019. **Journal of Virology**, v. 94, n. 1, p. 10.1128/jvi. 01623-19, 2019.

GOULD, E. A.; SOLOMON, T. Pathogenic flaviviruses. **The Lancet**, v. 371, n. 9611, p. 500-509, 2008.

GOULD, E.; PETTERSON, J.; HIGGS, S. et al. Emerging arboviruses: Why today? **One Health**, v. 4, p. 1-13, 2017.

KASBERGEN, L.M.R.; NIEUWENHUIJSE, D.F.; BRUIN, E. et al. The increasing complexity of arbovirus serology: An in-depth systematic review on cross-reactivity. **PLoS Neglected Tropical Diseases**, v. 17, n. 9, p. e0011651, 2023.

MALUKIEWICZ, J.**;** BOERE, V.; OLIVEIRA, M.A.B. et al. An introduction to the *Callithrix* genus and overview of recent advances in marmoset research. **ILAR Journal**, v. 61, n. 2-3, p. 110-138, 2020.

MORALES, M.A.; FABBRI, C.M.; ZUNINO, G.E. et al. Detection of the mosquito-borne flaviviruses, West Nile, dengue, Saint Louis encephalitis, Ilheus, Bussuquara, and yellow fever in free-ranging black howlers (*Alouatta caraya*) of northeastern Argentina. **PLoS Neglected Tropical Diseases**, v. 11, n. 2, p. e0005351, 2017.

MOREIRA-SOTO, A.; CARNEIRO, I.D.O.; FISCHER, C. et al. Limited evidence for infection of urban and peri-urban nonhuman primates with Zika and chikungunya viruses in Brazil. **Msphere**, v. 3, n. 1, p. 10.1128/msphere. 00523-17, 2018.

OMATSU, T.; MOI, M.L.; HIRAYAMA, T. et al. Common marmoset (*Callithrix jacchus*) as a primate model of dengue virus infection: development of high levels of viraemia and demonstration of protective immunity. **Journal of General Virology**, v. 92, n. 10, p. 2272-2280, 2011.

SANTOS, D.O.; OLIVEIRA, A.R.; LUCENA, F.P. et al. Histopathologic patterns and susceptibility of neotropical primates naturally infected with yellow fever virus. **Veterinary pathology**, v. 57, n. 5, p. 681-686, 2020.

SES-MG (Secretaria de Estado de Saúde de Minas Gerais). Boletim epidemiológico: Arboviroses urbanas (dengue, chikungunya e zika), Nº 264 – Semana epidemiológica 51/2022. 26 de dezembro de 2022. Available at: https://www.saude.mg.gov.br/images/2022/BO_ARBO264.pdf

SES-MG (Secretaria de Estado de Saúde de Minas Gerais). Boletim epidemiológico: Arboviroses urbanas (dengue, chikungunya e zika), Nº 302 – Semana epidemiológica 51/2023. 26 de dezembro de 2023. Available at: https://www.saude.mg.gov.br/images/1_noticias/06_2023/1-out-nov-dez/26-12-BO_ARBO302.pdf

STETTLER, K.; BELTRAMELLO, M.; ESPINOSA, D.A. et al. Specificity, cross-reactivity, and function of antibodies elicited by Zika virus infection. **Science**, v. 353, n. 6301, p. 823-826, 2016.

STRIER, K.B.; TABACOW, F.P.; POSSAMAI, C.B. et al. Status of the northern muriqui (*Brachyteles hypoxanthus*) in the time of yellow fever. **Primates**, v. 60, p. 21-28, 2019.

TERZIAN, A.C.B.; ZINI, N.; SACCHETTO, L. et al. Evidence of natural Zika virus infection in neotropical non-human primates in Brazil. **Scientific Reports**, v. 8, n. 1, p. 1-15, 2018.

VANCHIERE, J.A.; RUIZ, J.C.; BRADY, A.G. et al. Experimental Zika virus infection of neotropical primates. **The American Journal of Tropical Medicine and Hygiene**, v. 98, n. 1, p. 173, 2018.

WEAVER, S.C.; CHARLIER, C.; VASILAKIS, N. et al. Zika, chikungunya, and other emerging vector-borne viral diseases. **Annual Review of Medicine**, v. 69, p. 395-408, 2018.

CHAPTER 4

Antimicrobial resistance of *Staphylococcus* spp. isolated from free-ranging black-tuffed marmosets (*Callithrix penicillata*) in urban parks.

Abstract

Marmosets (*Callithrix* sp.), including black-tuffed marmosets (*C. penicillata*), are neotropical primates that can be highly adapted to urban environments living in parks and forested areas near cities. *Staphylococcus* spp. are part of the microbiota of many different host and lead to opportunistic severe infection. Isolates from wild animals can be resistant to antimicrobial drugs, however few studies evaluated *Staphylococcus* spp. in neotropical primates. The goal of this study was to evaluate *Staphylococcus* spp. isolated from free-ranging black-tuffed marmosets. Over 30% of captured individuals were positive for *Staphylococcus* spp., and *S. aureus* was the most isolated species followed by *S. sciuri*. Most isolates were susceptible to the antimicrobials used, however one isolate of *S. epidemidis* was resistant to multiple antimicrobials (penicillin, cefoxitin, ciprofloxacin, clindamycin and erythromycin). We considered *S. aureus* as the main staphylococci to colonize black-tuffed marmosets.

Keywords: neotropical primates, staphylococci, *S. aureus*, *S. sciuri*.

1. INTRODUCTION

Marmosets (*Callithrix* sp.) are neotropical primates native to Northeaster and Southeastern regions of Brazil, with six different species (Colman et al., 2020; Malukiewicz et al., 2020; Sales et al., 2024). Some of them are highly adapted to urban environments living in parks and forested areas near big cities, which leads to direct and indirect contact to humans (Goulart et al., 2010; Svensson et al., 2023; Sales et al., 2024). Black-tuffed marmosets (*Callithrix penicillata*) are present in the city of Belo Horizonte (Minas Gerais, Brazil), where they are frequently found in parks (Goulart et al., 2010; Silva et al., 2018; Pacheco et al., 2021).

Staphylococcus spp. are part of the microbiota of many different host and lead to opportunistic severe infection (Espinosa-Gongora et al., 2012; Godoy et al., 2016; Santana et al., 2022c; Souza et al., 2024). Also, interaction between humans and wild animals can induce changes in *Staphylococcus* spp. colonization profile in free-ranging animals and anthropization of forested areas facilitates the transmission of pathogens (Godoy et al., 2016; Sales et al., 2024; Suzuki et al., 2024).

Studies have shown *Staphylococcus* spp. isolates from wild animals can be resistant to antimicrobial drugs, supporting the hypothesis that wild animals can act as reservoirs of drug resistant *Saphylococcus* and transmit them to humans and other animals (Porrero et al., 2014; Radhouani et al., 2014; Vittecoq et al., 2016; Fessler et al., 2018; Heaton et al., 2020; Abdullahi et al., 2021; Santana et al., 2022c; Santana et al., 2023). In addition, callithrichids are genetically similar to humans, favoring interactions between bacterial organisms of the microbiota, in which multi-resistant organisms can be concerning (Colman et al., 2020; Malukiewicz et al., 2020; Sheh, 2020).

Despite the growing importance of wild animals in the epidemiology of antimicrobial-resistant pathogens, there are few studies that evaluate bacteria of the genus *Staphylococcus* in primates, especially in neotropical species. The goal of this study was to evaluate frequency, distribution and antimicrobial susceptibility of *Staphylococcus* spp. isolated from free-ranging black-tuffed marmosets (*Callithrix penicllata*) captured in parks in the city of Belo Horizonte (Minas Gerais, Brazil).

2. MATERIAL AND METHODS

2.1. Ethics

All procedures were authorized by the Ethics Committee on the Use of Animals of the Universidade Federal de Minas Gerais (CEUA/UFMG) under protocol number 78/2022, by Instituto Chico Mendes de Conservação de Biodiversiade under protocol number 81392, and by Fundação de Parques Municipais e Zoobotânica de Belo Horizonte (FPMZ-BH) under protocol numbers FU002/2022, and Sisgen under protocols ABB00DB and ACDCB02.

2.2. Animal procedures

Animals were captured in six parks in the city of Belo Horizonte (Minas Gerais, Brazil): *Parque das Mangabeiras* (PM), *Parque da Serra do Curral* (SC), *Parque Ursulina de Andrade Mello* (U), *Parque Aggeo Pio* (AP), *Parque Fazenda Lagoa do Nado* (LN), and Belo Horizonte Zoo (Z) (Figure 2.1).

Traps baited with bananas were placed in multiple places in each park and checked twice a day. When captured, animals were weighted and sedated with ketamine (IM, 20 mg/kg) and midazolam (IM, 1 mg/kg). During sedation, animals were clinically evaluated, and samples were collected. Clinical evaluation consisted of external exam, body condition, superficial lymph nodes palpation, abdominal palpation, respiratory frequency, heartbeats frequency and rectal temperature. Age was estimated based on weight, physical characteristics and reproductive condition. Infants weight less than 100 g, fur details are not fully defined, and tuffs are shorter. Young marmosets weighted between 100 and 250 g, fur details are also not fully defined, and tuffs are shorter. Adults weight more than 250 g, fur details are fully defined, and tuffs are longer. Pregnant and lactating females were also considered adults.

From each animal two swabs were collected, one from skin (axillary region) and one from the rectum, and placed in sterile microtubes. When available, feces samples were collected in sterile containers. All samples were refrigerated until analysis. All animals were tagged with subcutaneous microchips for later identification. After total recovery from sedation, animals were released near the same place of capture inside the parks. Dead marmosets found in the places of capture were sent to necropsy.

2.3. *Staphylococcus* spp. isolation and identification

Samples were plated in mannitol sal agar (Difco, USA) and incubated for 24 hours at 37°C. Colonies with morphology suggestive of *Staphylococcus* spp. were plated in Brain Heart Infusion (BHI) agar (Difco, USA) and identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with FlexControl MicroFlex LT mass spectrometer (Bruker Daltonics, USA) as previously described (Assis et al.., 2017; Santana et al.., 2023). Isolates with scores lower than 2,300 were identified by sequencing of *rpoB* and *16S rRNA* genes (Mellmann et al., 2006; Abellan-Schneyder et al., 2021). Isolates belonging to *Staphylococcus intermedius* group (SIG) were differentiated by PCR targeting the *nuc* gene (Sasaki et al., 2010).

2.4. Antimicrobial susceptibility and *mecA* gene detection

All *Staphylococcus* spp. isolates were submitted to antimicrobial susceptibility test by disk diffusion method according to Clinical and Laboratory Standards Institute documents M100-S30 and VET01S (CLSI, 2020a; CLSI, 2020b). The following antimicrobials and their concentration were tested: cefoxitin (30 µg), penicillin (10 UI), tetracycline (30 µg), sulfamethoxazole-trimethoprim (25 µg), chloramphenicol (30 µg), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), ciprofloxacin (5 µg), rifampicin (5 µg) (DME, Brazil). *Staphylococcus aureus* ATCC 25923 was used as control strain. Cefoxitin resistant *Staphylococcus* spp. isolates were submitted to DNA extraction according to Pitcher et al. (1989) and to PCR targeting *mecA* gene as previously described (Murakami et al., 1991) to evaluated methicillin resistance.

2.5. Anatomopathological analysis

Dead free-ranging black-tuffed marmosets submitted to necropsy were grossly evaluated and samples from several organs were collected in 10% formalin solution. Samples for bacterial isolation were collected with sterile swabs based on the presence of suggestive gross lesions. Formalin fixed samples were routinely processed by dehydration in increasing concentrations of alcohol, clarified in xylene and embedded in paraffin. Sections of 3-4 µm were stained with hematoxylin and eosin, and with Gram stain when necessary.

2.6. Statistical analysis

GraphPad Prism software (version 8.0.1) was used to analyze data. Frequencies were evaluated by Fisher's exact test or Chi-square test.

3. RESULTS

3.1. Isolation and identification of *Staphylococcus* spp.

Samples from 76 marmosets resulted in 30 *Staphylococcus* spp. isolates and a frequency of 31.57% (24/76) of *Staphylococcus* spp. positive individuals. From all *C. penicillata* evaluated, 34.88% (15/43) of the males and 27.27% (9/33) of females were positive for *Staphylococcus* sp. with no statistical difference between them (p = 0.6196). Regarding age, 30.65% (19/62) of the adults and 35.71% (5/14) of the young were positive for *Staphylococcus* sp. with no statistical difference (p = 0.7554). Animals were captured in multiple locations, PM had the most *Staphylococcus*-positive individuals with 37.5% (9/24) followed by Z with 25% (6/24) and LN with 20.83% (5/24). Table 4.1 details the *Staphylococcus* species isolated and their frequency by location of the captured *C. penicillata*.

Table 4.1. Distribution of *Staphylococcus* spp. isolated from free-ranging *Callithrix penicillata* captured in parks in Belo Horizonte (Minas Gerais, Brazil).

***Staphylococcus* species**	**Capture place of sampled *C. penicillata***						**Total (%)**
	PM	LN	Z	U	SC	AP	
S. aureus	7 (58.3)	0 (0.0)	3 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	10 (33.3)
S. sciuri	0 (0.0)	3 (60)	1 (11.1)	1 (33.3)	1 (100)	0 (0.0)	6 (20)
S. caprae	0 (0.0)	0 (0.0)	3 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	3 (10)
S. epidermidis	1 (8.3)	0 (0.0)	2 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)	3 (10)
S. warnieri	1 (8.3)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	2 (6.6)
S. intermedius	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.3)
S. saprophyticus	0 (0.0)	1 (20)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.3)
S. succinus	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.3)
S. xylosus	0 (0.0)	1 (20)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.3)
S. klossi	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	1 (3.3)
S. delphini	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.3)

PM: *Parque das* Mangabeiras; LN: *Parque Fazenda Lagoa do Nado*; Z: Belo Horizonte Zoo; U: *Parque Ursulina de Andrade* Mello; SC: *Parque da Serra do Curral*; AP: *Parque Aggeo Pio*;

Twenty-seven (90%) isolates were identified by MALDI-TOF, two (6.66%) were confirmed by sequencing of the *rpoB* and *16S r*RNA genes and one (3.33%) by sequencing of the *nuc* gene. Most positive individuals were colonized by only one species of *Staphylococcus* spp. (20/24) while three animals were colonized by two and one animal was colonized by three different species of *Staphylococcus* spp. *S. aureus* was the most isolated species with 33.33% (10/30) of the isolates, followed by *S. sciuri* representing 20% of the isolates. One of the animals infected by *S. aureus* presented extensive cutaneous lesions and was found in the Belo Horizonte Zoo. The animal was then evaluated by the Zoo's veterinary team, euthanized, and submitted to necropsy.

3.2. Antimicrobial resistance profile

Susceptibility to antimicrobials were evaluated in all 30 *Staphylococcus* spp. isolates. Twelve isolates (40%) were resistant to at least one antimicrobial and eighteen (60%) were sensible to all antimicrobials tested. Penicillin resistance was observed in most isolates (23.33%), followed by trimethoprim-sulfamethoxazole (6.66%) (Figure 4.1). All isolates were sensible to tetracyclin, chloramphenicol, gentamicin, nitrofurantoin and rifampicin. Methicilin resistance was evaluated through PCR targeting *mecA* gene, and no isolates were considered resistant. One isolated, identified as *S. epidermidis*, was resistant to multiple antimicrobials (penicillin, cefoxitin, ciprofloxacin, clindamycin and erythromycin) (Table 4.2).

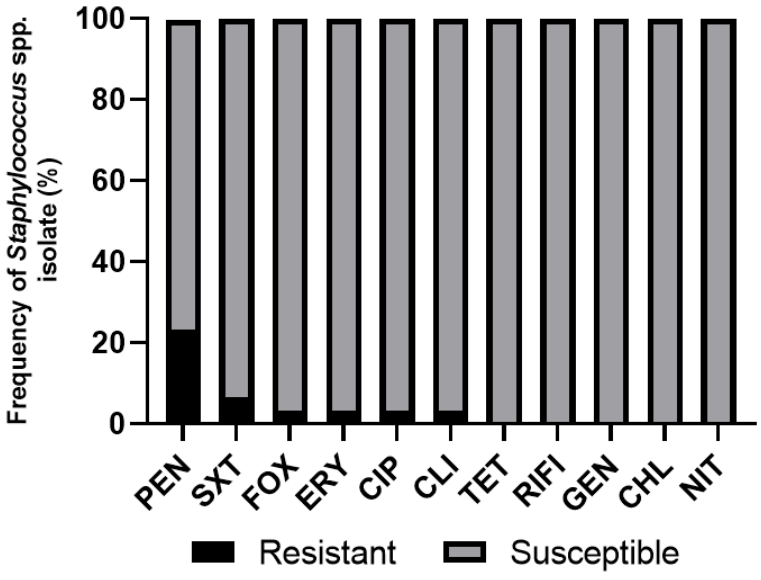

Figure 4.1. Frequency of antimicrobial resistance of *Staphylococcus* spp. isolated from free-ranging *Callithrix penicillata* by antimicrobial. PEN: penicillin, SXT: trimethoprim-sulfamethoxazole, FOX:

cefoxitin, ERY: erythromycin, CIP: ciprofloxacin, CLI: clindamycin, TET: tetracyclin, RIF: rifampicin, GEN: gentamicin, CHL: chloranfenicol, NIT: nitrofurantoin.

Table 4.2 shows information of marmosets positive for *Staphylococcus* spp. that presented antimicrobial resistance. *Staphylococcus* spp. with antimicrobial resistance were isolated from five marmosets, most animals were adult males (80%, 4/5). Most of the resistant isolates were from marmosets captured in PM and Z (40% each). The multi-resistant *S. epidermidis* was isolated from a marmoset captured at Z.

Table 4.2. Antimicrobial resistance of *Staphylococcus* spp. isolated from free-ranging *Callithrix penicillata* by animal.

ID	Sex	Age	Capture place	*Staphylococcus* species	Antimicrobial resistance
C009	**M**	**A**	**U**	*S. warneri*	PEN
C024	**M**	**A**	**PM**	*S. aureus*	PEN-SXT
				S. warneri	PEN
C031	**M**	**A**	**PM**	*S. succinus*	PEN
C044	**M**	**A**	**Z**	*S. epidermidis*	PEN-FOX-CIP-CLI-ERY
C045	**F**	**Y**	**Z**	*S. aureus*	PEN-SXT

M: male; F: female; A: adult; Y: young; U: *Parque Ursulina de Andrade* Mello; PM: *Parque das* Mangabeiras; Z: Belo Horizonte Zoo; PEN: penicillin, FOX: cefoxitin, CIP: ciprofloxacin, CLI: clindamycin, ERY: erythromycin; SXT: trimethoprim-sulfamethoxazole.

3.3. Anatomopathological evaluation of *S. aureus* positive *C. penicillata*

One free-ranging black-tuffed marmoset was found apathetic in Belo Horizonte Zoo. The marmoset was captured by the Zoo's veterinarians for clinical evaluation. The animal presented multiple finger fractures and cutaneous exudative lesions on the thoracic limbs and head. The animal was then euthanized and submitted to necropsy.

Grossly, in the skin of the thoracic limbs and head there were multiple perforations (Figure 4.2A). Adjacent subcutaneous showed moderate multifocal to coalescent hemorrhage (Figures 4.2B and C, red arrow). Skeletal muscles of the thoracic limbs had multiple areas of muscle loss associated with yellow-white exudate (Figure 4.2C, yellow arrow). The left salivary gland was adjacent to an area of skin perforation and had a focal extensive reddish area. The

external layer of abdominal wall muscle had a focal round area of discontinuity with mild white-yellowish exudate (Figure 4.2B).

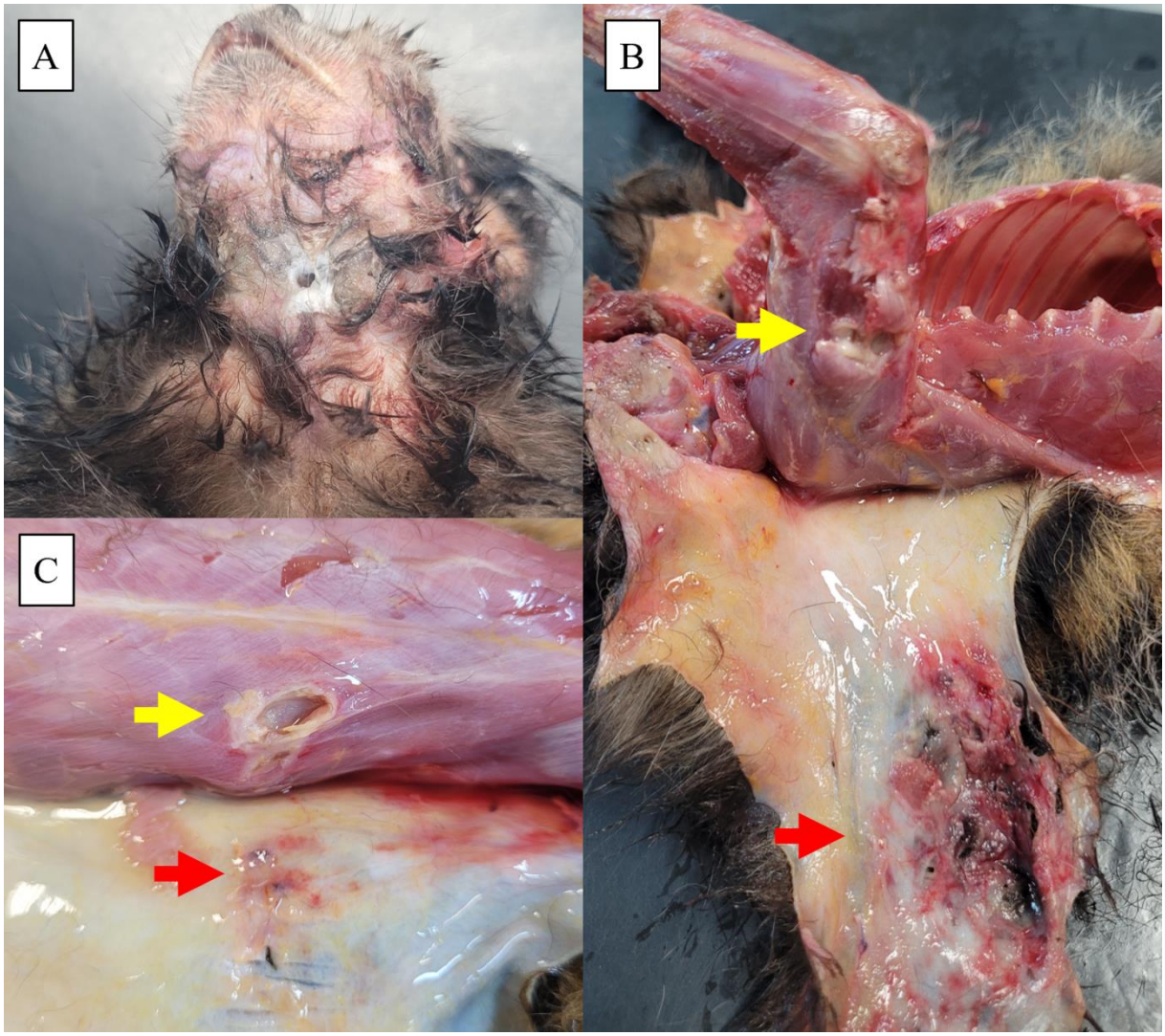

Figure 4.2. Gross lesions of *Staphylococcus aureus* infection in a free-ranging female adult black-tuffed marmoset (*Callithrix penicillata*). A. Submandibullary skin with focal area of perforation. B. Right thoracic limb, subcutaneous with multiple areas of skin perforation and hemorrhage (red arrow). Skeletal muscles of the arm with focal area of loss with yellow-white exudate (yellow arrow). C. Abdominal wall with focal area of discontinuity of the external muscle layer with mild yellow-white exudate (yellow arrow). Subcutaneous with multifocal mild hemorrhage (red arrow).

Histologically, skin lesions consisted of multifocal areas of coagulative necrosis with abundant Gram-positive cocci that extended from the epidermis to the subcutaneous (Figure 4.3A and B). A few vessels on the dermis were partially occluded by fibrin thrombi. Adjacent muscle had multifocal necrosis with neutrophilic and histiocytic intense infiltrate associated

with bacteria (Figure 4.3 E and F). The abdominal wall muscle had a focal area of discontinuity of the external layer with loss and necrosis of myocytes associated with neutrophilic and histiocytic infiltrate and abundant Gram-positive cocci. Salivary gland had a focally extensive area of necrosis with abundant Gram-positive cocci (Figure 4.3C and D). Other lesions included mild neutrophilic interstitial pneumonia and mild focal splenosis.

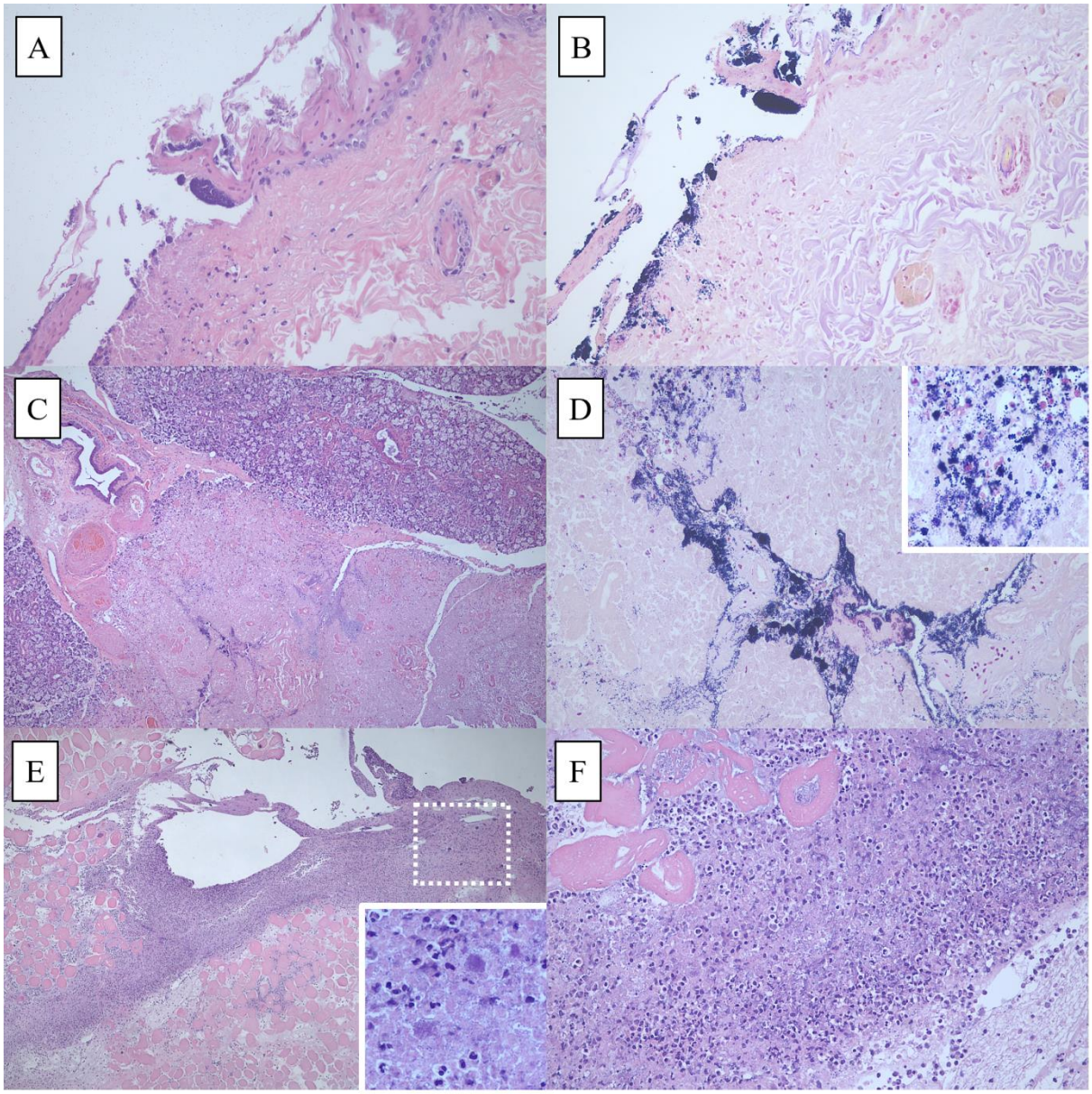

Figure 4.3. Histologic lesions of *Staphylococcus aureus* infection in a free-ranging female adult black-tuffed marmoset (*Callithrix penicillata*). A and B. Skin, focally extensive area of coagulative necrosis with multifocal abundant Gram-positive cocci. A. HE, 20x. B. Gram stain, 20x. C and D. Salivary gland, locally extensive area of necrosis with abundant Gram-positive cocci (inset). C. HE, 5x. D. Gram stain, 10x and 40x (inset). E and F. Skeletal muscle (thoracic

limb), locally extensive area of myocyte loss with intense neutrophilic infiltrate with abundant cocci (inset) and myocyte necrosis (F). E. HE, 5x and 40x (inset). F. HE, 20x.

Swabs from skin collected before death and swabs from skeletal muscle were submitted to bacterial isolation resulting in the identification of *S. aureus*. The isolate was sensible to all antimicrobials tested.

4. DISCUSSION

Studies about bacterial microbiota in free-ranging *Callithrix* sp. are rare, especially about *Staphylococcus* spp. This study described several species of *Staphylococcus* in free-ranging *C. penicillata*, as was also reported in other wild animals, like wild mammals, birds of prey, red-footed tortoise (*Chelonoidis carbonaria*) and amazonian manatees (*Trichechus inunguis*) and also in domestic animals (Weiss et al.., 2013; Sousa et al.., 2014; Sousa et al., 2016; Lee et al.., 2019; García et al., 2020; Lauková et al.., 2020; Santana et al., 2022c; Souza et al., 2024).

One third of the staphylococci isolated from *C. penicillata* in this study were *S. aureus*, the most important species of *Staphylococcus* genus, known to colonize humans and eventually leads to infection in multiple organs (Kwiecinski & Horswill, 2020; Hatlen & Miller, 2021; Chung, 2023). *S. aureus* have been reported in several wild animals including non-human primates (Porrero et al., 2014; Schaumburg et al.., 2015; Seinige et al., 2017; Fessler et al., 2018; Mätz-Rensing & Lowenstine, 2018; Molina et al., 2019; Chong et al.., 2020; Silva et al.., 2023; Suzuki et al., 2024).

Staphylococcus spp. were isolated from non-human primates (Schaumburg et al.., 2015; Chong et al.., 2020; Silva et al.., 2023). *S. aureus* was isolated from free-ranging *L. rosalia* and *Callithrix* sp. captured in preserved areas of Atlantic forest and urban areas of Rio de Janeiro (Brazil) with a higher frequency in *L. rosalia* (Sales et al., 2024). Captive golden lion tamarins (*Leontopithecus rosalia*), golden-headed lion tamarins (*L. chrysomelas*) and black lion tamarins (*L. chrysopygus*) can carry staphylococci as part of the vaginal microbiota, *S. simulans* was most frequently isolated but other species, including *S. aureus* was also present (Lilenbaum et al., 2006). These results support the hypothesis that *Staphylococcus aureus* is the main staphylococcal species in the microbiota of callithrichids, including *C. penicillata*, similar to what happens in humans and other wild animals (Albuquerque et al., 2020; Sales et al., 2024).

S. sciuri was the second most isolated staphylococci here, only after *S. aureus*. *S. sciuri* was the most isolated from free-ranging *Callithrix* sp. in a study in preserved and urbanized areas, and was also isolated from *L. rosalia* (Sales et al., 2024). Other studies described *S. sciuri* colonizing wild animals as red-footed tortoise (*Chelonoidis carbonaria*), amazonian manatees (*Trichechus inunguis*), pigeons (*Columba livia*), south american coatis (*Nasua nasua*) and other wild mammals (Sousa et al., 2016; Lee et al., 2019; Ruiz-Ripa et al., 2019; García et al., 2020; Santana et al., 2022c; Santana et al., 2022b; Souza et al., 2024). *S. sciuri* was also described associated with active infection in both humans and animals, including non-human primates (Carvalho et al., 2022; Sacramento et al., 2022; Thomson et al., 2022).

A high frequency of *Staphylococcus* spp. isolated here were resistant to penicillin which was also observed in previous studies in wild animals (Ruiz-Ripa et al., 2019; Santana et al., 2022c; Santana et al., 2023; Souza et al., 2024). This resistance may be associated with the intense use of beta-lactam antibiotics in both human and veterinary medicine, and staphylococci can acquire resistance determinants directly or indirectly (McEwen & Collignon, 2018; Loncaric et al., 2019b; Rebelo et al., 2021; Verstraete et al., 2022). Most isolates with antimicrobial resistance were isolated from marmosets captured in PM and the Zoo, and the multi-resistant strain of *S. epidermidis* was isolated from a marmoset captured in the Zoo. In both places there are hospitals nearby, near PM there is a human hospital and inside the Zoo there is a veterinary hospital to attend animals that reside in the Zoo. Isolates with antimicrobial resistance reported here could be associated with hospital environments.

Parque das Mangabeiras (PM) had the most frequency of staphylococci-positive *C. penicillata*. This park is the biggest urban park in the city of Belo Horizonte and receives many visitors throughout the years. In the same location other studies were conducted evaluating *Staphylococcus* spp. in free-ranging wild mammals, including rodents, marsupials, and coatis (*N. nasua*) (Santana et al., 2022a; Santana et al., 2023). The diversity of staphylococcal species described here can be associated with *C. penicillata* interaction with other animals and humans, as a direct or indirect result of the anthropization of forested areas (Carroll et al., 2015; Vittecoq et al., 2016; Sacristán et al., 2020; Sales et al., 2024).

One marmoset found in the Belo Horizonte Zoo presented cutaneous and muscular necrotic and inflammatory lesions associated with *S. aureus* infection. *S. aureus* is part of the microbiota of non-human primates (Mätz-Rensing & Lowenstine, 2018). Disease associated with *Staphylococcus* spp. usually start in the skin and can evolve to systemic infection. *Staphylococcus*-associated lesions were reported in free-ranging marmosets and capuchins with

meningitis, bronchopneumonia and bacteremia (Oliveira & Santos, 2023). In the marmoset reported here the lesions associated with *S. aureus* infection were limited to skin, subcutaneous, skeletal muscle and salivary gland. Since the animal was euthanized, the natural development of the diseases was interrupted. If not interrupted or treated the infection could evolve to a systemic infection.

Results suggest that black-tuffed marmosets are colonized by several species of *Staphylococcus*, mainly by *S. aureus*. Most isolates were sensible to most antimicrobials tested here, however one isolate was multi-resistant and others were resistant to penicillin and sulfonamide. One animal also presented cutaneous disease associated with *S. aureus* infection. More studies are needed to better understand the relationship between antimicrobial resistance and antropization of forested areas, and the epidemiology of antimicrobial resistance of *Staphylococcus* spp. in free-ranging wild animals.

5. REFERENCES

ABDULLAHI, I.N.; FERNÁNDEZ-FERNÁNDEZ, R.; JUÁREZ-FERNÁNDEZ, G. et al. Wild Animals Are Reservoirs and Sentinels of *Staphylococcus aureus* and MRSA Clones: A Problem with "One Health" Concern. **Antibiotics (Basel, Switzerland)**, v.10, p.1556, 2021.

ABELLAN-SCHNEYDER, I.; MATCHADO, M.S.; REITMEIER, S. et al. Primer, Pipelines, Parameters: Issues in 16S rRNA Gene Sequencing. **mSphere**, v.6, p.10.1128/msphere.01202-20, 2021.

ADIGUZEL, M.C.; SCHAEFER, K.; RODRIGUEZ, T. et al. Prevalence, Mechanism, Genetic Diversity, and Cross-Resistance Patterns of Methicillin-Resistant *Staphylococcus* Isolated from Companion Animal Clinical Samples Submitted to a Veterinary Diagnostic Laboratory in the Midwestern United States. **Antibiotics (Basel, Switzerland)**, v.11, p.609, 2022.

ALBUQUERQUE, D.D.A.; FIGUEIREDO, F.B.; BRANDÃO, M.L. et al. Microbiota oral e retal de calitriquídeos (*Callithrix* sp.) em área antropizada de Mata Atlântica, Rio de Janeiro, Brasil. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.72, p.1113–1121, 2020.

ASSIS, G.B.N.; PEREIRA, F.L.; ZEGARRA, A.U. et al. Use of MALDI-TOF Mass Spectrometry for the Fast Identification of Gram-Positive Fish Pathogens. **Frontiers in Microbiology**, v.8, p.1492, 2017.

CARROLL, D.; WANG, J.; FANNING, S. et al. Antimicrobial Resistance in Wildlife: Implications for Public Health. **Zoonoses and Public Health**, v.62, p.534–542, 2015.

CARVALHO, T.P.; MOREIRA, L.G.A.; VIEIRA, A.D.; et al. *Mammaliicoccus* (*Staphylococcus*) *sciuri*-induced suppurative meningoencephalitis and bacteremia in an infant western lowland gorilla (*Gorilla gorilla gorilla*). **Journal of Medical Primatology**, v.51, p.396–399, 2022.

CHONG, C.W.; ALKATHEERI, A.H.S.; ALI, N. et al. Association of antimicrobial resistance and gut microbiota composition in human and non-human primates at an urban ecotourism site. **Gut Pathogens**, v.12, p.14, 2020.

CHUNG, P.Y. Immunotherapies for the prevention and treatment of *Staphylococcus aureus* infections: updates and challenges. **Pathogens and Disease**, v.81, p.ftad016, 2023.

CLSI. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals. CLSI supplement VET01S (ISBN 978-1-68440-092-8 [Print];

ISBN 978-1-68440-093-5 [Electronic]). **Clinical and Laboratory Standards Institute,** 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2020a.

CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100, 19087. 2020b.

COLMAN, R.J.; CAPUANO, S.; BAKKER, J.et al.. Marmosets: Welfare, Ethical Use, and IACUC/Regulatory Considerations. **ILAR Journal**, v.61, p.167–178, 2020.

ESPINOSA-GONGORA, C.; CHROBAK, D.; MOODLEY, A.; et al. Occurrence and distribution of *Staphylococcus aureus* lineages among zoo animals. **Veterinary Microbiology**, v.158, p.228–231, 2012. DOI: 10.1016/j.vetmic.2012.01.027.

FESSLER, A.T.; THOMAS, P.; MÜHLDORFER, K. et al. Phenotypic and genotypic characteristics of *Staphylococcus aureus* isolates from zoo and wild animals. **Veterinary Microbiology**, v.218, p.98–103, 2018.

GARCÍA, L.A.; TORRES, C.; LÓPEZ, A.R. et al. *Staphylococcus* Spp. from Wild Mammals in Aragón (Spain): Antibiotic Resistance Status. **Journal of Veterinary Research**, v.64, p.373–379, 2020.

GODOY, I.; MORAES, D.F.S.D.; PITCHENIN, L.C. et al. Antimicrobial susceptibility profiles of *Staphylococcus* spp. from domestic and wild animals. **Ciência Rural**, v.46, p.2148–2151, 2016.

GOULART, V.D.L.R.; TEIXEIRA, C.P.; YOUNG, R.J. Analysis of callouts made in relation to wild urban marmosets (*Callithrix penicillata*) and their implications for urban species management. **European Journal of Wildlife Research**, v.56, p.641–649, 2010.

HATLEN, T.J.; MILLER, L.G. Staphylococcal Skin and Soft Tissue Infections. **Infectious Disease Clinics of North America**, v.35, p.81–105, 2021.

HEATON, C.J.; GERBIG, G.R.; SENSIUS, L.D. et al. *Staphylococcus aureus* Epidemiology in Wildlife: A Systematic Review. **Antibiotics (Basel, Switzerland)**, v.9, p.E89, 2020.

KWIECINSKI, J.M.; HORSWILL, A.R. *Staphylococcus aureus* bloodstream infections: pathogenesis and regulatory mechanisms. **Current Opinion in Microbiology**, v.53, p.51–60, 2020.

LAUKOVÁ, A.; BINO, E.; KUBAŠOVÁ, I. et al. Characterization of Faecal Staphylococci from Roe Deer (*Capreolus capreolus*) and Red Deer (*Cervus elaphus*) and Their Susceptibility to Gallidermin. **Probiotics and Antimicrobial Proteins**, v.12, p.302–310, 2020.

LEE, S.; HWANG, J.; KIM, J. et al. Biofilm production of coagulase-negative staphylococci isolated from rescued wild animals in the Republic of Korea. **Acta Veterinaria Scandinavica**, v.61, p.50, 2019.

LILENBAUM, W.; MORAES, I.A.; CARDOSO, V.S. et al. Antibiotic resistance in Staphylococci isolated from the vaginas of captive female *Leontopithecus* (Callitrichidae–Primates). **American Journal of Primatology: Official Journal of the American Society of Primatologists**, v. 68, n. 8, p. 825-831, 2006.

LONCARIC, I.; KÜBBER-HEISS, A.; POSAUTZ, A. et al. Characterization of mecC gene-carrying coagulase-negative *Staphylococcus* spp. isolated from various animals. **Veterinary Microbiology**, v.230, p.138–144, 2019a.

LONCARIC, I.; TICHY, A.; HANDLER, S. et al. Prevalence of Methicillin-Resistant *Staphylococcus* sp. (MRS) in Different Companion Animals and Determination of Risk Factors for Colonization with MRS. **Antibiotics**, v.8, p.36, 2019b.

MALUKIEWICZ, J.; BOERE, V.; OLIVEIRA, M.A.B. et al. An Introduction to the *Callithrix* Genus and Overview of Recent Advances in Marmoset Research. **ILAR Journal**, v.61, p.110–138, 2020.

MARTÍNEZ-SANTOS, V.I.; TORRES-AÑORVE, D.A.; ECHÁNIZ-AVILES, G. et al. Characterization of *Staphylococcus epidermidis* clinical isolates from hospitalized patients with bloodstream infection obtained in two time periods. **PeerJ**, v.10, p.e14030, 2022.

MÄTZ-RENSING, K.; LOWENSTINE, L.J. **New World and Old World Monkeys**. In: TERIO, K.A.; MCALOOSE, D.; LEGER, J.S. Pathology of Wildlife and Zoo Animals. Elsevier Inc : Cambridge. 2018.

MCEWEN, S.A.; COLLIGNON, P.J. Antimicrobial Resistance: a One Health Perspective. **Microbiology Spectrum**, v.6, 2018.

MELLMANN, A.; BECKER, K.; EIFF, C. VON. et al. Sequencing and staphylococci identification. **Emerging Infectious Diseases**, v.12, p.333–336, 2006.

MOLINA, C.V.; BUENO, M.G.; KIERULFF, M.C.M. et al. Spontaneous meningoencephalitis by *Staphylococcus aureus* in an infant golden-headed lion tamarin (*Leontopithecus chrysomelas*). **Journal of Medical Primatology**, v. 48, n. 6, p. 370-373, 2019.

MURAKAMI, K.; MINAMIDE, W.; WADA, K. et al. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. **Journal of Clinical Microbiology**, v.29, p.2240–2244, 1991.

OLIVEIRA, A.R.; SANTOS, R.L. Infectious diseases of neotropical primates. **Brazilian Journal of Veterinary Pathology**, v. 16, n. 1, p. 1-34, 2023.

PACHECO, F.S.; VITAL, O.V.; ÁVILA, L.V. et al. Novas ocorrências de *Callithrix* na Zona da Mata de Minas Gerais. **Revista Cientifica MG.Biota**, v.14, p.50–68, 2021.

PITCHER, D. G.; SAUNDERS, N. A.; OWEN, R. J. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. **Letters in Applied Microbiology**, v.8, p.151–156, 1989.

PORRERO, M.C.; MENTABERRE, G.; SÁNCHEZ, S. et al. Carriage of *Staphylococcus aureus* by free-living wild animals in Spain. **Applied and Environmental Microbiology**, v.80, p.4865–4870, 2014. DOI: 10.1128/AEM.00647-14.

RADHOUANI, H.; SILVA, N.; POETA, P. et al. Potential impact of antimicrobial resistance in wildlife, environment and human health. **Frontiers in Microbiology**, v.5, 2014.

REBELO, J.S.; DOMINGUES, C.P.F.; MONTEIRO, F. et al. Bacterial persistence is essential for susceptible cell survival in indirect resistance, mainly for lower cell densities. **PloS One**, v.16, p.e0246500, 2021.

RUIZ-RIPA, L.; ALCALÁ, L.; SIMÓN, C. et al. Diversity of *Staphylococcus aureus* clones in wild mammals in Aragon, Spain, with detection of MRSA ST130-mecC in wild rabbits. **Journal of Applied Microbiology**, v.127, p.284–291, 2019.

SACRAMENTO, A.G.; FUGA, B.; MONTE, D.F.M. et al. Genomic features of *mecA*-positive methicillin-resistant *Mammaliicoccus sciuri* causing fatal infections in pets admitted to a veterinary intensive care unit. **Microbial Pathogenesis**, v.171, p.105733, 2022.

SACRISTÁN, I.; ESPERÓN, F.; ACUÑA, F. et al. Antibiotic resistance genes as landscape anthropization indicators: Using a wild felid as sentinel in Chile. **Science of The Total Environment**, v.703, p.134900, 2020.

SALES, I.; VIEIRA-DA-MOTTA, O.; TAVARES, A. et al. Impact of human created environments in the pathogenic potential and antimicrobial resistance of staphylococci from wild neotropical primates in Brazil. **Comparative Immunology, Microbiology and Infectious Diseases**, v.104, p.102094, 2024.

SANTANA, J.A.; COLOMBO, S.A.; SILVA, B.A. et al. *Clostridioides difficile* and multi-drug-resistant staphylococci in free-living rodents and marsupials in parks of Belo Horizonte, Brazil. **Brazilian Journal of Microbiology**, v.53, p.401–410, 2022a.

SANTANA, J.A.; RAMOS, C.P.; SILVA, B.A. et al. Identification and characterization of pathogenic and multidrug-resistant bacteria in feral pigeons surrounding a veterinary hospital in Minas Gerais, Brazil. **Ciência Rural**, v.53, p.e20220244, 2022b.

SANTANA, J.A.; SILVA, B.A.; TREVIZANI, N.A.B. et al. Isolation and antimicrobial resistance of coagulase-negative staphylococci recovered from healthy tortoises in Minas Gerais, Brazil. **Ciência Rural**, v.52, 2022c.

SANTANA, J.A.; ZANON, I.P.; FREITAS, R.J.S.R. et al. DISTRIBUTION AND ANTIMICROBIAL RESISTANCE OF STAPHYLOCOCCI ISOLATED FROM FREE-LIVING SOUTH AMERICAN COATI (*Nasua nasua*) IN AN URBAN PARK IN MINAS GERAIS, BRAZIL. **Journal of Zoo and Wildlife Medicine**, v.54, p.578–583, 2023.

SASAKI, T.; TSUBAKISHITA, S.; TANAKA, Y. et al. Multiplex-PCR method for species identification of coagulase-positive staphylococci. **Journal of Clinical Microbiology**, v.48, p.765–769, 2010.

SCHAUMBURG, F.; PAULY, M.; ANOH, E. et al. *Staphylococcus aureus* complex from animals and humans in three remote African regions. **Clinical Microbiology and Infection**, v.21, p.345.e1-345.e8, 2015. DOI: 10.1016/j.cmi.2014.12.001.

SCHOENFELDER, S.M.K.; LANGE, C.; ECKART, M. et al. Success through diversity - how *Staphylococcus epidermidis* establishes as a nosocomial pathogen. **International Journal of Medical Microbiology: IJMM**, v.300, p.380–386, 2010.

SEINIGE, D.; VON ALTROCK, A.; KEHRENBERG, C. Genetic diversity and antibiotic susceptibility of *Staphylococcus aureus* isolates from wild boars. **Comparative Immunology, Microbiology and Infectious Diseases**, v.54, p.7–12, 2017.

SHEH, A. The Gastrointestinal Microbiota of the Common Marmoset (*Callithrix jacchus*). **ILAR Journal**, v.61, p.188–198, 2020.

SILVA, F.; MALUKIEWICZ, J.; SILVA, L.C. et al. A survey of wild and introduced marmosets (*Callithrix*: Callitrichidae) in the Southern and Eastern Portions of the State of Minas Gerais, Brazil. **Primate Conservation**, v.32, 2018.

SILVA, J.G.; CULUCHI, G.; PESTANA, C.P. et al. *Staphylococcus* nasal colonization in three species of non-human primates. **Brazilian Journal of Microbiology**, v.54, p.1267–1274, 2023.

SOUSA, M.; SILVA, N.; IGREJAS, G. et al. Genetic Diversity and Antibiotic Resistance Among Coagulase-Negative Staphylococci Recovered from Birds of Prey in Portugal. **Microbial Drug Resistance (Larchmont, N.Y.)**, v.22, p.727–730, 2016.

SOUSA, M.; SILVA, N.; IGREJAS, G. et al. Antimicrobial resistance determinants in *Staphylococcus* spp. recovered from birds of prey in Portugal. **Veterinary Microbiology**, v.171, p.436–440, 2014.

SOUZA, T.G.V.; XAVIER, R.G.C.; SANTANA, J.A. et al. Antimicrobial susceptibility of *Staphylococcus* sp. and *Escherichia coli* isolated from captive Amazonian manatee (*Trichechus inunguis*). **Ciência Rural**, v.54, p.e20230140, 2024.

SUZUKI, Y.; ISHITSUKA, T.; TAKAGI, M. et al. Isolation and genetic characterization of *Staphylococcus aureus* from wild animal feces and game meats. **Folia Microbiologica**, v.69, p.347–360, 2024.

SVENSSON, M.S.; NIJMAN, V.; SHEPHERD, C.R. Insights into the primate trade into the European Union and the United Kingdom. **European Journal of Wildlife Research**, v.69, p.51, 2023.

THOMSON, P.; GARCÍA, P.; MILES, J. et al. Isolation and Identification of *Staphylococcus* Species Obtained from Healthy Companion Animals and Humans. **Veterinary Sciences**, v.9, p.79, 2022.

VERSTRAETE, L.; VAN DEN BERGH, B.; VERSTRAETEN, N. et al. Ecology and evolution of antibiotic persistence. **Trends in Microbiology**, v.30, p.466–479, 2022.

VITTECOQ, M.; GODREUIL, S.; PRUGNOLLE, F. et al. Antimicrobial resistance in wildlife. **Journal of Applied Ecology**, v.53, p.519–529, 2016.

WEISS, S.; KADLEC, K.; FESSLER, A.T. et al. Identification and characterization of methicillin-resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Staphylococcus pettenkoferi* from a small animal clinic. **Veterinary Microbiology**, v.167, p.680–685, 2013.

XU, Z.; CAVE, R.; CHEN, L. et al. Antibiotic resistance and molecular characteristics of methicillin-resistant *Staphylococcus epidermidis* recovered from hospital personnel in China. **Journal of Global Antimicrobial Resistance**, v.22, p.195–201, 2020.