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

Ayisa Rodrigues de Oliveira

DISEASE INVESTIGATION IN FREE-RANGING NEOTROPICAL PRIMATES FROM THE BRAZILIAN ATLANTIC FOREST

Investigação de doenças que acometem primatas neotropicais de vida livre da Mata Atlântica Brasileira

Belo Horizonte
2022

Ayisa Rodrigues de Oliveira

DISEASE INVESTIGATION IN FREE-RANGING NEOTROPICAL PRIMATES FROM THE BRAZILIAN ATLANTIC FOREST

Investigação de doenças que acometem primatas neotropicais de vida livre da Mata Atlântica Brasileira

Versão final

Tese apresentada ao Programa de Pós-Graduação em Ciência Animal da Escola de Veterinária da Universidade Federal de Minas Gerais como requisito parcial para obtenção do grau de Doutora em Ciência Animal.

Área de concentração: Patologia Animal
Orientador: Prof. Dr. Renato de Lima Santos
Coorientadores: Prof.ª Dr.ª Tatiane Alves da Paixão e Prof. Dr. Marcelo Pires Nogueira de Carvalho

Belo Horizonte
2022

Oliveira, Ayisa Rodrigues de , 1987-
O48d Disease investigation in free-ranging neotropical primates from the Brazilian Atlantic Forest / Ayisa Rodrigues de Oliveira . - 2022 .
182 f.:il

Orientador: Renato de Lima Santos
Coorientadores: Tatiane Alves da Paixão
Marcelo Pires Nogueira de Carvalho
Tese (Doutorado) apresentada à Escola de Veterinária da Universidade Federal de Minas Gerais para obtenção do título de Doutora em Ciência animal.
Área de concentração: Patologia Animal.
Inclui Bibliografias.

1. Animais selvagens – Doenças - Teses - 2. Patologia - Teses – 3. Veterinária - Teses - I. Santo, Renato de Lima - II. Paixão, Tatiane Alves da – III. Carvalho, Marcelo Pires Nogueira de – IV. Universidade Federal de Minas Gerais, Escola de Veterinária – V. Título.

CDD – 636.089

Bibliotecária responsável Cristiane Patrícia Gomes – CRB2569
Biblioteca da Escola de Veterinária, Universidade Federal de Minas Gerais.

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

FOLHA DE APROVAÇÃO

AYISA RODRIGUES DE OLIVEIRA

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 05 de agosto de 2022, pela banca constituída pelos membros:

Dr.(a). Renato de Lima Santos - Presidente - Orientador(a)
Dr.(a). Karin Werther
Dr.(a). Jeann Leal
Dr.(a). Roosecelis Brasil Martines
Dr.(a). Josué Diaz Delgado

Documento assinado eletronicamente por **Renato de Lima Santos**, **Professor do Magistério Superior**, em 05/08/2022, às 18:38, 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 08/08/2022, às 09:18, 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 08/08/2022, às 10:23, 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 **Roosecelis Brasil Martines**, **Usuário Externo**, em 08/08/2022, às 20:40, 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 **Karin Werther**, **Usuária Externa**, em 09/08/2022, às 15:58, 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 **1630222** e o código CRC **3A07917F**.

AGRADECIMENTOS / ACKNOWLEDGMENT

"Part of the journey is the end."
Citando Tony Stark (filme *Vingadores: Ultimato*), chego no fim dessa jornada com o sentimento de plenitude e gratidão por todo o aprendizado, crescimento pessoal e acima de tudo, a oportunidade de poder ter divido esse momento da minha vida com pessoas tão especiais.

Agradeço ao meu marido, Moacir, por ter trilhado esse caminho comigo com tanto companheirismo e carinho. Sempre me incentivando, torcendo e vibrando com as minhas conquistas, mesmo quando o preço de cada decisão era estarmos a quilômetros de distância. Te amo muito, amore mio! Nossa jornada só começou...

Agradeço aos meus pais, Rosane e José, por sempre confiarem nas minhas escolhas e me ensinarem que o céu é o limite, mas que não importa o quão longe vamos, temos sempre para onde voltar. Vocês são meu porto seguro e minha inspiração!

Agradeço à minha irmã, Indira, minha grande conselheira e amiga! Sempre ao meu lado (as vezes via 5h de telefonema), acreditando no meu potencial e me apoiando de forma inabalável.

Agradeço a minha tia Solange, por me tirar do estresse do dia-dia, com o seu jeito divertido e sempre disposta a um "spa-day" com direito a massagens, tratamento de pele e muitas gargalhadas. Agradeço também a minha prima, Yasmin, por confiar muito na minha capacidade e sempre falar com orgulho da sua "prima veterinária"!

Agradeço aos grandes amigos que fiz nessa jornada, Daniel, Lucas e Thaynara. Obrigada pelo companheirismo, carinho e por dividirem comigo as insatisfações e conquistas dessa trajetória!

Agradeço aos amigos da UFMG, todos sempre dispostos a ajudar e tornando meu trabalho muito mais fácil e divertido! Em especial agradeço a Clarissa, Daniel, Izabela, Larissa, Mirtha, Monique, Nayara e Thaynara. Sentirei muitas saudades de compartilhar meu dia com vocês!

Agradeço à Leimar e à Valéria, mais do que técnicas, anjos do laboratório de histologia (205). Trabalhar com vocês é um grande prazer! Obrigada pelos ensinamentos, auxílios e conversas filosóficas!

Agradeço aos professores do Setor de Patologia Veterinária da Escola de Veterinária da UFMG, Felipe, Natália, Renato, Roberto, Rogéria e Roselene, por todos

os ensinamentos durante esse período. Em especial agradeço a Profa. Paula, que o pouco tempo que ficou conosco, somou muito para minha formação. Você é uma inspiração!

Agradeço ao meu orientador, Prof. Renato, por me guiar nessa jornada estimulando todo o meu potencial acadêmico e profissional. Obrigada pelo aprendizado, orientação e conselhos. O senhor é um exemplo de competência profissional e sou muito grata de ter tido a oportunidade de trabalhar ao seu lado!

Agradeço aos meus coorientadores, Profa. Tatiane e Prof. Marcelo, por todas as contribuições nesse trabalho e por acreditarem no meu potencial. Vocês são profissionais admiráveis!

I thank Jana, Rhonda, Roose and Tais for receiving me wonderfully, with so much affection and care, during the six months I spent in Atlanta. Thank you for the company, advice, help, and making me feel home - and for the waffles, coffees, and M&Ms as well!

I thank Jana for all the dedication to my project! Your contribution was and is being fundamental! I admire you so much and I am grateful to have had the chance to be mentored by you!

I also thank everyone at IDPB-CDC for their teachings and assistance in the development of this study. Special thanks to Amy, Brett, Chris, Cynthia, Felicia, Hannah, Jana, Jeffery, Josi, Julu, Luciana, Megan, Mitesh, Monica Spivey, Monica Peabody, Negar, Pamela, Rhonda, Roose, Sherif (*in memorian*) and Tais.

Agradeço à Fabiana Pizzolato e a todos do Instituto Municipal de Medicina Veterinária Jorge Vaitsman, que tornaram esse trabalho possível, tendo atuado com tamanha competência e dedicação na coleta e processamento dessas amostras.

Agradeço pôr fim aos órgãos financiadores desse projeto, CAPES, CNPq e Fresno Chaffee Zoo (Wildlife Conservation Fund), tornando o seu desenvolvimento possível.

“Sometimes the smallest step in the right direction ends up being the biggest step of your life. Tip toe if you must but take the step.”

- Naeem Callaway

RESUMO

Os primatas neotropicais são hospedeiros naturais e acidentais de várias doenças, inclusive zoonoses com grande importância na saúde pública, e, devido à antropização e fragmentação do seu habitat, estes animais são altamente expostos a inúmeros fatores antrópicos com impacto direto na sua sobrevivência. Estudos com foco em patologia de primatas não-humanos de cativeiro, principalmente de laboratórios, são comumente observados. Contudo, os estudos sobre as doenças que acometem as populações de vida livre são escassos. Neste contexto, este estudo objetivou investigar as doenças que afetam primatas neotropicais de vida livre da Mata Atlântica Brasileira, incluindo regiões periurbanas a urbanas e unidades de conservação, e que morreram naturalmente no período de janeiro de 2017 a junho de 2019. Fragmentos de órgãos, predominantemente fígado, baço, pulmão, rim e encéfalo, foram coletados nas necropsias dos primatas realizadas pelo Instituto Municipal de Medicina Veterinária Jorge Vaitsman do Estado do Rio de Janeiro (IJV/RJ) e foram avaliados por histopatologia, imuno-histoquímica, microscopia eletrônica de transmissão e análises moleculares de acordo com os achados histopatológicos observados e as suspeitas etiológicas. Portanto, neste estudo foi possível identificar e caracterizar os achados histopatológicos associados aos óbitos de PNM de vida-livre, com a identificação de importantes zoonoses como raiva, febre amarela e toxoplasmose. Foram também detectadas, pela primeira vez, infecção por *S. capitis*, *S. sanguinus, Moraxella* sp. e PIV-1 e 3. Adicionalmente, foram caracterizados os aspectos epidemiológicos e patológicos da platinossomíase e toxoplasmose em saguis (*Callithix* spp.) de vida-livre; e foi realizada uma descrição detalhada das doenças traumáticas em PNM de vida-livre, ressaltando o impacto do trauma nessas populações de vida-livre e com a descrição inédita de êmbolos não-trombóticos pulmonares causados por tecido hepático e nervoso. Por fim, foram apresentados resultados preliminares de doença renal em PNM de vida-livre com alta prevalência, identificação de imunomarcação glomerular para IgM e IgG e associação com maior frequência de hematopoiese extramedular.

Palavras-chave: Patologia. Animais selvagens. Primatas. Sagui. Bugio. Macaco-prego.

ABSTRACT

Neotropical primates are natural and accidental hosts for many infectious diseases, including zoonosis with high importance in public health; and, due to anthropization and fragmentation of their habitat, these animals are also highly exposed to innumerous anthropized factors that directly impact their survival. Studies focusing on pathology of captive, especially laboratory non-human primates are common. However, studies on diseases that affect wild populations are scarce. This study aimed to investigate the diseases that affect free-ranging neotropical primates of the Brazilian Atlantic Forest, including peri-urban to urbanized regions, and conservation units, that died naturally from January 2017 to June 2019. Tissues, mainly liver, spleen, lung, kidney and brain, were sampled during necropsies of primates at Municipal Institute of Veterinary Medicine Jorge Vaitsman of Rio de Janeiro State (IJV/RJ) and were evaluated by histopathology, immunohistochemistry, transmission electronic microscopy and molecular analyzes according to the histopathological findings and the suspected etiology. Therefore, we were able to identify the main pathological findings associated with death in free-ranging NWP with detailed histopathological features and immunophenotypic, ultrastructural and molecular characterization, detecting important zoonotic infections, such as rabies, YF and toxoplasmosis. Also, it was described for the first time in free-ranging NWP infection by *S. capitis*, *S. sanguinus, Moraxella* sp. and PIV-1 and 3. Additionally, we characterized the epidemiological and pathological aspects of platynosomiasis and toxoplasmosis infection in free-ranging marmosets (*Callithrix* spp.), both diseases with high importance in these animals. We also performed a detailed description of traumatic injuries in free-ranging NWP describing for the first time NTPE by liver and lung tissue in marmosets and highlighting the impact of trauma in free-ranging NWP population. Finally, we presented preliminary results of a high prevalent renal disease in free-ranging NWP with IgG and IgM glomerular immunolabelling and associated with high frequency of extramedullary hematopoiesis.

Keywords: Pathology. Wild animals. Primates. Marmoset. Howler-monkey. Capuchin.

FIGURE LIST

TABLE LIST

SUMMARY

INTRODUCTION

Neotropical primates (New World primates - NWP) are represented by five large families: Callithrichidae (tamarins and marmosets), Cebidae (capuchins and squirrel monkeys), Aotidae (owl monkeys), Pitheciidae (sakis, titi monkey and uakaris) and Atelidae (howler monkey, woolly monkey, muriqui and spider monkey), totalizing 204 species and sub-species (Rylands et al., 2011; Verona and Pissinatti, 2014). These groups have animals with variable sizes and weights, such as the small pigmy (*Cebuella pigmaea*) weighting 100 g and muriquis (*Brachyteles arachnoides*) weighting 14 kg (Shostell and Ruiz-Garcia, 2016). They have arboreal behavior and diversified eating habits, with species that consume predominantly fruits and leaves, while other species have diets based on invertebrates and small mammals (Verona and Pissinatti, 2014). These animals represent about 40% of all Amazon mammalian biomass, and its frugivorous behavior, together with other frugivorous animals, are responsible for the maintenance of about 80% of the neotropical plants (Shostell and Ruiz-Garcia, 2016).

All NWP are included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) indicating some degree of vulnerability of these species (Verona and Pissinatti, 2014). The Brazilian Atlantic Forest itself holds 24 species of NWP, being 20 endemic and nine critically endangered, according to IUCN Red List (Hirsh et al., 2006). There are many factors associated with this threatening, such as habitat loss and degradation, anthropization, illegal trade, hunting and emerging infectious diseases (Shostell and Ruiz-Garcia, 2016; Wilson et al., 2021; Ehlers et al., 2022). Importantly, NWP are natural and accidental hosts of various infectious agents, and due to the phylogenetic proximity with humans, these animals may often be considered reservoir and sentinels of important zoonoses, as exemplified in yellow fever (YF) outbreaks (Leite et al., 2008; Moreno et al., 2013).

Identification of diseases that affects free-ranging NWP, as well as the understanding of their pathogenesis, aids, in addition to the recognition of sentinels and potential reservoirs of infectious agents, also in the development of public health and *in situ* and *ex situ* conservation programs. Therefore, this study aimed to identify and characterize the pathological findings of free-ranging NWP that died from January 2017 to June 2019 in the state of Rio de Janeiro, through histochemical, ultrastructural, immunohistochemical and molecular techniques.

LITERATURE REVIEW

1. Neotropical primates (New World Primates - NWP)

NWP, also known as platyrrhines, are species from Primates Order with wide distribution in the Central and South America. These species differ from Old-World primates (OWP), or catarrhines, due to its flat nose and lateral nostrils (Verona and Pissinatti, 2014). There are some discussions in the literature about the taxonomy of the Platyrrhini Parvorder: some authors divide this group in five Families (Callithrichidae, Cebidae, Aotidae, Pitheciidae and Atelidae) (Rylands et al., 2011; Verona and Pissinatti, 2014); and others in three Families (Cebidae, Atelidae and Pitheciidae) (Verona and Pissinatti, 2014; Dumas and Mazzoleni, 2016), with 20 genus and 152 species (Rylands et al., 2011). In this study will be use the taxonomy described by Rylands et al. (2011).

Callithrichidae have seven genera, being four named as marmosets (*Callithrix*, *Cebuella*, *Callibella*, and *Mico*) and three as tamarins (*Saguinus*, *Leontopithecus*, and *Callimico*). This Family have the smallest primate's specie of the world, the 100 g-weight small pigmy (*Cebuella pigmaea*) and differ from the other Platyrrhini families due to its claws, instead of nails, useful to climb and access sap from the threes, an important feature from its diet; and due to the number of molars, two rather than three in each side of mandibular and maxillae. Also, the animals from this family usually have head ornaments such as tufts, crests, manes, and whiskers, and a long non-prehensible tail, are mainly arboreal, diurnal, and omnivorous, feeding from sap to insects and small vertebrates, according to availability (Verona and Pissinatti, 2014; Shostell and Ruiz-Garcia, 2016). Tamarins and marmoset are extensively used in biomedical research, being the common marmoset (*Callithrix jacchus*) the most widely NWP used in experimental laboratories, due to its small size, easy breeding and well adaptation to captivity (Whitney, 1995).

Cebidae holds two to three genera of monkeys: capuchins – *Cebus* and *Sapajus*; and squirrel monkeys - *Saimiri* (Verona and Pissinatti, 2014; Shostell and Ruiz-Garcia, 2016). The existence of *Sapajus* as a different genus from *Cebus* is still questionable (Shostell and Ruiz-Garcia, 2016). Capuchins are medium-size monkeys with 2.5 to 5 kg, with a semi prehensile tail and thick molars (Shostell and Ruiz-Garcia, 2016).

Squirrel monkeys are smallest than capuchins, weighting 900 g to 1 kg and have a white mask around their eyes (Shostell and Ruiz-Garcia, 2016). Capuchins and squirrel monkeys are also commonly used in laboratorial facilities (Whitney, 1995).

Atelidae have the largest monkeys from the Neotropics with four genera (*Ateles*, *Brachyteles*, *Alouatta*, and *Lagothrix*). They all have a prehensile long tail with mostly arboreal habitat and frugivorous diet. *Alouatta* has a very developed laryngeal and hyoid processes, significantly increasing its vocalization potential (Verona and Pissinatti, 2014; Shostell and Ruiz-Garcia, 2016). Species from the Atelidae and Pitheciidae families, are not well adapted to captivity, being hardly used in biomedical research (Whitney, 1995). In wildlife, populations of *Alouatta* have being giving more attention due to their high susceptibility to YF virus (YFV) infection, being considered an important sentinel to that disease (Santos et al., 2020).

Pitheciidae have four genera (*Cacajao*, *Callicebus*, *Chiropotes* and *Pithecia*). These animals do not have a prehensile tail and its diets vary from fruits and leaves to insects (Shostell and Ruiz-Garcia, 2016).

Aotidae have only one genus, *Aotus*, known as night monkey or owl monkey. These animals have big eyes and lack of a prehensile tail, weighting 570 g to 1.6 kg. They are the only nocturnal primate from de Neotropics, being more active at dawn and dusk. Their diet is based on fruits, flowers, leaves and insects and they are widely distributed throughout the rain forest areas of South America (Whitney, 1995; Shostell and Ruiz-Garcia, 2016). Owl monkeys became very important for research in antimalarial drug development and to immunological and ocular studies (Whitney, 1995).

2. Diseases affecting free-ranging NWP

NWP have been commonly raised in captivity through the past years, both in laboratory facilities and zoological gardens around the world. This high frequency in captivity leads to a rich supply of information available in the literature on the management, husbandry, and health of many of these animals (Mätz-Rensing and Lowenstine, 2018). However, information about free-ranging NWP is very scarce, especially focusing on its health, being the vast majority of reports associated with

serological diagnoses and outbreak events, mostly focusing on specific and pre-defined infectious agents (Bueno et al., 2017; Wilson et al., 2021; Wilson et al., 2022). Additionally, free-ranging animals usually deals with a greater environmental challenge, being extremally influenced by anthropic factors (Range-Negrín et al., 2014; Bueno et al., 2017; Correa et al., 2018; Ehlers et al., 2022; Wilson et al., 2021). These factor leads to a different profile of diseases between captive and free-ranging animals, even from the same species. Therefore, this review will focus especially on the diseases that occur or potentially occur in free-ranging populations of NWP.

2.1 Infectious diseases

Table 1 summarizes bacterial, viral, protozoan and fungal pathogens reported in free-ranging NWP.

2.1.1 Bacteria

Among all the infectious diseases, the ones caused by bacteria are the most commonly reported in free-ranging NWP (Ehlers et al., 2022), and it is often associated with history of trauma, which is an important predisposing factor or a consequence of it (Silva et al., 2020a; Ehlers et al., 2022).

2.1.1.1 Gram-positive cocci

***Staphylococcus* spp.** are Gram-positive cocci with zoonotic potential, being a part of the microbiota, but considered opportunistic pathogens. *Staphylococcus aureus* is the species most commonly reported. The infection usually starts as a skin lesion, evolving to cellulitis, lymphangitis, and bacteremia. Once bacteremia is established, suppurative inflammation with intralesional bacterial colonies can be found in multiple organs, causing suppurative pneumonia, hepatitis, meningitis, endocarditis, and nephritis (Mätz-Rensing and Lowenstine, 2018). Reports of infections in free-ranging NWP are rare. Molina et al. (2019a) described a single case of suppurative meningoencephalitis in an infant golden-headed lion tamarin (*Leontopithecus chrysomelas*), that, although was rescue from wildlife, stayed at a captive environment for 33 days before developing clinical signs. Diagnosis in that case was performed by the visualization of Gram-positive cocci by histopathology and was confirmed by bacterial culture.

Streptococcus pneumoniae is a Gram-positive coccus carried by asymptomatic animals and humans and transmitted by aerosol. Infections are enhanced by stressful factors and starts in the respiratory tract quickly progressing to bacteremia and multiple organs infections, being meningitis and arthritis a common consequence in NWP (Verona and Pissinatti, 2014; Mätz-Rensing and Lowenstine, 2018). Other species of *Streptococcus* have also been described in NWP, such as *Streptococcus pasteurianus* causing endocarditis and sepsis in a puerperal tamarin (*Saguinus imperator*) (Oliveira et al., 2022b) and *Streptococcus equi* subsp. *zooepidemicus* responsible for outbreaks in colonies of marmosets after being exposed by horse meat or keepers in contact with horses (Schiller et al., 1989; Mätz-Rensing et al., 2009). Diagnostic is performed based on identification of Gram-positive cocci at histopathology, confirmed by bacterial culture or PCR (Schiller et al., 1989; Mätz-Rensing et al., 2009; Mätz-Rensing and Lowenstine, 2018; Oliveira et al., 2022b). There is no information about the prevalence of *Streptococcus* species in free-ranging NWP.

2.1.1.2 Enterobacteriacea and others Gram-negative bacillus and coccobacillus

Salmonella enterica is a Gram-negative bacillus that causes salmonellosis, a zoonotic disease, usually asymptomatic for NWP, but responsible for sporadic outbreaks in captive primates (Verona and Pissinatti, 2014). In cases of symptomatic infection, affected animals usually progress rapidly to death (Mätz-Rensing and Lowenstine, 2018). The main clinical sign is watery to bloody diarrhea and histopathology shows necrotizing and suppurative enterocolitis with sepsis and systemic dissemination of the bacteria, often causing a suppurative to pyogranulomatous hepatitis and splenitis (Verona and Pissinatti, 2014; Mätz-Rensing and Lowenstine, 2018). The serovars frequently identified in NHP are Enteritidis and Typhimurium (Mätz-Rensing and Lowenstine, 2018). In wildlife, this bacterium is rarely described with one single report of *Salmonella*-induced enterocolitis in a free-ranging howler monkey (Ehlers et al., 2022). Usually, this organism is identified by histopathology, with Gram stain, and by IHC, being usually confirmed by bacterial culture and/or by DNA amplification and sequencing (Ehlers et al., 2022).

Escherichia coli is a Gram-negative bacillus associated with self-limiting to lethal diarrhea in NHP colonies, mainly of marmosets and tamarins (Thomson and Scheffler, 1996; Mansfield et al., 2001; Hayashimoto et al., 2016; Mätz-Rensing and

Lowenstine, 2018). The disease can be caused by enteropathogenic (EPEC), enterotoxigenic (ETEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC) and diffusely adherent (DAEC) serotypes (Mätz-Rensing and Lowenstine, 2018). However, EPEC is the most reported serotype in enzootic infections of captive NHP, frequently characterized by acute hemorrhagic diarrhea (Thomson and Scheffler, 1996; Mansfield et al., 2001; Hayashimoto et al., 2016; Mätz-Rensing and Lowenstine, 2018).

Histopathology shows neutrophilic colitis with hyperplasia of the intestinal crypt epithelium, increased mitotic index, loss of goblet cells, crypt abscesses and Gram-negative rods attached to the apical portion of the mucosal lining epithelium (Mansfield et al., 2001; Mätz- Rensing and Lowenstine, 2018). The identification of *E. coli* adhered to the intestinal epithelium is an important feature for the confirmation of the disease and can be performed by routine histological stains, hematoxylin and eosin, Gram stain and toluidine blue, or by electron microscopy (Ludlage and Mansfield, 2003). Besides enterocolitis, *E. coli* has been described as an important cause of septicemia for captive NWP (Ehlers et al., 2022). *E. coli* was identified as a cause of enterocolitis in one free-ranging howler monkey and was also associated with suppurative bronchopneumonia in free-ranging animals from this species (Ehlers et al., 2022).

Diagnosis is performed by bacterial culture, histopathology and IHC (Mätz-Rensing and Lowenstine, 2018; Ehlers et al., 2022). Molecular techniques, such as PCR of fecal samples, are important to monitoring the disease in a given population, since bacterial culture can underestimate the number of carrier animals (Mansfield et al., 2001). Importantly, *E. coli* is a commensal bacterium of digestive tract from healthy NHP, therefore, in cases of identification by PCR, it is extremely important to perform phylogenetic typification to assess the pathogenicity of the identified agent (McCoy et al., 2021). Additionally, Vásquez-Aguilar et al. (2020) detected antimicrobial resistance genes in *E. coli* isolates from free-ranging howler monkeys (*A. pigra*) and domestic animals (cattle, sheeps and horses) in a Mexican Fragmented Rainforest, confirming that wild animals exposed to anthropized environment are also susceptible to resistant bacteria, enhancing the impact of indiscriminate use of antibiotics.

***Shigella* spp**. is a Gram-negative bacillus that causes shigellosis, a severe zoonotic disease of the large intestine (cecum and colon) of all primates, including humans, but which is rarely described in NWP (Mansfield and Fox, 2019). Four

serogroups are identified: *Shigella flexneri*, *Shigella dysenteriae*, *Shigella boydii,* and *Shigella sonnei* (Mätz-Rensing and Lowenstine, 2018). Microscopically, infection is usually associated with ulcerative and necrotizing colitis/typhlitis with crypt abscesses, herniation of intestinal crypts into the intestine-associated lymphoid tissue, and exudation of neutrophils on the mucosal surface (Mätz-Rensing and Lowenstine, 2018). In OWP, *S. flexneri* is also associated with a linear ulcerative gingival syndrome (Mätz-Rensing and Lowenstine, 2018), with no reports in NWP. The diagnosis is confirmed through bacterial culture (Mätz-Rensing and Lowenstine, 2018), but it is usually difficult to distinguish from *E. coli* isolates, so the use of PCR becomes essential for accurate identification of this bacteria (Mansfield and Fox, 2019). There are no reports of *Shigella* infection in free-ranging NWP.

Yersinia enterocolitica and ***Yersinia pseudotuberculosis*** are Gram-negative coccobacilli that cause yersiniosis, a disease frequently reported causing outbreaks in NWP colonies with high morbidity and mortality (Bakker et al., 2007; Nakamura et al., 2010; Mätz-Rensing and Lowenstine, 2018). Wild birds and rodents are the reservoir and the source of infection, transmitting the bacteria through its feces, contaminating the water and food offered to these animals in captivity (Verona and Pissinatti, 2014; Mätz-Rensing and Lowenstine, 2018). Grossly, there is an ulcerative enterocolitis with multiple small yellowish nodules at liver and spleen, microscopically characterized by necro-suppurative enteritis, hepatitis and splenitis, with big intralesional colonies (Nakamura et al., 2010; Mätz-Rensing and Lowenstine, 2018). Diagnostic can be performed by histopathology and IHC, confirmed by bacterial culture (Bakker et al., 2007; Nakamura et al., 2010; Mätz-Rensing and Lowenstine, 2018). Co-infection with *E. coli* was recently described in nine wild caught marmosets (*Callithrix penicillata*) presenting diarrhea and 100% of lethality (Lemos et al., 2021). Although this disease is high relevant in captive NWP, the importance in free-ranging animals is unknown, with no reports in the literature.

Klebsiella pneumoniae is an encapsulated Gram-negative bacillus implicated in outbreaks in captive NWP, especially in marmosets, howler monkeys and tamarins, with lethal course, causing bacteremia with marked sinusoidal leukocytosis, suppurative splenitis, interstitial pneumonia, necrotizing adrenalitis, necrotizing myocarditis, peritonitis, and neutrophilic enteritis (Pisharath et al., 2005; Guerra et al., 2016; Anzai

et al., 2017; Mätz-Rensing and Lowenstine, 2018; Guerra et al., 2020). Intralesional and intravascular small bacilli surrounded by a clear halo can be observed. Transmission occurs via oral or respiratory secretions and the disease is associated with stress and debilitating co-infections, being infants and juveniles more susceptible (Mätz-Rensing and Lowenstine, 2018). Diagnoses is performed by bacterial culture, IHC and PCR (Pisharath et al., 2005; Mätz-Rensing and Lowenstine, 2018; Guerra et al., 2020), being important to identify the phenotype at bacterial culture, once hypermucoviscosity (HMV) phenotype are highly pathogenic and infective (Anzai et al., 2017; Mätz-Rensing and Lowenstine, 2018; Guerra et al., 2020).

Although it seems to be an important cause of sepsis and death in captive NWP, the incidence and importance of *K. pneumoniae* in free-ranging animals is unknown. One free-ranging tamarin from a translocation program developed a *Klebsiella*-induced pneumonia and septicemia during the 30-days-quarantine, when it was housed for clinical examination with other tamarins from the same group. In this case, the animal was prior negative for *Klebsiella* in fecal and blood samples, but another healthy contact tamarin was positive, being considered the reservoir and source of the infection (Bueno et al., 2015). Importantly, *Klebsiella* was observed as part of intestinal microbiota of healthy free-ranging tamarins, confirming that these animals can act as a natural carrier, developing the disease in stressful situations (Iovine et al., 2014).

***Pasteurella* spp.** is a Gram-negative bacillus responsible for necro-suppurative bronchopneumonia in captive NWP with reports of *Pasteurella multocida* co-infection with *K. pneumoniae* in some of these cases (Mätz-Rensing and Lowenstine, 2018). *Pasteurella* spp. was also identified causing suppurative bronchopneumonia in free-ranging NWP with history of dog attack (Silva et al., 2020a; Ehlers et al., 2022). Interestingly, *Pasteurella* sp., especially *Pasteurella canis*, is a commensal bacterium from the oral cavity of healthy dogs, being also reported in humans involved with dog accidents, such as biting and scratching (Bath et al., 2015). Diagnosis can be performed by bacterial culture and PCR for speciation (Silva et al., 2020a; Ehlers et al., 2022).

***Pseudomonas* spp**. is a Gram-negative bacillus associated with septicemia in humans and animals, being *Pseudomonas aeruginosa* the most pathogenic strain described. In NWP, *Pseudomonas simae* was reported as cause of death of a captive marmoset (*Callithrix geofroyi*) causing an acute bronchopneumonia and bacteremia

(Vela et al., 2006). In this case, in addition to bacterial culture, PCR and DNA sequencing have been performed to have a phylogenetic characterization of the isolate. Menezes-Costa et al. (2013) detected by blood PCR four phylotypes of *Pseudomonas* spp. in free-ranging howler monkeys and capuchins from different regions of Brazil, confirming the circulation of this bacterium in a wild population. Although *Pseudomonas* spp. is poorly described in captive or free-ranging NWP, it is one of the differential diagnoses for septicemia in primates.

Bordetella bronchiseptica is a Gram-negative coccobacillus that causes bordetellosis, a respiratory disease commonly reported in NWP colonies (Verona and Pissinatti, 2014; Mätz-Rensing and Lowenstine, 2018). *B. bronchiseptica* has an affinity to the respiratory tract, attaching to the ciliary epithelium of the airways, causing a necro-suppurative bronchopneumonia that is usually trigged by a stressful event (Mätz-Rensing and Lowenstine, 2018). Diagnosis can be confirmed by bacterial culture and PCR. Although it is very prevalent in captive NWP, the frequency and impact in free-ranging population have not been reported.

2.1.1.3 Anaerobic bacteria

Clostridioides difficile (previously known as *Clostridium difficile*) is a Gram-positive anaerobic bacillus from commensal microbiota of healthy mammals, including NWP, being considered an opportunistic pathogen. The disease occurs when a disruption of the microbiota happens and the *C. difficile* starts to overgrow, producing high concentrations of cytokine, responsible to induce a severe pseudomembranous colitis (Mätz-Rensing and Lowenstine, 2018). Usually, histopathology shows multifocal areas of fibrin and necrotic debris erupting from the intestinal mucosa, forming a characteristic “volcano” aspect, evolving to a diffuse pattern with a thick layer of fibrin, mucus, cell debris and neutrophils (Armstrong et al., 2019). However, in a case of lethal acute diarrhea associated with *C. difficile* toxin A and B in a buffy-tufted-ear marmoset (*Callithrix aurita*) it was observed just a mild neutrophilic colitis (unpublished data), warning that even with mild lesions, *C. difficile* must be considered in the differential diagnosis of diarrhea cases in NWP.

Stress, hospitalization, and prolonged use of antibiotics are the main predisposing factors (Keel and Songer et al., 2006; Mätz-Rensing and Lowenstine,

2018; Armstrong et al., 2019). Although there is no report of the disease in free-ranging NWP, it could potentially happen in wildlife, especially in free-ranging animals submitted to a high anthropogenic environment and translocation programs. The diagnose of *C. difficile* can be tricky, once the identification of the bacteria in IHC or bacterial culture is not enough to confirm that the agent is causing the disease. *C. difficile* toxins, such as CDT, TcdA, and TcdB, must be identified by cytokine neutralization assays, ELISA, or PCR, to establish a cause-effect association (Keel and Songer et al., 2006; Armstrong et al., 2019).

Clostridium tetani is an obligated anaerobic Gram-positive spore-forming bacillus that causes tetanus, a disease that affect must of mammal species. OWP and NWP are susceptible to this disease, developing characteristic clinical signs such as triad of trismus, opisthotonos and status epilepticus. Diagnosis is based on history and clinical signs, but to confirm is necessary to identify the neurotoxin tetanospasmin, produced by *C. tetani* (Verona and Pissinatti, 2014; Mätz-Rensing and Lowenstine, 2018). Once this bacterium is found in the soil and infection occurs by the contamination of skin wounds, it can potentially happen in free-ranging animals, even not being described yet. Importantly, pathological findings in this case are non-specific.

Clostridium botulinum is anaerobic Gram-positive spore-forming bacillus that causes botulism by producing a neurotoxin (A, B, C, D, E, F, and G) in the host organism that will cause a flaccid paralysis by inhibition of acetylcholine release from the presynaptic motor neuron terminal (Rao et al., 2021). Diagnosis is performed based on clinical signs and confirmed by the identification of the neurotoxin (Rao et al., 2021). Although botulism is a rarely reported disease in NHP, there are few reports of *C. botulinum* causing outbreaks in captive OWP and NWP (Lewis et al., 1990; Petit, 1990). Silva et al. (2018) described an outbreak of botulism in a rural area of Minas Gerais, Brazil, affecting chickens, dogs and one free-ranging marmoset (*C. penicillata*). In this outbreak all the species involved were found in the same region presenting flaccid paralysis progressing to death, and type C neurotoxin was identified in the stomach content and serum of two chickens and one dog by mouse neutralization test. Pathological findings are absent or non-specific (Rao et al., 2021).

2.1.1.4 Spirochete bacteria

***Leptospira* spp.** is an important zoonotic bacterium responsible for leptospirosis. It is uncommon to observe natural infections in captive NWP (Mätz-Rensing and Lowenstine, 2018), and it have been rarely described in free-ranging animals, with studies focusing mainly in serological and molecular evidence (Molina et al., 2014; Bueno et al., 2017; Molina et al., 2019b; Aliaga-Samanez et al., 2021). In two serological study of a free-ranging NWP population none of the animals showed positive serology anti-*Leptospira* by microagglutination test (MAT): the first study evaluated anti-*Leptospira* serology in 20 howler-monkeys (*A. caraya*) and 48 marmosets (*C. penicillata*) (Molina et al., 2014); and second in 48 capuchins (*S. flavius*) (Bueno et al., 2017). Also, in another study with 593 free-ranging tamarins (*L. chrysomelas*) using MAT, only two tamarins were positive (Molina et al., 2019b). Contrasting with the other previous studies, Aliaga-Samanez et al. (2021) found a high prevalence (43.3% to 61.5%) of *Leptospira* sp. antibodies in asymptomatic free-ranging tamarins (*L. weddelli* and *S. imperator*) from Peru. This high prevalence was also observed in a free-ranging capuchin (*S. apella nigritus*) population from São Paulo, Brazil, with 78% (39/50) of reactiveness by MAT (Girio et al., 2020). Together, these data suggest a difference of susceptibility and exposure between those studied species and confirms that *Leptospira* spp. circulates in the wild NWP population.

There are only two reported cases of leptospirosis-induced death in a free-ranging NWP, one in a howler monkey (*Alouatta guariba*) from Rio Grande do Sul, Brazil (Ehlers et al., 2022), and the other one in a black-tufted marmoset (*C. penicillata*) from Brasilia, Brazil (Wilson et al., 2021). Pathological findings reported by Wilson et al. (2021) were icterus, interstitial pneumonia with hemorrhage, edema, and fibrin exudation, interstitial nephritis with tubular degeneration and necrosis and hepatocyte cord dissociation and necrosis with sinusoid leukocytosis. Diagnosis in this case was based on observation of spirochetes through Warthin-Starling stain, specific anti-*Leptospira* IHC and real-time PCR targeting the lipL32 gene. Importantly, in this case, a great number of spirochetes were observed in the renal tubules, raising the possibility that marmosets may be important in the transmission of this zoonosis, especially because marmosets are well-adapted in urban environments increasing human-marmoset interactions (Wilson et al., 2021).

***Borrelia* sp.** is another spirochete that causes disease in humans and animals with an important zoonotic impact. In humans it is usually associated with Lyme disease and Brazilian Lyme-like disease, caused mainly by *Borrelia burgdorferi*, and transmitted to humans by ticks. NHP are used as experimental models for Lyme disease, reproducing all the three phases of the infection: localized, disseminated, and persistent; and developing persistent characteristic lesions, such as myocarditis (Cadavid et al., 2004; Crossland et al., 2018). *B. burgdorferi* was identified by PCR in 16% (32/200) free-ranging golden-headed lion tamarin (*L. chrysomelas*) from Rio de Janeiro, Brazil, confirming that this bacterium circulates in this region and suggesting that these tamarins may play a role in transmission of this pathogen to other animals or human beings (Santos et al., 2018).

Helicobacter pylori is a spiral bacterium (spirochete), commensal of the stomach, and associated with mild to moderate proliferative and erosive gastritis in immunosuppressed patients. Silver stain, such as Warthin-Starry impregnation method, and IHC are used to identify the bacteria in the tissue (Mätz-Rensing and Lowenstine, 2018). Although, *Helicobacter* spp. has been naturally identified in the stomach of marmosets, no association with specific pathological findings was observed (De Mello et al, 2005; Shen et al., 2015). There is no information on *Helicobacter* spp. in free-ranging NWP.

2.1.1.5 *Mycobacterium tuberculosis* complex (MTBC)

MTBC is a group of *Mycobacterium* species, such as *Mycobacterium tuberculosis*, *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium canettii*, and *Mycobacterium microti*, with the potential to cause tuberculosis, a zoonotic disease, in humans and other mammals. In captive primates this infection is usually associated with the proximity of these animals with humans, being considered an important anthropozoonosis (Mätz-Rensing and Lowenstine, 2018; Ehlers et al., 2020). In NHP this disease is mainly associated with *M. tuberculosis* and, although well described in captive primates, NWP seems to be more resistant to the infection, being uncommon in captive NWP and considered non-existent in free-ranging NWP with no human contact (Montali et al., 2001; Marieke et al., 2015; Mätz-Rensing and Lowenstine, 2018). Rosenbaum et al. (2015) found molecular evidence of *M. tuberculosis* complex in NWP, mainly from Atelidae and Cebidae family, from different

captive origin (pet, market, and zoological animals) in Peru. In that study, oral swabs were obtained from 220 individuals, with 13.6% of positive DNA amplification. Market origin had 5% (5/72) of positive animals. This group was represented by animals that were capture in wildlife and sold in the illegal market, being the closest reference of prevalence in free-ranging NWP described in the literature. In the contrast the prevalence in zoo primates was 22% (22/100), in agreement with the notion that this disease is highly associated with human contact.

Diagnosis is performed with necropsy and histopathology, with the identification of typical granulomas in multiples organs, but especially in the lungs, associated with variable amount of intralesional alcohol-acid resistant bacilli (Mätz-Rensing and Lowenstine, 2018; Ehlers et al., 2020). Typical tuberculosis granulomas are characterized by well-delimited nodules with a mineralized necrotic center surrounded by epithelioid macrophages, lymphocytes, plasma cells and multinucleated giant cells, usually of the Langham's-type (Mätz-Rensing and Lowenstine, 2018). This typical presentation is often observed in OWP, however, in NWP, it is also described a poorly delimited presentation with multifocal to coalescent granulomatous inflammation without central necrosis (Montali et al., 2001; Ehlers et al., 2020). IHC and PCR can be performed to confirm the intralesional agent (Ehlers et al., 2020). Bacterial culture, although confirmative, must be performed in a biological safety cabinet class 3, being not always accessible. Besides MTBC, ***Mycobacterium avium complex***, ***M. avium paratuberculosis*** and ***Mycobacterium leprae*** also infects captive NHP, however NWP are extremally less susceptible than OWP (Mätz-Rensing and Lowenstine, 2018), and there are no reports of infections in free-ranging NWP.

2.1.1.6 Hemotropic bacteria

Bartonella **spp.** is a facultative intracellular Gram-negative bacillus that infect erythrocytes and endothelial cells in a prolonged bacteremia. *Bartonella henselae* is responsible for the "cat scratch" disease, a zoonosis that has the cat as the most important reservoir and is transmitted by cat bite and scratches or by vectors (fleas or ticks). In humans it is responsible for causing endocarditis and other angioproliferative lesions, being associated to angiomatosis and hepatic/splenic peliosis (Psarros et al., 2012). *Bartonella* spp. has been poorly described in captive and free-ranging NHP (O'Rourke et al., 2005; Huang et al., 2011; Li et al., 2013). Bonato et al. (2015)

investigated *Bartonella* infection in 112 free-ranging capuchins and tamarins from São Luís, Brazil, by blood qPCR, but no positive animal was found. The authors, however, believes that the negative results may be due to a low bacteremia, once the animals from the study were all asymptomatic. Also, bartonellosis was investigated by PCR, Warthin-Starling stain and IHC in two cases of hepatic peliosis in captive owl monkeys (*A. infulatus*), but no evidence of bacteria was found (Souza et al., 2021).

***Mycoplasma* spp. (hemoplasmas or hemotropic mycoplasmas)** are bacteria that infect the surface of erythrocytes of a broad range of hosts, including primates, leading to hemolytic anemia by intra and extravascular hemolysis. Hemoplasmas have been detected in captive OWP and NWP, with new identified bacterial species, such as *Candidatus Mycoplasma kahanei* from squirrel monkeys (*Saimiri sciureus*) and *Candidatus Mycoplasma aoti* from owl monkeys (*Aaotus trivirgatus*) (Neimark et al., 2002; Baker et al., 2011; Sashida et al., 2014; Melo et al., 2019). There are some studies detecting hemoplasmas in free-ranging NWP as well (Santos et al., 2013; Bonato et al., 2015; Cubilla et al., 2017).

A free-ranging howler monkey was diagnosed with *Candidatus M. kahanei*-related hemoplasma by blood PCR and presented a regenerative anemia with low red blood cell count (RBC) and high mean corpuscular volume (MCV) (Santos et al., 2013). Another study investigating 112 healthy free-ranging NWP had molecular evidence of hemoplasmas in 35.7% of the evaluated animals, being represented by capuchins, squirrel monkeys and tamarins (Bonato et al., 2015). Cubilla et al. (2017) detected, by blood smears cytology and blood PCR, 20% to 25% (8-10/40), respectively, of hemoplasma-infected capuchins and howler monkeys. In this study, the authors also observed that wild-borne animals were more likely to test positive than captive-born animals and howler monkeys were 45 times more likely to test positive than capuchins and marmosets, presenting mild anemia when infected.

Erlichia canis is an obligate intracellular Gram-negative α-proteobacterium, that infects leukocytes and causes the canine monocytic ehrlichiosis, a potentially zoonotic disease that affects dogs and is transmitted by *Rhipicephalus sanguineus* bites (Vieira et al., 2011). *E. canis* was detected by blood PCR in one of 19 healthy free-ranging marmosets (Mafra et al., 2015). Interestingly, the genotype of the *E. canis* sequenced

from this marmoset was very similar to other genotypes identified in domestic dogs, indicating the overlap of habitats between these animals.

2.1.1.7 *Brucella* spp.

Brucella spp. is a facultative intracellular Gram-negative coccobacillus, that causes brucellosis, a recognize zoonotic disease, with high importance in public health, and a broad range of infected hosts, being the most important: *Brucella melitensis* (small ruminants), *Brucella abortus* (bovine), *Brucella suis* (swine),, and *Brucella canis* (canine) (Olsen and Palmer, 2014). *Brucella* spp. gained attention in NHP after being isolated a new species, named *Brucella papionis*, from stillbirth and retained placenta of two wild-caught baboons (*Papio* sp.) (Schlabritz-Loutsevitch et al., 2009; Whatmore et al., 2014). However, studies searching for serological evidence of this bacteria in free-ranging NWP had negative results, even in regions with brucellosis endemic herds (Ricciardi et al., 1976; Molina et al., 2014; Bueno et al., 2017), questioning the real important of this disease for an NWP population.

2.1.2 Viruses

There are many well-known viruses that cause disease in captive NWP, however, most of them are not reported in wildlife or were only observe in experimental conditions (Mätz-Rensing and Lowenstine, 2018). Viral diseases in free-ranging NWP are usually associated with outbreaks, and the main viruses described are the Herpesvirus simplex type I (HSV-I) and the yellow fever virus (YFV).

2.1.2.1 Human herpesvirus – HSV-I and II

HSV-I and II are alpha herpesvirus that have the human as primary host with high morbidity and mild or absent clinical signs. The virus is latent in the trigeminal and lumbosacral ganglia, with intermittent reactivation and viral shedding in periods of stress. In the primary host, lesions associated to viral infection consist of vesicles and ulcers on the oral (type I) or genital (type II) mucosa, sometimes associated with conjunctivitis, with rare cases of disseminated infections, usually associated with immunosuppression (Mätz-Rensing and Lowenstine, 2018). Histopathology shows a necrotizing and ulcerative lesion with multinucleated syncytial cells on the edge and eosinophilic intranuclear viral inclusion bodies (Mätz-Rensing and Lowenstine, 2018).

OWP can also be infected, developing a similar disease observed in humans. NWP are highly susceptible and usually develops fatal disseminated disease with high morbidity and high mortality (Juan-Sallés et al., 1997; Mätz-Rensing et al., 2003; Schrenzel et al., 2003; Hatt et al., 2004; Costa et al., 2011; Casagrande et al., 2014; Barnes et al., 2016; Wilson et al., 2022). Interestingly, the NWP that survives the outbreak develops a prolonged antibody response that last for at least four years, but do not extend to the offspring (Hatt et al., 2004), indicating that these outbreaks could have a cyclic pattern.

In free-ranging NWP populations, this disease has been reported mainly in peri-urban marmosets and it is usually associated with lethal outbreaks with monkey-to-monkey transmission after the virus has been introduce by the contact with secretions of the infected human host, and neurological signs are frequently reported (Bruno et al., 1997; Longa et al., 2011; Costa et al., 2011; Wilson et al., 2022). Pathological findings in NWP are similar to the ones described in humans, however, they are more severe and disseminated. Usually, is observed ulcerative and necrotizing lesions at mucocutaneous junctions with syncytial epithelial cells and nuclear viral inclusions, necro-ulcerative glossitis, and an acute marked encephalitis, characterized by mononuclear inflammation with variable amounts of neutrophils and severe necrosis, hemorrhage and vasculitis (Mätz-Rensing et al., 2003; Costa et al., 2011; Casagrande et al., 2014; Barnes et al., 2016; Wilson et al., 2022). Necrotizing hepatitis and conjunctivitis, although less common, is also observed (Wilson et al., 2022). Nuclear inclusions are also observed in neurons and astrocytes. Although the pathological findings are very characteristic of the HSV infection, PCR, electron microscopy and IHC are usually performed to confirm the diagnosis (Mätz-Rensing et al., 2003; Costa et al., 2011; Barnes et al., 2016; Wilson et al., 2022). Importantly, in a study with 16 cases of HSV in free-ranging marmosets, only HSV-type I was detected (Wilson et al., 2022).

There is one single report of a possible HSV-type II transmission from an asymptomatic infected howler monkey (*A. guariba*) to a human. After being bitten by the monkey, the human patient started to present a recurrent vesicular skin lesion in the site of the bite, being isolated HSV-II from the vesicle secretion (Lyra et al., 2018). Once HSV is a latent virus, it is possible that the howler monkey from this case got infected after being exposed to virus by a human source, and surprisingly did not die

with the initial infection, becoming a carrier and transmitting it to a human host, in an unusual monkey-to-human transmission.

2.1.2.2 Saimiriine Herpesvirus (SaHV-1 and 2)

SaHV-1 is an alpha herpesvirus that causes a disseminated necrotizing disease in NWP and SaHV-2 is a gamma herpesvirus T-lymphotropic related to Kaposi's sarcoma-associated herpesvirus (HHV-8) (Wachtman and Mansfield, 2012; Mätz-Rensing and Lowenstine, 2018). Both viruses are enzootic in captive squirrel monkeys, considered the primary host for these viruses, therefore, infected animals are usually asymptomatic. However, there is one report associating a leukemic histiocytic sarcoma with SaHV-2 in a squirrel monkey that was also infected with *Saimiri sciureus* lymphocryptovirus 2 and Squirrel monkey retrovirus, although it is difficult to establish a correlation between viral infection and the sarcoma (Buchanan et al., 2020). When transmitted to another susceptible NWP, such as tamarins, owl monkeys and marmoset, SaHV will lead to a systemic and lethal disease (Wachtman and Mansfield, 2012; Mätz-Rensing and Lowenstine, 2018). SaHV-1 causes a disseminated necrotizing disease affecting skin, oral mucosa, and parenchymal organs, with syncytial cells and nuclear inclusion bodies (Wachtman and Mansfield, 2012); and SaHV-2 causes an acute lymphoproliferative disorder, with CD3-CD8 positive T-lymphocytes proliferation in multiple organs, including GI tract, spleen, liver and kidney and leukemia (Mätz-Rensing and Lowenstine, 2018).

Others gammaherpesviruses have been described in NWP as well, such as herpesvirus ateles from spider monkeys and Callitrichine herpesvirus 3 (CalHV-3), that was isolated from spontaneous lymphoma in captive marmosets (Melendez et al., 1972; Albrecht, 2002; Ramer et al., 2000). Gammaherpesvirus was also detected by blood PCR in free-ranging golden-handed tamarin (*Saguinus midas*), white-faced saki (*Pithecia pithecia*), and squirrel monkey (*S. sciureus*), all from *Lymphocryptovirus* genus (Thoisy et al., 2003). Those studies brought a lot of contribution describing novel herpesvirus in NWP; however, little is known about the impact of these viruses in the health of those animals and the pathological features associated with these infections.

2.1.2.3 Flaviviruses

Yellow fever virus (YFV) is an arbovirus transmitted by mosquitos belonging to the genera *Sabethes* and *Haemagogus*, in the sylvatic cycle, and *Aedes*, in the urban cycle. This virus causes the yellow fever (YF), a disease of high importance in public health and NWP conservation. YF usually occurs as 10 years cyclic outbreaks in non-endemic regions with low vaccine coverage, and it usually starts in NWP population in the wild, extending to the adjacent human population, therefore NWPs are considered YF sentinels and important public health tools for the control and prevention of this disease (Litvoc et al., 2018).

The classic pathological findings of YF in NHP are jaundice with an enlarged and yellow liver characterized in histopathology by marked midzonal to massive hepatocellular necrosis with apoptotic hepatocytes (Councilman-Rocha Lima bodies), steatosis, and hemorrhage (Leal et al., 2016; Mätz-Rensing and Lowenstine, 2018; Fernandes et al., 2021a; Fernandes et al., 2021b). Official diagnosis is performed by liver histopathological evaluation with intracytoplasmic antigen immunolabeling in hepatocytes by IHC, confirmed by RT-qPCR (Giovanetti et al, 2020; Fernandes et al., 2021b). Viral isolation and immunofluorescence can also be performed, but is unusual (Almeida et al., 2012).

Santos et al. (2020) analyzed the histopathological findings of 57 positive NWP, including howler monkeys, marmosets, and capuchins. In this study the authors identified that there were differences in the pattern of liver injury of YFV-infected among different species of neotropical primates, being the howler monkey, the genus with the most aggressive pattern, characterized by the classic YFV histological features (Santos et al., 2020). In contrast, infected marmosets had unspecific findings, such as mild inflammatory infiltrate and occasional glycogenosis. This anatomopathological profile is compatible with the viral load identified in the tissues of those animals, where howler monkeys have a high viral load, proving to be good indicators of the disease, capuchins have a median viral load and marmosets have a low viral load (Fernandes et al., 2021b). Titi-monkeys (*Callicebus* spp.) is also highly susceptible to YFV, developing a massive necrotizing hepatitis with high viral loads (Fernandes et al., 2021a). Importantly, this high susceptibility of YFV in howler monkeys reflects directly

in its wild population that drastically decreases during YF outbreaks (Moreno et al., 2015).

The last outbreak happened in the Brazilian Southeast region, started by the end of 2016, and finished in 2019 and was considered the most severe over the past 80 years, with introduction of the virus in regions that it has never been reported before (Giovanetti et al, 2020; Jesus et al., 2020; Silva et al., 2020b). During this period there were more than 2,000 human cases with approximately 30% of lethality (Giovanetti et al, 2020). The wild population of NWP was extremally impacted by YFV, with thousands of positive lethal cases and an important reduction in its density, directly affecting conservation programs of species already threatened (Dietz et al., 2019; Strier et al., 2019). During this outbreak was also detected by RT-qPCR YFV-positive marmosets from urbanized regions of São Paulo, Brazil, increasing concern about the development of the urban cycle of the disease, which has not occurred in Brazil since 1942 (Cunha et al., 2020; Fernandes et al., 2020). In one non-autochthonous case the marmoset, raised as pet, presented clinical signs, such as fever, vomit, diarrhea, jaundice, difficulty in walking and loss of movement of pelvic members, for nine days before death. Considering this extremely fearful scenario, efforts have been made to use the human YF vaccine (17DD) in captive and free-living NWPs, with promising results observed in captive howler monkeys (Fernandes et al., 2021c).

Zika virus (ZIKV) was also investigate in free-ranging NWP from Brazil, and there was evidence of viral infection by RT-qPCR in marmosets and capuchins from peri-urban regions during YF outbreak (Terzian et al., 2018; Favoretto et al., 2019), suggesting that these species could play a role as a sylvatic reservoir of ZIKV, contributing to the maintenance of this virus in the environment (Han et al., 2019). Histopathology from 16 of the 32 positive animals revealed only nonspecific findings, such as pneumonia, cholangiohepatitis, splenic lymphoid reactive hyperplasia, interstitial nephritis, and myocarditis (Terzian et al., 2018). Squirrel monkeys, marmosets, and owl monkeys have been used as experimental models for ZIKV infections, being susceptible to the disease, with significant viremia and reproducing the congenital abnormalities and abortions commonly observed in humans (Alcantara et al., 2021).

Others flaviviruses have being investigated in wild NWP populations (Contigiani et al., 2000; Svoboda et al., 2014; Rocha et al., 2015; Loza-Rubio et al., 2016; Morales et al., 2017; Terzian et al., 2018; Dolz et al., 2019; Chaves et al., 2021). NWP are susceptible to most of flavivirus infections with human and veterinary importance in experimental conditions, being used as models to study this disease (Mätz-Rensing and Lowenstine, 2018; Alcantara et al., 2021).

However, little is known about the importance of the NWP as a reservoir of those viruses in wildlife and the real impact of these diseases in natural infections. Studies with NWP population from Costa Rica and Argentina observed molecular evidence and neutralizing antibodies for dengue virus (DENV), Saint Louis encephalitis virus (SLEV) and West Nile virus (WNV) in asymptomatic howler monkeys (Contigiani et al., 2000; Morales et al., 2017; Dolz et al., 2019; Chaves et al, 2021). DENV was also identified in free-ranging capuchins and squirrel monkeys from Costa Rica (Dolz et al., 2019; Chaves et al, 2021) and there is one report of positive serology for Equine eastern encephalitis virus (EEEV) in on spider monkey from Bolivia (Karesh et al., 1998). At Brazil, SLEV was observed in one free-ranging howler monkey and eight capuchins from Porto Rico County region, between the states of Paraná and Mato Grosso do Sul (Svoboda et al., 2014). Ilheus virus (ILHV) and Bussuquara virus (BSQV) was also detected in one free-ranging howler monkey from Argentina (Morales et al., 2017), although these two viruses have less importance in human medicine.

2.1.2.4 Rabies virus (RABV)

The rabies virus (RABV) is a Lyssavirus that causes rabies, a zoonosis with 100% of lethality that infects a wide range of mammal hosts and is transmitted by infected animal saliva. In Brazil RABV has been controlled by preventive vaccination programs focusing on domestic animals. However, currently, wild animals, especially vampire bats (*Desmodus rotundus*), are the main source of human infections. The role of NWP in the rabies cycle increased in the past years, mainly associated wild populations of common marmoset (*C. jacchus*) from Brazilian Northeast and Southeast regions, with 91 human exposures to infected common marmoset in the past 12 years (2008-2020) (Favoretto et al., 2001; Machado et al., 2012; Kotait et al., 2018; Moutinho et al., 2020; Benavides et al., 2022). Interestingly, outbreaks of RABV were initially concentrated in the states of Ceará and Pernambuco (up to 2012) but now extended to

other states, such as Piauí (since 2013), Bahia (2017), and Rio de Janeiro (2019) (Benavides et al., 2022).

A juvenile infected marmoset, that died with neurological signs, was recently reported in urbanized area from Niterói, Brazil (Moutinho et al., 2020). In this case the sequenced viral DNA showed characteristic of hematophagous bats *Desmodus rotundus* RABV strain (AgV3), similar to the one detected in the cases from Bahia. *C. jacchus* antigenic RABV strain was also identified in 20 reported cases from the Brazilian Northeast (Benavides et al., 2022). Capuchins (*Sapajus* sp.), although less frequent than marmosets, were also identified as a potential reservoir for RABV (Machado et al., 2012) with a confirmed symptomatic lethal case (Kobayashi et al., 2013). In this symptomatic case, the capuchin bitted a horse and had an aggressive and isolated behavior. The phylogenetic analysis from this case also showed a viral strain Chiroptera-related (Kobayashi et al., 2013).

Classical pathological finding is the non-suppurative encephalitis with round intracytoplasmic eosinophilic inclusion bodies (Negri-bodies) observed mainly in neurons including Purkinje cells (Mätz-Rensing and Lowenstine, 2018). Official diagnosis is performed by immunofluorescence and mouse inoculation test, but histopathology is highly indicative of the disease (Moutinho et al., 2020).

2.1.2.5 Parvoviruses

Parvoviral infections in NHP have being poorly described in free-ranging and captive OWP (Sharp et al., 2010; Adlhoch et al., 2012; Simon, 2008). Its relevance and role as a zoonotic agent in NWP is poorly known, especially in a wild environment. Chaves et al. (2020) investigated by blood PCR the prevalence of three parvovirus groups (Bocaparvovirus-HBoV, Erythroparvovirus-B19 and Tetraparvovirus-PARV4) in captive and free-ranging howler monkeys (*Alouatta palliata*), white-face monkeys (*Cebus imitator*), spider monkeys (*Ateles geoffroyi*) and squirrel monkeys (*Saimiri oerstedii*) from Central America for 15 years. In this study they found evidence of PARV4 infection, both in captive and free-ranging animals, in howler monkeys, capuchins and spider monkeys. HBoV and B19 were identified only in howler monkeys and capuchins, both from wildlife. The authors discussed that the identification of these viruses in the blood could indicate an active infection with viremia and the similarity

between the human and NWP strains from these cases may indicate a cross-species transmission with a zoonotic potential (Chaves et al., 2020).

In humans PARV4 is responsible for influenza-like symptoms, encephalitis, transient rash, hepatitis, and fetal hydrops; HBoV is also found in the respiratory tract, being associated respiratory diseases and B19 is a common cause of myocarditis, being also responsible for arthritis, glomerulonephritis and myelosuppression leading to anemia (Chaves et al., 2020). In OWP is described a macaque parvovirus (erythroparvovirus) that is also associated with anemia with identification of intranuclear inclusions in erythroid precursors in bone marrow (Mätz-Rensing and Lowenstine, 2018).

2.1.2.6 Simian foamy virus (SFV)

SFV are a complex zoonotic exogenous retrovirus that naturally infect OWP and NWP, with occasional reports in humans that have close contact with these primates (Muniz et al., 2015; Muniz et al., 2017; Pinto-Santini et al., 2017; Santos et al., 2019). It has been recently reported in free-ranging NWP, being the only known exogenous retrovirus naturally infecting this group and it is apparently non-pathogenic (Ghersi et al., 2015; Pinto-Santini et al., 2017; Muniz et al., 2018; Miranda et al., 2019; Santos et al., 2019), although co-infections with simian immunodeficient virus (SIV) accelerated SIV immunodeficiency-induced death (Pinto-Santini et al., 2017; Santos et al., 2019). SFV is transmitted through bites and grooming and is latent in red blood cells. It is believed that SFV primary infection occurs in blood and migratory cells, such as macrophages or leukocytes, carrying the virus to the basal epithelium of oropharyngeal tissues, with subsequent replication in differentiated epithelial cells (Santos et al., 2019). T lymphocyte differentiation and monocyte activation was observed in humans chronically infected with SFV (Gessain et al., 2020).

In captive NWP the prevalence ranges from 23% to 61%, being detected by serology and molecular evaluation (Muniz et al., 2015; Santos et al., 2019). SFV was detected in 34.8% (32/92) recently wild-caught tamarins from Rio de Janeiro, Brazil, by qPCR of saliva, with similar prevalence between sex and age (Miranda et al., 2019). Importantly, prevalence increased in animals with more than 7 months in captivity. This same profile was observed in NWP from Peru, where captive animals had a prevalence

of 47%, contrasting with 19% in free-ranging animals (Ghersi et al., 2015). Two distinct lineages of SFV co-circulating in this groups of tamarins, SFVlcm-1, known as infective for Cebiade family, and SFVlcm-2, infective for capuchins (*Sapajus xanthosternos*) and marmosets (*C. jacchus*) (Miranda et al., 2019).

2.1.2.7 Adenoviruses (AdV)

AdV are DNA viruses that infect most vertebrate animals, including humans and NHP. AdV infections in NWP have been described since 1970, when it was first detected by serology in captive squirrel monkeys and owl monkeys. Since then, this virus has been associated with asymptomatic to fatal infections, dependent of age and immunological status (Chen et al., 2011; Rogers et al., 2020). Although AdV is usually specie-specific, there are reports of cross-infection between different NHP species and even between NHP and humans, being considered a zoonotic pathogen (Chen et al., 2011; Yu et al., 2013). Importantly, free-ranging and captive marmosets and capuchins also showed neutralizing antibodies for human AdV in Brazil (Ersching et al., 2010) and Adv was detected by PCR in 17.9% (12/67) fecal samples of free-ranging howler monkeys (*Alouatta pigra*) from Mexico (Argüello-Sánchez et al., 2018). Pathological findings may be systemic with necrotizing lesions in the liver, intestine, pancreas, and spleen, but interstitial pneumonia is the main feature observed in NWP, causing fulminant respiratory outbreaks with high morbidity and lethality (Chen et al., 2011; Rogers et al., 2020). Intranuclear inclusion bodies can be observed in epithelial cells present in the borders of the necrotizing lesions (Rogers et al., 2020). AdV is eliminated in the feces. Therefore, PCR with template DNA extracted from fecal samples can be a good tool to detect a viral infection in primate colonies (Rogers et al., 2020).

2.1.2.8 Hepatitis A virus (HAV)

HAV was investigated by serology in 419 free-ranging and captive NWP from Brazilian Southeast region, and the results showed positive serology only in captive animals with a frequency of 5.2% (Setzer et al., 2014), contrasting with the high frequency of 22% to 37% observed in free-ranging OWP (Coursaget et al., 1981; Burke et al., 1984). In a free-ranging population of howler monkeys and capuchins was observed a similar prevalence of anti-A hepatitis antibodies (4.5% - 5/107), assessed by ELISA, and detected exclusively in capuchins (Svoboda et al., 2016). HAV is a

picornavirus, RNA that is transmitted by fecal-oral route and has the primates as the only natural host.

Clinical manifestations of symptomatic HAV infection in humans vary from mild, anicteric illness to fulminant hepatitis (Fiore, 2004). Naturally and experimental infections are reported in OWP and NWP, being described natural disease in owl monkeys, marmosets, and tamarins (Cullen and Lemon, 2018). Histopathology of the liver from infected marmosets and tamarins showed hepatitis with hepatocellular necrosis, ballooned hepatocytes, portal inflammation with piecemeal necrosis and proliferation of small-caliber bile ductules (Cullen and Lemon, 2018). Importantly, NHP HAVs are potentially zoonotic, and primates can become infected with human strains (Mätz-Rensing and Lowenstine, 2018).

2.1.2.9 Hepatitis B virus (HBV)

HBV is a hepadnavirus, being the major cause of hepatitis in humans and is transmitted by infected blood, saliva, and semen, leading to a persistent infection that causes chronic hepatitis and induces the development of hepatocellular carcinoma (Mätz-Rensing and Lowenstine, 2018). OWP are susceptible, especially cynomolgus monkeys, and the disease can be transmitted by humans. Pathological findings are periportal inflammation with lymphocytic cell infiltration progressing to cirrhosis (Mätz-Rensing and Lowenstine, 2018). A specific hepadnavirus was isolated from a captive wooly monkey (*Lagothrix* sp.) with a lethal fulminant hepatitis and nine others from the same institution tested positive by PCR, being seven also positive for anti-HBV serology (Lanford et al., 1998). This is the only report in NWP, with no data about this virus in wildlife.

2.1.2.10 Measles virus

Measles virus is a morbillivirus from the Paramyxoviridae family that have humans as primary hosts, but NHP are highly susceptible. Pathological findings in OWP are initially rash (maculopapular exanthema) progressing to a severe interstitial pneumonia rich in giant multinucleated syncytial cells with intranuclear and intracytoplasmic inclusions. In NWP the disease is more characterized by necrotizing gastroenteritis with multinucleated syncytia in various tissues including lymph nodes.

Once measles virus is strongly immunosuppressive, opportunistic co-infections are often observed (Mätz-Rensing and Lowenstine, 2018).

No evidence of measles virus in free-ranging NWP has been reported, however, measles virus was controlled by high vaccine coverage in human populations for years, reducing the circulation of the virus in most of countries. Over the last two decades the number of measles cases has been progressively increasing and, since 2018, vaccination coverage has been falling worryingly with high number of human cases, including in Brazil (Measles & Rubella Initiative, 2021). The COVID-19 pandemic has further undermined measles vaccination coverage, with vaccination levels reducing by up to 40% in the past two years (Silva et al., 2021; Shet et al., 2022). These data raise concerns regarding the re-emergence of a disease that can be controlled with vaccine in the human population and at the same time alerts about the possibility of infection of susceptible NWP that live in areas of high human density, such as marmosets.

2.1.2.11 Orthopoxvirus

Seroprevalence of vaccinia virus (VACV) in free-ranging capuchins and howler monkeys from Brazilian Amazon detected 25.3% (68/269) and 48.1% (13/27) of positive animals, respectively, indicating that this virus circulates in an NWP population in natural conditions (Abrahão et al., 2010). VACV is an Orthopoxvirus, closely related to cowpox virus, a virus that captive NWM, especially callitrichids, seems to be highly susceptible to infection (Mätz-Rensing and Lowenstine, 2018). Infection in NWP usually have lethal course and is characterized by vesicular and hemorrhagic to erosive-ulcerative dermal and mucous-cutaneous lesion with eosinophilic intracytoplasmic inclusion bodies in epithelial cells. Necrotizing lesions may be observed in multiple organs, such as liver, spleen, lymph nodes, stomach, and intestines. PCR and IHC can be performed to confirm the viral infection (Mätz-Rensing et al., 2006).

2.1.2.12 Oropouche virus (OROV)

OROV is an arbovirus from the family Bunyaviridae, which also comprises the genera Hantavirus, and is responsible to cause the Oropouche fever, a human disease that causes epidemics in Amazon region, being characterized by fever, headaches, chills, myalgia, arthralgia, retroocular pain, and, in a few cases, non-suppurative

meningitis or meningoencephalitis (Bastos et al., 2012). OROV have a sylvatic and urban cycle, depending on the vector. NWP are considered to play a role in the maintenance of this virus in the sylvatic cycle, with evidence of viral circulation in free-ranging capuchins and howler monkeys with positive serology to anti-OROV antibodies (Gibrail et al., 2016).

2.1.2.13 Papillomaviruses (PV)

PV are DNA viruses that infects epithelium and mucosa of a wide range of vertebrates, including humans, with 429 types, where 218 are found exclusively in humans (HPV). In humans and OWP, infections have been associated with dysplasia and neoplasia, being the most common cause of uterine cervical carcinoma in humans and causing typical papillomas with acanthosis and koilocytes in OWP (Köhler et al., 2011; Mätz-Rensing and Lowenstine, 2018). PV infection in NWP is scarcer, with reports in asymptomatic captive marmosets, squirrel monkeys, howler monkeys, spider monkeys and titi monkeys (Silvestre et al., 2016; Chen et al., 2018; D'arc et al., 2020). In wildlife PV was detected by PCR in oral swabs of two free-ranging NWP, one capuchin and one howler monkey, both from Argentina (Sanchez-Fernandez et al., 2022). HPV was investigated by IHC in a case of multicentric cutaneous keratoacanthomas in a free-ranging marmoset, but no viral antigen was detected (Díaz-Delgado et al., 2018b).

2.1.2.14 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

SARS-CoV-2 is an RNA virus responsible for the COVID-19 pandemic that started on December 2019 and is characterized by an acute respiratory syndrome with systemic inflammatory reaction. NHP, including common marmosets (*C. jacchus*) has been extensively used as experimental models for SARS-COV-2 and others respiratory coronavirus (MERS-COV and SARS-COV), reproducing the disease that is observed in human patients (Greenough et al., 2005; van Doremalen et al., 2015; Lu et al., 2020; Rockx et al., 2020; Singh et al., 2021). Therefore, two studies were performed searching for evidence of SARS-COV-2 in free-ranging NWP populations from the genera *Callithrix*, *Callicebus,* and *Alouatta*, all from hotspots for COVID-19 in Brazil (Abreu et al., 2021; Sacchetto et al., 2021). A total of 111 NWP was evaluated by RT-qPCR of oral and nasal swabs, blood and/or tissues and by PRNT, but no SARS-CoV-2 positive

samples were detected, regardless of NWP species or biome tested (Abreu et al., 2021; Sacchetto et al., 2021), raising questions about the susceptibility of these animals to SARS-COV-2 under natural conditions. However, there is a recent case of natural infection in a free-ranging black-tailed marmoset (*Mico melanurus*). This animal developed an interstitial pneumonia with identification of the viral spike protein in the lung tissue by IHC. Nasopharyngeal and oropharyngeal swabs were also positive to RT-PCR (Pereira et al., 2022).

2.1.2.15 Other viruses

Molina et al. (2019b) investigated by PCR hundreds free-ranging golden headed lion tamarin (*L. chrysomelas*) from Rio de Janeiro, Brazil, searching for hepatitis E virus genotype 3 (HEV-3), rotavirus A and norovirus GI and GII, but no positive animals was identified.

2.1.3 Parasites (Protozoan)

2.1.3.1 *Toxoplasma gondii*

Toxoplasmosis, caused by *T. gondii*, is an important and lethal disease for almost all NWP species, being frequently reported in captive animals, usually associated with outbreaks and an acute lethal evolution with sudden death (Paula et al., 2020; Santana et al., 2021). Animals are infected by ingesting food contaminated with infective oocysts, which are released in the feces of domestic and wild felines, considered the definitive hosts (Mätz-Rensing and Lowenstine, 2018). Pathological findings are represented by random necrotizing lesions in many organs, especially at liver, lungs, spleen, and brain, associated with intralesional tachyzoites, that are better observed by IHC (Grumann et al., 2017; Santos el al., 2017; Mätz-Rensing and Lowenstine, 2018; Paula et al., 2020; Santana et al., 2021). Another important feature of the *T. gondii* infection in NWP is the severe pulmonary edema and hemorrhage, in some cases associated with diffuse alveolar damage, characterized by alveolar hyaline membrane (Nishimura et al., 2019; Santana et al., 2021). There are only few reported cases of death by toxoplasmosis in free-ranging NWP, being three howler monkeys (*A. guariba*) (Ehlers et al., 2022) and one southern muriqui (*B. arachnoides*) (Santos et al., 2017). In both cases, intralesional tachyzoites were observed in multiple organs,

highlighted by IHC. Molecular studies are important to characterize the genotype of the *T. gondii*, which may play a role in the pathogenicity of the disease (Santos et al., 2017; Santana et al., 2021).

In captive, due to its acute lethal course, NWP usually dies from the infection before developing an immunological response (Paula et al., 2020), making serology a poorly efficient tool to evaluate the presence of the disease in these animals. This profile was also observed by Molina et al. (2017) in a serosurvey for toxoplasmosis in a free-ranging population of tamarins (*L. chrysomelas*) from Niteroi, Brazil, where 126 animals were tested by MAT and all were negative. However, some serological studies showed evidence of antibody anti-*T.gondii* in free ranging capuchins, howler monkeys and marmosets (Molina et al., 2014; Silva et al., 2014; Bueno et al., 2017; Niehaus et al., 2020), which indicate that some free-ranging NWP, although exposed to *T. gondii*, were able to survive to the acute phase. The severity of clinical toxoplasmosis in NWP may be associated with the protozoan (eg, inoculum, infective stage, genetic characteristics of the strain), host (eg, immune response, feeding behavior), and environment (eg, parasitic burden in soil and water) (Ajzenberg et al., 2004; Catão-Dias et al., 2013), justifying this contrasting results. In fact, it is known that capuchins are more resistant to infection than others NWP, even in similar exposure environment (Catão-Dias et al., 2013; Paula et al., 2020; Santana et al., 2021).

2.1.3.2 *Leishmania* spp.

Leishmaniosis, caused by *Leishmania* spp., has been reported in captive NWP from endemic regions with serology and molecular detection in asymptomatic animals, with few symptomatic cases, sometimes resulting in death (Malta et al., 2010, Lima et al., 2012; Lombardi et al., 2014; Oliveira et al., 2019; Santos and Oliveira, 2019). Pathological findings in a lethal case of a captive titi-monkey (*Callicebus nigrifrons*) infected by *L. infantum* were marked emaciation, severe pulmonary edema and hemorrhage, moderate splenomegaly, and lymphadenopathy, hepatic microgranulomas and lymphoplasmohytiocytic interstitial nephritis with macrophages containing amastigotes in all organs evaluated (Malta et al., 2010). IHC was performed to better visualize the amastigotes and PCR from the tissues confirmed the diagnosis. The other symptomatic NWP case reported in the literature was from a captive spider monkey (*A.*

paniscus) that presented weight loss and pale mucous membranes and blood PCR detected *Leishmania amazonensis* (Lima et al., 2012).

In wildlife there are serologic and molecular evidence of *Leishmania infantum, Leishmania mexicana, Leishmania shawi, L. amazonensis* and *Leishmania braziliensis* in a wide range of NWP from endemic areas (Herrer et al., 1976; Lainson et al., 1988; Lainson et al., 1989; Acardi et al., 2013; Rovirosa-Hernández et al, 2013; Bueno et al., 2017; Trueb et al., 2018; Medkour et al., 2019; Paiz et al., 2019; Santos and Oliveira, 2019; Cândido et al., 2021), but no symptomatic animals were identified. These findings could represent a public health concern once some species of NWP were competent in transmitting *L. infantum* to the invertebrate vector *Lutzomyia longipalpis* (Oliveira et al., 2019; Santos and Oliveira, 2019), being a potential reservoir of this parasite contributing to its maintenance in the environment.

2.1.3.3 *Trypanosoma* spp.

Trypanosoma cruzi is a mammal parasite, being the etiological agent of Chagas disease in humans, endemic throughout Latin America and transmitted by feces of blood-sucking triatomine bugs (Minuzzi-Souza et al., 2016). Pathological findings associated to *Trypanosoma* infections, especially for *T. cruzi*, are better known in captive primates, mainly reported in OWP. For *T. cruzi,* usually, it is observed a severe lymphoplasmacytic myocarditis with variable number of protozoan cysts filled with amastigotes in the cytoplasm of cardiomyocytes. Inflammation and amastigotes can be observed in other tissues, such as testis, but it is uncommon (DeLorenzo et al., 2019). Importantly, sometimes the amastigotes are not easily observed in the tissue, therefore IHC and ISH are important tools to confirm the diagnosis (DeLorenzo et al., 2019). PCR is also frequently use and is important to differentiate from *Leishmania* sp. and others *Trypanosoma* species (Cândido et al., 2021).

Serological evidence of *T. cruzi* was identified in 16% (8/48) of a free-ranging capuchin (*S. flavius*) population from the Brazilian Northern region, being five of these positive animals, also positive for *Leishmania* sp. (Bueno et al., 2017). Free-ranging populations of golden lion tamarin (*L. rosalia*) from Rio de Janeiro, Brazil, had a high prevalent (> 50%) and persistent (> five years) parasitemia, with identification of *T. cruzi* genotype II by hemoculture and serological assays, with no evidence of clinical

signs, being considered the most important wild reservoir for that genotype (Lisboa et al., 2004). However, mild cardiac alterations by electrocardiogram and hypergammaglobulinemia were identified by others studies in golden lion tamarin *T.cruzi*-infected, suggesting that, although difficult to identify, tamarins may have clinical signs and pathological disturbance similar to infected humans (Monteiro et al., 2006; Monteiro et al., 2010). Xenodiagnosis performed in two free-ranging squirrel monkeys (*Saimiri* sp.) infected by *T. cruzi* detected trypanosomes in the gut and salivary gland of the exposed triatomine (Ziccardi et al., 1997) and a study with captive NWP identified positive primates and bugs in the same enclosure (Minuzzi-Souza et al., 2016). Together, these data highlight the role of NWP in the trypanosome transmission, with direct impact in the public health, being an important key to the transmission and maintenance of this agent in wildlife.

Serology, blood smears cytology and blood PCR also detected *T. cruzi* and others *Trypanosoma* species in wild populations of marmosets (*Callithrix* sp. and *Mico melanurus*), howler monkeys (*Aloautta pigra, Alouatta caraya*, and *A. palliata*), spider monkeys (*A. geoffroyi*), tamarins (*Saguinus bicolor*) and capuchins (*Sapajus apella*) (Silva et al., 2008; Rovirosa-Hernández et al, 2013; Martinez et al., 2016; Coimbra et al., 2020; Cândido et al., 2021; Rovirosa-Hernández et al, 2021). In some of those studies, besides *T. cruzi*, *Trypanosoma minasense* and/or *Trypanosoma rangeli* were confirmed by DNA sequencing. These two species are primitive *Trypanosoma* species considered of low pathogenicity in vertebrates, but with a constant low parasitemia in NWP (Coimbra et al., 2020; Cândido et al., 2021). No symptomatic animals were identified in those studies.

2.1.3.4 *Plasmodium* spp.

Plasmodium spp. is extremally studied due to its importance on public health, being the causative agent of malaria, the deadliest human vector disease in the world. The species associated with this disease are *Plasmodium vivax* and *Plasmodium falciparum*, with cases of mixed infections (Rondon et al., 2019). There are 29 species of *Plasmodium* that parasitize NHP, with reports in free-ranging NWP of *P. falciparum, Plasmodium brazilianum* and *Plasmodium simium,* closely related to *P. vivax* and identified in human malaria outbreaks. *P. brazilianum* was also detected in humans,

being classified as quartan malaria parasite, which is considered harmless but have been associated with the development of renal disease (Lalremruata et al., 2015).

Free-ranging howler monkeys (*A. guariba clamitans*) are considered de main reservoir of malaria in the Atlantic Forest. Studies using blood and stool PCR have shown high prevalence (30 to 70%) of *Plasmodium* in free-ranging populations from fragmented and peri-urbanized areas, in some cases coinciding with human malarian outbreaks (Costa et al., 2014; Abreu et al., 2019; Nunes et al., 2019; Rondon et al., 2019; Chaves et al., 2022). Animals were infected with *P. simium*, *P. falciparuim,* and *P. brazilianum*, and at hematological and biochemical analysis the infected animals presented lymphocytosis, hypoalbuminemia, and high levels of ALT, that were even higher in mixed infections (Abreu et al., 2019; Nunes et al., 2019; Rondon et al., 2019). One symptomatic case presented inappetence, weakness, apathy, intermittent muscle tremors, dry and pale mucous membranes, mild dehydration and loss of muscle mass and body weight, with severe thrombocytopenia, anemia, and uremia (Costa et al., 2014). In another study, histopathology revealed hemozoin pigment at the spleen of the infected animals (Abreu et al., 2019).

In wildlife, from Amazon to Atlantic Forest, a wide range of NWP has been detected with *Plasmodium* spp., such. as capuchins (*Sapajus* sp., *Sapajus flavius,* and *Cebus vesicolor*) (Figueiredo et al., 2015; Bueno et al., 2017; Rondon et al., 2019; Chaves et al., 2022), spider monkeys (*Ateles* sp. and *Ateles hybridus*) (Rondon et al., 2019; Chaves et al., 2022), titi monkeys (*Callicebus dubius* and *Callicebus caligatus*) (Bueno et al., 2013), sakis (*Pithecia* sp.) (Bueno et al., 2013) and squirrel monkeys (*S. scireus*) (Chaves et al., 2022). Therefore, studied have been demonstrating the importance of NWP as potential reservoir of malaria parasite. However, studies about the impact of these parasites on the health of NWP are still scarce.

2.1.4 Parasites (Metazoan)

There are many studies of helminth fauna in free-ranging NWP, most of them evaluating feces from wild animals during capture and some performed during necropsies (Tavela et al., 2013; Solorzano-García et al., 2017; Rondon et al., 2017; Pereira et al., 2020a; Rondon et al., 2021; Catenacci et al., 2022; Zárate-Rendón et al., 2022). Free-ranging animals are usually parasitized and asymptomatic (Tavela et al.,

2013; Rondon et al., 2021; Catenacci et al., 2022). However in cases of any disturbance in the organism homeostasis, this balance is broken, and the animal may progress to clinical signs and death (Oliveira et al., 2017). Some parasites, such as *Platynosomum* sp. and *Prosthenorchis* sp., are well described in captive NWP, being extremally lethal and difficult to control (Sousa et al, 2008; Silva et al, 2012; Oliveira et al., 2021), but its impact in free-ranging animals is still unknown.

In general, the diagnostic of metazoan parasites is usually performed based on morphological features of the adult parasite and/or its eggs found in the feces and tissues of the animals. Unfortunately, there are only few studies focusing on molecular characterization of these parasites, therefore, there is scarce information on genomic database, making this a difficult tool to use in routine studies (Zárate-Rendón et al., 2022).

2.1.5 Fungi

Fungal infections in NWP have been rarely described, even in captive animals (Mätz-Rensing and Lowenstine, 2018).

2.1.5.1 *Aspergillus* spp.

Aspergillus sp. is a commensal fungus from NWP mucosa and is considered an opportunistic pathogen (Mätz-Rensing and Lowenstine, 2018). Guerra et al. (2021) described one single report of an *A. fumigatus* pulmonary infection in a free-ranging howler monkey infected by YFV. In this case the animal developed a necrosuppurative bronchopneumonia with angioinvasive fungal hyphae. CISH was performed to highlight the fungi in the tissue and PCR with DNA sequencing confirmed the diagnosis (Guerra et al., 2021).

2.1.5.2 Dermatophytosis

Dermatophytosis was investigated in 232 free-ranging tamarins (*L. chrysomelas*) with isolation of *Microsporum cookie* in one young healthy female (Neves et al., 2017). The diagnosis was performed by fungal culture on Sabouraud dextrose agar and phenotype identification. *Malassezia* spp. was isolated from 32.8% of a free-ranging tamarin population, being more frequently isolated from the haircoat than the ear canals

of these animals (Neves et al., 2017). No superficial cutaneous lesions or signs of external otitis were observed in the infected animals, indicating that *Malassezia* spp. may be part of the normal microbiota of its skin.

2.2 Non-infectious diseases

2.2.1 Trauma

Traumas are the main cause of death in free-ranging NWP (Ehlers et al., 2022), especially in NWP from periurban area, representing 20 to 90% of the cause of deaths (Ehlers et al., 2022; Oliveira et al., 2022). These traumas are usually due to roadkill, interspecific aggressions, and electrocutions (Lucena et al., 2017; Fernandes et al., 2019; Pereira et al., 2020b; Ehlers et al., 2022). Interspecific aggressions are mainly represented by dog and human attack, being the last one enhanced during the YF outbreak (Lucena et al., 2017; Fernandes et al., 2019; Ehlers et al., 2022; Oliveira et al., 2022).

Callithrix spp. (marmosets) are the main genus affected by traumatic events with blunt and sharp trauma representing more than 90% of the cases, and the head was the most affected site (Oliveira et al., 2022). NWP belonging to the Callithrichidae family, especially *Callithrix* spp., have a high ability to survive in urbanized fragmented habitats, adapting well to anthropogenic changes (Goulart et al, 2010). However, this also make them more exposed to anthropic traumatic risks factors, such as electrocution, roadkill, dog attack, and direct human aggression (Lucena et al., 2017; Teixeira et al., 2018; Fernandes et al., 2019; Pereira et al., 2020b). Electrocution was the main cause of death in marmosets from Brasilia, DF, Brazil, being mainly associated with electrified cables of urban regions. Besides the direct and more evident pathological features of trauma, such as bone fractures, skin injuries and marked hemorrhage, there are some secondary pathological findings associated to these traumatic cases, like non-thrombotic pulmonary embolization (Oliveira et al., 2022), pigmentary nephrosis caused by myoglobinuria (Fernandes et al., 2019) and secondary bacterial infections (Ehlers et al., 2022), interfering with a successful recovery of these animals.

2.2.2 Neoplasia

Neoplasia is usually described in captive NWP (Mätz-Rensing and Lowenstine, 2018). However, in free-ranging animals it is extremally rare, with few reports, such as: an intestinal lymphoma in a brown howler monkey (*A. guariba clamitans*) in a retrospective study with 59 animals (Ehlers et al., 2022), a retroperitoneal liposarcoma in a juvenile golden-headed lion tamarin (*L. chrysomelas*) (Díaz-Delgado et al., 2019), a hepatocellular carcinoma in a marmoset (*Callithrix* sp.) with liver flukes (Díaz-Delgado et al., 2018a), a multicentric cutaneous keratoacanthomas in a marmoset (*Callithrix* sp.) (Díaz-Delgado et al., 2018b) and a pulmonary adenosquamous carcinoma in a black capuchin monkey (*Sapajus nigritus*) (Díaz-Delgado et al., 2018c).

Table 1. List of bacterial, viral, protozoan, and fungal pathogens reported in free-ranging NWP.

Pathogen	Agent	Host genus	Host origin	Pathological findings	Diagnostic tools	References
Bacteria	*Escherichia coli*	*Alouatta*[1,40]	Brazil[1], Mexico[40]	Suppurative pneumonia[1] NP[40]	N[1], HP[1], IHC[1], BC[1,40]	Vásquez-Aguilar et al., 2020[40], Ehlers et al., 2021[1]
	Pasteurella spp.	*Alouatta*[1], *Mico*[8]	Brazil[1,8]	Suppurative pneumonia[1,8] Systemic leukocytosis[8]	N[1,8], HP[1,8], BC[1,8], PCR[8]	Ehlers et al., 2021[1], Silva et al., 2020[8]
	Pseudomonas spp.	*Alouatta*[41], *Cebus*[41]	Brazil[41]	NP[41]	Blood-PCR[41]	Menezes-Costa et al., 2013[41]
	Klebsiella pneumoniae	*Leontopithecus*[27]	Brazil[27]	Fibrinosuppurative bronchopneumonia[27] Fibrinosuppurative pericarditis[27] Splenic lymphoid depletion[27] Diffuse hepatic degeneration[27]	N[27], HP[27], BC[27]	Bueno et al., 2015[27]
	Staphylococcus aureus	*Leontopithecus*[7]	Brazil[7]	Suppurative meningoencephalitis[7]	N[7], HP[7], BC[7]	Molina et al., 2019[7]
	Leptospira spp.	*Alouatta*[1], *Ateles*[37], *Callithrix*[10], *Leontocebus*[19], *Leontopithecus*[12], *Saguinus*[19], *Sapajus*[46]	Bolivia[37], Brazil[1,10,12,46], Peru[19]	Interstitial pneumonia with hemorrhage, edema, and fibrin exudation[10] Interstitial nephritis with tubular degeneration and necrosis[10] Hepatocyte cord dissociation and necrosis with sinusoid leukocytosis[10] ND[1], NP[12,19,37,46]	N[1,10], HP[1,10], IHC[10], qPCR[10], PCR[12], MAT[12,19,37,46]	Karesh et al., 1998[37], Molina et al., 2019b[12], Girio et al., 2020[46], Aliaga-Samanez et al., 2021[19], Ehlers et al., 2021[1], Wilson et al., 2021[10]
	Borrelia burgdorferi	*Leontopithecus*[11]	Brazil[11]	NP[11]	Nested-PCR[11]	Santos et al., 2018[11]
	Clostridium botulinum type C toxin	*Callithrix*[42]	Brazil[42]	NP[42]	MNT[42]	Silva et al., 2018[42]
	Mycoplasma spp. (hemoplasmas)	*Alouatta*[43,45], *Saimiri*[44], *Saguinus*[44], *Sapajus*[44]	Brazil[43,44,45]	NP[43,44,45]	Blood-PCR[43,44,45], Blood-smears cytology[45]	Santos et al., 2013[43], Bonato et al., 2015[44], Cubilla et al., 2017[45]

Virus	Herpes simplex virus (HSV)	*Callithrix*[6,28,47,48]	Brazil[6,28,47,48]	Erosive and ulcerative lesions at skin and mucocutaneous junctions, conjunctivitis, nuclear inclusion bodies in epithelial cells that surrounds the vesicles or erosions[6,28,47,48] Ulcerative glossitis with syncytial cells and nuclear inclusion bodies[28,47] Necrotizing hepatitis with nuclear inclusion bodies[28] Lymphoplasmacytic to neutrophilic encephalitis with nuclear inclusion bodies[6,28,47,48] Lymphocytic adrenalitis, nephritis, and lymphoid hyperplasia[6,28]	N[6,28,47], HP[6,28,47,48], IHC[28,47], Nested PCR[6], qPCR[28], TEM[28,48]	Bruno et al., 1997[48], Costa et al., 2011[6], Longa et al., 2011[47], Wilson et al., 2022[28]
	Gammaherpesvirus	*Saguinus*[61], *Saimiri*[61], *Pithecia*[61]	French Guiana[61]	NP[61]	Blood-PCR[61]	Thoisy et al., 2003[61]
	Yellow fever virus (YFV)	*Alouatta*[1,9,25,50, 51,52,53], *Ateles*[37], *Callicebus*[25,49], *Callithrix*[25,50,51,52], *Leontopithecus*[25], *Sapajus*[25,51]	Bolivia[37], Brazil[1, 9, 25, 49, 50, 51, 52, 53]	Midzonal to diffuse hepatocellular necrosis with apoptotic bodies, mild mononuclear infiltrate and lipidosis[9,25,49,51] Lymphoid depletion[49] Acute renal tubular necrosis[49,51] NHF[25,51], ND[1,50,52,53], NP[37]	N[1,9,25,49,51], HP[1,9,25,49,51,53], IHC[1,9,25,49,50,51,53], RT-qPCR[9,25,49,50,52], VI[53], IFA[53], ND[37]	Karesh et al., 1998[37], Almeida et al., 2011[53], Leal et al., 2016[51], Cunha et al., 2020[50], Jesus et al., 2020[52], Santos et al., 2020[25], Ehlers et al., 2021[1], Fernandes et al., 2021[49], Guerra et al., 2021[9]
	Dengue virus (DENV)	*Alouatta*[13,14,15], *Cebus*[15], *Saimiri*[13]	Costa Rica[13,15], Argentina[14]	NP[13,14,15]	PRNT[13,14], RT-PCR[15]	Morales et al., 2017[14], Dolz et al., 2019[15], Chaves et al., 2021[13]
	Zika virus (ZIKV)	*Callithrix*[18,54], *Sapajus*[18,54]	Brazil[18,54]	NHF[18], NP[18,54]	HP[18], RT-qPCR[18,54], PRNT[54]	Terzian et al., 2018[18], Favoretto et al., 2019[54]

Saint Louis encephalitis virus (SLEV)	*Ateles*[37], *Alouatta*[13,14,16,17], *Sapajus*[17]	Argentina[14,16], Bolivia[37], Brazil[17] Costa Rica[13]	NP[13,14,16,17,37]	PRNT[13,14], HI[16,17], MNT[17], ND[37]	Karesh et al., 1998[37], Contigiani et al., 2000[16], Svoboda et al., 2014[17], Morales et al., 2017[14], Chaves et al., 2021[13]
West Nile virus (WNV)	*Alouatta*[13,14,15]	Costa Rica[13,15], Argentina[14]	NP[13,14,15]	PRNT[13,14], RT-PCR[15]	Morales et al., 2017[14], Dolz et al., 2019[15], Chaves et al., 2021[13]
Ilheus virus (ILHV)	*Alouatta*[14]	Argentina[14]	NP[14]	PRNT[14]	Morales et al., 2017[14]
Bussuquara virus (BSQV)	*Alouatta*[14]	Argentina[14]	NP[14]	PRNT[14]	Morales et al., 2017[14]
Eastern equine encephalitis virus (EEEV)	*Ateles*[37]	Bolivia[37]	NP[37]	ND[37]	Karesh et al., 1998[37],
Flavivirus (undetermined)	*Alouatta*[13,15], *Cebus*[15], *Saimiri*[15]	Costa Rica[13,15]	NP[13,15]	PRNT[13], ELISA[15]	Dolz et al., 2019[15], Chaves et al., 2021[13]
Tetraparvovirus (PARV4)	*Alouatta*[4], *Cebus*[4], *Ateles*[4]	Costa Rica[4], El Salvador[4]	NP[4]	Blood-PCR[4]	Chaves et al., 2019[4]
Bocaparvovirus (HBoV)	*Alouatta*[4], *Cebus*[4]	Costa Rica[4], El Salvador[4]	NP[4]	Blood-PCR[4]	Chaves et al., 2019[4]
Erythroparvovirus (B19)	*Alouatta*[4], *Cebus*[4]	Costa Rica[4], El Salvador[4]	NP[4]	Blood-PCR[4]	Chaves et al., 2019[4]
Rabies lyssavirus (RABV)	*Callithrix*[20,21], *Sapajus*[22,23]	Brazil[20,21,22,23,24]	NP[20,21,22,23,24]	DIF[20,21,22,24], MIT[20,22,24], RT-PCR[20,21,22,24], RFFIT[23]	Favoretto et al., 2001[21], Machado et al., 2012[23], Kobayashi et al., 2013[22], Kotait et al., 2018[24], Moutinho et al., 2019[20]
Simian foamy virus (SFV)	*Aotus*[56], *Ateles*[56], *Cebus*[56], *Lagothrix*[56], *Leontopithecus*[55], *Pithecia*[56]	Brazil[55], Peru[56]	NP[55,56]	EIA[56], WB[56], qPCR[55,56]	Ghersi et al., 2015[56], Miranda et al., 2019[55]
Hepatitis A virus (HAV)	*Sapajus*[57]	Brazil[57]	NP[57]	ELISA[57]	Svoboda et al., 2016[57]

	Papillomavirus (PV)	*Alouatta*[58], *Sapajus*[58]	Argentina[58]	NP[58]	PCR[58]	Sanchez-Fernandez et al., 2021[58]
	Adenovirus (AdV)	*Alouatta*[59], *Callithrix*[60], *Cebus*[60]	Brazil[60], Mexico[59]	NP[59, 60]	Stool-PCR[59], PRNT[60]	Ersching et al., 2010[60], Argüello-Sánchez et al., 2018[59]
	Vaccinia virus (orthopoxvirus)	*Alouatta*[62], *Sapajus*[62]	Brazil[62]	NP[62]	PRNT[62]	Abrahão et al., 2010[62]
	SARS-COV-2	*Mico*[85]	Brazil[85]	Interstitial pneumonia[85]	RT-PCR[85]; IHC[85]	Pereira et al., 2022[85]
Protozoan	*T. gondii*	*Alouatta*[1,26,64,65], *Brachyteles*[63], *Callithrix*[26], *Cebus*[65], *Sapajus*[2]	Brazil[1,2,26,63,64,65]	Necrotizing hepatitis, splenitis, lymphadenitis and nephritis[63] Non-suppurative meningoencephalitis[63] Observation of intralesional tachyzoites and bradyzoites in multiple organs[1,63] NP[2,26,64,65]	N[1,63], HP[1,63], IHC[1,63], MAT[2,26,64,65], PCR[63]	Ehlers et al., 2021[1], Molina et al., 2014[26], Silva et al., 2014[64], Bueno et al., 2017[2], Santos et al., 2017[63], Niehaus et al., 2020[65]
	Leishmania sp.	*Alouatta*[73,74,76], *Aotus*[30,66,67,70], *Callithrix*[71,72], *Chiropotes*[68,69], *Mico*[30], *Saguinus*[66,68,69], *Sapajus*[2,30]	Argentina[70,74], Brazil[2,30,68,69,71,72], French Guiana[73], Mexico[76], Panama[66,67]	NP[2,30,66,67,68,69,70,71,72,73,74,76]	ELISA[2,72,76], Blood-PCR[30,71,72,73], Skin-PCR[72,74], PCR-RFLP[70,74], PI[66,67,68,69], WB[76], IFA[76]	Herrer et al., 1976[67], Lainson et al., 1988[68], Lainson et al., 1989[69], Acardi et al., 2013[70], Rovirosa-Hernández et al, 2013[76], Bueno et al., 2017[2], Trueb et al., 2018[71], Medkour et al., 2019[73], Paiz et al., 2019[72], Martínez et al., 2020[74], Cândido et al., 2021[30]

	Trypanosoma sp.	*Alouatta*[75,76,77], *Ateles*[75], *Callithrix*[5], *Mico*[30], *Leontopithecus*[32,33,34], *Saguinus*[31], *Saimiri*[35], *Sapajus*[2,30]	Argentina[77], Brazil[2, 5, 30, 31, 32, 33, 34, 35], Mexico[75,76]	NP[2,5,30,31,32,33,34,35,75,76,77]	ELISA[75,76], TESA-blot[2], Blood-PCR[5,30,31,75,77], qPCR-HRM[75], Blood-smears cytology[5,31,32], IFA[32,33,34,76], HC[32,33,35], Xenodiagnosis[35]	Ziccardi et al., 1997[35], Lisboa et al., 2004[32], Monteiro et al., 2006[33], Silva et al., 2008[31], Monteiro et al., 2010[34], Rovirosa-Hernández et al, 2013[76], Martinez et al., 2016[77], Bueno et al., 2017[2], Coimbra et al, 2020[5], Cândido et al., 2021[30], Rovirosa-Hernández et al, 2021[75]
	Plasmodium spp.	*Alouatta*[39,78,79,81,83], *Ateles*[78,83], *Callicebus*[80], *Cebus*[78], *Pithecia*[80], *Saimiri*[83], *Sapajus*[2,82,83]	Brazil[2,39,79,80,81,82], Colombia[78], Costa Rica[83]	Hemozoin pigment intracytoplasmic in macrophages at red pulp from the spleen[39] NP[2,78,79,80,81,82,83]	HP[39], Blood-smears cytology[39,80,82], Blood-PCR[2,39,80,81,82,83], Stool-PCR[78,79], Nested-PCR[79], IFA[82]	Bueno et al., 2013[80], Costa et al., 2014[81], Figueiredo et al., 2015[82], Bueno et al., 2017[2], Abreu et al., 2019[39], Nunes et al., 2019[79], Rondon et al., 2019[78], Chaves et al., 2022[83]
	Entamoeba	*Alouatta*[84]	Mexico[84]	NP[84]	FPE[84], PCR[84]	Villanueva-García et al., 2017[84]
Fungal agent	*Aspergillus fumigatus*	*Alouatta*[9]	Brazil[9]	Necrosuppurative bronchopneumonia with angioinvasive fungal hyphae[9]	N[9], HP[9], CISH[9], PCR[9]	Guerra et al., 2021[9]
	Microsporum spp.	*Leontopithecus*[29]	Brazil[29]	NP[29]	FC[29]	Neves et al., 2017[29]
	Malassezia spp.	*Leontopithecus*[29]	Brazil[29]	NP[29]	PI[29], cytology[29]	Neves et al., 2017[29]

BC: bacterial culture, CISH: chromogenic *in situ* hybridization, DIF: direct immunofluorescence test, DPI: direct parasitological identification, EIA: enzyme immunoassay, FC: fungal culture, FPE: fecal parasitological examination, HC: hemoculture, HI: hemagglutination inhibition, HP: histopathology stained by hematoxylin and eosin, IFA: indirect immunofluorescence assay, IHC: immunohistochemistry, MIT: mouse inoculation test, MNT: mouse neutralization test, N: necropsy, ND: not described, NP: not performed, NHF: nonspecific histopathological findings, PI: parasite isolation, PRNT: plaque reduction neutralization test, RFFIT: rapid fluorescent focus inhibition test, TEM: transmission electron microscopy, VI: viral isolation.

REFERENCES

1. Abreu FVS de, Santos E dos, Mello ARL, et al. Howler monkeys are the reservoir of malarial parasites causing zoonotic infections in the Atlantic forest of Rio de Janeiro. *Plos Neglect Trop D*. 2019;**13**(12):e0007906.
3. Abreu FVS de, Macedo MV, Silva AJJ da, 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*. 2021;**18**(4):414–420.
4. Acardi SA, Rago MV, Liotta DJ, Fernandez-Duque E, Salomón OD. *Leishmania* (*Viannia*) DNA detection by PCR-RFLP and sequencing in free-ranging owl monkeys (Aotus azarai azarai) from Formosa, Argentina. *Vet Parasitol*. 2013;**193**(1–3):256-259.
5. Adlhoch, C, Kaiser M, Loewa A, et al. Diversity of parvovirus 4-like viruses in humans, chimpanzees, and monkeys in hunter-prey relationships. *Emerg Infect Dis*. 2012; **18**(5), 859–862.
6. Ajzenberg D, Bañuls AL, Su C, et al. Genetic diversity, clonality and sexuality in *Toxoplasma gondii*. *Int J Parasitol*. 2004; **34**:1185-1196.
7. Albrecht JC. Primary structure of the *Herpesvirus ateles* genome. *J Virol*. 2002; **74**:1033–1037.
8. Alcantara BN de, Imbeloni AA, Durans D de BS, et al. Histopathological lesions of congenital Zika syndrome in newborn squirrel monkeys. *Sci Rep-uk*. 2021;**11**(1):6099.
9. Aliaga-Samanez GG, Lescano J, Urday MJQ, et al. First detection of antibodies against *Leptospira* among free-ranging neotropical non-human primates in the Peruvian Amazon lowland rainforest. *Transbound Emerg Dis*. 2021. DOI: 10.1111/tbed.14112
10. Almeida MAB de, Santos E dos, Cardoso J da C, et al. Yellow fever outbreak affecting Alouatta populations in southern Brazil (Rio Grande do Sul State), 2008–2009. *Am J Primatol*. 2012;**74**(1):68–76.
11. Anzai EK, Júnior JCS, Peruchi AR, et al. First case report of non-human primates (*Alouatta clamitans*) with the hypervirulent *Klebsiella pneumoniae* serotype K1 strain ST 23: A possible emerging wildlife pathogen. *J Med Primatol*. 2017;**46**(6):337–342.
12. Argüello-Sánchez LE, Monteros AE de los, Santiago-Alarcon D, García-Sepúlveda CA. Detection and prevalence of adenoviruses from free-ranging black howler monkeys (Alouatta pigra). *Virus Genes*. 2018;**54**(6):818–822.
13. Bakker J, Kondova I, Groot CW De, Remarque EJ, Heidt PJ. A report on *Yersinia*-related mortality in a colony of New World Monkeys. *Lab Primate Newsletter*. 2007; **46**:11–6.
14. Baker EN, Helps CR, Neimark H, Peters IR, Peters W, Tasker S. A novel haemoplasma species identified in archived primate blood smears. *Vet. Microbiol*. 2011;**149**:478–481.
15. Barnes KJ, Garner MM, Wise AG, Persiani M, Maes RK, Kiupel M. Herpes simplex encephalitis in a captive black howler monkey (*Alouatta caraya*). *J Vet Diagn Invest*. 2016;**28**:76–8.
16. Bastos MS, Figueiredo LTM, Naveca FG, et al. Identification of Oropouche Orthobunyavirus in the cerebrospinal fluid of three patients in the Amazonas, Brazil. *Am J Trop Med Hyg.* 2012; **86**:732-735.
17. Bath S, Acharya PR, Biranthabail D, Rangnekar A, Shiragavi S. A case of lower respiratory tract infection with canine-associated *Pasteurella canis* in a patient with chronic obstructive pulmonary disease. *J Clin Diagn Res.* 2015;**9**(8):3-4.

18. Bonato L, Figueiredo MAP, Gonçalves LR, Machado RZ, André MR. Occurrence and molecular characterization of *Bartonella* spp. and hemoplasmas in neotropical primates from Brazilian Amazon, Comp. Immunol. Microbiol. *Infect. Dis*. 2015; **42**:15–20.
19. Bruno SF, Liebhold MM, Mätz-Rensing K, et al. Herpesvirus infections in free living black tufted ear marmosets (*Callithrix penicillata*, E. Geoffroyi 1812) at the State Park of Serra da Tiririca, Niterói, Rio de Janeiro, Brazil [in German]. *Berl Munch Tierarztl Wochenschr*. 1997;**110**:427–30.
20. Buchanan A, Díaz-Delgado J, Balamayooran G, Anguiano M, Groch K, Krol L. Leukemic histiocytic sarcoma in a captive common squirrel monkey (*Saimiri sciureus*) with Saimiriine Gammaherpesvirus 2 (Rhadinovirus), *Saimiri sciureus* lymphocryptovirus 2 (Lymphocryptovirus) and Squirrel monkey retrovirus (β-Retrovirus) coinfection. *J Med Primatol*. 2020;**49**(6):341–343.
21. Bueno MG, Rohe F, Kirchgatter K, et al. Survey of *Plasmodium* spp. in Free-Ranging Neotropical Primates from the Brazilian Amazon Region impacted by Anthropogenic Actions. *Ecohealth.* 2013;**10**(1):48–53.
22. Bueno MG, Iovine RO, Torres LN, et al. Pneumonia and bacteremia in a golden-headed lion tamarin (*Leontopithecus chrysomelas*) caused by *Klebsiella pneumoniae* subsp. *pneumoniae* during a translocation program of free-ranging animals in Brazil. J Vet Diag Investig. 2015; **27**(3):387–391.
23. Bueno MG, Catão-Dias JL, Laroque P de O, et al. Infectious Diseases in Free-Ranging Blonde Capuchins, *Sapajus flavius*, in Brazil. *Int J Primatol*. 2017;**38**(6):1017–1031.
24. Burke DS, Heisey GB: Wild malaysian cynomolgus monkeys are exposed to hepatitis A vırus. Am J 10 *Trop Med Hyg* 1984; **33**:940–4.
25. Cadavid D, Bai Y, Hodzic E, Narayan K, Barthold SW, Pachner AR. Cardiac involvement in non-human primates infected with the Lyme disease spirochete *Borrelia burgdorferi*. *Lab Invest*. 2004;**84**(11):1439–1450.
26. Cândido SL, Pavelegini LAD, Pacheco T dos A, et al. Molecular detection of trypanosomatids in neotropical primates in the state of Mato Grosso, Midwest, Brazil. *Revista Brasileira De Parasitol Veterinária*. 2021;**30**(2):e001321.
27. Casagrande RA, Pannuti CS, Kanamura C, Freire WS, Grespan A, Matushima ER. Fatal human herpesvirus 1 (HHV-1) infection in captive marmosets (*Callithrix jacchus* and *Callithrix penicillata*) in Brazil: clinical and pathological characterization. *Pesq Vet Bras*. 2014;**34**:1109–114.
28. Catão-Dias JL, Epiphanio S, Kierulff CM. Neotropical Primates and Their Susceptibility to Toxoplasma gondii: New Insights for an Old Problem. In: Brinkworth JF, Pechenkina K (ed). Primates, Pathogens, and Evolution. Springer: New York. 2013; 253-290.
29. Catenacci LS, Oliveira JBS, Vleeschouwer KMD, et al. Gastrointestinal parasites of Leontopithecus chrysomelas in the Atlantic Forest, Brazil. *Revista Brasileira De Parasitol Veterinária*. 2022;**31**(1):e013521.
30. Chaves A, Ibarra-Cerdeña CN, López-Pérez AM, et al. Bocaparvovirus, Erythroparvovirus and Tetraparvovirus in New World Primates from Central America. *Transbound Emerg Dis*. 2020;**67**(1):377–387.

31. Chaves A, Piche-Ovares M, Ibarra-Cerdeña CN, et al. Serosurvey of Nonhuman Primates in Costa Rica at the Human–Wildlife Interface Reveals High Exposure to Flaviviruses. *Insects*. 2021;**12**(6):554.
32. Chaves A, Dolz G, Ibarra-Cerdeña CN, et al. Presence and potential distribution of malaria-infected New World primates of Costa Rica. *Malaria J*. 2022;**21**(1):17.
33. Chen EC, Yagi S, Kelly KR, Mendoza SP, Tarara RP et al. Cross-species transmission of a novel adenovirus associated with a fulminant pneumonia outbreak in a new world monkey colony. *PLoS Pathog* 2011;**7**:e1002155.
34. Chen Z, Wood CE, Abee CR, Burk RD. Complete Genome sequences of three novel *Saimiri sciureus* papillomavirus types isolated from the cervicovaginal region of squirrel monkeys. Genome Announc. 2018; DOI: https://doi.org/10.1128/genomeA.01400-17
35. Coimbra DP, Penedo DM, Silva MOM, 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. *Parasitol Int.* 2020;**75**:101999.
36. Contigiani MS, Fernández C, Spinsanti LI, Díaz GE. Prevalence of Flavivirus antibodies in *Alouatta caraya* primate autochthonous of Argentina. *Medicina (B Aires)*. 2000;**60**(3):348-50.
37. Costa A, Luppi MM, Malta M de CC, et al. Outbreak of Human Herpesvirus Type 1 Infection in Nonhuman Primates (*Callithrix penincillata*). *J Wildlife Dis*. 2011;**47**(3):690–693.
38. Costa DC, Cunha VP da, Assis GMP de, et al. *Plasmodium simium/Plasmodium vivax* infections in southern brown howler monkeys from the Atlantic Forest. *Memórias Instituto Oswaldo Cruz*. 2014;**109**(5):641–643.
39. Coursaget P, Levesque B, Gretillat E, Eyraud M, Ferrara L, Germain M: Hepatitis A virus in primates outside captivity. *Lancet* 1981; **2**:929.
40. Crossland NA, Alvarez X, Embers ME. Late Disseminated Lyme Disease. *Am J Pathology*. 2018;**188**(3):672–682.
41. Cubilla MP, Santos LC, Moraes W, et al. Occurrence of hemotropic mycoplasmas in non-human primates (*Alouatta caraya, Sapajus nigritus* and *Callithrix jacchus*) of southern Brazil, *Comp Immonol Microbiol Infect Dis*. 2017; **52**: 6–13.
42. Cullen JM, Lemon SM. Comparative Pathology of Hepatitis A Virus and Hepatitis E Virus Infection. *Csh Perspect Med*. 2018;**9**(4):a033456.
43. D'arc M, Moreira FRR, Dias CA, et al. The characterization of two novel neotropical primate papillomaviruses supports the ancient within-species diversity model. *Virus Evol*. 2020;**6**(1):36.
44. DeLorenzo M, Carias E, Mustonen A, Gonzalez O, Dick EJ, Kumar S. In situ hybridization assay for the diagnosis of chagas myocarditis and orchitis in a rhesus macaque (*Macaca mulatta*): A case report. *J Med Primatol*. 2019;**48**(3):182–185.
45. Díaz-Delgado J, Sanches TC, Santos-Cirqueira C, Coimbra AAC, Guerra JM et al. Hepatocellular carcinoma in a free-living marmoset (*Callithrix* sp.) with concomitant biliary trematodiasis. *J Med Primatol*. 2018a; **47**(2):128-131.

46. Díaz-Delgado J, Sanches TC, Cirqueira CS, et al. Multicentric cutaneous keratoacanthomas in a free-living marmoset (*Callithrix sp*.). *J Med Primatol*. 2018b;47(3):205–208. Díaz-Delgado J, Guerra JM, Fernandes NCCA, et al.
47. Spontaneous pulmonary adenosquamous carcinoma in a free-living black capuchin monkey (*Sapajus nigritus*). *J Med Primatol*. 2018c; **47**(2):120-123.
48. Díaz-Delgado J, Molina CV, Catão-Dias JL, Kierulff MCM, Pissinatti A, Bueno MG. Spontaneous retroperitoneal liposarcoma in a free-ranging juvenile golden-headed lion tamarin (*Leontopithecus chrysomelas*). *J Med Primatol*. 2019;**48**(1):61–64.
49. Dietz JM, Hankerson SJ, Alexandre BR, et al. Yellow fever in Brazil threatens successful recovery of endangered golden lion tamarins. *Sci Rep-uk*. 2019;**9**(1):12926.
50. Dolz G, Chaves A, Gutiérrez-Espeleta GA, Ortiz-Malavasi E, Bernal-Valle S, Herrero MV. Detection of antibodies against flavivirus over time in wild non-human primates from the lowlands of Costa Rica. *Plos One*. 2019;**14**(7):e0219271.
51. Ehlers LP, Bianchi MV, Argenta FF, et al. Mycobacterium tuberculosis var. tuberculosis infection in two captive black capuchins (*Sapajus nigritus*) in Southern Brazil. *Braz J Microbiol*. 2020;**51**(4):2169–2173.
52. Ehlers LP, Slaviero M, Bianchi MV, et al. Causes of death in neotropical primates in Rio Grande do Sul State, Southern Brazil. *J Med Primatol*. 2022;**51**(2):85–92.
53. Ersching J, Hernandez MIM, Cezarotto FS. Neutralizing antibodies to human and simian adenoviruses in humans and New-World monkeys. *Virology*. 2010; **10**;407(1):1-6.
54. Favoretto SR, Mattos CC de, Morais NB, Araújo FAA, Mattos CA de. Rabies in marmosets (*Callithrix jacchus*), Ceará, Brazil. *Emerg Infect Dis*. 2001;**7**(6):1062–1065.
55. Favoretto SR, Araujo DB, Duarte NFH, et al. Zika Virus in Peridomestic Neotropical Primates, Northeast Brazil. *Ecohealth*. 2019;**16**(1):61–69.
56. Fernandes NCCA, Nascimento PM, Sánchez-Sarmiento AM, et al. Histopathological kidney changes and myoglobinuria in neotropical non-human primates attacked by dogs, Brazil. *J Med Primatol*. 2019;**49**(2):65–70.
57. Fernandes NCC de A, Guerra JM, Cunha MS, et al. Yellow fever surveillance challenge: investigation of a marmoset non-autochthonous case. *Acta Trop*. 2020;**212**:105702.
58. Fernandes AT da S, Moreira SB, Gaspar LP, et al. Safety and immunogenicity of 17DD attenuated yellow fever vaccine in howler monkeys (Alouatta spp.). *J Med Primatol*. 2021c;**50**(1):36–45.
59. Fernandes NCC de A, Cunha MS, Guerra JM, et al. Yellow Fever as Cause of Death of Titi Monkeys (*Callicebus* Spp.). *Vet Pathol*. 2021a;**58**(4):730–735.
60. Fernandes NCC de A, Guerra JM, Díaz-Delgado J, et al. Differential Yellow Fever Susceptibility in New World Nonhuman Primates, Comparison with Humans, and Implications for Surveillance. *Emerg Infect Dis*. 2021b;**27**(1):47–56.
61. Fiori AE. Hepatitis A transmitted by food. Clin Infect Dis. 2004; **38**:705-715.
62. Figueiredo MAP, Santi SMFD, Figueiredo TAP, Machado RZ. Natural *Plasmodium* infection in neotropical primates in the island of São Luís, state of Maranhão, Brazil. *Revista Brasileira De Parasitol Veterinária*. 2015;**24**(2):122–128.

63. Gessain A, Montange T, Betsem E, et al. Case-control study of the immune status of humans infected with zoonotic gorilla simian foamy viruses. *J Infect Dis*, 2020; DOI: 10.1093/infdis/jiz660 . pasteur-02313546v2
64. Ghersi BM, Jia H, Aiewsakun P, et al. Wide distribution and ancient evolutionary history of simian foamy viruses in New World primates. *Retrovirology*. 2015;**12**(1):89.
65. Gibrail MM, Fiaccadori FS, Souza M, et al. Detection of antibodies to Oropouche virus in non-human primates in Goiânia City, Goiás. *Rev Soc Bras Med Tro*. 2016;**49**(03):357–360.
66. Giovanetti M, Mendonça MCL de, Fonseca V, et al. Yellow Fever Virus Reemergence and Spread in Southeast Brazil, 2016–2019. *J Virol*. 2019;**94**(1).
67. Girio RJS, Andrade-Cruvinel TM, Vasconcellos SA, et al. Serological survey and DNA screening of *Leptospira* spp. in free-living adult tufted capuchin monkeys (*Cebus apella nigritus*) in a forest reserve Southeast São Paulo State, Brazil. *J Med Primatol*. 2021;**50**(1):3–8.
68. Greenough, TC, Carville A, Coderre J, et al. Pneumonitis and Multi-Organ System Disease in Common Marmosets (*Callithrix jacchus*) Infected with the Severe Acute Respiratory Syndrome-Associated Coronavirus. *Am J Pathol*. 2005; **167**, 455–463.
69. Grumann MR, Silva Z da, Filho JR da S, Costa MM, Vieira MIB, Motta AC da. Immunohistochemical and serological aspects of *Toxoplasma gondii* infection in Neotropical primates. *Semina Ciências Agrárias*. 2016;**38**(3):1375–1382.
70. Guerra MFL, Teixeira RHF, Ribeiro VL, et al. Suppurative peritonitis by *Klebsiella pneumoniae* in captive gold-handed tamarin (*Saguinus midas midas*). *J Med Primatol*. 2016;**45**(1):42–46.
71. Guerra JM, Fernandes NCC de A, Santos ALM dos, et al. Hypervirulent *Klebsiella pneumoniae* as Unexpected Cause of Fatal Outbreak in Captive Marmosets, Brazil. *Emerg Infect Dis*. 2020;**26**(12):3039–3043.
72. Guerra JM, Ferreira CS da S, Díaz-Delgado J, et al. Concurrent yellow fever and pulmonary aspergillosis due to *Aspergillus fumigatus* in a free-ranging howler monkey (*Alouatta* sp). *J Med Primatol*. 2021;**50**(3):201–204.
73. Han BA, Majumdar S, Calmon FP, et al. Confronting data sparsity to identify potential sources of Zika virus spillover infection among primates. *Epidemics-neth*. 2019;**27**:59–65.
74. Hatt JM, Grest P, Posthaus H, Bossart W. Serologic survey in a colony of captive common marmosets (*Callithrix jacchus*) after infection with herpes simplex type 1-like virus. *J Zoo Wildl Med*. 2004;**35**:387–90.
75. Hayashimoto N, Inoue T, Morita H, et al. Survey and Experimental Infection of Enteropathogenic *Escherichia coli* in Common Marmosets (Callithrix jacchus). *Plos One*. 2016;**11**(8):e0160116.
76. Herrer A, Christensen HA, Beumer RJ. Reservoir hosts of cutaneous leishmaniasis among Panamanian forest mammals. *Am J Trop Med Hyg*. 1973;**22**:585-591.
77. Herrer A, Christensen HA. Epidemiological patterns of cutaneous leishmaniasis in Panama III. Endemic persistence of the disease. *Am J Trop Med Hyg*. 1976;**25**:54-58.
78. Hirsh A, Dias LG, Martins LO, Resende NAT, Landau EC. Database of Georeferenced Occurrence Localities of Neotropical Primates. Department of Zoology, UFMG, Belo Horizonte. http://www.icb.ufmg.br/zoo/primatas/home bdgeoprim.htm. 2006.

79. Huang R, Liu Q, Li G, et al. *Bartonella quintana* infections in captive monkeys, China, *Emerg Infect Dis*. 2011; **17**:1707–1709.
80. Iovine RO, et al. *Klebsiella* spp. is part of the natural intestinal microbiota and a potential disease pathogen in wild golden-headed lion tamarins (*L. chrysomelas*). European Wildlife Disease Association Conference, Edinburgh, Scotland, August 2014:25–29.
81. Jesus JG de, Gräf T, Giovanetti M, et al. Yellow fever transmission in non-human primates, Bahia, Northeastern Brazil. *Plos Neglect Trop D*. 2020;**14**(8):e0008405.
82. Juan-Sallés C, Ramos-Vara JA, Prats N, Solé-Nicolás J, Segalés J, Marco AJ. Spontaneous herpes simplex virus infection in common marmosets (*Callithrix jacchus*). *J Vet Diagn Invest*. 1997;**9**:341–5.
83. Karesh WB, Wallace RB, Painter RLE, et al. Immobilization and Health Assessment of Free-Ranging Black Spider Monkeys (*Ateles paniscus chamek*). *Am J Primatol*. 1998; **44**:107–123.
84. Keel MK, Songer JG. The Comparative Pathology of Clostridium difficile-associated Disease. *Vet Pathol*. 2006;**43**(3):225–240.
85. Kobayashi Y, Sugimoto K, Mochizuki N, et al. Isolation of a phylogenetically distinct rabies virus from a tufted capuchin monkey (Cebus apella) in Brazil. *Virus Res*. 2013;**178**(2):535–538.
86. Köhler A, Gottschling M, Manning K, et al. Genomic characterization of ten novel cutaneous human papillomaviruses from keratotic lesions of immunosuppressed patients. J Gen Virol. 2011; **92**(7):1585-1594.
87. Kotait I, Oliveira R de N, Carrieri ML, et al. Non-human primates as a reservoir for rabies virus in Brazil. *Zoonoses Public Hlth*. 2019;**66**(1):47–59.
88. Lainson R, Shaw JJ, Braga RR, Sacawa E, Souza AA, Silveira FT. Isolation of *Leishmania* from monkeys in the Amazon region of Brazil. *Trans Roy Soc Trop Med Hyg*. 1988;**82**:132.
89. Lainson R, Braga RR, De Souza AA, Pôvoa MM, Ishikawa EA, Silveira FT *Leishmania (Viannia) shawi* sp., a parasite of monkeys, sloths and procyonids in Amazonian Brazil. *Ann Parasitol Hum Comp*. 1989;**64**(3):200-207.
90. Lalremruata A, Magris M, Vivas-Martínez S, et al. Natural infection of *Plasmodium brasilianum* in humans: Man and monkey share quartan malaria parasites in the Venezuelan Amazon. *Ebiomedicine*. 2015;**2**(9):1186–1192.
91. Lanford RE, Chavez D, Brasky KM, Burns RB, Rico-Hesse R. Isolation of a hepadnavirus from the woolly monkey, a New World primate. *Proc National Acad Sci*. 1998;**95**(10):5757–5761.
92. Leal SG, Romano APM, Monteiro RV, Melo CB de, Vasconcelos PF da C, Castro MB de. Frequency of histopathological changes in Howler monkeys (*Alouatta* sp.) naturally infected with yellow fever virus in Brazil. *Rev Soc Bras Med Tro*. 2016;**49**(1):29–33.
93. Lemos GAA, Pires BG, Mainardi RM, et al. Spontaneous outbreak of *Yersinia enterocolitica* infection and co-infection with *Escherichia coli* in black-tufted marmosets (*Callithrix penicillata*). *Braz J Vet Pathol*, 2021, **14**(3), 173 – 179.
94. Lewis J, Smith G, White V. An outbreak of botulism in captive hamadryas baboons (Papio hamadryas). *Vet Rec*. 1990;**126**(9):216.

95. Li H, Bai JY, Wang LY, et al. Genetic diversity of *Bartonella quintana* in macaques suggests zoonotic origin of trench fever, *Mol. Ecol*. 2013;**22**:2118–2127.
96. Lima VM, Santiago ME, Sanches LC, Lima BD. Molecular diagnosis of *Leishmania amazonensis* in a captive spider monkey in Bauru, São Paulo, Brazil. *J Zoo Wildl Med*. 2012;**43**(4):943-945.
97. Lisboa CV, Mangia RH, Lima NRCD, et al. Distinct patterns of *Trypanosoma cruzi* infection in *Leontopithecus rosalia* in distinct Atlantic Coastal Rainforest fragments in Rio de Janeiro – Brazil. *Parasitology*. 2004;**129**(6):703–711.
98. Litvoc MN, Novaes CTG, Lopes MIBF. Yellow fever. Rev Assoc Med Bras. 2018;**64**(2):106–113.
99. Lombardi MC, Turchetti AP, Tinoco HP, et al. Diagnosis of *Leishmania infantum* infection by polymerase chain reaction in wild mammals. *Pesq Vet Bras*. 2014;**34**:1243-1246.
100. Longa CS, Bruno SF, Pires AR, Romijn PC, Kimura LS, Costa CH. Human herpesvirus 1 in wild marmosets, Brazil, 2008. *Emerg Infect Dis*. 2011;**17**:1308–10.
101. Loza-Rubio E, Rojas-Anaya E, López-Ramírez RDC, Saiz JC, Escribano-Romero E. Prevalence of neutralizing antibodies against West Nile virus (WNV) in monkeys (*Ateles geoffroyi* and *Alouatta pigra*) and crocodiles (*Crocodylus acutus* and *C. acutus–C. moreletti hybrids*) in Mexico. *Epidemiol Infect*. 2016;**144**(11):2371–2373.
102. Lu S, Zhao Y, Yu W, et al. Comparison of nonhuman primates identified the suitable model for COVID-19. *Signal Transduct. Target. Ther*. 2020; **5**:1–9.
103. Lucena FP, De-Campos SN, Rodrigues RL. Maus tratos como causa de mortalidade em primatas não humanos recebidos pelo IJV, no estado do Rio de Janeiro, em surto de febre amarela. *Savannah J Res Dev*. 2017;**1**(suppl 1):92.
104. Ludlage E, Mansfield KG. Clinical care and diseases of the common marmoset (*Callithrix jacchus*). *Comp Med*. 2003; **53:369**-382.
105. Lyra MR, Oliveira LB, Silva EE da. Herpes simplex virus transmission following brown howler monkey (Alouatta guariba) bite. *Rev Soc Bras Med Tro*. 2019;**52**:e20180218.
106. Machado GP, Antunes JMA de P, Uieda W, et al. Exposure to rabies virus in a population of free-ranging capuchin monkeys (*Cebus apella nigritus*) in a fragmented, environmentally protected area in southeastern Brazil. *Primates*. 2012;**53**(3):227–231.
107. Mafra C, Barcelos RM, Mantovani C, et al. Occurrence of *Ehrlichia canis* in free-living primates of the genus *Callithrix*. *Revista Brasileira De Parasitol Veterinária*. 2014;**24**(1):78–81.
108. Malta M, Tinoco HP, Xavier MN, Vieira A, Costa EA, Santos RL. Naturally acquires visceral leishmaniasis in non-human primates in Brazil. *Vet Parasitol*. 2010;**169**:193-197.
109. Mansfield KG, Fox JG. Bacterial diseases. In: Marini R, Wachtman L, Tardif S, Mansfield K, Fox J (Eds). The Common Marmoset in Captivity and Biomedical Research. Elsevier: Missouri, 2019, 265–287.
110. Martínez MF, Kowalewski MM, Salomón OD, Schijman AG. Molecular characterization of trypanosomatid infections in wild howler monkeys (*Alouatta caraya*) in northeastern Argentina. *Int J Parasitol Parasites Wildl*. 2016;**5**(2):198–206.

111. Martínez MF, Kowalewski MM, Giuliani MG, Acardi SA, Salomón OD. Molecular identification of *Leishmania* in free-ranging black and gold howler monkeys (*Alouatta caraya*) in northeastern Argentina. *Acta Trop*. 2020;**210**:105534.

112. Mätz-Rensing K, Jentsch KD, Rensing S, Langenhuyzen S, Verschoor E, Niphuis H, et al. Fatal herpes simplex infection in a group of common marmosets (*Callithrix jacchus*). *Vet Pathol*. 2003;**40**:405–11.

113. Mätz-Rensing K, Ellerbrok H, Ehlers B, et al. Fatal Poxvirus Outbreak in a Colony of New World Monkeys. *Vet Pathol.* 2006; **43**:212–218.

114. Mätz-Rensing K, Winkelmann J, Becker T, et al. Outbreak of *Streptococcus equi* subsp. *zooepidemicus* infection in a group of rhesus monkeys (*Macaca mulatta*). *J Med Primatol*. 2009; **38**:328–334.

115. Mätz-Rensing K, Lowenstine. New World and Old World Monkeys. In: Terio KA, McAloose D, Leger JS. Pathology of Wildlife and Zoo Animals. Elsevier Inc: Cambridge. 2018; 343-374.

116. Measles & Rubella Initiative. Measles and Rubella Strategic Framework 2021–2030. 1st ed. Genebra: 2021.

117. Melendez LV, Hunt RD, King NW, et al. Herpesvirus ateles, a new lymphoma virus in monkeys. *Nat New Biol*. 1972; **235**:182–184.

118. Mello MFV de, Monteiro ABS, Fonseca EC, Pissinatti A, Ferreira AMR. Identification of *Helicobacter* sp. in gastric mucosa from captive marmosets (*Callithrix* sp.; callitrichidae, primates). *Am J Primatol*. 2005;**66**(2):111–118.

119. Melo CMF de, Daneze ER, Mendes NS, et al. Genetic diversity and hematological and biochemical alterations in *Alouatta* primates naturally infected with hemoplasmas in Brazil. *Comp Immunol Microbiol Infect Dis*. 2019;**63**:104–111.

120. Menezes-Costa A, Machado-Ferreira E, Voloch CM, et al. Identification of Bacterial Infection in Neotropical Primates. *Microbial Ecol*. 2013;**66**(2):471–478.

121. Minuzzi-Souza TTC, Nitz N, Knox MB, et al. Vector-borne transmission of *Trypanosoma cruzi* among captive Neotropical primates in a Brazilian zoo. *Parasite Vector*. 2016;**9**(1):39.

122. Miranda TS, Muniz CP, Moreira SB, et al. Eco-Epidemiological Profile and Molecular Characterization of Simian Foamy Virus in a Recently-Captured Invasive Population of *Leontopithecus chrysomelas* (Golden-Headed Lion Tamarin) in Rio de Janeiro, Brazil. *Viruses*. 2019;**11**(10):931.

123. Molina CV, Catão-Dias JL, Neto JSF, et al. Sero-epidemiological survey for brucellosis, leptospirosis, and toxoplasmosis in free-ranging *Alouatta caraya* and *Callithrix penicillata* from São Paulo State, Brazil. *J Med Primatol*. 2014;**43**(3):197–201.

124. Molina CV, Krawczak F da S, Bueno MG, et al. Negative serosurvey of *Toxoplasma gondii* antibodies in Golden-headed Lion Tamarin (*Leontopithecus chrysomelas*) from Niterói/RJ, Brazil. *Revista Brasileira De Parasitol Veterinária*. 2016;**26**(01):115–118.

125. Molina CV, Heinemann MB, Kierulff C, et al. Leptospira spp., rotavirus, norovirus, and hepatitis E virus surveillance in a wild invasive golden-headed lion tamarin (*Leontopithecus chrysomelas*; Kuhl,

1820) population from an urban park in Niterói, Rio de Janeiro, Brazil. *Am J Primatol*. 2019a;**81**(3):e22961.

126. Molina CV, Bueno MG, Kierulff MCM, et al. Spontaneous meningoencephalitis by *Staphylococcus aureus* in an infant golden-headed lion tamarin (*Leontopithecus chrysomelas*). *J Med Primatol*. 2019b;**48**(6):370–373.
127. Montali RJ, Mikota SK, Cheng LI. *Mycobacterium tuberculosis* in zoo and wildlife species. *Rev Sci Tech Off Int Epiz*. 2001; DOI: https://doi.org/10.20506/rst.20.1.1268
128. Monteiro RV, Baldez J, Dietz J, Baker A, Lisboa CV, Jansen AM. Clinical, biochemical, and electrocardiographic aspects of *Trypanosoma cruzi* infection in free-ranging golden lion tamarins (Leontopithecus rosalia). *J Med Primatol*. 2006;**35**(1):48–55.
129. Monteiro RV, Dietz JM, Jansen AM. The impact of concomitant infections by *Trypanosoma cruzi* and intestinal helminths on the health of wild golden and golden-headed lion tamarins. *Res Vet Sci*. 2010;**89**(1):27–35.
130. Morales MA, Fabbri CM, Zunino GE, 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 Neglect Trop D*. 2017;**11**(2):e0005351.
131. Moreno ES, Agostini I, Holzmann I, et al. Yellow fever impact on brown howler monkeys (*Alouatta guariba clamitans*) in Argentina: a metamodelling approach based on population viability analysis and epidemiological dynamics. *Memórias Instituto Oswaldo Cruz*. 2015;**110**(7):865–876.
132. Moutinho FFB, Andrade MGA de, Nunes VMA, et al. Rabies in *Callithrix* sp. in the urban area of Niterói, Rio de Janeiro, Brazil. *Rev Soc Bras Med Tro*. 2020;**53**:e20190402.
133. Muniz CP, Jia H, Shankar A, et al. An expanded search for simian foamy viruses (SFV) in Brazilian New World primates identifies novel SFV lineages and host age-related infections. *Retrovirology*. 2015;**12**(1):94.
134. Muniz CP, Cavalcante LTF, Jia H, et al. Zoonotic infection of Brazilian primate workers with New World simian foamy virus. *Plos One*. 2017;**12**(9):e0184502.
135. Muniz CP, Cavalcante LTF, Dudley DM, et al. First Complete Genome Sequence of a Simian Foamy Virus Infecting the Neotropical Primate *Brachyteles arachnoides*. *Microbiol Resour Announc*. 2018;**7**(2):e00839-18.
136. Nakamura S, Hayashidani H, Iwata T, Namai S, Une Y. Pathological changes in captive monkeys with spontaneous yersiniosis due to infection by *Yersinia enterocolitica serovar* O8. *J Comp Pathol*. 2010; **143**:150-6.
137. Neimark H, Barnaud A, Gounon P, Michel JC, Contamin H, The putative haemobartonella that influences *Plasmodium falciparum* parasitemia in squirrel monkeys is a haemotrophic *Mycoplasma*. *Microbes Infect*. 2002; **4**:693–698.
138. Neves JJ, Francelino M, Silva FG, et al. Survey of *Malassezia* sp and dermatophytes in the cutaneous microbiome of free-ranging golden-headed lion tamarins (Leontopithecus chrysomelas - Kuhl, 1820). *J Med Primatol*. 2017;**46**(3):65–69.
139. Niehaus C, Spínola M, Su C, et al. Environmental factors associated With *Toxoplasma gondii* Exposure in Neotropical Primates of Costa Rica. *Frontiers Vet Sci*. 2020;**7**:583032.

140. Nunes AJD, Alvarenga DAM de, Souza JC de, et al. Plasmodium infection and its association with biochemical and haematological parameters in free-living *Alouatta guariba clamitans* (Cabrera, 1940) (Primates: Atelidae) in Southern Brazil. *Memórias Instituto Oswaldo Cruz*. 2020;**114**:e190210.

141. Oliveira AR, Hiura E, Guião-Leite FL, et al. Pathological and parasitological characterization of *Prosthenorchis elegans* in a free-ranging marmoset Callithrix geofroyi from the Brazilian Atlantic Forest. *Pesquisa Veterinária Brasileira*. 2017;**37**(12):1514–1518.

142. Oliveira AR, Pinheiro GRG, Tinoco HP, et al. Competence of non-human primates to transmit *Leishmania infantum* to the invertebrate vector *Lutzomyia longipalpis*. *Plos Neglect Trop D*. 2019;**13**(4):e0007313.

143. Oliveira AR, Pereira FMAM, Santos DO, et al. Epidemiological, clinical and pathological aspects of lethal acanthocephalosis in captive neotropical primates. *J Med Primatol*. 2021;**50**(6):313–322.

144. Oliveira AR, Santos, DO, Lucena FP, et al. Non-thrombotic pulmonary embolism of brain, liver, or bone marrow tissues associated with traumatic injuries in free-ranging neotropical primates. *Vet Pathol.* 2022a; DOI: https://doi.org/10.1177/03009858221075595.

145. Oliveira AR, Castro M, Pimentel SP, et al. *Streptococcus pasteurianus* -induced valvular endocarditis and sepsis in a puerperal emperor tamarin (*Saguinus imperator*). *J Med Primatol*, 2022b (in press).

146. Olsen SC, Palmer MV. Advancement of knowledge of *Brucella* over the past 50 years. *Vet Pathol*. 2014; 51(6):1076-1089.

147. O'Rourke LG, Pitulle C, Hegarty BC, et al. *Bartonella quintana* in cynomolgus monkey (*Macaca fascicularis*). *Emerg Infect Dis*. 2005; **11**:1931–1934.

148. Paula NF de, Dutra KS, Oliveira AR de, et al. Host range and susceptibility to *Toxoplasma gondii* infection in captive neotropical and Old-world primates. *J Med Primatol*. 2020;**49**(4):202–210.

149. Pereira FV, Lucena FP, Rodrigues RL, et al. [Prevalence and spatial distribution of the occurrence of helminths in free-living nonhuman primates in the State of Rio de Janeiro, Brazil]. *Arquivo Brasileiro De Medicina Veterinária E Zootecnia*. 2020a;**72**(05):1705–1712.

150. Pereira AABG, Dias B, Castro SI, et al. Electrocutions in free-living black- tufted marmosets (*Callithrix penicillata*) in anthropogenic environments in the Federal District and surrounding areas, Brazil. *Primates.* 2020b;**61**(2):321–329.

151. Pereira AH, Vasconcelos AL, Silva VL, et al. Natural SARS-CoV-2 Infection in a Free-Ranging Black-Tailed Marmoset (*Mico melanurus*) from an Urban Area in Mid-West Brazil. *J Comp Pathol*. 2022; **194**:22-27.

152. Petit T. Seasonal outbreaks of botulism in captive South American monkeys, *Vet Rec*. 1991;**128**(13):311-312.

153. Pinto-Santini DM, Stenbak CR, Linial ML. Foamy virus zoonotic infections. *Retrovirology*. 2017;**14**(1):55.

154. Pisharath HR, Cooper TK, Brice AK, et al. Septicemia and peritonitis in a colony of common marmosets (*Callithrix jacchus*) secondary to *Klebsiella pneumoniae* infection. *Contemp Top Laboratory Animal Sci Am Assoc Laboratory Animal Sci*. 2005;**44**(1):35–37.

155. Psarros G, Riddell J, Gandhi T, Kauffman CA, Cinti SK. *Bartonella henselae* infections in solid organ transplant recipients: report of 5 cases and review of the literature. Medicine (Baltim) 2012; **91**:111–21.

156. Ramer JC, Garber RL, Steele KE, Boyson JF, O'Rourke C, Thomson JA. Fatal lymphoproliferative disease associated with a novel gammaherpesvirus in a captive colony of common marmosets. *Lab Anim Sci*. 2000;**60**:59–68.

157. Ricciardi ID, Nunes MP, Andrade CM, Da Silva AG. Anti-brucella agglutinins in bats and "Callithrix" monkeys. *J Wildl Dis.* 1976; **12**(1):52-4.

158. Rocha TC da, Batista PM, Andreotti R, et al. Evaluation of arboviruses of public health interest in free-living non-human primates (*Alouatta* spp., *Callithrix* spp., *Sapajus* spp.) in Brazil. *Rev Soc Bras Med Tro*. 2015;**48**(2):143–148.

159. Rockx B, Kuiken T, Herfst S, et al. Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model. *Science*. 2020; **368**:1012–1015.

160. Rogers DL, Ruiz JC, Baze WB, et al. Epidemiological and molecular characterization of a novel adenovirus of squirrel monkeys after fatal infection during immunosuppression. *Microb Genom*. 2020;**6**(9):mgen000395.

161. Rondón S, León C, Link A, González C. Prevalence of *Plasmodium* parasites in non-human primates and mosquitoes in areas with different degrees of fragmentation in Colombia. *Malaria J*. 2019;**18**(1):276.

162. Rosenbaum M, Mendoza P, Ghersi BM, et al. Detection of *Mycobacterium tuberculosis* Complex in New World Monkeys in Peru. *Ecohealth*. 2015;**12**(2):288–297.

163. Rovirosa-Hernández MDJ, Cortes-Ortíz L, García-Orduña F, et al. Seroprevalence of *Trypanosoma cruzi* and *Leishmania mexicana* in Free-Ranging Howler Monkeys in Southeastern Mexico. *Am J Primatol*. 2013;**75**(2):161–169.

164. Rovirosa-Hernández MJ, López-Monteon A, García-Orduña F, et al. Natural infection with *Trypanosoma cruzi* in three species of non-human primates in southeastern Mexico: A contribution to reservoir knowledge. *Acta Trop*. 2021;**213**:105754.

165. Rylands AB, Mittermeier RA, Silva JS. Neotropical primates: taxonomy and recently described species and subspecies. *Int Zoo Yearb*. 2011;**46**(1):11–24.

166. Sacchetto L, Chaves BA, Costa ER, 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;**13**(10):1933.

167. Sanchez-Fernandez C, Bolatti EM, Culasso ACA, et al. Identification and evolutionary analysis of papillomavirus sequences in New World monkeys (genera *Sapajus* and *Alouatta*) from Argentina. *Arch Virol*. 2022:1–12.

168. Santana CH, Oliveira AR, Santos DO, et al. Genotyping of *Toxoplasma gondii* in a lethal toxoplasmosis outbreak affecting captive howler monkeys (*Alouatta* sp.). *J Med Primatol*. 2021;**50**(2):99–107.

169. Santos LC, Cubilla MP, Moraes W, et al. Hemotropic mycoplasma in a free-ranging black howler monkey (*Alouatta caraya*) in Brazil. *J Wildl Dis*. 2013;**49**: 728–731.

170. Santos AVP dos, Souza AM de, Bueno MG, et al. Molecular detection of *Borrelia burgdorferi* in free-living golden headed lion tamarins (*Leontopithecus chrysomelas*) in Rio de Janeiro, Brazil. *Revista Instituto De Medicina Tropical De São Paulo*. 2018;**60**(0):e53.

171. Santos AF, Cavalcante LTF, Muniz CP, Switzer WM, Soares MA. Simian Foamy Viruses in Central and South America: A New World of Discovery. *Viruses*. 2019;**11**(10):967.

172. Santos RL, Oliveira AR. Leishmaniasis in non-human primates: Clinical and pathological manifestations and potential as reservoirs. *J Med Primatol*. 2020;**49**(1):34–39.

173. Santos DO dos, Oliveira AR de, Lucena FP de, et al. Histopathologic Patterns and Susceptibility of Neotropical Primates Naturally Infected With Yellow Fever Virus. *Vet Pathol*. 2020;**57**(5):681–686.

174. Sashida H, Suzuki Y, Rokuhara S, Nagai K, Harasawa R. Molecular demonstration of hemotropic mycoplasmas in wild Japanese monkeys (*Macaca fuscata*), *J Vet Med Sci*. 2014; **76**:97–101.

175. Shet A, Carr K, Danovaro-Holliday MC, et al. Impact of the SARS-CoV-2 pandemic on routine immunisation services: evidence of disruption and recovery from 170 countries and territories. *Lancet Global Heal*. 2021;**10**(2):e186–e194.

176. Schlabritz-Loutsevitch NE, Whatmore AM, Quance CR, et al. A novel *Brucella* isolate in association with two cases of still- birth in non-human primates – first report. *J Med Primatol*. 2009; **38**(1):70-3.

177. Schiller CA, Wolff MJ, Munson L, Montali RJ. *Streptococcus zooepidemicus* infections of possible horsemeat source in red-bellied tamarins and Goeldie‘s monkeys. *J Zoo Wildl Med*. 1989; **20**:322–327.

178. Schrenzel MD, Osborn KG, Shima A, Klieforth RB, Maalouf GA. Naturally occurring fatal herpes simplex virus 1 infection in a family of white-faced saki monkeys (*Pithecia pithecia pithecia*). *J Med Primatol*. 2003;**32**:7–14.

179. Setzer AP, Gaspar AMC, Sidoni M, Bueno MG, Catão-Dias JL. Serosurvey for hepatitis A in neotropical primates in southeast Brazil. *J Med Primatol*. 2014;**43**(3):202–205.

180. Shen Z, Feng Y, Sheh A, Everitt J, Bertram F, Paster BJ, Fox JG. Isolation and characterization of a novel *Helicobacter* species, *Helicobacter jaachi* sp. nov., from common marmosets (*Callithrix jachus*). *J Med Microbiol.* 2015;**64**:1063e73.

181. Silva FM, Naiff RD, Marcili A, Gordo M, D’Affonseca JA No, Naiff MF, et al. Infection rates and genotypes of *Trypanosoma rangeli* and *T. cruzi* infecting free-ranging *Saguinus bicolor* (Callitrichidae), a critically endangered primate of the Amazon Rainforest. *Acta Trop* 2008; **107**(2): 168-173.

182. Silva KSM, Silva RJ, Pereira WLA. Occurrence of infection by *Platynosomum illiciens* (Braun, 1901) in captive neotropical primates. *Primates.* 2012; **53**:79–82.

183. Silva MIV, Bento HJ, Maruyama FH, et al. *Pasteurella canis* infection in a non-human primate black-tailed marmoset (*Mico melanurus*) - A case report. *J Med Primatol*. 2020a;**49**(2):107–109.

184. Silva NIO, Sacchetto L, de Rezende IM, et al. Recent sylvatic yellow fever virus transmission in Brazil: the news from an old disease. *Virol J.* 2020b; **17**(1):9

185. Silva TMR da, Sá ACMGN de, Vieira EWR, Prates EJS, Beinner MA, Matozinhos FP. Number of doses of Measles-Mumps-Rubella vaccine applied in Brazil before and during the COVID-19 pandemic. *Bmc Infect Dis*. 2021;**21**(1):1237.

186. Silvestre RV, de Souza AJ, Júnior EC, Silva AK, de Mello WA, Nunes MR et al. First new world primate papillomavirus identification in the Atlantic Forest, Brazil: *Alouatta guariba papillomavirus* 1. *Genome Announc*. 2016; **4**(4):e00725-16

187. Simon M. Simian parvoviruses: Biology and implications for research. *Comp Med*. 2008; **58**(1), 47–50.

188. Shostell JM, Ruiz-Garcia M. An introduction to the biodiversity of the Neotropical Primates. In: Phylogeny, Molecular Population Genetics, Evolutionary Biology and Conservation of the Neotropical Primates. Ruiz-García M, Shostell JM Eds. Nova Science Publisher Inc.: New York, 2016: 5975.

189. Sousa MBC, Leão AC, Coutinho JFV, Ramos AMO. Histopathology findings in common marmosets (*Callithrix jacchus* Linnaeus, 1758) with chronic weight loss associated with bile tract obstruction by infestation with *Platynosomum* (Loos, 1907). *Primates*. 2008; **49**:283–287.

190. Souza AJS de, Coutinho LN, Silva WB da, et al. Hepatic lesions in captive owl monkeys (Aotus infulatus) with ultrasonographic "starry sky" liver. *J Med Primatol*. 2021;**50**(5):240–248.

191. Svoboda WK, Martins LC, Malanski L de S, et al. Serological evidence for Saint Louis encephalitis virus in free-ranging New World monkeys and horses within the upper Paraná River basin region, Southern Brazil. *Rev Soc Bras Med Tro*. 2014;**47**(3):280–286.

192. Svoboda WK, Soares MDCP, Alves MM, et al. Serological Detection Of Hepatitis A Virus In Free-Ranging Neotropical Primates (*Sapajus* spp., *Alouatta caraya*) From The Paraná River Basin, Brazil. *Revista Instituto De Medicina Tropical De São Paulo*. 2016;**58**:9.

193. Strier KB, Tabacow FP, Possamai CB de, et al. Status of the northern muriqui (*Brachyteles hypoxanthus*) in the time of yellow fever. *Primates*. 2019;**60**(1):21–28.

194. Singh DK, Singh B, Ganatra SR, et al. Responses to acute infection with SARS-CoV-2 in the lungs of rhesus macaques, baboons and marmosets. *Nat Microbiol*. 2021; **6**: 73–86.

195. Whatmore AM, Davison N, Cloeckaert A, et al. *Brucella papionis* sp. nov., isolated from baboons (*Papio* spp.). *Int J Syst Evol Micr*. 2014;**64**(Pt 12):4120–4128.

196. Tavela AO, Fuzessy LF, Silva VHD, et al. Helminths of wild hybrid marmosets (*Callithrix* sp.) living in an environment with high human activity. *Rev Bras Parasitol Vet*. 2013; **22** (3): 391-397.

197. Terzian ACB, Zini N, Sacchetto L, et al. Evidence of natural Zika virus infection in neotropical non-human primates in Brazil. *Sci Rep-uk*. 2018;**8**(1):16034.

198. Thoisy B, Pouliquen JF, Lacoste V, Gessain A, Kazanji1 M. Novel Gamma-1 Herpesviruses Identified in Free-Ranging New World Monkeys (Golden-Handed Tamarin [*Saguinus midas*], Squirrel Monkey [*Saimiri sciureus*], and White-Faced Saki [*Pithecia pithecia*]) in French Guiana. *J Virol*. 2003; 9099–9105.

199. Thomson JA, Scheffler JJ. Hemorrhagic typhlocolitis associated with attaching and effacing *Escherichia coli* in common marmosets. *Lab Anim Sci*. 1996; **46**(3):275-9.

200. Trüeb I, Portela RD, Franke CR, Carneiro IO, Ribeiro GJ Jr, Soares RP. Barrouin-Melo SM *Trypanosoma cruzi* and *Leishmania* sp. infection in wildlife from urban rainforest fragments in Northeast, Brazil. *J Wildl Dis*. 2018;**54**(1):76-84.

201. van Doremalen N, Munster VJ. Animal models of Middle East respiratory syndrome coronavirus infection. *Antivir Res*. 2015; **122**: 28–38.

202. Vásquez-Aguilar AA, Toledo-Manuel FO, Barbachano-Guerrero A, Hernández-Rodríguez D. Detection of Antimicrobial Resistance Genes in *Escherichia coli* Isolated from Black Howler Monkeys (*Alouatta pigra*) and Domestic Animals in Fragmented Rain-Forest Areas in Tabasco, Mexico. *J Wildlife Dis*. 2020;**56**(4):922–927.

203. Vela AI, Gutiérrez MC, Falsen E, et al. *Pseudomonas simiae* sp. nov., isolated from clinical specimens from monkeys (*Callithrix geoffroyi*). *Int J Syst Evol Micr*. 2006;**56**(11):2671–2676.

204. Verona CE, Pissinnatti A. Capítulo 34: Primates – Primatas do Novo Mundo (sagui, macaco-prego, macaco-aranha, bugio e muriqui). In.: Cubas ZS, Silva JCR, Catão-Dias JL (eds). Tratado de Animais Selvagens – Medicina Veterinária Volume 1. 2aEd, Editora Roca Ltda, São Paulo-SP, p. 723-743, 2014

205. Vieira RFC, Biondo AW, Guimarães MAS, et al. Ehrlichiosis in Brazil. *Rev Bras Parasitol Vet*. 2011; **20** (1):1-12.

206. Wilson TM, Ritter JM, Martines RB, et al. Pathology and One Health implications of fatal *Leptospira interrogans* infection in an urbanized, free-ranging, black-tufted marmoset (*Callithrix penicillata*) in Brazil. *Transbound Emerg Dis*. 2021;**68**(6):3207–3216.

207. Wilson TM, Ritter JM, Martines RB, et al. Fatal Human Alphaherpesvirus 1 Infection in Free-Ranging Black-Tufted Marmosets in Anthropized Environments, Brazil, 2012–2019. *Emerg Infect Dis*. 2022;**28**(4):802–811.

208. Wachtman L, Mansfield K. Viral diseases of nonhuman primates. In: Abee CR, Mansfield K, Tardif S, Morris T, Morris T, eds. Nonhuman Primates in Biomedical Research. London, UK: Academic Press; 2012: 7-26.

209. Whitney RA. Taxonomy. In: Bennett B, Abee C, Henrickson R, eds. Nonhuman primates in biomedical research. Biology and Management. Elsevier: Missouri. 1995.

210. Yu G, Yagi S, Carrion R, et al. Experimental Cross-Species Infection of Common Marmosets by Titi Monkey Adenovirus. *Plos One*. 2013;**8**(7):e68558.

211. Zárate-Rendón DA, Salazar-Espinoza MN, Catalano S, et al. Molecular characterization of *Dipetalonema yatesi* from the black-faced spider monkey (*Ateles chamek*) with phylogenetic inference of relationships among Dipetalonema of Neotropical primates. *Int J Parasitol Parasites Wildl*. 2022;**17**:152–157.

212. Ziccardi M, Lourenço-de-Oliveira R. The Infection Rates of Trypanosomes in Squirrel Monkeys at Two Sites in the Brazilian Amazon. *Memórias Instituto Oswaldo Cruz*. 1997;**92**(4):465–470.

CHAPTER I[1]

PATHOLOGY AND DISEASE INVESTIGATION OF FREE-RANGING NEW WORLD PRIMATES FROM THE BRAZILIAN ATLANTIC FOREST

SUMMARY

The recent COVID-19 pandemic raised a high concern in the society and scientific field about the impact of the emerging and re-emerging infectious diseases. Neotropical primates (New World primates - NWP) are natural and accidental hosts of many infectious agents and there is a scarce literature on the pathology and the diseases affecting free-ranging NWP, being the vast majority of reports associated with serological diagnoses and outbreak events. This study aimed to characterize through histochemical, ultrastructural, immunohistochemical and molecular assays, the pathological findings observed in free-ranging NWP from the Atlantic Forest biome at Rio de Janeiro state, between January 2017 to July 2019. More than a thousand animals were included in this study, covering species of the genera *Callithrix*, *Alouatta*, *Sapajus*, *Leontopithecus,* and *Callicebus* with a systematic evaluation of FFPE samples from brain, heart, lungs, liver, spleen, and kidneys. This study showed that free-ranging NWP are hosts of several diseases, including important zoonoses, being valuable tools for the development of strategies for disease control and prevention, both with a focus on public health and conservation of these species. Additionally, the study of histopathological alterations in these species may help recognizing specific and non-specific alterations (background), thus improving the accuracy of the diagnosis in these animals.

Keywords: marmoset; howler-monkey; capuchin; titi-monkey; tamarin; wildlife.

[1] Chapter formatted according to the guidelines of the *Journal of Comparative Pathology.*

1. INTRODUCTION

The recent COVID-19 pandemic raised a high concern in the society and scientific field about the impact of the emerging and re-emerging infectious diseases and how to early identify and prevent these diseases to emerge and spread worldwide. Most of the emerging infectious diseases are originally from wild animals, being transmitted to humans due to *spillover* events, favored by the increasing proximity of wild animal populations to domestic animals and humans (Bengis et al., 2004). Therefore, the study of the diseases that circulates in wild environment is essential to establish control strategies focusing on One Health and wildlife conservation.

Neotropical primates (New World primates - NWP) are natural and accidental hosts of many infectious agents, including viruses, such as arbovirus (Moreno et al., 2013; Favoretto et al., 2019; Santos et al., 2020); rabies virus (Favoretto et al., 2001), and human herpesvirus (Casagrande et al., 2014; Wilson et al., 2022); bacteria, such as *Klebsiella* sp. (Bueno et al., 2015), *Mycobacterium* spp. (Ehlers et al., 2020), and *Leptospira* spp. (Wilson et al., 2021); protozoan, such as *Toxoplasma gondii* (Paula et al., 2020), *Plasmodium* spp. (Figueiredo et al., 2017), and *Leishmania* spp. (Malta et al., 2010; Oliveira et al., 2019); and a wide variety of helminths (Tavela et al., 2013), among other agents of zoonotic potential and importance in public health.

The Brazilian Atlantic Forest holds more than 20 species of NWP, most of them endemic of Brazilian territory (Hirsh et al., 2006). The increase in density of human population associated with the reduction and fragmentation of wildlife habitats leads to a overlap of habitats between humans and NWP, dramatically increasing the potential for disease transmission (Gillespie et al., 2008; Aguirre, 2009; Brinkworth and Pechenkina, 2013; Bueno et al., 2017; Wilson et al., 2021) and also highly exposing these animals to innumerous anthropic threatens, such as illegal trade, hunting, roadkill, inter-specific aggressions, poisoning, among others (Shostell and Ruiz-Garcia, 2016; Ehlers et al., 2022; Wilson et al., 2021).

Diseases associated with captive NWP, mainly raised in experimental laboratories, are widely studied, however, there is a scarce literature on the pathology and the diseases affecting free-ranging NWP, being the vast majority of reports associated with serological diagnoses and outbreak events, mostly focusing on specific and pre-defined agents (Bueno et al., 2017). Herein, we aimed to characterize through

histochemical, ultrastructural, immunohistochemical and molecular assays, the pathological findings observed in a thousand free-ranging NWP from the Atlantic Forest biome at Rio de Janeiro state, from January 2017 to July 2019.

2. MATERIAL AND METHODS

2.1 Study design

This is a retrospective study with free-ranging NWP that were found dead from all mesoregions of the Rio de Janeiro state (RJ) and were necropsied at Instituto Municipal de Medicina Veterinária Jorge Vaitsman (IJV, Rio de Janeiro, Brazil) for Yellow Fever (YF) surveillance, between January 2017 to July 2019. Animals were previously tested for YF virus (YFV) and rabies virus (RABV) by the official Brazilian diagnostic service. For YFV it was performed a quantitative reverse transcription polymerase chain reaction (RT-qPCR) and immunohistochemistry (IHC) of fresh and formalin-fixed paraffin-embedded (FFPE) liver samples by Fundação Oswaldo Cruz (FIOCRUZ); and for RABV, fresh brain tissues were tested for direct fluorescent antibody test (DFAT) and mouse inoculation test (MIT) by IJV-RJ. Data about geographical origin, sex and age were collected by IJV. This study was authorized by the government environmental agency (ICMBio - Brazil) under the SISBIO license number 67014 and all procedures strictly adhered to humane care of animals and all applicable laws and regulations, including registration in the national system for management of genetic heritage and associated traditional knowledge by SISGEN code A2743E4.

2.2 Pathology

Animals were necropsied by veterinary pathologists from IJV and were evaluated by general aspect of the carcass, being included in the study all the animals necropsied and considered viable for histopathology. Samples of brain, heart, lung, liver, spleen, and kidney were systematically collected, fixed in 10% buffered formalin, and embedded in paraffin. Other organs such as adrenal, stomach, intestines, pancreas, ovary, uterus, testicle, and prostate, were collected randomly in some cases, and whenever it was observed gross alterations. FFPE tissue samples were sent to the Universidade Federal de Minas Gerais (UFMG, Belo Horizonte, Brazil), where they were sectioned (3–4 μm-thick) and stained with hematoxylin and eosin (HE) for further microscopic evaluation. All tissues were analyzed and, according to the pathological

findings, special histochemical stains were performed, such as Prussian blue, Gram, Warthin-Starling (WS), Periodic acid–Schiff (PAS), Masson's trichrome, Ziehl-Neelsen, Congo Red and Grocott methenamine silver (GMS) stains.

2.3 Immunohistochemistry (IHC)

IHC was performed in FFPE tissues samples at UFMG and at the Infectious Disease Pathology Branch, Centers for Disease Control and Prevention (IDPB-CDC, Atlanta, USA). Primary antibodies used in the study and their specifications are described at Table 1.1. Briefly, slides were deparaffinized, hydrated, exposed to antigenic retrieval, followed by endogenous peroxidase and nonspecific protein binding blockage, as needed, incubated in primary antibody and in the detection system and revealed with 3,3′-Diaminobenzidine-DAB (Envision®) for peroxidase-based system and Permanent Red (Cell Marque®) for phosphatase-based system. Slides were counterstained with Mayer's hematoxylin.

Table 1.1 Details about the primary antibodies used for immunohistochemistry.

Primary antibody	Clone/ Host	Dilution	Antigenic retrieval	Detection system	Laboratory (source)	Cross-reactivity*	Institution (performed)
Adenovirus	20-11/ Mouse	1:2000	PKD[a]	Mach4[b]	CDC	No detectable cross-reactivity	IDPB-CDC
CD3	F7.2.38/ Mouse	1:100	Heat; low pH[c]	Mach4[b]	DAKO	NWP[f]	IDPB-CDC
CD20	L26/ Mouse	1:200	Heat; high pH[d]	Mach4[b]	Leica Biosystems	NWP[f]	IDPB-CDC
CD163	10D6/ Mouse	1:50	Heat; low pH[c]	Mach4[b]	Leica Biosystems	NWP[f]	IDPB-CDC
E.coli H Pool	Polyclonal/ Rabbit	1:50	Heat; low pH[c]	Mach4[b]	SSI Diagnostica	*Clostridium*; *Shigella*; *Enterobacter; Salmonella; Serratia; Proteus; Moraxella.*	IDPB-CDC
Human herpesvirus 1 (HSV-1)	Polyclonal/ Rabbit	1:2000	PKD[a]	Mach4[b]	CDC	HSV-2; Macacine herpesvirus 1; *Mycobacterium.*	IDPB-CDC
Human herpesvirus 4 (HHV-4)	CS1-4/ Mouse	1:200	PKD[a]	Mach4[b]	DAKO	No detectable cross-reactivity	IDPB-CDC
Human herpesvirus 5 (HHV-5)	DDG9 & CCH2/ Mouse	1:25	Heat; low pH[c]	Mach4[b]	DAKO	No detectable cross-reactivity	IDPB-CDC

Human polyomavirus (SV40)	PAb416/ Mouse	1:200	Heat; low pH[c]	Mach4[b]	Calbiochem	No detectable cross-reactivity	IDPB-CDC
IBA-1	1022-5/ Mouse	1:50	Heat; high pH[d]	Envision[e]	Santa Cruz Biothecnology	NWP[f]	UFMG
Ki-67	MIB-1/ Mouse	1:50	Heat; low pH[c]	Envision[e]	DAKO	NWP[f]	UFMG
Leishmania	Polyclonal/ Dog	1:1000	Heat; low pH[c]	Mach4[b]	CDC	*Sarcocystis; T. cruzi; T. gondii; Staphylococcus; Histoplasma.*	IDPB-CDC
Myeloperoxidase (MPO)	A-5/ Mouse	1:100	Heat; high pH[d]	Envision[e]	Santa Cruz Biothecnology	NWP[f]	UFMG
Parainflueza (PIV)	Polyclonal/ Goat	1:200	Heat; low pH[c]	Mach4[b]	Meridian Life Sciences	Parainfluenza 2 and 3	IDPB-CDC
Staphylococcus spp.	Polyclonal/ Rabbit	1:500	PKD[a]	Mach4[b]	Biodesign	*Streptococcus; Haemophilus; Bartonella.*	IDPB-CDC
Streptococcus pneumoniae	128-390/ Mouse	1:500	PKD[a]	Mach4[b]	ThermoFisher	*Streptococcus*; *Haemophilus.*	IDPB-CDC
T. gondii	Tp3/ Mouse	1:100	Heat; high pH[d]	Envision[e]	Santa Cruz Biothecnology	No detectable cross-reactivity	UFMG

*Cross-reactivity shown in IDPB-CDC and UFMG lab tests; [a]PKD: proteinase K digestion (Roche Diagnostics); [b]Mach 4 Universal Alkaline Phosphatase (AP)-Polymer Kit® (Biocare Medical); [c]Reveal Decloaker (6.0 pH), Biocare Medical; [d]EDTA 9.0 pH buffer (1:50; DAKO); [e] Envision®- Indirect peroxidase polymeric detection kit (DAKO); [f]Anti-human antibody cross-reacting with NWP protein.

2.4 Molecular assays

Molecular analysis was performed at IDPB-CDC. First, FFPE tissues were sectioned in one scroll of 16 μm-thick, placed in a sterile 1.5 mL microtube and stored at 4°C until extraction.

RNA Extraction and conventional RT-PCR assays for Enterovirus and Flavivirus: RNA was extracted from FFPE heart tissues of myocarditis cases using an optimized RNA extraction protocol as previously described (Bhatnagar et al., 2012). Enterovirus and Flavivirus conventional RT-PCR assays were performed using a QIAGEN OneStep RT-PCR Kit (Valencia, CA, USA) and 5 μL of RNA template, according to the manufacturer's instructions (Guarner et al., 2007; Bhatnagar et al., 2012). PCR positive amplicons were identified by gel electrophoresis, extracted from the gel, and directly sequenced by Sanger sequencing on a GenomeLab GeXP sequencer (AB SCIEX LLC, Redwood City, CA, USA). The search for homologies to known sequences was performed by using the BLAST nucleotide database (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

RNA Extraction and quantitative RT-PCR assays for influenza A and B viruses (IFV), human parainfluenza viruses (PIV) types 1–4 and respiratory syncytial virus (RSV): fourteen cases from marmosets, that had had a moderate to marked acute interstitial pneumonia and/or bronchopneumonia, with alveolar fibrin deposition, were selected for this respiratory panel. RNA was extracted from FFPE lung tissues specimens using the EZ1 RNA Tissue Mini Kit, EZ1 RNA card and the EZ1 Advanced XL (Qiagen). Quantitative RT-PCR assays for influenza A and B viruses, human parainfluenza viruses (PIV) types 1–4 and respiratory syncytial virus (RSV) were performed using previously published primers and probes (Denison et al., 2011; Weinberg et al., 2013) and the Invitrogen Superscript III Platinum One-Step qRT-PCR Kit (ThermoFisher Scientific) on the Stratagene Mx3005P QPCR System (Agilent Technologies).

DNA extraction and PCR assays: DNA was extracted from FFPE tissue samples, using the QIAamp Ultra Clean Production (UCP) Pathogen DNA Mini Kit (Qiagen). According to the suspicious pathogen, samples were evaluated by: specific-qPCR assays for *S. pneumoniae* targeting the *piaB* gene, for methicillin-resistant *Staphylococcus aureus*; and for methicillin-sensitive *S. aureus*, as described previously (Trzcinski et al., 2003; Paddock et al., 2012); specific-PCR assays for *E. coli* targeting the *uidA* gene (Heininger et al., 1999) and for *Salmonella,* targeting *invA* gene (Shanmugasamy et al., 2011); and a rapid specific-seminested PCR for virulent *Shigella* sp. targeting the *ipaH* gene (Theron et al., 2001). Wide range 16S rRNA, 23S rRNA, and *Streptococcus* spp. PCR assays, followed by Sanger sequencing were also performed, as previously described (Harris and Hartley, 2003; Imrit et al., 2006; Paddock et al., 2012; Moore et al., 2013). For human herpesvirus-4 (HHV-4), extracted DNA was sent to be tested by the Division of Viral Diseases, CDC, Atlanta.

2.5 Transmission electron microscopy (TEM)

TEM was performed at IDPB-CDC. For TEM, areas of interest from FFPE blocks were selected based on results from HE and IHC. Samples for EM were removed from paraffin blocks using a 1-2 mm biopsy punch, deparaffinized using xylene, rehydrated using a decreasing ethanol series, and post-fixed in 2.5% glutaraldehyde. Samples were then post-fixed with 1% osmium tetroxide, en-bloc stained with 4% uranyl acetate, dehydrated using an increasing ethanol series and acetone, and embedded in Epon-Araldite resin. Samples were then ultrathin sectioned (~50 nm

thick), stained with uranyl acetate and lead citrate, and examined on a Tecnai Biotwin electron microscope.

2.6 Statistical analysis

Data were analyzed using the GraphPad Prism software (version 9.0). Descriptive statistics with 95% of confidence interval was used for general analysis. Frequency of histopathological lesions and other variables, such as age, sex and geographical distribution, were compared using Fisher's exact test.

3. RESULTS

A total of 1,078 NWP was included in this study, being 1,001 marmosets (*Callithrix* spp.), 43 howler-monkeys (*Alouatta* spp.), 29 capuchins (*Sapajus* spp.), three lion tamarins (*Leontopithecus* spp.), and two titi-monkeys (*Callicebus* spp.). Data about sex, age and origin are detailed at Table 1.2. For all species the frequency of adults necropsied was higher than juveniles, varying from 66.7% to 100%. Frequency of males and females varied according to the species of the study, being equally distributed in marmoset, 46.2% each (463/1,001; 43.2-49.3%), and with higher frequency of males in howler monkeys (32/43; 74.4%; CI 59.8-85.0%) and capuchins (21/29; 72.4%; CI 54.3-85.3%). According to geographical origin, marmosets and capuchins came more frequently from the Metropolitan region, 82.2% and 89.6%, respectively, while the origin of howler-monkeys, lion tamarin, and titi-monkey were mainly from the other mesoregions of RJ (Table 1.2).

3.1 Pathological findings in free-ranging marmosets

The main pathological findings observed in the necropsied marmosets were pulmonary edema (560/1,001; 56%; CI 52.8-59.0%), pulmonary hemorrhage (540/1,001; 54%; CI 50.8-57.0%), pneumonia (428/1,001; 42.8%; CI 39.7-45.8%), interstitial nephritis (381/1,001; 38.1%; CI 35.1-41.1%), splenic lymphoid hyperplasia (271/715; CI 37.9%; 34.4-41.5%), portal hepatitis (359/1,001; 35.9%; CI 32.9-38.9%), diffuse hepatocellular glycogen degeneration – DHGD (308/1,001; 30.8%; CI 28.0-33.7%), pulmonary anthracosis (200/1,001; 20%; CI 17.6-22.6%), hepatic portal fibrosis (191/1,001; 19.1%; CI 16.8-21.6%), and bile duct hyperplasia (186/1,001; 18.6%; CI 16.3-21.1%). Table 1.3 summarizes all the pathological findings observed in the free-ranging marmosets.

Table 1.2 Profile of free-ranging NWP from the Brazilian Atlantic Forest found dead during January 2017 to July 2019 and included in this study.

DATA	NWP species % (CI)					
	Marmoset (n = 1,001)	**Howler-monkey (n = 43)**	**Capuchin (n = 29)**	**Lion-tamarin (n = 3)**	**Titi-monkey (n = 2)**	**Total (n = 1,078)**
SEX						
Female	46.2 (43.2-49.3)	18.6 (9.7-32.6)	24.1 (12.2-42.1)	33.3 (1.7-88.1)	100.0 (17.8-100.0)	44.6 (41.7-47.6)
Male	46.2 (43.2-49.3)^{A}	74.4 (59.8-85)^{B}	72.4 (54.3-85.3)^{B}	66.7 (11.8-98.3)	-	48.0 (45.1-51.0)
No information	7.5 (6-9.3)	7.0 (2.4-18.6)	3.4 (0.2-17.2)	-	-	7.3 (5.9-9.0)
AGE						
Adult	78.2 (75.6-80.7)^{A}	76.7 (62.2-86.8)^{A}	79.3 (61.6-90.1)^{A}	66.7 (11.8-98.3)	100.0 (17.8-100.0)	78.2 (75.6-80.6)
Juvenile	18.7 (16.4-21.2)	23.3 (13.1-37.7)	10.3 (3.6-26.4)	33.3 (1.7-88.1)	-	18.6 (16.4-21.1)
No information	3.1 (2.2-4.4)	-	10.3 (3.6-26.4)	-	-	3.1 (2.3-4.3)
GEOGRAPHIC ORIGIN						
Metropolitan	82.2 (79.7-84.5)^{A}	9.3 (3.7-21.6)^{B}	89.6 (73.6-96.4)^{A}	33.3 (1.7-88.1)	-	79.2 (76.7-81.5)
Others	17.8 (15.5-20.3)	90.7 (78.4-96.3)	10.3 (3.6-26.4)	66.7 (11.8-98.3)	100.0 (17.8-100.0)	20.8 (18.5-23.3)

A, B: Different letters indicate statistically significant differences between species of NWP (Fisher's exact test, $p < 0.05$); NWP: New World primate; CI: Confidence interval; n: number of samples.

Table 1.3 Pathological findings in free-ranging marmosets (*Callithrix* spp.) from the Brazilian Atlantic Forest found dead during January 2017 to July 2019.

Organ (N)	Pathological findings	% (CI)	Intensity %		
			Mild	Moderate	Severe
Brain (794)	Encephalitis/ Meningoencephalitis	4.5 (3.3-6.2)	77.8 (28/36)	22.2 (8/36)	-
	Non suppurative	4.0 (2.9-5.6)	78.1 (25/32)	21.9 (7/32)	-
	Suppurative	0.5 (0.2-1.3)	75.0 (3/4)	25.0 (1/4)	-
	Hemorrhage	4.4 (3.2-6.1)	57.1 (20/35)	34.3 (12/35)	8.6 (3/35)
	Meningitis	1.0 (0.5-2.0)	50.0 (4/8)	37.5 (3/8)	12.5 (1/8)
	Non suppurative	0.8 (0.3-1.6)	50.0 (3/6)	50.0 (3/6)	-
	Suppurative	0.2 (0.0-0.9)	50.0 (1/2)	-	50.0 (1/2)
	Choroiditis	1.0 (0.5-2.0)	62.5 (5/8)	37.5 (3/8)	-
	Gliosis	0.4 (0.1-1.1)	66.7 (2/3)	33.3 (1/3)	-
	Thrombosis	0.2 (0.0-0.9)	50.0 (1/2)	50.0 (1/2)	-
	Extramedullary hematopoiesis	0.2 (0.0-0.9)	50.0 (1/2)	50.0 (1/2)	-
Heart (1,001)	Myocarditis	14.3 (12.2-16.6)	79.7 (114/143)	20.3 (29/143)	
	Lymphohistioplasmacytic	8.3 (6.7-10.2)	86.7 (72/83)	13.3 (11/83)	-
	Mixed	4.7 (3.5-6.2)	70.2 (33/47)	29.8 (14/47)	-
	Neutrophilic	1.2 (0.7-2.1)	66.7 (8/12)	33.3 (4/12)	-
	Granulomatous	0.1 (0.0-0.6)	100.0 (1/1)	-	-
	Fibrosis	3.0 (2.1-4.2)	73.3 (22/30)	26.7 (8/30)	-
	Necrosis	2.6 (1.8-3.8)	92.3 (24/26)	7.7 (2/26)	-
	Hemorrhage	1.8 (1.1-2.8)	55.5 (10/18)	44.5 (8/18)	-
	Thrombosis	0.4 (0.2-1.0)	100.0 (4/4)	-	-
	Arteriosclerosis	0.4 (0.2-1.0)	100.0 (4/4)	-	-
Lung (1,001)	Alveolar edema	56.0 (52.8-59.0)	28.7 (161/560)	50.7 (284/560)	20.6 (115/560)
	Alveolar hemorrhage	54.0 (50.8-57.0)	26.1 (141/540)	52.2 (282/540)	21.7 (117/540)
	Pneumonia	42.8 (39.7-45.8)	49.3 (211/428)	44.6 (191/428)	6.1 (26/428)
	Interstitial pneumonia	40.6 (37.6-43.6)	50.0 (203/406)	45.8 (186/406)	4.2 (17/406)
	Bronchopneumonia	1.7 (1.1-2.7)	29.4 (5/17)	23.5 (4/17)	47.1 (8/17)
	Granulomatous pneumonia	0.5 (0.2-1.2)	60.0 (3/5)	20.0 (1/5)	20.0 (1/5)
	Anthracosis	20.0 (17.6-22.6)	71.5 (143/200)	27.0 (54/200)	1.5 (3/200)
	Perivasculitis	18.5 (16.2-21.0)	70.8 (131/185)	26.5 (49/185)	2.7 (5/185)
	Extramedullary hematopoiesis	8.4 (6.8-10.3)	77.4 (65/84)	22.6 (19/84)	-
	Alveolar fibrin deposition	8.2 (6.6-10.0)	52.4 (43/82)	35.4 (29/82)	12.2 (10/82)
	Thrombosis	3.8 (2.8-5.2)	60.5 (23/38)	39.5 (15/38)	-

	Intravascular histiocytosis	3.7 (2.7-5.0)	5.5 (2/37)	56.7 (21/37)	37.8 (14/37)
	Non-thrombotic embolism	0.9 (0.5-1.7)	66.7 (6/9)	33.3 (3/9)	-
	Pleuritis	0.7 (0.3-1.4)	57.1 (4/7)	42.9 (3/7)	-
	Osseus metaplasia	0.7 (0.4-1.4)	100.0 (7/7)	-	-
	BALT hyperplasia	0.5 (0.2-1.2)	80.0 (4/5)	20.0 (1/5)	-
	Arteriosclerosis	0.4 (0.2-1.0)	100.0 (4/4)	-	-
	Bronchitis/ Bronchiolitis	0.2 (0.0-0.7)	50.0 (1/2)	50.0 (1/2)	-
	Multicentric B-cell lymphoma	0.1 (0.0-0.6)	-	-	100.0 (1/1)
	Nodular lymphoid hyperplasia	0.1 (0.0-0.6)	-	100.0 (1/1)	-
Liver (1,001)	Hepatitis	59.6 (56.5-62.5)	74.5 (444/596)	23.6 (141/596)	1.9 (11/596)
	Portal	35.9 (32.9-38.9)	69.3 (249/359)	28.4 (102/359)	2.3 (8/359)
	Random	23.7 (21.1-26.4)	82.3 (195/237)	16.4 (39/237)	1.3 (3/237)
	Diffuse glycogen degeneration	30.8 (28.0-33.7)	36.4 (112/308)	41.9 (129/308)	21.7 (67/308)
	Portal fibrosis	19.1 (16.8-21.6)	37.7 (72/191)	57.6 (110/191)	4.7 (9/191)
	Bile duct hyperplasia	18.6 (16.3-21.1)	69.9 (130/186)	26.3 (49/186)	3.8 (7/186)
	Necrosis	17.2 (15.0-19.4)	53.5 (92/172)	33.1 (57/172)	13.4 (23/172)
	Random	13.0 (11.0-15.2)	50.8 (66/130)	40.8 (53/130)	8.4 (11/130)
	Individual	2.9 (2.0-4.1)	89.6 (26/29)	10.4 (3/29)	-
	Massive	1.0 (0.5-1.8)	-	-	100.0 (10/10)
	Centrilobular	0.3 (0.1-0.9)	-	33.3 (1/3)	66.7 (2/3)
	Lipidosis	16.8 (14.6-19.2)	66.1 (111/168)	28.0 (47/168)	5.9 (10/168)
	Diffuse	9.4 (7.7-11.4)	63.8 (60/94)	26.6 (25/94)	9.6 (9/94)
	Random	7.4 (5.9-9.2)	68.9 (51/74)	29.7 (22/74)	1.4 (1/74)
	Sinusoidal leukocytosis	15.4 (13.3-17.7)	48.0 (74/154)	42.9 (66/154)	9.1 (14/154)
	Multinucleated hepatocytes	13.0 (11.0-15.2)	87.7 (114/130)	11.5 (15/130)	0.8 (1/130)
	Cholangiohepatitis	12.4 (10.5-14.6)	34.7 (43/124)	56.4 (70/124)	8.9 (11/124)
	Extramedullary hematopoiesis	6.5 (5.1-8.2)	40.0 (26/65)	46.1 (30/65)	13.9 (9/65)
	Iron storage (hemosiderosis)	5.1 (3.9-6.6)	70.6 (36/51)	23.5 (12/51)	5.9 (3/51)
	Portal granuloma	2.9 (2.0-4.1)	100.0 (29/29)	-	-
	Hemorrhage	2.4 (1.6-3.5)	56.0 (14/25)	32.0 (8/25)	12.0 (3/25)
	Megalocytosis	2.0 (1.3-3.1)	60.0 (12/20)	40.0 (8/20)	-
	Cholestasis	1.5 (0.9-2.5)	66.7 (10/15)	33.3 (5/15)	-
	Thrombosis	1.4 (0.8-2.3)	85.7 (12/14)	14.3 (2/14)	-
	Biliary lithiasis	0.6 (0.3-1.3)	66.6 (4/6)	16.7 (1/6)	16.7 (1/6)
	Multicentric B-cell lymphoma	0.1 (0-0.8)	-	-	100.0 (1/1)
	Glycogen intranuclear inclusion	0.1 (0-0.6)	-	-	100.0 (1/1)
Spleen (715)	Lymphoid hyperplasia	37.9 (34.4-41.5)	52.4 (142/271)	40.6 (110/271)	7.0 (19/271)
	Extramedullary hematopoiesis	12.4 (10.2-15.1)	64.0 (57/89)	28.1 (25/89)	7.9 (7/89)

	Splenitis	6.7 (5.1-8.8)	58.3 (28/48)	27.1 (13/48)	14.6 (7/48)
	Necrosis	2.9 (1.9-4.4)	19.0 (4/21)	66.7 (14/21)	14.3 (3/21)
	Hemosiderosis	2.2 (1.4-3.6)	62.5 (10/16)	31.2 (5/16)	6.3 (1/16)
	Lympholysis	1.8 (1.1-3.1)	7.7 (1/13)	53.8 (7/13)	38.5 (5/13)
	Follicular hyalinosis	1.8 (1.1-3.1)	61.6 (8/13)	30.8 (4/13)	7.8 (1/13)
	Lymphoid rarefaction	1.1 (0.6-2.2)	37.5 (3/8)	62.5 (5/8)	-
	Plasmacytosis	0.7 (0.3-1.6)	20.0 (1/5)	80.0 (4/5)	-
	Fibrosis	0.3 (0.0-1.0)	100.0 (2/2)	-	-
	Multicentric B-cell lymphoma	0.1 (0-0.8)	-	-	100.0 (1/1)
	Thrombosis	0.1 (0-0.8)	100.0 (1/1)	-	-
Kidney (1,001)	Interstitial nephritis	38.1 (35.1-41.1)	38.3 (146/381)	50.4 (192/381)	11.3 (43/381)
	Hyalin casts	15.8 (13.6-18.2)	68.4 (108/158)	27.8 (44/158)	3.8 (6/158)
	Glomerulopathy	11.6 (9.7-13.7)	37.9 (44/116)	56.9 (66/116)	5.2 (6/116)
	Membranous	10.9 (9.1-13.0)	38.5 (42/109)	56.9 (62/109)	4.6 (5/109)
	Proliferative	0.7 (0.3-1.4)	28.6 (2/7)	57.1 (4/7)	14.3 (1/7)
	Glomerulosclerosis	9.6 (7.9-11.6)	60.5 (58/96)	38.5 (37/96)	1.0 (1/96)
	Interstitial fibrosis	8.6 (7.0-10.5)	33.7 (29/86)	34.9 (30/86)	31.4 (27/86)
	Nephrocalcinosis	8.3 (6.7-10.2)	69.9 (58/83)	28.9 (24/83)	1.2 (1/83)
	Pigmentary nephrosis	4.2 (3.1-5.6)	54.8 (23/42)	42.9 (18/42)	2.3 (1/42)
	Tubular necrosis	3.9 (2.9-5.3)	53.8 (21/39)	35.9 (14/39)	10.2 (4/39)
	Retention cysts	1.8 (1.1-2.8)	89.0 (16/18)	5.5 (1/18)	5.5 (1/18)
	Cystic glomerular atrophy	1.3 (0.8-2.2)	76.9 (10/13)	23.1 (3/13)	-
	Tubular lipidosis	1.3 (0.8-2.2)	46.1 (6/13)	46.1 (6/13)	7.8 (1/13)
	Pyelonephritis	0.9 (0.5-1.7)	33.3 (3/9)	55.6 (5/9)	11.1 (1/9)
	Interstitial necrosis	0.5 (0.2-1.2)	20.0 (1/5)	60.0 (3/5)	20.0 (1/5)
	Uroliths	0.3 (0.1-0.9)	33.3 (1/3)	66.7 (2/3)	-
	Multicentric B-cell lymphoma	0.1 (0-0.6)	-	-	100.0 (1/1)
	Congenital cysts	0.1 (0-0.6)	-	100.0 (1/1)	-
	Glycogen intranuclear inclusion	0.1 (0-0.6)	-	-	100.0 (1/1)
Adrenal (349)	Adrenalitis	19.5 (15.7-24)	60.3 (41/68)	33.9 (23/68)	5.8 (4/68)
	Extramedullary hematopoiesis	12 (9-15.9)	52.4 (22/42)	38.1 (16/42)	9.5 (4/42)
	Hemorrhage	4.8 (3.1-7.7)	35.3 (6/17)	35.3 (6/17)	29.4 (5/17)
	Necrosis	4.6 (2.8-7.3)	43.7 (7/16)	43.7 (7/16)	12.6 (2/16)
	Thrombosis	0.3 (0-1.6)	-	100.0 (1/1)	-
	Multicentric B-cell lymphoma	0.3 (0-1.6)	-	-	100.0 (1/1)
Stomach (68)	Granulomatous gastritis	2.9 (0.5-10.1)	100.0 (2/2)	-	-
	Serositis	2.9 (0.5-10.1)	100.0 (2/2)	-	-

Intestines (7)	Enteritis	71.4 (35.9-94.9)	-	40.0 (2/5)	60.0 (3/5)
	Multicentric B-cell lymphoma	14.3 (0.7-51.3)	-	-	100.0 (1/1)
Pancreas (12)	Pancreatitis	25.0 (8.9-53.2)	-	33.3 (1/3)	66.7 (2/3)
Bladder (157)	Cystitis	2.54 (1.0-6.4)	75.0 (3/4)	25.0 (1/4)	-
	Perivasculitis	2.54 (1.0-6.4)	100.0 (4/4)	-	-
	Vasculitis	0.6 (0.0-3.5)	-	100.0 (1/1)	-
Ovary (140)	Hemosiderosis	2.1 (0.6-6.1)	100.0 (3/3)	-	-
	Follicular cyst	2.1 (0.6-6.1)	66.7 (2/3)	-	33.3 (1/3)
	Oophoritis	1.4 (0.2-5.0)	50.0 (1/2)	50.0 (1/2)	-
	Hemorrhage	1.4 (0.2-5.0)	50.0 (1/2)	-	50.0 (1/2)
Uterus (137)	Hemorrhage	5.1 (2.5-10.2)	14.3 (1/7)	71.4 (5/7)	14.3 (1/7)
	Endometritis	4.4 (2.0-9.2)	33.3 (2/6)	66.7 (4/6)	-
	Necrosis	4.4 (2.0-9.2)	66.7 (4/6)	33.3 (2/6)	-
	Thrombosis	2.9 (1.1-7.3)	-	75.0 (3/4)	25.0 (1/4)
	Hemosiderosis	2.2 (0.6-6.2)	33.3 (1/3)	66.7 (2/3)	-
	Cystic endometrial hyperplasia	1.5 (0.3-5.2)	50.0 (1/2)	50.0 (1/2)	-
	Adenomyosis	0.7 (0.0-4.0)	100.0 (1/1)	-	-
Testicle (221)	Degeneration	2.7 (1.2-5.8)	33.3 (2/6)	50.0 (3/6)	16.7 (1/6)
	Orchitis	0.9 (0.2-3.2)	50.0 (1/2)	50.0 (1/2)	-
Epididymis (221)	Epididymitis	0.4 (0.0-2.5)	-	100.0 (1/1)	-
Prostate (14)	Hyperplasia	14.2 (2.5-40.0)	50.0 (1/2)	50.0 (1/2)	-
	Prostatitis	7.1 (0.4-31.5)	-	100.0 (1/1)	-

CI: Confidence interval; N: number of samples.

Infectious diseases were detected in 214 cases (214/1,001; 21.4%; CI 18.9-24.0%), being 124 caused by parasites, 88 by bacteria, and 36 by viruses (Table 1.4). Importantly, in some cases co-infections was observed. Parasitic diseases were mainly represented by platynosomiasis (89/1,001; 8.9%; CI 7.3-10.8%), causing a proliferative and fibrosing cholangiohepatitis (Figure 1.1A); toxoplasmosis (16/1,001; 1.6%; CI 1.0-2.6%), causing systemic necrotizing and inflammatory lesion, especially at liver and spleen (Figure 1.1B), confirmed by IHC; microfilariosis (9/1,001; 0.9%; CI 0.5-1.7%), usually observed in blood vessels from multiple organs and eventually associated with inflammatory cells and microthrombosis (Figure 1.1C); and pulmonary metastrongylus - lungworm (7/1,001; 0.7%; CI 0.3-1.4%), causing a mild interstitial pneumonia with parasites observed at the airways (Figure 1.1D). Other metazoans were detected with low prevalence, such as hepatic capillariosis (Figure 1.1E), intravascular spirurid/ascarid, pulmonary trematode mesocercaria (Figure 1.1F), and intestinal/gastric acanthocephalan.

Bacterial etiology represented 41.1% (88/214) of the infectious disease. Bronchopneumonia with intralesional bacteria was detected in 13 cases, being six cases with intralesional Gram-negative bacteria and seven with Gram-positive bacteria. In most of these cases it was observe a necrotizing bronchopneumonia, moderate to marked, diffuse, with alveolar spaces filled with neutrophils, histiocytes, fibrin and cell debris (Figure 1.2A-B). One of the Gram-negative bronchopneumonia was confirmed as caused by *E. coli*, with positive immunoreaction to *E. coli* H Pool-IHC (Figure 1.2B) and confirmation by PCR and sequencing. In this case was also observed *E. coli*-bacteremia and hepatitis. In three cases there were Splendore-Hoeppli reaction, with intralesional Gram-positive cocci, immunoreactive to *S. pneumoniae*-IHC (Figure 1.2C), confirmed by PCR and sequencing as *Streptococcus* spp. in one case and as *S. sanguinis* in other one. One case the PCR was undetermined. In two of these cases, pleuritis and pericarditis was also observed, interpreted as an extension of the lung process. Another bronchopneumonia case was confirmed as *Staphylococcus* spp. by PCR and sequencing, having a distinct histopathological feature characterized by necrotizing perivasculitis, with myriads of Gram-positive cocci immunoreactive to *Staphylococcus* spp. IHC (Figure 1.2D). This case also had bacteremia and hepatitis associated with the pulmonary lesions. Granulomatous pneumonia was detected in five cases (5/1,001; 0.5%; CI 0.2-1.2%), but no intralesional organism was identified by Ziehl-Nielsen, PAS or GMS.

Bacteria were also observed causing suppurative meningoencephalitis with bacteremia in two cases: one with Gram-negative rods immunoreactive to *E. coli* H Pool-IHC and the other with Gram-positive cocci immunoreactive to *Staphylococcus* spp. confirmed by PCR and sequencing as *S. capitis* (Figure 1.3A-B). Bacterial cholangiohepatitis and random hepatitis was identified in 18 cases, being caused by a Gram-negative bacterium in 12 cases and by a Gram-positive bacterium in four cases, and in 13 cases was associated with intraductal trematode (*Platynosomum* sp.). Two cases had a severe cholangiohepatitis with peritonitis and a mixed population of bacteria, being one case associated with biliary lithiasis. Bacteremia was observed in 79.5% (70/88; CI 69.9-86.6%) of the bacterial cases, and in 50 cases, no primary site of infection was identified, with myriad of bacteria being observed in the cytoplasm of macrophages and neutrophils especially at sinusoids, pulmonary capillaries, and splenic red pulp. Gram-negative bacteria were identified in 80% (56/70; CI 69.2-87.7%) of the bacteremia cases and in 18.6% (13/70; CI 11.2-29.2%) was detected Gram-positive bacteria. In one case the Gram stain was undetermined. In all Gram-negative bacteremia cases was observed a mild to marked immunoreaction to *E. coli* H Pool-IHC, with detection of *Moraxella* spp. by PCR and sequencing in one case, being characterized by mild random hepatitis, with marked sinusoidal leukocytosis (Figure 1.3C) and moderate histiocytic splenitis.

Viral diseases were mainly represented by YFV, detected in 32 animals (32/1,001; 3.2%; 2.3-4.5%). Of these, two positive animals also had microfilariosis and bacteremia. Classical histopathological features, characterized by mediozonal to massive hepatic necrosis with apoptotic bodies (Councilman-Rocha Lima bodies) (Santos et al., 2020) was detected in ten cases (10/32; 31.2%; CI 17.9-48.6%). RABV was detected in one animal, a juvenile male, with mild non-suppurative encephalitis, but no identification of Negri-bodies. A total of 14 lungs that had a moderate to marked acute interstitial pneumonia and/or bronchopneumonia, with alveolar fibrin deposition, were selected for Adenovirus-IHC and molecular respiratory panel, resulting in three cases positive for PIV, being two for PIV-1 (Ct 22.84 and 30.1) and one for PIV-3 (Ct 32.5). No evidence of the virus was observed in the PIV-IHC or TEM. In one case positive for PIV-1 (Ct 22.84), a severe Gram-positive bacterial pneumonia was also observed (Figure 1.3D). All three animals were adults, being two females and one male.

Table 1.4 Infectious agents detected in the free-ranging NWP from the Brazilian Atlantic Forest found dead during January 2017 to July 2019.

ORGANISM	ORGANS AFFECTED	NWP SPECIE (%; CI)			
		Marmoset (n = 1,001)	Howler-monkey (n = 43)	Capuchin (n = 29)	Titi-monkey (n = 2)
BACTERIA					
Escherichia coli	Lung and liver	1 (0.1; 0.0-0.6)[a]	Not detectable[a]	Not detectable[a]	Not detectable[a]
Moraxella sp.	Systemic	1 (0.1; 0.0-0.6)	Not detectable[a]	Not detectable[a]	Not detectable[a]
Staphylococcus capitis	Brain, liver and adrenal	1 (0.1; 0.0-0.6)[a]	Not detectable[a]	Not detectable[a]	Not detectable[a]
Staphylococcus sp.	Liver and lungs	1 (0.1; 0.0-0.6)[a]	Not detectable[a]	1 (3.4; 0.2-17.2)[a]	Not detectable[a]
Streptococcus sanguinis	Lung, heart and liver	1 (0.1; 0.0-0.6)[a]	Not detectable[a]	Not detectable[a]	Not detectable[a]
Streptococcus sp.	Lung	1 (0.1; 0.0-0.6)[a]	Not detectable[a]	Not detectable[a]	Not detectable[a]
METAZOAN					
Acantocephalan (adult)	Stomach and intestines	4 (0.4; 0.2-1.0)[a]	Not detectable[a]	Not detectable[a]	Not detectable[a]
Ascarid (larvae)	Bladder	1 (0.1; 0.0-0.6)[a]	Not detectable[a]	Not detectable[a]	Not detectable[a]
Capillaria hepatica	Liver	1 (0.1; 0.0-0.6)[a]	Not detectable[a]	Not detectable[a]	Not detectable[a]
Filarial nematode	Lungs	Not detectable[a]	2 (4.6; 0.8-15.4)[b]	Not detectable[a,b]	Not detectable[a,b]
Mesocercaria (trematode)	Lungs	1 (0.1; 0.0-0.6)[a]	Not detectable[a]	Not detectable[a]	Not detectable[a]
Metastrongylus (lungworm)	Lungs	7 (0.7; 0.3-1.4)[a]	Not detectable[a]	Not detectable[a]	Not detectable[a]
Microfilariae	Systemic	9 (0.9; 0.5-1.7)[a]	5 (11.6; 5.1-24.5)[b]	1 (3.4; 0.2-17.2)[a,b]	2 (100.0; 17.8-100.0)[c]
Platynosomum sp.	Liver	89 (8.9; 7.3-10.8)[a]	Not detectable[b]	Not detectable[a,b]	Not detectable[a,b]
Spirurid (adult)	Pleura, lungs and spleen	3 (0.3; 0.1-0.9)[a]	Not detectable[a]	Not detectable[a]	Not detectable[a]
PROTOZOAN					
Toxoplasma gondii	Systemic	16 (1.6; 1-2.6)[a]	1 (2.3; 0.1-12.0)[a]	Not detectable[a]	Not detectable[a]
VIRUSES					
Parainfluenza virus	Lung	3 (0.3; 0.1-0.9)[a]	Not investigated	Not investigated	Not investigated
Rabies virus	Brain	1 (0.1; 0.0-0.6)[a]	Not detectable[a]	Not detectable[a]	Not detectable[a]
Yellow fever virus	Liver	32 (3.2; 2.3-4.5)[a]	22 (51.2; 36.7-65.4)[b]	1 (3.4; 0.2-17.2)[a]	2 (100.0; 17.8-100.0)[b]

a, b, c: Different letters indicate statistically significant differences between species of NWP (Fisher's exact test, $p < 0.05$). NWP: New World primate; CI: Confidence interval; n: number of samples.

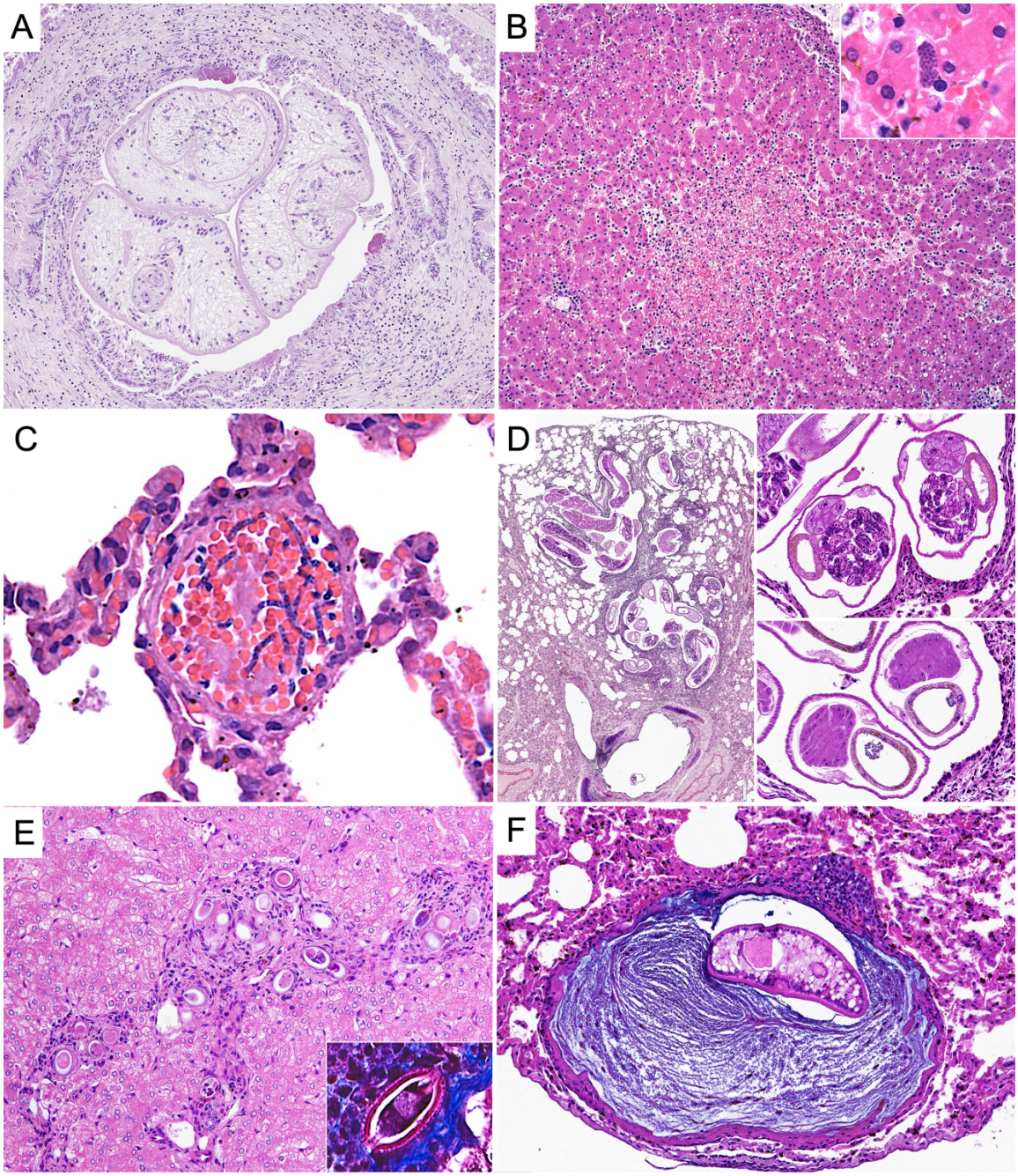


Figure 1.1 Parasitic diseases observed in free-ranging marmosets (*Callithrix* spp.). (A) Fibrosing and proliferative cholangiohepatitis with intraductal trematode. Liver, HE, 200x. (B) Random necrotizing hepatitis with intralesional tachyzoites (top right, HE, 400x). Liver, HE, 100x. (C) Myriad of intravascular microfilariae associated with mild fibrin clusters. Lung, HE, 400x. (D) Transversal and longitudinal sections of adult nematode at the airways (left frame, 50x), compatible with females (top right, 200x) and males (down right, 200x) of metastrongylus. Lung, HE. (E) Pyogranulomatous portal hepatitis with intralesional bi-operculated nematode eggs (down right, Masson's trichome, 400x), compatible with hepatic capillariosis. Liver, HE, 200x. (F) Pulmonary pseudocyst containing trematode mesocercaria immersed in a myxomatous matrix. Lung, HE, 200x.

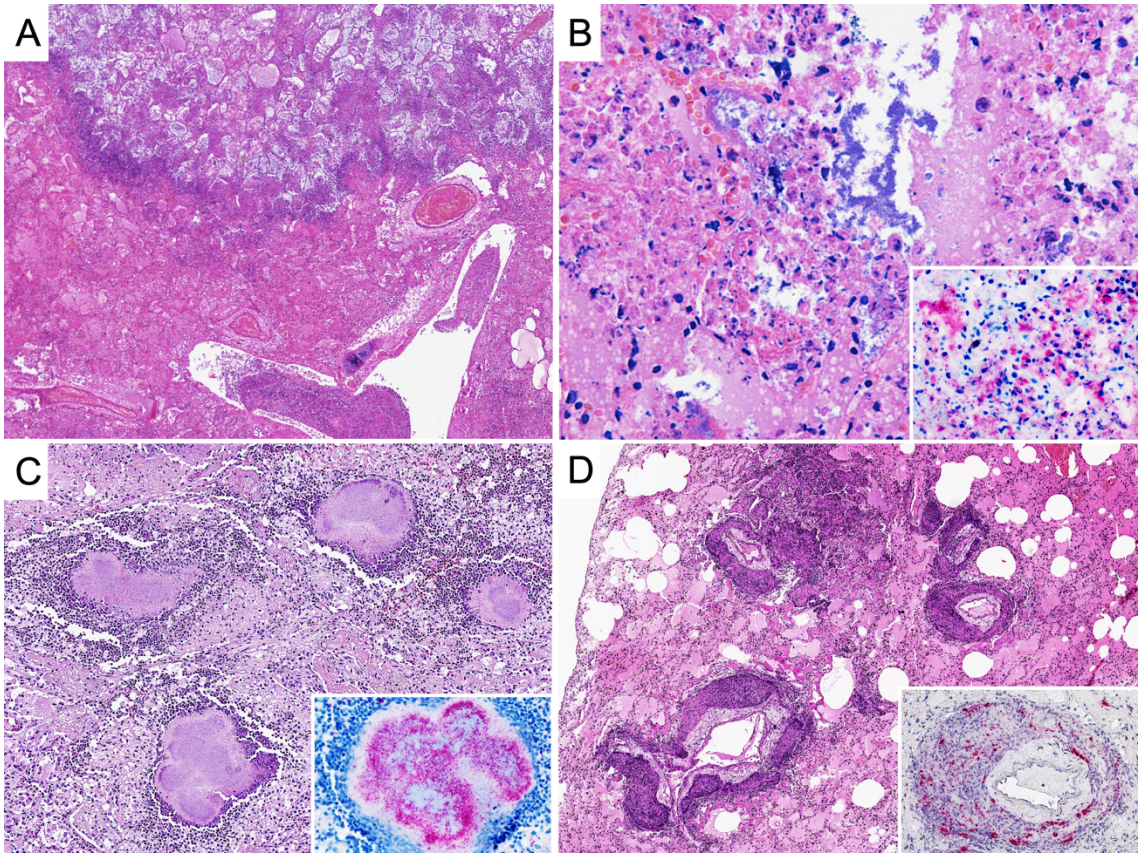


Figure 1.2 Bacterial diseases observed in free-ranging marmosets (*Callithrix* spp.). (A) Marked diffuse necrotizing bronchopneumonia. Lung, HE, 50x. (B) Alveoli filled with neutrophils, histiocytes, cell debris, fibrin, and myriad of bacteria, immunoreactive to *E. coli* H Pool-IHC (down right, Permanent Red, 200x). Lung, HE, 400x. (C) Necrotizing bronchopneumonia with Splendore-Hoeppli reaction and intralesional cocci immunoreactive to *S. pneumoniae*-IHC (down right, Permanent Red, 200x). Lung, HE, 100x. (D) Necrotizing bronchopneumonia with marked necrotizing perivasculitis with intralesional cocci immunoreactive to *Staphylococcus* spp.-IHC (down right, Permanent Red, 200x). Lung, HE, 50x.

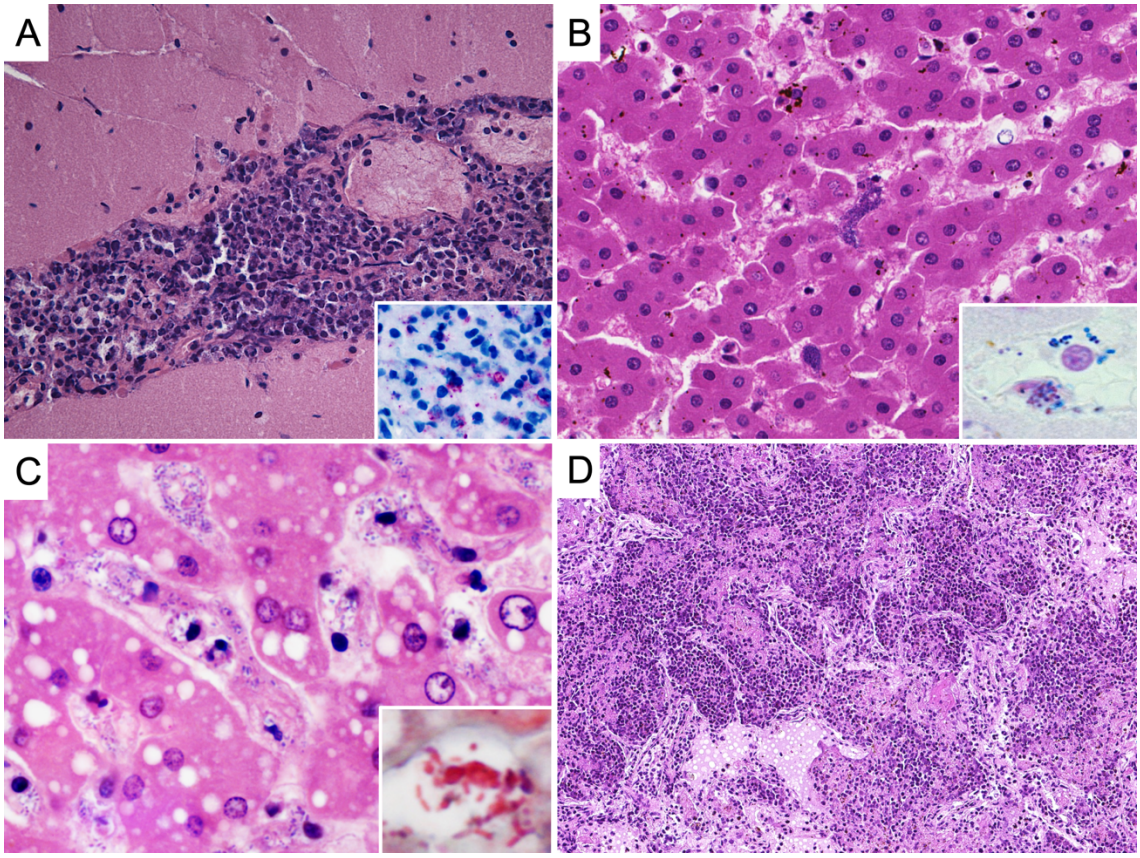


Figure 1.3 Bacterial diseases observed in free-ranging marmosets (*Callithrix* spp.). (A) Suppurative meningoencephalitis with intralesional cocci immunoreactive to *Staphylococcus* spp.-IHC (down right, Permanent Red, 400x). Brain, HE, 200x. (B) Sinusoidal leukocytosis with histiocytes filled with myriad of Gram-positive cocci (down right, Gram stain, 1000x). Liver, HE, 200x. (C) Sinusoidal leukocytosis with histiocytes filled with myriad of Gram-negative bacteria (down right, Gram stain, 1000x). Liver, HE, 400x. (D) Severe Gram-positive bacterial bronchopneumonia with alveoli filled with neutrophils and fibrin clusters. This case was also co-infected with PIV-1 Lung, HE, 100x.

Myocarditis was detected in 143 cases (143/1,001; 14.3%; CI 12.2-16.6%), most of them were characterized by a mild focal to multifocal myocardial infiltrate, usually perivascular, composed of lymphocytes and plasma cells, considered an unspecific found, and some others were mild to moderate associated with intralesional bacteria (18/143; 12.6%; CI 8.1-19.0%) or *T. gondii* zoites (7/143; 4.9%; CI 2.4-9.8%). In ten cases (10/143; 7%; CI 3.8-12.4%) the animals were also positive for YFV qRT-PCR. However, in 22 cases, this infiltrate was moderate multifocal to coalescent, with addition of histiocytes and neutrophils, in some cases with necrosis and fibroplasia, with no intralesional organism. These 22 cases were tested with a *Leishmania*-IHC that is known cross-react with *T. cruzi* and *T. gondii,* but no visible immunoreaction was

observed. These samples were also tested for flavivirus and enterovirus by PCR, with no positive samples, being the cause undetermined.

Interstitial nephritis was frequently observed in free-ranging marmosets (381/1,001; 38.1%; CI 35.1-41.1%), varying from mild focal to moderate multifocal interstitial inflammation, composed mainly by lymphocytes and plasma cells (Figure 1.4B). Suppurative pyelonephritis was observed in nine cases (9/1,001; 0.9%; CI 0.5-1.7%), three of them associated with calcium oxalate crystals (Figure 1.4C). No intralesional organisms were observed in any of these cases, with negative results to WS and Gram stains. Bacterial was also investigated by IHC for detection of *E. coli* H Pool, *S. pneumoniae*, and *Staphylococcus*, being negative in the three assays. Cases of severe chronical renal disease, characterized by marked diffuse interstitial inflammation, with fibrosis, severe membranous glomerulopathy, presence of hyalin casts, retention cysts and glomerulosclerosis, was identified in 47 animals (47/1,001; 4.7%; CI 3.5-6.1%) (Figure 1.4D). In addition to marked interstitial fibrosis and severe membranous glomerulopathy, one case had an intense anisokaryosis and karyomegaly of the tubular epithelium, interpreted as marked tubular regeneration (Figure 1.4E) with intranuclear inclusion bodies, PAS-positive and Ziehl-Neelsen negative, in the epithelium of the proximal tubules, also observed in hepatocytes, compatible with glycogen inclusions (Figure 1.4F). This case was tested by IHC for human herpesvirus (HSV-1 and 2), human herpesvirus 5 (HHV-5) and human polyomavirus (SV40), being negative.

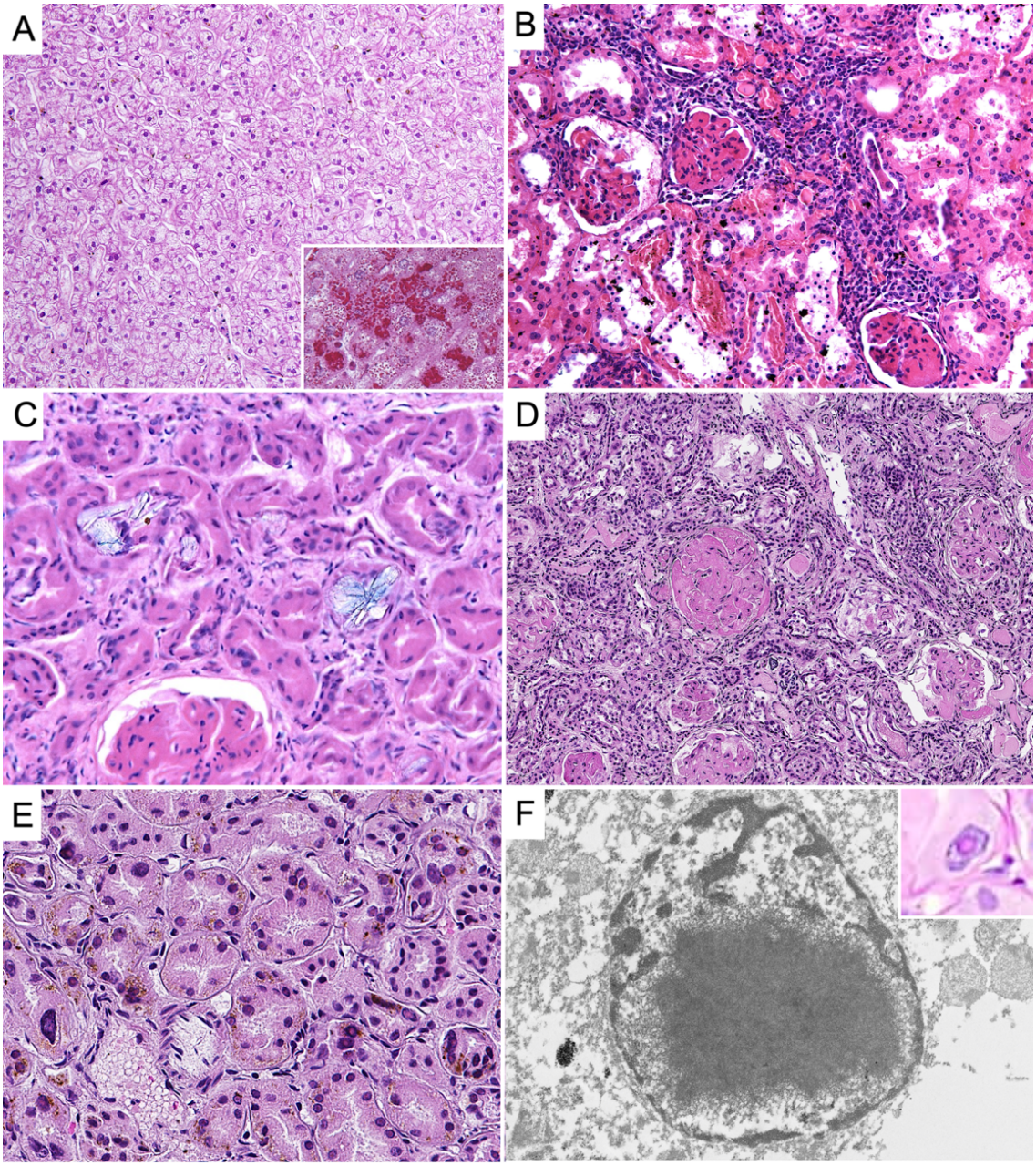


Figure 1.4 Non-infectious histopathological findings observed in free-ranging marmosets (*Callithrix* spp.). (A) Marked diffuse hepatocellular glycogen degeneration (DHGD), characterized by with PAS-positive material in the hepatocyte cytoplasm (down right, PAS, 100x). Liver, HE, 200x. (B) Multifocal interstitial nephritis composed by lymphocytes, plasma cells and histiocytes, with mild membranous glomerulopathy and hyalin casts. Kidney, HE, 100x. (C) Intratubular birefringent irradiated material, compatible with calcium oxalate crystals. Kidney, HE, 200x. (D) Severe chronical renal disease, with marked interstitial fibrosis and membranous glomerulopathy. Kidney, HE, 50x. (E) Marked diffuse anisocytosis with karyomegaly of the renal tubular epithelium with intranuclear inclusions. Kidney, HE, 200x. (F) Glassy PAS-positive intranuclear inclusion (top right, PAS, 400x) characterized at TEM by rough edges and round to amorphous shape, compatible with glycogen inclusion.

Non-infectious diseases were mainly represented by trauma, observed in 444 animals (444/1,001; 44.3%; CI 41.3-47.4%), with 323 cases of head trauma (323/444; 72.7%; CI 68.4-76.7%). Importantly, pulmonary hemorrhage and DHGD were more frequently observed in animals with gross lesions of trauma (Fisher's exact test, $p < 0.0001$). At total, DHGD was identified in 308 animals (30.8%; 308/1,001; CI 28-33.7%), being usually diffuse, mild to marked, with PAS-positive material in the hepatocyte cytoplasm (Figure 1.4A). Seven cases (7/1,001; 0.7%; CI 0.3-1.4%) had gross identification of "chumbinho" pellets in the stomach content. In five of these cases was observed mild to marked alveolar hemorrhage and in three moderate to marked alveolar edema.

Considering all the organs evaluated, extramedullary hematopoiesis (EH) was observed in 130 animals (130/1,001; 13%; CI 11.0-15.2%), being usually detected at liver (sinusoids) (Figure 1.5A), spleen (red pulp), adrenal (medullar), and lungs (alveolar wall). Atypical sites, such as choroid was also identified in the marmosets from this study (Figure 1.5B). Interestingly, animals with severe chronical renal disease also had a higher frequency of EH (Fisher's exact test, $p = 0.0015$). Neoplasia was observed in only one animal: an adult female with a multicentric B-cell lymphoma (positive to CD20-IHC and negative to CD3-IHC), characterized by a proliferation of monomorphic round cells at lungs, liver, adrenal and intestines (Figure 1.5C-D). In another case was observed a focally extensive lung nodular B-cell hyperplasia (positive to CD20-IHC and negative to CD3-IHC) (Figure 1.5E-F). Both cases were tested for human herpesvirus-4 (HHV-4) by IHC and PCR, being negative in all samples evaluated.

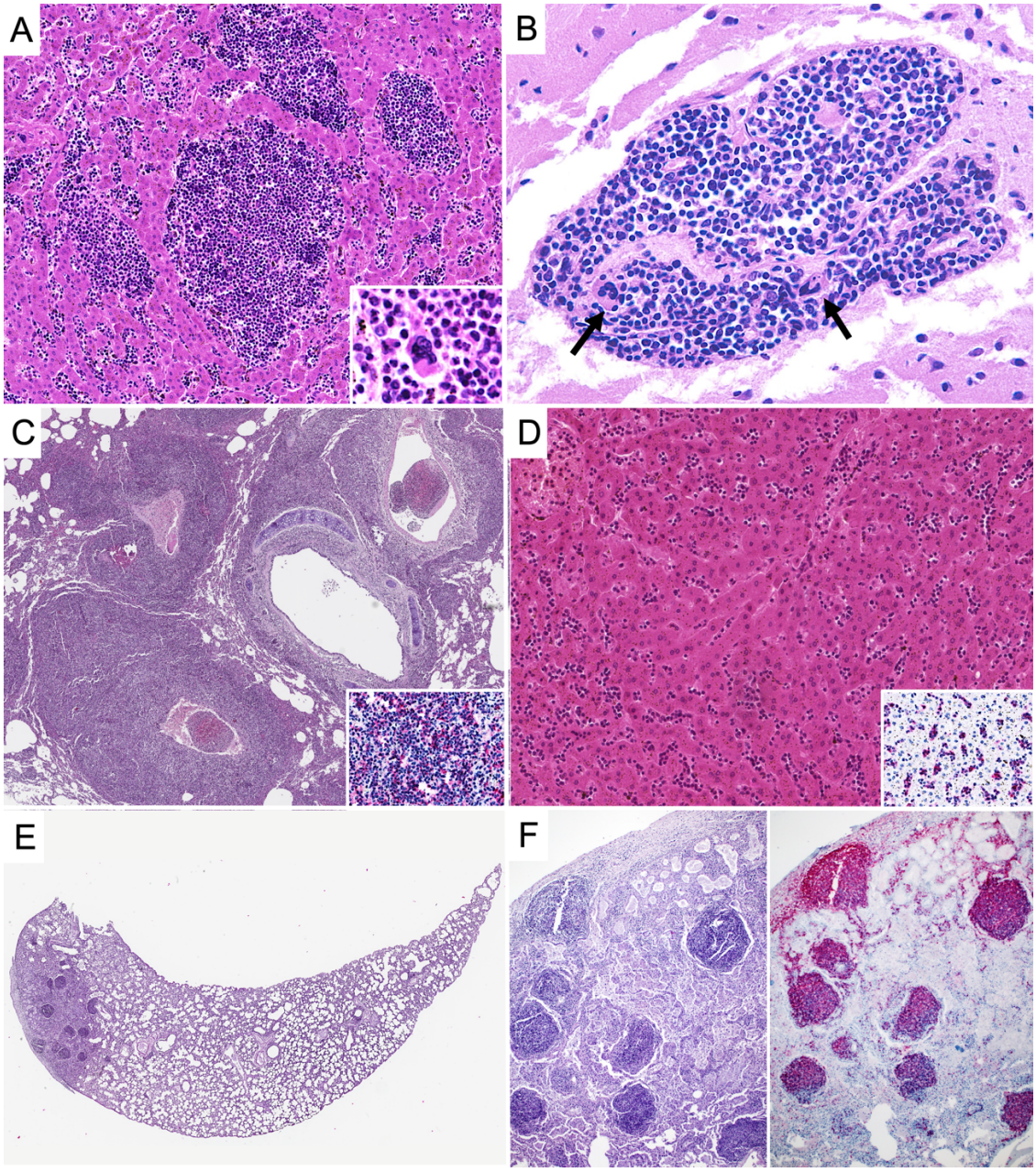


Figure 1.5 Non-infectious histopathological findings observed in free-ranging marmosets (*Callithrix* spp.). (A) Marked extramedullary hematopoiesis (EM) with enlarged sinusoids filled with hematopoietic cells, including megakaryocytes (down right, HE, 400x). Liver, HE, 100x. (B) Atypical EH at choroid. Arrows shows the megakaryocytes. Brain, HE, 200x. (C) B-cell lymphoma characterized by a sheet of round cells immunoreactive to CD20 (down right, Permanent Red, 200x). Lung, HE, 50x. (D) Sinusoids filled with monomorphic population of lymphocytes immunoreactive to CD20 (down right, Permanent Red, 200x). Liver, HE, 100x. (E) Pulmonary nodular B-lymphocyte hyperplasia. Lung, HE, submicroscopic view, (F) characterized by multifocal well differentiate lymphocytes forming multiple follicles (left frame, HE, 50x), with immunoreaction to CD20 (right frame, Permanent Red, 50x). Lung.

In the lungs of 37 cases (37/1,001; 3.7%; CI 2.7-5%) was observed mild to marked intravascular clusters of monomorphic round cells (Figure 1.6A-C). These cells had pale and abundant cytoplasm, in some cases vacuolized, with an eccentric cleaved nucleus and high mitotic index, evidenced by Ki67 immunolabeling (Figure 1.6A-E). In six representative cases, these cells were immunoreactive to IBA-1 and CD163 (Figure 1.6D-E) and negative for CD20, CD3 and myeloperoxidase (MPO). HE, IHC and TEM showed that most of these cells had morphology compatible with histiocytes, being interpreted as a pulmonary intravascular histiocytosis (PIVH) (Figure 1.6A-F). There was no evidence of viral particles in the sections evaluated by TEM and those six representative samples were tested for HHV-4-IHC being all negative.

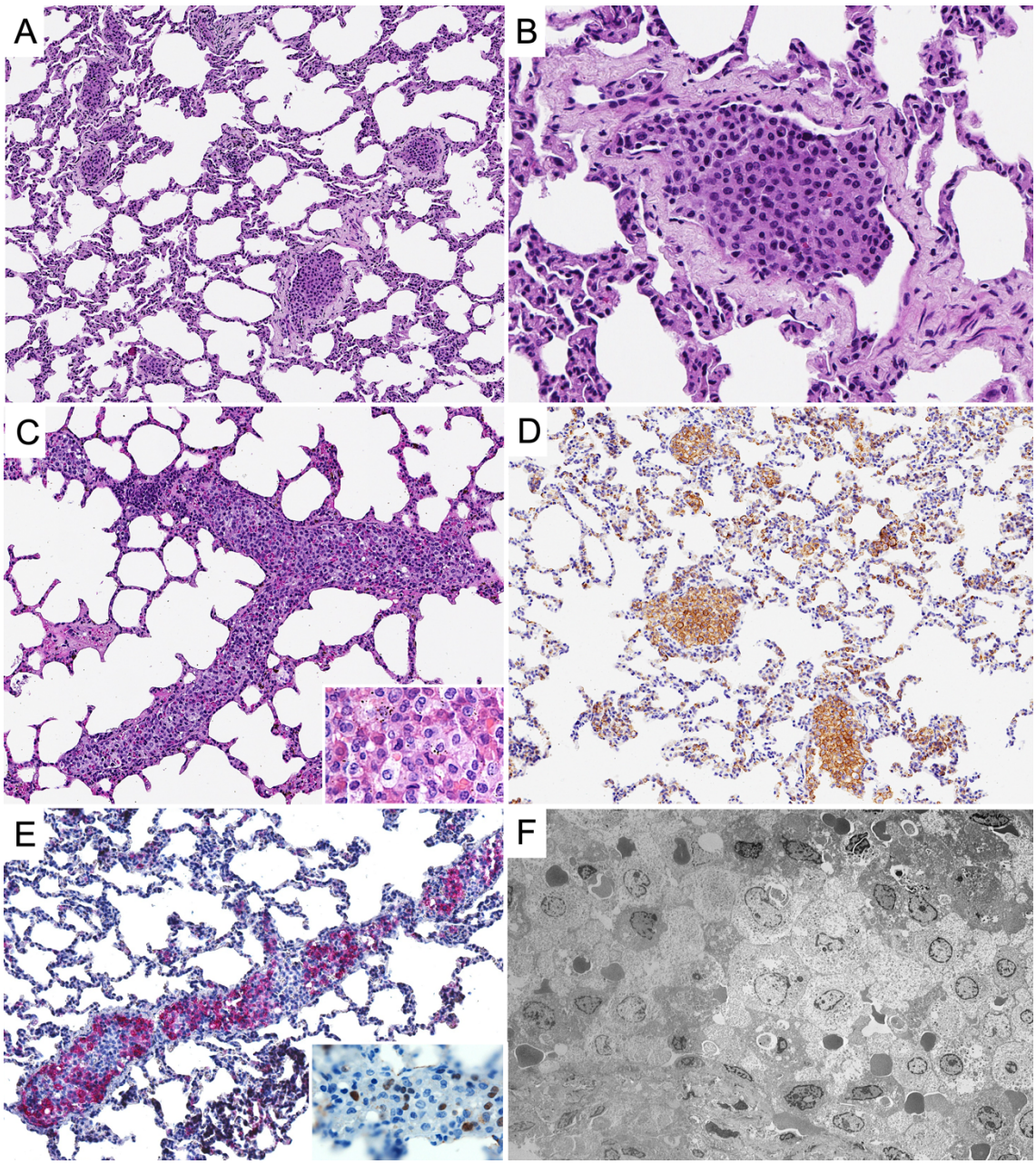


Figure 1.6 Pulmonary intravascular histiocytosis observed in free-ranging marmosets (*Callithrix* spp.). (A-C) Intravascular clusters of round cells, with pale and abundant cytoplasm, in some cases vacuolized (C- down right, HE, 400x), with an eccentric cleaved nucleus and few to moderate binucleated cells. HE, Lung, 50x (A), 200x (B), 100x (C). These cells were diffusely immunoreactive to IBA-1 (D - DAB, 50x) and CD163 (E - Permanent Red, 50x), with high immunolabeling to Ki-67 (E- down right, DAB, 400x). (F) TEM showed that most of these cells had morphology compatible with histiocytes, with no evidence of viral infection.

Importantly, this intravascular histiocytosis was always observed at lungs and rarely in other organs, being identified in the vessels from the meninges, kidney, and uterus in one case and at the liver of nine cases. Interestingly, all cases of PIVH were from the Metropolitan region of the Rio de Janeiro state, being more frequently observed in the animals from this region compared to the others (Fisher's exact test, $p = 0.0014$). No difference of frequencies was observed between sex (Fisher's exact test, $p = 0.4788$) or age (Fisher's exact test, $p = 0.1264$). Pulmonary anthracosis, observed in 200 animals (200/1,001; 20%; 17.6-22.6%), was also more frequently observed in animals from the Metropolitan region (Fisher's exact test, $p = 0.0051$)

Finally, multinucleated hepatocytes (MNH - more than three nuclei) (Figure 1.7A), was observed in 130 animals (130/1,1001; 13%; CI 11-15.2%), being 87.7% (114/130) mild (0 to 30 multinucleated hepatocytes per ten HPF), 11.5% (15/130) moderate (31-59 multinucleated hepatocytes per ten HPF) and 0.8% (1/130) marked (> 59 multinucleated hepatocytes per ten HPF). By TEM we observed that cytoplasm of MNH contained abundant mitochondria, but the cells were not necessarily larger than normal hepatocytes and no infectious agents were identified (Figure 1.7B).

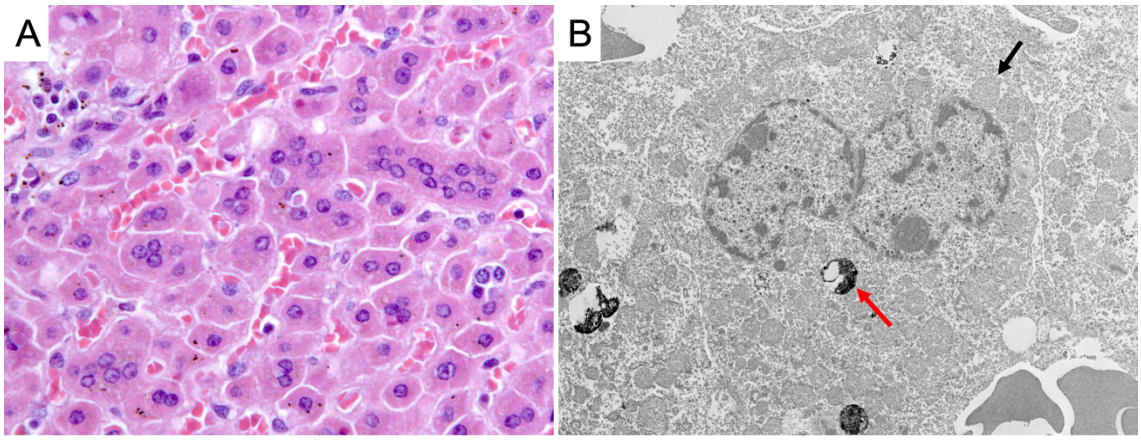


Figure 1.7 Multinucleated hepatocytes observed in free-ranging marmosets (*Callithrix* spp.). (A) Multinucleated hepatocytes (more than three nuclei). Liver, HE, 200x. (B) TEM showed that

multinucleated hepatocytes contained abundant mitochondria (black arrow) and mild to moderate lipofuscin (red arrow), with no evidence of viral infection.

3.2 Pathological findings in free-ranging howler-monkeys

The main pathological findings observed in the necropsied howler-monkeys were portal hepatitis (35/43; 81.4%; CI 67.4-90.3%), lipidosis (28/43; 65.1%; CI 50.2-77.6%), pulmonary edema (27/43; 62.8%; CI 47.9-75.6%), interstitial nephritis (26/43; 60.5%; CI 45.6-73.6%), hepatic necrosis (25/43; 58.9%; CI 28.4-5.7%), pneumonia (23/43; 53.5%; CI 38.9-67.5%), glomerulopathy (19/43; 44.2%; CI 30.4-58.9%), pulmonary hemorrhage (18/43; 41.7%; CI 28.4-56.7%), random hepatitis (14/43; 32.6%; CI 20.5-47.5%), and myocarditis (14/43; 32.6%; CI 20.5-47.5%). Table 1.5 summarizes all the pathological findings observed in the free-ranging howler-monkeys.

Infectious diseases were observed in 26 cases (26/43; 60.5%; CI 45.6-73.6%), being mainly caused by YFV (22/26; 84.6%; CI 66.4-93.8%). Classical hepatic histopathological lesions of YFV were observed in 21 animals (21/22; 95.4%; CI 78.2-99.7%). There were six cases of parasitic diseases: five caused by microfilariosis (5/43; 11.6%; CI 5.1-24.5%), with identification of the adult filariid nematode at lymphatic vessels of the lungs from two animals (2/43; 4.6%; CI 0.8-15.4%); and one case of toxoplasmosis (1/43; 2.3%; CI 0.1-12%), with mild diffuse interstitial pneumonia rich in alveolar foamy macrophages and moderate type-2 pneumocyte hyperplasia; random necrotizing hepatitis; mild histiocytic splenitis; and moderate interstitial nephritis. Intralesional tachyzoites and bradyzoites were observed at lungs, liver, spleen, and kidney, confirmed as *T. gondii* by IHC. Traumatic lesions were detected in ten animals (10/43; 23.3%; CI 13.1-37.7%). In general, besides the necrotizing hepatitis caused by YFV, trauma and the one case of toxoplasmosis, pathological findings in howler-monkeys were mild and not associated with the death of the animals, being most of them considered nonspecific.

Table 1.5 Pathological findings in free-ranging howler monkeys (*Alouatta* spp.) from the Brazilian Atlantic Forest found dead during January 2017 to July 2019.

Organ (N)	Pathological findings	% (CI)	Intensity %		
			Mild	Moderate	Severe
Brain (43)	Hemorrhage	9.3 (3.7-21.6)	100.0 (4/4)	-	-
	Meningitis	7.0 (2.4-18.6)	100.0 (3/3)	-	-
	Non suppurative	4.6 (0.8-15.4)	100.0 (2/2)	-	-
	Suppurative	2.3 (0.1-12.1)	100.0 (1/1)	-	-
	Encephalitis/ Meningoencephalitis	2.3 (0.1-12.1)	100.0 (1/1)	-	-
Heart (43)	Myocarditis	32.6 (20.5-47.5)	92.9 (13/14)	7.1 (1/14)	
	Lymphohistioplasmacytic	30.2 (18.6-45.1)	92.3 (12/13)	7.7 (1/13)	-
	Mixed	2.3 (0.1-12.1)	100.0 (1/1)	-	-
	Fibrosis	9.3 (3.7-21.6)	75.0 (3/4)	25.0 (1/4)	-
	Necrosis	7.0 (2.4-18.6)	66.7 (2/3)	33.3 (1/3)	-
Lung (43)	Alveolar edema	62.8 (47.9-75.6)	59.3 (16/27)	14.8 (4/27)	25.9 (7/27)
	Pneumonia	53.5 (38.9-67.5)	69.6 (16/23)	30.4 (7/23)	-
	Interstitial pneumonia	51.2 (36.7-65.4)	68.2 (15/22)	31.8 (7/22)	-
	Granulomatous pneumonia	2.3 (0.1-12.1)	100.0 (1/1)	-	-
	Alveolar hemorrhage	41.7 (28.4-56.7)	55.6 (10/18)	22.2 (4/18)	22.2 (4/18)
	Anthracosis	20.9 (11.4-35.2)	88.9 (8/9)	11.1 (1/9)	-
	Extramedullary hematopoiesis	7.0 (2.4-18.6)	100.0 (3/3)	-	-
	Pleuritis	7.0 (2.4-18.6)	100.0 (3/3)	-	-
	Alveolar fibrin deposition	4.6 (0.8-15.4)	100.0 (2/2)	-	-
	Thrombosis	4.6 (0.8-15.4)	100.0 (2/2)	-	-
	Perivasculitis	2.3 (0.1-12.1)	100.0 (1/1)	-	-
	Non-thrombotic embolism	2.3 (0.1-12.1)	100.0 (1/1)	-	-
Liver (43)	Portal hepatitis	81.4 (67.4-90.3)	68.6 (24/35)	22.8 (8/35)	8.6 (3/35)
	Lipidosis	65.1 (50.2-77.6)	53.6 (15/28)	32.1 (9/28)	14.3 (4/28)
	Diffuse	16.3 (8.1-30.0)	71.4 (5/7)	14.3 (1/7)	14.3 (1/7)
	Random	48.8 (34.6-63.2)	43.5 (10/21)	38.2 (8/21)	14.3 (3/21)
	Necrosis	58.9 (28.4-5.7)	8.0 (2/25)	12.0 (3/25)	80.0 (20/25)
	Random	7.0 (2.4-18.6)	66.7 (2/3)	33.3 (1/3)	-
	Massive	51.2 (36.7-65.4)	-	9.1 (2/22)	90.9 (20/22)
	Random hepatitis	32.6 (20.5-47.5)	71.4 (10/14)	21.5 (3/14)	7.1 (1/14)
	Iron storage (hemosiderosis)	18.6 (9.7-32.6)	100.0 (8/8)	-	-

	Sinusoidal leukocytosis	11.6 (5.1-24.5)	80.0 (4/5)	20.0 (1/5)	-
	Portal fibrosis	9.3 (3.7-21.6)	100.0 (4/4)	-	-
	Bile duct hyperplasia	7.0 (2.4-18.6)	100.0 (3/3)	-	-
	Cholestasis	7.0 (2.4-18.6)	100.0 (3/3)	-	-
	Diffuse glycogen degeneration	7.0 (2.4-18.6)	100.0 (3/3)	-	-
	Multinucleated hepatocytes	4.6 (0.8-15.4)	100.0 (2/2)	-	-
	Cholangitis	2.3 (0.1-12.1)	100.0 (1/1)	-	-
	Extramedullary hematopoiesis	2.3 (0.1-12.1)	-	100.0 (1/1)	-
Spleen (43)	Hemosiderosis	32.6 (20.5-47.5)	92.9 (13/14)	7.1 (1/14)	-
	Follicular hyalinosis	13.9 (6.6-27.3)	33.3 (2/6)	50.0 (3/6)	16.7 (1/6)
	Lymphoid rarefaction	13.9 (6.6-27.3)	33.3 (2/6)	66.7 (4/6)	-
	Extramedullary hematopoiesis	11.6 (5.1-24.5)	60.0 (3/5)	40.0 (2/5)	-
	Splenitis	11.6 (5.1-24.5)	80.0 (4/5)	20.0 (1/5)	-
	Lymphoid hyperplasia	7.0 (2.4-18.6)	66.7 (2/3)	33.3 (1/3)	-
Kidney (43)	Interstitial nephritis	60.5 (45.6-73.6)	80.8 (21/26)	19.2 (5/26)	-
	Glomerulopathy	44.2 (30.4-58.9)	89.5 (17/19)	10.5 (2/19)	-
	Membranous	41.7 (28.4-56.7)	88.9 (16/18)	11.1 (2/18)	-
	Proliferative	2.3 (0.1-12.1)	100.0 (1/1)	-	-
	Nephrocalcinosis	20.9 (11.4-35.2)	100.0 (9/9)	-	-
	Glomerulosclerosis	16.3 (8.1-30.0)	85.7 (6/7)	14.3 (1/7)	-
	Hyalin casts	16.3 (8.1-30.0)	100.0 (7/7)	-	-
	Interstitial fibrosis	7.0 (2.4-18.6)	100.0 (3/3)	-	-
	Tubular necrosis	7.0 (2.4-18.6)	66.7 (2/3)	33.3 (1/3)	-
	Pigmentary nephrosis	2.3 (0.1-12.1)	100.0 (1/1)	-	-
	Arteritis	2.3 (0.1-12.1)	100.0 (1/1)	-	-
Adrenal (11)	Adrenalitis	9.1 (0.5-37.7)	-	100.0 (1/1)	-
Uterus (05)	Hemorrhage	20.0 (1.0-62.4)	-	100.0 (1/1)	-
Testicle (13)	Degeneration	23.1 (8.2-50.3)	100.0 (3/3)	-	-

CI: Confidence interval; N: number of samples.

3.3 Pathological findings in free-ranging capuchins

The main pathological findings observed in the necropsied capuchins were pulmonary edema (18/29; 62.1%; CI 44-77.3%), splenic lymphoid hyperplasia (16/29; 55.2%; CI 37.5-71.6%), random hepatitis (15/29; 51.7%; CI 34.4-68.6%), interstitial pneumonia (13/29; 44.8%; CI 28.4-62.4%), pulmonary perivasculitis (12/29; 41.4%; CI 25.5-59.3%), hepatic lipidosis (12/29; 41.4%; CI 25.5-59.3%), DHGD (11/29; 37.9%; CI 22.7-56%), pulmonary hemorrhage (9/29; 31%; CI 17.3-49.2%), random hepatic necrosis (7/29; 24.1%; CI 12.2-42.1%), interstitial nephritis (6/29; 20.7%; CI 9.8-38.4%), and portal hepatitis (6/29; 20.7%; CI 9.8-38.4%). Table 1.6 summarizes all the pathological findings observed in the free-ranging capuchins.

Infectious diseases were observed in three animals (3/29; 10.3%; CI 3.6-26.4%), being: (1) one case of YFV in a male adult with head trauma and atypical pathological findings at liver, such as portal and random hepatitis with marked DHGD; (2) a female adult with random necrotizing hepatitis with intralesional Gram-positive cocci, immunoreactive to *Staphylococcus* spp. by IHC and confirmed as *Staphylococcus* sp. by PCR and sequencing (Figure 1.8A-C); (3) one case of microfilariosis in a female adult with head trauma, associated with neutrophilic interstitial pneumonia, leukocytosis and random lymphocytic and neutrophilic hepatitis. No co-infection was observed.

Traumatic lesions were observed in nine animals (9/29; 31%; CI 17.3-49.2%). Also, in four cases (4/29; 13.8%; CI 5.5-30.6%) there was gross identification of "chumbinho" pellets in the stomach content (Figure 1.8D). All four cases had mild to moderate pulmonary alveolar hemorrhage and mild to marked DHGD with mild random neutrophilic hepatitis. Two of them had moderate to marked diffuse pancreatic hemorrhage and one had an acute pancreatitis.

Testicular interstitial cell tumor was detected in one adult male (Figure 1.8E). This animal also had gross lesions of head and thoracic trauma, with a severe necrotizing myocarditis, marked hepatic centrilobular necrosis and multiples focuses of infarction in the kidney. Congenital cysts in the kidney and *rete ovarii* cysts in the ovary were observed in one adult female (Figure 1.8F).

Table 1.6 Pathological findings in free-ranging capuchins (*Sapajus* spp.) from the Brazilian Atlantic Forest found dead during January 2017 to July 2019

Organ (N)	Pathological findings	% (CI)	Intensity %		
			Mild	Moderate	Severe
Brain (29)	Hemorrhage	10.0 (3.5-25.6)	100.0 (3/3)	-	-
	Encephalitis/ Meningoencephalitis	3.5 (0.2-17.2)	100.0 (1/1)	-	-
Heart (29)	Myocarditis	17.2 (7.6-34.5)	60.0 (3/5)	40.0 (2/5)	
	Lymphohistioplasmacytic	6.9 (1.2-21.9)	100.0 (2/2)	-	-
	Mixed	6.9 (1.2-21.9)	-	100.0 (2/2)	-
	Neutrophilic	3.5 (0.2-17.2)	100.0 (1/1)	-	
	Hemorrhage	6.9 (1.2-21.9)	50.0 (1/2)	50.0 (1/2)	-
	Fibrosis	3.5 (0.2-17.2)	100.0 (1/1)	-	-
	Necrosis	3.5 (0.2-17.2)	100.0 (1/1)	-	-
Lung (29)	Alveolar edema	62.1 (44.0-77.3)	44.4 (8/18)	38.9 (7/18)	16.7 (3/18)
	Interstitial pneumonia	44.8 (28.4-62.4)	61.5 (8/13)	38.5 (5/13)	-
	Perivasculitis	41.4 (25.5-59.3)	58.3 (7/12)	41.7 (5/12)	-
	Alveolar hemorrhage	31.0 (17.3-49.2)	33.3 (3/9)	66.7 (6/9)	-
	Osseus metaplasia	10.0 (3.5-25.6)	100.0 (3/3)	-	-
	Alveolar fibrin deposition	6.9 (1.2-21.9)	100.0 (2/2)	-	-
	Thrombosis	6.9 (1.2-21.9)	100.0 (2/2)	-	-
	Anthracosis	3.5 (0.2-17.2)	100.0 (1/1)	-	-
	Extramedullary hematopoiesis	3.5 (0.2-17.2)	-	100.0 (1/1)	-
	BALT hyperplasia	3.5 (0.2-17.2)	-	100.0 (1/1)	-
Liver (29)	Random hepatitis	51.7 (34.4-68.6)	86.6 (13/15)	6.7 (1/15)	76.7 (1/15)
	Lipidosis	41.4 (25.5-59.3)	50.0 (6/12)	41.7 (5/12)	8.3 (1/12)
	Diffuse	31.0 (17.3-49.2)	55.5 (5/9)	33.3 (3/9)	11.2 (1/9)
	Random	10.0 (3.5-25.6)	33.3 (1/3)	66.7 (2/3)	-
	Diffuse glycogen degeneration	37.9 (22.7-56.0)	54.6 (6/11)	9.2 (1/11)	36.2 (4/11)
	Random necrosis	24.1 (12.2-42.1)	71.4 (5/7)	14.3 (1/7)	14.3 (1/7)
	Portal hepatitis	20.7 (9.8-38.4)	83.3 (5/6)	16.7 (1/6)	-
	Multinucleated hepatocytes	17.2 (7.6-34.5)	100.0 (5/5)	-	-
	Sinusoidal leukocytosis	13.7 (5.5-30.6)	100.0 (4/4)	-	-
	Iron storage (hemosiderosis)	6.9 (1.2-21.9)	100.0 (2/2)	-	-
	Portal fibrosis	3.5 (0.2-17.2)	100.0 (1/1)	-	-
	Bile duct hyperplasia	3.5 (0.2-17.2)	100.0 (1/1)	-	-

	Thrombosis	3.5 (0.2-17.2)	-	100.0 (1/1)	-
Spleen (29)	Lymphoid hyperplasia	55.2 (37.5-71.6)	56.2 (9/16)	31.2 (5/16)	12.6 (2/16)
	Follicular hyalinosis	3.5 (0.2-17.2)	100.0 (1/1)	-	-
	Extramedullary hematopoiesis	3.5 (0.2-17.2)	-	100.0 (1/1)	-
	Splenitis	3.5 (0.2-17.2)	-	100.0 (1/1)	-
Kidney (29)	Interstitial nephritis	20.7 (9.8-38.4)	83.3 (5/6)	16.7 (1/6)	-
	Glomerulosclerosis	10.0 (3.5-25.6)	66.7 (2/3)	33.3 (1/3)	-
	Membranous glomerulopathy	3.5 (0.2-17.2)	100.0 (1/1)	-	-
	Hyalin casts	3.5 (0.2-17.2)	100.0 (1/1)	-	-
	Interstitial fibrosis	3.5 (0.2-17.2)	100.0 (1/1)	-	-
	Congenital cysts	3.5 (0.2-17.2)	100.0 (1/1)	-	-
Adrenal (14)	Adrenalitis	21.4 (7.6-47.6)	66.7 (2/3)	33.3 (1/3)	-
	Hemorrhage	21.4 (7.6-47.6)	-	100.0 (3/3)	-
	Thrombosis	7.1 (0.4-31.5)	-	100.0 (1/1)	-
	Necrosis	7.1 (0.4-31.5)	-	100.0 (1/1)	-
Ovary (06)	Cysts (*rete ovarii*)	16.7 (0.8-56.3)	100.0 (1/1)	-	-
Testicle (11)	Interstitial cell tumor	9.1 (0.5-37.7)	100.0 (1/1)	-	-

CI: Confidence interval; N: number of samples.

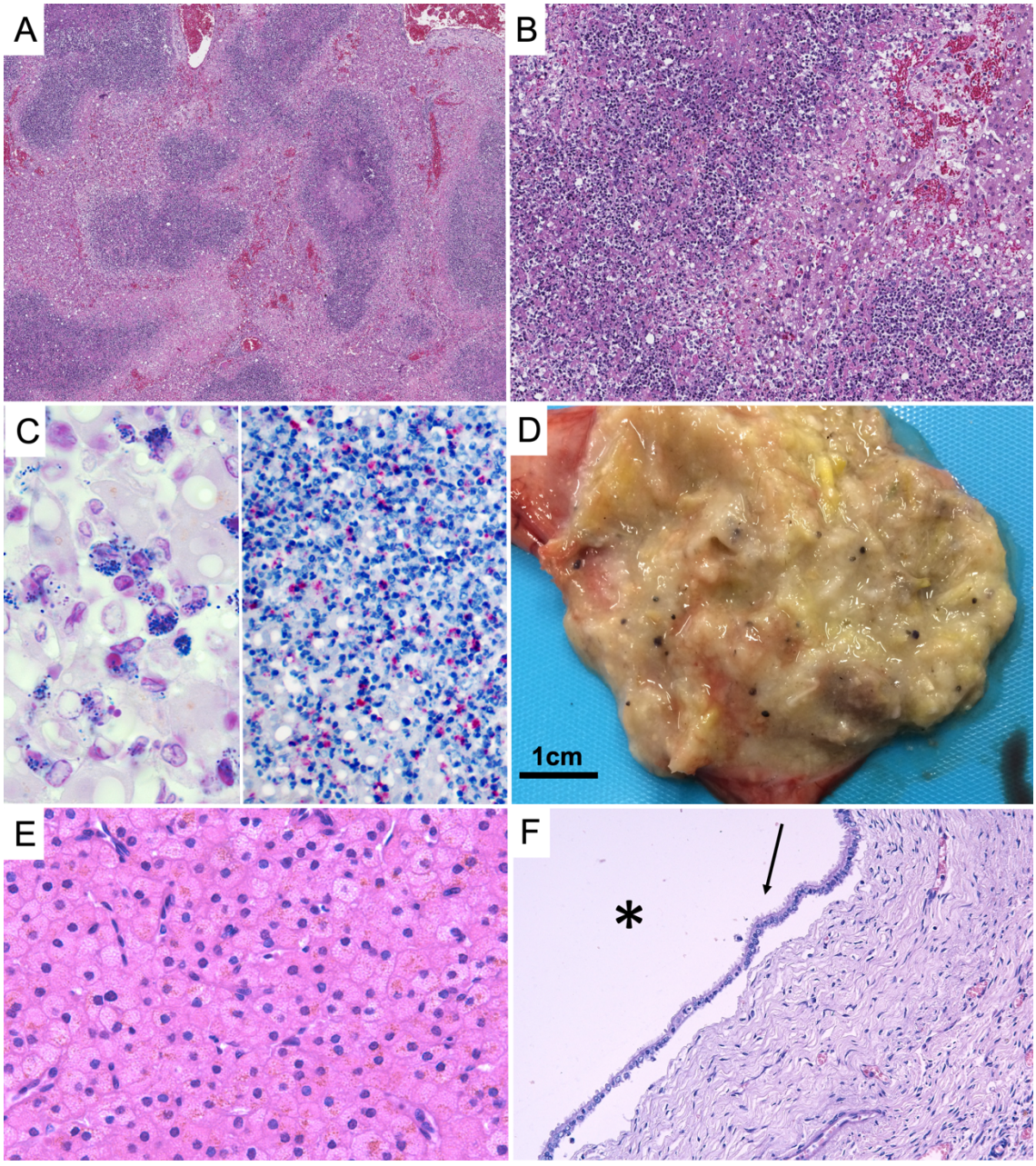


Figure 1.8 Pathological findings observed in free-ranging capuchins (*Sapajus* spp.). (A-B) Random necrotizing hepatitis. Liver, HE, 50x (A), 100x (B), with intralesional Gram-positive cocci (C- right frame, Gram stain, 1000x) immunoreactive to *Staphylococcus* spp. by IHC (C- left frame, Permanent Red, 200x). (D) Stomachal content with multiple millimetric black pellet compatible with "chumbinho". Scale bar = 1 cm. (E) Testicular interstitial cell tumor characterized by a sheet of well-differentiate round to polyhedric cells, with pale and vacuolized cytoplasm. Testicle, HE, 400x. (F) *Rete-ovarii*-cyst with the lumen (*) lined by a cuboidal and ciliated epithelium (arrow) surrounded by connective tissue with no muscular layer. Ovary, HE, 100x.

3.4 Pathological findings in free-ranging lion tamarins and titi-monkeys

Three lion tamarins were included in this study: two golden lion tamarins (*L. rosalia*) and one golden-headed lion tamarin (*L. chrysomelas*). One golden lion tamarin had severe polytraumatic gross lesions and marked autolysis. The other one was positive for YFV with classical hepatic pathological features, characterized by marked diffuse hepatocyte necrosis with moderate number of hepatocytes with eosinophilic cytoplasm and ghost nucleus (interpreted as apoptotic bodies - Councilman-Rocha Lima bodies). The remaining hepatocytes had marked lipidosis and moderate multifocal lymphohistioplasmocytic and neutrophilic infiltrate. Marked hemosiderosis was observed at spleen; and lungs had marked alveolar edema and mild alveolar hemorrhage. The golden-headed lion tamarin had diffused marked pulmonary hemorrhage and mild DHGD with mild random hepatitis. The cause was undetermined.

Two titi-monkeys were included in this study: both were positive for YFV, one with the same classical hepatic pathological features described above for golden lion tamarin (Figure 1.9A) and the other one had mild, mediozonal, multifocal, individual hepatocyte necrosis (Figure 1.9C). Also, both were parasitized with microfilariae, observed intravascular at heart, lungs, liver, spleen, kidneys and pancreas. In both cases, microfilariae were associated with intravascular neutrophils and microthrombus (Figure 1.9B-C), associated with mild to moderate neutrophilic interstitial pneumonia, mild to moderate random neutrophilic hepatitis and moderate multifocal neutrophilic splenitis. Importantly, microfilariosis was more frequently observed in titi-monkeys and howler monkeys, compared to capuchins and marmosets (Table 1.4). Additionally, in one case was observed hyalinosis of the vessels wall from the myometrium (Figure 1.9D).

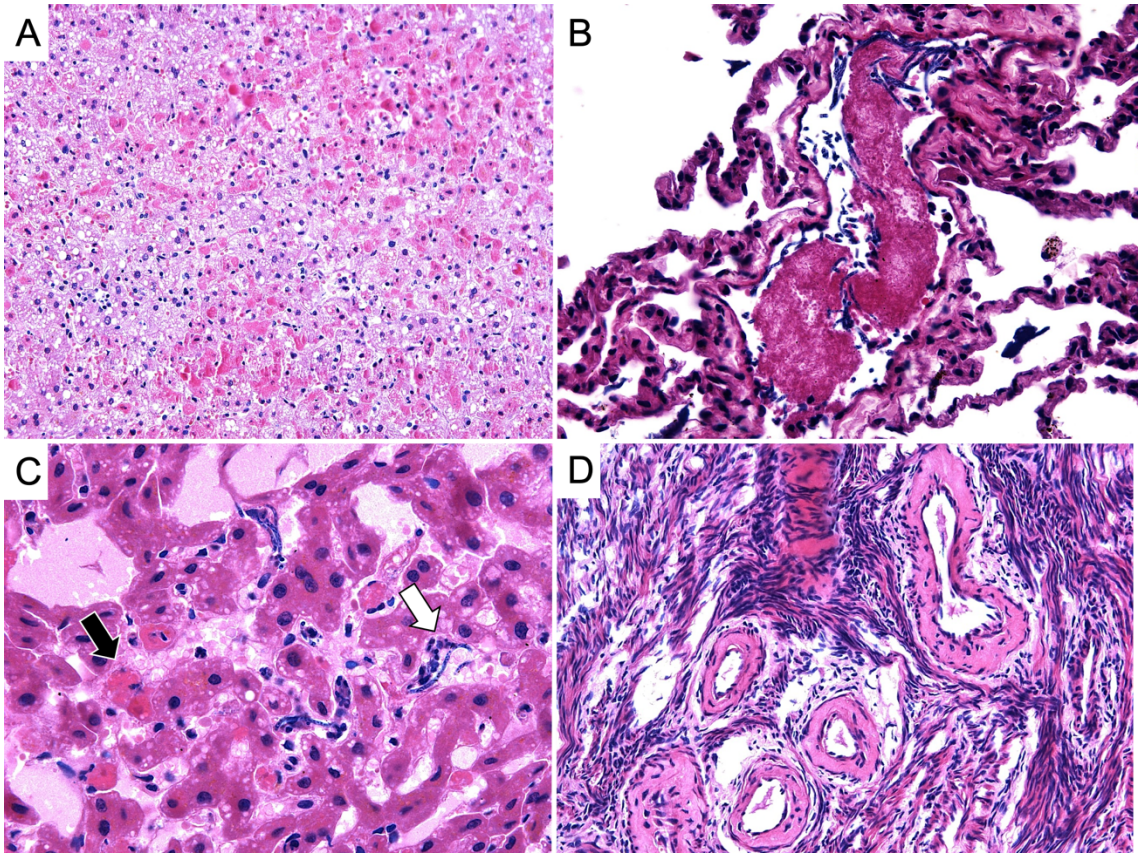


Figure 1.9 Pathological findings observed in free-ranging titi-monkeys (*Callicebus* spp.). (A) Marked diffuse necrotizing hepatitis with apoptotic hepatocytes and mild lipidosis, compatible with YFV infection. Liver, HE, 200x. (B) Lung vessel filled with microfilariae associated with clusters of fibrins (thrombus). Lung, HE, 200x. (C) Sinusoidal leukocytosis rich in neutrophils associated with microfilariae (white arrow). Individual hepatocyte necrosis, mainly observed in the mediozonal region (black arrow). Liver, HE, 400x. (D) Vessels from the myometrium with hyalinosis of the wall. Uterus, HE, 200x.

4. DISCUSSION

This is a comprehensive retrospective study focusing on pathology of free-ranging NWP with more than a thousand animals included and a systematic evaluation of FFPE samples from brain, heart, lungs, liver, spleen, and kidneys. Animals were found dead from different mesoregions of the RJ and were sent to IJV for YFV surveillance. During the period of the study, years 2017-2019, Brazilian Southeastern, including RJ, was facing one of the biggest outbreaks of sylvatic YF in the last 80 years (Possas et al., 2018; Santos et al., 2020), justifying the high prevalence of YFV in the animals evaluated in this study, especially in howler monkeys, a specie that is known to be highly susceptible to YF (Santos et al., 2020; Fernandes et al., 2021). Together with the YF outbreak, it was observed a high prevalence of trauma in this same population,

previous characterized by Oliveira et al. (2022), and affecting especially marmosets (*Callithrix* sp.).

In addition to YF, other infectious diseases were observed in the animals from this study. RABV was detected in one juvenile marmoset by the official diagnostic service, with the clinical and diagnostic report described by Moutinho et al. (2020). Briefly, this animal was found with neurological signs in an urbanized area of Niterói (a city from the RJ Metropolitan region) and naturally died testing positive for RABV by DFAT and MIT. PCR and sequencing showed that the genetic lineage of the virus detected in this animal had characteristic of hematophagous bats *Desmodus rotundus*. Pathological findings, described in our study, were mild, characterized by non-suppurative encephalitis, with no observation of Negri-bodies. This found highlight the importance of investigation of RABV in all free-ranging NWP, with minimal or even absent pathological findings, especially with history of neurological signs. Common marmosets (*C. jacchus*) are a recognized reservoir of RABV in the wild cycle of the disease, with increasing cases of human expositions in the Brazilian Northeastern and Southeastern in the past 12 years (Favoretto et al., 2001; Machado et al., 2012; Kotait et al., 2018; Moutinho et al., 2020; Benavides et al., 2022). The importance of other species of NWP in the Brazilian wild cycle of RABV is poorly known, with some evidence of infection in free-ranging capuchins (Machado et al., 2012; Kobayashi et al., 2013).

PIV-1 and 3 was diagnosed by RT-qPCR of FFPE lung tissue in three marmosets with moderate to marked acute interstitial to bronchointerstitial pneumonia and moderate to severe deposition of fibrin in the airways. This is the first report of PIV in free-ranging marmosets. PIV-1 was previously described in captive common marmosets causing an outbreak of mild to lethal respiratory disease with nasal discharge and interstitial pneumonia (Flecknell et al., 1983; Sutherland et al., 1986). Moustached tamarin (*Saguinus mystax*) experimentally infected with PIV-1 and PIV-3 developed respiratory clinical signs with natural transmission to other tamarins from the same cage (Hawthorne et al., 1982). PIV-1 and 3 are important cause of respiratory diseases in children, causing laringotracheobronchitis (PIV-1) and pneumonia with bronchiolitis (PIV-3) (Hawthorne et al., 1982). The replication of both viruses usually occurs in the upper respiratory tract (Hawthorne et al., 1982), which may explain why there were no viral antigens or particles at IHC and TEM from lung tissues in our cases. The three

cases of PIV were identified in animals that died between March and April from 2018 (PIV-3) and 2019 (PIV-1). Interestingly, this period coincides with seasons of high incidence of PIV infection in human population from the Brazilian Southeast region (SVS-MS, 2018; SVS-MS, 2019), being a possible source of infection for these animals.

Bacterial infections were diagnosed in marmosets and capuchins from this study, with prevalence of 41.1% and 33.3%, respectively, of all infectious cases. Bronchopneumonia, cholangiohepatitis, random hepatitis, meningoencephalitis, and bacteremia were the main lesions associated with bacteria in these cases. Although it was not possible to identify the bacteria specie associated with all cases, some agents, such as *Staphylococcus capitis* (causing suppurative meningoencephalitis with bacteremia), *Streptococcus sanguinis* (causing necrotizing bronchopneumonia with Splendore-Hoeppli reaction), and *Moraxella* sp. (causing bacteremia) were detected by PCR, being described for the first-time causing infection in free-ranging marmosets. *S. capitis* and *S. sanguinis* are components of the normal human microbiota, isolated in normal conditions from skin and dental biofilms, respectively (Alves et al., 2022; Elhusseiny et al., 2022), being both considered opportunistic agents, responsible for rare cases of bacterial endocarditis (Douedi et al., 2021; Alves et al., 2022). *Moraxella* sp. have a wide range of hosts, with species of great importance in veterinary medicine, such as *M. bovis* and *M. ovis*, responsible for the infectious keratoconjunctivitis in livestock (Seeger et al., 2021); and species that infects humans, such as *M. keratitis* and *M. catarrhalis* (Murphy and Parameswaran, 2009; McSwiney et al., 2019). The identification of these microorganisms, common in humans and domestic animals, in free-ranging marmosets, once again, raises a concern about the frequent circulation of pathogens between wildlife, domestic animals and humans, increasing the possibility of the emergence of new diseases.

E. coli was confirmed causing bronchopneumonia in one case. Ehlers et al. (2022) described *E. coli* as being an important opportunistic bacteria associated with suppurative bronchopneumonia and sepsis in captive howler-monkeys. Bacteremia was a frequent pathological finding observed in the marmosets from this study and it was mainly caused by Gram-negative agents. This high susceptibility of marmosets to bacterial infection and sepsis may be due to the limited variability of key loci in the MHC class II regions in this specie (Pisharath et al., 2005; Mätz-Rensing and

Lowenstine, 2018), contrasting with other NWP species, such as tamarins, that have abundant polymorphisms at the MHC class II region, being less susceptible to bacterial infections compared to common marmosets (Pisharath et al., 2005).

Toxoplasmosis was the only protozoal disease observed in this study, being identified in 16 marmosets and in one howler-monkey. Classical histopathological features characterized by necrotizing lesions in multiples organs with intralesional zoites (Paula et al., 2020; Santana et al., 2021) was observed in all cases. Platynosomiasis, although described in variable genus of NWP, such as *Callicebus, Chiropotes, Callimico, Saguinus,* and *Sapajus* (Kingston and Cosgrove, 1967; Sousa et al, 2008; Silva et al, 2012; Pereira et al., 2021), in our study was only observed in marmosets. Pulmonary metastrongylus (lungworms) were also observed exclusively in marmosets and were associated with mild to moderate interstitial pneumonia. Lungworms, usually from the genera *Filaroides* and *Filariopsis*, are commonly observed in captive NWP with no significant clinical or pathological findings (Wolff, 1993). Hepatic capillariosis was observed in one marmoset from our study. It is caused by *Capillaria hepatica* and is a globally distributed zoonotic disease, described in many species of mammals, including NHP, free-ranging or captive, with few cases in marmosets (Fuehrer, 2014).

Microfilariosis was observed in marmosets (0.9%), capuchins (3.4%), howler-monkeys (11.6%) and titi-monkeys (100%), with significant higher frequency in the last two species. In most of the cases few to moderate numbers of microfilariae were observed intravascular at lungs, liver, and spleen, among other organs, with no significant associated pathological features. However, there were few cases, including both titi-monkeys, in which the numbers of microfilariae were high forming intravascular parasite clusters associated with thrombosis and neutrophils in multiple organs. Filariid parasites are often described in NWP (Rondón et al., 2021), being observed in blood smears of free-ranging NWP with no clinical importance (Bueno et al., 2017; Rondón et al., 2021). This nematode belongs to Spirurida order and has an indirect life cycle, requiring an arthropod as intermediate host for development and transmission. *Mansonella* and *Dipetalonema* are the two main genera associated with infection in NWP, and the adult worm parasitizes the peritoneal or pleural cavity of their definitive hosts, while their microfilariae are found circulating in the bloodstream (Zárate-Rendón et al., 2022). The adults are usually observed at necropsy, often

associated with chronical mild inflammation (Karesh et al., 1998; Zárate-Rendón et al., 2022). Importantly, in our study, adult filariid forms were only observed in two howler-monkeys localized in the lymphatic vessel of the lungs. Although the pathogenicity and epidemiology of this parasite is poorly understood, our data indicates that this parasitic infection may have a greater importance in howler-monkey and titi-monkeys compared to the other species from the study.

Mild to marked interstitial pneumonia, characterized by the enlargement of the alveolar wall by lymphocytes, plasma cells and macrophages, sometimes with neutrophils, was frequently observed in marmosets (40.6%), howler-monkeys (51.2%) and capuchins (44.8%) from this study. This high frequency was also observed by Bleyer et al. (2017) in captive common marmosets, represented by 32.29% and in most of the case the cause was undetermined, with no intralesional agent detected, although no investigation of viral etiology was performed. Sepsis and endotoxemia are important causes of interstitial pneumonia in domestic animals, and in some cases the bacterial agent is not evident intralesional, making the final diagnosis a challenge (Caswell and Williams, 2016). Therefore, although we had 70 cases of confirmed bacteremia in marmosets, once the confirmation was based on the visualization of the organism in the affected tissue, it is possible that some cases of endotoxemia and sepsis went unnoticed. Also, besides infectious etiology there are many other causes of interstitial pneumonia with no specific pathological features, such as shock, massive/head trauma, pulmonary contusion, multi-organ failure, uremia, and inhalation of toxic agents (Caswell and Williams, 2016), being a challenge to confirm the etiology based only in FFPE tissue samples.

Pulmonary alveolar edema and hemorrhage was also prevalent in all species evaluated from our study. Causes of alveolar edema are variable being associated with direct and indirect injuries to the respiratory system (Caswell and Williams, 2016). Marmosets from our study had higher frequencies of alveolar hemorrhage in traumatized animals, which in most of the cases could be explained by the direct thoracic trauma. DHGD was another frequent pathological finding associated with trauma in marmosets. Hepatocellular glycogen degeneration in domestic animals is usually a consequence of systemic high levels of glucocorticoids, although the pathogenesis is uncertain (Cullen and Stalker, 2016). Trauma in wildlife is an important cause of abrupt increase in cortical levels, being the amplitude and length of serum

increase also influenced by pre-trauma (e.g., time of pursuit in cases of predation and hunting) and post-trauma (e.g., higher degree of pain experienced) situations (Gentsch et al., 2018). Therefore, the cases with DHGD associated with trauma may suggest a more stressful and agonic progression until lethal course. Additionally, DHGD has been frequently described in captive marmosets, being associated with laboratory diets in the past (Tucker, 1984; Kaspareit et al., 2006). This could be indicative of a more predisposition of marmosets to develop hepatic glycogen storage, being even considered a background lesion in these animals (Kaspareit et al., 2006).

PIVH was observed in 37 free-ranging marmosets from this study. The histiocytic phenotype of the cells with monomorphic pattern, nuclear pleomorphism and high mitotic index, raised the suspicious of a possible intravascular neoplastic disease, suggestive of a leukemic histiocytic sarcoma. However, the almost exclusively lung tropism, the high prevalence in the animals evaluated and the absence of primary parenchymatous foci of proliferation put in question this neoplastic hypothesis. A leukemic histiocytic sarcoma was observed in a captive squirrel monkey (*Saimiri sciureus*) co-infected with Saimiriine Gammaherpesvirus-2, *Saimiri sciureus* lymphocryptovirus-2 and Squirrel monkey retrovirus, however, although leukemic, tumor cells were observed infiltrating the parenchyma of multiple organs (Buchanan et al., 2020).

Intravascular histiocytosis was reported once in human as a rare presentation of hemophagocytic lymphohistiocytosis (HLH), a rare clinical syndrome of overreactive histiocytes and lymphocytes secondary to a chronical inflammatory stimulus (Zayed et al., 2019). In humans HLH is frequently associated to HHV-4 infection (Zayed et al., 2019), which was not detected in our cases. Also, an important feature of HLH is erythrophagocytosis (Zayed et al., 2019), rarely observed in the PIVH from our study. Similar pulmonary histopathological features observed in PIVH from our cases are described in the Jembrana disease (JD), a viral enzootic disease of Bali cattle (*Bos javanicus*) (Budiarso and Rikihisa, 1992). The main lesion in the lungs of cattle necropsied with this disease is the presence of large number of intravascular monocytes, sometimes occluding the lumen of small vessels (Budiarso and Rikihisa, 1992). JD is caused by a lentivirus genetically similar to bovine immunodeficiency virus (BIV) (Desport and Lewis, 2010). Therefore, a retroviral etiology associated with PIVH in free-ranging marmosets could be possible and is currently in pending investigation.

Importantly, all animals affected were from the Metropolitan region of RJ, suggesting that this condition may be favored by anthropic factors, such as exposition to human retroviruses.

Neoplasia was observed in only two animals: one B-cell lymphoma in a marmoset and one testicular interstitial cell tumor in a capuchin. Neoplasm in free-ranging NWP is rarely observed (Ehlers et al., 2022). To the authors knowledge this is the first report of interstitial cell tumor in a free-ranging capuchin. Lymphoma was previously diagnosed in a free-ranging howler monkey (Ehlers et al., 2022) and is commonly reported in laboratory marmoset colonies (David et al., 2009; Bleyer et al., 2017). Callitrichine herpesvirus-3, a virus related to HHV-4, has been associated to common marmoset B-cell lymphomas (Cho et al., 2001; Fogg et al., 2005). In our study we investigated HHV-4 co-infection by IHC and PCR, but no viral antigen or DNA was detected.

Interstitial nephritis with glomerulopathy was frequently observed in the NWP in this study, especially in marmosets, with cases of pathological findings compatible with severe chronical renal disease. Among renal lesions, glomerulopathies are frequently observed in captive NWP, especially marmosets (Isobe et al., 2012; Yamada et al., 2013; Burns and Wachtman, 2018; Kirejczyk et al., 2021). Glomerular lesions are usually membranoproliferative, membranous or sclerotic, accompanied by an interstitial inflammatory infiltrate, fibrosis, and tubular regeneration (Isobe et al., 2012; Yamada et al., 2013; Burns e Wachtman, 2018). Interstitial nephritis is a common lesion that can have an incidence above 80% in NWP colonies and is usually associated with glomerulopathies. Although it is often seen together, interstitial, and glomerular lesions can occur separately in some animals and usually progress to sclerosis of the renal parenchyma (Marini, 2019).

EH has been associated with these kidney injuries in callitrichids, which may indicate a secondary anemic condition due to erythropoietin deficiency (Burns and Wachtman, 2018). In our study animals with severe renal diseases had higher frequencies of EH. Pyelonephritis was observed in six marmosets, three of them had intratubular material compatible with calcium oxalate crystals. Plants are the usual source of oxalate poisoning in domestic animals (Cianciolo and Mohr, 2016). Vanselow et al. (2011) reported an oxalate nephropathy followed an accidental ingestion of

Eucalyptus viminalis in a colony of common marmosets. Unfortunately, the origin of the oxalate intake in the animals from our study was undetermined.

Finally, hepatocyte multinucleation can result from incomplete cell division or cell fusion, and in veterinary medicine was observed in association with hepatitis in newborn cats, foals, and piglets (Cullen and Stalker, 2016). MNH (more than three nuclei) have been considered a spontaneous background finding of laboratory-raised macaques with occasional reports as an incidental finding in macaques, chimpanzees, and gorillas (Lowenstine, 2003). Cynomolgus macaques have a prevalence of 61% of MNH, being considered a nonspecific reaction with unknown pathogenesis (Novilla et al., 2014). In captive marmosets MNH was associated with aflatoxin B1 toxicity (Lowenstine, 2003). In our study MNH had similar ultrastructural characteristics of normal hepatocytes, were observed in marmosets (13%), howler-monkeys (4.6%), and capuchins (17.2%), and were considered a nonspecific finding. Importantly, HSV infection must be considered as a differential diagnosis in free-ranging NWP with MNH, once it could lead to syncytial hepatocyte formation. However, in HSV cases, necrotizing hepatitis with intranuclear viral inclusions and viral particles are also observed (Wilson et al., 2022).

This study demonstrated that free-ranging NWP are hosts of several diseases, including important zoonoses, being valuable tools for the development of strategies for disease control and prevention, both with a focus on public health and conservation of these species. Additionally, it is very important to study the histopathological alterations in these species to help in the recognition of specific and non-specific alterations (background), thus improving the accuracy of the diagnosis in these animals.

REFERENCES

1. Aguirre AA (2009) Wild canids as sentinels of ecological health: a conservation medicine perspective. Parasite Vectors, 2, 1, 7.

2. Alves LA, Salvatierra GC, Freitas VA, Hofling JF, Bastos DC, Araujo TLS, Mattos-Graner RO (2022) Diversity in Phenotypes Associated with Host Persistence and Systemic Virulence in *Streptococcus sanguinis* Strains. Frontiers in microbiology, 18, 13, 875581.

3. Benavides JA, Raghavan RK, Boere V, Rocha S, Wada MY, Vargas A, Voietta F, Silva IO, Leal S, Castro A, Arruda MF, Peterson A, Megid J, Carrieri ML, Kotait I. Spatio-temporal dynamics of rabies and habitat suitability of the common marmoset *Callithrix jacchus* in Brazil. PLoS Neglected Tropical Diseases, 16, 3.

4. Bengis RG, Leighton FA, Fischer JR, Artois M, Mörner T, Tate CM (2004) The Role of Wildlife in Emerging and Re-Emerging Zoonoses. Revue scientifique et technique (International Office of Epizootics), 23, 2, 497-511

5. Bhatnagar J, Blau DM, Shieh W-J, Paddock CD, Drew C, Liu L, Jones T, Patel M, Zaki SR (2012) Molecular detection and typing of dengue viruses from archived tissues of fatal cases by rt-PCR and sequencing: diagnostic and epidemiologic implications. The American Journal of Tropical Medicine and Hygiene, 86, 335–40.

6. Bleyer M, Kunze M, Gruber-Dujardin E, Mätz-Rensing K (2017) Spontaneous lung pathology in a captive common marmoset colony (*Callithrix jacchus*). Primate Biology, 4, 17–25.

7. Brinkworth IF, Pechenkina K (2013) Primates, Pathogens and Evolution: An Introduction. In: Primates, Pathogens and Evolution. Brinkworth IF, Pechenkina K Eds, Springer, New York, pp. 1-14.

8. Bueno MG, Iovine RO, Torres LN, Catão-Dias JL, Pissinatti A, Kierulff MCM, Carvalho VM (2015). Pneumonia and bacteremia in a golden-headed lion tamarin (*Leontopithecus chrysomelas*) caused by *Klebsiella pneumoniae* subsp. *pneumoniae* during a translocation program of free-ranging animals in Brazil. Journal of Veterinary Diagnostic Investigation, 27, 3, 387–391.

9. Bueno MG, Catão-Dias JL, Laroque PO, Vasconcellos SA, Ferreira Neto JS, Gennari SM, Ferreira F, Laurenti MD, Umezawa ES, Kesper N, Kirchgatter K, Guimarães LO, Pavanato HJ, Valença-Montenegro MM (2017) Infectious Diseases in Free-Ranging Blonde Capuchins, *Sapajus flavius*, in Brazil. International Journal of Primatology, 38, 6, 1017–1031.

10. Buchanan A, Díaz-Delgado J, Balamayooran G, Anguiano M, Groch K, Krol L (2020) Leukemic histiocytic sarcoma in a captive common squirrel monkey (*Saimiri sciureus*) with *Saimiriine Gammaherpesvirus* 2 (*Rhadinovirus*), *Saimiri sciureus lymphocryptovirus* 2 (*Lymphocryptovirus*) and *Squirrel monkey retrovirus* (β-*Retrovirus*) coinfection. Journal of Medical Primatology, 49, 341–343.

11. Burns M, Wachtman L (2019) Physical Examination, Diagnosis, and Common Clinical Procedures In: The Common Marmoset in Captivity and Biomedical Research. Marini RP, Wachtman LM, Tardif SD, Mansfield K, Fox JG, Eds. Elsevier Inc.: Cambridge, 145.

12. Casagrande RA, Pannuti CS, Kanamura C, Freire WS, Grespan A, Matushima ER (2014) Fatal human herpesvirus 1 (HHV-1) infection in captive marmosets (*Callithrix jacchus* and *Callithrix penicillata*) in Brazil: clinical and pathological characterization. Pesquisa Veterinária Brasileira, 34, 1109–114.

13. Caswell JL, Williams KJ (2016) Respiratory System. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, Volume 2, Sixth Edition. Maxie MG Ed. Elsevier: Missouri, 465.

14. Cho Y, Ramer J, Rivailler P, Quink C, Garber RL, Beier DR, Wang F (2001) An Epstein-Barr-related herpesvirus from marmoset lymphomas. Proceedings of the National Academy of Sciences (Proceedings of the National Academy of Sciences of the United States of America), 98, 1224e9.

15. Cianciolo RE, Mohr FC (2016) Urinary System. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, Volume 2, Sixth Edition. Maxie MG Ed. Elsevier: Missouri, 376.

16. Cullen JM, Stalker MJ (2016) Liver and Biliary System. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, Volume 2, Sixth Edition. Maxie MG Ed. Elsevier: Missouri, 258.

17. David JM, Dick Jr EJ, Hubbard GB (2009) Spontaneous pathology of the common marmoset (*Callithrix jacchus*) and tamarins (*Saguinus oedipus, Saguinus mystax*). Journal of Medical Primatology, 38, 347–359.

18. Denison AM, Blau DM, Jost HA, Jones T, Rollin D, Gao R, Liu L, Bhatnagar J, Deleon-Carnes M, Shieh WJ, Paddock CD, Drew C, Adem P, Emery SL, Shu B, Wu KH, Batten B, Greer PW, Smith CS, Bartlett J, Montague JL, Patel M, Xu X, Lindstrom S, Klimov AI, Zaki SR (2011) Diagnosis of influenza from respiratory autopsy tissues: Detection of virus by real-time reverse transcription-PCR in 222 cases. Journal of Molecular Diagnose, 13, 123-8.

19. Desport M, Lewis J (2010) Jembrana Disease Virus: Host Responses, Viral Dynamics and Disease Control. Current HIV Research, 8, 1, 53–65.

20. Douedi S, Odak M, Ravin A, Campbell N (2021) *Staphylococcus capitis* Endocarditis of a Native Valve. Cureus,13, 6, e15738.

21. Ehlers LP, Bianchi MV, Argenta FF, Lopes BC, Taunde PA, Wagner PGC, Driemeier D, Pavarini SP, Mayer FQ, Siqueira FM, Sonne L (2020) *Mycobacterium tuberculosis* var. *tuberculosis* infection in two captive black capuchins (*Sapajus nigritus*) in Southern Brazil. Brazilian Journal of Microbiology, 51, 4, 2169–2173.

22. Ehlers LP, Slaviero M, Bianchi MV, Mello LS, Lorenzo C, Surita LE, Alievi MM, Driemeier D, Pavarini SP, Sonne L (2022) Causes of death in neotropical primates in Rio Grande do Sul State, Southern Brazil. Journal of Medical Primatology, 51, 2, 85–92.

23. Elhusseiny AM, Shamim MM, Sanders RN, Sallam AB (2022) Endogenous endophthalmitis caused by *Staphylococcus capitis*. American Journal of Ophthalmology Case Reports, 25, 101415.

24. Favoretto SR, Mattos CC de, Morais NB, Araújo FAA, Mattos CA de (2001) Rabies in marmosets (*Callithrix jacchus*), Ceará, Brazil. Emerging Infectious Diseases, 7, 6, 1062–1065.

25. Favoretto SR, Araujo DB, Duarte NFH, Oliveira DBL, Crus NG, Mesquita F, Leal F, Machado RRG, Gaio F, Oliveira WF, Zanotto PMA, Durigon EL (2019) Zika Virus in Peridomestic Neotropical Primates, Northeast Brazil. Ecohealth, 16, 1, 61–69.

26. Fernandes NCCA, Guerra JM, Díaz-Delgado J, Cunha MS, Saad L, Iglezias SD, Ressio RA, Cirqueira CS, Kanamura CT, Jesus IP, Maeda AY, Vasami FGS, Carvalho J, Araújo LJT, Souza RP,

Nogueira JS, Spinola RMF, Catão-Dias JL (2021) Differential Yellow Fever Susceptibility in New World Nonhuman Primates, Comparison with Humans, and Implications for Surveillance. Emerging Infectious Disease, 27, 1, 47–56.

27. Figueiredo MAP, Santi SMFD, Figueiredo TAP, Machado RZ (2015) Natural *Plasmodium* infection in neotropical primates in the island of São Luís, state of Maranhão, Brazil. Revista Brasileira de Parasitologia Veterinária, 24, 2, 122–128.

28. Flecknell PA, Parry R, Needham JR, Ridley RM, Baker HF, Bowes P (1983) Respiratory disease associated with parainfluenza Type I (Sendai) virus in a colony of marmosets (*Callithrix jacchus*). Laboratory Animals, 17, 2, 111-3.

29. Fogg MH, Carville A, Cameron J, Quink C, Wang F (2005) Reduced prevalence of Epstein-Barr virus-related lymphocryptovirus infection in sera from a new world primate. Journal of Virology, 79, 10069e72.

30. Fuehrer H (2014) An overview of the host spectrum and distribution of *Calodium hepaticum* (syn. *Capillaria hepatica*): part 2—Mammalia (excluding Muroidea). Parasitology Research, 113, 2, 641–651.

31. Gentsch RP, Kjellander P, Röken BO (2018) Cortisol response of wild ungulates to trauma situations: hunting is not necessarily the worst stressor. European Journal of Wildlife Research, 64, 11.

32. Gillespie TR, Nunn CL, Leendertz FH (2008) Integrative approaches to the study of primate infectious disease: implications for biodiversity conservation and global health. Yearbook of physical anthropology, 51, 53-69.

33. Guarner J, Bhatnagar J, Shieh WJ, Nolte KB, Klein D, Gookin MS, Peñaranda S, Oberste MS, Jones T, Smith C, Pallansch MA, Zaki SR (2007) Histopathologic, immunohistochemical, and polymerase chain reaction assays in the study of cases with fatal sporadic myocarditis. Human Pathology, 38, 9, 1412-9.

34. Hawthorne JD, Lorenz D, Albrecht P (1982) Infection of marmosets with parainfluenza virus types 1 and 3. Infection and Immunity, 37, 3, 1037-41.

35. Harris K, Hartley J (2003) Development of broad-range 16S rDNA PCR for use in the routine diagnostic clinical microbiology service. Medical Microbiology, 52, 685-691.

36. Heininger A, Binder M, Schmidt S, Unertl K, Botzenhart K, Döring G (1999) PCR and blood culture for detection of Escherichia coli bacteremia in rats. Journal of Clinical Microbiology, 37, 8, 2479-82.

37. Hirsh A, Dias LG, Martins LO, Resende NAT, Landau EC (2006) Database of Georeferenced Occurrence Localities of Neotropical Primates. Department of Zoology, UFMG, Belo Horizonte. http://www.icb.ufmg.br/zoo/primatas/home bdgeoprim.htm.

38. Imrit K, Goldfischer M, Wang J, Green J, Levine J, Lombardo J, Hong T (2006) Identification of bacteria in formalin-fixed, paraffin-embedded heart valve tissue via 16S rRNA gene nucleotide sequencing. Journal of Clinical Microbiology, 44, 2609-2611.

39. Isobe K, Adachi K, Hayashi S, Ito T, Miyoshi A, Kato A, Suzuki M (2012) Spontaneous glomerular and tubulointerstitial lesions in common marmosets (*Callithrix jacchus*). Veterinary Pathology, 49, 839–845.

40. Karesh WB, Wallace RB, Painter RLE, Rumiz D, Braselton WE, Dierenfeld ES, Puche H (1998) Immobilization and Health Assessment of Free-Ranging Black Spider Monkeys (*Ateles paniscus chamek*). American Journal of Primatology, 44, 107–123.

41. Kaspareit J, Friderichs-Gromoll S, Buse E, Habermann G (2006) Background pathology of the common marmoset (*Callithrix jacchus*) in toxicological studies. Experimental and Toxicologic Pathology, 57, 405–410.

42. Kingston N, Cosgrove GE (1967) Two new species of *Platynosomum* (Trematoda: Dicrocoeliidae) from South American monkeys. Helminthological Society of Washington, 34, 2, 147-151.

43. Kirejczyk S, Pinelli C, Gonzalez O, Kumar S, Dick Jr E, Gumber S (2021) Urogenital Lesions in Nonhuman Primates at Two National Primate Research Centers. Veterinary Pathology, 58, 1, 147–160.

44. Kobayashi Y, Sugimoto K, Mochizuki N, Segawa T, Itou T, Carvalho AAB, Nociti DP, Mello RM, Santos AKRA, Ito FH, Sakai T (2013) Isolation of a phylogenetically distinct rabies virus from a tufted capuchin monkey (*Cebus apella*) in Brazil. Virus Research, 178, 2, 535–538.

45. Kotait I, Oliveira R de N, Carrieri ML, Castilho JG, Macedo CI, Pereira PMC, Boere V, Montebello L, Rupprecht CE (2019) Non-human primates as a reservoir for rabies virus in Brazil. Zoonoses and Public Health, 66, 1, 47–59.

46. Lowenstine LJ (2003) A primer of primate pathology: lesions and non-lesions. Toxicologic Pathology, 31, 92–102.

47. Machado GP, Antunes JMA de P, Uieda W, Biondo AW, Cruvinel TMA, Kataoka AP, Martorelli LFA, Jong D, Amaral JMG, Hoppe EGL, Neto GG, Megid J (2012) Exposure to rabies virus in a population of free-ranging capuchin monkeys (*Cebus apella nigritus*) in a fragmented, environmentally protected area in southeastern Brazil. Primates, 53, 3, 227–231.

48. Malta M, Tinoco HP, Xavier MN, Vieira A, Costa EA, Santos RL (2010) Naturally acquires visceral leishmaniasis in non-human primates in Brazil. Veterinary Parasitology, 169, 193-197.

49. Marini RP (2019) Disease of the Urogenital System. In: The Common Marmoset in Captivity and Biomedical Research. Marini RP, Wachtman LM, Tardif SD, Mansfield K, Fox JG, Eds. Elsevier Inc.: Cambridge, 195.

50. Mätz-Rensing K, Lowenstine (2018) New World and Old World Monkeys In: Pathology of Wildlife and Zoo Animals. Terio KA, McAloose D, Leger JS Eds. Elsevier Inc: Cambridge, 343-374.

51. McSwiney TJ, Knowles SJ, Murphy CC. Clinical and microbiological characteristics of *Moraxella keratitis*. The British Journal of Ophthalmology, 103, 12, 1704-1709.

52. Moreno ES, Spinola R, Tengan CH, Brasil RA, Siciliano MM, Coimbra TLM, Silveira VR, Rocco IM, Bisordi I, Souza RP, Petrella S, Pereira LE, Maeda AY, Silva FG, Suzuki A (2013). Yellow fever epizootics in non- human primates, São Paulo state, Brazil, 2008-2009. Revista do Instituto de Medicina Tropical de São Paulo, 55, 1, 45–50.

53. Moore MS, McCann CD, Jordan JA (2013) Molecular detection of culture-confirmed bacterial bloodstream infections with limited enrichment time. Journal of Clinical Microbiology, 51, 11, 3720-5.

54. Moutinho FFB, Andrade MGA, Nunes VMA, Rubião ECN, Carvalho HB, Phyllis RB, Romijn C, Cattaneo CA, Oliveira FG, Oliveira RN, Gorga NML, Silvestre GR, Borges FVB, Bruno SF (2020) Rabies in *Callithrix* sp. in the urban area of Niterói, Rio de Janeiro, Brazil. Revista da Sociedade Brasileira de Medicina Tropical, 53, e20190402.

55. Murphy TF, Parameswaran G (2009) *Moraxella catarrhalis*, a human respiratory tract pathogen. Clinical Infectious Diseases, 49, 1, 124-131.

56. Novilla MN, Jackson MK, Reim DA, Jacobson SB, Nagata RA (2014) Occurrence of Multinucleated Hepatocytes in Cynomolgus Monkeys (*Macaca fascicularis*) From Different Geographical Regions. Veterinary Pathology, 51, 6.

57. Oliveira AR, Pinheiro GRG, Tinoco HP, Loyola ME, Coelho CM, Dias ES, Monteiro EM, Silva FOL, Pessanha AT, Souza AGM, Pereira NCL, Gontijo NF, Fujiwara RT, Paixão TA, Santos RL (2019) Competence of non-human primates to transmit *Leishmania infantum* to the invertebrate vector *Lutzomyia longipalpis*. Plos Neglected Tropical Disease, 13, 4, e0007313.

58. Oliveira AR, Santos, DO, Lucena FP, Mattos AS, Carvalho TP, Costa FB, Moreira LGA, Vasconcelos IMA, Paixão TA, Santos RL (2022) Non-thrombotic pulmonary embolism of brain, liver, or bone marrow tissues associated with traumatic injuries in free-ranging neotropical primates. Veterinary Pathology, 59, 3, 482-488.

59. Paddock CD, Liu L, Denison AM, Bartlett JH, Holman RC, Deleon-Carnes M, Emery SL, Drew CP, Shieh WJ, Uyeki TM, Zaki SR (2012) Myocardial injury and bacterial pneumonia contribute to the pathogenesis of fatal influenza B virus infection. Journal of Infectious Diseases, 15, 205, 6, 895-905

60. Paula NF de, Dutra KS, Oliveira AR, Santos DO, Rocha CEV, Vitor RWA, Tinoco HP, Costa MELT, Paixão TA, Santos RL (2020) Host range and susceptibility to *Toxoplasma gondii* infection in captive neotropical and Old-world primates. Journal of Medical Primatology, 49, 4, 202–210.

61. Pereira WLA, Conga DMF, Silva KSM, Silva RJ, Imbeloni AA (2021) Anatomopathological lesions of infection caused by *Platynosomum illiciens* (Braun, 1901) in Neotropical primates kept in captivity. Journal of Medical Primatology, 50, 1, 82-85

62. Pisharath HR, Cooper TK, Brice AK, Cianciolo RE, Pistorio AL, Wachtman LM, Mankowski JL, Newcomer CE (2005) Septicemia and peritonitis in a colony of common marmosets (*Callithrix jacchus*) secondary to *Klebsiella pneumoniae* infection. Contemporary topics in laboratory animal science / American Association for Laboratory Animal Science, 44, 1, 35–37.

63. Possas C, Lourenço-De-Oliveira R, Tauil PL, Pinheiro FP, Pissinatti A, Cunha RV, Freire M, Martins RM, Homma A (2008) Yellow fever outbreak in Brazil: the puzzle of rapid viral spread and challenges for immunization. Memórias do Instituto Oswaldo Cruz, 113, 10, 1-12.

64. Rondón S, Cavallero S, Renzi E, Link A, González C, D'Amelio S (2021) Parasites of Free-Ranging and Captive American Primates: A Systematic Review. Microorganisms, 9, 9, 12, 2546.

65. Santana CH, Oliveira AR, Santos DO, Pimentel SP, Souza LR, Moreira LGA, Braz HMB, Carvalho TP, Lopes CEB, Oliveira JBS, Paula NF, Carvalho MPN, Alves BF, Pena HFJ, Santos RL (2021) Genotyping of *Toxoplasma gondii* in a lethal toxoplasmosis outbreak affecting captive howler monkeys (*Alouatta* sp.). Journal of Medical Primatology, 50, 2, 99–107.

66. Santos DO dos, Oliveira AR de, Lucena FP, Mattos SA, Carvalho TP, Costa FB, Moreira LGA, Paixão TA, Santos RL (2020) Histopathologic Patterns and Susceptibility of Neotropical Primates Naturally Infected With Yellow Fever Virus. Veterinary Pathology, 57, 5, 681–686.

67. Secretaria de Vigilância em Saúde – Ministério da Saúde (SVS-MS). 2018. Informe Epidemiológico: Influenza: Monitoramento até a Semana Epidemiológica 52 de 2018. Access: https://www.gov.br/saude/pt-br/assuntos/saude-de-a-a-z/g/gripe-influenza/arquivos/informe-epidemiologico_influenza-2018-se-52.pdf. Accessed on July 5th, 2022.

68. Secretaria de Vigilância em Saúde – Ministério da Saúde (SVS-MS). 2019. Informe Epidemiológico: Influenza: Monitoramento até a Semana Epidemiológica 52 de 2019. Access: https://www.gov.br/saude/pt-br/assuntos/saude-de-a-a-z/g/gripe-influenza/arquivos/informe-epidemiologico_influenza-2019-se52.pdf. Accessed on July 5th, 2022.

69. Seeger MG, Corrêa LFD, Clothier KA, Loy JD, Cargnelutti JF. Isolation of *Moraxella* spp. from horses with conjunctivitis in Southern Brazil. Brazilian Journal of Microbiology, 52, 3, 1643-1648.

70. Shanmugasamy M, Velayutham T, Rajeswar T (2011) Inv A gene specific PCR for detection of Salmonella from broilers. Veterinary World, 4, 12, 562-564.

71. Shostell JM, Ruiz-Garcia M (2016) An introduction to the biodiversity of the Neotropical Primates. In: Phylogeny, Molecular Population Genetics, Evolutionary Biology and Conservation of the Neotropical Primates. Ruiz-García M, Shostell JM Eds. Nova Science Publisher Inc.: New York, 5975.

72. Silva KSM, Silva RJ, Pereira WLA (2012) Occurrence of infection by *Platynosomum illiciens* (Braun, 1901) in captive neotropical primates. Primates, 53, 79-82.

73. Sousa MBC, Leão AC, Coutinho JFV, Ramos AMO (2008) Histopathology findings in common marmosets (*Callithrix jacchus* Linnaeus, 1758) with chronic weight loss associated with bile tract obstruction by infestation with *Platynosomum* (Loos, 1907). Primates, 49, 283-287.

74. Sutherland SD, Almeida JD, Gardner PS, Skarpa M, Stanton J (1986) Rapid diagnosis and management of parainfluenza I virus infection in common marmosets (*Callithrix jacchus*). Laboratory Animals, 20, 2, 121-6.

75. Tavela AO, Fuzessy LF, Silva VHD, Silva FFR, Carretta Junior M, Silva IO, Souza VB (2013) Helminths of wild hybrid marmosets (*Callithrix* sp.) living in an environment with high human activity. Revista Brasileira de Parasitologia Veteterinária, 22, 3, 391-397.

76. Theron J, Morar D, Du Preez M, Brözela VS, Ventera SN (2001) A sensitive seminested PCR method for the detection of *Shigella* in spiked environmental water samples. Water Research, 35, 4, 869-874.

77. Trzciński K, Bogaert D, Wyllie A, Chu ML, van der Ende A, Bruin JP, van den Dobbelsteen G, Veenhoven RH, Sanders EA (2013) Superiority of trans-oral over trans-nasal sampling in detecting Streptococcus pneumoniae colonization in adults. PloS One, 8, 3, e60520.

78. Tucker MI (1984) A survey of the pathology of marmosets (*Callithrix jacchus*) under experiment. Laboratory Animals, 18, 351-358.

79. Vanselow BA, Pines MK, Bruhl JJ, Rogers LJ (2011) Oxalate nephropathy in a laboratory colony of common marmoset monkeys (*Callithrix jacchus*) following the ingestion of *Eucalyptus viminalis.* Veterinary Record, 169, 100.

80. Weinberg GA, Schnabel KC, Erdman DD, Prill MM, Iwane MK, Shelley LM, Whitaker BL, Szilagyi PG, Hall CB (2013) Field evaluation of Taqman Array Card (TAC) for the simultaneous detection of multiple respiratory viruses in children with acute respiratory infection. Journal of Clinical Virology, 57, 254-60.

81. Wilson TM, Ritter JM, Martines RB, Gonçalves AAB, Fair P, Galloway R, Weiner Z, Romano APM, Costa GRT, Melo CB, Zaki SR, Castro MB (2021) 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, 68, 6, 3207–3216.

82. Wilson TM, Ritter JM, Martines RB, Bullock HA, Fair P, Radford KW, Macêdo IL, Sousa DER, Gonçalves AAB, Romano AR, Passsos PHO, Ramos DG, Costa GRT, Cavalcante KRLJ, Melo CB, Zaki SR, Castro MB (2022) Fatal Human Alphaherpesvirus 1 Infection in Free-Ranging Black-Tufted Marmosets in Anthropized Environments, Brazil, 2012–2019. Emerging Infectious Diseases. 2022, 28, 4, 802–811.

83. Wolff PL (1993) Parasites of New World primates. In Zoo and Wild Animal Medicine, Volume 3. Fowler ME, Ed. Philadelphia: Saunders, 378- 389.

84. Yamada N, Sato J, Kanno T, Wako Y, and Tsuchitani M (2013) Morphological study of progressive glomerulonephropathy in common marmosets (*Callithrix jacchus*). Toxicologic Pathology, 41, 1106–1115.

85. Zárate-Rendón DA, Salazar-Espinoza MN, Catalano S, Sobotykc C, Rosenbaumf APMM, Verocaic G (2022) Molecular characterization of *Dipetalonema yatesi* from the black-faced spider monkey (*Ateles chamek*) with phylogenetic inference of relationships among Dipetalonema of Neotropical primates. International Journal for Parasitology: Parasites and Wildlife, 17, 152–157.

86. Zayed Y, Osman M, Kheiri B, Azher Q, Bachuwa G (2019) Bilateral pulmonary nodules and intravascular pulmonary histiocytosis: A rare presentation of hemophagocytic lymphohistiocytosis secondary to Epstein-Barr Virus infection. Respiratory Medicine Case Reports, 26, 11–13.

CHAPTER II[2]

PREVALENCE OF *PLATYNOSOMUM* SP. INFECTION AND ITS ASSOCIATION WITH BILIARY LITHIASIS AND SECONDARY BACTERIAL INFECTIONS IN FREE-RANGING MARMOSETS (*CALLITHRIX* SPP.) OF THE BRAZILIAN ATLANTIC FOREST

SUMMARY

Platynosomiasis is a parasitic disease caused by a trematode of the genus *Platynosomum* spp., a bile duct and gallbladder fluke that has been described in captive neotropical primates (NWP) with high morbidity and variable lethality. Although it is a high-concern disease in the handling of these animals in *ex situ* environment, there are only few studies describing platynosomiasis in free-ranging NWP. Therefore, the aim of this study was to characterize platynosomiasis in a free-ranging population of marmosets (*Callithrix* spp.) from the Brazilian Atlantic Forest, focusing on the epidemiological and pathological aspects. A total of 1,001 marmosets were evaluated and, based on general data analysis, histopathology, histochemistry, and immunohistochemistry, we concluded that *Platynosomum* sp. infection in free-ranging marmosets have a prevalence of 8.9%, being more frequently detected in the Metropolitan region of Rio de Janeiro state, causing a fibrosing and proliferative cholangiohepatitis associated to biliary lithiasis (3,0% of cases) and secondary bacterial infections (14,6% of cases).

Keywords: neotropical primates; nonhuman primates; metazoan; wildlife.

[2] Chapter submitted to the *Journal of Comparative Pathology.*

1. INTRODUCTION

Platynosomiasis is a parasitic disease caused by a trematode of the genus *Platynosomum* (Dicrocoeliidae family), a bile duct and gallbladder parasite commonly detected in domestic cats (Basu and Charles, 2014). During its life cycle, this parasite requires three intermediate hosts: being the first a mollusk, where the embryonated eggs develops into a sporocyst containing cercaria; the second a terrestrial isopod in which the cercaria is released and develops in to a metacercariae (infective form); and the third (paratenic host) a small vertebrate, usually *Anolis* spp. lizards, where the metacercariae will be held until predation by the definitive host (Maldonado, 1945; Eckerlin and Leigh, 1962; Basu and Charles, 2014; Pinto et al., 2014). There are reports of *Platynosomum* spp. infection in nonhuman primates (NHP) belonging to the genera *Pongo*, *Macaca*, *Callithrix*, *Callicebus*, *Chiropotes*, *Callimico*, *Cebuella, Sapajus*, and *Saguinus*, mostly affecting animals under human care (Kingston and Cosgrove, 1967; Shanta, 1970; Warren et al., 1998; Sousa et al., 2008; Silva et al., 2012; Pereira et al., 2021).

Platynosomiasis has been described in captive neotropical primates (NWP) with high morbidity and variable lethality. This disease is challenging to diagnose, due to the intermittent release of the parasite eggs in the host's feces, and to treat, due to the difficulty of antiparasitic drugs to act in the biliary tract, and it may be associated with debilitating conditions, such as wasting marmoset syndrome, hepatocellular carcinoma, and chronical inflammatory hepatic disease (Sousa et al., 2008; Silva et al., 2012; Mattioli et al., 2016; Díaz-Delgado et al., 2018; Pereira et al., 2021). Pathological findings are mainly observed in the liver and gallbladder, characterized by obstruction and hyperplasia of the bile ducts, cholestasis, ascending cholangitis and periportal fibrosis (Sousa et al., 2008; Pereira et al., 2021). In most cases, the parasite is detected at necropsy (Sousa et al., 2008; Pereira et al., 2021), being only rarely diagnosed by routine fecal examinations (Silva et al., 2012; Cullen and Brown, 2013; Mati et al., 2015) and occasionally by liver ultrasound (Mattioli et al., 2016).

Marmosets are NWP from the genus *Callithrix,* endemic to the Brazilian Atlantic Forest, and comprising six species: *Callithrix jacchus*, *Callithrix penicillata*, *Callithrix geoffroyi, Callithrix flaviceps*, *Callithrix aurita* and *Callithrix kuhlii*, all of them with decreasing populations and the last three species listed considered vulnerable to critically endangered (IUCN Red List). There are few studies describing

Platynosomum sp. infection in free-ranging marmosets (Melo, 2004; Díaz-Delgado et al., 2018; Pereira et al., 2020), so the impact of this parasite in these populations is poorly understood. Considering the importance of platynosomiasis in captive NWPs and the scarcity of information on this parasite in free-ranging populations, we aimed to characterize the burden of *Platynosomum* sp. infection in a free-ranging population of marmosets (*Callithrix* spp.) from the Brazilian Atlantic Forest, focusing on the epidemiological and pathological aspects of disease.

2. MATERIAL AND METHODS

2.1 Study design

This is a retrospective study with free-ranging marmosets (*Callithrix* spp.) that were found dead from all regions in the State of Rio de Janeiro, and were necropsied at Instituto Municipal de Medicina Veterinária Jorge Vaitsman (IJV, Rio de Janeiro, Brazil) for Yellow Fever (YF) surveillance, from January 2017 to July 2019. Animals were previously tested for YF virus (YFV) by quantitative reverse transcription polymerase chain reaction (RT-qPCR) and immunohistochemistry (IHC) by the official Brazilian diagnostic service. Data about geographical origin, sex and, age were collected by IJV. This study was authorized by the government environmental agency (ICMBio - Brazil) under the SISBIO license number 67014 and all procedures strictly adhered to humane care of animals and all applicable laws and regulations, including registration in the national system for management of genetic heritage and associated traditional knowledge by SISGEN code A2743E4.

2.2 Pathologic and parasitologic evaluation

Animals were necropsied by veterinary pathologists at the IJV. Conservation of carcasses were assessed and all the necropsied animals that were considered viable for histopathology were included in this study. Samples of the liver were fixed in 10% buffered formalin and embedded in paraffin. Formalin-fixed paraffin-embedded (FFPE) tissue samples were sent to the Universidade Federal de Minas Gerais (UFMG, Belo Horizonte, Brazil), cut in a microtome (3–4 μm-thick) and stained with hematoxylin and eosin (HE).

Liver samples from each animal were evaluated by light microscopy. Animals were considered parasitized when fragments of intraductal adult trematode or its eggs

were observed by histopathology. For better characterization of histopathological lesions, other stains, such as Prussian blue, Gram stain, Periodic acid–Schiff (PAS), Masson's trichrome and Hall's bilirubin stain were performed as needed.

2.3 Immunohistochemistry (IHC)

IHC was performed at the Infectious Disease Pathology Branch, Centers for Disease Control and Prevention (IDPB-CDC, Atlanta, USA) on FFPE liver samples from all parasitized animals to characterize bacterial co-infections. The primary antibody was a rabbit polyclonal antibody raised against *Escherichia coli* H pool serotype (SSI Diagnostica®, serial number 54396), and has been shown in our lab to cross-react with bacteria from the genera *Clostridium*, *Shigella*, *Enterobacter*, *Salmonella*, *Serratia*, and *Proteus*. FFPE liver sections were deparaffinized and hydrated, followed by heat induced antigen retrieval in a pressure cooker with 6.0 pH buffer (Reveal Decloaker, Biocare Medical®), and incubated in non-specific protein blockage buffer (Background Punisher, Biocare Medical®) for ten minutes at room temperature. Slides were incubated in primary antibody at room temperature for one hour, followed by detection with an alkaline phosphatase (AP)-antibody conjugate system (Mach 4 Universal AP-Polymer Kit, Biocare Medical®) and revealed with Permanent Red Chromogen (Permanent Red Chromogen kit, Cell Marque®). Slides were counterstained with Mayer's hematoxylin. For positive controls, slides containing *E. coli* cultures admixed with normal human tissue fragments were used, and for negative controls the primary antibody was replaced with wash buffer. Immunolabeling was classified as absent, rare, multifocal, extensive.

2.4 Statistical analysis

Data were analyzed using the GraphPad Prism software (version 9.0). Descriptive statistics with 95% of confidence interval was used for general analysis. Frequency of histopathological lesions and other variables, such as age, sex and geographical distribution, were compared between parasitized and non-parasitized animals using Fisher's exact test.

3. RESULTS

3.1 Epidemiology

A total of 1,001 marmosets (*Callithrix* spp.) were included in this study. Hepatic intraductal trematode compatible with *Platynosomum* sp. was detected by histopathologic evaluation of liver in 89 animals (8.9%; CI: 7.3-10.8%), including 48 females (53.9%); and 34 males (38.2%). In seven cases, the sex was not determined. Of the parasitized animals, 84.3% (76/89) were adults and 11.2% (9/89) were juveniles; age was not determined in 4 cases. No statistical difference in the presence of *Platynosomum* sp. infection was observed between sexes (Fisher's exact test, $p = 0.1322$) or age (Fisher's exact test, $p = 0.0828$). Considering all mesoregions of the State of Rio de Janeiro, parasitized marmosets were more often detected in animals from the Metropolitan region (Fisher's exact test, $p = 0.0007$; Figure 2.1), corresponding to 94.4% (CI: 87.5-97.6%) of all cases. Importantly, the majority of cases was detected in the city of Rio de Janeiro (69.7%; CI: 59.5-78.2%; Fisher's exact test, $p < 0.0001$). Additionally, parasitized animals were more often found during the second semester (July to December) (12.2%; CI: 8.6-17%) versus 7.8% (CI 6.1-10.0%) in the first semester (January-June) (Fisher's exact test, $p = 0.0492$). None of the parasitized animals was positive for YFV.

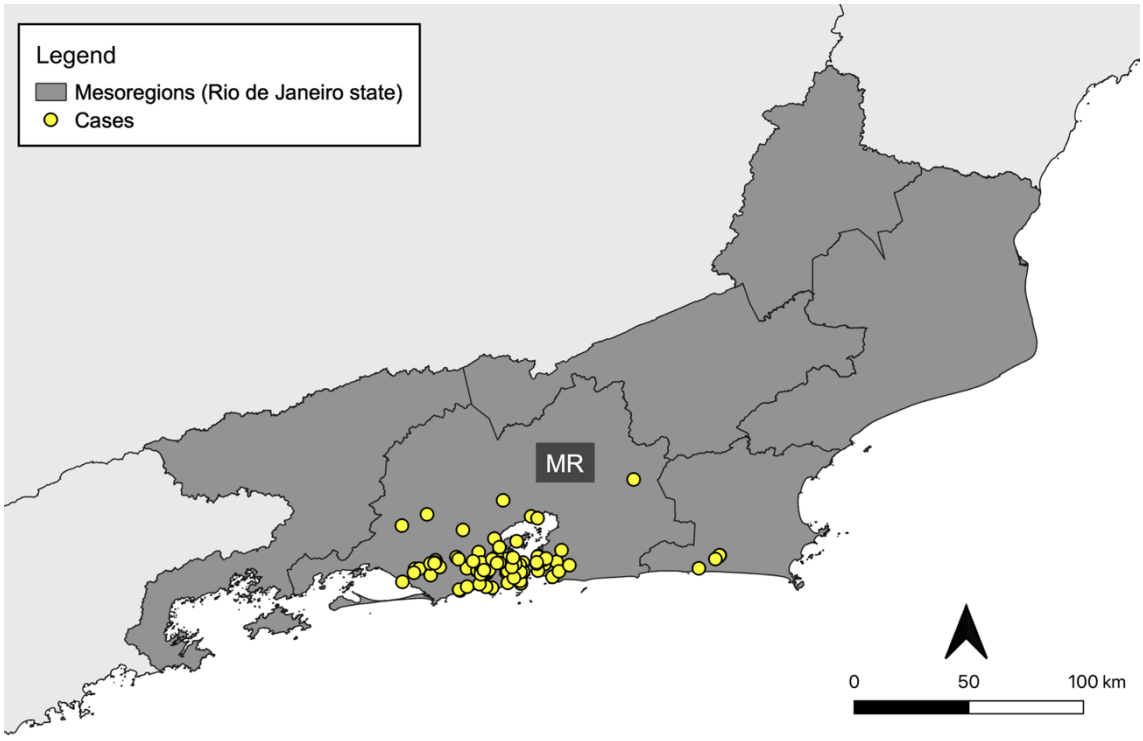


Figure 2.1 Distribution of *Platynosomum* sp. cases in free-ranging marmosets from the State of Rio de Janeiro (Brazil) from January 2017 to July 2019. Map of Rio de Janeiro mesoregions (IBGE 2021) with yellow dots indicating the distribution of cases with a high concentration in the Metropolitan Region (MR). SRC: EPCG 4674 – SIRGAS 2000 (QGIS Version 3.24.3).

3.2 Pathological findings

3.2.1 General lesions

Table 2.1 summarizes the pathological findings observed in the liver of the parasitized marmosets.

Table 2.1 Pathological findings in the liver of free-ranging marmosets parasitized by *Platynosomum* sp.

Pathological findings	% (CI)	Intensity (%)		
		Mild	Moderate	Marked
Portal fibrosis	92% (85-96%)[a]	21% (17/82)	73% (60/82)	6% (5/82)
Cholangiohepatitis	90% (82-94%)[a]	30% (29/80)	61% (49/80)	9% (7/80)
Bile duct hyperplasia	80% (70-87%)[a]	65% (46/71)	29% (21/71)	6% (4/71)
Diffuse glycogen degeneration	27% (19-37%)	26% (6/24)	37% (9/24)	37% (9/24)
Random necrosis	21% (14-31%)	37% (7/19)	42% (8/19)	21% (4/19)
Sinusoidal leukocytosis	15% (9-23%)	77% (10/13)	23% (3/13)	A
Random hepatitis	15% (9-23%)	85% (11/13)	15% (2/13)	A
Multinucleated hepatocytes	13% (8-22%)	50% (6/12)	42% (5/12)	8% (1/12)
Diffuse lipidosis	12% (7-21%)	69% (9/11)	8% (1/11)	23% (1/11)
Extramedullary hematopoiesis	8% (4-15%)	57% (4/7)	29% (2/7)	14% (1/7)
Portal granuloma	6% (2-12%)	NA	NA	NA
Iron storage in hepatocytes	6% (2-12%)	60% (3/5)	40% (2/5)	A
Portal hepatitis	4% (2-11%)	50% (2/4)	25% (1/4)	25% (1/4)
Cholestasis	4% (2-11%)	50% (2/4)	50% (2/4)	A
Biliary lithiasis	3% (1-9%)[b]	67% (2/3)	33% (1/3)	A

Fisher's exact test was performed to compare the frequencies of lesions between parasitized animals and non-parasitized animals: *green column*: specific findings (a: $p < 0.0001$; b: $p = 0.0158$); *yellow column*: non-specific findings ($p > 0.05$). CI: Confidence interval; NA: not applicable; A: absent

The most common morphologic diagnosis associated with the fluke infection was fibrosing and proliferative cholangiohepatitis with intraductal trematode (Figure 2.2A), observed in 74.1% (66/89; CI: 64.2-82.1%) of cases. Trematodes were considered morphologically compatible with *Platynosomum* sp. when detected in the bile duct and being characterized by transverse and longitudinal sections of a parasite without coelomatic cavity, and with the organs immersed in loosely connective tissue, and comprising some or all of the following: mature reproductive organs (testicles and uterus filled with eggs), digestive tract (cecum) and two visible suckers (Figure 2.2).

Portal fibrosis was usually lamellar and concentric with ectatic bile ducts (Figure 2.2B), but in severe cases, there was bridging of fibrous tissues connecting the portal

regions (Figure 2.2D). Cholangiohepatitis was mild to marked, characterized by an inflammatory infiltrate in the portal region composed of lymphocytes, plasma cells, histiocytes, neutrophils, and few eosinophils. Affected bile ducts were usually ectatic, with variable number of intraluminal neutrophils, and mild to marked hypertrophy of the ductal epithelium, which in some cases formed papillary projections into the lumen (Figure 2.2B). Bile duct hyperplasia was frequently observed (Figure 2.2C), in few cases associated with mild to moderate cholestasis. Erosions and ulcerations of the bile duct mucosa was also observed (Figure 2.2E), and, in some cases, there was brown dense pigment inside the bile duct lumen and sometimes associated with the site of insertion of the parasite in the duct mucosa, interpreted as fluke pigment (Figure 2.2F).

Other lesions, such as diffuse hepatocellular glycogen degeneration, necrosis, sinusoidal leukocytosis, random and portal hepatitis, lipidosis, syncytial hepatocytes, extramedullary hematopoiesis and iron storage in hepatocytes were also observed, but were considered non-specific findings, as parasitized and non-parasitized animals were similarly affected (Table 2.1).

3.2.2 Biliary lithiasis

Biliary lithiasis was observed by histopathology in three cases of the parasitized marmosets and its frequency was higher in parasitized animals when compared to the entire population (Fisher's exact test, $p = 0.0158$; Table 2.1). These stones were brown, compact, and sometimes adhered to the bile duct mucosa (Figure 2.3A). Additionally, in some cases, although a well-formed stone was not observed, there was a dense brown fluke pigment in the lumen of the bile duct (Figure 2.3B), which was often depositing and accumulating on the wall of trematode eggs (Figure 2.3C). This pigment had bile in its composition, staining green by Hall's stains for bile (Figure 2.3D).

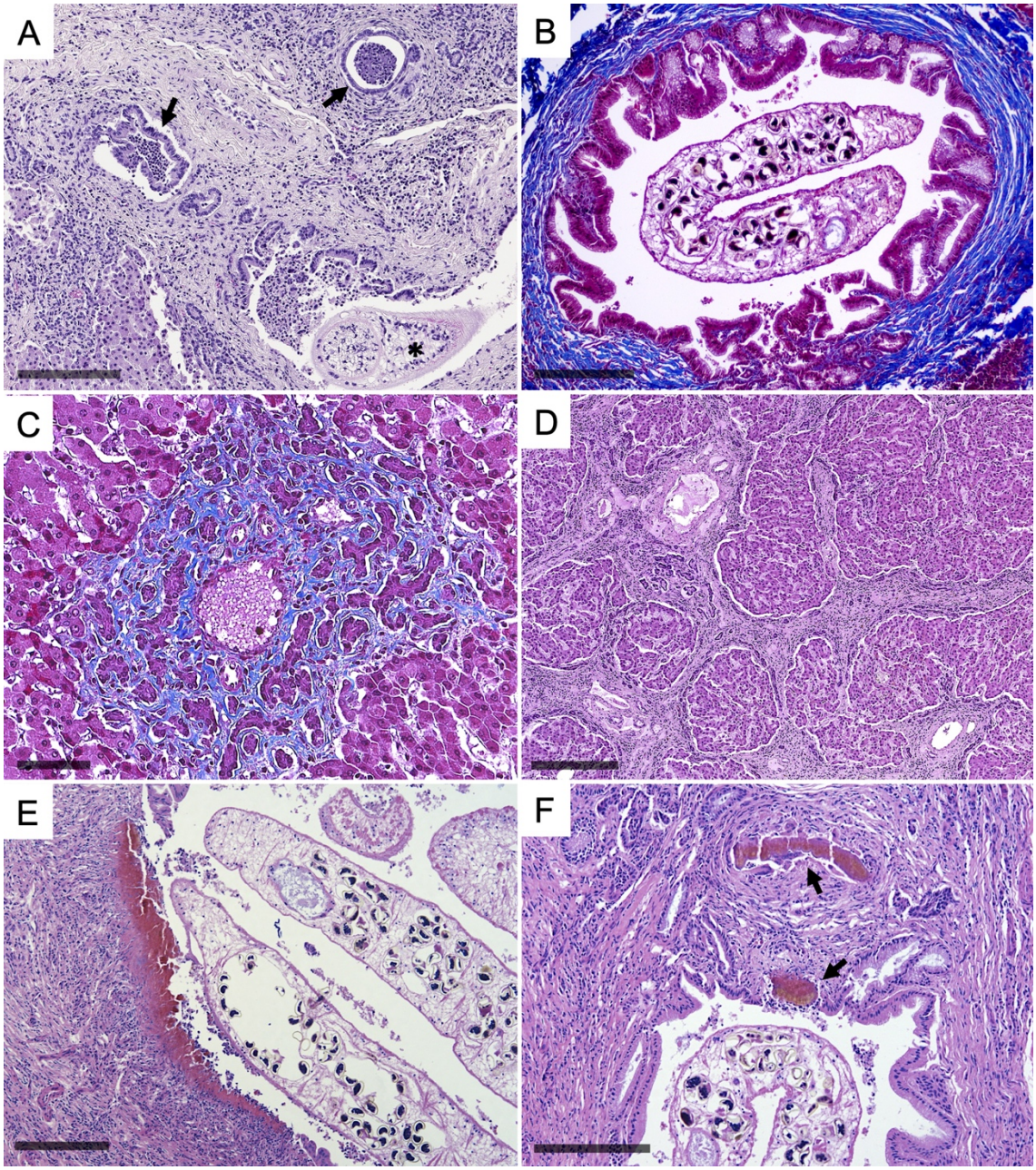


Figure 2.2 Pathological findings in the liver of the free-ranging marmosets parasitized by *Platynosomum* sp. (A) Fibrosing and proliferative cholangiohepatitis with intraductal trematode (*). Bile ducts are ectasic with intraluminal inflammatory cells (arrows). HE, scale bar = 200 μm. (B) Ectasic bile duct with intraluminal trematode. The epithelium, in red, is hypertrophic forming digital projections to the lumen and there is moderate lamellar and concentric fibrosis, in blue, adjacent to the duct. Masson's trichrome, scale bar = 200 μm. (C) Marked bile duct hyperplasia with fibrosis. Masson's trichrome, scale bar = 60 μm. (D) Marked portal fibrosis forming bridged of fibrous tissue connecting the portal tracts. HE, scale bar = 300 μm. (E) Focally extensive area of ulceration in the ductal mucosa associated with fluke brown pigment. HE, scale bar = 200 μm. (F) Fluke brown pigment is observed adhered to the mucosa and free in the duct lumen (arrow). HE, scale bar = 200 μm.

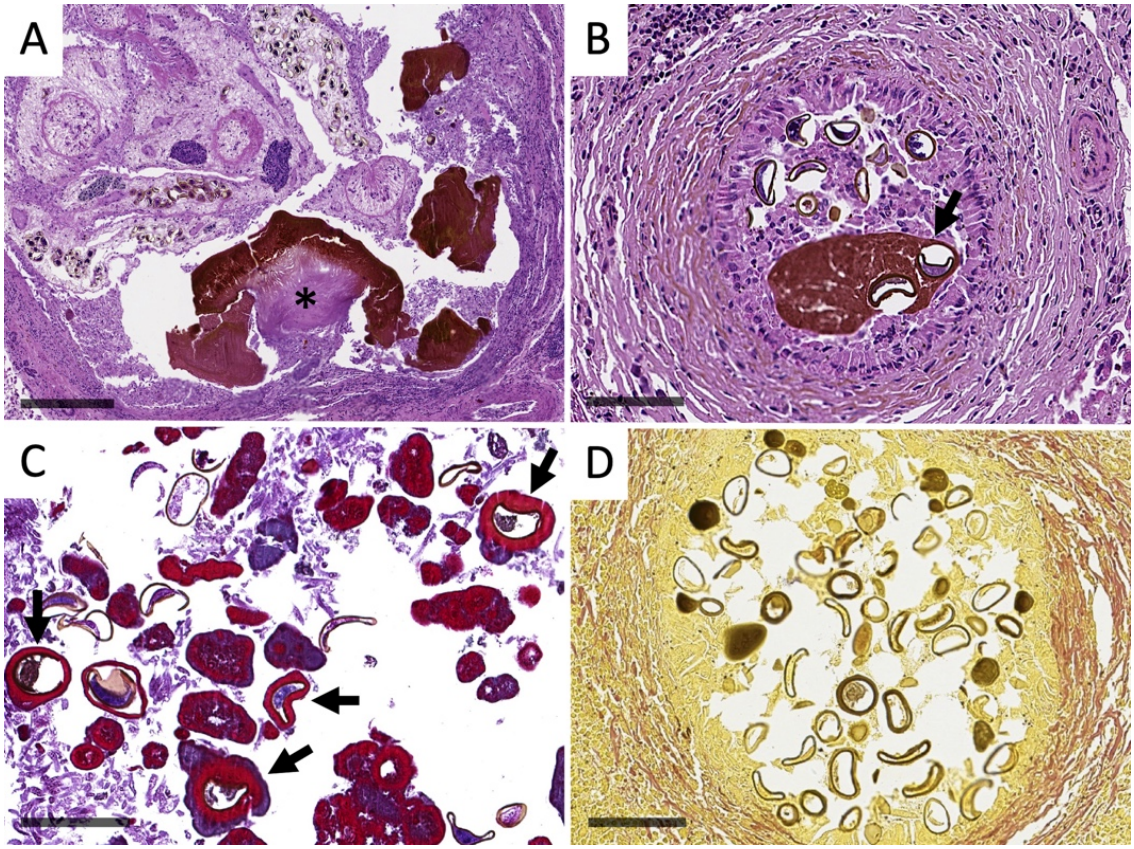


Figure 2.3 Biliary lithiasis in the liver associated with *Platynosomum* sp. infection in free-ranging marmosets. (A) Dense brown material (*) adhered to the ductal mucosa, interpreted as stones (biliary lithiasis). HE, scale bar = 300 μm. (B) Trematode eggs surrounded by dense fluke brown pigment (arrow). HE, scale bar = 80 μm. (C) In some cases the fluke pigment is observed depositing in the shell of trematode eggs (arrows), which act as an organic matrix for the formation of the biliary stones. Masson's trichrome, scale bar = 70 μm. (D) The pigment deposited in the eggshell of the trematode stains green, indicating the presence of bile in its composition. Hall's stain for bile, scale bar = 80 μm.

3.2.3 Bacterial co-infection

Among the 89 marmosets infected with *Platynosomum* sp., 13 cases (14.6%; CI: 8.7-23.4%) had intraductal bacteria associated with the parasitic cholangiohepatitis as demonstrated by HE; bacteria were highlighted by Gram stain in five cases and stained by *E. coli* IHC in 12 cases (Figure 2.4A-C). Bacterial colonies were often observed associated with areas of erosion and ulceration of the ductal mucosa, or free in the lumen, sometimes surrounded by the fluke pigment. Additionally, in five cases, bacteria were also observed in Kupffer cells within hepatic sinusoids (Figure 2.4D), compatible with bacteremia. Morphological and immunolabeling features of the organisms detected are described in Table 2.2.

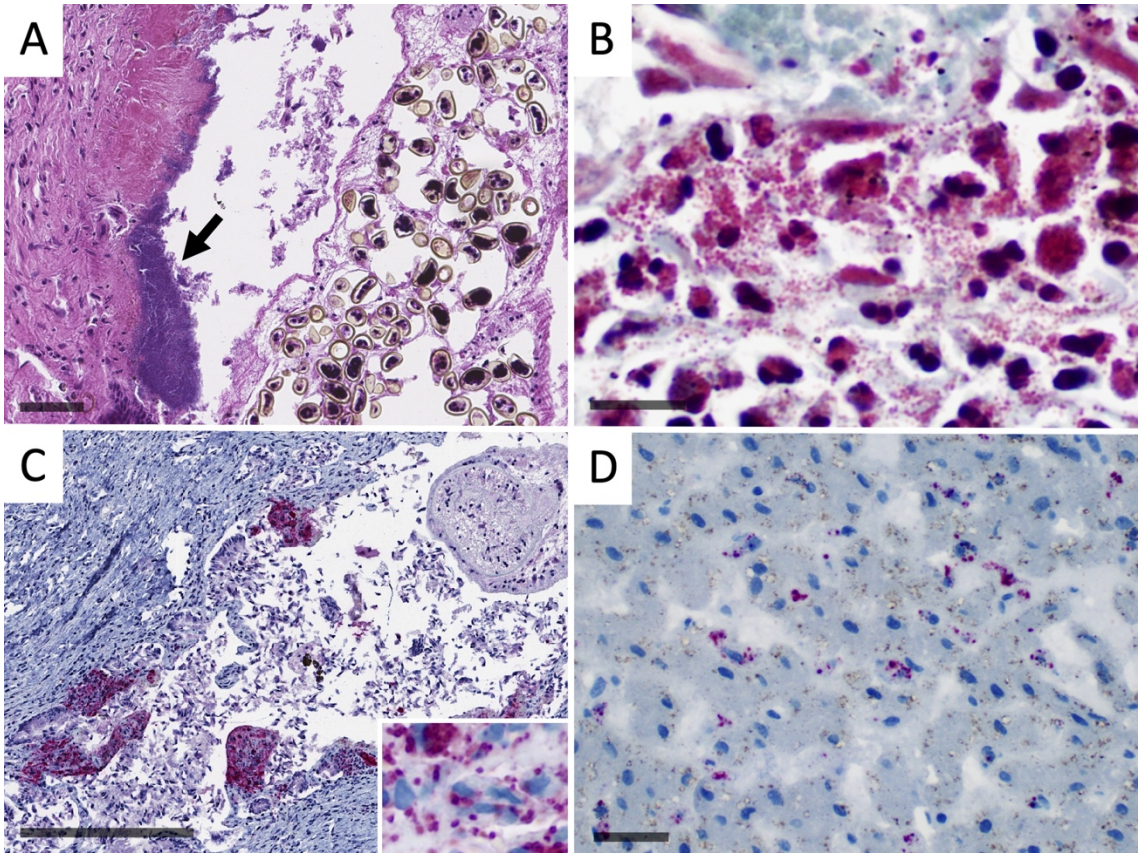


Figure 2.4 Secondary bacterial infection due to parasitic cholangiohepatitis caused by *Platynosomum* sp. in free-ranging marmosets. (A) Bacterial colonies adhered to areas of ulceration of the ductal epithelium (arrow). HE, scale bar = 70 μm. (B) In most cases of bacterial infections there were colonies of Gram-negative rods, associated with neutrophils and histiocytes. Gram stain, scale bar = 20 μm. (C) Immunoreactive stain for *E. coli* antibody, in red, showing myriads of bacteria attached to the ductal mucosa, with rod shape (down right). Mayer Hematoxylin, scale bar = 300 μm. (D) Immunoreactive stain for *E. coli* antibody, in red, showing myriad of bacteria in the cytoplasm of Kupffer cells at sinusoids, interpreted as bacteremia. Mayer Hematoxylin, scale bar = 60 μm.

Table 2.2 Morphological and immunolabeling features of the bacteria detected intraductal in parasitized marmosets.

ID	Morphology	Gram stain	IHC◊
01	Rods	Not performed*	Extensive
02	Cocci and rods	Mixed population	Rare
03	Rods	No visible staining*	Multifocal
04	Rods	No visible staining*	Extensive
05	Rods	Not performed*	Rare
06	Cocci	Gram-positive	Negative
07	Rods	Not performed*	Multifocal
08	Rods	Not performed*	Extensive

09	Rods	Gram-negative	Extensive
10	Rods	Not performed*	Extensive
11	Rods	Gram-negative	Extensive
12	Rods	Gram-negative	Extensive
13	Rods	Not performed*	Extensive

*Cases that no visible bacteria were detected at HE; ◊*Escherichia coli* antibody; IHC: Immunohistochemistry; ID: Individual numbers.

4. DISCUSSION

The main pathological findings associated with *Platynosomum* infection in free-ranging marmosets in this study were portal fibrosis, cholangiohepatitis and biliary duct hyperplasia. Those findings are in agreement with previous studies in parasitized captive NWP (Sousa et al., 2008; Pereira et al., 2021), and are consistent with lesions that have been described in association with bile duct trematode infections in other mammals (Cullen and Brown, 2013; Basu and Charles, 2014). In humans, biliary flukes, such as *Opisthorchis* and *Clonorchis*, usually cause inflammation and fibrosis of the biliary system due to direct mechanical irritation, toxic secretions, and immunogenic antigens from their eggs (Carpenter, 1998). Generally, in cases of partial and total obstruction of the bile ducts, there is marked cholestasis with severe accumulation of bilirubin in hepatocytes (Cullen and Brown, 2013). In this study, cholestasis was mild to moderate, and observed only in 4% of the parasitized animals. This difference may be because, unlike other flukes, *Platynosomum* feeds on bile acids (Kuntz, 1972), reducing bile accumulation and its potential toxicity.

In this study we were able to demonstrate a previously undocumented association between platynosomiasis and biliary lithiasis. In humans, biliary flukes have been identified as predisposing factors for cholelithiasis (Carpenter, 1998; Xia et al., 2015). In humans, rats, and sheep, fasciolosis is associated with intrahepatic stones, usually characterized as pigment stones (Valero et al., 2006; Katsoulos et al., 2011; Xia et al., 2015), similar to our findings in this study. *Clonorchis* and *Opisthorchis* infection are also known to predispose to cholecystolithiasis in humans, with detection of trematode eggs acting as an organic nucleus to gallstone formation with deposition of calcium, bilirubinate granules, and mucoid matter on their shells (Sripa et al., 2004; Qiao et al., 2012). Interestingly, in cases of lithiasis associated with trematode infection, the stones formed are usually pigmented, being mixed stones, with cholesterol stones

being less commonly observed (Sripa et al., 2004; Qiao et al., 2012). Furthermore, pigmented stones are observed more frequently in parasitized humans (Qiao et al., 2012). In this study, the frequency of biliary lithiasis was higher in parasitized marmosets, and it was possible to detect pigmented material with bile composition deposited on *Platynosomum* eggshells. These findings strongly support the hypothesis that platynosomiasis predisposes to cholelithiasis in NHP, similar to biliary flukes predisposing to lithiasis in humans, and our observations suggest that *Platynosomum* eggs may serve as a nidus for stone formation.

Importantly, 14.6% of the parasitized marmosets from this study also had secondary bacterial cholangitis, and five cases had evidence of bacteremia. Although it was not possible to identify these bacteria at the species level, considering the cross-reactivity of the IHC protocol employed in this study as well as bacterial morphology, most of these bacteria were likely members of the Enterobacteriaceae group. In humans, *Opisthorchis* is known to cause a modification in the biliary microbiome, increasing abundance of bacterial phylotypes such as *Selenomonas*, *Bacteroides*, *Rothia*, *Leptotrichia*, *Lactobacillus*, *Treponema*, and *Klebsiella* (Saltykova et al., 2016). Also, rats chronically infected with *Fasciola hepatica* had higher risk to develop biliary lithiasis and bacterial cholangitis, with isolation of *E. coli*, *Enterococcus faecalis*, and *Klebsiella pneumoniae* (Valero et al., 2003; Valero et al., 2006), the same species that are usually associated with bacterial cholangitis in human (Carpenter, 1998). Interestingly, in human schistosomiasis, it has been demonstrated that the release of immunosuppressive schistosome antigens that suppress immunity to microbial Toll-like receptor (TLR) ligands, such as lipopolysaccharides, which may increase host susceptibility to coinfecting pathogens and result in endotoxemia (Onguru et al., 2011). This immunosuppressive behavior has not yet been described for biliary flukes. However, five of the parasitized marmosets from our study had bacteria identified by *E. coli* IHC in Kupffer cells throughout the hepatic sinusoids, compatible with bacteremia secondary to the bacterial cholangiohepatitis.

In humans, total or partial chronic obstruction of the bile ducts, caused by trematode infection, may cause a decrease or even absence of biliary secretion into the duodenum. This reduction leads to poor digestion of lipids and low absorption of fat-soluble vitamins, causing severe nutritional deficiencies and immunosuppression, favoring secondary infections (Socha et al., 1997; Saron et al., 2009; Venkat et al.,

2014). Some marmoset species, such as common marmosets (*Callithrix jacchus*), are naturally susceptible to develop bacteremia and sepsis due to their limited variability of key loci in the MHC class II regions (Pisharath et al., 2005). Altogether, it is possible that *Platynosomum* infection may be an important predisposing factor to the development of systemic bacterial infection in free-ranging marmosets.

Cholangiocarcinoma and hepatocellular carcinoma are also associated with chronic trematode cholangiohepatitis (Carpenter, 1998; Xia et al., 2015), being detected in *Platynosomum* spp. infection in domestic cats and in marmosets (Andrade et al., 2012; Díaz-Delgado et al., 2018). In this study no parasitized animal had any hepatic neoplasia identified. Therefore, although the carcinogenic potential of liver flukes in humans is well-described (Carpenter, 1998; Andrade et al., 2012; Xia et al., 2015), it is not commonly observed in marmosets.

Callithrix spp. prey on small vertebrates and insects throughout the year, representing 11 to 20% of their daily diet (Passamani and Rylands, 2000). Among predated species are lizards of the genus *Anolis* and some species of mollusks (Passamani and Rylands, 2000), both of which have already been described as paratenic and intermediate hosts for *Platynosomum* spp. (Maldonado, 1945; Pinto et al., 2014). Direct ingestion of snails and isopods containing metacercaria by the definitive host does not result in infection (Maldonado, 1945; Warren et al., 1998), which requires ingestion of lizards (paratenic host) to complete the cycle, as described for domestic and wild cats (Basu and Charles, 2014). Terrestrial mollusks such as *Megalobulinus oblongus* and *Achatina fulica* have been suggested as intermediate hosts, completing the cycle in captive NWP in Brazil (Silva et al, 2012).

In this study, parasitized marmosets were more frequently observed in the Metropolitan region of Rio de Janeiro state. Ferreira et al. (1999) found a prevalence of 45% of *Platynosomum fastosum* infection in domestic cats from the city of Rio de Janeiro. Roaming cats are the main definitive host of *Platynosomum* spp. and this higher frequency in domestic cats from Rio de Janeiro is probably associated with the relatively high frequency in marmosets from the Metropolitan region in this study, because these two species share in the life cycle of this trematode, both preying the same paratenic host. Importantly, although several distinct species of *Platynosomum* have been described in mammals and birds, such as *Platynosomum amazonensis, P. fastosum* and *Platynosomum marmoseti* (Kingston and Cosgrove, 1967; Shanta, 1970;

Warren et al., 1998; Silva et al., 2012), some authors argue that all species described should be identified as *Platynosomum illiciens*, representing only morphological variation from the same trematode species infecting different hosts (Tantalean et al., 1990; Pinto et al., 2017; Assis et al., 2021; Pinto et al., 2021).

Our study characterized the epidemiological and pathological aspects of platynosomiasis in free-ranging marmosets, demonstrating that this infection is more frequently observed in the Metropolitan region, and it is usually associated with fibrosing and proliferative cholangiohepatitis that may predispose to biliary lithiasis or secondary bacterial infections and occasionally bacteremia. These findings are similar to those seen in humans with biliary trematodiasis and highlight the comparable disease pathogenesis among species.

REFERENCES

1. Andrade RLFS, Dantas AFM, Pimentel LA, Galiza GJN, Carvalho FKL (2012) *Platynosomum fastosum*-induced cholangiocarcinomas in cats. Veterinary Parasitology, 190, 277-280.

2. Assis RCP, Campos DR, Borges DA, Avelar BR, Pereira JASM et al. (2021) *Platynosomum illiciens* (Trematoda: Dicrocoeliidae) in a hybrid marmoset (*Callithrix* sp.) in the Municipality of Seropédica, RJ, Brazil – Case report. Brazilian Journal of Veterinary Parasitology, 30, 2, 1-6.

3. Basu AK, Charles AR (2014) A review of the cat liver fluke *Platynosomum fastosum* Kossack, 1910 (Trematoda: Dicrocoeliidae). Veterinary Parasitology, 200, 1-7.

4. Carpenter HA (1998) Bacterial and Parasitic Cholangitis. Mayo Clinic Proceedings, 73, 473-478.

5. Cullen JM, Brown DL (2013) Sistema hepatobiliar e pâncreas exócrino In: Bases da Patologia em Veterinária, Zachary JF, McGavin MD Eds., Elsevier Editora Ltda, Rio de Janeiro, pp 407-456.

6. Díaz-Delgado J, Sanches TC, Santos-Cirqueira C, Coimbra AAC, Guerra JM et al. (2018) Hepatocellular carcinoma in a free-living marmoset (*Callithrix* sp.) with concomitant biliary trematodiasis. Journal of Medical Primatology, 47, 2, 128-131.

7. Eckerlin RP, Leigh WH (1962) *Platynosomum fastosum* Kossack, 1910 (Trematoda: Dicrocoeliidae) in South Florida. Journal of Parasitology 48(2): 49.

8. Ferreira AMR, Almeida ECP, Labarthe NV (1999) Liver fluke infection (*Platynosomum concinnum*) in brazilian cats: prevalence and pathology. Feline Practice, 27, 19-22.

9. Katsoulos PD, Christodoulopoulos G, Karatzia MA, Pourliotis K, Minas A (2011) Liver flukes promote cholelithiasis in sheep. Veterinary Parasitology, 179, 262-265.

10. Kingston N, Cosgrove GE (1967) Two new species of *Platynosomum* (Trematoda: Dicrocoeliidae) from South American monkeys. Helminthological Society of Washington, 34, 2, 147-151.

11. Kuntz RE (1972) Trematodes of the intestinal tract and biliary passages In: Pathology of Simian Primates, Part II: Infectious and Parasitic Diseases, Fiennes RNTW, Ed., New York, 104-123.

12. Maldonado JF (1945) The life history and biology of *Platynosomum fastosum* Kossak, 1910 (Trematoda: Dicrocoeliidae). The Puerto Rico Journal of Public Health and Tropical Medicine, 21, 1, 17-40.

13. Mati VLT, Pinto HA, Melo AL (2015) Evaluation of Kato-Katz and spontaneous sedimentation methods for the diagnosis of platynosomiasis in Neotropical primates. Brazilian Journal of Veterinary Parasitology, 24, 1, 108-113.

14. Mattioli MP, Batista JS, Ferrari M, Paludo GR, Dias CA et al. (2016) Clinical, hematological, biochemical, and ultrasonographic aspects of *Platynosomum* sp. (Trematoda: Dicrocoeliidae) infection of captive *Callithrix penicillata.* Primates, 57, 279-287.

15. Melo AL (2004) Helminth Parasites of *Callithrix geoffroyi*. Laboratory Primate Newsletter, 43, 2, 7-9.

16. Onguru D, Liang Y, Griffith Q, Nikolajczyk B, Mwinzi P (2011) Short Report: Human Schistosomiasis Is Associated with Endotoxemia and Toll-Like Receptor 2- and 4-Bearing B Cells. American Journal of Tropical Medicine and Hygiene, 84, 2, 321-324.

17. Passamani M, Rylands AB (2000) Feeding Behavior of Geoffroy's Marmoset (*Callithrix geoffroyi*) in an Atlantic Forest Fragment of South-eastern Brazil. Primates, 41, 1, 27-38.

18. Pereira FV, Lucena FP, Rodrigues RL, Barros LA, Pires CA et al. (2020) Prevalência e distribuição espacial da ocorrência de helmintos em primatas não humanos de vida livre no estado do Rio de Janeiro, Brasil. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 72, 5, 1705-1712.

19. Pereira WLA, Conga DMF, Silva KSM, Silva RJ, Imbeloni AA (2021) Anatomopathological lesions of infection caused by *Platynosomum illiciens* (Braun, 1901) in Neotropical primates kept in captivity. Journal of Medical Primatology, 50, 1, 82-85.

20. Pinto HA, Mati VLT, Melo AL (2014) New insights into the life cycle of *Platynosomum* (Trematoda: Dicrocoeliidae). Parasitology Research, 113, 7, 2701-2707.

21. Pinto HA, Mati VLT, Pujoni DF, Melo AL (2017) *Platynosomum illiciens* (Trematoda: Dicrocoeliidae) in captive black-tufted marmoset *Callithrix penicillata* (Primates: Cebidae) from Brazil: a morphometric analyses with taxonomic comments on species of *Platynosomum* from nonhuman primates. Journal of Parasitology, 103, 1, 14-21.

22. Pinto HA, Melo AL, Mati VLT (2021) *Platynosomum illiciens*. Trends in Parasitology, 38, 2, 188-189.

23. Pisharath HR, Cooper TK, Brice AK, Cianciolo RE, Pistorio AL et al. (2005) Septicemia and peritonitis in a colony of common marmosets (*Callithrix jacchus*) secondary to *Klebsiella*

pneumoniae infection. Contemporary topics in laboratory animal science / American Association for Laboratory Animal Science, 44, 1, 35-37.

24. Qiao T, Ma R, Luo X, Luo Z, Zheng P (2012) Cholecystolithiasis is associated with *Clonorchis sinensis* infection. Plos one, 7, 8, 1-10.

25. Saron MLG, Godoy HT, Hessel G (2009) Nutritional status of patients with biliary atresia and autoimmune hepatitis related to serum levels of vitamins A, D and E. Arquivos de Gastroenterologia, 46, 1, 62-68.

26. Saltykova IV, Petrov VA, Logacheva MD, Ivanova PG, Merzlikin NV et al. (2016) Biliary Microbiota, Gallstone Disease, and Infection with *Opisthorchis felineus*. Plos Neglected Tropical Diseases, 10, 7, 1-15.

27. Shanta CS (1970) A species of *Platynosomum* from a monkey. Malaysian Veterinary Journal, 5, 1, 17-18.

28. Silva KSM, Silva RJ, Pereira WLA (2012) Occurrence of infection by *Platynosomum illiciens* (Braun, 1901) in captive neotropical primates. Primates, 53, 79-82.

29. Socha P, Koletzko B, Pawlowska J, Socha J (1997) Essential fatty acid status in children with cholestasis, in relation to serum bilirubin concentration. The Journal of Pediatrics, 131, 5, 700-706.

30. Sousa MBC, Leão AC, Coutinho JFV, Ramos AMO (2008) Histopathology findings in common marmosets (*Callithrix jacchus* Linnaeus, 1758) with chronic weight loss associated with bile tract obstruction by infestation with *Platynosomum* (Loos, 1907). Primates, 49, 283-287.

31. Sripa B, Kanla P, Sinawat P, Haswell-Elkins MR (2004) Opisthorchiasis-associated biliary stones: Light and scanning electron microscopic study. World Journal of Gastroenterology, 10, 22, 3318-3321.

32. Tantalean M, Gozalo A, Montoya E (1990) Notes on some helminthparasites from Peruvian monkeys. Laboratory Primate Newslatter, 29, 6–8.

33. Valero MA, Santana M, Morales M, Hernandez JL, Mas-Coma S (2003) Risk of gallstone disease in advanced chronic phase of fasciolosis: an experimental study in a rat model. Journal of Infectious Diseases, 188, 787-793.

34. Valero MA, Navarro M, Garcia-Bodelon MA, Marcilla A, Morales M et al. (2006) High risk of bacterobilia in advanced experimental chronic fasciolosis. Acta Tropica, 100, 17-23.

35. Venkat VL, Shneider BL, Magee JC, Turmelle, Y, Arnon R, et al. (2014) Total serum bilirubin predicts fat-soluble vitamin deficiency better than serum bile acids in infants with biliary atresia. Journal of Pediatric Gastroenterology and Nutrition, 59, 6, 702-707.

36. Warren KS, Swan RA, Hobbs RP, Heriyanto Kuhn EM, Heeney JL (1998) *Platynosomum fastosum* in ex-captive orangutans from Indonesia. Journal of Wildlife Disease, 34, 3, 644-646.

37. Xia J, Jiang S, Peng H (2015) Association between Liver Fluke Infection and Hepatobiliary Pathological Changes: A Systematic Review and Meta-Analysis. Plos One, 10, 7, 1-19.

CHAPTER III[3]

PATHOLOGY AND EPIDEMIOLOGY OF FATAL TOXOPLASMOSIS IN FREE-RANGING MARMOSETS (*Callithrix* spp.) FROM THE BRAZILIAN ATLANTIC FOREST

ABSTRACT

Toxoplasmosis is an important zoonotic disease that affects a wide range of warm-blooded host species. New World Primates (NWP) are highly susceptible, developing a lethal acute systemic disease. Toxoplasmosis in free-ranging NWP is poorly described, with only a few studies based on serosurveys. Herein we performed a retrospective study focusing on the epidemiology and pathology of toxoplasmosis among 1,001 free-ranging marmoset (*Callithrix* spp.) deaths from the Brazilian Atlantic Forest. This study included marmosets necropsied at the Instituto Municipal de Medicina Veterinaria Jorge Vaitsman (IJV) from January 2017 to July 2019, which were found dead from all regions in the State of Rio de Janeiro. Histopathology, immunohistochemistry, and transmission electron microscopy were performed to better characterize toxoplasmosis in this free-ranging population. All samples were also tested for yellow fever virus (YFV) by the official diagnostic service. A total of 1,001 free-ranging marmosets were included in this study, with 16 (1.6%) cases of lethal *T. gondii* infections identified both as individual cases and in outbreaks. Presence of infection was not associated with sex, age, geographical distribution, or year of death, and no co-infection with YFV was observed. The main pathological feature in these cases was random necrotizing hepatitis with detection of intralesional *T. gondii* zoites in all infected cases. Interstitial pneumonia rich in alveolar foamy macrophages and fibrin deposition, necrotizing myocarditis and necrotizing splenitis were also pathological features in affected marmosets. Toxoplasmosis was responsible for the deaths of 1.6% of free-ranging marmosets, also including some associated with outbreaks. Necrotizing random hepatitis is the main pathological finding associated with infection, making the liver the optimal tissue for pathologic diagnosis of toxoplasmosis in marmosets.

Keywords: neotropical primates; nonhuman primates; zoonosis; pathology; wildlife.

[3] Chapter submitted to the journal *Plos Neglected Tropical Diseases.*

1. INTRODUCTION

Toxoplasmosis is a worldwide zoonotic disease, caused by *Toxoplasma gondii*, a coccidian parasite that infects a wide range of warm-blooded animals. Domestic and wild cats are the definitive hosts, in which the sexual form of the coccidia develops, generating infective oocysts that are shed in the feces (Mätz-Rensing and Lowenstine, 2018). Neotropical primates (New World Primates; NWP) are highly susceptible to toxoplasmosis (Mätz-Rensing and Lowenstine, 2018; Paula et al., 2020). In captive NWP, toxoplasmosis is characterized by a hyperacute to acute, necrotizing disease with difficult treatment approach, that evolves quickly to death, being sometimes associated with outbreaks with high lethality (Grumann et al., 2017; Santos et al., 2018; Mätz-Rensing and Lowenstine, 2018; Paula et al., 2020; Santana et al., 2021; Moreira et al., 2022). There are differences in susceptibility between NWP species, with capuchins being considered more resistant than other neotropical species (Paula et al., 2020; Santana et al., 2021).

Studies of free-ranging NWP are mainly based on serological analysis using microscopic agglutination test (MAT), with some positive serosurveys in wild populations of marmosets (*Callithrix penicillata*), howler-monkeys (*Alouatta caraya* and *A. palliata*), and capuchins (*Sapajus flavius* and *Cebus imitator*) (Molina et al., 2014; Silva et al., 2014; Bueno et al., 2017; Molina et al., 2016; Niehaus et al., 2020). However, due to its acute lethal course, captive NWPs usually die from the infection before developing a humoral response (Paula et al., 2020), making serology a limited tool to evaluate the presence of the disease in these animals. In fact, in a study of seroprevalence of toxoplasmosis in a free-ranging population of golden-headed lion tamarins (*Leontopithecus chrysomelas*), which are known to be highly susceptible, none of the animals was serologically positive (Molina et al., 2017), supporting the notion that serology may not be a reliable tool to assess circulation of *T. gondii* in NWP populations.

Furthermore, there are only a few reports of death associated with toxoplasmosis in free-ranging NWP, including three howler monkeys (*A. guariba*) (Ehlers et al., 2022) and one southern muriqui (*Brachyteles arachnoides*) (Santos et al., 2018). All these cases are individual reports, with no characterization of the disease profile in the wild population. Therefore, although well-described in captive animals, the behavior of this disease in free-ranging NWP warrants further study. To this end, we conducted a three-

year-retrospective study focusing on the epidemiology and pathology of toxoplasmosis among 1,001 free-ranging marmosets (*Callithrix* spp.) from the Brazilian Atlantic Forest.

2. MATERIAL AND METHODS

2.1 Study design

This was a retrospective study of free-ranging marmosets (*Callithrix* spp.) that included 1,001 animals necropsied at the Instituto Municipal de Medicina Veterinária Jorge Vaitsman (IJV, Rio de Janeiro, Brazil) for yellow fever (YF) surveillance, during January 2017 to July 2019, that were found dead from all regions in the State of Rio de Janeiro. Animals were previously tested for YF virus (YFV) by quantitative reverse transcription polymerase chain reaction (RT-qPCR) and immunohistochemistry (IHC) by the official Brazilian diagnostic service. Data about geographical origin, sex and age were collected and informed by IJV. This study was authorized by the government environmental agency (ICMBio - Brazil) under the SISBIO license number 67014. All procedures strictly adhered to humane care of animals and all applicable laws and regulations, including registration in the national system for management of genetic heritage and associated traditional knowledge by SISGEN code A2743E4.

2.2 Necropsy and histopathology

All animals were necropsied by veterinary pathologists at the IJV. Animals were evaluated by gross examination of the carcass and all that were considered suitable for histopathology were included in this study. Samples of brain, heart, lungs, liver, spleen, and kidney were fixed in 10% buffered formalin and embedded in paraffin by routine histology. Formalin-fixed paraffin-embedded (FFPE) tissue samples were sectioned using a microtome (3–4 μm thick) and stained with hematoxylin and eosin (HE) for microscopic evaluation. Other stains, such as Prussian blue, Gram stain and Periodic acid–Schiff (PAS) were performed as needed. HE and immunohistochemistry (IHC) concordance were evaluated to assess the ability of histopathological diagnosis among the affected organs. The sensitivity of HE for the diagnosis of toxoplasmosis in different tissues was also evaluated, using IHC as the gold standard.

2.3 Immunohistochemistry (IHC)

IHC was performed on all suspected toxoplasmosis cases: cases with intralesional zoites seen by HE stains, or cases with histopathological lesions compatible with *T. gondii* infection as previously described (Paula et al., 2020; Santana et al., 2021). FFPE tissue sections were deparaffinized, hydrated, and subjected to antigen retrieval in a pressure cooker at pH 9.0 buffer (Envision®). Endogenous peroxidase activity was blocked with 3.5% hydrogen peroxide and to block non-specific protein bindings, slides were incubated in 6% powdered skim milk. A mouse primary monoclonal antibody (IgG2a) targeting p30 membrane of *T. gondii* tachyzoite (clone Tp3; Santa Cruz Biotechnology) was used at a dilution of 1:100. First, slides were incubated in the primary antibody, overnight, at 4°C and then, incubated with an indirect peroxidase polymeric detection kit (Envision®) for 30 minutes at room temperature, followed by revelation with 3,3′-Diaminobenzidine (DAB) solution. Slides were counterstained with hematoxylin. Positive controls included sections of liver from a NWP with confirmed *T. gondii* infection (Paula et al., 2020). Negative controls had the primary antibody replaced by wash buffer.

2.4 Transmission electron microscopy (TEM)

TEM was performed in one case *T. gondii* infection at the Infectious Diseases Pathology Branch at the Centers for Disease Control and Prevention (IDPB-CDC, Atlanta, USA). For TEM, areas of interest from FFPE blocks were selected based on results from HE and IHC. Samples for EM were removed from paraffin blocks using a 1-2 mm biopsy punch, deparaffinized using xylene, rehydrated using a decreasing ethanol series, and post-fixed in 2.5% glutaraldehyde. Samples were then post-fixed with 1% osmium tetroxide, en-bloc stained with 4% uranyl acetate, dehydrated using an increasing ethanol series and acetone, and embedded in Epon-Araldite resin. Samples were then ultrathin sectioned (~50nm thick), stained with uranyl acetate and lead citrate, and examined on a Tecnai Biotwin electron microscope.

2.5 Statistical analysis

Data were analyzed using the GraphPad Prism software (version 9.0). Descriptive statistics with 95% confidence interval was used for general analysis. Frequency of histopathological lesions and other variables, such as age, sex and geographical distribution, were compared between infected and non-infected animals

using the Fisher's exact test. Kappa Coefficient was calculated to analyze the concordance between HE and IHC assays to detect *T. gondii* zoites in the tissue.

3. RESULTS

3.1 Epidemiology

A total of 1,001 free-ranging marmoset (*Callithrix* spp.) were included in this study, including 463 females and 463 males (in 75 cases sex was not determined), and 783 adults and 187 juveniles (in 31 cases age was not determined). Toxoplasmosis was diagnosed in 16 animals (1.6%; CI: 1.0-2.6%) (Table 3.1) from different geographic locations in the State of Rio de Janeiro, Brazil (Figure 3.1), with 11 cases from the Metropolitan mesoregion (68.7%; 11/16; CI: 44.4-85.8%). In all cases, the animals were found dead in urbanized areas. Eight cases were reported in 2017 (8/304 – 2.6%; CI: 1.3-5.1%), six in 2018 (6/605 – 1.0%; CI: 0.4-2.1%), and two in 2019 (2/92 – 2.2%; CI: 0.4-7.6%). All these animals were adults, being 12 females (75%; 12/16; CI: 50.5-89.2%) and four males (25%; 4/16; CI: 10.2-49.5%).

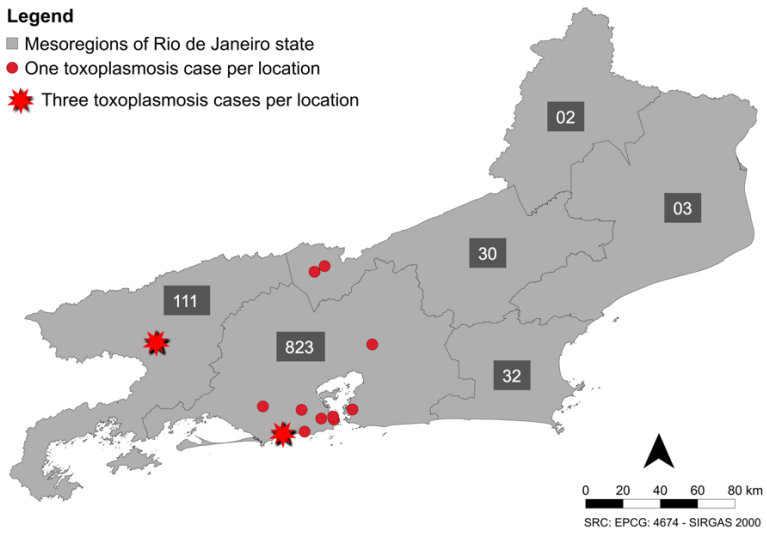


FIGURE 3.1 Distribution of toxoplasmosis cases in free-ranging marmosets from Rio de Janeiro state from January 2017 to July 2019. Map from Rio de Janeiro mesoregions (IBGE 2021) with total number of animals with by each mesoregion (grey box). SRC: EPCG 4674 – SIRGAS 2000 (QGIS Version 3.24.3).

No statistical difference in the frequency of toxoplasmosis between sex (Fisher's exact test, $p = 0.0743$), age (Fisher's exact test, $p = 0.0526$), geographical origin

(Fisher's exact test, p = 0.1819) or years (Fisher's exact test, p > 0.05) was noted. Most incidents were of only one positive individual found dead, however there were two situations (ID3-5 and ID6-8; Table 3.1) with three positive individuals from the same location, found dead at the same month, constituting an outbreak in that specific population. None of the animals with toxoplasmosis were positive for YFV by testing at the official diagnostic laboratory.

3.2 Pathological findings

Individual pathological findings are described in Table 3.1. Based on Kappa statistics, concordance between HE and IHC to detect *T. gondii* zoites varied according to the organ analyzed, with perfect agreement in the liver (κ = 1), spleen (κ = 0.87) and heart (κ = 0.87), substantial agreement in the lungs (κ = 0.80) and kidney (κ = 0.61), and moderate agreement in the brain (κ = 0.48). Additionally, considering IHC as the gold standard for the diagnosis of toxoplasmosis in FFPE tissue samples, microscopic examination of HE stained sections yielded sensitivities of 100% in the liver, 87% in the spleen, 83% in the heart, 75% in the lung, 20% in the brain, and 14% in the kidney. Frequencies of positivity in different tissues through examination of HE or IHC stained sections are demonstrated in Figure 3.2.

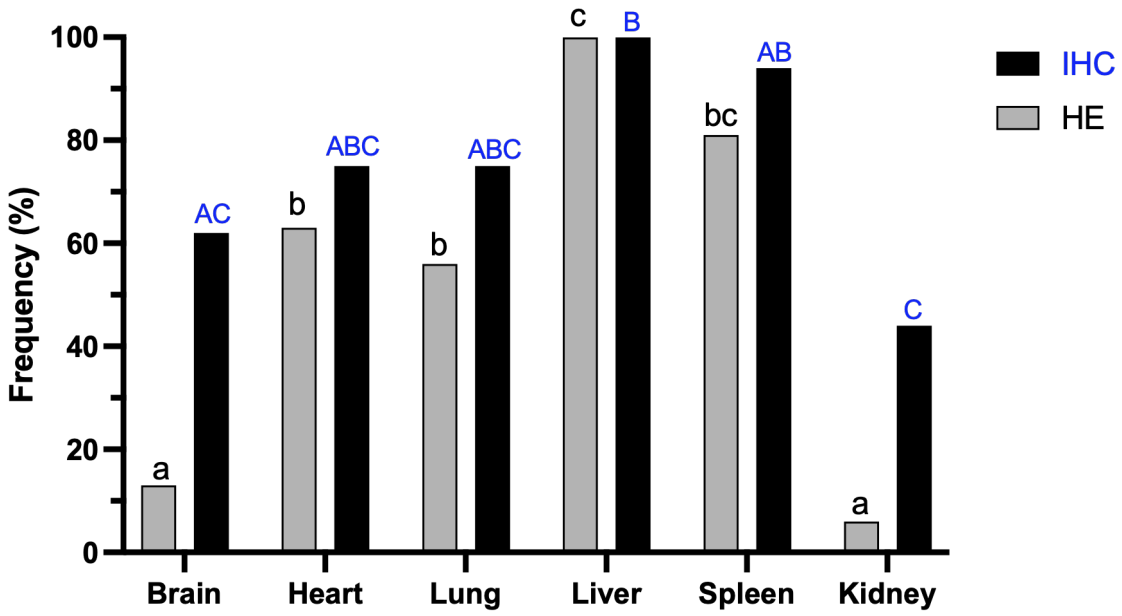


FIGURE 3.2 Frequency of intralesional *T. gondii* zoites per organ evaluated by histopathology (HE) and immunohistochemistry (IHC). Fisher's exact test was used to compare the distribution frequency of tachyzoites and bradyzoites in the different organs evaluated by HE stained sections (a,b,c: $p < 0.05$) or by IHC (A,B,C: $p < 0.05$).

TABLE 3.1 General data and pathological findings of free-ranging marmosets (*Callithrix* spp.) from the State of Rio de Janeiro (Brazil) with toxoplasmosis from January 2017 to July 2019.

ID	Age	Sex	Date	Origin (city)	Morphologic diagnosis
1	Adult	Female	March, 2017	Rio de Janeiro	Heart: zoites in the cytoplasm of cardiomyocytes, multifocal, moderate. Liver: NH, LHP, random, marked, with intralesional and intracytoplasmic zoites; SPCH, multifocal, moderate, with intraductal trematode. Spleen: splenitis, histiocytic, multifocal, mild, with few intralesional and intracytoplasmic zoites. Kidney: IN, LHP, multifocal, mild, with few intracytoplasmic zoites.
2	Adult	Female	April, 2017	Niterói	Heart: cardiomyocytes necrosis, multifocal, mild, with rare intralesional and intracytoplasmic zoites. Lung: IP, LH, diffuse, mild with moderate multifocal AE and AH, and intralesional and intracytoplasmic zoites. Liver: NH, LHP, random, moderate, with few intralesional intracytoplasmic zoites. Spleen: splenitis, histiocytic, diffuse, moderate, with rare intralesional and intracytoplasmic zoites.
3	Adult	Male	June, 2017	Volta Redonda	Lung: AH, diffuse, moderate; AE, multifocal, mild; moderate amount of alveolar FM with few intralesional intracytoplasmic zoites. Liver: NH, H&N, random, moderate, with intralesional intracytoplasmic zoites and mild random lipidosis. Spleen: splenitis, histiocytic, diffuse, moderate, with intralesional and intracytoplasmic zoites and marked lymphoid hyperplasia. Adrenal: hemorrhage, diffuse, marked.
4	Adult	Female	June, 2017	Volta Redonda	Brain: ME, non-suppurative, multifocal, mild with intralesional intracytoplasmic zoites. Heart: zoites in the cytoplasm of cardiomyocytes, multifocal, rare. Lung: IP, H&N, multifocal, mild with moderate diffuse AE and AH, moderate amount of alveolar fibrin deposition, FM and intralesional and intracytoplasmic zoites. Liver: NH, H&N, random, moderate, with intralesional intracytoplasmic zoites. Spleen: NS, H&N, diffuse, mild, with intralesional and intracytoplasmic zoites and moderate lymphoid hyperplasia. Kidney: IN, LHP, multifocal, moderate, with moderate membranous glomerulopathy and nephrocalcinosis. Adrenal: adrenalitis, LHP&N, multifocal, mild.
5	Adult	Female	June, 2017	Volta Redonda	Heart: NM, multifocal, mild with intralesional intracytoplasmic zoites. Lung: IP, H&N, diffuse, mild with moderate multifocal AE and AH, large number of FM and few intralesional and intracytoplasmic zoites. Liver: NH, H&N, random, moderate, with intralesional intracytoplasmic zoites. Spleen: NS, histiocytic, diffuse, mild, with few intralesional and intracytoplasmic zoites. Adrenal: adrenalitis, LHP&N, multifocal, mild.

6	Adult	Male	July, 2017	Rio de Janeiro	Heart: myocarditis, H&N, multifocal, mild with intralesional intracytoplasmic zoites. Lung: IP, H&N, multifocal, mild with diffuse mild AH, large number of FM and few intralesional and intracytoplasmic zoites. Liver: NH, H&N, random, moderate, with intralesional intracytoplasmic zoites, moderate random lipidosis and moderate SL. Spleen: splenitis, histiocytic, diffuse, mild, with few intralesional and intracytoplasmic zoites.
7	Adult	Male	July, 2017	Rio de Janeiro	Heart: rare zoites in the cytoplasm of cardiomyocytes, multifocal, moderate. Lung: IP, H&N, multifocal, mild with diffuse moderate AE and AH, large number of FM and few intralesional and intracytoplasmic zoites. Liver: NH, H&N, random, moderate, with intralesional intracytoplasmic zoites, moderate random lipidosis and mild SL. Spleen: splenitis, H&N, diffuse, moderate, with few intralesional and intracytoplasmic zoites. Kidney: pigmentary nephrosis, multifocal, moderate, with mild glomerulosclerosis and moderate nephrocalcinosis.
8	Adult	Female	July, 2017	Rio de Janeiro	Heart: myocarditis, H&N, multifocal, moderate with rare intralesional intracytoplasmic zoites. Lung: IP, LH&N, diffuse, moderate with multifocal moderate AE, diffuse marked AH, large number of FM and few intralesional and intracytoplasmic zoites. Liver: NH, H&N, random, moderate, with intralesional intracytoplasmic zoites, marked random lipidosis and mild SL; SPCH, multifocal, moderate, with intraductal trematode. Spleen: NS, H&N, diffuse, mild, with few intralesional and intracytoplasmic zoites. Kidney: IN, LP, multifocal, mild.
9	Adult	Female	February, 2018	Rio de Janeiro	Lung: BIP, H&N, multifocal, moderate with mild multifocal AE and AH, and few intralesional and intracytoplasmic zoites. Liver: NH, H&N, random, moderate, with intralesional intracytoplasmic zoites and moderate random lipidosis. Spleen: splenitis, H&N, diffuse, mild, with few intralesional and intracytoplasmic zoites. Kidney: IN, LP, multifocal, mild. Adrenal: adrenalitis, LHP&N, multifocal, moderate, with multifocal moderate hemorrhage and few intralesional and intracytoplasmic zoites.
10	Adult	Male	March, 2018	Paraíba do Sul	Heart: cardiomyocytes necrosis, multifocal, mild, with rare intralesional and intracytoplasmic zoites. Lung: AE, diffuse, marked and AH, multifocal, moderate. Liver: NH, H&N, random, moderate, with intralesional intracytoplasmic zoites; SCH, multifocal, moderate. Testicle: tubular degeneration, multifocal, moderate.
11	Adult	Female	May, 2018	Rio de Janeiro	Lung: IP, LH&N, diffuse, mild with multifocal mild AH. Liver: NH, H&N, random, moderate, with intralesional intracytoplasmic zoites and moderate random lipidosis; SPCH, multifocal, moderate. Spleen: splenitis, histiocytic, diffuse, mild, with few intralesional and intracytoplasmic zoites.

					Small intestine: enteritis, histiocytic, diffuse, moderate, with intralesional and intracytoplasmic zoites.
12	Adult	Female	May, 2018	Rio de Janeiro	Brain. Gliosis, multifocal, mild, with rare intralesional and intracytoplasmic zoites. Heart: NM, multifocal, mild with rare intralesional intracytoplasmic zoites. Lung: BIP, H&N, diffuse, mild with diffuse moderate AE. Liver: NH, H&N, random, moderate, with intralesional intracytoplasmic zoites; SPCH, multifocal, moderate, with intraductal trematode. Spleen: splenitis, histiocytic, multifocal, mild, with few intralesional and intracytoplasmic zoites. Kidney: IN, LHP, multifocal, moderate, with membranous glomerulopathy and glomerulosclerosis, multifocal, mild.
13	Adult	Female	July, 2018	Rio de Janeiro	Lung: IP, LH, multifocal, mild with multifocal mild AE and multifocal marked AH. Liver: NH, H&N, random, moderate, with intralesional intracytoplasmic zoites; marked iron storage in hepatocytes. Kidney: IN, LHP, multifocal, moderate, with glomerulosclerosis, multifocal, moderate.
14	Adult	Female	July, 2018	Guapimirim	Heart: myocarditis, multifocal, mild. Lung: IP, LH, diffuse, mild with multifocal mild necrosis, multifocal mild AE, and few intralesional intracytoplasmic zoites. Liver: necrosis, random, moderate with intralesional intracytoplasmic zoites and marked EH. Spleen: Few intralesional intracytoplasmic zoites; lymphoid hyperplasia, mild; EH, mild. Kidney: IN, LP, multifocal, mild. Adrenal: necrotizing adrenalitis, LH, multifocal, moderate, with intralesional intracytoplasmic zoites.
15	Adult	Female	January, 2019	Três Rios	Heart: myocarditis, multifocal, mild. Lung: IP, LH&N, multifocal, mild with multifocal mild AE. Liver: NH, H&N, random, moderate, with rare intralesional intracytoplasmic zoites. Kidney: IN, LP, multifocal, mild.
16	Adult	Female	February, 2019	Campo Grande	Heart: myocarditis, H&N, multifocal, moderate with rare intralesional intracytoplasmic zoites. Lung: IP, LH&N, diffuse, moderate with diffuse marked AE and multifocal mild AH. Liver: NH, H&N, random, moderate, with rare intralesional intracytoplasmic zoites, mild random lipidosis; SPCH, multifocal, moderate, with intraductal trematode. Spleen: splenitis, histiocytic, multifocal, mild, with few intralesional and intracytoplasmic zoites. Kidney: IN, LP, multifocal, mild.

ID: Individual numbers; Pathological abbreviations: AE (alveolar edema); AH (alveolar hemorrhage); BIP (bronchoIP); EH (extramedullary hematopoiesis); FM (foamy macrophages); H&N (histiocytic and neutrophilic); IN (interstitial nephritis); IP (interstitial pneumonia); LH (lymphohistiocytic); LH&N (lymphohistiocytic and neutrophilic); LHP (lymphohistioplasmacytic); LHP&N (lymphohistioplasmacytic and neutrophilic); LP (lymphoplasmacytic); ME (meningoencephalitis); NH (necrotizing hepatitis); NM (necrotizing myocarditis); NS (necrotizing splenitis); SCH (sclerosing cholangiohepatitis); SL (sinusoidal leukocytosis); SPCH (sclerosing and proliferative cholangiohepatitis.

Liver: Multifocal random lytic hepatocellular necrosis was observed in all infected cases (100%; 16/16; CI: 80.6-100%), and together with multifocal random hepatitis (93.8%; 15/16; CI: 71.7-99.7%) and multifocal random lipidosis (43.8%; 7/16; CI: 23.1-66.8%), were the main changes associated with toxoplasmosis in these cases (Fisher's exact test, $p < 0.0001$, $p < 0.0001$, and $p = 0.0002$, respectively). In most of the cases there was a mild to marked random necrotizing hepatitis with mild to marked multifocal lipidosis, usually observed in the hepatocytes adjacent to necrotic areas (Figure 3.3A). Intralesional tachyzoites and bradyzoites were observed in all cases (100%; 16/16; CI: 80.6-100%), both in HE and IHC stained sections, with significantly higher frequency compared to the other organs (Figure 3.2). Bradyzoites were identified in the cytoplasm of hepatocytes in areas without inflammation or necrosis, and tachyzoites were observed in Kupffer cells, hepatocytes, and free in the necrotic areas (Figure 3.3B). Iron storage in hepatocytes, evidenced by Prussian blue stain, was observed in 18.8% (3/16; CI: 6.6-43%) of the cases, but was not necessarily associated with the *T. gondii* infection (Fisher's exact test, $p = 0.0728$). Extramedullary hematopoiesis (12.5%; 2/16. CI: 2.2-36%), sinusoidal leukocytosis (18.8%; 3/16; CI: 6.6-43%) and multinucleated hepatocytes (18.8%; 3/16; CI: 6.6-43%) were also observed in these cases but were considered non-specific findings (Fisher's exact test, $p = 0.37$, $p > 0.9999$ and $p = 0.7299$, respectively). Importantly, there was a significantly higher frequency of cholangiohepatitis with intraductal trematodes, compatible with *Platynosomum* spp., in toxoplasmosis cases (25.0%; 4/16) compared to the frequency of *Platynosomum* spp. in non-infected animals (8.6%; 5/985; Fisher's exact test, $p = 0.0441$).

Spleen: splenitis was observed in 75.0% (12/16; CI: 50.5-89.8%) of the cases, being characterized by mild to moderate inflammatory infiltrate composed mainly of neutrophils and histiocytes, associated with mild necrosis and fibrin deposition in 18.7% (3/16; CI: 6.6-43.0%) of the cases (Figure 3.3C). Splenitis and necrosis were both features associated with *T. gondii* infection (Fisher's exact test, $p < 0.0001$ and $p = 0.0113$, respectively), and in most cases necrosis was accompanied by mild to moderate deposition of fibrin in the red pulp. Intralesional tachyzoites and bradyzoites were observed in 81.3% (13/16; CI: 57.0-93.4%) of the cases in HE stained sections and in 93.7% (15/16; CI: 71.7-99.7%) by IHC, frequently associated with histiocytes or free at the red pulp (Figure 3.2 and 3.3D). Lymphoid hyperplasia (31.2%; 5/16; CI: 14.2-

55.6%) and extramedullary hematopoiesis (12.5%; 2/16; CI: 2.2-36.0%) was also observed but were not considered specific features of infected animals (Fisher's exact test, $p = 0.4326$ and $p > 0.9999$, respectively).

Heart: Myocarditis and myocardial necrosis were observed, each in 43.8% (7/16; CI: 23.1-66.8%) of the infected animals, making both a frequent lesion in these toxoplasmosis cases, compared with non-infected animals (Fisher's exact test, $p = 0.0141$ and $p < 0.0001$ for myocarditis or myocardial necrosis, respectively). Myocarditis and myocardial necrosis were not always observed together; five cases of myocarditis had necrosis, two cases of myocarditis did not have necrosis, and two cases had only myocardial necrosis with no significant inflammatory component. Myocarditis was usually characterized by mild to moderate mixed inflammatory infiltrate composed of lymphocytes, plasma cells, histiocytes, and neutrophils. The myocardial necrosis was mild, focal to multifocal, lytic, and in most cases associated with intralesional tachyzoites, observed free or in the cytoplasm of interstitial histiocytes and cardiomyocytes. Bradyzoites were also observed in the cytoplasm of cardiomyocytes, in most of the cases with no inflammatory process associated. *T. gondii* zoites in the heart tissue were observed in ten cases (62.5%; 10/16; CI: 38.6-81.5%) in HE stained sections and in 12 cases by IHC (75%; 12/16; CI: 50.5-89.8%) (Figure 3.2).

Lungs: Pneumonia (81.2%; 13/16; CI: 57.0-93.4%) with large number of intra-alveolar foamy macrophages (50.0%; 8/16; CI: 28.0-70.0%) and fibrin deposition (31.2%; 5/16; CI: 14.2-55.6%) were the main pulmonary findings in infected animals (Fisher's exact test: $p = 0.0029$, $p < 0.0001$, and $p = 0.0088$ for pneumonia, alveolar foamy macrophages, and alveolar fibrin deposition, respectively). Pneumonia was usually interstitial and broncho-interstitial, mild to moderate, in some cases associated with multifocal areas of interstitial necrosis and mild to marked alveolar edema and hemorrhage (Figure 3.3E). Intralesional tachyzoites and bradyzoites were observed in nine cases (56.3%; 9/16; CI: 36.2-76.9%) in HE stained sections and in 12 cases by IHC (75%; 12/16; CI: 50.5-89.8%) (Figure 3.2), usually in the cytoplasm of alveolar macrophages and type II pneumocytes (Figure 3.3E and F).

Kidney: No specific findings were observed in the kidneys of infected animals. All had mild to moderate interstitial nephritis composed mainly of lymphocytes, plasma cells and histiocytes; however, this was also a frequent lesion in non-infected animals and was considered a non-specific finding (Fisher's exact test, $p > 0.9999$). Tachyzoites

and bradyzoites were observed in in the cytoplasm of histocytes in one animal (6.3%; 1/16; CI: 0.3-28.3%) in HE stained sections and in seven animals (43.7%; 7/16; CI: 23.1-66.8%) by IHC Together with brain, kidney was the tissue with the least frequency of intralesional zoites (Figure 3.2).

Brain: Lesions in the brain were rarely observed in these cases. There was one case of mild non-suppurative meningoencephalitis (6.3%; 1/16; CI: 0.3-28.3%) and one case of mild multifocal gliosis (6.3%; 1/16; CI: 0.3-28.3%). Intralesional tachyzoites and bradyzoites were observed in both cases by HE. IHC detected *T. gondii* zoites in ten cases (Figure 3.2), although in eight, they were not associated with any significant pathological findings.

Other tissues: Adrenalitis was observed in four animals, being associated with intralesional tachyzoites in two cases. There was one case with diffuse histiocytic enteritis with myriad intralesional tachyzoites.

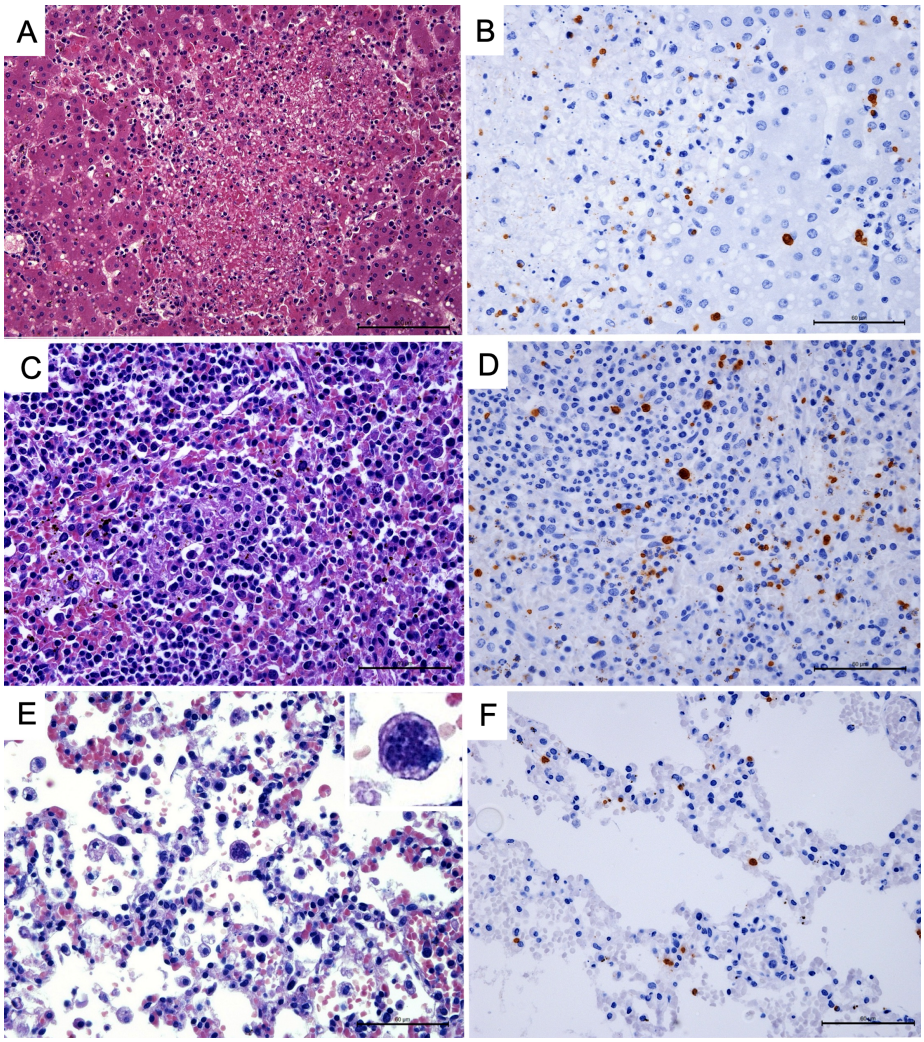


FIGURE 3.3 Pathological findings associated with toxoplasmosis in free-ranging marmosets (*Callithrix* spp.). (A) Random lytic necrotizing hepatitis with adjacent mild lipidosis, Liver, HE, Scale bar = 100 μm. (B) Immunostaining for *T. gondii* zoites, Liver, DAB, Scale bar = 60 μm. (C) Splenitis, histiocytic, with fibrin deposition in red pulp, Spleen, HE, Scale bar = 60 μm. (D) Immunostaining for *T. gondii* zoites, Spleen, DAB, Scale bar = 60 μm. (E) Interstitial pneumonia, characterized by alveolar walls expanded by histiocytes, lymphocytes and plasma cells, with moderate number of foamy macrophages, mild alveolar hemorrhage, diffuse congestion, and intracytoplasmic *Toxoplasma gondii* zoites (inset), Lung, HE, Scale bar = 60 μm. (F) Immunostaining for *T. gondii* zoites, Lung, DAB, Scale bar = 60 μm.

3.3 Transmission electron microscopy (TEM)

One of the *T. gondii* IHC positive cases (ID14; Table 3.1) was analyzed by TEM for ultrastructural characterization. Intralesional tachyzoite forms were identified as ovoid-shaped organisms with sizes ranging from 1.6 to 2.5 μm x 1.3 to 1.4 μm and characterized by a round nuclei, dense intracytoplasmic granules, and the presence of a conoid (Figure 3.4). No bradyzoites were observed.

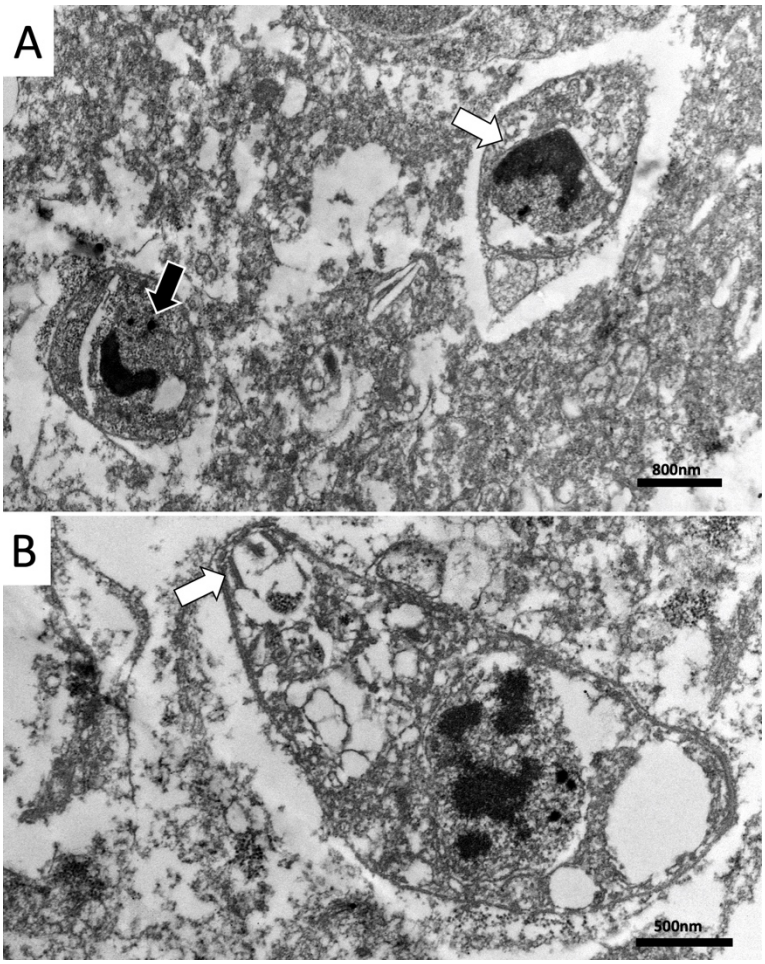


FIGURE 3.4 Transmission electron microscopy from the formalin-fixed paraffin-embedded lung of an infected marmoset. Tachyzoites of *Toxoplasma gondii*, (A) Two ovoid organisms with 1.6 to 2.5 μm x 1.3 to 1.4 μm, a distinct nucleus (white arrow) and electron-dense granules (black arrow), Scale bar = 800 nm. (B) Tachyzoite with one central nucleus and a visible conoid (white arrow), Scale bar = 500 nm.

4. DISCUSSION

This study describes the epidemiological and pathological aspects of fatal toxoplasmosis in a free-ranging population of marmosets (*Callithrix* spp.) in Brazil. The prevalence of fatal toxoplasmosis in the population from this study was 1.6%, and was not influenced by sex, age, geographical distribution, or year of death. Additionally, fatal toxoplasmosis in free-ranging marmosets was detected as single cases (cases from different geographical regions) and as outbreaks (multiple cases from the same geographical location found dead in the same month) (Figure 1), a similar profile to previously reported toxoplasmosis in captive NWP (Epiphanio et al., 2003; Paula et al., 2020; Santana et al., 2021).

Pathological findings of fatal toxoplasmosis in this study were characterized by a multifocal random necrotizing hepatitis, associated with random lipidosis; interstitial pneumonia rich in alveolar foamy macrophages and fibrin deposition; necrotizing myocarditis; and necrotizing splenitis. These findings agree with what has been described in cases of NWP lethal infections (Epiphanio et al., 2003; Grumann et al., 2017; Santos et al., 2018; Mätz-Rensing and Lowenstine, 2018; Paula et al., 2020; Santana et al., 2021). Necrotizing lesions are an important feature in *T. gondii* infection and are caused by the direct rupture of the cells by the tachyzoite forms during replication and exit from the host cell, leading to cell death and necrosis surrounded by an acute inflammatory response (Bhopale, 2003). *T. gondii* tachyzoites also invade and replicate in epithelial respiratory cells, endothelial cells, fibroblasts, and macrophages, causing a disruption in the pulmonary epithelia and capillary barrier, leading to an acute respiratory disease (Parker et al., 1981). Lung injuries are usually described in lethal NWP toxoplasmosis, being characterized as an acute interstitial pneumonia, in some cases associated with diffuse alveolar damage and detection of alveolar hyaline membranes (Epiphanio et al., 2003; Nishimura et al., 2019; Paula et al., 2020; Santana et al., 2021).

After being ingested by an intermediate host, e.g., NWP, the oocysts sporulate in the intestinal lumen by enzymatic degradation and the sporozoites released invade the host cells, changing to tachyzoites that disseminate through blood and lymphatic vessels to most organs (Bhopale, 2003). *T. gondii* zoites were observed in the liver from all cases of this study, followed by spleen, heart, and lung, and were less commonly observed in the brain and kidney. These data highlight the importance of liver and spleen in *T. gondii* detection, and together with the prevalence of pathological findings, indicate that liver is the organ of choice to be sampled for the diagnosis of toxoplasmosis by histopathology in marmosets (*Callithrix* spp.). Additionally, the concordance between HE and IHC was stronger in the liver, spleen, and heart, indicating that in these tissues, *T. gondii* zoites are easily observed, even by standard histopathological evaluation based on HE only. Brain, kidney, and lungs had moderate to substantial agreement, with detection being enhanced by IHC technique.

Random hepatic lipidosis was a frequent feature in toxoplasmosis cases from this study and was also described in previous captive NWP lethal cases (Paula et al., 2020; Santana et al., 2021). In our cases, it was usually observed adjacent to the necrotic areas, being interpreted as a degenerative cellular response to the inflammatory reaction and necrosis caused by the infection. Hemosiderosis has been considered a predisposing factor for toxoplasmosis in NWP (Epiphanio et al., 2003), being frequently reported in lethal cases (Epiphanio et al., 2003; Paula et al., 2020). However, in this study, hemosiderosis, characterized by iron storage within hepatocytes, was a nonspecific finding, being observed with similar prevalence in infected and non-infected animals.

The increase in density of human population associated with the reduction and fragmentation of wildlife habitats leads to proximity and habitat overlap among humans, domestic animals, and wild primates, dramatically increasing the potential for disease transmission and spillover (Gillespie et al., 2008; Aguirre, 2009; Brinkworth and Pechenkina, 2013). These inter-species transmission events can directly impact both the survival of wild animal populations and human public health (Brinkworth and Pechenkina, 2013). Marmosets are known for their ability to survive in urban and peri-urban environments, adapting to anthropogenic changes, often consuming human food remains, and exposing themselves to environmental risks of anthropized areas (Goulart et al., 2010). Importantly, all animals from this report were found dead in urbanized areas, where contact with domestic feline feces is thought to be extremely high,

enhancing the exposure of these NWP to *T. gondii* infective oocysts. Interestingly, *T. gondii* infected marmosets, in our study, had a higher frequency of *Platynosomum* spp. parasitism compared to non-infected animals. Domestic cat is considered the main definitive host in the *Platynosomum* spp. life cycle, as in toxoplasmosis (Basu and Charles, 2014). These data therefore reinforce that marmosets and domestic cats coinhabit the same environments in peri-urban and urbanized areas, and indicate that domestic cats, especially feral and roaming animals, are a potential source of zoonotic pathogens of One Health importance, to both wildlife and humans. Additionally, a serosurvey of toxoplasmosis in asymptomatic free-ranging marmosets from São Paulo, Brazil, found a prevalence of 16.6% (Molina et al., 2014). Based on the prevalence of 1.6% of fatal toxoplasmosis observed in our study, we hypothesize that lethality rates in exposed marmoset populations could be approximately 10%, i.e., one of ten marmosets exposed to *T. gondii* will probably have a lethal outcome.

Finally, marmosets are sentinels and hosts of other important zoonotic disease with great impact in public health and higher concern in One Health studies, such as YF. During the period of this study the Brazilian Southeast region underwent a major sylvatic YF outbreak (Santos et al., 2020; Fernandes et al., 2021b). Therefore, YF was an important differential diagnosis in these cases, especially because all these cases coincided with the YF outbreak. Grossly, there are no significant differences between these diseases: both are associated with icterus and an enlargement of the liver that is usually diffusely pale to yellowish, with multifocal reddish areas compatible with hemorrhage (Epiphanio et al., 2003; Mätz-Rensing and Lowenstine, 2018; Paula et al., 2020; Santana et al., 2021). Therefore, histopathology is essential to differentiate these two important zoonotic diseases. YF in susceptible NWP is characterized by a midzonal to massive necrotizing hepatitis with coagulative necrosis, scarce inflammatory cells, and apoptotic hepatocytes, which are traditionally called Councilman-Rocha Lima bodies (Leal et al., 2016; Santos et al., 2020; Fernandes et al., 2021a; Fernandes et al., 2021b). Animals infected with toxoplasmosis, usually develop a multifocal random necrotizing hepatitis with lytic necrosis, variable inflammatory infiltrate and intralesional zoites, as was observed in our cases (Epiphanio et al., 2003; Grumann et al., 2017; Santos et al., 2018; Mätz-Rensing and Lowenstine, 2018; Paula et al., 2020; Santana et al., 2021). Importantly, in agreement with histopathological findings, no

infected marmoset from this study was co-infected with YFV, based on negative RT-qPCR and IHC testing for YFV performed at the official diagnostic laboratory.

REFERENCES

1. Mätz-Rensing K, Lowenstine. New World and Old World Monkeys. In: Terio KA, McAloose D, Leger JS. Pathology of Wildlife and Zoo Animals. Elsevier Inc: Cambridge. 2018; 343-374.

2. Paula NF, Dutra KS, Oliveira AR, Santos DO, Rocha CEV, Vitor RWA, Tinoco HP, Costa MEL, Paixão TA, Santos RL. Host range and susceptibility to Toxoplasma gondii infection in captive neotropical and Old-world primates. *J Med Primatol*. 2020;49(4):202–210.

3. Grumann MR, Silva Z da, Filho JR da S, Costa MM, Vieira MIB, Motta AC. Immunohistochemical and serological aspects of *Toxoplasma gondii* infection in neotropical primates. *Semina Ciências Agrárias*. 2016;38(3):1375–1382.

4. Santos SV, Pena HFJ, Talebi MG, Teixeira RHF, Kanamura CT, Diaz-Delgado J, Gennari SM, Catão-Dias JL. Fatal toxoplasmosis in a southern muriqui (*Brachyteles arachnoides*) from São Paulo state, Brazil: Pathological, immunohistochemical, and molecular characterization. *J Med Primatol*. 2018;47:124-127.

5. Santana CH, Oliveira AR, Santos DO, Pimentel SP, Souza LR, Moreira LGA, Braz HMB, Carvalho TP, Lopes CEB, Oliveira JBS, Paula NF, Carvalho MPN, Alves BF, Pena HFJ, Santos RL. Genotyping of *Toxoplasma gondii* in a lethal toxoplasmosis outbreak affecting captive howler monkeys (*Alouatta* sp.). *J Med Primatol*. 2021;50(2):99–107.

6. Moreira SB, Pereira AHB, Pissinatti TA, Arruda IF, Azevedo RRM, Schiffler FB, Outbreak Workgroup, Amendoeira MRR, Santos AFA, Pissinatti A, Ubiali DG. Subacute multisystemic toxoplasmosis in a captive blackand-gold howler monkey (*Alouatta caraya*) indicates therapy challenging. *J Med Primatol*. 2022; DOI: 10.1111/jmp.12600

7. Molina CV, Catão-Dias JL, Neto JSF, Vasconcellos SA, Gennari SM, Valle RDR, Souza GO, Morais ZM, Vitaliano SN, Strefezzi RF, Bueno MG. Sero-epidemiological survey for brucellosis, leptospirosis, and toxoplasmosis in free-ranging *Alouatta caraya* and *Callithrix penicillata* from São Paulo State, Brazil. *J Med Primatol*. 2014;43(3):197–201.

8. Silva RC, Machado GP, Cruvinel TMA, Cruvinel CA, Langoni H. Detection of antibodies to *Toxoplasma gondii* in wild animals in Brazil. *J Venom Anim Toxins Incl Trop Dis.* 2014; 20(41):1-4.

9. Bueno MG, Catão-Dias JL, Laroque P de O, Vasconcellos AS, Ferreira Neto JS, Gennari SM, Ferreira F, Laurenti MD, Umezwa ES, Kesper N, Kirchgatter K, Guimarães LO, Pavanato HJ, Valença-Montenegro MM. Infectious Diseases in Free-Ranging Blonde Capuchins, *Sapajus flavius*, in Brazil. *Int J Primatol*. 2017;38(6):1017–1031.

10. Molina CV, Krawczak F da S, Bueno MG, Soares HS, Genari SM, Pissinatti A, Kierulff MCM, Silva TF, Freitas DG, Caneli LC, Catão-Dias JL. Negative serosurvey of *Toxoplasma gondii* antibodies in

Golden-headed Lion Tamarin (*Leontopithecus chrysomelas*) from Niterói/RJ, Brazil. *Rev Bras Parasitol Vet*. 2016;26(01):115–118.

11. Niehaus C, Spínola M, Su C, Rojas N, Rico-Chavez O, Ibarra-Cerdeña CN, Foley J, Suzán G, Gutiérrez-Espeleta GA, Chaves A. Environmental factors associated with *Toxoplasma gondii* Exposure in Neotropical Primates of Costa Rica. *Frontiers Vet Sci*. 2020;7:583032.

12. Ehlers LP, Slaviero M, Bianchi MV, Mello LS, Lorenzo C, Surita LE, Alievi MM, Driemeier D, Pavarini SP, Sonne L. Causes of death in neotropical primates in Rio Grande do Sul State, Southern Brazil. *J Med Primatol*. 2022;51(2):85–92.

13. Epiphanio S, Sinhorini IL, Catão-Dias JL. Pathology of toxoplasmosis in captive New World primates. *J Comp Pathol.* 2003;129:196–204.

14. Bhopale GM. Pathogenesis of toxoplasmosis. *Comp Immunol Microbiol Infect Dis*. 2003;26(4):213-222.

15. Parker GA, Langloss JM, Dubey JP, Hoover EA. Pathogenesis of acute toxoplasmosis in specific-pathogen-free cats. *Vet Pathol.* 1981 Nov;18(6):786-803

16. Nishimura M, Goyama T, Tomikawa S, Fereig RM, El-Alfy EN, Nagamune K, Kobayashi Y, Nishikawa Y. Outbreak of toxoplas- mosis in four squirrel monkeys (*Saimiri sciureus*) in Japan. *Parasitol Int*. 2019;68(1):79-86.

17. Gillespie TR, Nunn CL, Leendertz FH. Integrative approaches to the study of primate infectious disease: implications for biodiversity conservation and global health. *Yearb Phys Anthropol.* 2008;51 53-69.

18. Aguirre AA. Wild canids as sentinels of ecological health: a conservation medicine perspective. *Parasit Vectors*. 2009;2(1):7.

19. Brinkworth IF, Pechenkina K. Primates, Pathogens and Evolution: An Introduction. In: Brinkworth IF, Pechenkina K. Primates, Pathogens and Evolution. Springer: New York. 2013; 1-14.

20. Goulart VDLR, Teixeira CP, Young RJ. Analysis of callouts made in relation to wild urban marmosets (*Callithrix penicillata*) and their implications for urban species management. *Eur J Wildlife Res*. 2010;56(4):642-649.

21. Basu AK, Charles AR. A review of the cat liver fluke *Platynosomum fastosum* Kossack, 1910 (Trematoda: Dicrocoeliidae). *Vet Parasitol*. 2014;200:1-7.

22. Leal SG, Romano APM, Monteiro RV, Melo CB de, Vasconcelos PF da C, Castro MB. Frequency of histopathological changes in Howler monkeys (*Alouatta* sp.) naturally infected with yellow fever virus in Brazil. *Rev Soc Bras Med Tro*. 2016;49(1):29–33.

23. Santos DO, Oliveira AR, Lucena FP, Matto AS, Carvalho TP, Costa FB, Moreira LGA, Paixão TA, Santos RL. Histopathologic Patterns and Susceptibility of Neotropical Primates Naturally Infected With Yellow Fever Virus. *Vet Pathol*. 2020;57(5):681–686.

24. Fernandes NCC de A, Cunha MS, Guerra JM, Diaz-DelgadoJ, Ressio RA, Cirqueira CS, Kanamura CT, Fuentes-Castilho D, Catão-Dias JL. Yellow Fever as Cause of Death of Titi Monkeys (*Callicebus* spp.). *Vet Pathol*. 2021a;58(4):730–735.

25. Fernandes NCCA, Guerra JM, Díaz-Delgado J, Cunha MS, Saad L, Iglezias SD, Ressio RA, Cirqueira CS, Kanamura CT, Jesus IP, Maeda AY, Vasami FGS, Carvalho J, Araújo LJT, Souza RP, Nogueira JS, Spinola RMF, Catão-Dias JL. Differential Yellow Fever Susceptibility in New World Nonhuman Primates, Comparison with Humans, and Implications for Surveillance. *Emerg Infect Dis*. 2021b;27(1):47–56.

CHAPTER IV[4]

NON-THROMBOTIC PULMONARY EMBOLISM OF BRAIN, LIVER, OR BONE MARROW TISSUES ASSOCIATED WITH TRAUMATIC INJURIES IN FREE-RANGING NEOTROPICAL PRIMATES

ABSTRACT

From 2016 to 2019, Southeastern Brazil faced an outbreak of yellow fever (YF) affecting both humans and New World primates (NWP). The outbreak was associated with a marked increase in traumatic lesions in NWP in the affected regions. Non-thrombotic pulmonary embolization (NTPE) can be a consequence of massive traumatic events, and it is rarely reported in human and veterinary medicine. Here, we describe NTPE of the brain, liver, and bone marrow in free-ranging NWP, highlighting the epidemiological aspects of these findings and the lesions associated with this condition, including data on traumatic injuries in wild NWP populations during the course of a recent YF outbreak. A total of 1078 NWP were necropsied from January 2017 to July 2019. Gross traumatic injuries were observed in 444 marmosets (44.3%), 10 howler monkeys (23.2%), 9 capuchins (31.0%), 1 titi-monkey (50.0%), and 1 golden lion tamarin (33.3%). NTPE was observed in 10 animals, including 9 marmosets (2.0%) and 1 howler monkey (10.0%). NTPE was identified in the lung and comprised hepatic tissue in 1 case, brain tissue in 1 case, and bone marrow tissue in 8 cases. Although uncommon, it is important to consider NTPE with pulmonary vascular occlusion during the critical care of traumatized NWP. In addition, this study highlights the importance of conservational strategies and environmental education focusing on One Health, not only to protect these free-ranging NWP populations but also to maintain the efficacy of epidemiological surveillance programs.

Keywords: marmoset, howler monkey, capuchin, lion tamarin, titi-monkey, embolism

[4] Chapter published in the journal *Veterinary Pathology* (Doi: 10.1177/03009858221075595)

1. INTRODUCTION

Traumatic injuries are well characterized in veterinary medicine, with a particular focus on forensic pathology (Finnie, 2016; Ressel et al., 2016; Siqueira et al; 2016; Wohlsein et al., 2016). In free-ranging New World (neotropical) primates (NWP), traumatic injuries are important and prevalent, usually associated with periurban NWP populations and anthropogenic factors, and often have a negative impact on NWP conservation strategies (Fernandes et al., 2019; Lucena et al., 2017; Pereira et al., 2020; Teixeira et al., 2018).

Non-thrombotic pulmonary embolism (NTPE) may be a consequence of trauma, but it is less common than pulmonary thromboembolism. NTPE is defined as the occlusion of pulmonary arteries and capillaries by non-thrombotic material, including adipose tissue, bone marrow, foreign material, gas, neoplastic cells, or infectious agents (Montagnana et al., 2010). Both in humans and in animals, NTPE caused by brain, liver, or bone marrow tissues is uncommon and is usually associated with massive traumatic injuries (Caswell et al., 2016; Husain et al., 2021; Michalodimitrakis et al., 1998; Neto et al., 2020; Warren and Goodhue, 2013).

From 2016 to 2019, an outbreak of yellow fever (YF) took place in Southeastern Brazil affecting humans and NWP. This outbreak resulted in high lethality rates in NWP populations, especially howler monkeys, due to their high susceptibility to this disease (Fernandes et al., 2021; Mares-Guia et al., 2020; Santos et al., 2020). This YF outbreak was associated with a marked increase in trauma affecting free-ranging NWP from the regions affected by the YF outbreak. Due to misinformation and fear among the human population, and the misconception that NWP were responsible for transmitting YF, hundreds of NWP were brutally killed (Lucena et al., 2017; Teixeira et al., 2018).

Here, we describe a series of cases of NTPE caused by fragments of brain, liver, and bone marrow tissues in traumatized free-ranging NWP, highlighting the epidemiological aspects of these findings and the lesions associated with this condition, including additional data on traumatic injuries affecting these wild NWP populations. The diagnosis of embolic lesions as described in this study may allow identification of traumatized animals even when examining an incomplete set of tissue samples in the absence of a comprehensive gross report. Importantly, although previously recognized in humans, with the exception of bone marrow and fat embolism in experimental

cynomolgus macaques (Fong et al., 2011), these embolic lesions have not been reported in free-ranging nonhuman primates.

2. MATERIAL AND METHODS

2.1 Animals

This study included NWP necropsied between January 2017 and July 2019 at the Instituto Municipal de Medicina Veterinária Jorge Vaitsman (IJV, Rio de Janeiro, Brazil), including wild NWP that were found dead from all regions in the State of Rio de Janeiro. Data about geographic origin, sex, age, and gross findings were provided by the IJV. All animals were tested for YF virus infection by quantitative reverse transcription polymerase chain reaction (RT-qPCR) by the official diagnostic service. This study was authorized by the government environmental agency (ICMBio, Brazil) under the SISBIO license number 67014. All procedures strictly adhered to humane care of animals and all applicable laws and regulations, including registration in the national system for management of genetic heritage and associated traditional knowledge by SISGEN code A2743E4. This was a retrospective study based on diagnostic samples submitted for laboratory analysis.

2.2 Necropsy and Histopathology

Necropsies of NWP were performed by a veterinary pathologist at the IJV. Samples of lung, liver, heart, kidney, spleen, and brain were fixed in 10% buffered formalin and processed for paraffin embedding. Additional organ samples were collected in cases with gross changes in any organ or tissue. Paraffin-embedded tissue sections (3–4 μm thick) were stained with hematoxylin and eosin (HE) and analyzed under light microcopy.

2.3 Immunohistochemistry

Anti-hepatocyte-specific antigen (Hep Par 1) monoclonal antibody was used in cases of NTPE suspected to be composed of liver tissue; anti–glial fibrillary acidic protein (GFAP) and anti–neurofilament protein were used for NTPE suspected to be composed of brain tissue; and anti-myeloperoxidase (MPO) was used for NTPE suspected to be composed of bone marrow tissue. Antigen retrieval protocols and information about primary antibodies are detailed in Table 4.1. Negative controls included sections incubated with phosphate-buffered saline instead of the primary antibody.

Table 4.1 Primary antibodies and protocols used for immunohistochemistry in this study.

Target Antigen	Clone	Dilution	Antigen Retrieval	Source (code)
Hepatocyte-specific antigen (Hep Par-1)	OCH1E5	1:100	Pressurized heat (120ºC/10min); High pH	Cell Marque
Glial fibrillary acidic protein (GFAP)	Polyclonal	Ready-to-use	Pressurized heat (120ºC/10min); High pH	Dako Denmark
Neurofilament Protein	2F11	Ready-to-use	Pressurized heat (120ºC/10min); High pH	Dako Denmark
Myeloperoxidase (MPO)	A-5	1:100	Not performed	Santa Cruz Biotechnology
von Willebrand factor (VWF)	C-12	1:500	Pressurized heat (120ºC/10min); High pH	Santa Cruz Biotechnology
Glycophorin A (GA)	JC159	1:100	Pressurized heat (120ºC/10min); High pH	Santa Cruz Biotechnology

Briefly, lung samples with tissue embolism were selected based on evaluation of HE-stained slides. Lung sections (3–4 μm thick) were mounted on silanized slides, deparaffinized in xylene, and hydrated in decreasing ethanol concentrations. Endogenous peroxidase activity and nonspecific protein anti- body binding were blocked using a blocking solution provided with a commercially available kit (Envision®; Dako). After incubation with the primary antibodies, slides were rinsed and incubated with a secondary anti-mouse/anti-rabbit antibody conjugated with a polymer for 30 minutes at room temperature (Envision®; Dako). Reaction was revealed with a 3,3′-diaminobenzidine (DAB) solution, and sections were then counter- stained with Mayer's hematoxylin. Between all steps mentioned before, slides were washed with wash buffer provided by a commercially available kit (Envision®; Dako).

2.4 Statistical Analysis

Data were analyzed using the GraphPad Prism software (version 9.0). Descriptive statistics and Fisher's exact test for non- parametric data were performed.

3. RESULTS

3.1 Epidemiologic data on traumatic cases

A total of 1078 NWP were necropsied from January 2017 to July 2019, including 1001 marmosets (*Callithrix* sp.), 43 howler monkeys (*Alouatta* sp.), 29 capuchins (*Sapajus* sp.), 3 lion tamarins (*Leontopithecus* sp.), and 2 titi-monkeys (*Callicebus* sp.). Grossly identifiable traumatic injuries were observed in 444 marmosets (44.3%; 444/1001), 10 howler monkeys (23.2%; 10/43), 9 capuchins (31.0%; 9/29), 1 titi- monkey (50.0%; 1/2), and 1 golden lion tamarin (33.3%; 1/3).

Gross traumatic lesions were grouped into blunt or sharp trauma (contusions, lacerations, perforations, organ ruptures, and fractures) or thermal injuries (burns) (Table 4.2). There were no lesions attributable to gun shots, and no firearm projectiles were detected in any of the animals. Considering all genera included in this study, more than 90% of traumatic lesions were due to blunt or sharp trauma, affecting the head, thorax, abdomen, arms, legs, and tail. In addition, there were cases with a single traumatic injury and cases characterized as polytraumatized (multiple affected sites) (Table 4.2). In marmosets, gross traumatic injuries were observed more often in the head ($p < 0.01$) and less frequently in the limbs and tail ($p < 0.01$). Howler monkeys and capuchins did not have statistically significant differences in the frequency of affected sites. The titi-monkey had a fissure in the occipital bone with focal subcutaneous hematoma and a submeningeal blood clot, whereas the lion tamarin had severe head trauma with multiple fractures of the skull, severe loss of brain tissue, fractures of several ribs with laceration of intercostal muscles, and rupture of the diaphragm and the liver.

Gross traumatic lesions were observed more frequently in marmosets than in howler monkeys ($p < 0.01$). Traumatized juvenile marmosets were more frequent than adults ($p < 0.01$), with no statistically significant difference between males and females. Among howler monkeys, traumatized males were more frequent than females ($p < 0.001$). Traumatic lesions were not associated with YF virus infection in marmosets ($p = 0.2559$), howler monkeys ($p = 0.2661$), or capuchin ($p = 0.2917$). Additional details about these cases are provided in Table 4.3.

3.2 NTPE Cases

NTPE was observed in 10 animals (cases 1–10; Table 4.4), including 9 marmosets (2.0%, 9/444) and 1 howler monkey (10.0%, 1/10), with no statistically significant difference in the frequency of NTPE between these 2 genera. NTPE was only observed in the lung. In marmosets, the NTPE was composed of liver, brain, or

bone marrow tissues, whereas the only case affecting a howler monkey involved bone marrow embolism.

NTPE of hepatic tissue was observed in only 1 animal (case 1), an adult female marmoset, polytraumatized, with multiple sites of rupture of the liver, lungs, and spleen. Microscopically, there were multiple emboli measuring 200 to 800 μm in diameter, occluding arteries in the pulmonary parenchyma. The emboli were characterized by cords of epithelial cells with morphology compatible with hepatocytes delimiting vascular spaces similar to hepatic sinusoids (Fig. 4.1A). All emboli in this case had a strong and diffusely positive cytoplasmic immunolabeling for Hep Par 1, confirming the hepatic origin of the emboli (Fig. 4.1B). There was moderate multifocal alveolar edema and hemorrhage, but these changes were not restricted to areas affected by embolism.

NTPE of brain tissue (case 2) was observed in only 1 animal, an adult female marmoset, also polytraumatized, with multiple fractures of the skull, jaw, and ribs, as well as brain evisceration and laceration, with severe meningeal hemorrhage. Microscopically, emboli occluded 2 pulmonary arteries, measuring 250 to 300 μm in diameter. Emboli in this case were characterized by a hypocellular tissue with a web pattern and rare cells with small nuclei, morphologically compatible with oligodendrocytes, suggestive of white matter (Fig. 4.2A). The emboli had a strong and diffusely positive immunolabeling for both GFAP and neurofilament protein (Fig. 4.2B, C). There was marked congestion particularly affecting larger arterioles, which was probably due to vascular occlusion by the emboli. In addition, there was a mild multifocal interstitial pneumonia, which was not associated with embolism.

Eight of the NTPE cases diagnosed in this study were composed of bone marrow tissue (cases 3–10). Seven of these cases affected marmosets and 1 case was observed in a howler monkey. All were associated with blunt trauma. Bone fractures were observed in all marmosets with this kind of embolism, but not in the affected howler monkey (Table 4.4). Histopathology from these cases revealed a few to several small pulmonary arteries filled with a tissue composed of a heterogeneous population of myeloid cells interspersed with adipose tissue, ranging from 50 to 200 μm in diameter (Fig. 4.3A). By immunohistochemistry, the embolic tissues had positive heterogeneous immunolabeling for MPO, compatible with a polymorphic population of myeloid cell lineage, supporting the morphologic identification of the emboli as bone marrow tissue (Fig. 4.3B).

Pulmonary alveolar hemorrhage and edema was observed in all cases with embolism, but it was not restricted to sites of embolism. In addition, the howler monkey with NTPE was also positive for YF virus, presenting the typical massive necrotizing hepatitis with Councilman-Rocha Lima bodies. Gross and histopathological findings from these 10 cases are detailed in Supplemental Table 4.4.

Table 4.2 Types and distribution of gross traumatic lesions identified in the period of January 2017 to July 2019 in five different genera of free-ranging NWP.

	Marmoset (n = 444)		Howler monkey (n = 10)		Capuchin (n = 9)		Titi-monkey (n = 1)		Lion tamarin (n = 1)	
Types of trauma	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Blunt and sharp trauma	429	96.6	9	90.0	9	100.0	1	100.0	1	100.0
Head	323	72.7[a]	2	20.0[a]	6	66.7[a]	1	100.0	1	100.0
Thorax	114	25.6[b]	5	50.0[a]	2	22.2[a]	0	0.0	1	100.0
Abdomen	104	23.4[b]	6	60.0[a]	0	0.0[a]	0	0.0	1	100.0
Members	37	8.3[c]	4	40.0[a]	3	33.3[a]	0	0.0	0	0.0
Tail	6	1.3[d]	0	0.0[a]	0	0.0[a]	0	0.0	0	0.0
Thermal injuries	21	4.7	1	10.0	1	11.1	0	0.0	0	0.0
Number traumatic sites										
Single (01)	301	70.2[a]	4	44.4[a]	5	62.5[a]	1	100.0	0	0.0
Polytraumatized (> 1)	128	29.8[a]	5	55.6[a]	3	37.5[a]	0	0.0	1	100.0

Legend: different letters in the same column indicate statistically significant differences (Fisher's exact test $p < 0.01$). Abbreviation: n, number of animals.

Table 4.3 Epidemiological data from the cases with gross traumatic lesions identified in the period of January 2017 to July 2019 in five genera of free-ranging New World primates.

	Marmoset (n = 1001)		Howler monkey (n = 43)		Capuchin (n = 29)		Titi-monkey (n = 2)		Lion tamarin (n = 3)	
General	**T (%)**	**NT (%)**	**T (%)**	**NT (%)**	**T (%)**	**NT (%)**	**T (%)**	**NT (%)**	**T (%)**	**NT (%)**
	44.35^{A}	55.65	23.25^{B}	76.75^{b}	$31.03^{A,B}$	$68.97^{a,b}$	50.00	50.00	33.33	66.67
Official YF diagnosis										
Positive	4.12^{a}	2.55^{a}	40.00	64.00^{a}	14.29^{a}	0.00^{a}	100.00	100.00	0.00	50.00
Negative	95.88^{a}	97.45^{a}	60.00	36.00^{a}	85.71^{a}	100.00^{a}	0.00	0.00	100.00	50.00
Sex										
Male	52.96^{a}	48.68^{a}	100.00^{a}	77.27^{b}	77.78^{a}	72.22^{a}	0.00	0.00	100.00	50.00
Female	47.04^{a}	51.32^{a}	0.00^{a}	22.73^{b}	22.22^{a}	27.78^{a}	100.00	100.00	0.00	50.00
Age										
Adult	76.62^{a}	84.04^{b}	80.00^{a}	76.00^{a}	75.00^{a}	94.12^{a}	100.00	100.00	0.00	100.00
Juvenile	23.38^{a}	15.96^{b}	20.00^{a}	24.00^{a}	25.00^{a}	5.88^{a}	0.00	0.00	100.00	0.00
Geographic distribution in the State of Rio de Janeiro										
Metropolitan	79.95^{a}	84.22^{a}	0.00^{a}	8.00^{a}	77.78^{a}	94.74^{a}	0.00	0.00	0.00	50.00
Others*	20.05^{a}	15.78^{a}	100.00^{a}	92.00^{a}	22.22^{a}	5.26^{a}	100.00	100.00	100.00	50.00

Different uppercase letters in the first row indicate statistically significant differences among genera, whereas different lowercase letters indicate statistically significant differences between T and NT animals in each genus according to yellow fever diagnosis, sex, age, and geographic distribution (Fisher's exact test; $p < 0.01$). Abbreviations: n, total number of primates; T, traumatized; NT, non-traumatized.

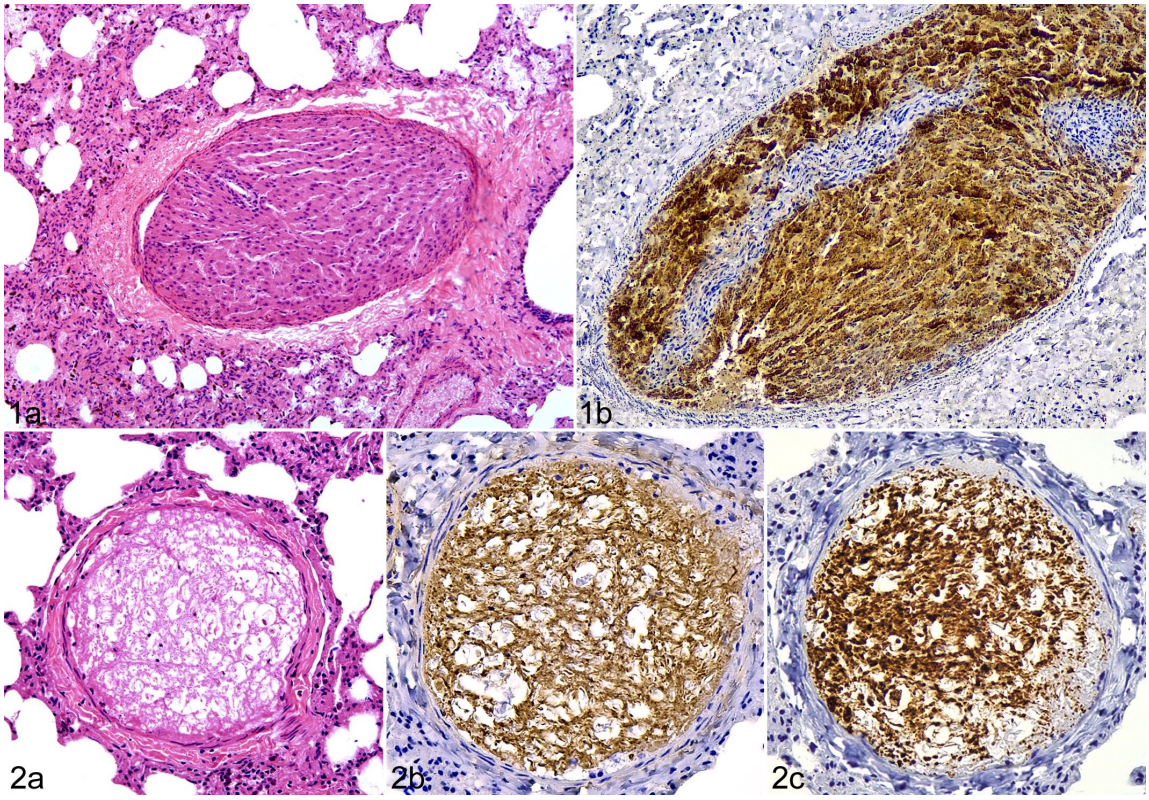
Table 4.4 Epidemiological and anatomopathological data from the ten cases of pulmonary embolism in free-ranging New World primates, associated with traumatic injuries in the period of January 2017 to July 2019.

ID	Specie	Sex	Age	Region	Official YF diagnose	Type of embolism	Gross findings	Histopathology*
1	*Callithrix* sp.	Female	Adult	Low Coast	Negative	Hepatic	Mild epistaxis Left rib fractures Multiple ruptures of the lungs, liver, and spleen Hemoperitoneum	Lungs: alveolar hemorrhage and edema, multifocal, moderate Spleen: lymphoid hyperplasia, moderate. Liver: LHP and neutrophilic portal hepatitis, multifocal, mild
2	*Callithrix* sp.	Female	Adult	Metropolitan	Negative	Brain	Multiple fractures of the skull, jaw, and ribs Brain evisceration and laceration with severe hemorrhage Rupture of the spleen Hemoperitoneum	Lungs: LHP interstitial pneumonia, multifocal, mild and severe, diffuse, congestion.
3	*Callithrix* sp.	Female	Adult	Metropolitan	Negative	Bone marrow	Multiple fractures of the skull Brain evisceration Multiple ruptures of the lungs Hemothorax	Lung: LP and neutrophilic interstitial pneumonia, multifocal, mild, with alveolar hemorrhage and edema, multifocal, moderate Liver: LHP and neutrophilic random hepatitis, multifocal, mild Kidney: LHP and neutrophilic interstitial nephritis, multifocal, mild

4	*Callithrix* sp.	Female	Adult	Fluminense South	Negative	Bone marrow	Moderate hemorrhage in meninges Left femur fracture Thoracic subcutaneous hematoma Hemopericardium Large intestine rupture	Lung: alveolar hemorrhage and edema, multifocal, moderate Liver: LP and neutrophilic portal and random hepatitis, multifocal, moderate
5	*Callithrix* sp.	Female	Adult	Fluminense Center	Negative	Bone marrow	Multiple fractures of the skull	Lung: alveolar edema, diffuse, severe, with alveolar hemorrhage, multifocal, mild Liver: LP and neutrophilic random hepatitis, multifocal, mild, with glycogen storage, diffuse, moderate
6	*Callithrix* sp.	Female	Adult	Low Coast	Negative	Bone marrow	Multiple fractures of the skull with flattening of the head and loss of brain mass	Lung: alveolar edema, diffuse moderate Liver: LHP and neutrophilic random hepatitis, multifocal, mild, with glycogen storage, diffuse, moderate
7	*Callithrix* sp.	Male	Adult	Metropolitan	Negative	Bone marrow	Femur fracture Hemoperitoneum	Lung: alveolar hemorrhage and edema, multifocal, severe Liver: sclerosant and proliferative portal hepatitis, multifocal, moderate Kidney: fibrosing LHP interstitial nephritis, multifocal to coalescent, severe, with membranous glomerulopathy and glomerulosclerosis, multifocal, moderate, and multiple retention cysts.

8	*Callithrix* sp.	Male	Juvenile	Metropolitan	Negative	Bone marrow	Occipital bone fracture	Lung: LHP interstitial pneumonia, multifocal, moderate, with alveolar edema and hemorrhage, multifocal, moderate Liver: LHP and neutrophilic random hepatitis, multifocal, mild
9	*Callithrix* sp.	Female	Adult	Metropolitan	Negative	Bone marrow	Parietal bone fracture Hemothorax	Lung: alveolar edema, diffuse, mild Liver: LP and neutrophilic random hepatitis, multifocal, mild, with glycogen storage, diffuse, mild, and sclerosant and proliferative cholangiohepatitis, multifocal, moderate
10	*Alouatta* sp.	Male	Adult	Fluminense Center	Positive	Bone marrow	Liver diffusely yellow and friable with multiple ruptures Hemoperitoneum Subcutaneous hematoma in inguinal region	Lung: alveolar hemorrhage, diffuse, severe, and alveolar edema, multifocal, mild Liver: necrotizing hepatitis, diffuse, severe, with Councilman-Rocha Lima bodies and portal lipidosis, diffuse, severe Kidney: LHP and neutrophilic interstitial nephritis with tubular necrosis, multifocal, mild

*abbreviations: ID: Individual numbers; LP: lymphoplasmacytic; LHP: lymphohistioplasmacytic.



Figures 4.1 Non-thrombotic pulmonary embolism composed of hepatic tissue, lung, marmoset. Figure 4.1. (A) Pulmonary artery occluded by hepatic tissue, with preservation of a trabecular pattern. Hematoxylin and eosin (HE). (B) Strong and diffuse cytoplasmic immunolabeling. Immunohistochemistry (IHC) for Hep Par 1. **Figure 4.2 Non-thrombotic pulmonary embolism composed of brain tissue, lung, marmoset.** (A) Pulmonary artery occluded with brain tissue morphologically compatible with white matter. HE. (B) Strong and diffuse immunolabeling. IHC for glial fibrillary acidic protein. (C) Strong and diffuse immunolabeling. IHC for neurofilament protein.

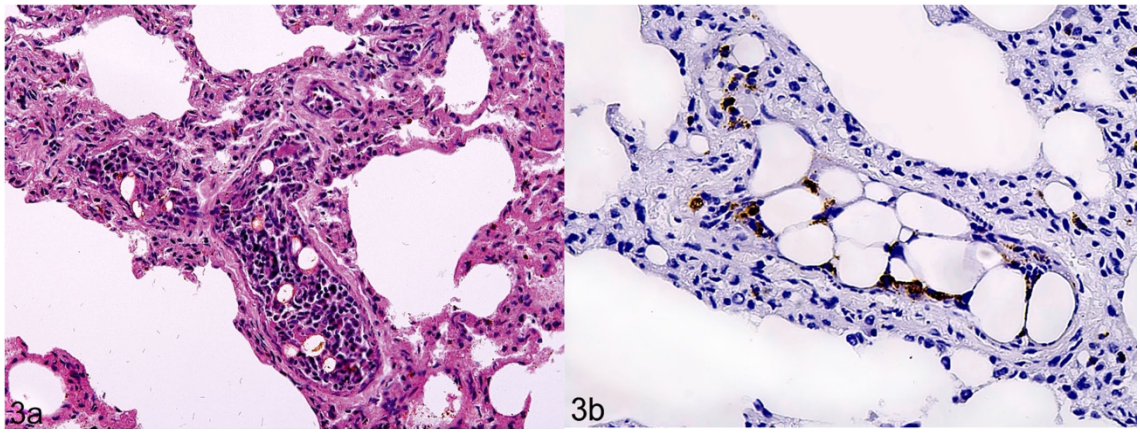


Figure 4.3 Non-thrombotic pulmonary embolism composed of bone marrow tissue, lung, marmoset. (A) Pulmonary artery occluded with bone marrow and fat tissue. Hematoxylin eosin. (B) Heterogeneous immunolabeling. Immunohistochemistry for myeloperoxidase.

4. DISCUSSION

NWP belonging to the Callitrichidae family, especially *Callithrix* spp., have a high ability to survive in urbanized fragmented habitats, adapting well to anthropogenic changes (Goulart et al., 2010). However, this also makes them more exposed to anthropogenic trauma, such as electrocution, roadkill, dog attack, and direct human aggression (Fernandes et al., 2019; Lucena et al., 2017; Pereira et al., 2020; Teixeira et al., 2018). Importantly, in our study, *Callithrix* spp. (marmosets) were the main genus affected by traumatic events, and although it was not possible to precisely identify the cause of the trauma, blunt and sharp trauma represented more than 90% of the cases, and the head was the most commonly affected site in marmosets. The higher frequency of traumatic lesions in marmosets than in howler monkeys is likely explained by their social behavior as marmosets are highly susceptible to anthropogenic influence when compared with howler monkeys, which rarely get in close proximity to humans. Under these conditions, juvenile marmosets are supposedly less experienced and/or less agile, and therefore tend to be more susceptible to human-induced trauma.

There was no correlation between trauma and YF infection, indicating that many NWP deaths reported during the course of the YF outbreak were not associated with YF infection itself. Therefore, although the YF outbreak resulted in a great impact on wild NWP populations (Dietz et al., 2019; Mares-Guia et al., 2020; Strier et al., 2019), especially among highly susceptible hosts such as howler monkeys (Fernandes et al., 2021; Santos et al., 2020), the indirect impact caused by high numbers of traumatic events during the same period is also very concerning and highlights the urgent need for environmental education and better strategies for wild NWP conservation. Furthermore, preservation of free-ranging NWP populations is not only an environmental conservation need, but it also has a significant public health relevance, as their phylogenetic proximity with humans makes nonhuman primates sentinels for certain zoonotic diseases such as YF (Leite et al., 2008; Moreno et al., 2013). Thus, impairment of their natural habitat may lead to impairment of epidemiological surveillance programs.

In forensic pathology, NTPE composed of hepatic tissue is rare; it has been reported in humans involved in motor vehicle accidents, with multiple lacerations of the liver, symptoms related to acute pulmonary occlusion and rapid progressing to death (Michalodimitrakis et al., 1998; Tozzini et al., 2003). The one marmoset with NTPE

composed of liver tissue in this study also had multiple lacerations of the liver with severe hemoperitoneum, rupture of the lungs and spleen, as well as multiple fractures in the ribs. Cases of pulmonary embolism caused by brain tissue are also uncommon, and they are usually associated with massive skull damage and cerebral laceration, with an incidence ranging from 1.9% to 10.0% in human cases of death by head trauma (Collins et al., 1994; Ogilvy et al., 1988; Warren and Goodhue, 2013). In animals, cases of NTPE composed of brain tissue are reported in cattle subjected to slaughter using captive bolt guns (Caswell et al., 2016), and rarely in dogs and birds with severe head trauma (JPC, 2014; Neto et al., 2020). Therefore, head injury with a severe brain tissue involvement is the cause of this type of NTPE as observed in a marmoset in this study.

In contrast, bone marrow embolism of the lung is more commonly reported. In addition to traumatic injuries, this kind of embolism is also associated with other causes of bone marrow disruption, such as bone infarction secondary to sickle cell anemia or bone marrow neoplasia (Hussain, 2021; Husebye et al., 2006). There is only 1 previous report of NTPE in nonhuman primates caused by bone marrow and fat tissues, which included 5 cases affecting cynomolgus macaques (*Macaca fascicularis*) with clinical and histological evidence of pulmonary embolism that were thought to result from multiple bone marrow biopsies (Fong et al, 2011). In our cases, all marmosets with bone marrow embolism had bone fractures. In contrast, the one affected howler monkey had lesions of blunt trauma, including inguinal hematoma, ruptured liver, and hemoperitoneum. Therefore, we hypothesize that this howler monkey may have had incomplete fractures or microfractures, which were not grossly detectable but sufficient to cause bone marrow embolism. Importantly, this howler monkey was also positive for YF virus, with massive necrotizing hepatitis, as the probable cause of death. Thrombotic pulmonary embolism or NTPE is not reported as a characteristic lesion in YF-infected NWP (Fernandes et al., 2021; Ferreira et al., 2020; Santos et al., 2020), and no correlation between the infection and traumatic lesions was observed.

In our study, there were 444 traumatized marmosets and 10 traumatized howler monkeys. However, pulmonary embolism was observed in only 9 marmosets (2.0%; 9/444) and in 1 howler monkey (10.0%; 1/10), which may suggest that pulmonary embolism is less frequent in traumatized NWP than in traumatized human patients (Husain, 2021; Husebye et al., 2006). One required condition for pulmonary tissue embolism is that the heart must remain functional after the traumatic tissue laceration,

allowing the tissue emboli to reach pulmonary vessels (Neto et al., 2020). In cases of severe trauma to the brain or liver, the patient may die before pulmonary embolism is established. Therefore, emboli of nervous or hepatic tissues are more rarely observed. In contrast, trauma that leads to bone marrow injury is less likely to be lethal, and therefore embolism of marrow to pulmonary vessels is more frequently observed.

This study reports cases of NTPE composed of liver, brain, or bone marrow tissue in free-ranging NWP that were associated with traumatic lesions, often anthropogenic, occurring during a YF outbreak. Although uncommon, it is important to consider NTPE with pulmonary occlusion in the critical care of traumatized NWP. In addition, this study highlights the importance of conservational strategies and environmental education focusing on One Health, not only to protect free-ranging NWP populations but also to maintain the efficiency of epidemiological surveillance programs.

REFERENCES

1. Caswell JL, Williams KJ. Respiratory system. In: Maxie MG, ed. Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. 6th ed. Elsevier; 2016.

2. Collins KA, Davis GJ. A retrospective and prospective study of cerebral tissue pulmonary embolism in severe head trauma. J Forensic Sci. 1994;39:624–628.

3. Dietz JM, Hankerson SJ, Alexandre BR, et al. Yellow fever in Brazil threatens successful recovery of endangered golden lion tamarins. Sci Rep. 2019;9:12926.

4. Fernandes NCCA, Cunha MS, Guerra JM, et al. Yellow fever as cause of death of titi monkeys (Callicebus Spp.). Vet Pathol. 2021;58(4):730–735.

5. Fernandes NCCA, Guerra JM, Díaz-Delgado J, et al. Differential yellow fever susceptibility in new world nonhuman primates, comparison with humans, and implications for surveillance. Emerg Infect Dis. 2021;27(1):47–56.

6. Fernandes NCCA, Nascimento PM, Sánchez-Sarmiento AM, et al. Histopathological kidney changes and myoglobinuria in neotropical non- human primates attacked by dogs, Brazil. J Med Primatol. 2019;49(2):65–70.

7. Ferreira MS, Bezerra Júnior PS, Cerqueira VD, et al. Experimental yellow fever virus infection in the squirrel monkey (Saimiri spp.) I: gross anatomical and histopathological findings in organs at necropsy. Mem Inst Oswaldo Cruz. 2020;115:e190501.

8. Finnie JW. Forensic pathology of traumatic brain injury. Vet Pathol. 2016;53(5):962–978.

9. Fong DL, Murnane RD, Hotchkiss CE, et al. Pulmonary embolization of fat and bone marrow in cynomolgus macaques (Macaca fascicularis). Comp Med. 2011;61(1):86–91.

10. Goulart VDLR, Teixeira CP, Young RJ. Analysis of callouts made in rela- tion to wild urban marmosets (Callithrix penicillate) and their implications for urban species management. Eur J Wildlife Res. 2010;56(4):642–649.

11. Husain AN. The lung. In: Kumar V, Abbas AK, Aster JC, eds. Robbins and Cotran Pathologic Basis of Disease. 10th ed. Elsevier; 2021.

12. Husebye E, Lyberg T, Roise O. Bone marrow fat in the circulation: clinical entities and pathophysiological mechanisms. Injury. 2006;37:8–18.

13. Joint Pathology Center. Veterinary pathology service. In: Wednesday Slide Conference Proceedings 2013-2014 (Conf. 16, case II accessed). Published 2014. Accessed August 10, 2021. https://www.askjpc.org/wsco/wsc/wsc13/ WSCProceedings2013-2014.pdf.

14. Leite TNB, Maja TA, Ovando TM, et al. Ocorrência de infecção por Leishmania spp. e Toxoplasma gondii em macacos-prego (Cebus apella) de Campo Grande, MS. Rev Bras Parasitol Vet. 2008;17(1):307–310.

15. Lucena FP, De-Campos SN, Rodrigues RL. Maus tratos como causa de mor- talidade em primatas não humanos recebidos pelo IJV, no estado do Rio de Janeiro, em surto de febre amarela. Savannah J Res Dev. 2017;1(suppl 1):92.

16. Mares-Guia MAMM, Horta MA, Romano A, et al. Yellow fever epizootics in non-human primates, Southeast and Northeast Brazil (2017 and 2018). Parasit Vectors. 2020;13(1):90.

17. Michalodimitrakis M, Tsatsakis A. Massive pulmonary embolism by liver tis- sue. Med Sci Law. 1998;38(1):85–87.

18. Montagnana M, Cervellin G, Franchini M, et al. Pathophysiology, clinics and diagnostics of non-thrombotic pulmonary embolism. J Thromb Thrombolysis. 2010;31(4):436–444.

19. Moreno ES, Spinola R, Tengan CH, et al. Yellow fever epizootics in non- human primates, São Paulo state, Brazil, 2008-2009. Rev Inst Med Trop Sao Paulo. 2013;55(1):45–50.

20. Neto RLALT, Vieson MD. Brain tissue pulmonary embolism due to severe blunt force head trauma in a dog. J Comp Path. 2020;175:75–78.

21. Ogilvy CS, McKee AC, Newman NJ, et al. Embolism of cerebral tis- sue to lungs: report of two cases and review of the literature. Neurosurg. 1988;23(4):511–516.

22. Pereira AABG, Dias B, Castro SI, et al. Electrocutions in free-living black- tufted marmosets (Callithrix penicillata) in anthropogenic environments in the Federal District and surrounding areas, Brazil. Primates. 2020;61(2):321–329.

23. Ressel L, Hetzel U, Ricci E. Blunt force trauma in veterinary forensic pathology. Vet Pathol. 2016;53(5):941–961.

24. Santos DO, Oliveira AR, Lucena FP, et al. Histopathologic patterns and sus- ceptibility of neotropical primates naturally infected with yellow fever virus. Vet Pathol. 2020;57(5):1–6.

25. Siqueira A, Campusano Cuevas SE, Salvagni FA, et al. Forensic veterinary pathology: sharp injuries in animals. Vet Pathol. 2016;53(5):979–987.

26. Strier KB, Tabacow FP, Possamai CB, et al. Status of the northern muriqui (Brachyteles hypoxanthus) in the time of yellow fever. Primates. 2019;60(1):21–28.

27. Teixeira RHF, Buti TEM, Costa ALM. Exames post mortem em primatas não humanos durante epizootia de febre amarela na Região Metropolitana de Sorocaba (RMS) / SP, Brasil. Clin Vet. 2018;23(137):32–48.

28. Tozzini S, Anichini C, Gori F, et al. Pulmonary embolism by liver tissue. Am J Forensic Med Pathol. 2003;24(4):87.

29. Warren M, Goodhue W. Cerebral tissue pulmonary embolism after severe head trauma in an infant. Am J Forensic Med Pathol. 2013;34(1):9–10.

30. Wohlsein P, Peters M, Schulze C, et al. Thermal injuries in veterinary forensic pathology. Vet Pathol. 2016;53(5):1001–1017.

CHAPTER V[5]

PATHOLOGICAL AND IMMUNOPHENOTYPICAL CHARACTERIZATION OF GLOMERULOPATHY AND INTERSTITIAL NEPHRITIS IN FREE-RANGING NEOTROPICAL PRIMATES FROM THE BRAZILIAN ATLANTIC FOREST - *Preliminary results*

ABSTRACT

Background: Kidneys are considered vital organs for the maintenance of homeostasis. Therefore, renal damage can have systemic and severe consequences. In captive NWP renal diseases have high prevalence, being frequently reported cases of glomerulopathies and interstitial nephritis. **Methods:** This is a retrospective study with histopathological and immunohistochemistry evaluation of kidneys from free-ranging marmosets (*Callithrix* sp.), howler monkeys (*Alouatta* sp.), and capuchins (*Sapajus* sp.). These animals were from the State of Rio de Janeiro and were necropsied by veterinary pathologists from Instituto Municipal de Medicina Veterinária Jorge Vaitsman (IJV-RJ) in the period of January 2017 to June 2019. All animals were also tested for Yellow Fever viruses by qRT-PCR. Renal diseases were graded (0-4) and IgM/IgG glomerular deposits were scored by IHC **Results:** A total of 799 kidneys from free-ranging NWP were included in this study, being 733 marmosets (*Callithrix* sp.), 37 howler monkeys (*Alouatta* sp.), and 29 capuchins (*Sapajus* sp.). High frequency of RD (> 30%) was observed in the three genera of the study. Immunolabeling for IgM and IgG was also observed in all grades of RD from the three genera evaluated. **Conclusions:** This study characterized for the first time glomerular and interstitial lesions observed in a free-ranging NWP, highlighting the importance of these renal diseases that have been widely studied in captive animals, but are also common in free-ranging animals.

Keywords: marmosets; howler-monkeys; capuchins; new world primates; kidney.

[5] Chapter formatted according to the *Journal of Medical Primatology*

1. INTRODUCTION

Kidneys are considered vital organs for the maintenance of homeostasis in the animal body. Therefore, renal damage can have systemic and severe consequences. However, for a patient to have renal failure > 75% of the renal parenchyma must be compromised (Cline et al., 2012). Retrospective studies of species from the genus *Callithrix* in captive environment indicate that there is a 75% chance of identifying a kidney lesion in the necropsy of individuals over the age of 8 years and in 16.7% of these animals this lesion is the cause of death (Cline et al., 2012; Burns and Wachtman, 2018).

Among renal lesions, glomerulopathies are frequently observed in captive New World primates (NWP), such as the squirrel monkey (Cline et al., 2012), owl monkey (Cline et al., 2012), and marmosets (Isobe et al., 2012; Yamada et al., 2013; Burns and Wachtman, 2018; Kirejczyk et al., 2021). Glomerular lesions are usually membranoproliferative, membranous or sclerotic, accompanied by an interstitial inflammatory infiltrate, fibrosis, and tubular regeneration (Isobe et al., 2012; Yamada et al., 2013; Burns e Wachtman, 2018). In captive animals, interstitial nephritis is a common lesion that can have an incidence above 80% in NWP colonies and is usually associated with glomerulopathies. Although it is often seen together, interstitial, and glomerular lesions can occur separately in some animals and usually progress to sclerosis of the renal parenchyma (Cline et al., 2012; Marini, 2018).

The present study aimed to characterize, by histopathology and immunohistochemistry, the glomerular and tubulo-interstitial diseases in kidneys of free-ranging NWP from the Brazilian Atlantic Forest.

1. MATERIAL AND METHODS

2.1 Study design

This is a retrospective study with histopathological evaluation of kidneys from free-ranging marmosets (*Callithrix* spp.), howler-monkeys (*Alouatta* spp.) and capuchins (*Sapajus* spp.). These animals were from the State of Rio de Janeiro and were necropsied by veterinary pathologists from Instituto Municipal de Medicina Veterinária Jorge Vaitsman (IJV-RJ) in the period of January 2017 to June 2019. In addition to the kidney, samples of spleen, lung, heart, brain, and liver were systematically collected, fixed in 10% buffered formalin, paraffin embedded and sent to Universidade Federal de

Minas Gerais (UFMG) for further investigation. Because the studied animals died during the yellow fever (YF) outbreak in Brazil, all animals were tested for YF virus (YFV) infection by RT-qPCR by the official Brazilian surveillance agency. This study was authorized by the government environmental agency (ICMBio - Brazil) under the SISBIO license number 67014 and all procedures strictly adhered to humane care of animals and all applicable laws and regulations, including registration in the national system for management of genetic heritage and associated traditional knowledge by SISGEN code A2743E4.

2.2 Histopathology

Were included in the study kidneys with low autolytic grade and with all histological regions (cortical and medullary) preserved. For histopathological evaluation kidney slides were stained with hematoxylin and eosin (HE), Congo red, Masson's trichome and methenamine-PAS (M-PAS). Graduation of renal lesions was adapted from Yamada et al. (2013) considering glomerular alterations (1-3), tubular lesions (0-2), interstitial inflammatory infiltrate (0-3), and interstitial fibrosis (0-2) (Table 5.1). The final sum of the scores were grouped in grade 0 - no lesion (1), grade 1 - mild (2-3), grade 2 – mild to moderate (4-5), grade 3 – moderate to severe (6-7), and grade 4 – severe (8-10). Extramedullary hematopoiesis was evaluated in the liver and spleen and classified as mild, moderate, and marked. The other organs were analyzed focusing on extrarenal lesions of chronical renal disease.

Table 5.1 Histopathological criteria used for grading glomerular, tubular, and interstitial renal lesions adapted from Yamada et al., 2013

Glomeruli	**Score**
- No lesion.	1
- Increased of mesangial matrix mainly in glomerular hilum; focal or multifocal (affecting < 80% of the evaluated renal fragment).	2
- Global mesangial proliferation; diffuse (affecting > 80% of the evaluated renal fragment).	3
Tubule (tubular regeneration and/or intratubular hyalin casts)	**Score**
- No lesion.	0
- Focal to multifocal (affecting < 80% of the evaluated renal fragment).	1
- Diffuse (affecting > 80% of the evaluated renal fragment).	2
Interstitial infiltrate	**Score**
- No lesion.	0
- Sporadic in the glomerular hilum or perivascular.	1

- Focal to multifocal (affecting < 80% of the evaluated renal fragment).	2
- Diffuse (affecting > 80% of the evaluated renal fragment).	3
Interstitial fibrosis	**Score**
- No lesion.	0
- Focal to multifocal (affecting < 80% of the evaluated renal fragment).	1
- Diffuse (affecting > 80% of the evaluated renal fragment).	2
Grade	**Sum of scores**
0	1
1	2-3
2	4-5
3	6-7
4	8-10

2.3 Immunohistochemistry (IHC) anti-IgG and anti-IgM

FFPE kidneys from each histopathological grade of each genus (*Callithrix*, *Alouatta*, and *Sapajus*), were sectioned (3 μm thick), mounted on silanized slides, deparaffinized in xylene, and hydrated in decreasing alcohol dilutions. Antigenic retrieval was performed by pressurized humid heat in high pH (50x Tris/EDTA buffer, pH 9, Envision, Dako). Peroxidase block was performed by incubation in 3.5% hydrogen peroxide, for 30 minutes in room temperature, and for protein block, slides were incubated in 6% skim powdered milk, for 30 minutes in room temperature. Then, the slides were incubated overnight at 4°C with specific monoclonal primary antibody: anti-human IgG (1:100; clone D-1; mouse; Santa Cruz Biotechnology) and anti-human IgM (1:100; clone R1/69; mouse; Santa Cruz Biotechnology), followed by incubation with secondary antibody (Envision, Dako), for 30 minutes, at room temperature. Revelation was performed with diaminobenzidine (DAB). Glomerular immunolabeling was graded by as mild (grade 1: sparce granular deposition at mesangium), moderate (grade 2: multifocal deposition at mesangium forming clusters), and severe (grade 3: diffuse deposition at mesangium).

2.4 Statistical analysis

Data were analyzed using the GraphPad Prism software (version 9.0). Descriptive statistics with 95% of confidence interval was used for general analysis. Frequency of RD grades, IHC scores and other variables, such as age, sex and

geographical distribution, were compared using Fisher's exact test. Kruskal-Wallis was performed to compare frequency of lesions according to RD grades and IHC scores.

2. RESULTS

3.1 Profile of renal disease (RD) in free-ranging NWP

A total of 799 free-ranging NWP were included in this study, being 733 marmosets, 37 howler monkeys, and 29 capuchins. High frequency of RD (> 30%) was observed in the three genus evaluated in the study (Figure 5.1).

***Callithrix* spp.**

Considering all 733 animals included in this study, 387 (52.8%; CI 49.2-56.4%) had some grade of RD (Figure 5.1A), being 41.7% grade 1 (162/387; CI 39.9-46.7%, Figure 5.2A); 28.1% grade 2 (109/387; CI 23.8-32.8%, Figure 5.2B); 18% grade 3 (70/387; CI 14.5-22.2%, Figure 5.2C); and 12.1% grade 4 (47/387 CI 9.2-15.7%, Figure 5.2D). Glomerular and tubular lesions were rarely observed in grade 1, with significant increase ($p < 0.0001$) in grade 2, and higher frequencies ($p < 0.0001$) in grades 3 and 4 compared with grades 0 to 2 (Figure 5.1B). Interstitial lesions (inflammation and/or fibrosis) were frequently observed in all grades (Figure 5.1B). RD were associated with higher frequency of extramedullary hematopoiesis (84/387; 21.4%; CI 17.6-25.8%) when compared to kidneys without RD ($p < 0.0001$), especially in cases with severe interstitial inflammation (score 2 and 3).

RD were observed more frequently in adults (350/387; 90.4%; CI 87.1-93.0%) than in juveniles (26/387; 6.7%; CI 4.6-9.7%; $p < 0.0001$); in 11 animals the age was undetermined. No difference was observed between the frequency of RD in males (174/387; 44.9%; CI 40.1-49.9%) and females (190/387; 49.1%; CI 44.1-54.1%); in 23 animals the sex was undetermined. Curiously, RD was more frequently observe in marmosets from Metropolitan region of the Rio de Janeiro state (322/387; 83.2%; CI 79.1-86.6%; $p = 0.0207$). Thirteen animals (13/387; 3.4%; CI 2.0-5.7%) with RD were positive for YFV. Besides YFV, other infectious agents were observed infecting the animals with RD. Bacterial diseases was observed in 39 cases of RD, toxoplasmosis in nine cases and microfilariosis in five cases. However, there was no difference of RD frequency between infected and non-infected animals ($p > 0.05$).

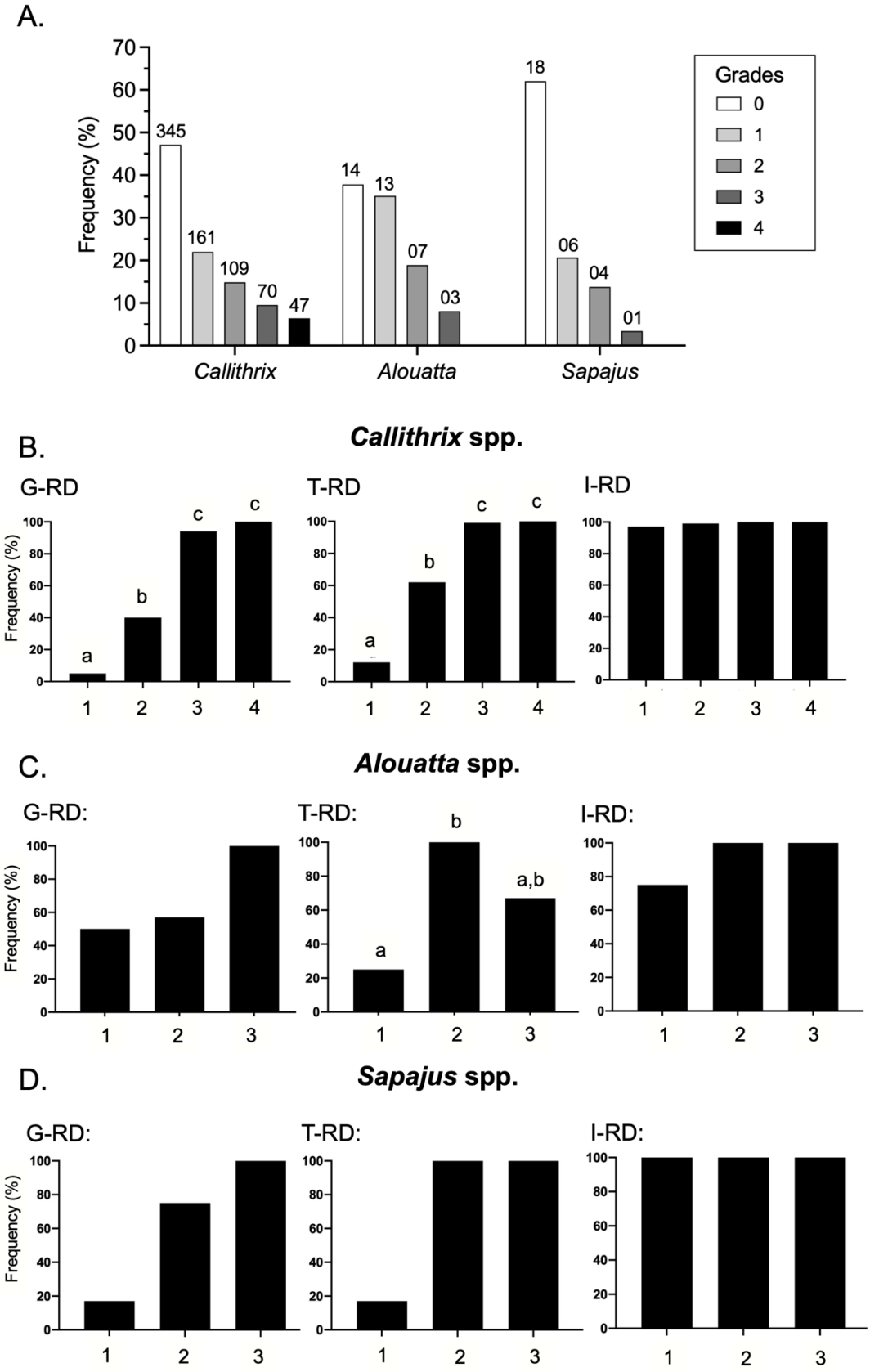


Figure 5.1 Renal diseases (RD) in free-ranging NWP. (A) Frequency of RD in marmosets (*Callithrix*), howler-monkeys (*Alouatta*) and capuchins (*Sapajus*). RD were graded as 0 (absent); 1 (mild); 2 (mild to moderate); 3 (moderate to severe); and 4 (severe). (B-D) Frequency of glomerular (G-RD), tubular (T-

RD) and interstitial (I-RD) renal diseases in *Callithrix* spp. (B), *Alouatta* spp. (C) and *Sapajus* (D). Exact Fisher's test, a,b,c: $p < 0.05$; no letters means that there is no significant difference between columns.

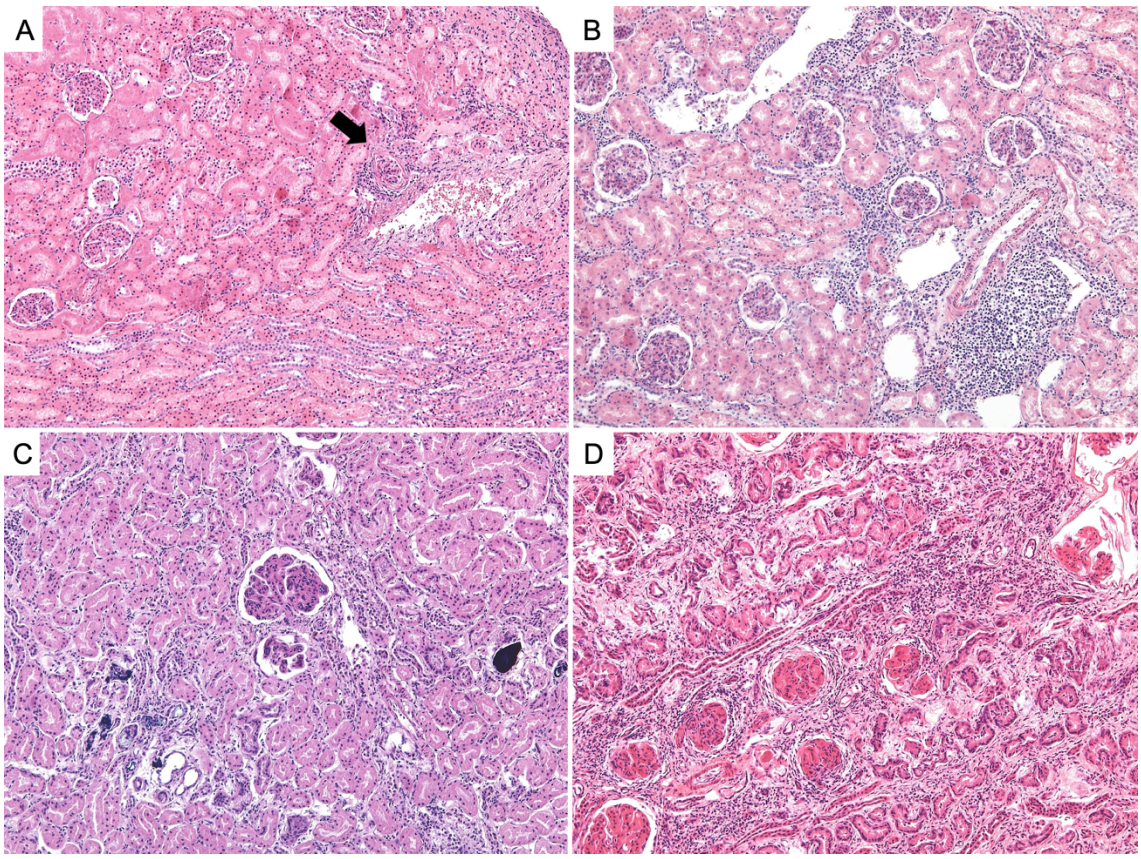


Figure 5.2. Grades of renal disease (RD) in free-ranging NWP. (A) Grade 1. Mild to multifocal interstitial infiltrate (arrow), composed mainly by lymphocytes and plasma cells, usually perivascular, and in some cases associated with focal glomerulosclerosis or hyalin casts. Kidney, HE, 100x. (B) Grade 2. Moderate multifocal to coalescent interstitial infiltrate that is usually associated with mild multifocal membranous glomerulopathy, glomerulosclerosis and hyalin casts. Kidney, HE, 100x. (C) Grade 3. Moderate multifocal to coalescent interstitial infiltrate, associated with marked tubular changes (hyalin casts, mineralization, necrosis, and regeneration), mild to moderated interstitial fibrosis and moderate to marked membranous glomerulopathy and glomerulosclerosis. Kidney, HE, 100x. (D) Grade 4. Diffuse interstitial infiltrate, associated with marked tubular changes (hyalin casts, mineralization, necrosis, and regeneration), marked interstitial fibrosis and moderate to marked membranous glomerulopathy and glomerulosclerosis. Kidney, HE, 100x.

Alouatta spp.

From the 37 kidneys evaluated 23 (62.2%; CI 46.1-75.9%) had RD (Figure 5.1A), being 56.5% grade 1 (13/23; CI 36.8-74.4%); 30.4% grade 2 (7/23; CI 15.6-50.8%); and 13% grade 3 (3/23; CI 4.5-32.1%). No howler-monkey was identified with grade 4 of RD. Glomerular and interstitial lesions were observed with similar frequency in all three grades (Figure 5.1C). Tubular lesions were less observed in grade 1

compared with grade 2 ($p < 0.0001$) (Figure 1C). As observed in marmosets, RD was more frequently observed in adults (21/23; 91.3%; CI 73.2-98.4%) than in juveniles (2/23; 8.7%; CI 1.5-26.8%; $p = 0.0361$). Sixteen were male (16/23; 69.6%; CI 49.1-84.4%) and six were females (6/23; 26.1%; CI 12.5-46.5%), however there were no statistically significant difference in frequencies between these groups. In one animal de sex was undetermined. Only two animals (2/23; 8.6%; 1.5-26.7%) were from Metropolitan area and twelve animals (12/23; 52.2%; CI 32.9-70.8%) with RD were positive for YFV. Toxoplasmosis was observed in one case of RD. There was no difference of RD frequency between infected and non-infected animals ($p > 0.05$).

***Sapajus* spp.**

From the 29 kidneys evaluated eleven (37.9%; CI 22.7-56.0%) had RD (Figure 5.1A), being 54.5% grade 1 (6/11; CI 28.0-78.7%); 36.4% grade 2 (4/11; CI 15.2-64.6%); and 9.1% grade 3 (1/11; CI 0.5-37.7%). As in howler monkeys no animal was identified with grade 4 of RD. Glomerular, tubular, and interstitial lesions were observed with similar frequency in all three grades (Figure 5.1D). Eight animals were adults (8/11; 72.7%; CI 43.4-90.2%), and one was juvenile (1/11; 9.1%; CI 0.5-37.7%). In two cases the age was undetermined. Four animals were females (4/11; 36.3%; CI 15.2-64.6%) and seven were males (7/11; 63.7%; CI 35.4-84.8%) and all animals were from Metropolitan region of Rio de Janeiro state. No animal with RD was positive for YFV. Bacterial hepatitis was observed in one case of RD. There was no difference of RD frequency between infected and non-infected animals ($p > 0.05$).

2.2 Anti-IgM and anti-IgG immunolabeling – *Preliminary results*

In this preliminary study, four to six random cases of each grade of RD were selected from howler-monkeys, marmosets, and capuchins, to evaluate the presence of immunoglobulins at the glomeruli. Immunolabeling anti-IgM and anti-IgG was observed in all grades of RD from the three genera evaluated (Table 5.2; Figure 5.3).

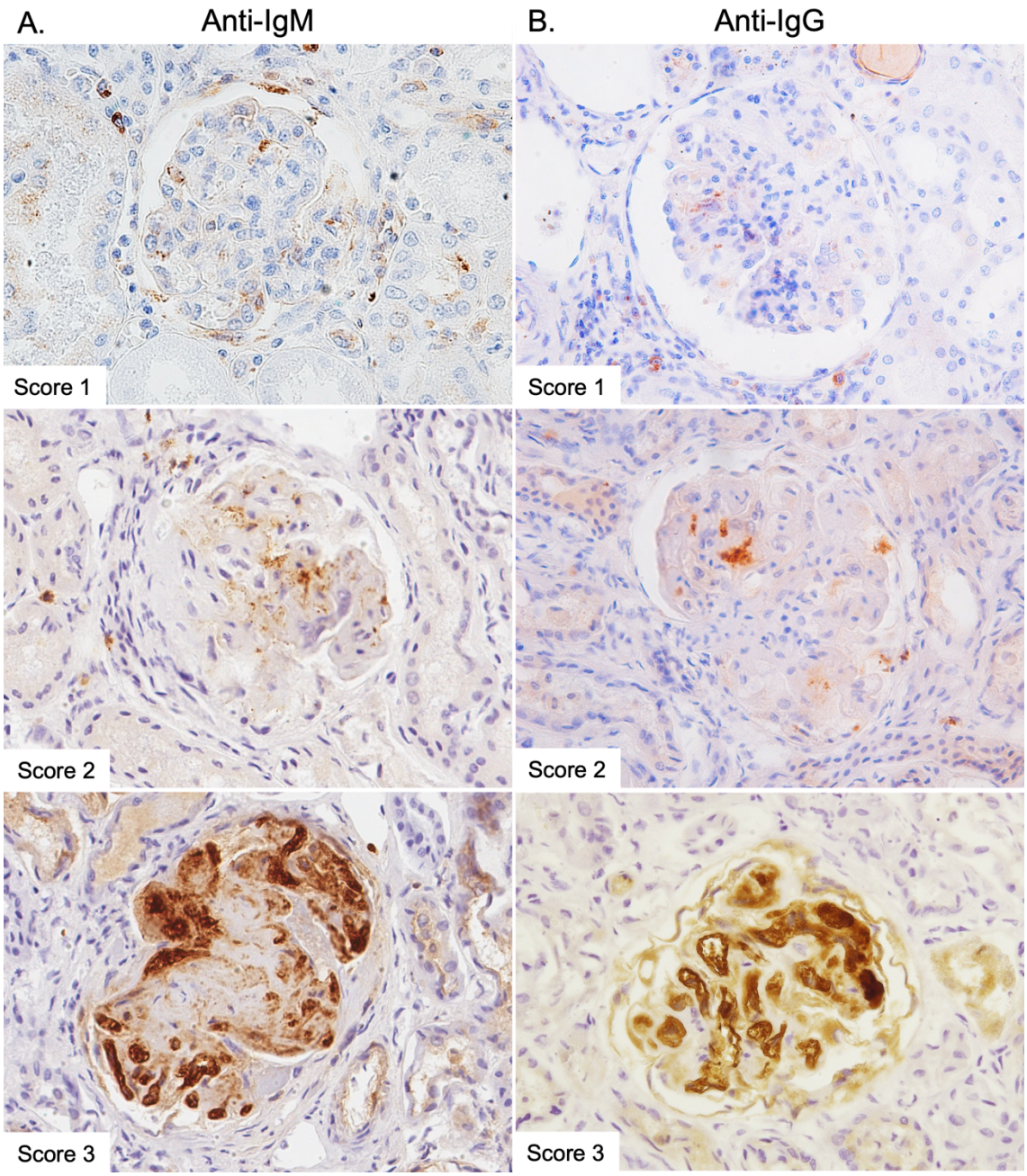


Figure 5.3. Anti-IgM and Anti-IgG glomerular immunolabeling in the kidney of NWP. Score 1-3 of anti-IgM (A) and anti-IgG (B), Kidney, DAB, 200x.

Table 5.2 Immunolabeling score of anti-IgM and anti-IgG in the glomeruli of free-ranging *Callithrix* spp., *Alouatta* spp., and *Sapajus* spp. in the different grades of renal disease (RD).

Grades (RD)	Anti-IgM								Anti-IgG							
	K1	K2	K3	K4	K5	K6	**MD**	Score 2-3* (%)	K1	K2	K3	K4	K5	K6	**MD**	Score 2-3* (%)
***Callithrix* spp.**																
G0	0	0	0	0	1	2	**0.0**	33.3	1	0	1	0	0	-	**0.0**	0.0
G1	0	2	1	0	1	0	**0.5**	16.6	0	0	1	1	0	-	**0.0**	0.0
G2	3	2	1	1	1	2	**1.5**	50.0	0	1	1	2	1	3	**1.0**	33.3
G3	0	2	0	0	2	3	**1.0**	50.0	0	1	0	1	1	2	**1.0**	16.6
G4	0	3	0	3	3	-	**3.0**	60.0	1	1	1	2	2	-	**1.0**	40.0
***Alouatta* spp.**																
G0	0	1	0	0	1	3	**0.5**	16.6	1	1	1	1	1	3	**1.0**	16.6
G1	2	1	3	0	1	-	**1.0**	40.0	1	1	0	0	0	-	**0.0**	0.0
G2	2	1	1	0	2	-	**1.0**	40.0	2	3	0	2	3	-	**2.0**	80.0
G3	0	3	1	-	-	-	**1.0**	33.3	3	3	3	-	-	-	**3.0**	100.0
***Sapajus* spp.**																
G0	1	1	2	3	-	-	**1.5**	25.0	0	0	3	0	-	-	**0.0**	25.0
G1	0	2	0	0	1	0	**0.0**	16.6	0	1	0	0	1	2	**0.5**	16.6
G2	0	0	2	-	-	-	**0.0**	33.3	1	1	1	-	-	-	**1.0**	0.0
G3	1	-	-	-	-	-	**1.0**	0.0	1	-	-	-	-	-	**1.0**	0.0

G: Grades; K: Kidney; MD: Median. *Frequency of IHC scores 2-3 in each grade of RD.

There was no significative statistical difference between the RD grades (G0-4) and glomerular immunoglobulin IHC scores (1-3) ($p > 0.05$), probably because of the low numbers of samples evaluated. However, it was possible to observe that, in the three genera evaluated, high scores of anti-IgM immunolabeling (scores 2-3) were detected with similar frequency in all grades of RD ($p > 0.05$; Table 5.2), including in kidneys with no microscopic lesions (G0); as for IgG, scores 2-3 were observed with similar frequency in all grades of RD, for *Callithrix* spp. and *Sapajus* spp. ($p > 0.05$; Table 5.2), but for *Alouatta* spp. these scores had higher frequency on grades 2-3 ($p = 0.0012$).

3. DISCUSSION

Although glomerulopathies and interstitial nephritis have been widely studied in captive NWP, there are no studies characterizing these diseases in free-ranging populations. The present study evidenced the high frequency of RD in wildlife NWP,

with prevalence similar of what is observed in captive animals (Cline et al., 2012; Marini, 2018) raising a concern about the impact of RD in the health of these wild population.

In captive, most of renal lesions studied in NWP, especially in marmosets, are characterized as a spontaneous progressive primary glomerulopathy (SPPG), which is already identified in one-year-old animals and worsens as age increases (Brack et al., 1999; Yamada et al., 2013; Yamada et al., 2018). In our study, RD was more frequently observed in adults NWP, although it was also observed in some juvenile animals. The literature describes that the initial glomerular changes in the SPPG are mild and can only be identified by ultramicroscopy, being characterized by an obliteration of the podocyte extensions and a partial thickening of the glomerular basement membrane, resulting in an extravasation of proteins (Yamada et al., 2018), triggering tubulointerstitial lesions (inflammation, fibrosis and tubular necrosis), that is the first lesion observed at histopathology (Isobe et al., 2012). These reports agreed with our findings, where glomerulopathies were visible mainly in more severe renal lesions (grade 3 and 4) and inflammatory infiltrate and tubular hyalin casts were observed even in mild RD (grade 1 and 2).

Yamada et al. (2021) identified a reduction in nephrin expression, which correlates with the progression of glomerulopathies in marmosets. Nephrin is one of the key molecules expressed in podocytes and is responsible for the functional health of the podocyte cytoskeleton and consequently for the efficiency of glomerular filtration. In humans, reduced nephrin expression is directly associated with proteinuric kidney diseases (Yu et al., 2018). Also, in SPPG, there was identification of IgM deposits in the mesangium already in the initial stages of the diseases with subsequent deposit of IgA and IgG as the glomerulopathy progresses (Yamada et al., 2018). The identification of these deposits of immunocomplexes has also been described in studies of glomerulopathies of squirrel monkeys and captive prosimians (Burkholder, 1981; Borda et al, 2000; Leary et al., 1981; Nimri and Lamners, 1994; Burns and Wachtman, 2019). Importantly, similar profile was observed in our preliminary study evaluating immunoglobulin glomerular deposits by IHC: IgM immunolabeling was observed in high scores regardless of RD grade, being detected even in cases with no microscopic lesion. While IgG had higher IHC scores in advanced RD grades. The etiology of glomerulopathy due to deposition of immune complexes in NWP is still poorly understood.

In humans, an IgM deposit nephropathy is described, considered a neglected nephropathy, despite the relevant morbidity in young and adult patients from developing countries (incidence of 2-18.5%) (Vanikar, 2013). This IgM nephropathy is speculated to be associated with environmental and food antigens, which would incite a recurrent IgM response, leading to the formation of glomerular deposits (Vanikar, 2013). Despite presenting inconclusive results, some studies in callitrichids have correlated the presence of glomerulopathies with IgM deposits to high levels of circulating IgM that bind to soy, cereal and milk protein antigens, suggesting a possible food etiology (Brack et al., 1999). Interestingly, in our study marmosets from the Metropolitan region of Rio de Janeiro state, where it could easily access human foods, were more frequently affected by RD. However, additional studies are necessary to better understand the origin of these lesions.

In general, in humans, the factors that can trigger the development of glomerulopathies are numerous, ranging from systemic infectious diseases to autoimmune diseases, with congenital cases being rare (Miller et al., 2019). Some infectious agents such as filarial nematodes, *Plasmodium* sp. and bacterial agents that cause systemic infections (eg, *Staphylococcus aureus* and *Streptococcus* sp.) may contribute to the development of these lesions in NHP (Markowitz, 1969; Stills and Bullock, 1981). In our cases no association between RD and knowing systemic diseases, such as microfilariasis, toxoplasmosis, YF and bacteremia, was observed. Additionally, the increase in vascular permeability and consequently in the glomerular filtration rate caused by diabetes mellitus is also considered a predisposing factor for the development of membranous glomerulopathies and glomerulosclerosis, in addition to secondary tubulointerstitial lesions (Jonasson et al., 1985; Sasseville et al., 2012).

In callitrichids there is a correlation between the observation of these glomerular lesions, when in more chronic and severe stages, with the presence of clinical signs such as progressive weight loss, anemia, azotemia and calcium and phosphorus imbalance (Burns and Wachtman, 2018). Hepatic extramedullary hematopoiesis has been associated with these kidney injuries, which may indicate a secondary anemic condition due to erythropoietin deficiency (Cline et al., 2012). In this study a positive correlation was observed between extramedullary hematopoiesis and renal disease, indicating a clinical impact in the animals affected.

This study characterized for the first time glomerular and interstitial lesions observed in a free-ranging NWP, highlighting the importance of these renal diseases that have been widely studied in captive animals, but, as we reported in this study, are also common in free-ranging animals.

REFERENCES

1. Cline JM, Brignolo L, Ford EW. Urogenital System. In: Abee C, Mansfield K, Tardif S, Morris T. Nonhuman Primates in Biomedical Research Second Edition, 2nd edit. Elsevier: St. Louis. 2012; 483-562.
2. Burns M, Wachtman L. Physical Examination, Diagnosis, and Common Clinical Procedures. In: Marini R, Wachtman L, Tardif S, Mansfield K, Fox J. The Common Marmoset in Captivity and Biomedical Research, 1st edit. Elsevier:St. Louis. 2018; 145-175.
3. Isobe K, Adachi K, Hayashi S, Ito T, Miyoshi A, Kato A, Suzuki M. Spontaneous glomerular and tubulointerstitial lesions in common marmosets (*Callithrix jacchus*). *Vet Pathol*. 2012; 49:839–845.
4. Yamada N, Sato J, Kanno T, Wako Y, Tsuchitani M. Morphological study of progressive glomerulonephropathy in common marmosets (*Callithrix jacchus*). *Toxicol Pathol*. 2013; 41:1106–1115.
5. Kirejczyk S, Pinelli C, Gonzalez O, Kumar S, Dick Jr E, Gumber S. Urogenital Lesions in Nonhuman Primates at Two National Primate Research Centers. *Vet. Pathol.* 2021; 58(1):147-160.
6. Marini RP. Diseases of the Urogenital System. In: Marini R, Wachtman L, Tardif S, Mansfield K, Fox J. The Common Marmoset in Captivity and Biomedical Research, 1st edit. Elsevier: St. Louis. 2018; 195-212.
7. Brack M, Schroeder C, Fooke M, Schlumberger W. IgM/IgA Nephropathy in Callitrichids: Antigen Studies. *Nephron.* 1999; 82(3):221-31.
8. Yamada N, Hashimoto N, Kamiie J, Doi T, Sato J, Inoue T, Shirota K, Tsuchitani M. Relationship between immunoglobulin deposition and early lesions of progressive glomerulonephropathy in young common marmosets. *Vet Pathol*. 2018; 55: 173–176.
9. Yamada N, Doi T, Sato J, Inoue T, Tsuchitani M, Kobayashi Y. Morphological analyses of nephrin expression in progressive glomerulonephropathy of common marmosets. *J Toxicol Pathol*. 2021; 34(1):83-88.
10. Burkholder PM. Glomerular disease in captive galagos. *Vet Pathol.* 1981;18:6e22.
11. Borda JT, Idiart JR, Negrette MS. Glomerular lesions in renal biopsies of *Saimiri boliviensis* (primate) examined by light and electron microscopy and immunohistochemistry. *Vet Pathol*. 2000; 37:409-414.
12. Leary SL, Sheffield WD, Strandberg JD. Immune complex glomerulonephritis in baboons (*Papio cynocephalus*) with indwelling intravascular catheters. *Lab Anim Sci*. 1981; 31:416-420.
13. Nimri LF, Lanners NH. Immune complexes and nephropathies associated with *Plasmodium inui* infection in the rhesus monkey. *Am J Trop Med Hyg*. 1994; 51:183-189.
14. Vanikar A. IgM nephropathy; can we still ignore it? *J Nephropathol*. 2013. 2(2): 98–103.

15. Miller M, Klinger M, Dziemianko I, Kazimierczak K, Drabczyk R. Glomerular Diseases. McMaster Textbook of Internal Medicine. Kraków: Medycyna Praktyczna. https://empendium.com/mcmtextbook/chapter/B31.II.14.3. Accessed April 01, 2021.

CONCLUSION

In this study we were able to identify the main pathological findings associated with death in free-ranging NWP with detailed histopathological features and immunophenotypic, ultrastructural and molecular characterization, and detection of important zoonotic infections, such as rabies, YF and toxoplasmosis. Also, it was described for the first time in free-ranging NWP infection by *S. capitis*, *S. sanguinus, Moraxella* sp. and PIV-1 and 3.

Additionally, we characterized the epidemiological and pathological aspects of platynosomiasis and toxoplasmosis infection in free-ranging marmosets (*Callithrix* spp.), and we performed a detailed description of traumatic injuries in free-ranging NWP describing for the first time NTPE by liver and lung tissue in marmosets, highlighting the impact of trauma in free-ranging NWP population.

Finally, we presented preliminary results of a high prevalent renal disease in free-ranging NWP with IgG and IgM glomerular immunolabelling and associated with high frequency of extramedullary hematopoiesis.