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**Bárbara Braga Ferreira Marta**

# PREVALÊNCIA E CARACTERIZAÇÃO MOLECULAR DE *Giardia* spp. EM AMOSTRAS FECAIS DE CAPIVARAS (*Hydrochoerus hydrochaeris*) EM ÁREAS URBANAS

**ARAÇATUBA/SP**
**2021**

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Dissertação apresentada à Faculdade de Medicina Veterinária de Araçatuba, UNESP, Campus de Araçatuba, como parte do Programa de Pós-Graduação em Ciência Animal, Nível Mestrado, Área de concentração em Medicina Veterinária Preventiva e Produção Animal, Linha de pesquisa Epidemiologia, Etiopatogenia, Diagnóstico e Controle das Enfermidades dos Animais

Orientador: Professor Marcelo Vasconcelos Meireles

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## CERTIFICADO DE APROVAÇÃO

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

**Madre Teresa de Calcutá**

MARTA, B.B.F. **Prevalência e caracterização molecular de *Giardia* spp. em amostras fecais de capivaras (*Hydrochoerus Hydrochaeris*) em áreas urbanas**. 2021. 64f. Dissertação (Mestrado) Faculdade de Medicina Veterinária, Universidade Estadual Paulista, Araçatuba, 2021.

## RESUMO

A giardíase é a causa mais comum de diarreia em humanos e animais em todo o mundo. Atualmente, existem oito espécies de *Giardia* spp., dentre elas a *Giardia duodenalis,* que infecta a maioria dos vertebrados. A capivara (*Hydrochoerus hydrochaeris*) é o maior roedor herbívoro do mundo, no entanto existem poucos estudos referente ao potencial zoonótico desses animais. O objetivo deste trabalho foi determinar a prevalência e realizar a caracterização molecular de *Giardia* spp. em populações de capivaras presentes em áreas urbanas, bem como correlacionar a presença de *Giardia* spp. com a faixa etária do animal e a estação climática. Foram coletadas 247 amostras de capivaras na Lagoa Maior no município de Três Lagoas – Mato Grosso do Sul e no Lago do Amor e no Parque das Nações Indígenas, ambos localizados no município de Campo Grande – Mato Grosso do Sul. Três protocolos de *nested* PCR foram utilizados para amplificação de fragmentos parciais dos genes 18S rRNA, GDH e TPI, seguidos por sequenciamento genético, em 183 e 64 amostras fecais colhidas nos períodos chuvoso e de seca, respectivamente. Cento e trinta e três amostras (54%) pertenciam a capivaras adultas, 61 a filhotes (25%) e 53 a capivaras juvenis (21%). Todas as amostras (n=247) de fezes apresentaram resultado negativo para *Giardia* spp. Neste trabalho não foi detectada a presença de *Giardia* spp. em amostras fecais de capivaras, sugerindo que nas áreas analisadas esses animais não são um reservatório importante de *Giardia* spp. para os seres humanos.

**Palavras-chave:** Zoonoses. Vertebrados. Prevalência. Reação em cadeia da polimerase.

MARTA, B.B.F. **Prevalence and molecular characterization of *Giardia* spp. in fecal samples of capybaras (*Hydrochoerus Hydrochaeris*) in urban areas**. 64f. Dissertação (Mestrado) Faculdade de Medicina Veterinária, Universidade Estadual Paulista, Araçatuba, 2021.

## ABSTRACT

Giardiasis is the most common cause of diarrhea in humans and animals worldwide. Currently, there are eight species of *Giardia* spp., including *Giardia duodenalis*, which infects most vertebrates. Capybara (*Hydrochoerus hydrochaeris*) is the largest herbivorous rodent in the world, however there are few studies regarding the zoonotic potential of these animals. Our aim were to determine the prevalence and perform the molecular characterization of *Giardia* spp. in populations of capybaras present in urban areas, as well as to correlate the presence of *Giardia* spp. with age and seasons of the year. A total of 247 fecal samples of capybaras were collected in the state of Mato Grosso do Sul, at the municipalities of Três Lagoas (Lagoa maior) and Campo Grande (Lago do Amor and Parque das Nações Indígenas). Nested PCR targeting the 18S rRNA, GDH and TPI genes, followed by genetic sequencing, was performed for detection and species caracterization of *Giardia* spp. A total of 183 samples were collected in the rainy season and 64 in the dry season. One hundred and thirty-three samples (54%) originated from adults, 61 from offspring (25%) and 53 from juvenile capybaras (21%). All fecal samples (n=247) were negative for *Giardia* spp. *Giardia* spp. was not detected in fecal samples of capybaras, suggesting that in the areas analyzed capybaras are not an important reservoir of *Giardia* spp. for humans.

**Keywords:** Zoonoses.Vertebrates.Prevalence.Polymerase chain reaction.

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# SUMÁRIO

# 1 INTRODUÇÃO GERAL

A giardíase é a causa mais comum de diarreia em humanos e animais em todo o mundo. *Giardia* spp. infecta cerca de 280 milhões de seres humanos anualmente e é responsável pelo óbito de crianças menores de cinco anos e indivíduos imunocomprometidos em países em desenvolvimento (SULAIMAN et al., 2003; EINARSSON; MA'AYEH; SVÄRD, 2016; SANTIN, 2020)**.**

A transmissão de *Giardia* ocorre por via fecal-oral, quando o hospedeiro entra em contato com humanos e animais infectados, ou pela ingestão de água e alimentos contaminados com cistos (SANTIN, 2020).

Os sinais clínicos da giardíase em humanos variam desde casos assintomáticos até quadros de diarreia, cólica, distensão abdominal, náuseas, perda de peso e má absorção (FENG; XIAO, 2011; RYAN; CACCIÒ, 2013; EINARSSON; MA'AYEH; SVÄRD, 2016).

Há descrição de oito espécies de *Giardia*, dentre elas *Giardia microti*, *Giardia cricetidarum* e *Giardia muris*, que infectam roedores; *Giardia ardeae* e *Giardia psittaci*, identificadas em aves; *Giardia agilis* em anfíbios, *Giardia peramelis* em marsupiais e *Giardia duodenalis*, que infecta a maioria dos vertebrados, incluindo os seres humanos (SULAIMAN et al., 2003; MALONEY et al., 2020; SANTIN, 2020).

*Giardia duodenalis* é constituída por oito *assemblages* identificados como A a H; apesar de serem morfologicamente semelhantes, os oito assemblages possuem singularidade genética. Os *assemblages* A e B apresentam potencial zoonótico e infectam humanos e uma grande variedade de animais; os outros *assemblages* são específicos para o hospedeiro, sendo que os *assemblages* C e D infectam caninos e os assemblages E, F, G e H infectam artiodáctilos, felinos, roedores e mamíferos marinhos, respectivamente (BERRILLI et al., 2004; MALONEY et al., 2020; SANTIN, 2020). No entanto, embora exista especificidade em relação ao hospedeiro, os *assemblages* C, D, E e F eventualmente são observados em humanos e os *assemblages* A e B já foram isolados em animais de estimação; desse modo, ainda há controvérsias sobre

quais *assemblages* estão relacionados à transmissão zoonótica desse parasito (MALONEY et al., 2020; CAPEWELL et al., 2021).

## 1.1 Ciclo Biológico

A giardíase é transmitida pela ingestão de água e alimentos contaminados com cistos ou pelo contato com pessoas (transmissão antroponótica) e animais (transmissão zoonótica) infectados (KOEHLER et al., 2014; SANTIN, 2020).

O ciclo de vida de *Giardia* apresenta dois estágios evolutivos: o trofozoíto, que é responsável pela colonização do epitélio intestinal, e o cisto, considerado a forma infectante e resistente ao ambiente (GEURDEN; VERCRUYSSE; CLAEREBOUT, 2010; EINARSSON; MA'AYEH; SVÄRD, 2016).

Após o hospedeiro ingerir os cistos de *Giardia*, ocorre o processo de desencistação, decorrente da ação do ácido gástrico no estômago e da bile e tripsina presentes no duodeno, resultando na liberação de quatro trofozoítos no intestino delgado. Os trofozoítos aderem nas células epiteliais do intestino, por meio do seu disco ventral, e se multiplicam rapidamente por divisão binária (MOREIRA et al., 2020; SANTIN, 2020). A estrutura do trofozoíto consiste em dois núcleos, disco adesivo ou ventral, corpo mediano e quatro pares de flagelos, que são importantes para a motilidade (ROXSTRÖM-LINDQUIST et al., 2006; EINARSSON; MA'AYEH; SVÄRD, 2016)

Algumas características no ambiente intestinal estimulam o processo de encistação, como a redução do nível de colesterol, pH alcalino e presença de sais biliares. Durante a encistação, o trofozoíto modifica sua estrutura, formando uma parede resistente e, por fim, é eliminado nas fezes, fechando assim o ciclo do parasito (GEURDEN; VERCRUYSSE; CLAEREBOUT, 2010; FENG; XIAO, 2011; EINARSSON; MA'AYEH; SVARD, 2016; MOREIRA et al., 2020; SANTIN, 2020).

Os cistos de *Giardia* são resistentes a produtos químicos utilizados nas etapas de tratamento de água, bem como permanecem infectantes por meses em ambiente frio e úmido (FENG; XIAO, 2011; EINARSSON; MA'AYEH; SVARD, 2016).

## 1.2 Sinais Clínicos

*Giardia* spp. promove alterações fisiológicas e estruturais no intestino delgado do hospedeiro, como atrofia das microvilosidades, que resulta em má absorção de proteínas, de vitaminas e de eletrólitos e hipersecreção de cloreto em seres humanos; com isso, ocorre o acúmulo de líquido no lúmen intestinal, ocasionando distensão das alças intestinais, aumento do peristaltismo e diarreia (CACCIÒ; LALLE; SVÄRD, 2018; MOREIRA et al., 2020)**.**

O início dos sintomas em seres humanos ocorre entre nove e 15 dias após a infecção e os hospedeiros podem manifestar a doença nas formas assintomática, aguda ou crônica. A forma aguda é evidenciada pela presença de diarreia aquosa com odor fétido, esteatorreia**,** náuseas, dor epigástrica e perda de peso; a manifestação crônica está associada à deficiência de crescimento e distúrbios nutricionais (CACCIÒ; LALLE; SVÄRD, 2018; HOOSHYAR et al., 2019)**.**

A patogênese da giardíase em animais não é definida, porém sabe-se que é semelhante à que ocorre em seres humanos, com alterações das microvilosidades do intestino delgado, deficiências de enzimas, aumento da permeabilidade epitelial, diminuição de reabsorção de água, eletrólitos e nutrientes, ocasionando diarreia (GEURDEN; VERCRUYSSE; CLAEREBOUT, 2010)**.**

*Giardia* é o parasito mais presente nas criações de ovinos, bovinos e caprinos. Os animais jovens são importantes fontes de transmissão, pois podem eliminar cistos intermitentemente, mesmo que haja desenvolvimento da imunidade. Há relatos de casos assintomáticos, mas comumente ocorrem quadros severos de diarreia, perda de peso, depressão e mortalidade, ocasionando grandes prejuízos para o produtor (GEURDEN; VERCRUYSSE; CLAEREBOUT, 2010; RYAN; ZAHEDI, 2019).

Existem poucas informações referentes aos efeitos clínicos da giardíase em animais selvagens, porém já foram descritos casos de infecções assintomática, leve ou grave, dependendo do hospedeiro (APPELBEE; THOMPSON; OLSON, 2005; RYAN; ZAHEDI, 2019).

## 1.3 Epidemiologia

*Giardia* está amplamente distribuída no mundo. Em seres humanos, estima-se prevalência de 2% a 7% em países desenvolvidos e 30% em países em desenvolvimento. Desde 2004, a giardíase é considerada uma doença negligenciada pela Organização Mundial da Saúde (HOOSHYAR et al., 2019; FANTINATTI et al., 2020).

A giardíase tem um grande impacto em saúde pública e em medicina veterinária devido à sua alta prevalência e capacidade de causar surtos, além de prejudicar o crescimento e ocasionar alterações cognitivas em crianças, bem como, mortalidade em animais (YAOYU; XIAO, 2011; RYAN; ZAHEDI, 2019).

Quando infectados, os humanos e animais podem eliminar uma grande quantidade de cistos de *Giardia* no meio ambiente, sendo $2x10^6$, $1,7x10^6$, $4,7x10^9$, $2,1x10^4$, $2,3x10^5$ cistos por grama de fezes para humanos, bovinos, ovinos, suínos e cães, respectivamente (SMITH et al., 2006; RYAN; ZAHEDI, 2019).

Os cistos de *Giardia* pode permanecer infectantes por muitos dias no meio ambiente. Em amostras de água de lago, foi relatada uma taxa de sobrevivência de 56 dias em uma temperatura de 0° C a 7° C e 28 dias em temperatura de 17° C a 20° C; em água de rio, observou-se maior taxa sobrevivência, correspondendo a 84 dias (0° C a 4° C) e 28 dias (20° C a 28° C). Em água de torneira, os cistos podem permaner viáveis de 14 dias (20° C a 28° C) a 56 dias (0° C a 4° C) (FENG; XIAO, 2011).

A giardíase apresenta baixa dose infectante, ou seja, menos de 10 cistos administrados oralmente são capazes de causar doença clínica. Por isso, a ocorrência de surtos de giardíase em creches, piscinas comunitárias e por ingestão de água potável contaminada é relevante (ERICKSON; ORTEGA, 2006; KOEHLER et al., 2014).

*Giardia duodenalis* é a única espécie que causa infecção em humanos e na maioria dos animais, e é subdividida em *assemblages* (A-H) e *subassemblages* (AI, AII, AIII, BIII e BIV), que são identificados por análises moleculares (RYAN; ZAHEDI, 2019; MALONEY; MOLOKIN; SANTIN, 2020; SANTIN, 2020).

Existem relatos de animais e humanos compartilhando *assemblages* e *subassemblages*, porém na maioria dos casos o potencial zoonótico de *Giardia* spp. não foi determinado (MALONEY; MOLOKIN; SANTIN, 2020; CAPEWELL et

al., 2021). Na maioria dos estudos, não foram identificadas infecções mistas e não houve determinação do potencial zoonótico dos *assemblages* identificados. Ainda, diferenças relacionadas a dose infectante, idade e a outros marcadores de patogenicidade dificultam a comparação de estudos (CACCIÒ; LALLE; SVÄRD, 2018; RYAN; ZAHEDI, 2019; FANTINATTI et al., 2020).

A maioria dos estudos de genotipagem de *Giardia* é realizada em humanos, animais domésticos e animais de produção. Portanto, sabe-se pouco sobre a distribuição, diversidade genética e o potencial zoonótico da giardíase em animais selvagens (RYAN; ZAHEDI, 2019; FANTINATTI et al., 2020).

Um estudo realizado no Brasil com animais selvagens e exóticos de cativeiro detectou os *assemblages B* de *G. duodenalis* na maioria das amostras analisadas, indicando que esses animais são um possível reservatório e um risco de transmissão da giardíase para os seres humanos (SOARES et al., 2011).

Os animais da ordem Rodentia podem ser importantes reservatórios de vários patógenos, dentre eles, vírus, bactérias e parasitos, incluindo *G. muris*, *G. microti* e os *assemblages* zoonóticos A e B de *G. duodenalis* (HELMY et al., 2018). A presença de *Giardia* spp. já foi relatada em fezes de capivaras (*Hydrochoerus hydrochaeris*), porém não foi realizada a genotipagem dessas amostras; desse modo, as informações sobre a possível transmissão zoonótica a partir desses animais ainda são escassas (REGINATTO et al., 2008; RODRÍGUEZ-DURÁN; BLANCO PALMA; PEÑA FLÓREZ, 2015).

## 1.4 Capivara

A capivara é o maior roedor herbívoro do mundo, pertence à ordem Rodentia e família Hydrochoeridae. Seu tamanho varia conforme a região geográfica; no centro-oeste do Brasil, as capivaras podem pesar até 100kg (BONUTI et al., 2002; NOGUEIRA-FILHO; NOGUEIRA, 2018). Possui habitat em ambientes semiaquáticos, próximos de leitos e margens de rios, lagoas, pântano e manguezais, com vegetação arbustiva e pastagens. A água é importante para seu consumo, chafurdação, proteção contra predadores e reprodução. Também necessitam de terra para descansar e buscar alimentos (HERRERA et al., 2011; ALMEIDA; BIONDI, 2014).

São animais sociais e podem viver em grupos de quatro a 16 indivíduos em média. Conforme a estação do ano, o grupo pode conter 40 indivíduos que

permanecem juntos por meses ou anos (HERRERA et al., 2011; NOGUEIRA-FILHO et al., 2017). Durante a estação chuvosa é comum cada grupo permanecer isolado, mas na estação seca, as capivaras se reúnem e formam grandes grupos com mais de 100 animais (HERRERA et al., 2011; NOGUEIRA-FILHO; NOGUEIRA, 2018). O grupo é liderado por um macho dominante e segue uma hierarquia; o líder é o macho mais velho, com maior dimensão corporal e com a glândula olfativa no focinho mais avantajada. Quando ocorre a remoção do líder, o próximo macho da fila assume a posição (HERRERA et al., 2011).

As capivaras possuem alta plastificidade fenotípica, ou seja, conseguem explorar e se adaptar em diversos habitats, dentre eles em ambientes antropogênicos, conseguindo sobreviver nos grandes centros urbanos (HERRERA et al., 2011; ALMEIDA; BIONDI, 2014).

Ainda, esses animais são caçados para consumo de carne ou pelo couro, em comunidades tradicionais distribuídas em vários países da América do Sul (HERRERA et al., 2011; NOGUEIRA-FILHO; NOGUEIRA, 2018). Com isso, destaca-se a possibilidade de contato com o homem não somente em áreas urbanas compartilhadas, mas também por contato direto.

A criação de capivaras no Brasil teve início em 1990, quando o sistema semiconfinado foi desenvolvido e expandido no país. Com o avanço dos criatórios em cativeiro e a crescente presença das capivaras em áreas alagadas e antrópicas, como praças, parques e represas, estudos das parasitoses em capivaras despertaram interesse (VERDADE; FERRAZ, 2006; FERRAZ; BONACH; VERDADE, 2005; ALMEIDA et al., 2013; ALMEIDA; BIONDI, 2014; NOGUEIRA-FILHO; NOGUEIRA, 2018).

A bibliografia acerca da presença de *Giardia* em capivaras é escassa e a maioria dos estudos com esses animais está relacionada à pesquisa de *Trypanosoma evansi* (EBERHARDT et al., 2014)*, Plasmodium* spp*.* (SANTOS et al., 2009)*,* de parasitos da classe Nematoda (EBERHARDT et al., 2019) e *Neospora caninum* (TRUPPEL et al., 2009).

## 1.5 Diagnóstico da Giardíase

Existem inúmeras técnicas para identificação de *Giardia*, dentre elas destaca-se a microscopia tradicional, métodos com anticorpos imunofluorescentes e a detecção molecular (THOMPSON; ASH, 2019).

A microscopia é utilizada para visualizar cistos e trofozoítos de *Giardia*, após a realização de técnicas de concentração e coloração, no entanto, essa técnica possui baixa sensibilidade e depende do grau de infecção e da experiência do analista (KOEHLER et al., 2014; HOOSHYAR et al., 2019; CAPEWELL et al., 2021).

Os métodos imunológicos apresentam maior sensibilidade e atuam complementando a microscopia no diagnóstico da giardíase. A detecção de anticorpos é um indicador útil e identifica infecções recentes, ou seja, antes da eliminação de cistos pelo hospedeiro, no entanto os anticorpos permanecem por muito tempo na corrente sanguínea, mesmo após o tratamento. A detecção de antígenos ocorre em fezes frescas ou preservadas com formalina e sua sensibilidade varia de 95 a 100%. Como desvantagem, os métodos imunológicos não identificam as espécies ou *assemblages* de *Giardia* (KOEHLER et al., 2014; HOOSHYAR et al., 2019).

As técnicas de biologia molecular possibilitam identificar as espécies de *Giardia* e os *assemblages* e *subassemblages* de *G. duodenalis*. A técnica de biologia molecular mais utilizada é a reação em cadeia pela polimerase (PCR), na qual há amplificação específica de fragmentos de diversos genes de *Giardia* spp. (KOEHLER et al., 2014; HOOSHYAR et al., 2019), incluindo o gene da glutamato desidrogenase (gdh), beta-giardina (bg), fator de alongamento 1-alfa, triose-fosfato-isomerase (tpi) e o gene que codifica a subunidade menor do RNA ribossomal (SSU rRNA) (LYU et al., 2018; MALONEY; MOLOKIN; SANTIN, 2020).

O gene SSU rRNA é o gene de escolha para detecção de *Giardia* spp., pois possui grande quantidade de cópias por genoma (KOEHLER et al., 2014; CAPEWELL et al., 2021).

## 1.6 Tratamento

Os medicamentos mais utilizados para tratamento da giardíase são os da família do 5-nitroimidazol (5-NI), dentre eles o metronidazol, tinidazol, ornidazol e secnidazol (MIYAMOTO; ECKMANN, 2015; ARGÜELLO-GARCÍA et al., 2020).

O metronidazol é o mais administrado e possui taxa de cura de 80 a 95% com dose de 250mg, duas a três vezes por dia, por um período de cinco a 10 dias. Esse fármaco age provocando estresse oxidativo, ruptura na fita de DNA e consequentemente morte do trofozoíto (ROSSIGNOL, 2010; MIYAMOTO; ECKMANN, 2015; ARGÜELLO-GARCÍA et al., 2020).

A eficácia do tinidazol varia conforme a carga parasitária, apresentando em média 90% de cura. O ornidazol possui eficácia similar à do tinidazol, variando de 90 a 100%. A absorção do secnidazol é mais lenta e sua taxa de cura é 80% a 98%. Esses fármacos agem de maneira similar ao metronidazol, no entanto, promovem poucos efeitos colaterais e o tempo de tratamento é menor, sendo indicados em dose única (ROSSIGNOL, 2010; MIYAMOTO; ECKMANN, 2015; ARGÜELLO-GARCÍA et al., 2020).

Existem casos de cepas resistentes aos fármacos da família do 5-nitroimidazol (5-NI), sendo importante a busca por medicamentos alternativos (EINARSSON; MA'AYEH; SVÄRD, 2016; MMBAGA; HOUPT, 2017).

A nitazoxanida é uma alternativa para o tratamento da giardíase. Sua ação compromete a integridade celular do parasito e apresenta eficácia de 70 a 80% quando administrada por três dias (MIYAMOTO; ECKMANN, 2015; ARGÜELLO-GARCÍA et al., 2020).

Os benzimidazóis são usados para tratamento de helmintíases, mas também podem ser uma alternativa para infecções por *Giardia*. Sua eficácia varia de 25% a 90%, dependendo do tempo e da dosagem; o mecanismo de ação desses fármacos consiste em diminuir a capacidade do parasito em captar glicose. Embora alguns estudos demonstrem a eficácia dos benzimidazóies, existem relatos de falha na eliminação da *Giardia* (ROSSIGNOL, 2010; MIYAMOTO; ECKMANN, 2015; ARGÜELLO-GARCÍA et al., 2020).

A paromomicina é uma alternativa para mulheres grávidas, pois o fármaco é excretado pelas fezes sem ser metabolizado e atua diminuindo a síntese de proteínas do parasito. Sua taxa de eficácia é de 60 a 70% e o tempo de duração do tratamento é 10 dias (ROSSIGNOL, 2010; ARGÜELLO-GARCÍA et al., 2020)

A furazolidona é outra opção para o tratamento da giardíase e a sua eficácia

depende da idade do paciente. Possui muitos efeitos colaterais como, vômitos, dor de cabeça, reações de hipersensibilidade, hipotensão, erupções cutâneas e urticária. Seu uso deve ser por um período de sete a 10 dias e seu mecanismo de ação é desconhecido (ARGÜELLO-GARCÍA et al., 2020).

## 1.7 Objetivo

O objetivo deste trabalho foi determinar a prevalência e realizar a caracterização molecular de *Giardia* spp. em populações de capivaras presentes em áreas urbanas, bem como correlacionar a presença de *Giardia* spp. com a faixa etária do animal e a estação climática.

## 1.8 Material e Métodos

### 1.8.1 Áreas de Estudo

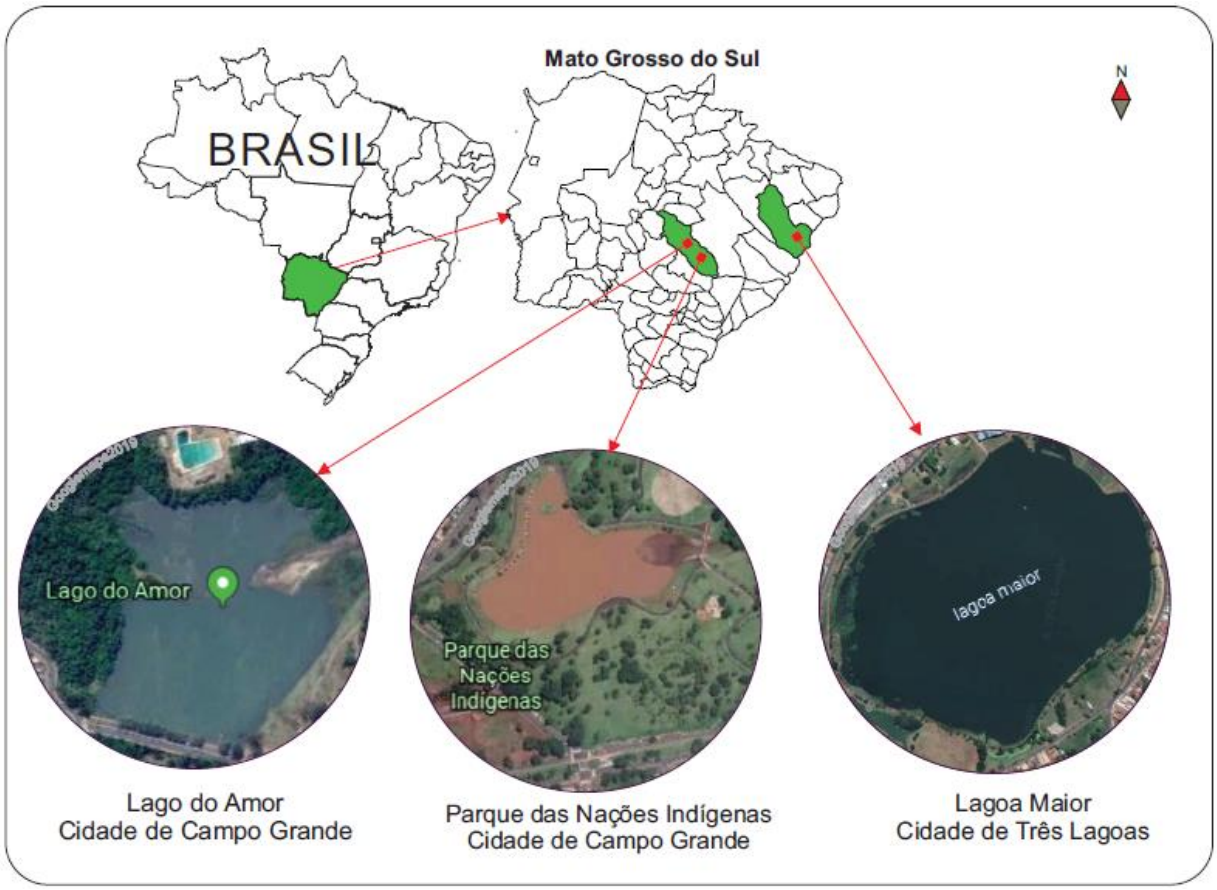
As amostras de fezes de capivaras foram colhidas nos municípios de Campo Grande e Três Lagoas, ambos no estado do Mato Grosso do Sul (Figura 1). As áreas para coleta foram escolhidas por serem áreas urbanas e por estarem ligadas a corpos de águas importantes desses municípios. Além disso, são áreas com populações expressivas de capivaras, de fácil acesso e que recebem visitas constantes da população.

No município de Campo Grande, as colheitas de amostras foram realizadas em duas áreas. A 1ª área corresponde ao Lago do Amor (20°30'10.7"S 54°37'02.0"W), que é um reservatório de água do município, pertence à reserva particular do patrimônio natural da Universidade Federal do Mato Grosso do Sul (UFMS) e está localizado no campus da UFMS, na confluência dos córregos Bandeiras e Cabaça. O segundo local de coleta foi o entorno do lago do Parque das Nações Indígenas (20°27'22.5"S 54°34'52.5"W). Esse parque é considerado o maior parque urbano do município, possui uma área de 4.810,6773 m², com áreas para atividades de lazer, recreação e esportes e está próximo do centro de Campo Grande (Mato Grosso do Sul, 2011).

Na cidade de Três Lagoas, as colheitas ocorreram no entorno da Lagoa Maior (20°46'56.0"S 51°42'58.9"W), situada no centro da cidade. Essa lagoa faz

parte da bacia hidrográfica do córrego da onça. Em suas proximidades estão localizados quiosques, quadras esportivas, academia ao ar livre, residências e restaurantes.

**Figura 1- Localização do Lago do Amor, do Parque das Nações Indígenas e da Lagoa Maior. Mapas adaptados do Instituto Brasileiro de Geografia e Estatística (IBGE)-2010.**




### 1.8.2 Amostras Fecais

Para colheita das amostras fecais foram obtidas autorizações da Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária da UNESP (PROCESSO FOA nº 917-2019), do Instituto de Meio ambiente de Mato Grosso do Sul (IMASUL) e do Sistema de Autorização e Informação em Biodiversidade (SISBio) número 70987-1, considerando a Instrução Normativa ICMBio nº 03/2014, que regulamenta a coleta de material biológico para fins científicos e didáticos (no âmbito do ensino superior) e a execução de pesquisa em unidades de conservação e cavernas.

As colheitas das amostras foram realizadas durante os períodos chuvoso e de seca, com início em 30/11/2019 e término em 22/07/2020. De acordo com os dados disponíveis no Instituto Nacional de Meteorologia (INMET), as regiões correspondentes às cidades de Campo Grande e Três Lagoas possuem períodos chuvosos entre os meses de novembro e janeiro e de seca entre junho e agosto.

As colheitas foram divididas entre as seguintes faixas etárias: filhote, juvenil e adulto. O critério para essa classificação foi baseado na biometria, incluindo as características físicas e comportamentais dos animais. Os filhotes são considerados animais muito pequenos e ainda em fase de aleitamento, geralmente acompanhados pelas fêmeas e formando “creches” (RODRIGUES et al., 2013). Os juvenis são os animais em fase intermediária entre filhotes e adultos, ou seja, não estão em fase de aleitamento, não atingiram a maturidade sexual e possuem tamanho reduzido (VERDADE; FERRAZ, 2006) (Figura 2). Os animais adultos possuem dimensão e peso corporal maior que os filhotes e juvenis, entretanto, não há dimorfismo sexual aparente em relação às dimensões do corpo (FERRAZ; BONACH; VERDADE, 2005). Foram considerados adultos todos os animais de grande porte e fêmeas amamentando.

O cálculo do número de amostras para determinação da prevalência de *Giardia* spp. nas populações deste estudo foi realizado com o uso do programa OpenEpi versão 3.0.1 (DEAN et al., 2013), com índice confiança de 95%, erro absoluto de 5% e prevalência esperada de 50% (Quadro 1). O número de indivíduos (n=480) considerado para o cálculo do número de amostras foi baseado em informações fornecidas pelos profissionais responsáveis pelos locais de coleta (Secretarias Municipais do Meio Ambiente).

Os animais se dividiam em grupos bem definidos com mais de 30 indivíduos. No Parque das Nações Indígenas havia cinco grupos de capivaras; no Lago do Amor e na Lagoa Maior havia dois grupos em cada.

A fim de minimizar o risco de colheita de amostras do mesmo indivíduo, foi realizada uma varredura no local para identificação e retirada das fezes preexistentes. No momento da coleta, foi feita uma contagem prévia e identificação dos indivíduos por faixa etária.

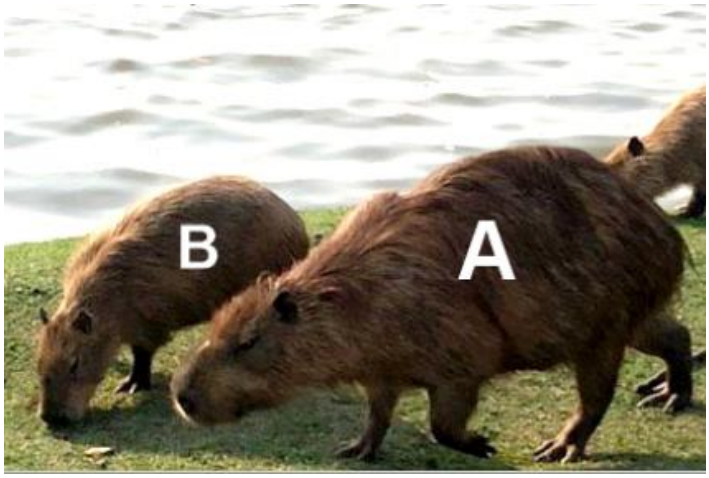
Aproximadamente 10 gramas de fezes foram colhidos logo após a defecação, com auxílio de espátula de madeira descartável. Apenas os péletes

da região superior dos montículos foram colhidos, a fim de evitar contaminação com cistos presentes no solo. As amostras foram armazenadas em frascos contendo bicromato de potássio 2,5% a 4º C e encaminhadas para análise na Faculdade de Medicina Veterinária da Unesp, Campus de Araçatuba.

**Tabela 1- Origem das amostras fecais de capivaras, número de amostras colhidas e número aproximado da população local de capivaras.**

| Local de coleta | nº aproximado da população | nº de amostras |
|---|---|---|
| Lagoa Maior /Três Lagoas – MS | 200 | 102 |
| Lago do Amor/Campo Grande – MS | 80 | 29 |
| Parque das Nações Indígenas/Campo Grande - MS | 200 | 116 |
| Total | 480 | 247 |

**Figura 2- Capivaras de diferentes faixas etárias no entorno da Lagoa Maior na cidade de Três Lagoas. A: adulto; B: jovem.**




### 1.8.3 Purificação e Concentração dos cistos

As amostras foram homogeneizadas, diluídas em água deionizada com Tween 20 0,1%, coadas em peneiras de plástico descartáveis e submetidas à concentração por centrífugo-sedimentação em água-éter.

Para purificação e concentração dos cistos, 5 g de fezes foram coados em peneiras de plástico descartáveis usando água deionizada/tween 20 0,1%. Um volume de 30 mL foi transferido para um tubo Falcon de 50 mL, no qual foi adicionado éter etílico até o volume de 40 ml. A amostra foi vortexada, centrifugada a 2.000 g por 8 m e o sobrenadante foi descartado. O sedimento resultante desse processo foi transferido para um tubo de 2 mL e os resíduos de éter foram retirados por um processo de adição de água destilada e centrifugação a 10.000 g, por 3 m, por 4 vezes. O sedimento resultante do processo de purificação foi armazenado a -20º C até a extração do DNA genômico.

### 1.8.4 Classificação Molecular de *Giardia* spp.

#### 1.8.4.1 Extração de DNA dos cistos de *Giardia* spp.

A extração de DNA foi realizada em todas as amostras, com utilização do "ZR Fecal DNA MiniPrep™" (Zymo Research), de acordo com o protocolo sugerido pelo fabricante.

#### 1.8.4.2 *Nested* PCR e Sequenciamento

Foram realizados três protocolos de *nested* PCR para detecção e classificação molecular de *Giardia* spp. Para amplificação de fragmento parcial do gene SSU rRNA, foram utilizados os *primers* 5′AAGTGTGGTGCAGACGGACTC3′ e 5′CTGCTGCCGTCCTTGGATGT3′ (497bp) (APPELBEE et al., 2003), na reação primária, e 5′CATCCGGTCGATCCTGCC3′ e 5′-GTCGAACCCTGATTCTCCGCCAGG-3′ (292 bp) (HOPKINS et al., 1997) na reação secundária.

As amostras com tamanho da banda amplificada pela *nested* PCR para o gene SSU rRNA, sugestiva de *Giardia* spp., foram submetidas à *nested* PCR para amplificação de fragmento parcial do gene GDH, com utilização dos *primers* 5′TCAACGTYAAYCGYGGYTTCCGT3′ e 5′GTTRTCCTTGCACATCTCC3′, para a reação primária, e 5′CAGTACAACTCYGCTCTCGG3′ e 5′GTTRTCCTTGCACATCTCC3′ para a reação secundária (READ; MONIS; THOMPSON, 2004). Para o gene TPI, foram utilizados os *primers*

5′AAATIATGCCTGCTCGTCG3′ e 5′CAAACCTTITCCGCAAACC3′, para a reação primária, e 5′CCCTTCATCGGIGGTAACTT3′ e 5′GTGGCCACCACICCCGTGCC3′ para a reação secundária (SULAIMAN et al., 2003).

Como controle positivo da *nested* PCR foram utilizadas amostras de DNA genômico de *G. duodenalis*. Água ultrapura foi utilizada como controle negativo. Os fragmentos amplificados foram visualizados por eletroforese em gel de agarose 1,5% corado com GelRed (Biotium).

Os fragmentos com banda de tamanho sugestivo para *Giardia* spp. foram purificados utilizando o ExoSAP-IT® PCR *Product Cleanup Reagent* (Termofisher Scientific) e submetidos a sequenciamento bidirecional, com o “ABI Prism® Dye Terminator 3.1”.

## 1.9 Resultados

Dentre as 247 amostras, 183 foram coletadas durante o período chuvoso nos três locais de estudo (Lagoa Maior, no município de Três Lagoas – MS, e no Lago do Amor e no Parque das Nações Indígenas, no município de Campo Grande - MS). O restante das amostras (n=64) foi coletado durante o período de seca, somente na Lagoa Maior.

Neste estudo, 133 amostras (54%) pertenciam a capivaras adultas, 61 a filhotes (25%) e 53 a capivaras juvenis (21%). Todos os animais estavam aparentemente saudáveis no momento da coleta.

Dezesseis amostras (n=16) apresentaram na eletroforese bandas de DNA sugestivas de *Giardia* spp. pela PCR para o gene SSU rRNA. No entanto, o sequenciamento genético foi inconclusivo e sugestivo de amplificação inespecífica. Todas as amostras com bandas sugestivas de *Giardia* spp. pela PCR para o gene SSU rRNA foram submetidas à PCR para os genes TPI e GDH e todas foram negativas para *Giardia* spp. Portanto, todas as amostras (n=247) de fezes de capivara apresentaram resultado negativo para *Giardia* spp.

## 1.10 Discussão

*Giardia* é um parasito entérico amplamente distribuído no mundo, encontrado em vertebrados, incluindo humanos e várias espécies de animais (FANTINATTI et al., 2020). A capivara é hospedeira de vários parasitos de

importância para saúde pública, dentre eles, helmintos e protozoários (SOUZA et al., 2021).

Neste trabalho, observamos ausência de *Giardia* spp. em 247 amostras de fezes de capivara por meio de três protocolos de *nested* PCR. Um estudo realizado em áreas antroponizadas do Estado de São Paulo e em áreas naturais no Estado do Mato Grosso e Mato Grosso do Sul analisou 113 amostras de capivaras e destacou a presença de *Eimeria* spp. em 76,1% (86) e dos parasitos da superfamília *Trichostrongyloidea* em 53,1%(60) em ambos locais de estudo (SOUZA et al., 2021). Outra pesquisa realizada em Curitiba – Paraná analisou 53 amostras de fezes de capivara e constatou que 92,4% dos animais estavam parasitados, principalmente por parasitos do Filo Nematoda (TRUPPEL, 2009). Em ambos os estudos, foi realizada a técnica de microscopia e não houve a presença de *Giardia* nas amostras de capivaras, corroborando com nosso resultado.

No entanto, (REGINATTO et al., 2008) relataram, após análise microscópica por centrífugo-flutuação com sulfato de zinco, em três amostras de cutias e três amostras de capivaras assintomáticas criadas em cativeiro no Rio Grande do Sul, a presença de cistos de *Giardia* spp. e oocistos de *Cryptosporidium* spp. e de *Eimeria* spp. em todas as amostras analisadas. Como o número de amostras examinadas por esses autores correspondem a uma baixa amostragem e os animais eram mantidos em cativeiro, não há como comparar os resultados aos deste trabalho.

Um estudo realizado na Colômbia com amostras de 360 capivaras que viviam em seu habitat natural identificou a presença de *Giardia* e correlacionou os resultados com as estações do ano. No verão e no inverno, 1,1% (4/360) e 0,6% (2/360) das amostras revelaram presença de *Giardia* spp. por meio de microscopia, respectivamente. Os autores sugeriram que a baixa prevalência de infecção por *Giardia* no inverno pode estar relacionada com a estação chuvosa, devido à maior disponibilidade de nutrientes para as capivaras nessa época, e, consequentemente, ao aumento do potencial biótico dos protozoários ciliados e possível competição com outros protozoários (RODRÍGUEZ-DURÁN et al., 2015). No entanto, neste trabalho não foi possível corroborar essa hipótese, pois todas as amostras foram negativas para *Giardia* spp., independentemente do período de colheita.

Em relação à prevalência de *Giardia* spp. em diferentes faixas, não há nenhum trabalho publicado com amostras de capivaras. No entanto, com referência a roedores, TIJJANI et al. (2020) analisaram a prevalência de parasitos em ratos selvagens da Malásia por meio da técnica de concentração com formalina-éter seguida por microscopia e correlacionaram os resultados com a idade dos animais. *Giardia* spp. estava presente em 16% dos ratos adultos (8/56) e 12,8% dos ratos jovens (5/39). Apesar de não haver diferença estatística significativa, os autores relataram que, de modo geral, os ratos jovens (17/95;18,3%) foram infectados com mais frequência que os adultos (14/95;15,3%), devido ao fato de eles serem mais ativos e explorarem o ambiente (TIJJANI et al., 2020). Neste trabalho todas as amostras provenientes de capivaras de adultas, juvenis e filhotes foram negativas.

As capivaras pesquisadas neste estudo viviam em seu habitat natural, próximo a lagos e parques localizados no centro da cidade. Apesar do contato indireto com as pessoas, não foi identificada infecção nas capivaras por espécies zoonóticas de *Giardia* spp. No entanto, em um zoológico da Croácia, dentre duas amostras de capivaras, uma apresentou resultado positivo para *Giardia* spp. pela imunoflorescência direta; em outras quatro amostras de animais da ordem Rodentia: esquilo de prevost (1), lebre saltadora (2), e lebre da patagônia (1), a análise molecular revelou a presença de *G. duodenalis assemblage* B (BECK et al., 2011).

Apesar de nenhuma amostra ter apresentado positividade para *Giardia* neste trabalho, existe a possibilidade de outros roedores apresentarem infecção por *Giardia* spp. Coppola et al. (2020) observaram a presença de *G. duodenalis* em 48% (25/52) das amostras de porco-espinho-de-crista, bem como, identificou os *assemblages* B (n=12), BIV (n=1) e AII (n=2), destacando a possibilidade desses animais serem transmissores zoonóticos de *Giardia*. O baixo potencial zoonótico de roedores em relação a *G. duodenalis foi* discutido por Helmy et al. (2018), que relataram maior prevalência de *G. microti* (358/314/87,7%) e *G. muris* (358/36/9,8%) em roedores na Alemanha. Somente 1,4% (358/5) das amostras examinadas continham *os assemblages* A e B de *G. duodenalis*.

Em todos os relatos de *Giardia* em capivaras descritos em literatura, foram utilizadas técnicas de microscopia (REGINATTO et al., 2008; RODRÍGUEZ-DURÁN et al., 2015) ou imunoflorescência (BECK et al., 2011), ou seja, não

houve a identificação da espécie e *assemblages* por técnicas moleculares. Portanto, este é o primeiro trabalho em que o potencial zoonótico referente à giardíase nesses animais pôde ser determinado e demonstra, juntamente com os resultados de outros trabalhos, que a capivara provavelmente não é um reservatório importante de *Giardia* spp. para seres humanos.

## 1.11 Conclusão

Neste trabalho, observamos ausência de *Giardia* spp. em amostras fecais oriundas de capivaras de diferentes faixas etárias e estações do ano nas três áreas urbanas examinadas.

# 2 CAPÍTULO 1 - PREVALENCE OF *Giardia* spp. IN FECAL SAMPLES OF CAPYBARAS (*Hydrochoerus hydrochaeris*) IN URBAN AREAS

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## 2.1 Resumo

A giardíase é a causa mais comum de diarreia em humanos e animais em todo o mundo. Atualmente, existem oito espécies de Giardia spp., Incluindo Giardia duodenalis, que infecta a maioria dos vertebrados. A Capivara (Hydrochoerus hydrochaeris) é o maior roedor herbívoro do mundo, porém existem poucos estudos a respeito do potencial zoonótico desses animais. O objetivo deste trabalho foi determinar a prevalência e realizar a caracterização molecular de Giardia spp. em populações de capivaras presentes em áreas urbanas, bem como correlacionar a presença de Giardia spp. entre a faixa etária do animal e as mudanças climáticas durante as diferentes estações. Foram coletadas 247 amostras de capivaras na Lagoa Maior em Três Lagoas - Mato Grosso do Sul e no Lago do Amor e Parque das Nações Indígenas, ambos localizados em Campo Grande - Mato Grosso do Sul. Nested PCR foi realizada com os genes da subunidade menor do RNA ribossomal (SSU rRNA), Glutamato Desidrogenase (GDH) e Triosefosfato Isomerase (TPI), seguido de sequenciamento genético. 183 amostras foram coletadas na estação chuvosa e 64 na estação seca. Cento e trinta e três amostras (54%) pertenciam a capivaras adultas, 61 a descendentes (25%) e 53 a capivaras juvenis (21%). Todas as amostras de fezes (n = 247) foram negativas para Giardia spp. Neste trabalho, a presença de Giardia spp. em amostras fecais de capivaras, sugerindo que nas áreas

[1]O artigo está nas normas da revista Veterinary Research Communications. Vide Anexo 1.

analisadas esses animais não são um importante reservatório de Giardia spp. para humanos.
**Palavras-chave:** *Giardia*. Roedores. Prevalência. Reação em cadeia da polimerase

## 2.2 Abstract

Giardiasis is the most common cause of diarrhea in humans and animals worldwide. Currently, there are eight species of *Giardia* spp., including *Giardia duodenalis,* which infects most vertebrates. The Capybara (*Hydrochoerus hydrochaeris*) is the largest herbivorous rodent in the world, however there are few studies regarding the zoonotic potential of these animals. The objective of this work was to determine the prevalence and carry out the molecular characterization of *Giardia* spp. in populations of capybaras present in urban areas, as well as to correlate the presence of *Giardia* spp. between the animal's age range and climate change during different seasons. 247 samples of capybaras were collected in Lagoa Maior in Três Lagoas – Mato Grosso do Sul and in Lago do Amor and Parque das Nações Indígenas, both located in Campo Grande - Mato Grosso do Sul. Nested PCR was performed with the genes of the ribosomal RNA minor subunit (SSU rRNA), Glutamate Dehydrogenase (GDH) and Triosephosphate Isomerase (TPI), followed by genetic sequencing. 183 samples were collected in the rainy season and 64 in the dry season. One hundred and thirty-three samples (54%) belonged to adult capybaras, 61 to offspring (25%) and 53 to juvenile capybaras (21%). All stool samples (n=247) were negative for *Giardia* spp. In this work, the presence of *Giardia* spp. in fecal samples of capybaras, suggesting that in the areas analyzed these animals are not an important reservoir of *Giardia* spp. for humans.
**Keywords:** *Giardia*. Rodents. Prevalence. Polymerase chain reaction.

## 2.3 Introduction

Giardiasis is the most common cause of diarrhea in humans and animals worldwide. An estimated 280 million humans are infected with *Giardia* spp. annually, leading to death of children under the age of five and

immunocompromised individuals in developing countries (Sulaiman et al. 2003; Einarsson et al. 2016; Santin 2020).

*Giardia* transmission occurs via the fecal-oral route, in other words, when the host comes into contact with infected humans and animals, or by ingesting water and food contaminated with cysts (Santin 2020). The life cycle of *Giardia* spp. occurs in two stages: the trophozoite, responsible for colonization of the intestinal epithelium, and the cyst, considered the infective form, resistant to the environment (Geurden et al. 2010; Einarsson et al. 2016).

The clinical signs of giardiasis in humans range from asymptomatic cases to diarrhea, colic, nausea, weight loss and malabsorption (Feng and Xiao 2011; Ryan and Cacciò 2013; Einarsson et al. 2016). In wild animals, there is little information regarding the clinical effects of giardiasis, but cases of asymptomatic, mild or severe infections, depending on the host, have been described (Appelbee et al. 2005; Ryan and Zahedi 2019).

There are descriptions of eight species of *Giardia*, among them *Giardia microti*, *Giardia cricetidarum* and *Giardia muris*, which infect rodents; *Giardia ardeae* and *Giardia psittaci*, identified in birds; *Giardia agilis* found in amphibians, *Giardia peramelis* in marsupials and *Giardia duodenalis*, which infects most vertebrates, including humans (Sulaiman et al. 2003; Maloney et al. 2020; Santin 2020).

*Giardia duodenalis* consists of eight assemblages identified as A to H. Assemblages A and B have zoonotic potential and infect humans and a wide variety of animals; the other assemblages are host-specific, with assemblages C and D infecting canines and assemblages E, F, G, and H infecting artiodactyls, felines, rodents, and marine mammals, respectively (Berrilli et al. 2004; Maloney et al. 2020; Santin 2020).

Rodent animals can be important reservoirs of several pathogens, including viruses, bacteria and parasites, including G. *muris*, *G. microti* and the zoonotic assemblages A and B of G. *duodenalis* (Helmy et al. 2018). The presence of *Giardia* spp. has already been reported in feces of capybaras, but the genotyping of these samples was not performed; therefore, information on possible zoonotic transmission from these animals is still scarce (Reginatto et al. 2008; Rodríguez-Durán et al. 2015).

The capybara (*Hydrochoerus hydrochaeris*) is the largest herbivorous rodent in the world. They are animals that live in semi-aquatic environments, i.e. they need water for drinking, wallowing, protection from predators and reproduction; they also need land to rest and forage for food (Bonuti et al. 2002; Herrera et al. 2011; Almeida and Biondi 2014; Nogueira-Filho and Nogueira 2018). They have the ability to explore and adapt to different habitats, including anthropogenic environments, managing to survive in large urban centers (Herrera et al. 2011; Almeida and Biondi 2014). Also, with the advance of capybara breeding and the growing presence of capybaras in flooded and anthropogenic areas, studies of parasitosis in these animals aroused interest (Verdade and Ferraz, 2006; Ferraz et al. 2005; Almeida et al. 2013; Almeida and Biondi, 2014; Nogueira-Filho and Nogueira, 2018).

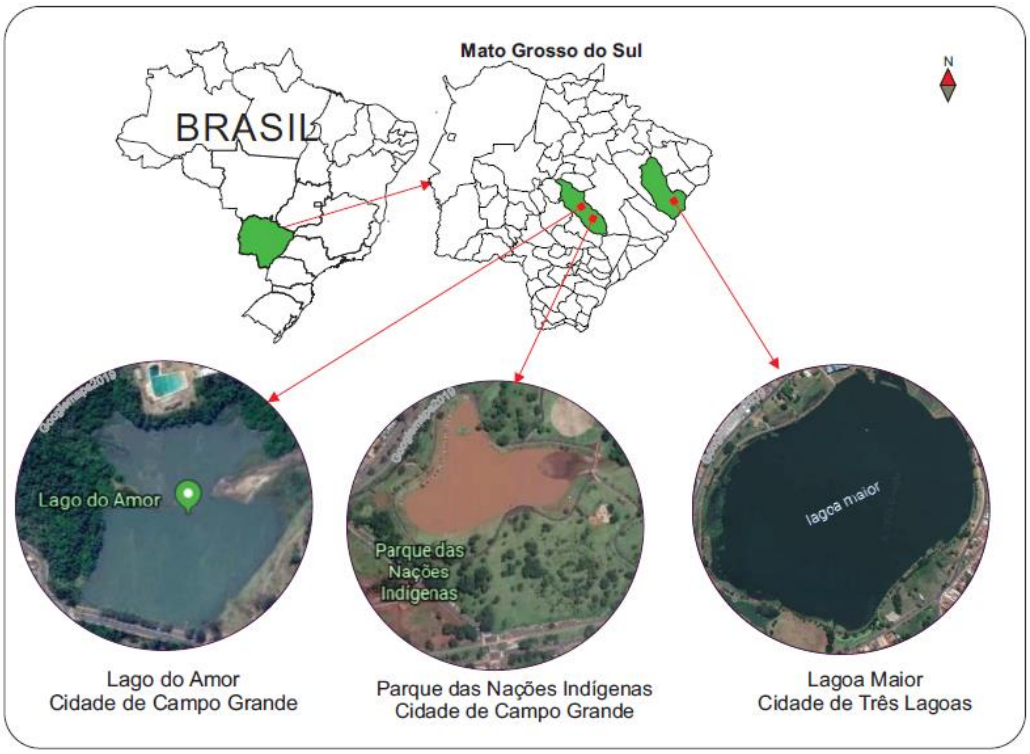
## 2.4 Material and Methods

Capybara feces samples were collected in the municipalities of Campo Grande and Três Lagoas, both in the state of Mato Grosso do Sul (Fig 1) and containing expressive populations of capybaras, are easily accessible and receive constant visits from the population.

In the municipality of Campo Grande-MS, sample collections were carried out in two areas. The first area corresponds to Lago do Amor (20°30'10.7"S 54°37'02.0"W) and the second collection site was in the Parque das Nações Indígenas (20°27'22.5"S 54°34'52.5" W). In the city of Três Lagoas-MS, sample collections took place in the surroundings of Lagoa Maior (20°46'56.0"S 51°42'58.9"W), located in the center of the city.

Fig 1 Location of Lago do Amor, Parque das Nações Indígenas and Lagoa Maior. Maps adapted from the Brazilian Institute of Geography and Statistics

(IBGE)-2010.




Sample collections were carried out during the rainy and dry periods, starting on 11/30/2019 and ending on 07/22/2020. According to data available at the National Institute of Meteorology (INMET), the regions corresponding to the cities of Campo Grande and Três Lagoas have rainy periods between the months of November and January and dry periods between June and August.

The animals were divided into the offspring, juvenile and adult age groups; the criteria used to determine the age groups were based on biometrics and on the physical and behavioral characteristics of the animals. Capybara pups were considered very small animals and still in the suckling stage, usually accompanied by females and forming “nursery” (Rodrigues et al. 2013). Juveniles are animals in an intermediate stage between offspring and adults, i.e. they are not suckling, have not reached sexual maturity and have a reduced size (Verdade and Ferraz 2006). Adult animals have larger body size and weight than pups and young capybaras, however, there is no apparent sexual dimorphism in relation to body dimensions (Ferraz et al. 2005). All large animals and nursing females were considered adults.

Calculating the number of samples to determine the prevalence of *Giardia* spp. in the populations of this study was carried out using the OpenEpi version 3.0.1 program (Dean et al. 2013), with a confidence index of 95%, an absolute

error of 5% and an expected prevalence of 50% (Table 1). The number of individuals (n=480) considered to calculate the number of samples was based on information provided by the professionals responsible for the collection sites (Municipal Departments of the Environment).

In order to minimize the risk of collecting samples from the same individual, an on-site scan was performed to identify and remove pre-existing stools. At the time of collection, a previous count and identification of individuals by age group was performed.

Approximately 10 grams of feces were collected right after defecation, using a disposable wooden spatula. Only the pellets from the upper surface of the feces were collected in order to avoid contamination. The samples were stored in flasks containing 2.5% potassium bichromate at 4º C and sent for analysis at the Faculty of Veterinary Medicine, UNESP, Araçatuba. The samples were subjected to concentration by centrifugal sedimentation in water-ether and genomic DNA extraction using the ZR Fecal DNA MiniPrep™ kit (Zymo Research).

Table 1 Origin of fecal samples of capybaras, number of samples collected and approximate number of local capybara population.

| **Collection location** | **Approximate population** | **Number of samples** |
|---|---|---|
| Lagoa Maior /Três Lagoas - MS | 200 | 102 |
| Lago do Amor/Campo Grande – MS | 80 | 29 |
| Parque das Nações Indígenas/Campo Grande - MS | 200 | 116 |
| Total | 480 | 247 |

For screening of *Giardia* spp. DNA the partial fragment of the gene encoding the small subunit of ribosomal RNA (SSU rRNA) was amplified using primers 5′AAGTGTGGTGCAGACGGACTC3′ and 5′CTGCTGCCGTCCTTGGATGT3′ (497bp) (Appelbee et al. 2003), in the primary reaction, and 5′CATCCGGTCGATCCTGCC3′ and

5′GTCGAACCCTGATTCTCCGCCAGG3′ (292 bp) (Hopkins et al. 1997), in the secondary reaction.

Samples with band size amplified by nested PCR for the SSU rRNA gene, suggestive of *Giardia* spp., were submitted to nested PCR for amplification of a partial fragment of the glutamate dehydrogenase (GDH) gene, using primers 5′TCAACGTYAAYCGYGGYTTCCGT3′ and 3′GTTRTCCTTGCACATCTCC3′, for the primary reaction, and 5′CAGTACAACTCYGCTCTCGG3′ and 5′GTTRTCCTTGCACATCTCC3′, for the secondary reaction (Read et al. 2004), and of the triosephosphate isomerase (TPI) gene, with primers 5′AAATIATGCCTGCTCGTCG3′ and 5′ CAAACCTTITCCGCAAACC 3′, for the primary reaction, and 5′CCCTTCATCGGIGGTAACTT3′ and 5′GTGGCCACCACICCCGTGCC3′ for the secondary reaction (Sulaiman et al. 2003).

As a positive control for *nested* PCR, genomic DNA from *G. duodenalis* was used and ultrapure water for negative control. The amplified fragments were visualized by electrophoresis in a 1.5% agarose gel stained with GelRed (Biotium).

Fragments with bands of suggestive size for *Giardia* spp. were purified using the ExoSAP-IT® PCR Product Cleanup Reagent (Termo Fisher Scientific) and submitted to bidirectional sequencing, with the “ABI Prism® Dye Terminator 3.1”.

## 2.5 Results

Among the 247 samples, 183 were collected during the rainy season in the three study sites (Lagoa Maior, in the municipality of Três Lagoas – MS, and in Lago do Amor and Parque das Nações Indígenas, in the municipality of Campo Grande – MS). The remaining samples (n=64) were collected during the dry season, only in Lagoa Maior.

In this study, 133 samples (54%) belonged to adult capybaras, 61 to offspring (25%) and 53 to young capybaras (21%). All animals were apparently healthy at the time of collection.

Sixteen samples (n=16) showed DNA bands suggestive of *Giardia* spp. by PCR for the SSU rRNA gene and were submitted to nested PCR for amplification of the TPI and GDH genes. However, genetic sequencing was inconclusive and

suggestive of nonspecific amplification. Therefore, all samples (n=247) of capybara feces were negative for *Giardia* spp.

## 2.6 Discussion

*Giardia* is an enteric parasite widely distributed in the world, found in vertebrates, including humans and several animal species (Fantinatti et al. 2020). Capybara is host to several parasites of public health importance, including helminths and protozoa (Souza et al. 2021).

In this work, we observed the absence of *Giardia* spp. in 247 capybara stool samples using three nested PCR protocols. A study carried out in anthroponized areas in the State of São Paulo and in natural areas in the State of Mato Grosso and Mato Grosso do Sul analyzed 113 samples of capybaras and highlighted the highest prevalence of *Eimeria* spp. (86/76.1%) and Phylum Nematoda parasites (60/53.1%) in both study sites (Souza et al. 2021). In this study, the microscopy technique was performed and there was no presence of *Giardia* in the capybara samples, corroborating our result.

However, Reginatto et al. (2008) reported, after microscopic analysis by centrifugal flotation with zinc sulfate, in three samples of agouti and three samples of asymptomatic capybaras raised in captivity in Rio Grande do Sul, the presence of cysts of *Giardia* spp. and oocysts of *Cryptosporidium* spp. and *Eimeria* spp. in all analyzed samples. As the number of samples examined by these authors corresponds to a low sampling and the animals were kept in captivity, it was not possibe to compare the results with those in this work.

A study carried out in Colombia with samples of 360 capybaras that lived in their natural habitat identified the presence of *Giardia* and correlated the results with the seasons. In summer and winter, 4/360 (1.1%) and 2/360 (0.6%) of the samples revealed the presence of *Giardia* spp. through microscopy, respectively. The authors suggested that the low prevalence of *Giardia* infection in winter may be related to the rainy season, due to the greater availability of nutrients for capybaras at that time, and, consequently, to the increase in the biotic potential of ciliated protozoa and possible competition with other protozoa (Rodríguez-Durán et al. 2015). However, in this work it was not possible to corroborate this hypothesis, as all samples were negative for *Giardia* spp., regardless of the collection period.

Regarding the prevalence of *Giardia* spp. in different age groups of capybaras, there is no published work with these animals. However, with reference to rodents, Tijjani et al. (2020) analyzed the prevalence of parasites in wild Malaysian rats using the formalin-ether concentration technique followed by microscopy and correlated the results with the age of the animals. *Giardia* spp. it was present in 16% of adult rats (8/56) and 12.8% of young rats (5/39). Although there was no statistically significant difference, the authors reported that, in general, young rats (17/95;18.3%) were infected more frequently than adults (14/95;15.3%), by be more active and explore the environment (Tijjani et al. 2020). However, in this work all samples from adult, young and offspring capybaras were negative.

In this study, capybaras lived in their natural habitat near lakes and parks located in the city center. Despite indirect contact with people, there was no evidence of zoonotic transmission of *Giardia* spp. However, in a Croatian zoo, among two samples of capybaras, one was positive for *Giardia* spp. by direct immunofluorescence; in another four rodent animals samples: prevost squirrel (1), jumping hare (2), and patagonia hare (1), molecular analysis revealed the presence of G. *duodenalis* assemblage B (Beck et al. 2011).

Although no sample was positive for *Giardia* in this study, there is a possibility that other rodents may have an infection with *Giardia* spp. Coppola et al. (2020) observed the presence of G. *duodenalis* in 48% (25/52) of the crested porcupine samples, as well as identified the assemblages B (n=12), BIV (n=1) and AII (n=2), highlighting the possibility of these animals being zoonotic transmitters of *Giardia*. The low zoonotic potential of rodents in relation to G. *duodenalis* was discussed by Helmy et al. (2018), who reported a higher prevalence of G. *microti* (358/314/87.7%) and G. *muris* (358/36/9.8%) in rodents in Germany. Only 1.4% (358/5) of the examined samples contained G. *duodenalis* assemblages A and B.

In all reports of *Giardia* in capybaras, microscopy techniques (Reginatto et al. 2008; Rodríguez-Durán et al. 2015) or immunoflorescence (Beck et al. 2011) were used, i.e. there was no identification of the species and assemblages by molecular techniques. Therefore, this is the first study in which the zoonotic potential for giardiasis in these animals could be determined and it demonstrates,

along with the results of other works, that capybara is probably not an important reservoir of *Giardia* spp. for humans.

In this work, we observed the absence of *Giardia* spp. in fecal samples from capybaras of different age groups and seasons in the three urban areas examined.

## 2.7 Statements

### 2.7.1 Financing

Not applicable

### 2.7.2 Conflicts of interest / Competing interests

Authors have no conflict of interest to declare that they are relevant to the content of this article.

### 2.7.3 Availability of Data and Materials

Not applicable

### 2.7.4 Code Availability

Not applicable

### 2.7.5 Author Contributions

All authors made fundamental contributions to the elaboration of this research, as well as critically reviewing the content of this work.

### 2.7.6 Ethics Approval

Authorizations were obtained from the Ethics Committee in the Use of Animals of the Faculty of Veterinary Medicine, UNESP (PROCESS FOA No. 917-2019), the Institute of Environment of Mato Grosso do Sul (IMASUL) and the Authorization and Information System on Biodiversity ( SISBio) number 70987-1, considering ICMBio Normative Instruction 03/2014, which regulates the collection of biological material for scientific and educational purposes (in the context of higher education) and the execution of research in conservation units and caves.

### 2.7.7 Consent to Participate

All authors participated and helped voluntarily in the research.

### 2.7.8 Consent for Publication

All authors read and approved the fnal manuscript.

## 2.8 Bibliography


ALMEIDA A. M. R.; ARZUA, M.; TRINDADE P. W. S; SILVA JUNIOR, A. Capybaras (*Hydrochoerus hydrochaeris*, Linnaeus, 1766) (Mammalia: Rodentia) in green areas of Curitiba, Parana State, Brazil. **Estudos de Biologia**, v. 35, n. 84, p. 9-16, 2013. http://dx.doi.org/10.7213/estud.biol.7845

ALMEIDA, A. R.; BIONDI, D. Area of use by *Hydrochoerus hydrochaeris* L. in an urban environment. **Ciência Animal Brasileira**, v.15, p. 369-376, 2014. https://doi.org/10.1590/1809-6891v15i319663

APPELBEE, A. J.; THOMPSON, R. C. A.; OLSON, M. E. *Giardia* and *Cryptosporidium* in mammalian wildlife - Current status and future needs. **Trends in Parasitology**, v. 21, n. 8, p. 370-376, 2005. https://doi.org/10.1016/j.pt.2005.06.004

BERRILLI, F.; CAVE, D.D.; LIBERATO, C.D.; FRANCO, A.; SCARAMOZZINO, P.; ORECCHIA, P. Genotype characterisation of *Giardia duodenalis* isolates from domestic and farm animals by SSU-rRNA gene sequencing. **Veterinary Parasitology**, v.122, p.193–199, 2004. https://doi.org/10.1016/j.vetpar.2004.04.008

BECK, R.; SPRONG, H.; BATA, I.; LUCINGER, S.; POZIO, E.; CACCIÒ, S.M. Prevalence and molecular typing of *Giardia* spp. in captive mammals at the zoo of Zagreb, Croatia. **Veterinary Parasitology**, v. 175, p.40-46, 2011.

BONUTI, M. R.; NASCIMENTO, A. A.; MAPELLI, E. B.; ARANTES, I. G. Gastrintestinal helminths of capybara (*Hydrochoerus hydrochaeris*) from the Paiaguás subregion, in the floodplain of "Mato Grosso do Sul", Brazil. **Semina: Ciências Agrárias**, v. 23, n. 1, p. 57-62, 2002.

CACCIÒ, S. M.; LALLE, M.; SVÄRD, S. G. Host specificity in the *Giardia duodenalis* species complex. **Infection, Genetics and Evolution**, v. 66, n. October 2017, p. 335–345, 2018. https://doi.org/10.1016/j.meegid.2017.12.001

COPPOLA, F.; MAESTRINI, M.; BERRILLI, F.; PROCESI, I.G.; FELICIOLI, A.; PERRUCCI, S. First report of *Giardia duodenalis* infection in the crested porcupine ( *Hystrix cristata* L ., 1758 ). **International Journal for Parasitology: Parasites and Wildlife**, v. 11, p. 108–113, 2020. https://doi.org/10.1016/j.ijppaw.2020.01.006

DEAN, A. G.; SULLIVAN, K. M.; SOE, M. M. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Versão. www.OpenEpi.com, atualizado 2013/04/06, acessado em 2019/03/16.

EINARSSON, E.; MA'AYEH, S.; SVÄRD, S. G. An up-date on *Giardia* and *Giardia*sis. **Current Opinion in Microbiology**, v. 34, p. 47–52, 2016. https://doi.org/10.1016/j.mib.2016.07.019

FANTINATTI, M.; GONÇALVES-PINTO, M.; LOPES-OLIVEIRA, L.A.P.; DA-CRUZ, A.M. Epidemiology of *Giardia duodenalis* assemblages in brazil: There is still a long way to go. **Memórias do Instituto Oswaldo Cruz**, v. 115, n. 11, p. 1–12, 2020. https://doi.org/10.1590/0074-02760200431

FENG, Y.; XIAO, L. Zoonotic potential and molecular epidemiology of *Giardia* species and *Giardia*sis. **Clinical Microbiology Reviews**, v. 24, n. 1, p. 110-140, 2011. https://doi.org/10.1128/CMR.00033-10

FERRAZ, K.M.P.M.B.; BONACH, K.; VERDADE, L.M. Relationship between body mass and body length in capybaras (*Hydrochoerus hydrochaeris*). **Biota neotropica**, v. 5, n.1, 2005. https://doi.org/10.1590/S1676-06032005000100020

GEURDEN, T.; VERCRUYSSE, J.; CLAEREBOUT, E. Is *Giardia* a significant pathogen in production animals? **Experimental Parasitology**, v. 124, n. 1, p. 98–106, 2010.

https://doi.org/10.1016/j.exppara.2009.03.001

HELMY, Y. A.; SPIERLING, N.G.; SCHMIDT, S.; ROSENFELD, U.M.; REIL, D.; IMHOLT, C.; JACOB, J.; ULRICH, R.G.; AEBISCHER, T.; KLOTZ, C. Occurrence and distribution of *Giardia* species in wild rodents in Germany. **Parasites and Vectors**, v. 11, n. 1, p. 213, 2018. DOI:10.1186/s13071-018-2802-z

HERRERA, E. A.; SALAS, V.; CONGDON, E.R.; CORRIALE, M.J.; TANG-MARTI´NEZ, Z. Capybara social structure and dispersal patterns: variations on a theme. **Journal of Mammalogy**, v. 92, n. 1, p. 12–20, 2011. https://doi.org/10.1644/09-MAMM-S-420.1

HOPKINS, R.M., MELONI, B.P., GROTH, D.M., WETHERALL, J.D., REYNOLDSON, J.A., THOMPSON R.C. Ribosomal RNA sequencing reveals differences between the genotypes of *Giardia* isolates recovered from humans and dogs living in the same locality. **Journal of Parasitology**, v.83, p. 44–51, 1997. DOI: 10.2307/3284315

MALONEY, J. G.; MOLOKIN, A.; SANTIN, M. Assessment of next generation amplicon sequencing of the beta-giardin gene for the detection of *Giardia duodenalis* assemblages and mixed infections. **Food and Waterborne Parasitology**, v. 21, 2020. https://doi.org/10.1016/j.fawpar.2020.e00098

NOGUEIRA-FILHO, S. L. G.; NOGUEIRA, S. S. C. Capybara meat: An extraordinary resource for food security in South America**. Meat Science**, v.145, p.329-333, 2018. https://doi.org/10.1016/j.meatsci.2018.07.010

NOGUEIRA-FILHO, S. L. G.; LOPES, P.C.; FERREIRA, D.N.; NOGUEIRA, S.S.C. Flexibility in the social behavior of captive female capybaras (Mammalia, Rodentia). **Behavioural Processes**, v.142, p. 29-32, 2017. https://doi.org/10.1016/j.beproc.2017.05.018

READ, C. M.; MONIS, P. T.; THOMPSON, R. C. A. Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. **Infection, Genetics and Evolution**, v. 4, p. 125–130, 2004. https://doi.org/10.1016/j.meegid.2004.02.001

REGINATTO, A. R.; FARRET, M.H.; FANFA, V.R.; SILVA, A.S.; MONTEIRO, S.G. Infection by Giardia spp. and Cystoisospora spp. in capybara and agouti in southern Brazi. **Revista Portuguesa de Ciências Veterinárias**, v. 103, p. 96–99, 2008.

RODRÍGUEZ-DURÁN, A.; BLANCO PALMA, L. C.; PEÑA FLÓREZ, R. Main gastrointestinal protozoa in wild capybara (*Hydrochoerus hydrochaeris*) in a village in the municipality of Arauca, Colombia. **Zootecnia Tropical**, v. 33, n. 3, p. 261–268, 2015.

RODRIGUES, M. V.; PAULA, T.A.R.; FERREIRA, L.B.C.; ÁVILA, E.C.; SILVA, L.C.; SOUZA, V.B. Behavior of a group of capybaras in an urban area. **Acta Veterinária Brasilica**, v.7, p.212-217, 2013.
RYAN, U.; CACCIÒ, S.M. Zoonotic potential of *Giardia*. **International Journal for Parasitology**, v. 43, p. 943-956, 2013. https://doi.org/10.1016/j.ijpara.2013.06.001

RYAN, U.; ZAHEDI, A. Molecular epidemiology of giardiasis from a veterinary perspective. **Advances in Parasitology**, v. 106, p. 209–254, 2019. https://doi.org/10.1016/bs.apar.2019.07.002

SANTIN, M. *Cryptosporidium* and *Giardia* in Ruminants. **Veterinary Clinics of North America - Food Animal Practice**, v. 36, n. 1, p. 223–238, 2020. https://doi.org/10.1016/j.cvfa.2019.11.005

SOUZA S.L.P.; BENATTI H.R.; LUZ H.R.; COSTA F.B.; PACHECO R.C.; LABRUNA M.B. Endoparasites of capybaras (*Hydrochoerus hydrochaeris*) from anthropized and natural areas of Brazil. **Brazilian Journal of Veterinary Parasitology**, v. 30, n. 2, 2021. https://doi.org/10.1590/S1984-29612021049

SULAIMAN, I.M.; FAYER, R.; BERN, C.; GILMAN, R.H.; TROUT, J.M.; SCHANTZ, P.M.; DAS, P.; LAL, A.A.; XIAO, L. Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. **Emerging Infectious Diseases**, v. 9, n. 11, 2003. doi: 10.3201/eid0911.030084

TIJJANI, M.; MAJID, R. A.; ABDULLAHI, S. A.; UNYAH, N. Z. Detection of rodent-borne parasitic pathogens of wild rats in Serdang, Selangor, Malaysia: A potential threat to human health. **International Journal for Parasitology: Parasites and Wildlife**, v. 11, p. 174–182, 2020. https://doi.org/10.1016/j.ijppaw.2020.01.008

VERDADE, L. M, FERRAZ, K. M. P. B. Capybaras in an anthropogenic habitat in southeastern Brazil. **Brazilian Journal of Biology**. p. 371-378, 2006. https://doi.org/10.1590/S1519-69842006000200019

# APÊNDICE A. Referências da Introdução Geral

ALMEIDA, A. R.; BIONDI, D. Área de uso de *Hydrochoerus hydrochaeris* em ambiente urbano. **Ciência Animal Brasileira**, v.15, p. 369-376, 2014.

ALMEIDA A. M. R.; ARZUA, M.; TRINDADE P. W. S; SILVA JUNIOR, A. Capivaras (*Hydrochoerus hydrochaeris*, Linnaeus, 1766) (Mammalia: Rodentia) em áreas verdes do município de Curitiba-PR. **Estudos de Biologia**, v. 35, n. 84, p. 9-16, 2013.

APPELBEE, A. J.; FREDERICK, L. M.; HEITMAN, T. L.; OLSON, M. E. Prevalence and genotyping of *Giardia duodenalis* from beef calves in Alberta, Canada. **Veterinary Parasitology,** v. 112, p. 289–294, 2003.

APPELBEE, A. J.; THOMPSON, R. C. A.; OLSON, M. E. *Giardia* and *Cryptosporidium* in mammalian wildlife - Current status and future needs. **Trends in Parasitology**, v. 21, n. 8, p. 370-376, 2005.

ARGÜELLO-GARCÍA, R.; LEITSCH, D.; SKINNER-ADAMS, T.; ORTEGA-PIERRES, M.G. Drug resistance in *Giardia*: Mechanisms and alternative treatments for Giardiasis. **Advances in Parasitology**, v. 107, p. 201–282, 2020.

BECK, R.; SPRONG, H.; BATA, I.; LUCINGER, S.; POZIO, E.; CACCIÒ, S.M. Prevalence and molecular typing of *Giardia* spp. in captive mammals at the zoo of Zagreb, Croatia. **Veterinary Parasitology**, v. 175, p.40-46, 2011.

BERRILLI, F.; CAVE, D.D.; LIBERATO, C.D.; FRANCO, A.; SCARAMOZZINO, P.; ORECCHIA, P. Genotype characterisation of *Giardia duodenalis* isolates from domestic and farm animals by SSU-rRNA gene sequencing. **Veterinary Parasitology**, v.122, p.193–199, 2004.

BONUTI, M. R.; NASCIMENTO, A. A.; MAPELLI, E. B.; ARANTES, I. G. Helmintos gastrintestinais de capivaras (*Hydrochoerus hydrochaeris*) na sub-região de Paiaguás, Pantanal do Mato Grosso do Sul, Brasil. **Semina: Ciências Agrárias**, v. 23, n. 1, p. 57-62, 2002.

CACCIÒ, S. M.; LALLE, M.; SVÄRD, S. G. Host specificity in the *Giardia duodenalis* species complex. **Infection, Genetics and Evolution**, v. 66, n. October 2017, p. 335–345, 2018.

CAPEWELL, P.; KRUMRIE, S.; KATZER, F.; ALEXANDER, C.L.; WEIR, W. Molecular Epidemiology of  infections in the genomic era. **Trends in Parasitology**, v. 37, n. 2, p. 142–153, 2021.

COPPOLA, F.; MAESTRINI, M.; BERRILLI, F.; PROCESI, I.G.; FELICIOLI, A.; PERRUCCI, S. Parasites and Wildlife First report of *Giardia duodenalis* infection in the crested porcupine ( Hystrix cristata L ., 1758 ). **International Journal for Parasitology: Parasites and Wildlife**, v. 11, p. 108–113, 2020.

DEAN, A. G.; SULLIVAN, K. M.; SOE, M. M. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Versão. www.OpenEpi.com, atualizado 2013/04/06, acessado em 2019/03/16.

EBERHARDT, A.T.; MONJE, L.D; ZURVERA, D.A.; BELDOMENICO, P.M. Detection of Trypanosoma evansi infection in wild capybaras from Argentina using smear microscopy and real-time PCR assays. **Veterinary Parasitology**, 2014.

EBERHARDT, A.T.; ROBLES, M.R.; MONJE, L.D.; BELDOMENICO, P.M.; CALLEJÓN, R. A new Trichuris species (Nematoda: Trichuridae) from capybaras: Morphological-molecular characterization and phylogenetic relationships. **Acta Tropica**, v. 190, p. 244–252, 2019.

EINARSSON, E.; MA'AYEH, S.; SVÄRD, S. G. An up-date on *Giardia* and Giardiasis. **Current Opinion in Microbiology**, v. 34, p. 47–52, 2016.

ERICKSON, M. C.; ORTEGA, Y. R. Inactivation of protozoan parasites in food, water, and environmental systems. **Journal of Food Protection**, v. 69, n. 11, p. 2786–2808, 2006.

FANTINATTI, M.; GONÇALVES-PINTO, M.; LOPES-OLIVEIRA, L.A.P.; DA-CRUZ, A.M. Epidemiology of *Giardia duodenalis* assemblages in brazil: There is still a long way to go. **Memórias do Instituto Oswaldo Cruz**, v. 115, n. 11, p. 1–12, 2020.

FENG, Y.; XIAO, L. Zoonotic potential and molecular epidemiology of *Giardia* species and Giardiasis. **Clinical Microbiology Reviews**, v. 24, n. 1, p. 110-140, 2011.

FERRAZ, K.M.P.M.B.; BONACH, K.; VERDADE, L.M. Relationship between body mass and body length in capybaras (*Hydrochoerus hydrochaeris*). **Biota neotropica**, v. 5, n.1, 2005.

GEURDEN, T.; VERCRUYSSE, J.; CLAEREBOUT, E. Is *Giardia* a significant pathogen in production animals? **Experimental Parasitology**, v. 124, n. 1, p. 98–106, 2010.

HELMY, Y. A.; SPIERLING, N.G.; SCHMIDT, S.; ROSENFELD, U.M.; REIL, D.; IMHOLT, C.; JACOB, J.; ULRICH, R.G.; AEBISCHER, T.; KLOTZ, C. Occurrence and distribution of *Giardia* species in wild rodents in Germany. **Parasites and Vectors**, v. 11, n. 1, p. 213, 2018.

HERRERA, E. A.; SALAS, V.; CONGDON, E.R.; CORRIALE, M.J.; TANG-MARTI´NEZ, Z. Capybara social structure and dispersal patterns: variations on a theme. **Journal of Mammalogy**, v. 92, n. 1, p. 12–20, 2011.

HOPKINS, R.M., MELONI, B.P., GROTH, D.M., WETHERALL, J.D., REYNOLDSON, J.A., THOMPSON R.C. Ribosomal RNA sequencing reveals differences between the genotypes of *Giardia* isolates recovered from humans

and dogs living in the same locality. **Journal of Parasitology**, v.83, p. 44–51, 1997.

HOOSHYAR, H.; ROSTAMKHANI, P.; ARBABI, M.; DELAVARI, M. *Giardia lamblia* infection: review of current diagnostic strategies. **Gastroenterology and Hepatology From Bed to Bench.** v.12, n.1, p.3-12, 2019.

KOEHLER, A. V.; JEX, A.R.; HAYDON, S.R.; STEVENS, M.A.; GASSER, R.B. *Giardia*/Giardiasis - A perspective on diagnostic and analytical tools. **Biotechnology Advances**. v.32, p.280-289, 2014.

LYU, Z.; SHAO, J.; XUE, M.; YE, Q.; CHEN, B.; QIN, Y.; WEN, J. A new species of *Giardia* Künstler, 1882 (Sarcomastigophora: Hexamitidae) in hamsters. **Parasites & Vectors**, v.11, 2018.

**MATO GROSSO DO SUL**. Plano de Manejo do Parque Estadual do Prosa - Secretaria de Estado de Meio Ambiente, do Planejamento, da Ciência e Tecnologia (SEMAC). Campo Grande, 2011.

MALONEY, J. G.; MOLOKIN, A.; SANTIN, M. Assessment of next generation amplicon sequencing of the beta-giardin gene for the detection of *Giardia duodenalis* assemblages and mixed infections. **Food and Waterborne Parasitology**, v. 21, 2020.

MIYAMOTO, Y.; ECKMANN, L. Drug development against the major diarrhea-causing parasites of the small intestine, *Cryptosporidium* and *Giardia.* **Frontiers in Microbiology**, v.6, 2015.

MOREIRA, A.S.; MARTINS, N.S.; MOTTA S.P.; SANTOS, C.C.; MACEDO, M.R.P.; RUAS, J.L. Potencial Zoonótico Da Giardiose: Uma Revisão / Zoonotic Potential of Giardiasis: a Review. **Brazilian Journal of Development**, v. 6, n. 10, p. 79856–79871, 2020.

MMBAGA, B. T.; HOUPT, E. R. *Cryptosporidium* and *Giardia* infections in children: a review. **Pediatric Clinics of North America**, v. 64, p. 837–850, 2017.

NOGUEIRA-FILHO, S. L. G.; NOGUEIRA, S. S. C. Capybara meat: An extraordinary resource for food security in South America**. Meat Science**, v.145, p.329-333, 2018.

NOGUEIRA-FILHO, S. L. G.; LOPES, P.C.; FERREIRA, D.N.; NOGUEIRA, S.S.C. Flexibility in the social behavior of captive female capybaras (Mammalia, Rodentia). **Behavioural Processes**, v.142, p. 29-32, 2017.

READ, C. M.; MONIS, P. T.; THOMPSON, R. C. A. Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. **Infection, Genetics and Evolution**, v. 4, p. 125–130, 2004.

REGINATTO, A. R.; FARRET, M.H.; FANFA, V.R.; SILVA, A.S.; MONTEIRO, S.G. Infecção por *Giardia* spp . e *Cystoisospora* spp . em capivara e cutia no sul

do Brasil. **Revista Portuguesa de Ciências Veterinárias**, v. 103, p. 96–99, 2008.

RODRÍGUEZ-DURÁN, A.; BLANCO PALMA, L. C.; PEÑA FLÓREZ, R. Main gastrointestinal protozoa in wild capybara (*Hydrochoerus hydrochaeris*) in a village in the municipality of Arauca, Colombia. **Zootecnia Tropical**, v. 33, n. 3, p. 261–268, 2015.

RODRIGUES, M. V.; PAULA, T.A.R.; FERREIRA, L.B.C.; ÁVILA, E.C.; SILVA, L.C.; SOUZA, V.B. Comportamento de um grupo de capivaras em uma área urbanizada. **Acta Veterinária Brasilica**, v.7, p.212-217, 2013.

ROSSIGNOL, J. F. *Cryptosporidium* and *Giardia*: Treatment options and prospects for new drugs. **Experimental Parasitology**, v. 124, p. 45–53, 2010.

ROXSTRÖM-LINDQUIST, K.; PALM, D.; REINER, D.; RINGQVIST, E.; SV Ä RD, S.G. *Giardia* immunity - An update. **Trends in Parasitology**, v. 22, n. 1, p. 26–31, 2006.

RYAN, U.; CACCIÒ, S.M. Zoonotic potential of *Giardia*. **International Journal for Parasitology**, v. 43, p. 943-956, 2013.

RYAN, U.; ZAHEDI, A. Molecular epidemiology of Giardiasis from a veterinary perspective. **Advances in Parasitology**, v. 106, p. 209–254, 2019.

SANTIN, M. *Cryptosporidium* and *Giardia* in Ruminants. **Veterinary Clinics of North America - Food Animal Practice**, v. 36, n. 1, p. 223–238, 2020.

SANTOS, L.C.; CUROTTO, S.M.R.; MORAES, W.; CUBAS, Z.S.; COSTA-NASCIMENTO, M.J.; FILHO, I.R.B.; BIONDO, A.W.; KIRCHGATTERD, K. Detection of Plasmodium sp. in capybara. **Veterinary Parasitology**, v.163, p.148–151, 2009.

SOARES, R. M.; SOUZA, S. L. P.; SILVEIRA, L. H.; FUNADA, M .R.; RICHTZENHAIN, L. J.; GENNARI, S. M. Genotyping of potentially zoonotic *Giardia duodenalis* from exotic and wild animals kept in captivity in Brazil. **Veterinary Parasitology**, v. 180, p. 344–348, 2011.

SOUZA S.L.P.; BENATTI H.R.; LUZ H.R.; COSTA F.B.; PACHECO R.C.; LABRUNA M.B. Endoparasites of capybaras (*Hydrochoerus hydrochaeris*) from anthropized and natural areas of Brazil. **Brazilian Journal of Veterinary Parasitology**, v. 30, n. 2, 2021.

SMITH, H. V.; CACCIO, S.M.; TAIT, A. MCLAUCHLIN, J.; THOMPSON, R.C.A.Tools for investigating the environmental transmission of *Cryptosporidium* and *Giardia* infections in humans. **Trends in Parasitology**, v. 22, n. 4, p. 160–167, 2006.

SULAIMAN, I.M.; FAYER, R.; BERN, C.; GILMAN, R.H.; TROUT, J.M.; SCHANTZ, P.M.; DAS, P.; LAL, A.A.; XIAO, L. Triosephosphate Isomerase Gene

Characterization and Potential Zoonotic Transmission of *Giardia duodenalis*. **Emerging Infectious Diseases**, v. 9, n. 11, 2003.

TIJJANI, M.; MAJID, R. A.; ABDULLAHI, S. A.; UNYAH, N. Z. Detection of rodent-borne parasitic pathogens of wild rats in Serdang, Selangor, Malaysia: A potential threat to human health. **International Journal for Parasitology: Parasites and Wildlife**, v. 11, p. 174–182, 2020.

THOMPSON, R. C. A.; ASH, A. Molecular epidemiology of *Giardia* and *Cryptosporidium* infections – What's new?. **Infection, Genetics and Evolution,** v.75 , 2019.

TRUPPEL, J. H. **Avaliação do parasitismo em capivaras (*Hydrochaeris Hydrochaeris*) e sua atuação como hospedeiro intermediário de *Neospora caninum* e *Toxoplasma gondii*.** 2009. 162 f. Dissertação (Mestrado)- Departamento de Patologia Básica e Departamento de Patologia Médica, Universidade Federal do Paraná, Curitiba, 2009.

VERDADE, L. M, FERRAZ, K. M. P. B. Capybaras in an anthropogenic habitat in southeastern Brazil. **Brazilian Journal of Biology**. p. 371-378, 2006.

YAOYU, F.; XIAO, L. Zoonotic potential and molecular epidemiology of *Giardia* species and Giardiasis. **Clinical Microbiology Reviews**, v. 24, n. 1, p. 110–140, 2011.

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Transparency in authors' contributions and responsibilities to promote integrity in scientific publication, McNutt at all, PNAS February 27, 2018

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A Graduate Student's Guide to Determining Authorship Credit and Authorship Order, APA Science Student Council 2006

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• This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University B (Date.../No. ...).

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– The International Council for Laboratory Animal Science (ICLAS) provides ethical guidelines for researchers as well as editors and reviewers

– The Association for the study of Animal Behaviour describes ethical guidelines for the treatment of animals in research and teaching

– The International Association of Veterinary Editors' Consensus Author Guidelines on Animal Ethics provide guidelines for authors on animal ethics and welfare

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• All procedures performed in the study were in accordance with the ARVO Statement for Use of Animals in Ophthalmic Vision and Research. The ethical principles established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8523, revised 2011) were followed. The research protocol was approved by the Ethics Committee on Animal Use (Protocol No. 06174/14) of FCAV/Unesp, Jaboticabal.

• This study involved a questionnaire-based survey of farmers as well as blood sampling from their animals.

The study protocol was assessed and approved by Haramaya University, research and extension office. Participants provided their verbal informed consent for animal blood sampling as well as for the related survey questions. Collection of blood samples was carried out by veterinarians adhering to the regulations and guidelines on animal husbandry and welfare.

• All brown bear captures and handling were approved by the Ethical Committee on Animal Experiments, Uppsala, Sweden (Application C18/15) and the Swedish Environmental Protection Agency in compliance with Swedish laws and regulations.

• The ethics governing the use and conduct of experiments on animals were strictly observed, and the experimental protocol was approved by the University of Maiduguri Senate committee on Medical Research ethics. Proper permit and consent were obtained from the Maiduguri abattoir management, before the faecal samples of the cattle and camels slaughtered in this abattoir were used for this experiment.

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• All experiments have been conducted as per the guidelines of the Institutional Animal Ethics Committee, Department of Zoology, Utkal University, Bhubaneswar, Odisha, India. However, the insect species used in this study is reared for commercial production of raw silk materials, as a part of agro-based industry. Therefore, use of this animal in research does not require ethical clearance. We have obtained permission from the office of Research officer sericulture, Baripada, Orissa, India for the provision of infrastructure and support for rearing of silkworm both in indoor and outdoor conditions related to our study to promote sericulture practices.

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EMBL Nucleotide Sequence Database (ENA)

DNA and RNA sequencing data NCBI Trace Archive
NCBI Sequence Read Archive (SRA)

Genetic polymorphisms dbSNP
dbVar

European Variation Archive (EVA)

Linked genotype and phenotype data dbGAP
The European Genome-phenome Archive (EGA)

Macromolecular structure Worldwide Protein Data Bank (wwPDB)
Biological Magnetic Resonance Data Bank (BMRB)

Electron Microscopy Data Bank (EMDB)

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ArrayExpress

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