**MARCOS ANTÔNIO BEZERRA SANTOS**

# PARASITES OF *Didelphis aurita* OPOSSUMS CAPTURED IN URBAN ENVIRONMENTS FROM SOUTHEASTERN BRAZIL

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Medicina Veterinária, para obtenção do título de *Magister Scientiae*.

Orientador: Artur Kanadani Campos

Coorientadores: Rafael Antonio do Nascimento Ramos
Ricardo Seiti Yamatogi

**VIÇOSA - MINAS GERAIS**
**2019**

**Ficha catalográfica preparada pela Biblioteca Central da Universidade Federal de Viçosa - Câmpus Viçosa**

T
S237p
2019

Santos, Marcos Antônio Bezerra, 1990-
Parasites of *Didelphis aurita* opossums captured in urban environments from Southeastern Brazil / Marcos Antônio Bezerra Santos. – Viçosa, MG, 2019.
115 f. : il. (algumas color.) ; 29 cm.

Texto em inglês.
Orientador: Artur Kanadani Campos.
Dissertação (mestrado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Didelphidae. 2. Carrapatos. 3. Pulgas. 4. Helminto. 5. Protozoário. 6. Parasitologia veterinária. I. Universidade Federal de Viçosa. Departamento de Veterinária. Programa de Pós-Graduação em Medicina Veterinária. II. Título.

CDD 22. ed. 599.276

MARCOS ANTONIO BEZERRA SANTOS

**PARASITES OF *Didelphis aurita* OPOSSUMS CAPTURED IN URBAN ENVIRONMENTS FROM SOUTHEASTERN BRAZIL**

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Medicina Veterinária, para obtenção do título de *Magister Scientiae*.

APROVADA: 31 de outubro de 2019.

Assentimento:

Marcos Antonio Bezerra Santos
Autor

Artur Kanadani Campos
Orientador

**AKNOWLEDGEMENTS**

To my advisor Dr. Artur Kanadani, and my co-advisors Dr. Rafael Ramos and Dr. Ricardo Yamatogi, for their support during the research project. I am very thankfull for all the opportunities provided and all the knowledge I acquired with all of you.

To the professors and technicians of the Veterinary Medicine Department of the Universidade Federal de Viçosa, specially Dr. Jackson Araújo and Dr. Abelardo Silva Junior for the support with equipments and materials used in my research.

To CAPES, CNPq, FAPEMIG, UFV and the Veterinary Medicine Post-Graduate Program for the support provided, which allow us to perform our research.

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001

To Rosineia Cunha for her valuable time and availability to attend us, always with a smile, at the Secretary of the Post-Graduate program.

To Dr. Gílcia Carvalho, for her availability to participate in the evaluation commetee of this dissertation. It is an honour to be evaluated by one of the persons who have taught me about research in parasitology in a very responsible way.

To Dr. Gisele Del Giudice for providing traps to capture animals used in my research.

To Dr. Elida Rabelo for the help with the molecular analysis of some nematodes.

To my family that always give me support in my decisions, which make it easier for me to choose the way I want to follow, even if it means being away from them.

To all my friends, with a special thanks to Lucia Macedo, who has always been supporting me whenever I need.

To Bárbara Nogueira and Carolina Fontes for the help during the research experiment and for holding all those beautiful opossums, even the angry ones, without any fear.

To all individuals that have contributed to my research, either directly or indirectly.

To God, who have always guided my steps and provided me with the best opportunities.

## ABSTRACT

SANTOS, Marcos Antônio Bezerra, M.Sc., Universidade Federal de Viçosa, October, 2019. **Parasites of *Didelphis aurita* opossums captured in urban environments from Southeastern Brazil.** Adviser: Artur Kanadani Campos. Co-advisers: Rafael Antônio do Nascimento Ramos and Ricardo Seiti Yamatogi.

*Didelphis* spp. are marsupials well adapted to anthropogenic activity, playing important role in the dissemination of pathogens to humans and domestic animals due to their circulation between the wild and the urban environment. Several parasite species, consisting of vectors of zoonotic pathogens (i.e. ticks and fleas), helminths and protozoa affect these marsupials. The aim of this study was to evaluate the parasitic fauna of *D. aurita*, and identify arthropod borne pathogens transmitted by ticks and fleas. From January to June 2019, 58 *D. aurita* were captured and ectoparasites, blood, spleen and fecal samples were collected for molecular and parasitological tests. DNA of ticks, blood and spleen samples was extracted and screened for *Rickettsia*, *Borrelia*, *Babesia* spp. and Anaplasmataceae by conventional Polymerase Chain Reaction (PCR). Blood and spleen DNA samples of 57 animals were further screened for *Toxoplasma gondii* by real time PCR (qPCR). Two tick species were identified, *Ixodes loricatus* in 41.38% (24/58) and *Amblyomma sculptum* in 1.72% (1/58) of the animals. For fleas, *Ctenocephalides felis felis* was detected in 60.34% (35/58) of the animals, and *Xenopsyla cheopis* in 5.17% (3/58). PCR analysis detected Anaplasmataceae DNA in 34.04% (n = 16/47) pool samples of *C. felis felis*, and in 66.66% (n = 2/3) pool samples of *X. cheopis*. Sequence analysis of the products revealed *Wolbachia pipientis* symbiont in all positive samples. Tick, blood and spleen samples scored negative for other pathogens assessed by conventional PCR. *T. gondii* DNA was detected in 26.32% (15/57) of the animals and BLAST analysis demonstrated 100% homology with sequences available in the GenBank database. Fecal samples were evaluated by parasitological procedures. Eggs and oocysts were analyzed at different magnifications (400x and 1000x), and their identification, together with adult nematodes, was established on morphological and morphometric features. Of all samples analyzed, 87.76% (n = 43/49) scored positive for at least one gastrointestinal parasite, being 83.67% (41/49) for helminths, and 65.30% (32/49) for protozoa. For

*Cryptosporidium*, only 13 samples were evaluated due to insufficient amount of feces obtained of some animals. A frequency of 23.08% (3/13) was reported for this parasite. Samples positive for *Eimeria* spp. oocysts were allowed to sporulate in 2.5% potassium dichromate ($K_2Cr_2O_7$), and detailed morphometric analyses were performed to determine the species present. Opossums were infected with from one to five *Eimeria* spp. Four of the eimerians were discovered, described and named by others: *E. auritanensis*, *E. caluromydis*, *E. gambai*, and *E. philanderi*. Additionally, sporulated oocysts of a species new to science, herein named as *Eimeria vicoensis* n. sp., were detected. Data herein obtained demonstrated the parasitism in *D. aurita* by some species of ectoparasites, including those commonly found in domestic animals (*C. felis felis* and *A. sculptum*), and high infection rates by several helminths and protozoa species, including those with zoonotic potential. These findings imply that these opossums may be involved in zoonotic cycles of these parasites in urban environments; however, further studies are needed to elucidate the extent to which these animals are involved in the dissemination of these pathogens.

Keywords: Didelphidae. Ticks. Fleas. Helminths. Protozoa.

## RESUMO

SANTOS, Marcos Antônio Bezerra, M.Sc., Universidade Federal de Viçosa, outubro de 2019. **Parasites of *Didelphis aurita* opossums captured in urban environments from Southeastern Brazil.** Orientador: Artur Kanadani Campos. Coorientadores: Rafael Antonio do Nascimento Ramos e Ricardo Seiti Yamatogi.

*Didelphis* spp. são marsupiais bem adaptados à atividade antrópica, apresentando importante papel na disseminação de patógenos para humanos e animais domésticos devido sua presença em ambientes selvagens e urbanos. Várias espécies de parasitos, incluindo artrópodes vetores de patógenos zoonóticos (ex. carrapatos e pulgas), helmintos e protozoários acometem estes marsupiais. O objetivo deste estudo foi avaliar a fauna parasitária em *D. aurita*, e identificar patógenos transmitidos por pulgas e carrapatos. De janeiro a junho de 2019, 58 *D. aurita* foram capturados e amostras de ectoparasitos, sangue, baço e fezes foram coletadas para testes moleculares e parasitológicos. DNA dos carrapatos, sangue e baços foram extraídos e testados para a presença de *Rickettsia*, *Borrelia*, *Babesia* spp. e Anaplasmataceae através de Reação em Cadeia de Polimerase convencional (PCR). Adicionalmente, amostras de sangue e baço de 57 animais foram testados para presença de *Toxoplasma gondii* através da PCR em tempo real (qPCR). Duas espécies de carrapatos foram identificadas como *Ixodes loricatus* em 41.38% (24/58) e *Amblyomma sculptum* em 1.72% (1/58) dos animais. Para pulgas, *Ctenocephalides felis felis* foi detectada em 60.34% (35/58) e *Xenopsyla cheopis* em 5.17% (3/58) dos animais. DNA de Anaplasmataceae foi detectado através da PCR em 34.04% (16/47) das amostras de *C. felis felis* e em 66.66% (2/3) das amostras de *X. cheopis*. A análise do sequenciamento dos produtos identificou *Wolbachia pipientis* em todas as amostras positivas. Amostras de carrapatos, sangue e baço foram negativas para todos os patógenos testados por PCR convencional. DNA de *T. gondii* foi detectado em 26.32% (15/57) dos animas e a análise do produto sequenciado demonstrou 100% de homologia com sequências disponíveis no banco de dados do GenBank. Amostras fecais foram avaliadas através de métodos parasitológicos. Ovos e oocistos foram analisados em diferentes objetivas (400x e 1000x), e a identificação dos mesmos e dos nematódeos adultos foi estabelecida através de características morfológicas e morfométricas. De todas as amostras analisadas, 87.76% (43/49) foram positivas para pelo menos um parasito gastrointestinal, sendo 83.67% (41/49) para helmintos e 65.30% (32/49) para protozoários. Para detecção de *Cryptosporidium*, apenas 13 amostras foram avaliadas devido a quantidade insuficiente de fezes coletadas em alguns animais. Foi observada uma frequência de 23.08% (3/13) para este parasito. Amostras positivas para oocistos de *Eimeria* spp. foram encubadas para esporular em dicromato de potássio ($K_2Cr_2O_7$) a 2.5% e análises morfométricas detalhadas foram realizadas para determinação das espécies presentes. Os animais apresentaram infecção consistindo de uma a cinco espécies de *Eimeria*. Quatro destas espécies foram descobertas, descritas e nomeadas em outros estudos: *E. auritanensis*, *E. caluromydis*, *E. gambai*, e *E. philanderi*. Adicionalmente, oocistos de uma nova espécie, nomeada como *Eimeria vicoensis* n. sp., foram detectados. Os resultados obtidos no presente estudo demonstraram o parasitismo em *D. aurita* por algumas espécies de ectoparasitos, incluindo alguns comumente encontrados em animais domésticos (*C. felis felis* e *A. sculptum*), e uma alta taxa de infecção por várias espécies de helmintos e protozoários, incluindo aquelas com potencial zoonótico. Estes achados indicam que gambás podem estar envolvidos em ciclos zoonóticos destes parasitos em áreas urbanas; no entanto, mais estudos são necessários para elucidar o grau de envolvimento destes animais na disseminação destes patógenos.

Palavras-chave: Didelphidae. Carrapatos. Pulgas. Helmintos. Protozoários.

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

UFV – Universidade Federal de Viçosa

ECAE – Ethics Committee for Animal Experimentation

SISBIO – Biodiversity Information and Authorization System

IBAMA – Brazilian Institute of Environment and Renewable Natural Resources

CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico

FAPEMIG – Fundação de Amparo à Pesquisa do Estado de Minas Gerais

DNA – Deoxyribonucleic acid

rRNA – Ribosomal ribonucleic acid

PCR – Polymerase Chain Reaction

qPCR – Real Time Polymerase Chain Reaction

BLAST – Basic Local Alignment Search Tool analysis

$K_2Cr_2O_7$ – Potassium dichromate

EDTA – Ethylenediaminetetraacetic acid

Cwa – Humid Subtropical Climate

## SUMMARY

## 1. INTRODUCTION

Marsupials of the genus *Didelphis* consist of six known species with occurrence limited to the American continent. Five of those species belonging to two groups occur in South America: *D. marsupialis* and *D. aurita* of the *D. marsupialis*-group, and *D. albiventris*, *D. pernigra*, and *D. imperfecta* of the *D. albiventris*-group. A sixth species of this genus is the *D. virginiana* opossum, which up to date is only reported in North and Central America, from Canada to Costa Rica (Garder, 2008). In Brazil, four species of this genus are described: *D. albiventris* occurring primarily in the biom Cerrado, *D. aurita* in the Atlantic forest, and *D. imperfecta* that along with *D. marsupialis* inhabits in the Amazon region (Faria and Melo, 2017; Gardner, 2008).

These opossums are well adapted to human dwellings, and are frequently found on the roof of houses, hollows of trees and other shelters within the cities and peripheral areas. They have twilight and nocturnal habits, and are considered synanthropic animals due to the adaptive efficiency developed over history (Jansen, 2002). *Didelphis* spp. co-inhabits with humans and domestic animals in the rural and urban environments, which makes these marsupials potential disseminators of zoonotic pathogens (Melo et al., 2016; Muller et al., 2005).

Parasites found in *Didelphis* spp. are very diverse, consisting of arthropods, helminths and protozoa. Many of these parasites affect the health of their hosts causing disease or even death of the animal. Some of them, are also important for public health, since they may be vectors of zoonotic pathogens (i.e. ticks and fleas), or be the etiological agent of human diseases (i.e., gastrointestinal helminths and protozoa) (Castro et al., 2017; Muller et al., 2005).

Among the arthropods found parasitizing these opossums, ticks and fleas are frequently detected, and due the capacity of these ectoparasites to transmit pathogens, they play important role on the dissemination of zoonotic agents among humans, wild and domestic animals (Linardi, 2006; Muller et al., 2005). Arthropod-borne pathogens that occur in *Didelphis* spp. and are transmitted by ticks and fleas, include *Rickettsia* spp., *Borrelia* spp., *Ehrlichia* spp. and *Anaplasma* spp., which are epidemiologically important to public health, since they may infect and cause diseases in humans (Peniche-Lara et al., 2016; Melo et al., 2016; Rojero-Vázquez et al., 2017).

Gastrointestinal parasites of *Didelphis* spp. are represented by a great variety of helminths (Chero et al., 2017; Aragón-Pech et al., 2018; Pinto et al., 2014), and protozoa (Duszynski, 2016; Zanette et al., 2008). Some of these parasites have zoonotic potential. For instance, *Toxocara cati*, the agent of the Larva migrans visceral in humans, was detected in *D. albiventris* opossums (Pinto et al., 2014). Other reports found *Ancylostoma* spp., *Toxocara* spp., *Trichuris* spp., *Ascaris* spp., and *Capillaria* spp. in *D. virginiana* (Aragón-Pech et al., 2018), and protozoa of the genus *Giardia* and *Cryptosporidium* in *D. albiventris* (Zanette et al., 2008).

Given the importance of endo and ectoparasites to One Health, as well as the role of opossums as reservoirs and amplifiers of pathogens, the aim of this study was to evaluate the parasitic fauna of *D. aurita*, and identify arthropod borne pathogens transmitted by ticks and fleas.

# 2. LITERATURE REVIEW

## 2.1 *Didelphis aurita*

*Didelphis aurita*, the black-eared opossum, is found within the eastern Neotropical region of Brazil to southeastern Paraguay and northeastern Argentina (Figure 1). This species is widely distributed in the State of Minas Gerais, being the most frequent representative of this genus occurring within this region (Gardner, 2008; Cáceres and Monteiro-Filho, 2001). As the other species of the genus *Didelphis*, this opossum is well adapted to the anthropogenic activity, being frequently seen in public places within urban centers and peripheral areas (Jansen, 2002).

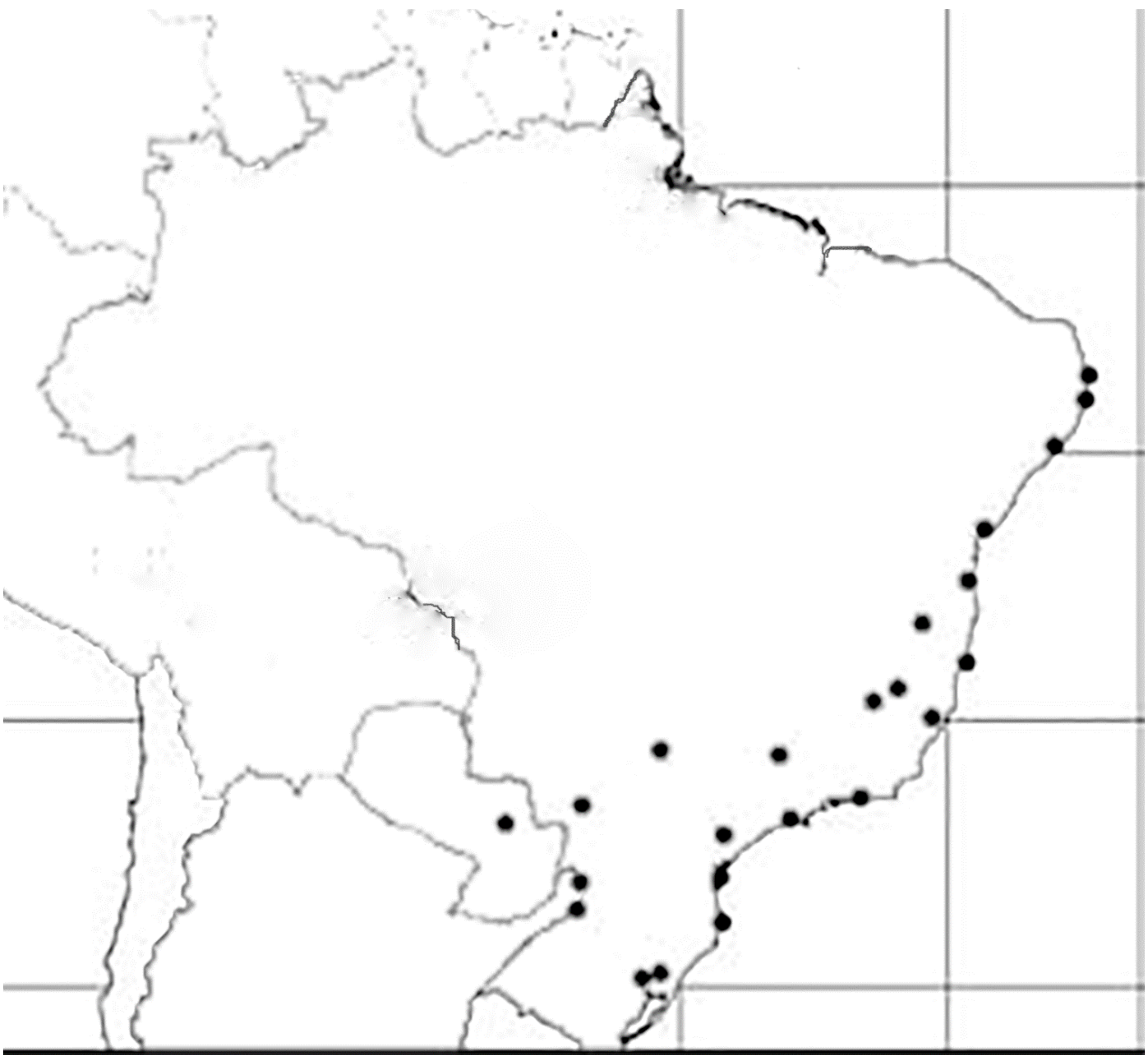

**Figure 1.** Distribution of *D. aurita* with marginal localities (Adapted from Gardner, 2008).

This opossum presents an opportunistic omnivorous food habit, with a diet consisting of small vertebrates, invertebrates, seeds and fruits (Ceotto et al., 2009; Cáceres and Monteiro-Filho, 2001). The category of food item consumed by these animals varies among the seasons of the year, depending on the availability of the resource found in each period. For instance, during the rainy season they have higher food intake of fruits and vertebrate animals. Additionally, the diet of these animals may differ according to the environment where they are mostly inserted, being possible to record fruits and rubbish remnants of human consumption, as well as food only available inside forest fragments (Cáceres and Monteiro-Filho, 2001).

The reproduction season of *D. aurita* is from July to January. They have an estrus cycle of approximately thirty days, and copulate from one to two days before ovulation. The gestational period is of about thirteen days and the offspring born are blind, deaf and with vestigial tail and posterior limbs (Figure 2). This marsupial present a high mortality rate per litter, since the number of pups born (about 22) is higher than the thirteen teats present inside the female marsupium (Jansen, 2002). However, due to the ability to control the sex-ratio of their offspring, they present a high reproductive efficiency, which may explain their good adaptability to different ecological conditions (Austad and Sunquist, 1986; Austad, 1988). According to Jansen (2002), after 55 days inside the marsupium, pups become more independent and start leaving this pouch, passing most of the time on the back of their mother (Figure 3).

Overall, marsupials of the genus *Didelphis* have important ecological role in nature, including the dispersion of seeds and predation of venomous animals such snakes (Voss and Jansa, 2012; Cáceres et al., 2009). The propagation of seeds by these animals contribute to the ecology of various species of plants as consequence of the mutualistic interactions between them. This behavior makes *Didelphis* spp. to play a key role in the natural reforestation process in disturbed forest fragments (Cantor et al., 2010). Regarding the ability to consume venomous snakes, these opossums are naturally immune to the toxins present in the venom of these reptiles, which allow them to prey such animals present in their geographical range (Voss and Jansa, 2012).

Despite the ecological importance of the opossums, there is also a potential risk associated to the presence of these animals in anthropogenic environments, since they are hosts of many zoonotic pathogens. For instance, *Didelphis* spp. may be amplifier reservoirs of *Rickettsia rickettsii*, an arthropod borne bacterium that causes The Brazilian Spotted Fever (Horta et al., 2010).

**Figure 2.** Undeveloped pups inside the pouch of *D. aurita*.

**Figure 3.** Developed pups of *D. aurita* outside their mothers pouch.

## 2.2 Ticks

Ticks are among the vectors of major importance in the transmission of pathogens to humans and animals. The main agents transmitted by these ectoparasites include *Babesia* spp., *Ehrlichia* spp., *Rickettsia* spp., and *Borrelia* spp. (Massard and Fonseca, 2004). All of these pathogens present zoonotic potential, which consequently, make them public health issues.

Approximately 955 tick species are identified worldwide. From those,  about 218 belong to the family Argasidae, 736 to the family Ixodidae, and a single species, known as *Nuttalliella Namaqua*, represents the family Nutalliellidae (Dantas-Torres et al., 2019; Muñoz-Leal et al., 2017, 2016; Labruna et al., 2016; Wolf et al., 2016; Barros-Battesti et al., 2015; Venzal et al., 2015; Dantas-Torres et al., 2012a; Health, 2012; Apanaskevich et al., 2011; Guglielmone et al., 2010). Within these species, at least 222 have been reported parasitizing humans, and about 28 are vectors of pathogens of medical concern (Dantas-Torres et al., 2012b; Nava et al., 2009; Anderson and Magnarelli, 2008). In Brazil, the number of tick species described have been gradually increasing over the years, passing from 61 species divided in nine genera in 2009 to 71 distributed in ten genera in 2019. From those, 48 belong to the family Ixodidae and 23 to the family Argasidae (Dantas-Torres et al., 2019; Martins et al., 2019).

Ticks have been identified parasitizing a great variety of wild animals, including many Brazilian mammals. From the marsupial species found in Brazil, the opossums *D. albiventris*, *D. aurita, D. marsupialis* and *D. imperfecta* present infestation by various hard tick species. On the other hand, regarding soft ticks, only species of the genus *Ornithodoros* were reported in *D. albiventris* and *D. marsupialis* (Table 1).

**Table 1.** Checklist of ticks reported in *Didelphis* spp.

| | Tick species | Reference |
|---|---|---|
| ***D. albiventris*** | **Ixodidae** | |
| | *Amblyomma fuscum* | Martins et al., 2009 |
| | *Amblyomma* sp. | Martins et al., 2009; Sponchiado et al., 2015; Massini et al., 2019; Muller et al., 2005; De Sá et al., 2018; Silva et al., 2017; Horta et al., 2007; Krawczak et al., 2016; Fontalvo et al., 2017 |
| | *A. coelebs* | Witter et al., 2015; Sponchiado et al., 2015; De Sá et al., 2018; Lamatina et al., 2018 |
| | *A. sculptum* | Sponchiado et al., 2015; De Sá et al., 2018; Silva et al., 2017; Wolf et al., 2016 |
| | *A. dubitatum* | Sponchiado et al., 2015; Massini et al., 2019; De Sá et al., 2018; Silva et al., 2017; Horta et al., 2007 |
| | *A. ovale* | Sponchiado et al., 2015; Silva et al., 2017; Blanco et al., 2016; Krawczak et al., 2016 |
| | *A. parkeri* | Sponchiado et al., 2015 |
| | *A. aureolatum* | Muller et al., 2005 |
| | *A. auriculatum* | Fontalvo et al., 2017 |
| | *Ixodes loricatus* | Muller et al., 2005; De Sá et al., 2018; Silva et al., 2017; Horta et al., 2007; Krawczak et al., 2016 |
| | **Argasidae** | |
| | *Ornithodoros mimon* | Sponchiado et al., 2015; Silva et al., 2017 |
| ***D. aurita*** | **Ixodidae** | |
| | *A. dubitatum* | Horta et al., 2007 |
| | *A. incisum* | Lamantina et al., 2018b |
| | *Amblyomma* sp. | Martins et al., 2009; Horta et al., 2007 |
| | *A. aureolatum* | Luz et al., 2018; Salvador et al., 2007 |
| | *A. brasiliense* | Lamantina et al., 2018a; Szabó et al., 2013; Lamantina et al., 2018b |
| | *A. coelebs* | Lamantina et al., 2018a; Saraiva et al., 2012; Lamantina et al., 2018b |

*Table 1 Continued...*

| | | |
|---|---|---|
| | *A. ovale* | Lamantina et al., 2018a; Szabó et al., 2013; Lamantina et al., 2018b |
| | *A. fuscum* | Szabó et al., 2013; Martins et al., 2009 |
| | *A. sculptum* | Szabó et al., 2013; Saraiva et al., 2012; Salvador et al., 2007 |
| | *A. geayi* | Oliveira et al., 2014 |
| | *A. scutatum* | Oliveira et al., 2014 |
| | *Haemaphysalis juxtakochi* | Lamantina et al., 2018a; Lamantina et al., 2018b |
| | *Ixodes loricatus* | Szabó et al., 2013; Saraiva et al., 2012; Oliveira et al., 2014; Luz et al., 2018; Horta et al., 2007; Salvador et al., 2007 |
| | *I. amarali* | Oliveira et al., 2014 |
| | *I. auritulus* | Oliveira et al., 2014 |
| | *I. didelphidis* | Oliveira et al., 2014 |
| | *I. luciae* | Oliveira et al., 2014 |
| ***D. marsupialis*** | **Ixodidae** | |
| | *Amblyomma* sp. | Witter et al., 2015; Dominguez et al., 2019; Bermúdez et al., 2017; Abel et al., 2000; Troyo et al., 2016 |
| | *A. coelebs* | Witter et al., 2015; Binetruy et al., 2019 |
| | *A. humerale* | Witter et al., 2015; Soares et al., 2014; Labruna et al., 2005; Binetruy et al., 2019 |
| | *A. parkeri* | Witter et al., 2015 |
| | *A. geayi* | Soares et al., 2014 |
| | *A. pacae* | Soares et al., 2014; Labruna et al., 2005 |
| | *A. sculptum* | Lemos et al., 1996 |
| | *A. triste* | Lemos et al., 1996 |
| | *A. dissimile* | Dominguez et al., 2019 |
| | *A. mixtum* | Dominguez et al., 2019 |
| | *A. oblongoguttatum* | Dominguez et al., 2019 |
| | *A. ovale* | Dominguez et al., 2019 |
| | *A. sabanerae* | Dominguez et al., 2019 |
| | *A. cajennense* sensu stricto | Binetruy et al., 2019 |
| | *Haemaphysalis juxtakochi* | Dominguez et al., 2019 |
| | *Ixodes* sp. | Figueiredo et al., 1999; Dominguez et al., 2019; Abel et al., 2000 |
| | *I. luciae* | Labruna et al., 2005; Dominguez et al., 2019; Díaz et al., 2009; Binetruy et al., 2019; Bermúdez et al., 2017; Bermúdez et al., 2010 |
| | *I. loricatus* | Abel et al., 2000 |
| | *I. didelphidis* | Abel et al., 2000 |
| | **Argasidae** | |
| | *Ornithodoros* sp. | Abel et al., 2000 |
| ***D. virginiana*** | | |
| | *A. americanum* | Pung et al., 1994; Lavender and Oliver, 1996; Kollars, 1993; Durden and Wilson, 1990; Oliver et al., 1999 |
| | *Dermacentor variabilis* | Pung et al., 1994; Lavender and Oliver, 1996; Kollars and Kengluecha, 2001; Kollars, 1993; Durden and Wilson, 1990; Smith et al., 2019; Durden and Richardson, 2013; Oliver et al., 1999; Fish and Dowler, 1989 |
| | *I. dammini* | Fish and Dowler, 1989 |
| | *I. cookei* | Fish and Dowler, 1989 |

| | | ***Table 1 Continued...*** |
|---|---|---|
| | *I. scapularis* | Pung et al., 1994; Lavender and Oliver, 1996; Kollars, 1993; Oliver et al., 1999 |
| | *I. tetanus* | Pung et al., 1994; Fish and Dowler, 1989 |
| | *I. affins* | Lavender and Oliver, 1996 |
| | *Rhipicephalus sanguineus* | Kollars, 1993 |
| ***D. imperfecta*** | **Ixodidae** | |
| | *A. cajennense* sensu stricto | Binetruy et al., 2019 |

### 2.2.1 Tick borne helminths

Few helminths are known to be transmitted by ticks, and most studies involve the genus *Cercopithifilaria*, which consists of 28 described species that parasitize various mammal hosts, including marsupials (Bain et al., 2008). These helminths were reported infecting dogs in many parts of the world (Cortes et al., 2014), and in Brazil, *Cercopithifilaria* spp. were recorded in dogs from Northeastern and Southeastern regions (Santos et al., 2018; Santos et al., 2017; Ramos et al., 2016; Almeida and Vicente, 1984). In american opossums, *Cercopithifilaria didelphis* was described in *D. marsupialis* (Bain et al., 1982); however, studies regarding the parasitism by these nematodes in *Didelphis* spp. are needed.

Up to date, *Cercopithifilaria* spp. do not present a clearly defined pathogenic relevance. However, histological findings suggest that these parasites may cause edematous changes in epidermal and subepidermal tissues, as well as an interstitial and perivascular dermatitis with presence of neutrophils, eosinophils and lymphocytes (Otranto et al., 2012). In addition, they may be associated to chronic polyarthritis, as filarial larva of this genus were detected in the joints of an animal presenting this problem (Gabrielli et al., 2014).

### 2.2.2 Tick borne protozoa

Ticks have central role in the spreading of many protozoa species, and among these endoparasites, *Babesia*, *Theileria* and *Hepatozoon* spp. are important representatives due to the diseases they cause. These protozoa may be transmitted to a vertebrate host by ixodid ticks during the blood meal (i.e. *Babesia* and *Theileria* spp.), or by the ingestion of an infected vector by a susceptible host (i.e. *Hepatozoon* spp.) (Criado-Fornelio et al., 2003).

Blood protozoa of the genus *Babesia* are responsible for a disease known as Babesiosis or Piroplasmosis, which depending on the parasite species, may cause health problems in humans and animals (Hunfeld et al., 2008). Human babesiosis is considered an emerging zoonosis, and the infections may vary from asymptomatic to severe depending on the immune status of the host. In immune-depressed patients, newborn or elderly, infections may be aggravated by hemolysis, hemodynamic instability, acute respiratory problems, and multiple failure of organs, which may lead to death (Schnittger et al., 2012; Keirans et al., 1996; Gray et al., 2010; Leiby, 2011). Zoonotic *Babesia* are mainly represented by *B. microti*, *B. divergens*, *B. duncani* and *B. venatorum*, having rodents, cattle and wild animals as the main reservoirs (Ord and Lobo, 2015).

Domestic animals are affected by a wide variety of *Babesia* spp. In cattle *B. bovis*, *B. bigemina* and *B. divergens* are related to important economic losses, due to mortality, abortion, reduction in meat and milk production, control measures, and trade restrictions (Schnittger et al., 2012). *B. bovis* and *B. bigemina* are found in tropical and subtropical regions where ticks of the genus *Rhipicephalus*, their main vectors, are present. On the other hand, *B. divergens* are mainly associated to livestock from Europe and North Africa, being transmitted by *Ixodes ricinus* ticks (Bock et al., 2004). In dogs, the species that present major importance are *B. canis vogeli*, transmitted by *R. sanguineus*; *B. canis canis*, transmitted by *Dermacentor* spp. and *R. sanguineus*; *B. canis rossi*, transmitted by *Haemaphysalis* spp.; *B. conradae*, to which vectors are unknown; and *B. gibsoni*, transmitted by *Haemaphysalis longicornis* (Schnittger et al., 2012; Uilenberg, 2006).

*Theileria* spp. are other important tick borne protozoa that cause disease in vertebrate hosts. Domestic animals are mainly affected by *T. parva* and *T. equi*, which cause severe illnesses in cattle and horses, respectively (Bowman, 2010). *T. parva* is transmitted by *R. appendiculatus* ticks and occurs in eastern, central and southern African regions, where it causes a disease known as East Coast Fever (Gachohi et al., 2012), whereas, *T. equi* is vectored by *Hyalomma*, *Dermacentor* and *Rhipicephalus* spp. and have a worldwide distribution, being endemic in tropical and subtropical regions (Onyiche et al., 2019).

Other important tick borne protozoa are the coccidia of the genus *Hepatozoon.* These parasites consist of more than 300 species that affect several hosts worldwide, including wildlife and domestic animals (Sumrandee et al., 2015; Smith, 1996). Differently from the majority of vector borne protozoa, which infect their vertebrate hosts through the salivary glands of the hematophagous vectors during a blood meal, *Hepatozoon* spp. are transmitted via the ingestion of arthropods infected with sporulated oocysts (Baneth, 2011). Additionally, some species may also be transmitted by predation of a vertebrate host over another (i.e. snakes and rodents), by the ingestion of tissues containing first stages of the oocysts (Smith, 1996).

Among the domestic animal species susceptible to *Hepatozoon* spp., dogs are considered the most affected due to the development of clinical signs, which depending on the protozoa species, may range from mild to severe. Two species of this genus infect these animals: *H. canis* that causes an infection that may be asymptomatic to severe, according to the health status of the dog, but most of the time, mild disease is observed in these hosts; and *H. americanum* that causes a debilitating and frequently fatal infection (Baneth et al., 2003). In Brazil, only *H. canis* is currently known infecting dogs and it is mainly associated to the ingestion of *R. sanguineus* and *A. ovale* ticks. Nevertheless, information about the epidemiology, pathogenicity and occurrence of other species are limited (Bowman, 2010).

### 2.2.3 Tick borne bacteria

Bacteria of the genera *Rickettsia*, *Borrelia*, *Ehrlichia* and *Anaplasma* are among the most relevant pathogens transmitted by ticks, due to the potential that some species have to cause severe disease in humans and animals. *Rickettsia* spp. are intracellular obligate gram-negative bacteria divided in four groups: the ancestral group (*R. belli* and *R. canadenses*), the typhus group (*R. prowazekii* and *R. typhi*), the transitional group (*R. australis*, *R. akari* and *R. felis*), and the spotted fever group (*R. rickettsii, R. conorii* and *R. massiliae*). From those species, *R. rickettsii* is particularly relevant to public health, as it causes the Spotted Fever, the most severe form of rickettsiosis in humans (Faccini-Martínez et al., 2014; Mansueto et al., 2012; Merhej and Raoult, 2011; Renvoisé et al., 2009; Cunha et al., 2009). In Brazil, ticks of the *A. cajennense* complex are the main vectors of this bacterium (Martins et al., 2016; Oliveira et al., 2000; Dias and Martins, 1939). However, many other tick species are reported harboring *R. rickettsii* and are suggested to have potential role in the spreading of the pathogen among humans, wild and domestic animals (Harris et al., 2017; Eremeeva et al., 2011; Cunha et al., 2009).

Other important tick borne zoonotic bacteria are those of the genus *Borrelia*. Representatives of these organisms are divided in two taxonomic groups. The first consists of species of the *B. burgdorferi* sensu lato complex, which are the etiological agents of Lyme disease and have ticks of the genus *Ixodes* as vectors, and the second group involves *Borrelia* spp. that are associated to the human relapsing fever, being primarily transmitted by soft ticks (Telford et al., 2015).

Lyme disease is characterized by diverse dermatological, neurological, rheumatic and cardiac problems. Ticks of the *I. ricinus* complex (*I. ricinus, I. scapularis* and *I. pacificus*) transmit the bacteria that normally circulates among small mammals and birds (Steere et al., 2016; Mead, 2015). On the other hand, the human relapsing fever normally causes nonspecific symptoms such as fever, headache, myalgia, arthralgia and abdominal pain. The *Borrelia* spp. responsible for causing this disease are transmitted by soft ticks during their blood meal, or yet, by the contact with the hemolymph of the human louse, *Pediculus humanus* (Dworkin et al., 2008). In Brazil, relapsing fever is endemic and the soft tick *Ornithodoros brasiliensis* is the potential vector of the spirochetes that cause this disease (Lopez et al., 2016).

Lastly, *Ehrlichia* and *Anaplasma* spp. are gram-negative bacteria that cause a tick borne disease known as ehrlichiosis (Dagnone et al., 2003). The infection by these pathogenic microorganisms is given via tick saliva while these arthropods are attached to their hosts. The most important species among these genera are *E. ewingii*, *E. chaffeensis* and *A. phagocitophilum* due to their capacity to cause disease in both humans and animals (Dahlgren et al., 2011). In the definitive host, these bacteria are normally found in leucocytes, and as intracellular obligate microorganisms, the immune response of the host against the infection may fail. Additionally, this factor brings complications to the therapy with antimicrobials (Cohn, 2003).

## 2.3 Fleas

Parasites of the order Siphonaptera, commonly known as fleas, are wingless insects that parasitize many vertebrate species, including humans. These ectoparasites are involved in the transmission of zoonotic bacteria such *Yersinia pestis*, *R. typhi* and *R. felis*. Furthermore, fleas are intermediate hosts of *Dipylidium caninum*, a cestode species that occasionally cause disease in humans (Bitam et al., 2010; Poo-munoz et al., 2016).

Currently, approximately 3000 flea species are known. They are divided in 16 families that englobes 246 genera (Whiting et al., 2008). In Brazil, there are 8 families, 20 genera and 62 species, and 18 of those are endemic (Linardi and Guimarães, 2000; Linardi, 2011). In *Didelphis* spp., many flea species have been reported (Table 2), with prevalences varying according to the host species, season and geographical area.

**Table 2.** Checklist of fleas reported in *Didelphis* spp.

| | Flea species | Reference |
|---|---|---|
| ***D. albiventris*** | *Ctenocephalides felis felis* | Urdapilleta et al., 2019; Fontalvo et al., 2017; Silva et al., 2017; Horta et al., 2007 |
| | *Polygenis* sp. | Silva et al., 2017 |
| | *Polygenis atopus* | Lareschi et al., 2006; Horta et al., 2007 |
| | *Tunga penetrans* | Saraiva et al., 2011 |
| ***D. aurita*** | *C. felis felis* | Horta et al., 2007 |
| | *Polygenis rimatus* | Urdapilleta et al., 2019 |
| | *Polygenis occidentalis* | Pinto et al., 2009 |
| | *Polygenis atopus* | Moraes et al., 2003; Horta et al., 2007 |
| | *Adoratopsylla intermedia* | Salvador et al., 2007; Pinto et al., 2009 |
| | *A. antiquorum* | Pinto et al., 2009 |
| | *Leptopsylla segnis* | Salvador et al., 2007 |
| | *Xenopsylla cheopis* | Salvador et al., 2007 |
| ***D. marsupialis*** | *C. felis felis* | Beaucournu et al., 1998 |
| | *Polygenis beebei* | Beaucournu et al., 1998 |
| | *Polygenis klagesi* | Beaucournu et al., 1998 |
| | *A. intermediata* | Beaucournu et al., 1998 |
| | *A. copha* | Troyo et al., 2016 |
| ***D. virginiana*** | *C. felis felis* | Reeves et al., 2005; Pung et al., 1994; Maina et al., 2016; Eremeeva et al., 2012; Durden and Wilson, 1990 |
| | *Orchopeas howardi* | Pung et al., 1994; Durden and Wilson, 1990 |
| | *Pulex simulans* | Pung et al., 1994; Durden and Richardson, 2013 |
| | *Polygenis gwyni* | Pung et al., 1994 |
| | *Pulex irritans* | Eremeeva et al., 2012 |
| | *Xenopsylla cheopis* | Eremeeva et al., 2012 |
| | *Echidnophaga gallinaceae* | Eremeeva et al., 2012 |
| | *Cediopsylla simplex* | Durden and Wilson, 1990 |
| | *Ctenophthalmus pseudagyrtes* | Durden and Wilson, 1990 |

### 2.3.1 Flea borne helminths

Among the helminth species transmitted by fleas, *Dipylidium caninum* and *Acanthocheilonema reconditum* are particularly important due to their zoonotic potential and the disease they cause in companion animals. *D. caninum* is a gastrointestinal parasite transmitted by fleas of the genus *Ctenocephalides* (Beugnet et al., 2018). Infections caused by this cestode in humans are rare, being most commonly found in children that accidently ingest infected fleas. The infection is usually subclinical, but clinical signs may be observed in some individuals, i.e. anal itching, diarrhea, abdominal pain, loss of appetite, indigestion and other gastrointestinal disorders (Jiang et al., 2017).

*Acanthocheilonema reconditum* is a worldwide distributed nematode vectored by several flea species (*C. canis, C. felis, Pulex irritans, P. simulans, Echidnophaga gallinae*) and lices (*Heterodoxus spiniger, Linognathus setosus*). Differently from *D. caninum* that have only part of its life cycle in the intermediate hosts, *A. reconditum* have a complete development inside its arthropod vector (Otranto et al., 2013). In dogs, this parasite have a high prevalence and are usually located beneath the subcutaneous tissues (Brianti et al., 2012). In contrast to what is observed in dogs, reports on this filarioid in humans are scarce and it has been recorded parasitizing the human eye (Huynh et al. 2001).

### 2.3.2 Flea borne bacteria

Fleas are vectors of some bacteria species of great relevance to human and animal health. Among these pathogens, the most important are *Yersinia pestis, R. typhi* and *R. felis*, the etiological agents of plague, murine typhus and rickettsiosis felis, respectively (Bitam et al., 2010).

*Yersinia pestis* is a pathogen that primarily infects rodents and wild mammals, but may infect humans that have contact with fleas and diseased animals. Approximately 31 flea species are able to transmit this bacterium; among them, the ones with major importance are *Xenopsylla cheopis* (cosmopolitan), *X. brasiliensis* (Africa, India, and South America), *X. astia* (Indonesia and Asia), *X. vexabilis* (Pacific Islands), and *Nosopsyllus fasciatus* (Temperate climates). Overall, fleas become infected with *Y. pestis* through the ingestion of blood of infected hosts (Bitam et al., 2010). The transmission to the vertebrate host occurs through the bite of fleas that harbor the bacterium; however, there are other routes of transmission such the direct contact with infected animals, the consumption of uncooked meat and the inhalation of aerosols of people and animals that develop the pneumonic form of the disease (Bitam et al., 2010; Stenseth et al., 2008).

The bacterium *R. typhi* is the causing agent of the murine typhus, also known as endemic typhus, which is a zoonosis distributed worldwide. The life cycle of this *Rickettsia* usually involves rodents of the species *Rattus rattus* and *Rattus norvegicus* as vertebrate hosts, and *X. cheopis* fleas as vectors. Yet, *R. typhi* have been reported through molecular methods in *Ctenocephalides felis*, *Leptopsylla segnis*, *Ctenophthalmus congeneroides* and *Rhadinopsylla insolita* (Peniche-Lara et al., 2015). The transmission happens from rodents to humans via contaminated feces of infected fleas. In the vertebrate host, the bacterium invades the endothelial cells and cause unspecific clinical signs such as fever, headache, muscle pain, weakness, nausea and cutaneous eruptions that may present macular, maculopapular and petechial shapes (Bitam et al., 2010). Despite the rodents being the main hosts of *R. typhi*, opossums are implicated as potential reservoirs of this bacterium in the United States, and are believed to have important role on the epidemiology of the disease (Brown and Macaluso, 2016; Sorvillo et al., 1993; Williams et al., 1992).

Other important disease transmitted by fleas is the rickettsiosis felis, caused by *R. felis*, an important agent of the transitional group of rickettsia, which presents worldwide distribution. This bacterium may be transmitted by several flea species, but have the *C. felis felis* as its main vector (Angelakis et al., 2016). The clinical signs of this disease are unspecific and similar to the ones of other rickettsioses (fever, headache, muscle pain, and cutaneous eruptions), which cause confusion in the diagnostic (Brown and Macaluso, 2016). Just like in the murine typhus, marsupials are reported as potential reservoirs of *R. felis*, and since they are present in urban environments, they may be involved in the spreading of these bacterium among humans (Brown and Macaluso, 2016; Boostrom et al., 2002).

### 2.4 Endoparasites

*Didelphis* spp. opossums have an omnivorous and opportunistic diet, which predispose these animals to infections by a great variety of endoparasites, including gastrointestinal helminths and protozoa (Elsheikha et al., 2004). Furthermore, species of this genus harbor important blood parasites, which are also associated to their food habit, particularly the predatory behavior (Legey et al., 2003).

#### 2.4.1 Helminths

Parasitic helminths are mainly classified as cestodes, trematodes and nematodes. Cestodes (tapeworms) and trematodes (flukes) are members of the phylum Platyhelminthes. While parasites of the class Cestoda have an elongated and flattened body consisting of segments known as proglottids, the trematodes present a dorsoventrally flattened leaf-shaped body. Differently from Platyhelminthes, nematodes have a cylindrical body, making them commonly known as roundworms (Castro, 1996).

Cestodes, trematodes and nematodes parasitizing opossums of the genus *Didelphis* have been widely reported in the geographical range of these marsupials (Table 3). Among the helminths of the class Trematoda, the most common genera found in *Didephis* spp. are *Rhopalias* and *Brachylaima* (Costa-Neto et al., 2018; Acosta-Virgen et al., 2015; Richardson and Campo, 2005; Silva et al., 1999). The class Cestoda have less representatives reported in these opossums, with the species *Mesocestoides* sp. and *Thaumasioscolex didelphidis* mostly described in *D. marsupialis* and *D. virginiana* (Acosta-Virgen et al., 2015; Richardson and Campo, 2005; Cañeda-Guzmán et al., 2001). In contrast to Platyhelminthes, the class Nematoda is represented by a great diversity of species parasitizing *Didelphis* spp., with *Cruzia tentaculata* and *C. americana* being most frequently found (Castro et al., 2017; Acosta-Virgen et al., 2015; Richardson and Campo, 2005; Gomes et al., 2003).

Interestingly, most studies on parasitic helminths of *Didelphis* spp. have described the adult form of the parasites, whereas, works related to egg identification are scarce and have recently been performed in *D. virginiana* in México, and in *D. albiventris* and *D. aurita* in Brazil (Aragón-Pech et al., 2018; Teodoro et al., 2018). However, the detection of these immature forms, allow to identify only *C. tentaculata* and *C. americana* at species level (Li, 2019; Adnet et al., 2009); thus, for other parasites, the evaluation of eggs allow their classification only up to genus level. It is particularly important to notice that, in order to study the adult forms of most of these helminths, usually they have to be recovered from dead animals through necropsy. Parasitic eggs and larva, on the other hand, are easily recovered from feces via parasitological methods (Lima et al., 2018; Rezende et al., 2015).

**Table 3.** Checklist of helminths reported in *Didelphis* spp.

| | Helminth | Reference |
|---|---|---|
| ***D. albiventris*** | **Acanthocephala** | |
| | *Oligacanthorhynchus microcephalus* | Schmidt, 1977 |
| | **Cestoda** | |
| | *Mathevotaenia argentinensis n.* sp. | Campbell et al., 2003 |
| | **Nematoda** | |
| | *Aspidodera* spp. | Silva and Costa, 1999; Muller et al., 2004 |
| | *Didelphostrongylus hayesi* | Martinez et al., 1999; Muller et al., 2004 |
| | *Turgida turgida* | Silva and Costa, 1999; Muller et al., 2004 |
| | *Viannaia* spp. | Silva and Costa, 1999; Muller et al., 2004 |
| | *Trichuris* spp. | Muller et al., 2004; Silva and Costa, 1999 |
| | *Cruzia* spp. | Travassos, 1922; Silva and Costa, 1999; Muller et al., 2004 |
| | *Capillaria* sp. | Silva and Costa, 1999; Muller et al., 2004 |
| | *Gnathostoma* sp. | Muller et al., 2004 |
| | *Gongylonema* sp. | Silva and Costa, 1999 |
| | *Travassostrongylus* spp. | Muller et al., 2004; Silva and Costa, 1999; Muller et al., 2004 |
| | **Trematoda** | |
| | *Rhopalias* spp. | Silva and Costa, 1999 |
| | *Brachylaema migrans* | Silva and Costa, 1999 |
| | *Schistosoma mansoni* | Kawazoe et al., 1978 |
| ***D. aurita*** | **Acanthocephala** | |
| | *Hamanniella* sp. | Travassos, 1945 |
| | *Oligacanthorhynchus microcephalus* | Costa-Neto et al., 2018 |
| | **Nematoda** | |
| | *Aspidodera* spp. | Travassos, 1913; Proença, 1937; Gomes et al., 2003; Chagas-Moutinhoet al., 2007; Chagas-Moutinho et al., 2014; Castro et al., 2017; Costa-Neto et al., 2018 |
| | *Cruzia* spp. | Travassos, 1945; Travassos, 1922; Gomes et al., 2003; Adnet et al., 2009; Castro et al., 2017; Costa-Neto et al., 2018 |
| | Trichostrongylidae | Travassos, 1945 |
| | *Viannaia hamata* | Gomes et al., 2003; Costa-Neto et al., 2018 |
| | *Gongylonema sp.* | Travassos, 1945 |
| | *Gongylonemoides marsupialis* | Gomes et al., 2003 |
| | *Gnathostoma* sp. | Albuquerque et al., 2007. |
| | *Lagochilascaris turgida* | Sprent, 1982 |

***Table 3 Continued...***

| | | |
|---|---|---|
| | *Nematodirus (Mecistocirrus) didelphis* | Vicente et al., 1997 |
| | *Turgida* spp. | Travassos, 1945; Travassos, 1920; Gomes et al., 2003; Castro et al., 2017; Costa-Neto et al., 2018 |
| | *Trichuris* spp. | Costa-Neto et al., 2018 |
| | *Globocephalus marsupialis* | Costa-Neto et al., 2018 |
| | *Heterostrongylus heterostrongylus* | Costa-Neto et al., 2018 |
| | *Travassostrongylus orloffi* | Costa-Neto et al., 2018 |
| | **Trematoda** | |
| | *Duboisiella proloba* | Costa-Neto et al., 2018 |
| | *Brachylaima advena* | Costa-Neto et al., 2018 |
| | *Rhopalias coronatus* | Costa-Neto et al., 2018 |
| ***D. marsupialis*** | **Acanthocephala** | |
| | *Gigantorhynchus lutzi* | Tantaleán et al., 2005; 2010 |
| | *Oncicola luehei* | Acosta-Virgem et al., 2015 |
| | **Cestoda** | |
| | *Thaumasioscolex didelphidis* | Cañeda-Guzmán et al., 2001; Acosta-Virgem et al., 2015 |
| | **Nematoda** | |
| | *Aspidodera raillieti* | Sarmiento et al., 1999; Chero et al., 2017 |
| | *Cruzia* spp. | Sarmiento et al., 1999; Arrojo, 2002; Tantaleán et al., 2010; Chero et al., 2017; Acosta-Virgem et al., 2015 |
| | *Cercopithifilaria didelphis* | Bain et al., 1982. |
| | *Gnathostoma turgidum* | Miyazaki et al., 1978; Sarmiento et al., 1999; Acosta-Virgem et al., 2015. |
| | *Gongylonemoides marsupialis* | Chero et al., 2017 |
| | *Physaloptera mirandai* | Tantaleán et al., 2010; Chero et al., 2017 |
| | *Turgida turgida* | Tantaleán et al., 2010; Acosta-Virgem et al., 2015; Chero et al., 2017 |
| | *Trichuris* spp. | Tantaleán et al., 2010; Acosta-Virgem et al., 2015; Chero et al., 2017 |
| | *Viannaia* sp. | Acosta-Virgem et al., 2015; Chero et al. 2017 |
| | **Trematoda** | |
| | *Duboisiella proloba* | Miyazaki et al., 1978 |
| | *Bursotrema tetracotyloides* | Kifune and Uyema, 1982 |
| | *Fibricola gradosi* | Kifune and Uyema, 1982 |
| | *Plagiorchis didelphidis* | Miyazaki et al., 1978; Tantaleán et al., 1992; Chero et al., 2017 |
| | *Microphallus garciai* | Tantaleán et al., 1992 |
| | *Rhopalias* spp. | Miyazaki et al., 1978; Tantaleán et al., 1992; Kifune and Uyema, 1982; Tantaleán et al., 1992; Tantaleán and Chávez, 2004; Chero et al. 2017; Acosta-Virgem et al., 2015 |
| | *Amphimerus* spp. | Kifune and Uyema, 1981; Tantaleán et al., 1992; Acosta-Virgem et al., 2015 |
| | *Philandrophilus magnacirrus* | Acosta-Virgem et al., 2015 |
| ***D. virginiana**** | **Acanthocephala** | |
| | *Centrorynchus* spp. | Miller and Harkema, 1970; Richardson, 1993 |
| | *Macracanthorhynchus ingens* | Sherwood et al., 1969 |
| | *Oligacanthorhynchus* spp. | Babero, 1957; Babero, 1960; Krupp and Quillin, 1964; Stewart and Dean, 1971; Wong et al, 1979; Richardson, 1993; Richardson, 1993; Blumenthal and Kirkland, 1976; Alden, 1995; Acosta-Virgem et al., 2015 |
| | *Porrorchis nickoli* | Acosta-Virgem et al., 2015 |

***Table 3 Continued...***

| | |
|---|---|
| **Cestoda** | |
| *Anoplocephala* sp. | Krupp and Quillin, 1964 |
| *Hymenolepis* sp. | Leigh, 1940; Krupp and Quillin, 1964 |
| *Mesocestoides* spp. | Dikmans, 1931; Mueller, 1930; Rausch and Tiner, 1949; Voge, 1953; Blumenthal and Kirkland, 1976; Alden, 1995; Byrd and Ward, 1942; Byrd and Ward, 1943; Babero, 1957; Babero, 1960; Miller and Harkema, 1970; Stewart and Dean, 1971; Feldman et al., 1972; Richardson and Campo, 2005. |
| *Mathevotaenia* sp. | Acosta-Virgem et al., 2015 |
| *Thaumasioscolex didelphidis* | Acosta-Virgem et al., 2015 |
| *Oochoristica* sp. | Leigh, 1940 |
| *Spirometra mansonoides* | Corkum, 1966 |
| **Nematoda** | |
| *Anatrichosoma buccalis* | Pence and Little, 1972; Kinsell and Winegarner, 1975 |
| *Aspidodera* spp. | Chandler, 1932; Acosta-Virgem et al., 2015 |
| *Capillaria* spp. | Sherwood et al., 1969; Prestwood et al., 1977; Brow, 1988; Miller and Harkema, 1970; Feldman et al., 1972; Feldman and Self, 1973; Nettles et al., 1975; Butterworth and Beverley-Burton, 1977; Snyder et al., 1991; Alden, 1995; Babero, 1960; Feldman et al., 1972; Richardson and Campo, 2005 |
| *Cruzia* spp. | Chandler, 1932; Leigh, 1940; Crites, 1956; Babero, 1957; Babero, 1960; Holloway and Dowler, 1963; Holloway, 1966; Miller and Harkema, 1970; Stewart and Dean, 1971; Feldman et al., 1972; Feldman and Self, 1973; Nettles et al., 1975; Blumenthal and Kirkland, 1976; Prestwood et al., 1977; Snyder et al., 1991; Canavan, 1929; Dikmans, 1931; Canavan, 1931; Chandler, 1932; Reiber and Byrd, 1942; Rausch and Tiner, 1949; Sherwood et al., 1969; Lamothe et al., 1981; Richardson and Campo, 2005; Nichelason et al., 2008; Acosta-Virgem et al., 2015 |
| *Didelphonema longispiculata* | Hill, 1939b; Stewart and Dean, 1971 |
| *Didelphostrongylus hayesi* | Prestwood, 1976; Prestwood et al., 1977; Anderson et al., 1980; Brown, 1988; Duncan et al., 1989; Nichelason et al., 2008; Acosta-Virgem et al., 2015 |
| *Dipetalonema* spp. | Babero, 1960; Feldman et al., 1972; Esslinger and Smith, 1979; Blumenthal and Kirkland, 1976. |
| *Dirofilaria* sp. | Babero, 1960; Feldman et al., 1972 |
| *Dracunculus* sp. | Crichton and Beverley-Burton, 1973 |
| *Gnathostoma* spp. | Dikmans, 1931; Chandler, 1932; Babero, 1960; Stewart and Dean, 1971; Canavan, 1929; Canavan, 1931; Babero, 1960; Flores-Barroeta et al., 1961; Babero, 1960; Blumenthal and Kirkland, 1976; Acosta-Virgem et al., 2015. |
| *Gongylonema* spp. | Babero, 1960; Acosta-Virgem et al., 2015. |
| *Lagochilascaris* spp. | Bowman et al., 1983; Canavan, 1931. |
| *Longistriata didelphis* | Dikmans, 1931; Leigh, 1940; Reiber and Byrd, 1942; Dikmans, 1943; Babero, 1957; Babero, 1960; Miller and Harkema, 1970; Stewart and Dean, 1971; Feldman et al., 1972; Feldman and Self, 1973; Alden, 1995. |
| *Oesophagostomum* sp. | Dikmans, 1931 |

***Table 3 Continued...***

| | |
|---|---|
| *Physaloptera turgida* | Canavan, 1929; Dikmans, 1931; Canavan, 1931; Chandler, 1932; Haley, 1938; Hill, 1939a; Leigh, 1940; Reiber and Byrd, 1942; Stoner, 1945; Rausch and Tiner, 1949; Babero, 1957; Babero, 1960; Krupp, 1962; Holloway and Dowler, 1963; Krupp and Quillin, 1964; Holloway, 1966; Sherwood et al., 1969; Miller and Harkema, 1970; Stewart and Dean, 1971; Feldman et al., 1972; Nettles et al., 1975; Blumenthal and Kirkland, 1976; Prestwoodet al., 1977; Lamothe et al., 1981; Gray and Anderson, 1982; Duncan et al., 1989; Snyder et al, 1991; Alden, 1995; Richardson and Campo, 2005. |
| *Strongyloides* sp. | Little, 1966 |
| *Toxocara canis* | Blumenthal and Kirkland, 1976 |
| *Trichinella spiralis* | Zimmerman et al., 1956; Zimmerman et al., 1959; Solomon and Warner, 1969; Scholtens and Norman, 1971; Schadet al., 1984; Leibyetal., 1988. |
| *Trichostrongylns* sp. | Dikmans, 1931 |
| *Trichuris* spp. | Dikmans, 1931; Miller and Harkema, 1970; Feldman et al., 1972; Feldman and Self, 1973; Babero, 1960; Stewart and Dean, 1971; Babero, 1960; Krupp and Quilin, 1964; Acosta-Virgem et al., 2015. |
| *Turgida turgida* | Nichelason et al., 2008; Acosta-Virgem et al., 2015. |
| *Viannaia* spp. | Miller and Harkema, 1970; Feldman et al., 1972; Feldman and Self, 1973; Dikmans, 1943; Acosta-Virgem et al., 2015. |
| **Trematoda** | |
| *Alaria marcianiae* | Shoop and Corkum, 1981a. |
| *Amphimerus* spp. | Leigh, 1940; Acosta-Virgem et al., 2015. |
| *Brachylaima* spp. | Premvati and Bair, 1979; Dickerson, 1930; Dikmans, 1931; Chandler, 1932; Krull, 1935; Leigh, 1940; Byrd et al., 1942a; Rausch and Tiner, 1949; Babero, 1957; Babero, 1960; Holloway and Dowler, 1963; Kaplan, 1964; Holloway, 1966; Miller and Harkema, 1970; Feldman et al., 1972; Feldman and Self, 1973; Nettles et al., 1975; Blumenthal and Kirkland, 1976; Prestwood et al., 1977; Shoop and Corkum, 1981b; Shoop and Corkum, 1982; Alden, 1995; Richardson and Campo, 2005; Acosta-Virgem et al., 2015. |
| *Didelphodiplostomum variabile* | Chandler, 1932; Leigh, 1940; Byrd et al., 1942a; Babero, 1957; Babero, 1960; Miller and Harkema, 1970; Feldman et al., 1972; Premvati and Bair, 1979; Alden, 1995; Richardson and Campo, 2005. |
| *Echinostoma trivolvis* | Dikmans, 1931; Park, 1936; Leigh, 1940; Byrd et al., 1942a; Rausch and Tiner, 1949; Feldman et al., 1972; Blumenthal and Kirkland, 1976; Alden, 1995. |
| *Fibricola* spp. | Byrd et al., 1942a; Chandler and Rausch, 1946; Rausch and Tiner, 1949; Premvati and Bair, 1979; Shoop and Corkum, 1981b; Shoop and Corkum, 1982; LaRue and Bosma, 1927; Dikmans, 1931; Park, 1936; Byrd et al., 1942a; Babero, 1957; Lumsden and Zischke, 1961; Kaplan, 1964; Premvati and Bair, 1979; Shoop and Corkum, 1982. |
| *Heterobilharzia americana* | Kaplan, 1964; Shoop and Corkum, 1981b |
| *Linstowiella szidati* | Lumsden and Winkler, 1962; Shoop and Corkum, 1982 |
| *Maritreminoides nettae* | Miller and Harkema, 1970 |

| | | |
|---|---|---|
| | | ***Table 3 Continued...*** |
| | *Paragonimus* spp. | McKeever, 1958; Sherwood et al., 1969; Feldman et al., 1972; Shoop and Corkum, 1982; Lamothe et al., 1981; Lamothe et al., 1986; Byrd, 1941; Byrdetal., 1941; Byrdetal., 1942b; Alden, 1995. |
| | *Phagicola lageniformis* | Premvati and Bair, 1979 |
| | *Rhopalias* spp. | Dikmans, 1931; Chandler, 1932; Leigh, 1940; Byrdetal., 1942a; Self and McKnight, 1950; Babero, 1957; Babero, 1960; Lumsden and Zischke, 1961; Miller and Harkema, 1970; Stewart and Dean, 1971; Feldman et al., 1972; Feldman and Self, 1973; Premvati and Bair, 1979; Shoop and Corkum, 1981b; Shoope and Corkum, 1982; Alden, 1995; Kinsella and Heard, 1974; Denton, 1944; Acosta-Virgem et al., 2015. |

* Data regarding helminths of *Didelphis virginiana* was updated from the study of Alden (1995).

### 2.4.2 Protozoa

The kingdom Protozoa consists of unicellular microorganisms, which in their majority are free-living species. However, parasitic protozoa infect all classes of vertebrate and some invertebrate individuals. These organisms are currently classified into eight phyla (Amoebozoa, Percolozoa, Euglenozoa, Parabasalla, Fornicata, Preaxostyla, Apicomplexa and Ciliophora), from which the Euglenozoa (*Leishmania* and *Trypanosoma* spp.) and the Apicomplexa (i.e. *Plasmodium* and *Toxoplasma* spp.) have the most important pathogenic representatives (Taylor et al., 2016; YaEger, 1996). Parasitic protozoa are particularly important due to their wide host range, which include humans, domestic and wild animals. Additionally, many of these parasites are responsible for important economic losses related to farm animals, and for causing serious diseases in humans (Kaltung and Musa, 2013; Sahinduran, 2012).

In opossums, few studies have reported protozoa species; however, it is known that the genus *Eimeria* is the most abundant and diverse in *Didelphis* spp. (Duszynski, 2016; Teixeira et al., 2007). Apart from eimerians, most studies on protozoa infecting these marsupials described blood and gastrointestinal species of public health importance, such as *Trypanosoma*, *Toxoplasma*, *Leishmania* and *Cryptosporidium* spp. These studies suggest that opossums may act as reservoirs that have important role in the zoonotic cycle of these pathogens (Torres-Castro et al., 2016; Cantillo-Barraza et al., 2015; Quintal et al., 2011; Zanette et al., 2008; Santiago et al., 2007).

Investigations on the pathogenic effect that protozoa have in *Didelphis* spp. are limited, and only few studies have been performed for coccidia, i.e. *Besnoitia darling* and *E. marmosops*, demonstrating histopathological lesions in *D. virginiana* and *D. marsupialis*, respectively. However, no apparent clinical signs were noticed in these hosts (Chinchilla et al., 2015; Elsheikha et al., 2003). In fact, the dietary habit of *Didelphis* spp. make protozoa infections more prone to happen. Their predatory behavior over insects, for instance, increases the risk of acquiring *Trypanosoma* spp. infections (Jansen et al., 2017; Jansen et al., 2015). The same happens for infections by *Sarcocystis* spp., when opossums feed on birds and even on road-killed raccoons (Stabenow et al., 2012; Dubey et al., 2001).

The human risk of acquiring protozoa infections from *Didelphis* spp. may come from direct or indirect contact with these animals. Gastrointestinal protozoa, for example, may be spread through contaminated feces of opossums in food and water (Oates et al., 2012). Another infectious path for protozoa found in these marsupials, is through arthropod vectors, i.e. Phlebotomines, the main vectors of *Leishmania* spp., are commonly found in opossum nests close to human dwellings, which consequently increase the risk of leishmaniosis in people (Andrade et al., 2015; Cutolo et al., 2014). Lastly, in some regions, opossums are considered food source, and the consumption of undercooked meat of this animals have been implicated as potential risks for the transmission of *Toxoplasma gondii* and *Trypanosoma cruzi* (Alvarado-Esquivel et al., 2016; Sangenis et al., 2016; 2015; Carvalho et al., 2003).

## REFERENCES

ABEL, I. S.; MARZAGÃO, G.; YOSHINARI, N. H.; SCHUMAKER, T. T. S. *Borrelia*-like Spirochetes Recovered from Ticks and Small Mammals Collected in the Atlantic Forest Reserve, Cotia County, State of São Paulo, Brazil. **Memórias do Instituto Oswaldo Cruz**, v. 95, n. 5, p. 621-624, 2000.

ACOSTA-VIRGEN, K.; LÓPEZ-CABALLERO, J., GARCÍA-PRIETO, L.; MATA-LÓPEZ, R. Helminths of three species of opossums (Mammalia, Didelphidae) from Mexico. **ZooKeys**, v. 511, p. 131-152, 2015.

ADNET, F. A. O.; ANJOS, D. H. S.; MENEZES-OLIVEIRA, A.; LANFREDI, R. M. Further description of *Cruzia tentaculata* (Rudolphi, 1819) Travassos, 1917 (Nematoda: Cruzidae) by light and scanning electron microscopy. **Parasitology Research**, v. 104, p. 1207-1211, 2009.

ALBUQUERQUE, G. R.; LANFREDI, R. M.; TEIXEIRA FILHO, W. L.; RODRIGUES, M. L. A.; LOPES, C. W. G. Gnathostomiasis in *Didelphis aurita* and its importance in public health. **Revista Brasileira de Medicina Veterinária**, v. 29, n. 4, p. 168-170, 2007.

ALDEN, K. J. Helminths of the Opossum, *Didelphis virginiana*, in Southern Illinois, with a Compilation of All Helminths Reported from This Host in North America. **Journal of the Helminthological Society of Washington**, v. 62, n. 2, p. 197-208, 1995.

ALMEIDA, G. L. G.; VICENTE, J. J. *Cercopithifilaria bainae* sp. n. parasita de *Canis familiaris* (L.) (Nematoda, Filarioidea). **Atas da Sociedade de Biologia do Rio de Janeiro**, v. 24, p.18, 1984.

ALVARADO-ESQUIVEL, C.; SALCEDO-JAQUEZ, M.; SANCHEZ-ANGUIANO, L. F.; HERNANDEZ-TINOCO, J.; RABAGO-SANCHEZ, E.; BERISTAIN-GARCIA, I.; LIESENFELD, O., ESTRADA-MARTINEZ, S.; PEREZ-ALAMOS, A. R.; ALVARADO-SOTO, E. Association Between *Toxoplasma gondii* Exposure and Heart Disease: A Case-Control Study. **Journal of Clinical Medicine Research**, v. 8, n. 5, p. 402–409, 2016.

ANDERSON, J.F.; MAGNARELLI, L.A. Biology of ticks. **Infectious Disease Clinics of North America**, v. 22, p.195-215, 2008.

ANDERSON, R. C.; LITTLE, M. D.; STRELIVE, U. R. The unique lungworms (Nematoda: Metastrongyloidea) of the opossum (*Didelphis marsupialis* Linnaeus). **Systematic Parasitology**, v. 2, p. 1-8, 1980.

ANDRADE, A. J.; GURGEL-GONÇALVES, R. New Record and Update on the Geographical Distribution of *Pintomyia Monticola* (Costa Lima, 1932) (Diptera: Psychodidae) in South America. **Check List**, v. 11, n. 2, 2015.

ANGELAKIS, E.; MEDIANNIKOV, O.; PAROLA, P.; RAOULT, D. *Rickettsia felis*: The Complex Journey of an Emergent Human Pathogen. **Trends in Parasitology**, v. 32, p. 554-564, 2016.

APANASKEVICH, D. A.; HORAK, I. G.; MATTHEE, C. A.; MATTHEE, S. A new species of *Ixodes* (Acari: Ixididae) from South African mammals. **The Journal of Parasitology**, v. 97, n. 3, p. 389-398, 2011.

ARAGÓN-PECH, R. A.; RUIZ-PIÑA, H. A.; RODRÍGUEZ-VIVAS, R. I.; CUXIM-KOYOC, A. D.; Reyes-Novelo, E. Prevalence, Abundance and Intensity of Eggs and Oocysts of Gastrointestinal Parasites in the Opossum *Didelphis Virginiana* Kerr, 1792 in Yucatan, Mexico. **Helminthologia** (Poland), v. 55, n. 2, p. 119-126, 2018.

ARROJO, L. Parásitos de animales silvestres en cautiverio en Lima, Perú. **Revista Peruana de Biología**, v. 9, p. 118-120, 2002.

AUSTAD, S. N. The adaptable opossum. **Scientific American**, v. 258, n. 2, p. 98-105, 1988.

AUSTAD, S. N., SUNQUIST, M. E. Sex-ratio manipulation in the common opossum. **Nature**, v. 324, p. 58-60, 1986.

BABERO, B. B. Further studies on helminths of the opossum, *Didelphis virginiana*, with a description of a new species from this host. **Journal of Parasitology**, v. 46, p. 455-463, 1960.

BABERO, B. B. Some helminths from Illinois opossums. **Journal of Parasitology**, v. 43, p. 232, 1957.

BAIN, O.; BAKER, M.; CHABAUD, A. G. Nouvelles données sur la lignée *Dipetalonema* (Filarioidea, Nematoda). **Annales de Parasitologia (Paris).** v. 57, n. 6, p. 593-620.

BAIN, O.; CASIRAGHI, M.; MARTIN, C.; UNI, S. The nematoda Filarioidea: critical analysis linking molecular and traditional approaches. **Parasite**, v. 15, p. 342-348, 2008.

BANETH, G. Perpectives on canine and feline hepatozoonosis. **Veterinary Parasitology**, v. 181, p. 3-11, 2011.

BANETH, G.; MATHEW, J. S.; SHKAP, V.; MACINTIRE, D. K.; BARTA, J. R.; EWING, S. A. Canine Hepatozoonosis: Two Disease Syndromes Caused by Separate *Hepatozoon* spp.**Trends in Parasitology**, v. 19, n. 1, p. 27-31, 2003.

BARROS-BATTESTI, D. M.; LANDULFO, G. A.; LUZ, H. R.; MARCILI, A.; ONOFRIO, V. C.; FAMADAS, K. M. *Ornithodoros faccinii* n. sp. (Acari: Ixodida: Argasidae) parasitizing the frog *Thoropa miliaris* (Amphibia: Anura: Cycloramphidae) in Brazil. **Parasites & Vectors**, v. 8, p. 268-279, 2015.

BEAUCOURNU, J. C.; REYNES, J. M.; VIÉ. J. C. Fleas in French Guiana (Insecta: Siphonaptera). **Journal of Medical Entomology**, v. 35, n. 1, p. 3-10, 1998.

BERMÚDEZ S. E.; MIRANDA, R. J.; SMITH, D. Ticks Species (Ixodida) in the Summit Municipal Park and Adjacent Areas, Panama City, Panama. **Experimental and Applied Acarology**, v. 52, n. 4, p. 439-448, 2010.

BERMÚDEZ, S. E.; GOTTDENKER, N.; KRISHNVAJHALA, A.; FOX, A.; WILDER, H. K.; GONZÁLEZ, K.; SMITH, D.; LÓPEZ, M.; PEREA, M.; RIGG, C.; MONTILLA, S.; CALZADA, J. E.; SALDAÑA, A.; CABALLERO, C. M.; LOPEZ, J. E. Synanthropic Mammals as Potential Hosts of Tick-Borne Pathogens in Panama. **PLoS ONE**, v. 12, n. 1, p. 1-9, 2017.

BINETRUY, F.; CHEVILLON, C.; THOISY, B.; GARNIER, S.; Duron, O. Survey of Ticks in French Guiana. **Ticks and Tick-Borne Diseases**, v. 10, n. 1, p. 77–85, 2019.

BITAM, I.; DITTMAR, K.; PAROLA, P.; WHITING, M.F.; RAOULT, D. Fleas and flea-borne diseases. **International Journal of Infectious Diseases**, v. 14, n. 8, p. 667-676, 2010.

BLANCO, C. M.; TEIXEIRA, B. R.; DA SILVA, A. G.; DE OLIVEIRA, R. C.; STRECHT, L.; OGRZEWALSKA, M.; DE LEMOS, E. R. S. Microorganisms in Ticks (Acari: Ixodidae) Collected on Marsupials and Rodents from Santa Catarina, Paraná and Mato Grosso Do Sul States, Brazil. **Ticks and Tick-Borne Diseases**, v. 8, n. 1, p. 90-98, 2017.

BLUMENTHAL, E. M.; KIRKLAND, Jr. G.L. The biology of the opossum, *Didelphis virginiana* in south central Pennsylvania. **Proceedings of the Pennsylvania Academy of Science**, v. 50, p. 81-85, 1976.

BOCK, R.; JACKSON, L.; JORGENSEN, W. Babesiosis of Cattle. **Parasitology**, v. 129, S247-269, 2004.

BOOSTROM, A.; BEIER, M. S.; MACALUSO, J. A.; MACALUSO, K. R.; SPRENGER, D.; HAYES, J. Geographic association of *Rickettsia felis*-infected opossums with human murine typhus, Texas. **Emerging Infectious Diseases**, v. 8, n. 6, p. 549–554, 2002.

BOWMAN, D. D. **Georgis – Parasitologia Veterinária**. 9. ed. Rio de Janeiro: Elsevier, 2010. 432p.

BOWMAN, D. D.; Smith, J. L.; Little, M. D. *Lagochilascaris sprenti* sp. n. (Nematoda: Ascarididae) from the opossum, *Didelphis virginiana* (Marsupialia: Didelphidae). **Journal of Parasitology**, v. 69, p. 754-760, 1983.

BRIANTI, E.; GAGLIO, G.; NAPOLI, E.; GIANNETTO, S.; DANTAS-TORRES, F.; BAIN O.; OTRANTO, D. New Insights into the Ecology and Biology of *Acanthocheilonema Reconditum* (Grassi, 1889) Causing Canine Subcutaneous Filariosis. **Parasitology**, v. 139, n. 4, p. 530–36, 2012.

BROWN, C. C. Endogenous lipid pneumonia in opossums from Louisiana. **Journal of Wildlife Diseases**, v. 24, p. 214-219, 1988.

BROWN, D. L.; MACALUSO, K. R. *Rickettsia felis*, an Emerging Flea-Borne Rickettsiosis. **Current Tropical Medicine Reports**, v. 3, p. 27-39, 2016.

BUTTERWORTH, E. W.; BEVERLEY-BURTON, M. *Capillaria didelphis* n. sp. (Nematoda: Trichuroidea) from the opossum, *Didelphis virginiana* L. in Georgia. **Canadian Journal of Zoology**, v. 55, p. 616-619, 1977.

BYRD, E. E. The opossum, *Didelphis virginiana* Kerr, a new host for *Paragonimus* in Tennessee. **Science**, v. 93, p. 542, 1941.

BYRD, E. E.; REIBER, R. J.; PARKER, M. V. Mammalian trematodes. I. Trematodes from the opossum, *Didelphis virginiana* Kerr. **Journal of the Tennessee Academy of Science**, v. 17, p. 130-142, 1942a.

BYRD, E. E.; REIBER, R. J.; Parker, M. V. The anatomy of a lung fluke from the opossum (*Didelphis virginiana* Kerr). **Journal of the Tennessee Academy of Science**, v. 17, p. 116-129, 1942b.

BYRD, E. E.; REIBER, R. J.; PARKER, M. V. The opossum, *Didelphis virginiana*, a new host for *Paragonimus westermani* in the United States. **Journal of the Tennessee Academy of Science**, v. 16, p. 356-357, 1941.

BYRD, E. E.; WARD, J. A. Observations on the segmental anatomy of the tapeworm, *Mesocestoides variabilis* Mueller, 1928, from the opossum. **Journal of Parasitology**, v. 29, p. 217-226, 1943.

BYRD, E. E.; WARD, J. A. Segmental anatomy of an opossum cestode, *Mesocestoides* sp. **Journal of the Tennessee Academy of Science**, v. 17, p. 341-342, 1942.

CÁCERES, N. C., PRATES, L. Z.; GHIZONI-JR, I. R.; GRAIPEL, M. E. Frugivory by the black-eared opossum *Didelphis aurita* in the Atlantic Forest of the southern Brazil: Roles of sex, season and sympatric species. **Biotemas**, v. 22, n. 3, p. 203-211, 2009.

CÁCERES, N. C.; MONTEIRO-FILHO, E. L. A. Food Habits, Home Range and Activity of *Didelphis aurita* (Mammalia, Marsupialia) in a Forest Fragment of Southern Brazil. **Studies on Neotropical Fauna and Environment**, v. 36, n. 2, p. 85-92, 2001.

CAMPBELL, M. L.; GARDNER, S. L.; NAVONE, G. T., A New species of *Mathevotaenia* (Cestoda: Anoplocephalidae) and other tapeworms from marsupials in Argentina. **Journal of Parasitology**, v. 89, n. 6, p. 1181-1185, 2003.

CANAVAN, W. P. N. Nematode parasites of vertebrates in the Philadelphia Zoological Garden and vicinity. **Parasitology**, v. 21, p. 63-102, 1929.

CANAVAN, W. P. N. Nematode parasites of vertebrates in the Philadelphia Zoological Garden and vicinity. II. **Parasitology**, v. 23, p. 196-229, 1931.

CAÑEDA-GUZMÁN, I. C.; CHAMBRIER, A.; SCHOLZ, T. *Thaumasioscolex didelphidis* n. gen., n. sp. (eucestoda: proteocephalidae) from the black-eared opossum *Didelphis marsupialis* from Mexico, the first proteocephalidean tapeworm from a mammal. **Journal of Parasitology**, v. 87, n. 3, v. 639-646, 2001.

CANTILLO-BARRAZA, O.; GARCÉS, E.; GÓMEZ-PALACIO, A.; CORTÉS, L. A.; PEREIRA, A.; MARCET, P. L.; JANSEN, A. M.; TRIANA-CHÁVEZ, O. Eco-Epidemiological Study of an Endemic Chagas Disease Region in Northern Colombia Reveals the Importance of *Triatoma maculata* (Hemiptera: Reduviidae), Dogs and *Didelphis Marsupialis* in *Trypanosoma cruzi* Maintenance. **Parasites & Vectors**, v. 8, n. 1, p. 1-10, 2015.

CANTOR, M.; FERREIRA, L.A.; SILVA, W.R., SETZ, E. Z. F. Potential seed dispersal by *Didelphis albiventris* (Marsupialia, Didelphidae) in highly disturbed environment. **Biota Neotropica**, v. 10, n. 2, 45-51, 2010.

CASTRO, G. A. Helminths: Structure, Classification, Growth, and Development. *In.* Baron, S. (ed.). **Medical Microbiology.** 4. ed. Galveston (TX): University of Texas Medical Branch at Galveston, 1996. Available from: https://www.ncbi.nlm.nih.gov/books/NBK8282/

CASTRO, R.G.B.M.; COSTA NETO, S.F.; MALDONADO JUNIOR, A.M.; GENTILE, R. Ecological aspects of nematode parasites of *Didelphis aurita* (Didelphimorphia, Didelphidae) in urban-sylvatic habitats in Rio de Janeiro, Brazil. **Oecologia Australis**, v. 21, n. 1, p. 54-61, 2017.

CEOTTO, P.; FINOTTI, R.; SANTORI, R.; CERQUEIRA, R. Diet variation of the marsupails *Didelphis aurita* and *Philander frenatus* (Didelphimorphia, Didelphidae) in a rural area of Rio de Janeiro State, Brazil. **Mastozoologia Neotropical**, v. 16, n. 1, p. 49-58, 2009.

CHAGAS-MOUTINHO, V. A.; OLIVEIRA-MENEZES, A.; CÁRDENAS, M. Q.; LANFREDI, R. M. Further description of *Aspidodera raillieti* (Nematoda: Aspidoderidae) from *Didelphis marsupialis* (Mammalia: Didelphidae) by light and scanning electron microscopy. **Parasitology Research**, v. 101, p. 1331–1336, 2007.

CHAGAS-MOUTINHO, V. A.; SANT'ANNA, V.; OLIVEIRA-MENEZES, A.; SOUZA, W. New Aspidoderidae species parasite of *Didelphis aurita* (Mammalia: Didelphidae): A light and scanning electron microscopy approach. **Acta Tropica**, v. 130, p. 162-166, 2014.

CHANDLER, A. C. Notes on the helminth parasites of the opossum (*Didelphis virginiana*) in southeast Texas, with descriptions of four new species. **Proceedings of the United States National Museum**, v. 81, p. 1-15, 1932.

CHANDLER, A. C.; Rausch, R. A study of strigeids from Michigan mammals, with comments on the classification of mammalian strigeids. **Transactions of the American Microscopical Society**, v. 65, p. 328-337, 1946.

CHERO, J. D.; SÁEZ, G.; MENDONZA-VIDAURRE, C.; IANNACONE, J.; CRUCES, C. L. Helminths of the common opossum *Didelphis marsupialis* (Didelphimorphia: Didelphidae), with a checklist of helminths parasitizing marsupials from Peru. **Revista Mexicana de Biodiversidad**, v. 88, p. 560-571, 2017.

CHINCHILLA, M.; VALERIO, I.; DUSZYNSKI, D. Endogenous Life Cycle of *Eimeria marmosopos* (Apicomplexa: Eimeriidae) from the Opossum, *Didelphis marsupialis* (Didelphimorphia: Didelphidae) in Costa Rica. **Journal of Parasitology**, v. 101, n. 4, p. 436–443, 2015.

COHN, L. A. Ehrlichiosis and related infections. **Veterinary Clinic Small Animal**, v. 33, p. 863-884, 2003.

CORKUM, K. C. Sparganosis in some vertebrates of Louisiana and observations on a human infection. **Journal of Parasitology**, v. 52, p. 444-448, 1966.

CORTES, H. C. E.; CARDOSO, L.; GIANNELLI, A.; LATROFA, M. S.; DANTAS-TORRES, F.; OTRANTO, D. Diversity of *Cercopithifilaria* species in dogs from Portugal. **Parasites & Vectors**, v. 7, p. 261-265, 2014.

COSTA-NETO, S. F.; CARDOSO, T. S.; BOULLOSA, R. G.; Maldonado, A.; Gentile, R. Metacommunity Structure of the Helminths of the Black-Eared Opossum *Didelphis aurita* in Peri-Urban, Sylvatic and Rural Environments in South-Eastern Brazil. **Journal of Helminthology**, v. 93, n. 6, p. 720-731, 2018.

CRIADO-FORNELIO, A.; MARTINEZ-MARCOS, A.; BULING-SARAÑA, A.; BARBA-CARRETERO, J. C. Molecular Studies on *Babesia*, *Theileria* and *Hepatozoon* in Southern Europe: Part I. Epizootiological Aspects. **Veterinary Parasitology**, v. 113, p. 189-201, 2003.

CRICHTON, V. F.; Beverley-Burton, M. *Dracunculus lutrae* n. sp. (Nematoda: Dracunculoidea) from the otter, *Lutra canadensis*, in Ontario, Canada. **Canadian Journal of Zoology**, v 51, p. 521-529, 1973.

CRITES, J. L. A redescription of *Cruzia americana*, a nematode parasitic in the opossum, *Didelphis marsupialis virginiana*. **Journal of Parasitology**, v. 42, p. 68-72, 1956.

CUNHA, N. C.; FONSECA, A. H.; REZENDE, J.; ROZENTAL, T.; FAVACHO, A. R. M.; BARREIRA, J. B.; MASSARD, C. L.; LEMOS, E. R. S. First identification of natural infection of *Rickettsia rickettsii* in the *Rhipicephalus sanguineus* tick, in the State of Rio de Janeiro. **Pesquisa Veterinária Brasileira**, v. 29, n. 2, p. 105-108, 2009.

CUTOLO, A. A.; TEODORO, A. K. M.; OVALLOS, F. G.; ALLEGRETTI, S. M.; GALATI, E. A. B. Sandflies (Diptera: Psychodidae) Associated with Opossum Nests at Urban Sites in Southeastern Brazil: A Risk Factor for Urban and Periurban Zoonotic *Leishmania* Transmission? **Memorias do Instituto Oswaldo Cruz**, v. 109, n. 3, p. 391-393, 2014.

DA SILVA, M. R. L., FORNAZARI, F., DEMONER, L. C., TEIXEIRA, C. R., LANGONI, H., O'DWYER, L. H. *Didelphis albiventris* Naturally Infected with *Hepatozoon canis* in Southeastern Brazil. **Ticks and Tick-Borne Diseases**, v. 8, n. 6, p. 878-881, 2017.

DAGNONE, A. S.; MORAIS, H. S. A.; VIDOTTO, M. C.; JOJIMA, F. S.; VIDOTTO, O. Ehrlichiosis in anemic, thrombocytopenic, or tick-infested dogs from a Hospital population in South Brazil. **Veterinary Parasitolology**, v. 117, p. 285-290, 2003.

DAHLGREN, F. S.; Mandel, E. J.; Krebs, J. W.; Massung, R. F.; McQuiston, J. H. Increasing Incidence of *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* in the United States, 2000-2007. **American Journal of Tropical Medicine and Hygiene**, v. 85, n. 1, p. 124-131, 2011.

DANTAS-TORRES, F.; CHOMEL, B. B.; OTRANTO, D. Ticks and tick-borne diseases: a One Health perspective. **Trends in Parasitology**, v. 28, n. 10, p. 437-446, 2012b.

DANTAS-TORRES, F.; MARTINS, T. F.; MUÑOZ-LEAL, S.; ONOFRIO, V. C.; BARROS-BATTESTI, D. M. Ticks (Ixodida: Argasidae, Ixodidae) of Brazil: Updated Species Checklist and Taxonomic Keys. **Ticks and Tick-Borne Diseases**, v. 10, n. 6, 2019.

DANTAS-TORRES, F.; VENZAL, J. M.; BERNARDI, L. F. O.; FERREIRA, R. L.; ONOFRIO, V. C.; MARCILI, A.; BERMÚDEZ, S. E.; RIBEIRO, A. F.; BARROS-BATTESTI, D. M.; LABRUNA, M. B. Description of a new species of bat-associated Argasid tick (Acari: Argasidae) from Brazil. **The Journal of Parasitology**, v. 98, n. 1, p. 36-45, 2012a.

DE CARVALHO, E. M.; SILVA, R. A.; BARATA, J. M. S.; DOMINGOS, M. F.; CIARAVOLO, R. M. C.; ZACHARIAS, F. Soroepidemiologia Da Tripanosomíase Americana Na Região Do Litoral Sul, São Paulo. **Revista de Saúde Pública**, v. 37, n. 1, p. 49-58, 2003.

DE LEMOS, E. R.; MACHADO, R. D.; COURA, J. R.; GUIMARÃES, M. A.; FREIRE, N. M. Infestation by Ticks and Detection of Antibodies to Spotted Fever Group Rickettsiae in Wild Animals Captured in the State of São Paulo, Brazil. A Preliminary Report. **Memórias Do Instituto Oswaldo Cruz**, v. 91, n. 6, p. 701-702, 1996.

DE MORAES, L. B.; BOSSI, D. E. P.; LINHARES, A. X. Siphonaptera Parasites of Wild Rodents and Marsupials Trapped in Three Mountain Ranges of the Atlantic Forest in Southeastern Brazil. **Memórias Do Instituto Oswaldo Cruz**, v. 98, n. 8, p. 1071-1076, 2003.

DE SÁ, E. F. G. G.; RODRIGUES, V. S.; GARCIA, M. V.; ZIMMERMANN, N. P.; RAMOS, V. N.; BLECHA, I. M. Z.; DUARTE, P. O.; MARTINS, T. F.; BORDIGNON, M. O.; ANDREOTTI, R. Ticks on *Didelphis albiventris* from a Cerrado Area in the Midwestern Brazil. **Systematic and Applied Acarology**, v. 23, n. 5, p. 935-945, 2018.

DENTON, J. F. The occurrence of *Eurytrema allentoshi* (Foster, 1939) in the opossum in Texas. **Proceedings of the Helminthological Society of Washington**, v. 11, p. 54-55. 1944.

DIAS, E.; MARTINS, A. V. Spotted fever in Brazil: A summary. **American Journal of Tropical Medicine**, v.19, p.103-108, 1939.

DÍAZ, M. M.; NAVA, S.; GUGLIELMONE A. A. The Parasitism of *Ixodes luciae* (Acari: Ixodidae) on Marsupials and Rodents in Peruvian Amazon. **Acta Amazonica**, v. 39, n. 4, p. 997-1002, 2009.

DICKERSON, L. M. A new variety of *Harmostomum opisthotrias* from the North American opossum, *Didelphys virginiana*, with a discussion of its possible bearing on the origin of its host. **Parasitology**, v. 22, p. 37-46, 1930.

DIKMANS, G. A new nematode worm, *Viannaia bursobscura*, from the opossum with a note on other parasites of the opossum. **Proceedings of the United States National Museum**, v. 79, n. 1-4, 1931.

DIKMANS, G. The occurrence of *Viannaia viannai* Travassos (Nematoda: Heligmosomidae) in opossums in North America. **Proceedings of the Helminthological Society of Washington**, v. 10, p. 6-7, 1943.

DOMÍNGUEZ, L., MIRANDA, R. J., TORRES, S., MORENO, R., ORTEGA, J., BERMÚDEZ, S. E. Hard Tick (Acari: Ixodidae) Survey of Oleoducto Trail, Soberania National Park, Panama. **Ticks and Tick-Borne Diseases**, v. 10, n. 4, p. 830-837, 2019.

DUBEY, J. P.; SAVILLE, W. J. A.; STANEK, J. F.; LINDSAY, D. S.; ROSENTHAL, B. M.; OGLESBEE, M. J.; ROSYPAL, A. C.; NJOKU, C. J.; STICH, R. W.; KWOK, O. C.; SHEN, S. K.; HAMIR, A. N.; REED, S. M. *Sarcocystis Neurona* Infections in Raccoons (*Procyon lotor*): Evidence for Natural Infection with *Sarcocysts*, Transmission of Infection to Opossums (*Didelphis virginiana*), and Experimental Induction of Neurologic Disease in Raccoons. **Veterinary Parasitology**, v. 100, v. 3-4, p. 117-129, 2001.

DUNCAN, R. B.; REINEMEYER, C. R.; FUNK, R. S. Fatal lungworm infection in an opossum. **Journal of Wildlife Diseases**, v. 25, p. 266-269, 1989.

DURDEN, L. A.; RICHARDSON, D. J. Ectoparasites of the *Virginia Opossum* (Didelphis Virginiana), Raccoon (*Procyon lotor*), and Striped Skunk (*Mephitis mephitis*) from Keith County, Nebraska. **Transactions of the Nebraska Academy of Sciences**, v. 33, p. 21-24, 2013.

DURDEN, L. A.; WILSON, N. Ectoparasitic and Phoretic Arthropods of Virginia Opossums (*Didelphis Virginiana*) in Central Tennessee. **The Journal of Parasitology**, v. 76, n. 4, p. 581-583, 1990.

DUSZYNSKI, D. W. **The biology and identification of the coccidia (Apicomplexa) of marsupials of the world**. London: Elsevier/Academic Press Inc.. 2016.

DWORKIN, M. S.; SCHWAM, T. G.; ANDERSON, D. E.; BORCHARDT, S. M. Tick-borne relapsing fever. **Infectious Disease Clinics of North America**, v. 22, n. 3, p. 449-468, 2008.

ELSHEIKHA, H. M.; FITZGERALD, S. D.; ROSENTHAL, B. M.; MANSFIELD L. S. Concurrent Presence of *Sarcocystis neurona* Sporocysts, *Besnoitia darlingi* Tissue Cysts, and *Sarcocystis inghami Sarcocysts* in Naturally Infected Opossums (*Didelphis virginiana*). **Journal of Veterinary Diagnostic Investigation**, v. 16, n. 4, p. 352-356, 2004.

ELSHEIKHA, H. M.; MANSFIELD, L. S.; FITZGERALD, S. D.; SAEED, M. A. Prevalence and Tissue Distribution of *Besnoitia darlingi* Cysts in the Virginia Opossum (*Didelphis virginiana*) in Michigan. **Veterinary Parasitology**, v. 115, n. 4, p. 321-327, 2003.

EREMEEVA, M. E.; KARPATHY, S. E.; KRUEGER, L.; HAYES, E. K.; WILLIAMS, A. M.; ZALDIVAR, Y.; BENNETT, S.; CUMMINGS, R.; TILZER, A.; VELTEN, R. K.; KERR, N.; DASCH, G. A.; HU, R. Two Pathogens and One Disease: Detection and Identification of Flea-Borne Rickettsiae in Areas Endemic for Murine Typhus in California. **Journal of Medical Entomology**, v. 49, n. 6, 1485-1494, 2013.

EREMEEVA, M. E.; ZAMBRANO, M. L.; ANAYA, L.; BEATI, L.; KARPATHY, S. E.; SANTOS-SILVA, M. M.; SALCEDA, B.; MACBETH, D.; OLGUIN, H.; DASCH, G. A.; ARANDA, C. A. *Rickettsia rickettsii* in *Rhipicephalus* Ticks, Mexicali, Mexico. **Journal of Medical Entomology**, v. 48, n. 2, p. 418-421, 2011.

ESSLINGER, J. H.; SMITH, J. L. *Dipetalonema* (*Acanthocheilonema*) *didelphis* sp. n. (Nematoda: Filarioidea) from opossums, with a redescription of *D.* (*A.*) *pricei* (Vaz and Pereira 1934). **Journal of Parasitology**, v. 65, p. 928-933, 1979.

FACCINI-MARTÍNEZ, A. A.; GARCIA-ÁLVAREZ, L.; HIDALGO, M.; OTEO, J. A. Syndromic classification of rickettsioses: an approach for clinical practice. **International Journal of Infectious Diseases**, v. 28, p. 126-139, 2014.

FARIA, B. F.; MELO, F. R. *Didelphis imperfecta*, Didelphimorphia, Didelphidae (Mondolfi and Pérez-Hernández, 1984): a new record in the Brazilian Amazon. **Boletim da Sociedade Brasileira de Mastozoologia**. v. 79, p. 44-46, 2017.

FELDMAN, D. B.; MOORE, J. A.; HARRIS, M. W.; SELF, J. L. Characteristics of common helminths of the Virginia opossum (*Didelphis virginiana*) from North Carolina. **Laboratory Animal Science**, v. 22, p. 183-189, 1972.

FELDMAN, D. B.; SELF, J. L. Establishment of a helminth-free opossum colony. **Laboratory of Animal Science**, v. 23, p. 855-857, 1973.

FIGUEIREDO, L. T. M.; BADRA, S. J.; PEREIRA, L. E.; SZABÓ, M. P. J. Report on Ticks Collected in the Southeast and Mid-West Regions of Brazil: Analyzing the Potential Transmission of Tick-Borne Pathogens to Man. **Revista Da Sociedade Brasileira de Medicina Tropical**, v. 32, n. 6, p. 613–619, 1999.

FISH, D.; DOWLER, R. C. Host Associations of Ticks (Acari: Ixodidae) Parasitizing Medium-Sized Mammals in a Lyme Disease Endemic Area of Southern New York. **Journal of Medical Entomology**, v. 26, n. 3, p. 200-209, 1989.

FLORES-BARROETA, L.; HILDAGO-ESCALANTE, E.; GARCIA-TORRES, F. Nematodos de aves y mamiferos, III datos adicionales de *Gnathostoma* em huespedes norte americanos. **Anales de la Escuela Nacional de Cienas Biologicas**, v. 10, p. 107-111, 1961.

FONTALVO, M. C.; MENDONÇA FAVACHO, A. R.; ARAUJO, A. C.; SANTOS, N. M.; OLIVEIRA, G. M. B.; AGUIAR, D. M.; LEMOS, E. R. S.; HORTA, M. C. *Bartonella* Species Pathogenic for Humans Infect Pets, Free-Ranging Wild Mammals and Their Ectoparasites in the Caatinga Biome, Northeastern Brazil: A Serological and Molecular Study. **Brazilian Journal of Infectious Diseases**, v. 21, n. 3, p. 290-296, 2017.

GABRIELLI, S.; GIANNELLI, A.; BRIANTI, E.; DANTAS-TORRES, F.; BUFALINI, M.; FRAULO, M.; TORRE, F. L.; RAMOS, R. A. N.; CANTACESSI, C.; LATROFA, M. S.; CANCRINI, G.; OTRANTO, D. Chronic polyarthritis associated to *Cercopithifilaria bainae* infection in a dog. **Veterinary Parasitology**, v. 205, p. 401-404, 2014.

GACHOHI, J.; SKILTON, R.; HANSEN, F.; NGUMI, P.; KITALA, P. Epidemiology of East Coast Fever (*Theileria parva* Infection) in Kenya: Past, Present and the Future. **Parasites and Vectors**, v. 5, n. 1, 1-13, 2012.

GARDNER, A. L. **Mammals of South America, Volume I. Marsupials, xenarthrans, shrews, and bats**. Chicago and London: The University of Chicago Press, 2008. 669 p.

GOMES, D. C.; DA CRUZ, R. P.; VICENTE, J. J.; PINTO, R. M. Nematode parasites of marsupials and small rodents from the Brazilian Atlantic Forest in the State of Rio de Janeiro, Brazil. **Revista Brasileira de Zoologia**, v. 20, p. 699–707, 2003.

GRAY, J. B.; ANDERSON, R. C. Observations on *Turgida turgida* (Rudolphi, 1819) (Nematoda: Physalopteroidea) in the American opossum: (*Didelphis virginiana*). **Journal of Wildlife Diseases**, v. 18, p. 279-285, 1982.

GRAY, J.; ZINTL, A.; HILDEBRANDT, A.; HUNFELD, K.; WEISS, L. Zoonotic babesiosis: overview of the disease and novel aspects of pathogen identity. **Ticks and Tick-borne Diseases**, v. 1, n. 1, p. 3-10, 2010.

GUGLIELMONE, A. A.; ROBBINS, R. G.; APANASKEVICH, D. A.; PETNEY, T. N.; ESTRADA-PEÑA, A.; HORAK, I. G.; SHAO, R.; BARKER, S. C. The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world: a list of valid species names. **Zootaxa**, v. 2528, p. 1-28, 2010.

HALEY, J. S. Parasites in some wild fur bearers. **Veterinary Medicine**, v. 33, p. 291, 1938.

HARRIS, E. K.; VERHOEVE, V. I.; BANAJEE, K. H.; MACALUSO, J. A.; AZAD, A. F.; MACALUSO, K. R. Comparative Vertical Transmission of *Rickettsia* by *Dermacentor variabilis* and *Amblyomma maculatum*. **Ticks and Tick-Borne Diseases**, v. 8, n. 4, p. 598-604, 2017.

HEALTH, A. C. G. A new species of soft tick (Ixodoidea: Argasidae) from the New Zealand lesser short-tailed bat, *Mystacina tuberculata* Gray. **Tuhinga**, v. 23, p. 29-37, 2012.

HILL, W. C. *Physaloptera ackerti* n. sp. (Nematoda). **Transactions of the American Microscopical Society**, v. 58, p. 285-291, 1939a.

HILL, W. C. *Spirocerca Longispiculata* n. sp. American Midland. **Naturalist**, v. 21, 636-640, 1939b.

HOLLOWAY, H. L. Helminths of rabbits and opossums at Mountain Lake, Virginia. **Bulletin of the Wildlife Disease Association**, v. 2, p. 38-39, 1966.

HOLLOWAY, H. L.; DOWLER, J. L. The helminths of opossums in western Virginia. **Virginia Journal of Science**, v. 14, p. 203, 1963.

HORTA, M. C.; LABRUNA, M. B.; PINTER, A.; LINARDI, P. M.; SCHUMAKER, T. T. S. *Rickettsia* Infection in Five Areas of the State of São Paulo, Brazil. **Memorias Do Instituto Oswaldo Cruz**, v. 102, n. 7, p. 793-801, 2007.

HORTA, M. C.; SABATINI, G. S.; MORAES-FILHO, J.; OGRZEWALSKA, M.; CANAL, R. B.; PACHECO, R. C.; MARTINS, T. F.; MATUSHIMA, E. R.; LABRUNA, M. B. Experimental Infection of the Opossum *Didelphis aurita* by *Rickettsia felis*, *Rickettsia bellii*, and *Rickettsia parkeri* and Evaluation of the Transmission of the Infection to Ticks *Amblyomma cajennense* and *Amblyomma dubitatum.* **Vector-Borne and Zoonotic Diseases***,* v. 10, n. 10, p. 959-967, 2010.

HUNFELD, K. P.; HILDEBRANDT, A.; GRAY, J. S. Babesiosis: Recent Insights into an Ancient Disease. **International Journal for Parasitology**, v. 38, n. 11, p. 1219-1237, 2008.

HUYNH, T.; THEAN, J.; MAINI, R. *Dipetalonema reconditum* in the human eye. **British Journal of Ophthalmology**, v. 85, p. 1384-1393, 2001.

JANSEN, A. M. Marsupiais Didelfídeos: gambás e cuícas. In: ANDRADE, A.; PINTO, S.C; OLIVEIRA, R.S. **Animais de Laboratório: criação e experimentação [online].** Rio de Janeiro: FIOCRUZ, 2002. 388 p.

JANSEN, A. M.; XAVIER, S. C. C.; ROQUE, A. L. R. Ecological Aspects of *Trypanosoma cruzi*: Wild Hosts and Reservoirs. *In.* TELLERIA, J.; TIBAYRENC, M. (ed.). **American Trypanosomiasis Chagas Disease: One Hundred Years of Research.** 2 ed. Elsevier Inc., 2017. 243-264 p. https://doi.org/10.1016/B978-0-12-801029-7.00011-3

JANSEN, A. M.; XAVIER, S. C. C.; ROQUE, A. L. R. The Multiple and Complex and Changeable Scenarios of the *Trypanosoma cruzi* Transmission Cycle in the Sylvatic Environment. **Acta Tropica**, v. 151, n. 1, 1-15, 2015.

JIANG, P.; ZHANG, X.; LIU, R. D.; WANG, Z. Q.; CUI, J. A. Human Case of Zoonotic Dog Tapeworm, *Dipylidium caninum* (Eucestoda: Dilepidiidae), in China. **Korean Journal of Parasitology**, v. 55, p. 61-64, 2017.

KALTUNG, B. Y.; MUSA, I. W. A Review of Some Protozoan Parasites Causing Infertility in Farm Animals. **ISRN Tropical Medicine**, v. 2013, p. 1-6, 2013.

KAPLAN, E. H. *Heterobilharzia americana* Price, 1929, in the opossum from Louisiana. **Journal of Parasitology**, v. 50, p. 797, 1964.

KAWAZOE, U.; DIAS, L. C. S.; PIZA, J. T. Infecção natural de pequenos mamíferos por *schistosoma mansoni*, na represa de Americana (São Paulo, Brasil). **Revista de Saúde Pública de São Paulo**, v. 12, p. 200-208, 1978.

KEIRANS, J. E.; HUTCHESON, H. J.; DURDEN, L. A.; KLOMPEN, J. S. *Ixodes* (*Ixodes*) *scapularis* (Acari:Ixodidae): redescription of all active stages, distribution, hosts, geographical variation, and medical and veterinary importance. **Journal of Medical Entomology**, v. 33, p. 297-318, 1996.

KIFUNE, T.; UYEMA, N. Reports of Fukuoka University Scientific Expedition to Peru, 1976. Part 2. Taxonomical studies on two species of the genus *Amphimerus* from opossums with a description of a new species (Trematoda: Opisthorchiidae). **Medical Bulletin of Fukuoka University**, v. 8, p. 393-400, 1981.

KIFUNE, T.; UYEMA, N. Reports of Fukuoka University Scientific Expedition to Peru, 1976. Part 3. Taxonomical studies on trematodes from marsupials and rodents with records of two crabs. **Medical Bulletin of Fukuoka University**, v. 9, p. 241-256, 1982.

KINSELLA, J. M.; HEARD, R. W. Morphology and life cycle of *Stictodora cursitans* n. comb. (Trematoda: Heterophyidae) from mammals in Florida salt marshes. **Transactions of the American Microscopical Society**, v. 93, p. 408-412, 1974.

KINSELLA, J. M.; WINEGARNER, C. E. A field study of *Anatrichosoma* infections in the opossum, *Didelphis virginiana*. **Journal of Parasitology**, v. 61, p. 779-781, 1975.

KOLLARS JR., T. M.; KENGLUECHA, A. Spotted Fever Group *Rickettsia* in *Dermacentor variabilis* (Acari: Ixodidae) Infesting Raccoons (Carnivora: Procyonidae) and Opossums (Marsupialia: Didelphimorphidae) in Tennessee. **Journal of Medical Entomology**, v. 38, n. 4, p. 601-602, 2001.

KOLLARS, T. M. Ticks (Acari: Ixodidae) Infesting Medium-Sized Wild Mammals in Southwestern Tennessee. **Journal of Medical Entomology**, v. 30, n. 5, p. 896–900, 1993.

KRAWCZAK, F. S.; BINDER, L. C.; OLIVEIRA, C. S.; COSTA, F. B.; MORAES-FILHO, J.; MARTINS, T. F.; SPONCHIADO, J.; MELO, G. L.; GREGORI, F.; POLO, G.; OLIVEIRA, S. V.; LABRUNA, M. B. Ecology of a Tick-Borne Spotted Fever in Southern Brazil. **Experimental and Applied Acarology**, v. 70, n. 2, p. 219-229, 2016.

KRULL, W. H. Some observations on the life history of *Brachylaemus virginiana* (Dickerson) Krull, N. 1934. 1935. **Transactions of the American Microscopical Society**, v. 54, p. 118-134, 1935.

KRUPP, J. H. Treatment of opossums with *Physaloptera* infections. **Journal of the American Veterinary Medical Association**, v. 141, p. 369-370, 1962.

KRUPP, J. H.; QUILLIN, R. A review of the use of the opossum for research-husbandry, experimental techniques and routine health measures. **Laboratory Animal Care**, v. 14, p. 189-194, 1964.

LABRUNA, M. B.; CAMARGO, L. M. A.; TERRASSINI, F. A.; FERREIRA, F.; SCHUMAKER, T. T. S.; CAMARGO, E. P. Ticks (Acari: Ixodidae) from the State of Rondonia, Western Amazon, Brazil. **Systematic and Applied Acarology**, v. 10, n. 17, 2004.

LABRUNA, M. B.; NAVA, S.; MARCILI, A.; BARBIERI, A. R. M.; NUNES, P. H.; HORTA, M. C.; VENZAL, J. M. A new argasid tick species (Acari: Argasidae) associated with the rock cavy, *Kerodon rupestris* Wied-Neuwied (Rodentia: Caviidae), in a semiarid region of Brazil. **Parasites & Vectors**, v. 9, p. 511-526, 2016.

LABUSCHAGNE, M.; BEUGNET, F.; REHBEIN, S.; GUILLOT, J.; FOURIE, J.; CRAFFORD, D. Analysis of *Dipylidium caninum* Tapeworms from Dogs and Cats, or Their Respective Fleas: Part 1. Molecular Characterization of *Dipylidium caninum*: Genetic Analysis Supporting Two Distinct Species Adapted to Dogs and Cats. **Parasite**, v. 25, 2018.

LAMATTINA, D.; COSTA, S. A.; ARRABAL, J. P.; VENZAL, J. M.; GUGLIELMONE, A. A.; NAVA, S. Factors Associated with Hard Tick (Acari: Ixodidae) Parasitism in Medium-Sized Mammals in the Atlantic Rainforest Region of Argentina. **Ticks and Tick-Borne Diseases**, v. 9, n. 6, p. 1451–1458, 2018.

LAMATTINA, D.; VENZAL, J. M.; COSTA, S. A.; ARRABAL, J. P.; FLORES, S.; BERROZPE, P. E.; GONZÁLEZ-ACUÑA, D.; GUGLIELMONE, A. A.; NAVA. S. Ecological Characterization of a Tick Community across a Landscape Gradient Exhibiting Differential Anthropogenic Disturbance in the Atlantic Forest Ecoregion in Argentina. **Medical and Veterinary Entomology**, v. 32, n. 3, p. 271-281, 2018.

LAMOTHE, A. R.; ALONSO, J. L.; LOPEZ, R. Una neuva zona endemica de paragonimiasis em Mexico. **Anales del Centre de Ciencias del Mar y Limnologia. Universidad Nacional Autonoma de Mexico, 57 Serie Zoologica**, v. 2, p.415-418, 1986.

LAMOTHE, A. R.; PINEDA, R. L.; MEAVE, O. G. Infeccion natural de *Paragonimus mexicanus* en *Didelphis virginiana* Califórnia, em Colima. **Anales del Centro de Ciencias del Mar y Limnologia. Universidad Nacional Autonoma de Mexico, 52 Serie Zoologica**, v. 1, p. 45-50, 1981.

LARESCHI, M.; VENZAL, J. M.; ARZUA, M.; GONZÁLEZ, E. Fleas of Small Mammals in Uruguay, with New Host and Distribution Records. **Comparative Parasitology**, v. 73, n. 2, p. 263-268, 2006.

LARUE, G. R.; BOSMA, N. J. Studies on the trematode family Strigeidae (Holostomidae) *Neodiplostomum lucidum* n. sp. **Journal of Parasitology**, v. 14, p. 124-125, 1927.

LAVENDER, D. R.; OLIVER, J. H. Ticks (Acari: Ixodidae) in Bulloch County, Georgia. **Journal of Medical Entomology**, v. 33, n. 2, p. 224-231, 1996.

LEGEY, A. P.; PINHOL, A. P.; XAVIER, S. C. C.; MARCHEVSKY, R.; CARREIRA, J. C.; LEON, L. L.; JANSEN, A. M. *Trypanosoma cruzi* in Marsupial Didelphids (*Philander frenata* and *Didelhis marsupialis*): Differences in the Humoral Immune Response in Natural and Experimental Infections. **Revista Da Sociedade Brasileira de Medicina Tropical**, v. 36, n. 2, p. 241-248, 2003.

LEIBY, D. A., SCHAD, G. A.; DUFFY, C. H.; MURRELL, K. D. *Trichinella spiralis* in an agricultural ecosystem. III. Epidemiological investigations of *Trichinella spiralis* in resident wild and feral animals. **Journal of Wildlife Diseases**, v. 24, p. 606-609, 1988.

LEIBY, D.A. Transfusion-transmitted *Babesia* spp.: Bull's-eye on *Babesia microti*. **Clinical Microbiology Review**, v. 24, p. 14-28, 2011.

LEIGH, W. H. Preliminary studies on parasites of upland game birds and fur-bearing mammals in Illinois. **Bulletin of the Illinois State Natural History Survey**, v. 21, p. 185-194, 1940.

Li, L. Redescription of *Cruzia americana* Maplestone, 1930 (Nematoda: Kathlaniidae) a Parasite of *Didelphis virginiana* (Kerr) (Mammalia: Didelphidae) in the USA. **Systematic Parasitology**, v. 96, n. 4-5, p. 433-440, 2019.

LIMA, J. A. S.; REZENDE, H. H. A.; ROCHA, T. M. D. D.; CASTRO, A. M. Analysis of the Accuracy of Different Laboratory Methods for the Diagnosis of Intestinal Parasites from Stray and Domiciled Cats (*Felis catus domesticus*) in Goiânia, Goiás, Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 27, n. 1, 95-98, 2018.

LINARDI, P. M. Checklist de Siphonaptera (Insecta) Do Estado de São Paulo. **Biota Neotropica**, v. 11, n. 1, p. 607-117, 2011.

LINARDI, P.M. Os ectoparasitos de marsupiais brasileiros. In: Cáceres, N. C.; Monteiro-Filho, E. L. A. **Os marsupiais do Brasil: biologia, ecologia e evolução**. Campo Grande: Editora da Universidade Federal do Mato Grosso do Sul, 2006; p. 37-52.

LITTLE, M. D. Seven new species of *Strongyloides* (Nematoda) from Louisiana. **Journal of Parasitology**, v. 52, p. 85-97, 1966.

LOPEZ, J. E.; KRISHNAVAHJALA, A.; GARCIA, M. N.; BERMUDEZ, S. Tick-Borne Relapsing Fever Spirochetes in the Americas. **Veterinary Sciences**, v. 3, n. 3, p. 16-34, 2016.

LUMSDEN, R. D.; WINKLER, C. A. The opossum, *Didelphis virginiana* (Kerr), a host for the cyathocotylid trematode *Linstowiella szidati* (Anderson, 1944) in Louisiana. **Journal of Parasitology**, v. 48, p. 503, 1962.

LUMSDEN, R. D.; ZISCHKE, J. A. Seven trematodes from small mammals in Louisiana. **Tulane Studies in Zoology and Botany**, v. 9, p. 87-98, 1961.

LUZ, H. R.; NETO, S. F. C.; WEKSLER, M.; GENTILE, R.; FACCINI, J. L. H. Ticks Parasitizing Wild Mammals in Atlantic Forest Areas in the State of Rio de Janeiro, Brazil. **Revista Brasileira de Parasitologia Veterinaria**, v. 27, n. 3, p. 409-414, 2018.

MAINA, A. N.; FOGARTY, C.; KRUEGER, L.; MACALUSO, K. R.; ODHIAMBO, A.; NGUYEN, K.; FARRIS, C. M.; LUCE-FEDROW, A.; BENNETT, S.; JIANG, J.; SUN, S.; CUMMINGS, R. F.; RICHARDS, A. L. Rickettsial Infections among *Ctenocephalides felis* and Host Animals during a Flea-Borne Rickettsioses Outbreak in Orange County, California. **PLoS ONE**, v. 11, n. 8, p. 1-13, 2016.

MANSUETO, P.; VITALE, G.; CASCIO, A.; SEIDITA, A.; PEPE, I.; CARROCCIO, A. New insight into immunity and immunopathology of rickettsial diseases. **Clinical and Developmental Immunology**, v. 2012, p. 1-26, 2012.

MARTINEZ, F. A.; TROIANO, J. C.; SANCHES-NEGRETE, M.; GAUANA-ANASCO, L. *Didelphostrongylus hayesi* Prestwood, 1976 in Argentina. **Revista de Medicina Veterinária, Buenos Aires**, v. 80, n. 6, 478-479, 1999.

MARTINS, T. F.; BARBIERI, A. R. M.; COSTA, F. B.; TERASSINI, F. A.; CAMARGO, L. M. A.; PETERKA, C. R. L.; PACHECO, R. C.; DIAS, R. A.; NUNES, P. H.; MARCILI, A.; SCOFIELD, A.; CAMPOS, A. K.; HORTA, M. C.; GUILLOUX, A. G. A.; BENATTI, H. R.; RAMIREZ, D. G.; BARROS-BATTESTI, D. M.; LABRUNA, M. B. Geographical distribution of *Amblyomma cajennense* (*sensu lato*) ticks (Parasitiformes: Ixodidae) in Brazil, with description of the nymph of *A. cajennense* (*sensu stricto*). **Parasites & Vectors**, v. 9, p. 186-200, 2016.

MARTINS, T. F.; DANTAS-TORRES, F.; NIERI-BASTOS, F. A.; MARCILI, A.; DE SIQUEIRA, D. B.; ALÉSSIO, F. M.; MAUFFREY, J. F.; MARVULO, M. F. V.; DA SILVA, J. C. R.; LABRUNA, M. B. Host Records for the Immature Stages of the South American Tick, *Amblyomma fuscum* (Acari: Ixodidae). **Entomological News**, v. 120, n. 4, p. 370-374, 2009.

MARTINS, T. F.; LUZ, H. R.; MUÑOZ-LEAL, S.; RAMIREZ, D. G.; MILANELO, L.; MARQUES, S.; SANCHES, T. C.; ONOFRIO, V. C.; ACOSTA, C. L.; BENATTI, H. R.; MATURANO, R.; DE OLIVEIRA, P. B.; ALBUQUERQUE, G. R.; MARCII, A.; FLAUSTINO, W.; SILVEIRA, L.F.; MCINTOSH, D.; FACCINI, J. H. L.; LABRUNA, M. B. A New Species of *Amblyomma* (Acari: Ixodidae) Associated with Monkeys and Passerines of the Atlantic Rainforest Biome, Southeastern Brazil. **Ticks and Tick-Borne Diseases**, v. 10, n. 6, 101259, 2019.

MASNY, A.; GOŁĄB, E.; CIELECKA, D.; SAŁAMATIN, R. Vector-Borne Helminths of Dogs and Humans - Focus on Central and Eastern Parts of Europe. **Parasites & Vectors**, v. 6, n. 1, p. 1-14, 2013.

MASSARD, C. L.; FONSECA, A. H. Carrapatos e doenças transmitidas comuns ao homem e aos animais. **A Hora Veterinária**, v. 135, n. 1, p. 15-23, 2004.

MASSINI, P. F.; DROZINO, R. N.; OTOMURA, F. H.; MONGRUEL, A. C. B.; VALENTE, J. D. M.; TOLEDO, M. J. O.; MARTINS, T. F.; VIDOTTO, O.; VIEIRA, T. S. W. J.; VIEIRA, R. F. C. Detection of Hemotropic Mycoplasma sp. in White-Eared Opossums (*Didelphis albiventris*) from Southern Brazil. **Revista Brasileira de Parasitologia Veterinária**, 2019. Doi: https://doi.org/10.1590/S1984-29612019058

MCKEEVER, S. Observations on *Paragonimus kellicotti* Ward from Georgia. **Journal of Parasitology**, v. 44, p. 324-327, 1958.

MEAD, P. S. Epidemiology of Lyme Disease. **Infectious Disease Clinics of North America**, v. 29, n. 2, p. 187-210, 2015.

MELO, A. L. T.; AGUIAR, D. M.; SPOLIDORIO, M. G.; YOSHINARI, N. H.; MATUSHIMA, E. R.; LABRUNA, M. B.; HORTA, M. C. Serological evidence of exposure to tick-borne agents in opossums (*Didelphis* spp.) in the state of São Paulo, Brazil. **Brazilian Journal of Veterinary Parasitology***,* v. 25, n. 3, p. 348-352, 2016.

MERHEJ, V.; RAOULT, D. Rickettsial evolution in the light of comparative genomics. **Biological Reviews**, v. 86, p. 379–405, 2011.

MILLER, G. C.; HARKEMA, R. Helminths of the opossum (*Didelphis virginiana*) in North Carolina. **Proceedings of the Helminthological Society of Washington**, v. 37, p. 36-39, 1970.

MIYAZAKI, I.; KIFUNE, T.; HABE, S.; UYEMA, N. Reports of Fukuoka University Scientific Expedition to Peru, 1976. Part 1. General account of the Expedition and records of helminth parasites of wild mammals mollusks and insects. **Department of Parasitology, School of Medicine, Fukuoka University** Occ. Publ. ii xi, p. 1-28, 1978.

MUELLER, J. F. Cestodes of the genus *Mesocestoides* from the opossum and the cat. **American Midland Naturalist**, v. 12, p.81-91, 1930.

MULLER, G.; BRUM, J. G. W.; LANGONE, P. Q.; MICHELS, G. H.; SINKOC, A. L.; RUAS, J. L.; BERNE, M. E. A. *Didelphis albiventris* Lund, 1841 Parasitado Por *Ixodes loricatus* Neumann, 1899 e *Amblyomma aureolatum* (Pallas, 1772) (Acari: Ixodidae) No Rio Grande Do Sul. **Arquivos do Instituto Biológico**, v. 72, n. 3, p. 319-324, 2005.

MULLER, G.; LANGONI, P. Q.; MICHELS, G. H.; BERNE, M. E. A.; BRUM, J. G. W. Nematódeos em *Didelphis albiventris* (gambás) da região sul do Rio Grande do Sul. **Revista Brasileira de Parasitologia Veterinária**, v. 13, p. 259, 2004.

MUÑOZ-LEAL, S.; TOLEDO, L. F.; VENZAL, J. M.; MARCILI, A.; MARTINS, T. F.; COSTA, I. C. L.; PINTER, A.; LABRUNA, M. B. Description of a new soft tick species (Acari: Argasidae: *Ornithodoros* associated with stream-breeding frogs (Anura: Cycloramphidae: Cycloramphus) in Brazil. **Ticks and Tick-borne Diseases**, v. 8, p. 682-692, 2017.

MUÑOZ-LEAL, S.; VENZAL, J. M.; GONZÁLEZ-ACUÑA, D.; NAVA, S.; LOPES, M. G.; MARTINS, T. F.; FIGUEROA, C.; FERNÁNDEZ, N.; LABRUNA, M. B. A new species of *Ornithodoros* (Acari: Argasidae) from desert areas of northern Chile. **Ticks and Tick-borne Diseases**, v. 7, p. 901-910, 2016.

NAVA, S.; GUGLIELMONE, A. A.; MANGOLD, A. J. An overview of systematics and evolution of ticks. **Frontiers in Bioscience**, v. 14, n. 1, p. 2857-2877, 2009.

NETTLES, V. F.; PRESTWOOD, A. K.; DAVIDSON, W. R. Severe parasitism in an opossum. **Journal of Wildlife Diseases**, v. 11, p. 419-420, 1975.

OATES, S. C.; MILLER, M. A.; HARDIN, D.; CONRAD, P. A.; MELLI, A.; JESSUP, D. A.; DOMINIK, C.; ROUG, A.; TINKER, M. T.; MILLER, W. A. Prevalence, Environmental Loading, and Molecular Characterization of *Cryptosporidium* and *Giardia* Isolates from Domestic and Wild Animals along the Central California Coast. **Applied and Environmental Microbiology**, v. 78, n. 24, p. 8762–8772, 2012.

OLIVEIRA, H. H.; GOMES, V.; AMORIM, M.; GAZÊTA, G. S.; SERRA-FREIRE, N. M.; QUINELATO, I. P. F.; MORELLI-AMARAL, V. F.; ALMEIDA, A. B.; CARVALHO, R. W.; CARVALHO, A. G. Diversidade de Ixodida Em Roedores e Marsupiais Capturados No Parque Estadual Da Pedra Branca, Rio de Janeiro, Brasil. **Arquivo Brasileiro de Medicina Veterinaria e Zootecnia**, v. 66, n. 4, p. 1097-1104, 2014.

OLIVEIRA, P. R.; BORGES, L. M. F.; LOPES, C. M. L.; LEITE, R. C. Population dynamics of the free-living stages of *Amblyomma cajennense* (Fabricius, 1787) (Acari:Ixodidade) on pastures of Pedro Leopoldo, Minas Gerais State, Brazil. **Veterinary Parasitology**, v. 92, p. 295-301, 2000.

OLIVER, J. H.; MAGNARELLI, L. A.; HUTCHESON, H. J.; ANDERSON J. F. Ticks and Antibodies to *Borrelia burgdorferi* from Mammals at Cape Hatteras, NC and Assateague Island, MD and VA. **Journal of Medical Entomology**, v. 36, n. 5, p. 578-587, 1999.

ONYICHE, T. G. E.; SUGANUMA, K.; IGARASHI, I.; YOKOYAMA, N.; XUAN, X.; THEKISOE, O. A Review on Equine Piroplasmosis: Epidemiology, Vector Ecology, Risk Factors, Host Immunity, Diagnosis and Control. **International Journal of Environmental Research and Public Health**, v. 16, n. 10, 2019.

ORD, R. L.; LOBO, C. A. Human Babesiosis: Pathogens, Prevalence, Diagnosis, and Treatment. **Current Clinical Microbiology Reports**, v. 2, n. 4, p. 173–181, 2015.

OTRANTO, D.; BRIANTI, E.; ABRAMO, F.; GAGLIO, G.; NAPOLI, E.; LATROFA, M. S.; RAMOS, R. A. N.; DANTAS-TORRES, F.; BAIN, O. Cutaneous distribution and localization of *Cercopithifilaria* sp. microfilariae in dogs. **Veterinary Parasitology**, v. 190, n. 1, p. 143-150, 2012.

PARK, P. J. The miracidium of *Neodiplostomum lucidum*. LaRue and Bosma. **Transactions of the American Microscopical Society**, v. 55, p. 49-54, 1936.

PENCE, D. B.; LITTLE, M. D. **Anatrichosoma buccalis** sp. n. (Nematoda: Trichosomoididae) from the buccal mucosa of the common opossum, *Didelphis marsupialis* L. **Journal of Parasitology**, v. 58, p. 767-773, 1972.

PENICHE-LARA, G.; DZUL-ROSADO, K.; PÉREZ-OSORIO, C.; ZAVALA-CASTRO, J. *Rickettsia typhi* in Rodents and *R. felis* in Fleas in Yucatán as a Possible Causal Agent of Undefined Febrile Cases. **Revista do Instituto de Medicina Tropical de São Paulo**, v. 57, p. 129-132, 2015.

PINTO H. A.; MARI V. L. T.; MELO A. L. *Toxocara cati* (Nematoda: Ascarididae) in *Didelphis albiventris* (Marsupialia: Didelphidae) from Brazil: a case of pseudoparasitism. **Brazilian Journal of Veterinary Parasitology**, v. 23, n. 4, p. 522-525, 2014.

PINTO, I. S.; BOTELHO, J. R.; COSTA, L. P.; LEITE, Y. L. R.; LINARDI, P. M. Siphonaptera Associated With Wild Mammals From the Central Atlantic Forest Biodiversity Corridor in Southeastern Brazil. **Journal of Medical Entomology**, v. 46, n. 5, p. 1146-1151, 2009.

POO-MUNOZ, D. A.; ELIZONDO-PATRONE, C.; ESCOBAR, L. E.; ASTORGA, F.; BERMUDEZ, S. E.; MARTINEZ-VALDEBENITO, C.; ABARCA, K.; MEDINA-VOGEL, G. Fleas and Ticks in Carnivores from a Domestic–Wildlife Interface: Implications for Public Health and Wildlife. **Journal of Medical Entomology**, v. 53, n. 6, p. 1-11, 2016.

PREMVATI, G.; BAIR, T. D. Trematode parasites of the opossum, *Didelphis virginiana*, from Florida. **Proceedings of the Helminthological Society of Washington**, v. 46, p. 207-212, 1979.

PRESTWOOD, A. K. *Didelphostrongylus hayesi* genet sp. n. (Metastrongyloidea: Filaroididae) from the opossum, *Didelphis marsupialis*. **Journal of Parasitology**, v. 62, p. 272-275, 1976.

PRESTWOOD, A. K.; NETTLES, V. F.; FARRELL, R. L. Pathologic manifestations of experimentally and naturally acquired lungworm infections in opossums. **American Journal of Veterinary Research**, v. 38, p. 529- 532, 1977.

PROENÇA, M. C. Revisão do gênero *Aspidodera* Railliet and Henry, 1912: (Nematoda: Subuluroidea). **Memórias do Instituto Oswaldo Cruz**, v. 32, n. 3, p. 427-438, 1937.

PUNG, O. J.; DURDEN, L. A.; BANKS, C. W.; JONES, D. N. Ectoparasites of Opossums and Raccoons in Southeastern Georgia. **Journal of Medical Entomology**, v. 31, n. 6, p. 915-919, 1994.

QUINTAL, A. P. N.; RIBEIRO, E. S.; RODRIGUES, F. P.; ROCHA, F. S.; FLOETER-WINTER, L. M.; NUNES, C. M. *Leishmania* spp. in *Didelphis albiventris* and *Micoureus paraguayanus* (Didelphimorphia: Didelphidae) of Brazil. **Veterinary Parasitology**, v. 176, n. 2-3, p. 112-119, 2011.

RAMOS, R. A. N.; DO RÊGO, A. G. O.; FIRMINO, E. D. F.; RAMOS, C. A. N.; DE CARVALHO, G. A.; DANTAS-TORRES, F.; OTRANTO, D.; ALVES, L. C. Filarioids infecting dogs in Northeastern Brazil. **Veterinary Parasitology**, v. 226, p. 26-29, 2016.

RAUSCH, R.; TINER, J. D. Studies on the parasitic helminths of the north central states. II. Helminths of voles (*Microtus* spp.) preliminary report. **American Midland Naturalist**, v. 41, p. 665-694, 1949.

REEVES, W. K.; NELDER, M. P.; KORECKI, J. A. *Bartonella* and *Rickettsia* in Fleas and Lice from Mammals in South Carolina, U.S.A. **Journal of Vector Ecology : Journal of the Society for Vector Ecology**, v. 30, n. 2, p. 310-315, 2005.

REIBER R. J.; BYRD, E. E. Some nematodes from mammals of Reelfoot Lake in Tennessee. **Journal of the Tennessee Academy of Science**, v. 17, p. 78-89, 1942.

RENVOISÉ, A.; RAOULT, D. An update on rickettsiosis. **Médecine et Maladies Infectieuses**, v. 39, n. 2, p. 71-81, 2009.

REZENDE, H. H. A.; AVELAR, J. B.; STORCHILO, H. R.; VINAUD, M. C.; DE CASTRO, A. M. Evaluation of the Accuracy of Parasitological Techniques for the Diagnosis of Intestinal Parasites in Cats. **Revista Brasileira de Parasitologia Veterinária**, v. 24, n. 4, p. 471-474, 2015.

RICHARDSON, D. J. Acanthocephala of the Virginia opossum (*Didelphis virginiana*) in Arkansas, with a note on the life history of *Centrorhynchus wardae* (Centrorhynchidae). **Journal of the Helminthological Society of Washington**, v. 60, p. 128-130, 1993.

RICHARDSON, D. J.; CAMPO, J. D. Gastrointestinal Helminths of the Virginia Opossum (*Didelphis virginiana*) in South-Central Connecticut, U.S.A. **Comparative Parasitology**, v. 72, n. 2, p. 183-185, 2005.

ROJERO-VÁZQUEZ, E.; GORDILLO-PÉREZ, G.; WEBER, M. Infection of *Anaplasma phagocytophilum* and *Ehrlichia* spp. in Opossums and Dogs in Campeche, Mexico: The Role of Tick Infestation. **Frontiers in Ecology and Evolution**, v. 5, p. 161, 2017.

SAHINDURAN, S. Protozoan Diseases in Farm Ruminants. *In*. PEREZ-MARIN, C. C. (ed.) **A Bird's-Eye View of Veterinary Medicine**, 2012. https://doi.org/10.5772/30251.

SALVADOR, C. H.; CARVALHO-PINTO, C.; CARVALHO, R.; GRAIPEL, M. E.; SIMÕES-LOPES, P. C. Interação parasito-hospedeiro entre ectoparasitos (Ixodida & Siphonaptera) e gambás *Didelphis aurita* Wied-Neuwied, 1826 (Mammalia: Didelphimorphia), no continente e em ilhas do litoral de Santa Catarina, Sul do Brasil. **Biotemas**, v. 20, n. 4, p. 81-90, 2007.

SANGENIS, L. H. C.; NIELEBOCK, M. A. P.; SANTOS, C. S.; SILVA, M. C. C.; BENTO, G. M. R. Transmissão Da Doença de Chagas Por Consumo de Carne de Caça: Revisão Sistemática. **Revista Brasileira de Epidemiologia**, v. 19, n. 4, p. 803-811, 2016.

SANGENIS, L. H. C.; SARAIVA, R. M.; GEORG, I.; DE CASTRO, L.; LIMA, V. S.; ROQUE, A. L. R.; XAVIER, S. C.; SANTOS, L. C.; FERNADES, F. A.; SARQUIS, O.; LIMA, M. M.; CARVALHO-COSTA, F. A.; BÓIA, M. N. Autochthonous Transmission of Chagas Disease in Rio de Janeiro State, Brazil: A Clinical and Eco-Epidemiological Study. **BMC Infectious Diseases**, v. 15, n. 1, p. 1-12, 2015.

SANTIAGO, M. E. B.; VASCONCELOS, R. O.; FATTORI, K. R.; MUNARI, D. P.; MICHELIN, A. F.; LIMA, V. M. F. An Investigation of *Leishmania* spp. in *Didelphis* spp.

from Urban and Peri-Urban Areas in Bauru (São Paulo, Brazil). **Veterinary Parasitology**, v. 150, n. 4, p. 283-290, 2007.

SANTOS, M. A. B.; MACEDO, L. O.; OTRANTO, D.; RAMOS, C. A. N.; REGO, A. G. O.; GIANNELLI, A.; ALVES, L. C.; CARVALHO, G. A.; RAMOS, R. A. N. Screening of *Cercopithifilaria bainae* and *Hepatozoon canis* in ticks collected from dogs of Northeastern Brazil. **Acta Parasitologica**, v. 63, n. 3, p. 606-609, 2018.

SANTOS, M. A. B.; SOUZA, I. B.; MACEDO, L. O.; RAMOS, C. A. N.; REGO, A. G. O.; ALVES, L. C.; RAMOS, R. A. N.; CARVALHO, G. A. *Cercopithifilaria bainae* in *Rhipicephalus sanguineus* sensu lato ticks from dogs in Brazil. **Ticks and Tick Borne Diseases**, v. 8, p. 623-625, 2017.

SARAIVA, D. G.; FOURNIER, G. F. S. R.; MARTINS, T. F.; LEAL, K. P. G.; VIEIRA, F. N.; CÂMARA, E. M. V. C.; COSTA, C. G.; ONOFRIO, V. C.; BARROS-BATTESTI, D. M.; GUGLIELMONE, A. A.; LABRUNA, M. B. Ticks (Acari: Ixodidae) Associated with Small Terrestrial Mammals in the State of Minas Gerais, Southeastern Brazil. **Experimental and Applied Acarology**, v. 58, n. 2, p. 159-166, 2012.

SARAIVA, D.; FOURNIER, G. S. R.; OLIVEIRA, S. P.; OGRZEWALSKA, M.; CAMARA, E. M. V. C.; COSTA, C. G.; BOTELHO, J. R. Ectoparasites from Small Mammals from the Cerrado Region in the Minas Gerais State, Brazil. **UNED Research Journal**, v. 4, n. 1, p. 21-29, 2012.

SARMIENTO, L.; TANTALEÁN, M.; HUIZA, A. Nematodos parásitos delhombre y de los animales en el Perú. **Revista Peruana de Parasitología**, v. 14, p. 9-65, 1999.

SCHAD, G. A.; LEIBY, D. A.; MURRELL, K. D. Distribution, prevalence and intensity of *Trichinella spiralis* infection in furbearing mammals of Pennsylvania. **Journal of Parasitology**, v. 70, p. 372-377, 1984.

SCHMIDT, G. D. *Oncicola martini* sp. n. and other Archiacantocephala for de Chaco Boreal, Paraguai. **Journal of Parasitology**, v. 63, n. 3, p. 508-510, 1977.

SCHNITTGER, L.; RODRIGUEZ, A. E.; FLORIN-CHRISTENSEN, M.; MORRISON, D. A. *Babesia*: A world emerging. **Infection, Genetics and Evolution**, v. 12, p. 1788-1809, 2012.

SCHOLTENS, R. G.; NORMAN, L. *Trichinella spiralis* in Florida wildlife. **Journal of Parasitology**, v. 57, p. 1103, 1971.

SELF, J. T.; MCKNIGHT, T. J. Platyhelminthes from fur bearers in the Wichita mountains wildlife refuge, with especial reference to *Oochoristica* spp. **American Midland Naturalist**, v. 43, n. 58-61, 1950.

SHERWOOD, B. F.; ROWLANDS, D. T.; HACKEL, P. B.; LEMAY, J.C. The opossum, *Didelphis virginiana*, as a laboratory animal. **Laboratory Animal Care**, v. 19, p. 494-499. 1969.

SHOOP, W. L.; CORKUM, K. C. Additional trematodes of mammals in Louisiana with a compilation of all trematodes reported from wild and domestic mammals in the state. **Tulane Studies in Zoology and Botany**, v. 23, p. 109-122, 1982.

SHOOP, W. L.; CORKUM, K. C. Epidemiology of *Alaria amarcianae* mesocercariae in Louisiana. **Journal of Parasitology**, v, 67, p. 928-931, 1981a.

SHOOP, W. L.; CORKUM, K. C. Some trematodes of mammals in Louisiana. **Tulane Studies in Zoology and Botany**, v. 22, p. 109-121, 1981b.

SILVA, M. G. Q.; COSTA, H. M. A. Helminths of White-Bellied Opossum from Brazil. **Journal of Wildlife Diseases**, v. 35, n. 2, p. 371-374, 1999.

SILVA, M. G. Q.; COSTA, H. M. A. Helminths of white-bellied opossum from Brazil. **Journal of Wildlife Disease**, v. 35, n. 2, p. 371-374, 1999.

SMITH, K. A.; OESTERLE, P. T.; JARDINE, C. M.; DIBERNARDO, A.; HUYNH, C.; LINDSAY, R.; PEARL, D. L.; NEMETH, N. M. Tick Infestations of Wildlife and Companion Animals in Ontario, Canada, with Detection of Human Pathogens in *Ixodes scapularis* Ticks. **Ticks and Tick-Borne Diseases, v.** 10, n. 1, p. 72-76, 2019.

SMITH, T. G. The genus *Hepatozoon* (Apicomplexa: Adeleina). **Journal of Parasitology**, v. 82, p. 565-585, 1996.

SNYDER, D. E.; HAMIR, A. N.; HANLON, C. A.; RUPPRECHT, C. E. Lung lesions in an opossum (*Didelphis virginiana*) associated with *Capillaria didelphis*. **Journal of Wildlife Diseases**, v. 27, p. 175-177, 1991.

SOARES, H. S.; BARBIERI, A. R. M.; MARTINS, T. F.; MINERVINO, A. H. H.; DE LIMA, J. T. R.; MARCILI, A.; GENNARI, S. M.; LABRUNA, M. B. Ticks and Rickettsial Infection in the Wildlife of Two Regions of the Brazilian Amazon. **Experimental and Applied Acarology**, v. 65, n. 1, p. 125-140, 2015.

SOLOMON, G. B.; WARNER, G. S. *Trichinella spiralis* in mammals at Mountain Lake, Virginia. **Journal of Parasitology**, v. 55, p. 730-732, 1969.

SORVILLO, F. J.; GONDO, B.; EMMONS, R.; RYAN, P.; WATERMAN, S. H.; TILZER, A. A suburban focus of endemic typhus in Los Angeles County: association with seropositive domestic cats and opossums. **American Journal of Tropical Medicine and Hygiene**, v. 48, n. 2, p. 269–773, 1993.

SPONCHIADO, J.; MELO, G. L.; MARTINS, T. F.; KRAWCZAK, F. S.; LABRUNA, M. B.; CÁCERES, N. C. Association Patterns of Ticks (Acari: Ixodida: Ixodidae, Argasidae) of Small Mammals in Cerrado Fragments, Western Brazil. **Experimental and Applied Acarology**, v. 65, n. 3, p. 389-401, 2015.

SPRENT, J. F. A. Ascaridoid nematodes of South American mammals with a definition of a new genus. **Journal of Helminthology**, v. 56, n. 3, p. 275-295, 1982.

STABENOW, C. S.; EDERLI, N. B.; LOPES, C. W. G.; OLIVEIRA, F. C. R. *Didelphis aurita* (Marsupialia: Didelphidae): A New Host for *Sarcocystis lindsayi* (Apicomplexa: Sarcocystidae) . **Journal of Parasitology**, v. 98, n. 6, p. 1262-1265, 2012.

STEERE, A. C.; STRLE, F.; HU, L. T.; BRANDA, J. A.; HOVIUS, J. W.; LI, X.; MEAD, P. S. Lyme borreliosis. **Nature Reviews Disease Primers**, v. 15, n. 2, p. 16090, 2016.

STENSETH, N. C.; ATSHABAR, B. B.; BEGON, M.; BELMAIN, S. R.; BERTHERAT, E.; CARNIEL, E. Plague: past, present, and future. **PLoS Medicine**, v. 5, n. 1, p. 9-13, 2008.

STEWART, T. B.; DEAN, D. *Didelphonema longispiculata* (Hill, 1939) Wolfgang, 1953 (Nematoda: Spiruroidea) and other helminths from the opossum (*Didelphis marsupialis virginiana*) in Georgia. **Journal of Parasitology**, v. 57, p. 687-688, 1971.

STONER, D. Further remarks on the opossum in New York. **Journal of Mammalogy**, v. 26, p. 192, 1945.

SUMRANDEE, C.; BAIMAI, V.; TRINACHARTVANIT, W.; AHANTARIG, A. *Hepatozoon* and *Theileria* detected in ticks collected from mammals and snakes in Thailand. **Ticks and tick-borne diseases**, v. 6, n. 3, p. 309-315, 2015.

SZABÓ, M. P. J.; NIERI-BASTOS, F. A.; SPOLIDORIO, M. G.; MARTINS, T. F.; BARBIERI, A. M.; LABRUNA, M. B. In Vitro Isolation from *Amblyomma ovale* (Acari: Ixodidae) and Ecological Aspects of the Atlantic Rainforest *Rickettsia*, the Causative Agent of a Novel Spotted Fever Rickettsiosis in Brazil. **Parasitology**, v. 140, n. 6, p. 719-728, 2013.

TANTALEÁN, M.; CHÁVEZ, J. Wild animal endoparasites (Nemathelminths and Plathelminths) from the Manu Biosphere Reserve, Peru. **Revista Peruana de Biología**, v. 11, p. 219-222, 2004.

TANTALEÁN, M.; DÍAZ, M.; SÁNCHEZ, N.; PORTOCARRERO, H. Endoparasitos de micromamíferos del noroeste de Peru: helmintos de marsupiales. **Revista Peruana de Biología**, v. 17, p. 207-213, 2010.

TANTALEÁN, M.; SÁNCHEZ, L.; GÓMEZ, L.; HUIZA, A. Acantocéfalos del Peru. **Revista Peruana de Biologia**, v. 12, p. 83-92, 2005.

TANTALEÁN, M.; SARMIENTO, L.; HUIZA, A. Digéneos (Trematoda) del Perú. **Boletín de Lima**, v. 80, p. 47-84, 1992.

TAYLOR, M. A.; COOP, R. L.; WALL, R. L. **Veterinary Parasitology 4th Edition**. John Wiley and Sons, 2016.

TEIXEIRA, M.; RAUTA, P. D.; ALBUQUERQUE, G. R.; LOPES, C. W. G. *Eimeria auritanensis* n. sp. and *E. gambai* Carini, 1938 (Apicomplexa: Eimeriidae) from the opossum *Didelphis aurita* Wied-newied, 1826 (Marsupialia: Didelphidae) from southeastern Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 16, p. 83-86, 2007.

TELFORD, S. R.; GOETHERT, H. K.; MOLLOY, P. J.; BERARDI, V. P.; CHOWDRI, H. R.; GUGLIOTTA, J. L.; LEPORE, T. J. *Borrelia miyamotoi* Disease: Neither Lyme Disease Nor Relapsing Fever. **Clinics in Laboratory Medicine**, v. 35, p. 867-882, 2015.

TEODORO, A. K. M.; CUTOLO, A. A.; MOTOIE, G.; MEIRA-STREJEVITCH, C. S.; PEREIRA-CHIOCCOLA, V. L.; MENDES, T. M. F.; ALLEGRETTI, S. M. Gastrointestinal, Skin and Blood Parasites in *Didelphis* spp. from Urban and Sylvatic Areas in São Paulo State, Brazil. **Veterinary Parasitology: Regional Studies and Reports**, v. 16, 100286, 2019.

TORRES-CASTRO, M.; NOH-PECH, H.; PUERTO-HERNÁNDEZ, R.; REYES-HERNÁNDEZ, B.; PANTI-MAY, A.; HERNÁNDEZ-BETANCOURT, S.; YEH-GOROCICA, A.; GONZÁLEZ-HERRERA, L.; ZAVALA-CASTRO, J.; PUERTO, F. I. First Molecular Evidence of *Toxoplasma gondii* in Opossums (*Didelphis virginiana*) from Yucatan, Mexico. **Open Veterinary Journal**, v. 6, n. 1, p. 57-61, 2016.

TRAVASSOS, L. Contribuição para o conhecimento da fauna helmintológica brasileira. XVI. *Cruzia tentaculata* (Rud. 1819). **Memórias do Instituto Oswaldo Cruz**, v. 14, p. 88-94, 1922.

TRAVASSOS, L. Contribuições para o conhecimento da fauna helmintológica brasileira. X. Sobre as espécies do gênero *Turgida*. **Memórias do Instituto Oswaldo Cruz**, v. 12, p. 73-77, 1920.

TRAVASSOS, L. Relatório da excursão realizada no vale do rio Itaúnas, norte do estado do Espírito Santo, nos meses de setembro e outubro de 1944. **Memórias do Instituto Oswaldo Cruz**, v. 42, n. 3, p. 487-502, 1945.

TRAVASSOS, L. Sobre as espécies brasileiras da subfamília *Heterakinae* Railliet & Henry, 1912. **Memorias do Instituto Oswaldo Cruz**, v. 5, p. 271-318, 1913.

TROYO, A.; MOREIRA-SOTO, R. D.; CALDERON-ARGUEDAS, O.; MATA-SOMARRIBAS, C.; ORTIZ-TELLO, J.; BARBIERI, A. R. M.; AVENDAÑO, A.; VARGAS-CASTRO, L. E.; LABRUNA, M. B.; HUN, L.; TAYLOR, L. Detection of Rickettsiae in Fleas and Ticks from Areas of Costa Rica with History of Spotted Fever Group Rickettsioses. **Ticks and Tick-Borne Diseases**, v. 7, n. 6, p. 1128-1134, 2016.

UILENBERG, G. *Babesia* – A historical overview. **Veterinary Parasitology**, v. 138, p. 3-10, 2006.

URDAPILLETA, M.; LINARDI, P. M.; LARESCHI, M. Fleas Associated with Sigmodontine Rodents and Marsupials from the Paranaense Forest in Northeastern Argentina. **Acta Tropica**, v. 193, 71-77, 2019.

VENZAL, J. M.; GONZÁLEZ- ACUÑA, D.; MUÑOZ-LEAL, S.; MANGOLD, A. J.; NAVA, S. Two new species of *Ornithodoros* (Ixodida, Argasidae) from the Southern Cone of South America. **Experimental Applied Acarology**, v. 66, n. 1, p. 127-139, 2015.

VICENTE, J. J.; RODRIGUES, H. O.; GOMES, D. C.; PINTO, R. M. Nematóides do Brasil. Parte V: Nematóides de mamíferos. **Revista Brasileira de Zoologia**, v. 14, p. 1-452, 1997.

VOGE, M. New host records for *Mesocestoides* (Cestoda: Cyclophyllidea) in California. **American Midland Naturalist**, v. 49, p. 249-251, 1953.

VOSS, R. S.; JANSA, S. A. Sanke-venom resistance as a mammalian trophic adaptation: lessons from didelphid marsupials. **Biological Reviews**, v. 87, p. 822-837, 2012.

WHITING, M. F.; WHITING, A. S.; HASTRITER, M. W.; DITTMAR, K. A molecular phylogeny of fleas (Insecta: Siphonaptera): origins and host associations. **Cladistics**, v. 24, p. 677-707, 2008.

WILLIAMS, S. G.; SACCI JR, J. B.; SCHRIEFER, M. E.; ANDERSEN, E. M.; FUJIOKA, K. K.; SORVILLO, F. J. Typhus and typhuslike rickettsiae associated with opossums and their fleas in Los Angeles County, California. **Journal of Clinical Microbiology**, v. 30, n. 7, p.1758-1762, 1992.

WITTER, R.; MARTINS, T. F.; CAMPOS, A. K.; MELO, A. L. T.; CORRÊA, S. H. R.; MORGADO, T. O.; WOLF, R. W.; MAY-JÚNIOR, J. A.; SINKOC, A. L.; STRÜSSMANN, C.; AGUIAR, D. M.; ROSSI, R. V.; SEMEDO, T. B. F.; CAMPOS, Z.; DESBIEZ, A. L. J.; LABRUNA, M. B.; PACHECO, R. C. Rickettsial Infection in Ticks (Acari: Ixodidae) of Wild Animals in Midwestern Brazil. **Ticks and Tick-Borne Diseases**, v. 7, n. 3, p. 415–423, 2016.

WOLF, R. W.; ARAGONA, M.; MUÑOZ-LEAL, S.; PINTO, L. T.; MELO, A. L. T.; BRAGA, I. A.; COSTA, J. S.; MARTINS, T. F.; MARCILI, A.; PACHECO, R. C.; LABRUNA, M. B.; AGUIAR, D. M. Novel *Babesia* and *Hepatozoon* agents infecting non-volant small mammals in the Brazilian Pantanal, with the first record of the tick *Ornithodoros guaporensis* in Brazil. **Ticks and Tick-borne Diseases**, v. 7, p. 449-456, 2016.

WONG, B. S.; MILLER, D. M.; DUNAGAN, T. T. Electrophysiology of acanthocephalan body wall muscles. **Journal of Experimental Biology**, v. 82, p. 273-280, 1979.

YAEGER, R. G. Protozoa : Structure, Classification, Growth, and Development. *In.* Baron, S. (ed.). **Medical Microbiology.** 4. ed. Galveston (TX): University of Texas Medical Branch at Galveston, 1996. Available from: https://www.ncbi.nlm.nih.gov/books/NBK8385/

YAMAGUTI, S. **Systema helminthum. Vol. III. The nematodes of vertebrates, pt. I & II**. New York, NY: Interscience Publishers Inc.,1961.

ZANETTE, R. A.; SILVA, A. S.; LUNARDI, F.; SANTURIO, J. M.; MONTEIRO, S. G. Occurrence of gastrointestinal protozoa in *Didelphis albiventris* (opossum) in the central region of Rio Grande do Sul state. **Parasitology International**, v. 57, p. 217-218, 2008.

ZIMMERMAN, W. J.; HUBBARD, E. D.; BIESTER, H. E. Studies on trichiniasis in Iowa wildlife (1955-56 and 1956-57 seasons). **Journal of Parasitology**, v. 45, p. 87-90, 1959.

ZIMMERMAN, W. J.; SCHWARTE, L. H.; BIESTER, H. E. Incidence of trichiniasis in swine, pork products, and wildlife in Iowa. **American Journal of Public Health**, v. 46, p. 313-319, 1956.

# OBJECTIVES

## General objective

Evaluate the parasites of *Didelphis aurita* and detect zoonotic pathogens these opossums may transmit to humans and domestic animals.

## Specific objectives

- Identify the diversity of ectoparasites in *Didelphis aurita*.
- Detect via molecular methods the presence of arthropod borne pathogens with zoonotic potential.
- Detect via molecular biology, animals positive to pathogens with zoonotic potential.
- Identify the diversity of gastrointestinal helminths in *D. aurita*.
- Identify the diversity of gastrointestinal protozoa in *D. aurita.*

# CHAPTER 1

**Article: Ectoparasite fauna of *Didelphis aurita* and molecular detection of *Wolbachia pipientis* endosymbiont in fleas**

Submitted to the Medical and Veterinary Entomology Journal

**Abstract**

Ticks and fleas are important vectors of pathogens that affect humans and animals. Among their hosts, *Didelphis aurita* play important role in public health due to their ability to circulate between urban centers and forests. This study aimed to assess the ectoparasite fauna of *D. aurita* (Didelphimorphia: Didelphidae), as well as the presence of pathogens transmitted by ticks and fleas. Fifty-eight Opossums were captured, and ectoparasites, blood and spleen samples were collected for molecular processing. Samples were screened for Anaplasmataceae, *Rickettsia* spp. (Rickettsiales: Rickettsiaceae), *Borrelia* spp. (Spirochaetales: Spirochaetaceae), and *Babesia* spp. (Piroplasmorida: Babesiidae). *Ixodes loricatus* (Acari: Ixodidae) was identified in 41.38% and *Amblyomma sculptum* (Acari: Ixodidae) in 1.72% of the animals. *Ctenocephalides felis felis* (Siphonaptera: Pulicidae) and *Xenopsylla cheopis* (Siphonaptera: Pulicidae) were detected in 60.34% (35/58) and 5.17% (3/58) of the animals, respectively. PCR analysis detected Anaplasmataceae in 34.04% pool samples of *C. felis felis*, and in 66.66% of *X. cheopis*. Sequence analysis revealed *Wolbachia pipientis* (Rickettsiales: Rickettsiaceae) in all positive samples. Tick, blood and spleen samples scored negative for the pathogens assessed. This study contributes to the knowledge of the ectoparasite fauna in *D. aurita* in urban areas of Brazil, discussing the importance of these findings to public health.

**Keywords:** Opossums; ticks; siphonaptera; Ixodidae; vectors; Anaplasmataceae

## 1. Introduction

Marsupials of the genus *Didelphis* are mammals geographically restricted to the American continent. In South America these animals are represented by five species divided in two groups, the *D. marsupialis*-group (*D. marsupialis* and *D. aurita*) and the *D. albiventris*-group (*D. albiventris*, *D. pernigra*, and *D. imperfecta*). The only *Didelphis* species that up to date is not found in this region is *D. virginiana*, which occurs from Canada to Costa Rica (Gardner, 2008). In Brazil, four species of this genus are reported, *D. imperfecta* (Guianan White-eared Opossum) and *D. marsupialis* (Common Opossum) in the Amazon rainforest, *D. albiventris* (White-eared Opossum) mostly in the Cerrado biome, and *D. aurita* (Black-eared Opossum) in the Atlantic forest (Faria & Melo, 2017; Gardner, 2008).

Species of the genus *Didelphis* are well adapted to the anthropogenic activity, being found on the roof of houses, hollows of trees and other shelters within urban centers (Jansen, 2002). Due to this synanthropic behavior, the possibility of transmission of pathogens (i.e. *Rickettssia* spp. and *Borrelia* spp.) vectored by ectoparasites to humans and domestic animals may occur (Muller et al., 2005).

It is known that ticks and fleas are commonly reported infesting *Didelphis* spp. (Muller et al., 2005). For instance, studies of the ectoparasite fauna of *D. aurita* have reported the occurrence of ticks of the genus *Amblyomma* and *Ixodes* (Luz et al., 2018; Gonzalez et al., 2017; Acosta et al., 2016; Oliveira et al., 2014; Saraiva et al., 2012; Dantas-Torres et al., 2012), and fleas of the genus *Adoratopsylla, Polygenis, Leptopsylla, Xenopsylla* and *Ctenocephalides* (Urdapilleta et al., 2019; Pinto et al., 2009; Salvador et al., 2007; Horta et al., 2007). Interestingly, specimens of the genus *Amblyomma* and *Ctenocephalides* are commonly found in domestic animals (i.e. dogs, cats, horses) (Dantas-Torres & Otranto, 2014), which may imply that these species have major importance in the epidemiological life cycle of pathogens of medical and veterinary concern (Muller et al., 2005). The aim of this study was to assess the ectoparasite fauna of *D. aurita*, as well as the presence of pathogens transmitted by ticks and fleas.

## 2. Material and Methods

### *2.1 Study area and ethical aspects*

The study was conducted in the municipality of Viçosa (20°45´14” South and 42°52´54” West), located in the State of Minas Gerais, Southeastern Brazil. The climate in this region is classified as Cwa (Köppen climate classification), mesothermic, with hot and rainy summers and cold and dry winters. The area is 650 meters above sea level and presents an annual average temperature varying from 20 to 22 °C.

All procedures herein performed were approved by the Ethics Committee for Animal Experimentation (ECAE) of the Universidade Federal de Viçosa (license number: 80/2018) and by the Biodiversity Information and Authorization System (SISBIO) of the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) under the license number 64930-1.

*2.2 Animals and sampling*

Animals were captured through Tomahawk live traps (0.45x0.21x0.21m), which were armed and checked daily (5PM and 7AM, respectively), from January to June 2019, totaling a sampling effort of 516 trap-nights in urban environments of the study area (Figure 1). A mix of corn flour, canned fish and banana were used as bait. After capture, the animals were classified by sex, age group (pups, subadults and adults), and marked with a small V cut at the right ear to identify recaptures. Additionally, animals found dead were necropsied and fragments of spleen collected (n = 9).

Initially, animals were individually inspected for a period of approximately 10 minutes for the presence of ectoparasites. Specimens collected were stored in plastic vials (ticks in empty tubes and fleas in tubes containing 70% alcohol) for morphological identification and molecular procedures. In addition, blood samples were collected via venipuncture and stored at -20 $^{o}$C until molecular processing.

*2.3 Laboratorial procedures*

Ectoparasites were quantified, separated according to the stage and gender, and morphologically identified using dichotomy keys (Barros-Battesti et al., 2006; Linardi & Guimarães, 2000). Afterwards, 58 pools of ticks (up to 3 specimens) and 50 pools of fleas (up to 4 specimens) were formed for DNA extraction.

For molecular procedures, genomic DNA of blood and spleen were extracted using a commercial kit for blood and spleen (Illustra tissue and cells genomicPrep Mini Spin Kit, GE Healthcare Life Sciences) following the manufacturer’s instructions. On the other hand, DNA extraction of ectoparasites were performed following a protocol previously described (Ramos et al., 2015).

Each DNA sample was screened for the *gltA* gene of *Rickettsia* spp., the flagellin gene of *Borrelia* spp., the 16S rRNA gene for Anaplasmataceae, and for the 18S gene for *Babesia* spp. (Table 1). Amplifications were performed using Taq Pol Master Mix Green 2X following the manufacturer’s recommendations, 400 nmol of each primer, 5 µL of DNA sample and nuclease free water until complete 25 µL volume. DNA of *Rickettsia vini* (Preventive Veterinary Medicine and Animal Health Department of the University of São Paulo), *Borrelia garini* (Epidemiology and Public Health Department of the Federal Rural University of Rio de Janeiro), *Ehrlichia canis* and *Babesia canis* (Veterinary Medicine

Department of the Federal University of Viçosa) were used as positive control, and nuclease free water as negative control.

All amplicons obtained were purified using PCR Purification Kit (Cellco Biotec) according to manufacturer's recommendations. Sanger's method was performed for sequencing amplicons in both directions in an automated sequencer AB 3500 Genetic Analyzer. DNA sequences were aligned using Mega7 and compared with sequences from GenBank using the BLAST search tool.

**Table 1.** Primers used for molecular analysis.

| Pathogen | Gene | Amplicon size (bp) | Primer sequence |
|---|---|---|---|
| *Rickettsia* spp. | gltA | 300-380 | 5'-GGGGGCCTGCTCACGGCGG-3' |
| | | | 5'-ATTGCAAAAAGTACAGTGAACA-3' |
| *Borrelia* spp. | Fla | 482 | 5'-AGAGCAACTTACAGACGAAATTAAT-3' |
| | | | 5'-CAAGTCTATTTTGGAAAGCACCTAA-3' |
| Anaplasmataceae | 16S | 345 | 5'-GGTACCYACAGAAGAAGTCC-3' |
| | | | 5'-TAGCACTCATCGTTTACAGC-3' |
| *Babesia* spp. | 18S | 411-452 | 5'-GTCTTGTAATTGGAATGATGG-3' |
| | | | 5'-TAGTTTATGGTTAGGACTACG-3' |

*2.4 Data analysis*

Descriptive statistical analysis was performed to calculate the relative and absolute frequency, as well as mean intensity and abundance of infestation by ticks and fleas. The normality of data was checked using the Lilliefors test. Additionally, the Chi-square test with Yates correction ($\chi^2$) was used to compare the occurrence of these ectoparasites according to the gender and age. The significance level was set up at 5% and all analyzes were carried out using the BioEstat 5.3 software.

The sites of capture of opossums were geoprocessed with the geographic information system program QGIS 3.4.12 (qgis.org). Digital maps of the Brazil, the State of Minas Gerais and the municipality of Viçosa were used as cartographic basis, and the SIRGAS2000 and the UTM coordinates system as ellipsoid of reference. The layers and points were automatically converted by the system (extension on the fly). The complement QuickMapServices was used to obtain the satellite images (Google Satellite).

## 3. Results

Fifty-eight animals were captured during the whole study in the study area, being 50% (29/58) males and 50% (29/58) females. In particular, 3.45% (2/58) were classified as pup, 46.55% (27/58) as subadults and 50% (29/58) as adults. A total of 69 ticks and 121 fleas were collected on the animals, which correspond to frequencies of infestation of 41.38% (24/58) and 60.34% (35/58), respectively (Table 2). No significant difference was observed for tick and flea infestation according to the gender and age of the opossums ($p > 0.05$).

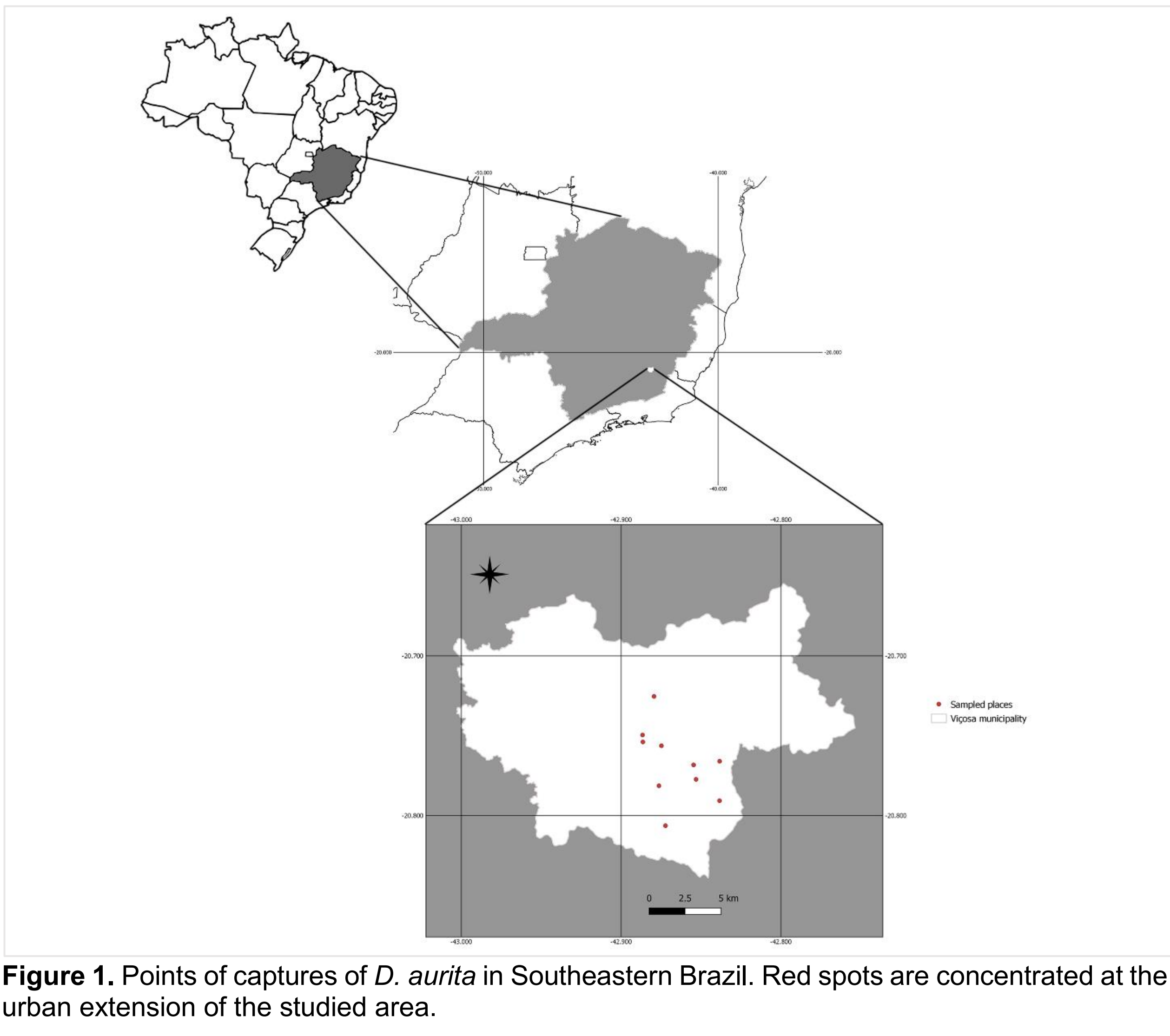


**Figure 1.** Points of captures of *D. aurita* in Southeastern Brazil. Red spots are concentrated at the urban extension of the studied area.

**Table 2.** Ectoparasites found in *Didelphis aurita* from Southeastern Brazil

| Ectoparasite | M | F | Ny | L | Infested animals | [1] Mean abundance | [2] Mean intensity | RF % | AF/N |
|---|---|---|---|---|---|---|---|---|---|
| **Ticks (n = 69)** | | | | | | | | | |
| *Ixodes loricatus* | 16 | 42 | 1 | 9 | 24 | 1.17 | 2.83 | 41.38 | 24/58 |
| *Amblyomma sculptum* | 0 | 0 | 1 | 0 | 1 | 0.02 | 0.04 | 1.72 | 1/58 |
| **Fleas (n = 121)** | | | | | | | | | |
| *Ctenocephalides felis felis* | 22 | 95 | 0 | 0 | 34 | 2.02 | 3.34 | 58.62 | 34/58 |
| *Xenopsylla cheopis* | 1 | 3 | 0 | 0 | 3 | 0.07 | 0.11 | 5.17 | 3/58 |

[1]Number of ectoparasites per total of examined animals. [2]Number of ectoparasites per total of infested animals. M – male; F – female; Ny – nymph; L – Larva; RF – relative frequency; AF – absolute frequency; N – number of captured animals.

The PCR analysis detected Anaplasmataceae DNA in 34.04% (n = 16/47) pool samples of *C. felis felis*, and in 66.66% (n = 2/3) pool samples of *X. cheopis*. The sequence analysis of these products and comparisons in the GenBank database revealed *Wolbachia pipientis* with query Cover of 100% and identity of 98% as compared with BLAST results (Accession numbers: KP114101.1, KP165047.1, MF944223.1, EF121345.1, and AY026912.1). Tick, blood and spleen samples scored negative for the other pathogens assessed.

## 4. Discussion

This study assessed the occurrence of ectoparasites as well as the detection of microorganisms infecting ticks and fleas of *D. aurita* from Southeastern Brazil. *I. loricatus* was the most frequent tick species found, infesting 41.38% of the animals. In fact, most studies on ectoparasites of *Didelphis* spp. found the adult stage of *I. loricatus* to be the most predominant tick species in these marsupials (Luz et al., 2018; Gonzalez et al., 2017; Oliveira et al., 2014; Saraiva et al., 2012; Dantas-Torres et al., 2012, Muller et al., 2005). In contrast with these studies, when compared with *I. loricatus*, *A. auricularium* was reported with a higher frequency on *D. albiventris* in a study performed in Northeastern Brazil (Lopes et al., 2018). Additionally, Acosta et al. (2016) have reported only immature stages of *Amblyomma* spp. in *D. aurita* from the State of Espirito Santo, Brazil; however, few opossums of this species were inspected in that study.

*Amblyomma sculptum* was detected with a very low frequency (1.72%) in the animals analyzed in the present study. This tick species presents great public health importance, as it is the main vector of *Rickettsia rickettsii*, the etiological agent of the Brazilian Spotted Fever (Parola et al., 2013). The presence of *Amblyomma* spp. in *D. aurita* captured in urban areas has a great epidemiological importance, since this opossum act as an amplifier host for *R. rickettsii* infection to *A. sculptum* ticks (Horta et al., 2009). In our study, a single animal was found parasitized by an *A. sculptum* nymph. Indeed, most studies reported that larvae and nymphs of *Amblyomma* are the most common life stages retrieved in opossums (Lopes et al., 2018; Acosta et al., 2016).

Regarding the flea species found in our study, *C. felis felis* was predominantly identified in *D. aurita*, with a frequency of 60.34%. This species, known as the cat flea, has been reported with high prevalence rates in *Didelphis* spp. (Horta et al., 2007; Boostrom et al., 2002; Barros-Battesti & Arzua, 1997). However, Salvador et al. (2007) studying ectoparasites of *D. aurita* in the state of Santa Catarina, Brazil, found only *Adoratopsylla intermedia*, *Leptopsylla segnis* and *Xenopsylla cheopis*. The authors of that study suggested that the absence of *C. felis felis* in the studied population was due to the lack of human habitations in the studied area, which consequently reduce the contact of the wildlife with domestic animals. In fact, it is known that *C. felis felis* is the most prevalent flea species in dogs and cats worldwide (Durden & Hinkle, 2019). In our study, the animals were captured in urban areas, and the high frequency of *C. felis felis* may suggest the exchange of this ectoparasite species between companion animals and *D. aurita*.

The detection of *X. cheopis* in the present study is a relevant finding. Salvador et al. (2007) found a higher rate of *D. aurita* infested by this flea species in the State of Santa Catarina, Brazil, with a frequency of 15.1%. Rodents are the main hosts of *X. cheopis*, and this species is involved in the transmission of important zoonotic pathogens such as the bacteria *Yersinia pestis* and *Rickettsia typhi*, the causative agents of Plague and Murine Typhus, respectively (Durden & Hinkle, 2019; Civen & Ngo, 2008). In addition, the presence of this flea in *Didelphis* spp. may have important implications in public health, as it is known that these opossums are probably involved in the zoonotic cycles of pathogens such as *R. typhi* and *R. felis* (Boostrom et al., 2002).

Interestingly, DNA of *W. pipientis* was detected in *C. felis felis* and *X. cheopis*. It is known that this bacterium is an endosymbiont that infects a great number of insect species worldwide, which includes various flea species, i.e. *C. felis*, *C. canis*, *Tunga penetrans*, *Polygenis gwyni*, *Orchopeas howardi*, *Pulex irritans*, *P. simulans*, *Echidnophaga gallinacea*, and *X. cheopis* (Onder et al., 2019; Heukelbach et al., 2004; Dittmar & Whiting, 2004; Gorham et al., 2003). In fact, Dittmar & Whiting (2004) claim that there is a potentially widespread association between these bacterial symbionts with fleas in general. In addition, *W. pipientis* has influence on reproduction, sex determination, speciation and behavior of arthropods, being a potential candidate to biological control of insect vectors (LePage & Bordenstein, 2013).

This study reports the occurrence of ticks and fleas in *D. aurita* opossums, as well as the infection by *W. pipientis* in *C. felis felis* and *X. cheopis* collected in these animals in Southeastern Brazil. Data herein obtained demonstrates the parasitism in *D. aurita* by some species of ectoparasites commonly found in domestic animals (*C. felis felis* and *A. sculptum*). These findings suggest that these arthropods may have important role in the cycle of zoonotic pathogens among opossums, humans and companion animals.

**Acknowledgements**

Authors would like to thank the Professors Dr. Marcelo Bahia Labruna and Dr. Adivaldo Henrique da Fonseca who kindly donated the positive controls for *Rickettsia* and *Borrelia* spp., respectively. Secondly, authors acknowledge Professor Gisele Mendes Lessa del Giúdice for providing traps to capture the animals used in our study. Finally, the authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), and the Graduate Program in Veterinary Medicine of the Universidade Federal de Viçosa (UFV) for the support provided.

Authors declare they have no conflict of interest. Additionally, there are no disputes over the ownership of the data presented in the paper and all contributions have been

attributed appropriately, via coauthorship or acknowledgement, as appropriate to the situation.

**References**

Acosta, I.C.L., Martins, T.F., Marcili, A. *et al.* (2016) Ticks (Acari: Ixodidae, Argasidae) from humans, domestic and wild animals in the state of Espírito Santo, Brazil, with notes on rickettsial infection. *Veterinary Parasitology: Regional Studies and Reports*, **3**, 66-69.

Barros-Battesti, D. & Arzua, M. (1997) Geographical Distribution by Biomes of some Marsupial Siphonaptera from the State of Paraná, Brazil. *Memorias do Instituto Oswaldo Cruz*, **92**, 485-486.

Barros-battesti, D.M., Arzua, M. & Bechara, G.H. (2006) Carrapatos de Importância Médico-Veterinária da Região Tropical: Um guia ilustrado para identificação de espécies. Vox/ICTTD-3/Butantan, São Paulo.

Boostrom, A., Beier, M.S., Macaluso, J.A. *et al.* (2002) Geographic Association of *Rickettsia felis*-Infected Opossums with Human Murine Typhus, Texas. *Emerging and Infectious Disease*, **8**, 549-554.

Civen, R. & Ngo, V. (2008) Murine Typhus: An Unrecognized Suburban Vectorborne Disease. *Clinical Infectious Diseases*, **46**, 913-918.

Dantas-Torres, F., Aléssio, F.M., Siqueira, D.B. *et al.* (2012) Exposure of small mammals to ticks and rickettsiae in Atlantic Forest patches in the metropolitan area of Recife, North-eastern Brazil. *Parasitology*, **139**, 83-91.

Dantas-Torres, F. & Otranto, D. (2014) Dogs, cats, parasites, and humans in Brazil: opening the black box. *Parasites & Vectors*, **7**, 22.

Dittmar, K. & Whiting, M.F. (2004) New *Wolbachia* Endosymbionts from Nearctic and Neotropical Fleas (Siphonaptera). *Journal of Parasitology*, **90**, 953-957.

Durden, L.A. & Hinkle, N.C. (2019) Fleas (Siphonaptera). *Medical and Veterinary Entomology* (ed. By G.R. Mullen and L.A. Durden). Academic Press, https://doi.org/10.1016/B978-0-12-814043-7.00010-8.

Faria, B.F. & Melo, F.R. (2017) *Didelphis imperfecta*, Didelphimorphia, Didelphidae (Mondolfi & Pérez-Hernández, 1984): a new record in the Brazilian Amazon. *Boletim da Sociedade Brasileira de Mastozoologia*, **79**, 44-46.

Gardner, A.L. (2008) *Mammals of South America, Volume I. Marsupials, xenarthrans, shrews, and bats*. The University of Chicago Press, Chicago and London.

Gonzalez, I.H.L., Labruna, M.B., Chagas, C.R.F. *et al.* (2017) Ticks infesting captive and free-roaming wild animal species at the São Paulo Zoo, São Paulo, Brazil. *Brazilian Journal of Veterinary Parasitology*, **26**, 496-499.

Gorham, C.H., Fang, Q.Q. & Durnen, L.A. (2003) *Wolbachia* endosymbionts in fleas (Siphonaptera). *Journal of Parasitology*, **89**, 283-289.

Heukelbach, J., Bonow, I., Witt, L., Feidmeier, H. & Fischer, P. (2004) High infection rate of *Wolbachia* endobacteria in the sand flea *Tunga penetrans* from Brazil. *Acta Tropica*, **92**, 225-230.

Horta, M.C., Labruna, M.B., Pinter, A., Linardi, P.M. & Schumaker, T.T.S. (2007) *Rickettsia* infection in five areas of the state of São Paulo, Brazil. *Memorias do Instituto Oswaldo Cruz*, **102**, 793-801.

Horta, M.C., Moraes-Filho, J., Casagrande, R.A. *et al.* (2009) Experimental Infection of Opossums *Didelphis aurita* by *Rickettsia rickettsii* and Evaluation of the Transmission of the Infection to Ticks *Amblyomma cajennense*. *Vector-Borne and Zoonotic Diseases*, **9**, 109-117.

Jansen, A.M., 2002. Marsupiais Didelfídeos: gambás e cuícas. *Animais de Laboratório: criação e experimentação [online]* (ed. by A. Andrade, S.C. Pinto and R.S. Oliveira), pp. 167-173, FIOCRUZ, Rio de Janeiro.

LePage, D. & Bordenstein, S. (2013) *Wolbachia*: can we save lives with a great pandemic? *Trends in Parasitology*, **29**, 385-393.

Linardi, P.M. & Guimarães, L.R. (2000) *Sifonápteros do Brasil*. Museu de Zoologia, USP/Fapesp, São Paulo.

Lopes, M.G., Muñoz-Leal, S., De Lima, J.T.R. *et al.* (2018) Ticks, rickettsial and erlichial infection in small mammals from Atlantic forest remnants in northeastern Brazil. *International Journal for Parasitology: Parasites and Wildlife*, **7**, 380-385.

Luz, H.R., Neto, S.F.C., Weksler, M., Gentile, R. & Faccini, J.L.H. (2018) Ticks parasitizing wild mammals in Atlantic Forest areas in the state of Rio de Janeiro, Brazil. *Brazilian Journal of Veterinary Parasitology*, **27**, 409-414.

Muller, G., Brum, J.G.W., Langone, P.Q. *et al.* (2005) *Didelphis albiventris* Lund, 1841, parasitado por *Ixodes loricatus* Neuman, 1899, e *Amblyomma aureolatum* (Pallas, 1772) (Acari: Ixodidae) no Rio Grande do Sul. *Arquivos do Instituto Biológico*, **72**, 319-324.

Oliveira, H.H., Gomes, V., Amorim, M. *et al.* (2014) Diversidade de ixodida em roedores e marsupiais capturados no Parque Estadual da Pedra Branca, Rio de Janeiro, Brasil. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia*, **66**, 1097-1104.

Onder, z., Ciloglu, A., Duzlu, O. *et al.* (2019) Molecular detection and identification of *Wolbachia* endosymbiont in fleas (Insecta: Siphonaptera). *Folia Microbiologica*, https://doi.org/10.1007/s12223-019-00692-5

Parola, P., Paddock, C.D., Socolovschi, C. *et al.* (2013) Update on tick-borne rickettsioses around the world: a geographic approach. *Clinical Microbiology Reviews*, **26**, 657-702.

Pinto, I.S., Botelho, J.R., Costa, L.P., Leite, Y.L.R. & Linardi, P.M. (2009) Siphonaptera Associated with Wild Mammals From the Central Atlantic Forest Biodiversity Corridor in Southeastern Brazil. *Journal of Medical Entomology*, **46**, 1146-1151.

Ramos, R.A.N., Campbell, B.E., Whittle, A. *et al.* (2015) Occurrence of *Ixodiphagus hookeri* (Hymenoptera: Encyrtidae) in *Ixodes ricinus* (Acari: Ixodidae) in Southern Italy. *Ticks and Tick-Borne Diseases*, **6**, 234-236.

Salvador, C.H., Carvalho-Pinto, C., Carvalho, R., Graipel, M.E. & Simões-Lopes, P. (2007) Interação parasito-hospedeiro entre ectoparasitos (Ixodida & Siphonaptera) e gambás *Didelphis aurita* Wied-Neuwied, 1826 (Mammalia: Didelphimorphia), no continente e em ilhas do litoral de Santa Catarina, Sul do Brasil. *Biotemas*, **20**, 81-90.

Saraiva, D.G., Fournier, G.F.S.R., Martins, T.F. *et al.* (2012) Ticks (Acari: Ixodidae) associated with small terrestrial mammals in the state of Minas Gerais, southeastern Brazil. Experimental and Applied Acarology, **58**, 159-166.

Urdapilleta, M., Linardi, P.M. & Lareschi, M. (2019) Fleas associated with sigmodontine rodents and marsupials from the Paranaense Forest in Northeastern Argentina. Acta Tropica, **193**, 71-77.

# CHAPTER 2

**Article: Gastrointestinal parasites in the opossum *Didelphis aurita*: Are they a potential threat to human health?**

Submitted to the Parasitology International Journal

**Abstract**

Zoonotic pathogens account for a great proportion of diseases that affect humans, and agents originated from wildlife cause many of these illnesses. In this sense, synanthropic animals such as *Didelphis* spp. play important role in the dissemination of pathogens due to their proximity to human dwellings. These marsupials are affected by many gastrointestinal parasites, including those with zoonotic potential. The aim of this study was to assess the diversity of gastrointestinal parasites infecting *D. aurita* captured in urban areas of Southeastern, Brazil. In addition, the potential risk for the human population based on the One Health perspective has been discussed. From January to June 2019, 49 animals were captured by using Tomahawk live traps (0.45x0.21x0.21m). Fecal samples of the trapped opossums were collected from the cloaca as the animal defecated. Afterwards, samples were evaluated by parasitological procedures. Eggs and oocysts were analyzed at different magnifications (400x and 1000x), and their identification, together with adult nematodes, was established on morphological and morphometric features. Of all samples analyzed, 87.76% (n = 43/49) scored positive for at least one gastrointestinal parasite, being 83.67% (41/49) for helminths, and 65.30% (32/49) for protozoa. For *Cryptosporidium*, only 13 samples were evaluated due to insufficient amount of feces obtained of some animals. A frequency of 23.08% (3/13) was reported for this parasite. PCR analysis revealed Ancylostomatidae eggs to belong to the genus *Ancylostoma*. Our results demonstrated that multiparasitism is frequently found in these animals and a high percentage of potentially zoonotic parasites are observed, implying that *D. aurita* may be involved in zoonotic cycles in urban environments.

**Keywords:** Synanthropic animals; nematodes; trematodes; protozoa; zoonosis; parasitic infections.

## 1. Introduction

Currently, it is estimated that 60% of the emerging infectious human diseases are zoonotic, with more than 71% of those, originated from wild animals [1]. Over the time, several wildlife species have developed the synanthropic behavior, and animals (e.g., opossums, canids, birds) previously seen only in forest areas have been frequently observed in urban centers. The proximity among humans, domestic animals and synanthropic species has constantly increased, and the risk of transmission of zoonotic pathogens has been considered one of the most important issues debated on the One Health perspective [2, 3, 4].

Amongst the synanthropic animals, the species *Didelphis aurita* Wied-Newied, 1826 (Didelphimorphia: Didelphidae) are marsupials well adapted to the anthropogenic activity. This species has semi arboreal behavior and occurs primarily in the Atlantic forest regions, being commonly found near human dwellings [5]. The diet of this opossum is very diverse including fruits, insects and a great variety of small vertebrates. This opportunistic feeding behavior exposes these animals to a great number of gastrointestinal parasites, including those of zoonotic concern such as the genera *Ancylostoma*, *Toxocara*, *Trichuris*, *Ascaris*, *Capillaria*, *Giardia* and *Cryptosporidium* [6, 7, 8, 9, 10].

Studies on gastrointestinal parasites in opossums are scarce, and information regarding host-parasite interactions between Didelphidae and their parasitic fauna are limited. The multiparasitism observed in these animals may affect their health, since the interactions among parasites are possibly linked to host susceptibility, duration of infection, risk of spreading, and clinical signs [11]. Thus, the description of those organisms in a given species is extremely important in order to better understanding the relationship they have to each other, and with their hosts. Therefore, the aim of this study was to assess the diversity of gastrointestinal parasites infecting *D. aurita* captured in urban areas of Southeastern, Brazil. In addition, the potential risk for the human population based on the One Health perspective has been discussed.

## 2. Material and Methods

### *2.1 Study area and ethical aspects*

This study was performed in urban and periurban areas of the municipality of Viçosa (Latitude 20°45´14”South and Longitude 42°52´54”West), State of Minas Gerais, Brazil. The area is featured by a Cwa climate, mesothermic, with hot and rainy summers and cold and dry winters. Annual average temperature varies from 20 to 22 °C and the region is 650 meters above sea level.

The Ethics Committee for Animal Experimentation (ECAE) of the Universidade Federal de Viçosa (license number: 80/2018) and the Biodiversity Information and Authorization System (SISBIO) (license number: 64930-1) of the Brazilian Institute of

Environment and Renewable Natural Resources (IBAMA) approved all procedures herein performed.

*2.2 Animals and sampling*

From January to June 2019 animals were captured through Tomahawk live traps (0.45x0.21x0.21m), which were armed and checked daily (5PM and 7AM, respectively), totaling a sampling effort of 516 trap-nights (Figure 1). A mix of corn flour, canned fish and banana were used as bait. After capture, each individual was mechanically contained, classified by sex, age group (pups, subadults and adults), and marked with a small V cut at the right ear to identify recaptures [12]. Fresh fecal samples were collected from the trap or directly from the cloaca as soon as the animals defecated. After sampling procedures, each animal was released at the same site of capture.

*2.3 Laboratorial procedures*

Samples were individually evaluated by the simple flotation technique of Willis-Mollay [13]. On the other hand, the search for *Cryptosporidium* spp. oocysts was carried out by malachite green negative staining as previously described [14]. Positive samples for *Eimeria* spp. oocysts were placed in Petri dishes containing 2.5% Potassium dichromate ($K_2Cr_2O_7$) and stored at 24°C for seven days in order to allow sporulation [15]. Eggs and oocysts were analyzed at different magnifications (400x and 1000x) and measured with an Olympus CX31 microscope attached to a camera connected to the ToupView software version 3.7. The identification of eggs and oocysts was established on morphological and morphometric features previously described [15, 16, 17, 18]. Adult nematodes recovered from feces of some animals were identified according to Adnet et al. [19].

Molecular analysis was further performed for Ancylostomatidae. Individual eggs were isolated [20] and genomic DNA was extracted according to Lake et al. [21]. Afterwards, DNA samples were screened by duplex PCR for *Ancylostoma* spp. and *Necator americanus* using primers that amplify a region of internal transcribed spacer 2 and the 28S ribosomal RNA (ITS2-28S rRNA). Forward primers AD1 (5’ CGA CTT TAG AAC GTT TCG GC 3’) and NA (5’ ATG TGC ACG TTA TTC ACT 3’), and the reverse primer NC2 (5’ TTA GTT TCT TTT CCT CCG CT 3’) were used for DNA amplification, resulting in 250 bp if positive for *N. americanus*, and in 130 bp if positive for *Ancylostoma* spp. [22]. Reactions were performed using 0.2 µM of each primer, 1 U of Taq DNA polymerase (Phoneutria, Brasil), 200 µM of deoxynucleoside triphosphate (dNTPs), 1X reaction buffer, 5 µL of DNA sample, and ultrapure water to complete a 10 µL final volume. DNA of *N. americanus* and *Ancylostoma* spp. previously extracted from adult worms [20, 23] were used as positive control, and nuclease free water as negative control. Electrophoresis in 1% agarose gel with 0.5 TAE buffer and GelRed ™ (Biotium, EUA) was performed to visualize the amplified products.

*2.4 Data analysis*

Descriptive statistics was performed to calculate the relative and absolute frequency of helminths and protozoa infections. The normality of data was checked using the Lilliefors test. Additionally, Chi-square or Fisher exact test were used to analyze gastrointestinal parasite infections, as well as sex and ages of the infected animals using the R (Studio 2012) software. A 5% significance level was considered to all parameters tested ($p<0.05$).

Sites of capture of opossums were geoprocessed with the geographic information system program QGIS 3.4.12 (qgis.org). Digital maps of the Brazil, the State of Minas Gerais and the municipality of Viçosa were used as cartographic basis, and the SIRGAS2000 and the UTM coordinates system as ellipsoid of reference. The layers and points were automatically converted by the system (extension on the fly). The complement QuickMapServices was used to obtain the satellite images (Google Satellite).

**3. Results**

Forty-nine animals (40 alive and 9 found dead) were captured during the whole study period in nine collection points. From those, 28.57% (14/49) were classified as adult female, 20.40% (10/49) as adult male, 24.49% (12/49) as subadult female, 24.49% (12/49) as adult male, and 2.04% (1/49) as a pup. Of all samples analyzed, 87.76% (n = 43/49) scored positive for at least one gastrointestinal parasite (Tables 1 and 2). Eggs of helminths were detected in 83.67% (41/49) of the samples (Figure 2), whereas protozoa oocysts were observed in 65.30% (32/49) of the animals (Figure 3). The *Cryptosporidium* search was performed only in 13 samples due to the insufficient amount of feces obtained of some animals. A frequency of 23.08% (3/13) was reported for this parasite. Additionally, adult nematodes (n = 53) recovered from feces were identified as C*ruzia tentaculata* (Figure 4).

In particular, PCR analysis of Ancylostomatidae eggs amplified a product of 130 bp, revealing *Ancylostoma* spp. in all samples analyzed. None of the parameters statistically checked presented significance, except for the parasitism by *Ancylostoma* spp. between male and females (p = 0.0233), and for the frequency between *Cruzia tentaculata* and *Ancylostoma* spp. (p = 0.04211).

**Table 1.** Absolute and relative frequencies for gastrointestinal parasites detected in *Didelphis aurita*

| Parasite | AF/N | RF% |
|---|---|---|
| *Ancylostoma* spp. | 32/49 | 65.30[a] |
| Ascaridoidea | 10/49 | 20.41[ab] |
| Spiruroidea | 2/49 | 4.08 [ab] |
| Trematoda | 8/49 | 16.33 [ab] |
| *Cruzia tentaculata* | 36/49 | 73.47 [b] |
| *Strongyloides* spp. | 7/49 | 14.29 [ab] |
| *Trichuris* spp. | 14/49 | 28.57 [ab] |
| *Capillaria* spp. | 6/49 | 12.24 [ab] |
| *Eimeria* spp. | 32/49 | 65.30 [ab] |
| *Isospora* spp. | 2/49 | 4.08 [ab] |
| *Cryptosporidium* spp. | 3/13* | 23.08 [ab] * |

AF – Absolute frequency; RF – Relative frequency; N – number of animals evaluated
***Cryptosporidium* spp. diagnosis was performed in 13 animals
[ab]Different letters in the same collunm represent $p<0.05$

**Table 2.** Single and multiple infections by helminths and protozoa in *Didelphis aurita*

| Parasites | RF% | AF/n |
|---|---|---|
| *Ancylostoma* spp. | 2.33 | 1/43 |
| *Cruzia tentaculata* | 4.65 | 2/43 |
| *Eimeria* spp. | 4.65 | 2/43 |
| *Ancylostoma* spp. + Ascaridoidea + Spiruroidea + *Eimeria* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Strongyloides* spp. + *Trichuris* spp. + *Ancylostoma* spp. + Trematoda + *Eimeria* spp. | 4.65 | 2/43 |
| *C. tentaculata* + *Strongyloides* spp. + *Capillaria* spp. + *Ancylostoma* spp. + Trematoda | 2.33 | 1/43 |
| *C. tentaculata* + *Ancylostoma* spp. + *Eimeria* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Trichuris* spp. + *Ancylostoma* spp. + Trematoda | 2.33 | 1/43 |
| *C. tentaculata* + *Ancylostoma* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Trichuris* spp. + *Ancylostoma* spp. + *Eimeria* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Trichuris* spp. + *Capillaria* spp. + *Ancylostoma* spp. + Ascaridoidea | 2.33 | 1/43 |
| *C. tentaculata* + *Ancylostoma* spp. + Ascaridoidea + *Eimeria* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Trichuris* spp. + *Capillaria* spp. + *Ancylostoma* spp. + Ascaridoidea + *Eimeria* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Strongyloides* spp. + *Ancylostoma* spp. + *Eimeria* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Ancylostoma* spp. + *Eimeria* spp. | 2.33 | 1/43 |
| *C. tentaculata* + Ascaridoidea | 2.33 | 1/43 |
| *C. tentaculata* + *Capillaria* spp. + *Ancylostoma* spp. + Ascaridoidea + *Eimeria* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Trichuris* spp. + *Ancylostoma* spp. + Ascaridoidea + *Eimeria* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Trichuris* spp. + *Eimeria* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Ancylostoma* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Trichuris* spp. + *Eimeria* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Ancylostoma* spp. + *Eimeria* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Trichuris* spp. + *Ancylostoma* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Ancylostoma* spp. + Trematoda + *Eimeria* spp. | 4.65 | 2/43 |
| *C. tentaculata* + *Capillaria* spp. + Trematoda + *Eimeria* spp. + *Isospora* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Eimeria* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Ancylostoma* spp. + *Eimeria* spp. | 11.63 | 5/43 |
| *C. tentaculata* + Ascaridoidea + *Eimeria* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Strongyloides* spp. + Ascaridoidea +*Eimeria* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Ancylostoma* spp. + Ascaridoidea + Spiruroidea + Trematoda + *Eimeria* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Ancylostoma* spp. | 2.33 | 1/43 |
| *C. tentaculata* + *Strongyloides* spp. + *Trichuris* spp. + *Ancylostoma* spp. + *Eimeria* spp. | 2.33 | 1/43 |
| *Strongyloides* spp. + *Trichuris* spp. + *Ancylostoma* spp. + *Eimeria* spp. + *Isospora* spp. | 2.33 | 1/43 |
| *Trichuris* spp. + *Capillaria* spp. + *Ancylostoma* spp. + *Eimeria* spp. | 2.33 | 1/43 |
| *Trichuris* spp. + *Ancylostoma* spp. + *Eimeria* spp. | 2.33 | 1/43 |
| **Total %** | 100.0 | 43/43 |

RF – Relative frequency; AF – Absolute frequency; n – positivite samples

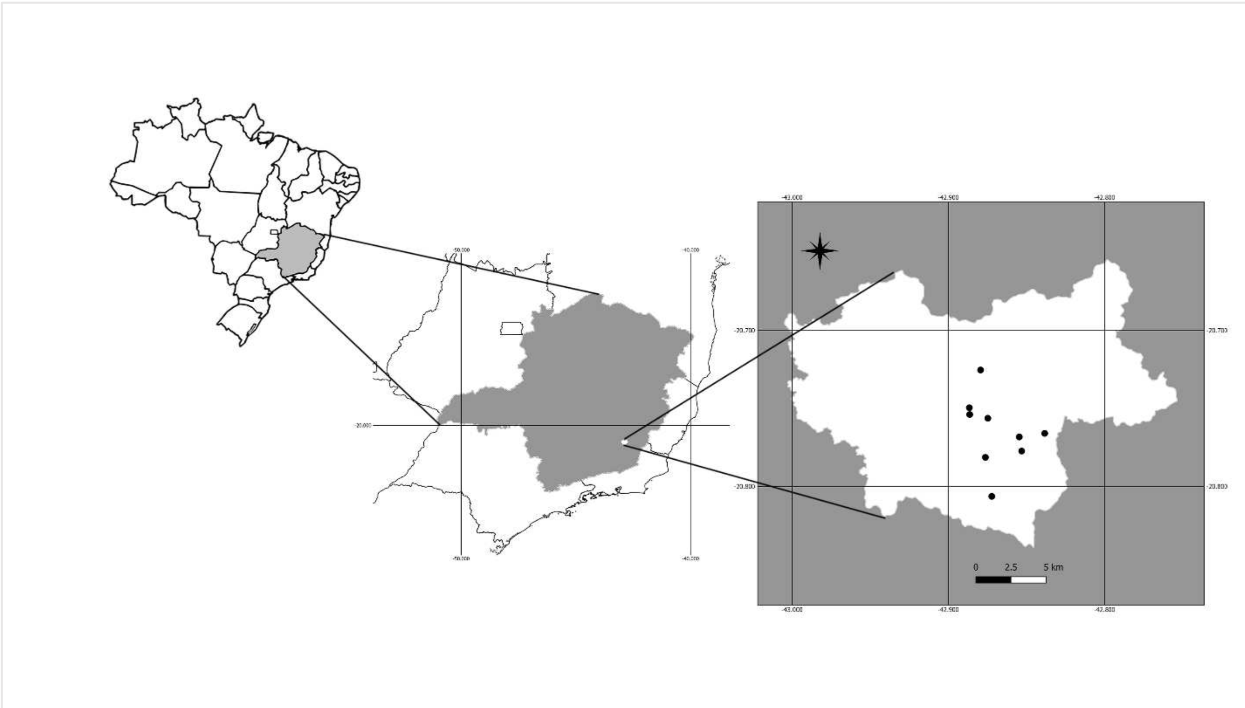


**Figure 1.** Points of capture of opossums positive for gastrointestinal parasites. The area where the black spots are concentrated is the urban extension of the municipality of Viçosa, Minas Gerais, Brazil.

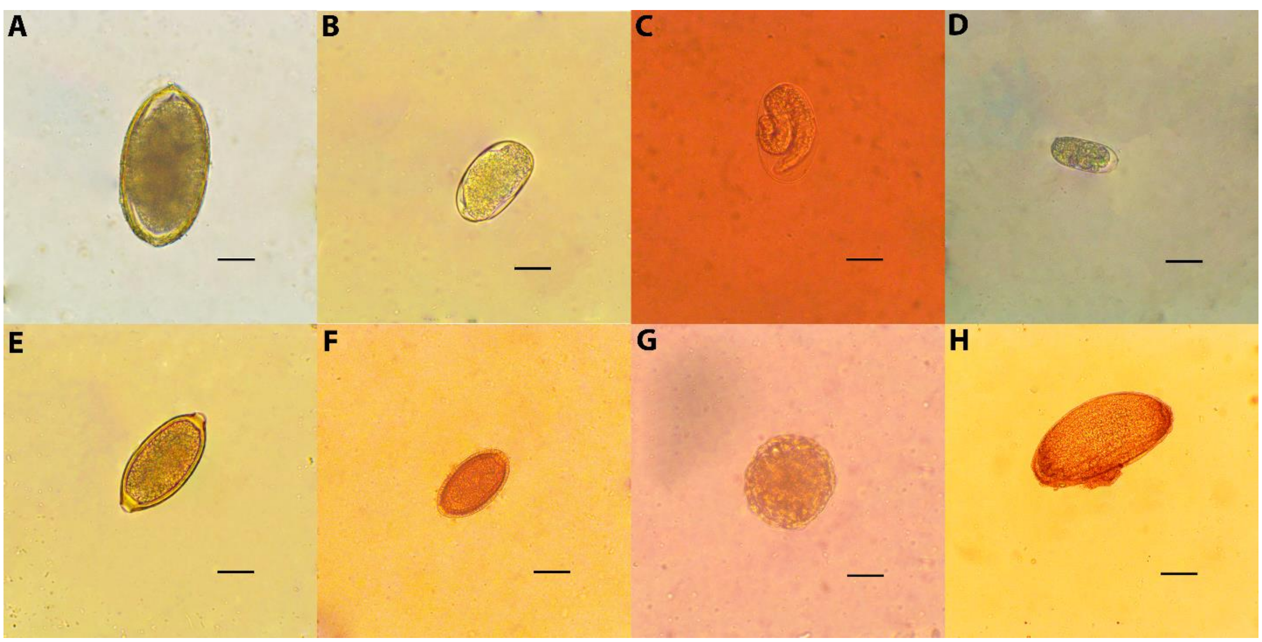


**Figure 2.** Helminth eggs detected in fecal samples from *Didelphis aurita*. A. *Cruzia tentaculata*; B. *Ancylostoma* sp.; C. *Strongyloides* sp.; D. Spiruroidea; E. *Trichuris* sp.; F. *Capillaria* sp.; G. Ascaridoidea; H. Trematoda. Scale bar 25µm.

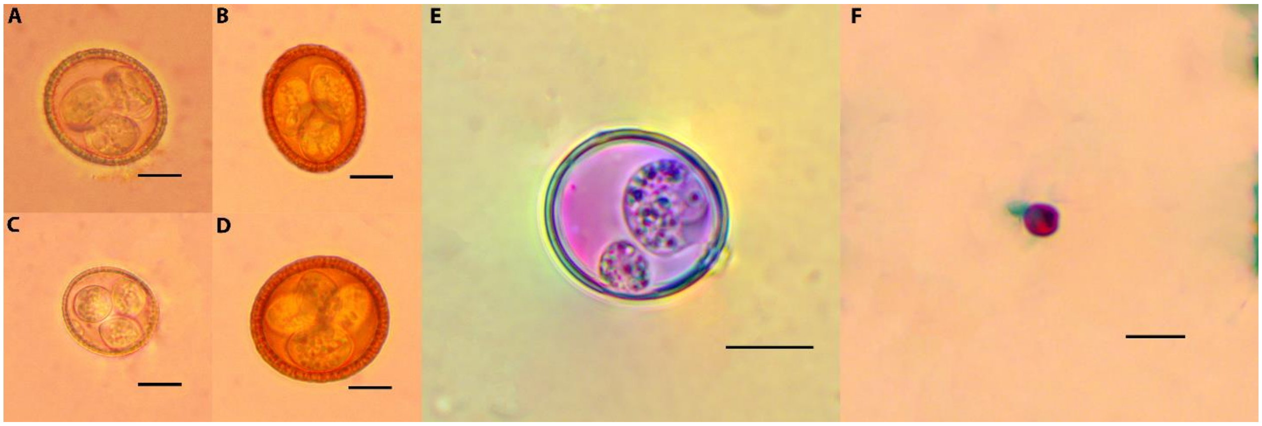


**Figure 3.** Protozoa oocysts detected in fecal samples from *Didelphis aurita*. A. *Eimeria auritanensis*; B. *E. gambai*; C. *E. philanderi*; D. *E. caluromydis*; E. *Isospora* sp.; F. *Cryptosporidium* sp. Scale bar 10µm.

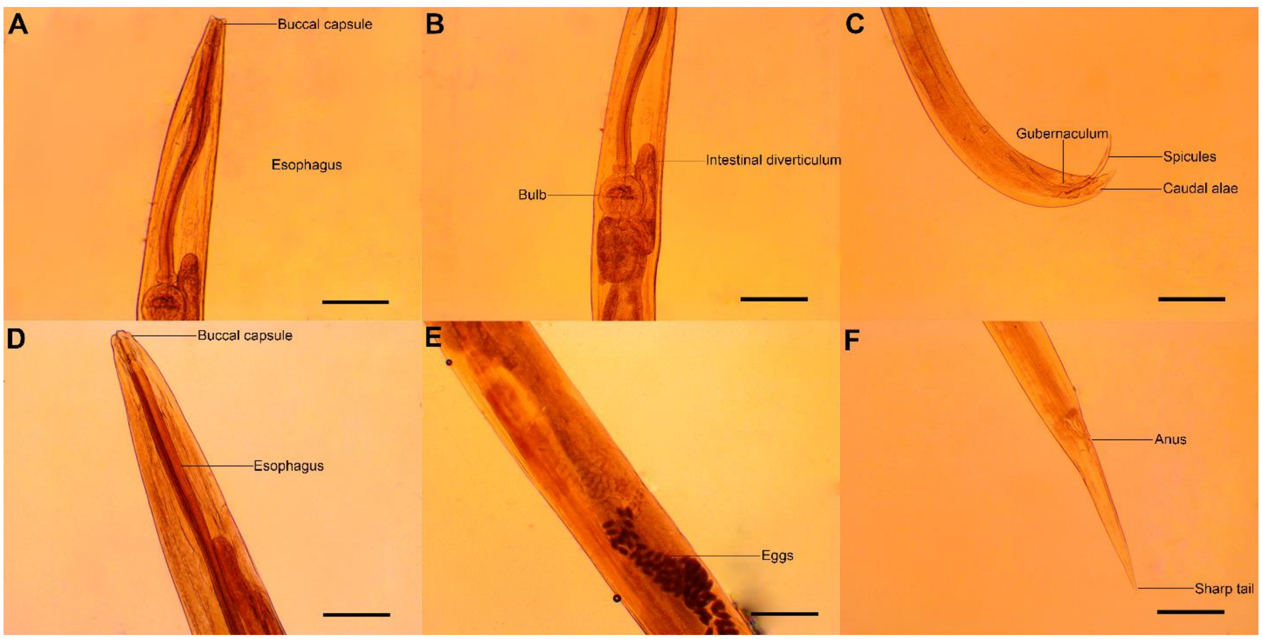


**Figure 4.** *Cruzia tentaculata* adult male (A, B, C) and female (D, E, F) recovered from *Didelphis aurita* feces. A - Anterior view of male showing buccal capsule, and esophagus; B - Esophagus with bulb and intestinal diverticulum common in male and female; C - Posterior region of male showing one pair of spicules, gubernaculum and caudal alae; D - Anterior region of female showing buccal capsule and esophagus; E - Mid region of female filled with eggs; F - Posterior end of female showing anus and sharp caudal end. Scale bar 500µm.

## 4. Discussion

This study reports a wide diversity of gastrointestinal parasites infecting *D. aurita* opossums from Southeastern Brazil. A very high frequency of helminths and protozoa was detected in the animals herein evaluated. This finding may be related to the omnivorous and opportunistic diet observed in *Didelphis* spp. (i.e. fruits, small vertebrates, invertebrates, seeds and rubbish remnants of human consumption), which may vary according to the environment (i.e. urban or forest fragments) where they are mostly inserted [9, 24]. The great variety of gastrointestinal parasites herein observed, include some species that may present zoonotic potential [25, 26, 27, 28, 29, 30, 31, 32]. Furthermore, to our knowledge, the protozoa *Cryptosporidium* sp., *Isospora* sp., and the nematode *Strongyloides* sp. are new records for *D. aurita.*

The presence of potentially zoonotic nematodes in opossums of the genus *Didelphis* was previously reported in America [6, 7, 33]. In the present study, immature forms resembling *Ancylostoma* spp. were detected in 65.30% of *D. aurita*. Teodoro et al. [6] recently identified immature parasitic forms of the family Ancylostomatidae with a frequency of 41.07% and 100.0% in *D. albiventris* and *D. aurita*, respectively. Similarly, Aragón-Pech et al. [7], evaluating gastrointestinal parasites in *D. virginiana* in Mexico, found 84.50% of the animals to be positive for *Ancylostoma* sp. These data suggest that *Didelphis* spp. opossums are commonly infected by this parasite. This genus includes the species *A. caninum*, which is commonly found in dog feces, and causes a disease known as *Cutaneous larva migrans* in humans [34]. Since the animals herein evaluated were all captured in urban sites where domestic animals, such as stray dogs are present, it is possible that opossums are involved in the cycle of *A. caninum* in urban environments.

Parasites of the family Trichuridae were frequently detected in the opossums herein evaluated. The genera *Trichuris* and *Capilaria* were previously reported in *Didelphis* spp. as *T. didelphis*, *T. marsupialis*, *T. minuta*, *C. didelphis* and *C. longicauda* [35, 36, 37]. Both genera have representative species with zoonotic potential; however, it is not clear whether the species described in *Didelphis* spp. may infect humans. In our study, the specimens were classified as *Trichuris* sp. and *Capillaria* sp. according to morphological and morphometric analysis. The same was applied to *Strongyloides* sp. eggs, which are described with an ellipsoid shape presenting a thin wall and a larva [38].

*Cruzia tentaculata* was the most prevalent parasite detected in the animals. Similar results were observed in other studies performed in Brazil with the same opossum species, identifying frequencies of approximately 76.0% for this nematode [6, 35]. These findings suggest that *C. tentaculata* is the most prevalent gastrointestinal parasite in *D. aurita* opossums. However, information about the ecology of this species in this marsupial are limited, and further studies are needed in order to determine their role regarding pathogenicity and effects on other gastrointestinal parasite populations found in these animals. In the present study, male and female adult forms of the parasite were directly recovered from feces of four opossums. Since the transmission of *Cruzia* spp. is given by ingestion of eggs [39], it is not clear what the releasing of alive adult forms through feces means in the parasite life cycle.

Trematode eggs were found in eight animals in our study. Among the trematodes previously reported in *Didelphis* spp., *Schistosoma mansoni* found in *D. albiventris* [40], and *Brachylaima* sp. found in *D. aurita* [35] are potentially zoonotic [41, 42]. Eggs detected in the present work did not seem to be of none of these parasites. In fact, all the immature forms presented morphological and morphometric characteristics that resembled *Rhopalias* spp. [43].

Oocysts belonging to three genera of protozoa were herein detected. *Eimeria* spp. are the most prevalent coccidia in opossums, as reported in a previous study [7]. In fact, there are many species of this genus infecting *Didelphis* spp.; however, the extent to which most eimerians affect the health of these animals is unknown or nor clear [44]. *Isospora* sp. is herein reported for the first time in *D. aurita*. This parasite was previously described in *D. virginiana* [45], and in *D. marsupialis* [46]. A third protozoan, herein identified as *Cryptosporidium* sp. is also a new record for *D. aurita*. However, the presence of this coccidian in the black eared opossum were hypothetically known, as it was already recorded in *D. albiventris* [10], and due to the ubiquitous behavior this coccidia have [47].

The pathological effects of many parasites of wildlife are unknown or not investigated, and most of these parasites co-inhabit in their hosts with other parasites [11]. The interactions among these parasites are hardly studied; however, it is known that these relationships may be antagonistic (when an organism inhibit the infection and/or development of other organism), or synergistic (when one parasite facilitates the entry and/or development of other parasites) [48]. In our study, most animals presented multiparasitism, and within this variety of parasites, it is difficult to know the role that each individual have in animal health. Nevertheless, it is important to highlight that since many of those organisms have zoonotic potential, further studies regarding their identification at species level, as well as their potential to cause human disease, should be performed in order to find out what is the public health impact they have.

In the last decades, the concept of one health has been applied in many areas that involve humans, animals and the environment. In this aspect, *Didelphis* spp. are particularly important since these animals play relevant ecological role in nature, but they also are reservoirs of important zoonotic pathogens [49, 50]. The synanthropic habit observed in these animals is a reflex of the human activity in terms of deforestation, which consequently increases the opossum population in urban centers due to the destruction of their natural habitat. *D. aurita* in particular, inhabits mostly the Atlantic Forest remnants [5]; however, this marsupial has been frequently found within the urban environments, as observed in our study. This finding is of substantial importance within the One Health concept, since the environmental degradation caused by anthropic activity, causes imbalance in the *Didelphis* spp. population [51, 52]. As a result, the animals seek for new habitats, finding the human dwellings the perfect place to live due to the availability of food and shelter, which leads to the emergence of zoonotic pathogens from the wild to the cities.

## 5. Conclusion

This study provides data on the gastrointestinal parasites in *D. aurita* opossums, highlighting the species with zoonotic potential. Results herein obtained demonstrated that multiparasitism is commonly detected in these animals, and that *C. tentaculata* is the most prevalent nematode found parasitizing them. Additionally, our study indicated a high percentage of opossums infected by potentially zoonotic parasites such as *Ancylostoma* spp., Ascaridoidea and *Trichuris* spp. implying that *D. aurita* may be involved in zoonotic cycles in urban environments.

## Acknowledgements

The authors acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), and the Graduate Program in Veterinary Medicine of the Universidade Federal de Viçosa (UFV) for the support provided. Finally, authors would like to thank Professor Gisele M. L. del Giúdice for providing traps to capture the animals, Professor Elida M. L. Rabelo and Dr. Luis F. V. Furtado for the support with the molecular analysis.

**Declarations of interest:** none.

**References**

[1] S.J. Cutler, A.R. Fooks, W.H.M. Van der Poel, Public health threat of new, reemerging, and neglected zoonoses in the industrialized world. Emerg. Infect. Dis. 16 (1) (2010) 1-7.

[2] R.C.A. Thompson, Parasite zoonoses and wildlife: One health, spillover and human activity. Int. J. Parasitol. 43 (2013) 1079-1088.

[3] R.O. McFarlane, A. Sleigh, T. McMichael, Synanthropy of Wild Mammals as a Determinant of Emerging Infectious Diseases in the Asian–Australasian Region. Eco Healt. 9 (2012) 24-35.

[4] C.M. Pinto, S. Ocaña-Mayorga, M.S. Lascano, M.J. Grijalva, Infection by trypanosomes in marsupials and rodents associated with human dwellings in Ecuador. J. Parasitol. 92 (6) (2006) 1251-1255.

[5] A.L. Gardner, Mammals of South America, Volume I. Marsupials, xenarthrans, shrews, and bats. The University of Chicago Press, Chicago and London, 2008.

[6] A.K.M. Teodoro, A.A. Cutolo, G. Motoie, C.S. Meira-Strejevitch, V.L. Pereira-Chioccola, T.M.F. Mendes, S.M. Allegretti, 2019. Gastrointestinal, skin and blood parasites in *Didelphis* spp. from urban and sylvatic areas in São Paulo state, Brazil. Vet. Parasitol. Reg. Stud. Reports. 16, 100286.

[7] R.A. Aragón-Pech, H.Á. Ruiz-Piña, R.I. Rodríguez-Vivas, A.D. Cuxim-Koyoc, E. Reyes-Novelo, Prevalence, abundance and intensity of eggs and oocysts of gastrointestinal parasites in the opossum *Didelphis virginiana* Kerr, 1792 in Yucatan, Mexico. Helminth. 55 (2) (2018) 119-126.

[8] H.A. Pinto, V.L.T. Mari, A.L. Melo *Toxocara cati* (Nematoda: Ascarididae) in *Didelphis albiventris* (Marsupialia: Didelphidae) from Brazil: a case of pseudoparasitism. Braz J Vet Parasitol 23 (4) (2014) 522-525.

[9] P. Ceotto, R. Finotti, R. Santori, R. Cerqueira, Diet variation of the marsupails *Didelphis aurita* and *Philander frenatus* (Didelphimorphia, Didelphidae) in a rural area of Rio de Janeiro State, Brazil. Mastozool. Neotrop. 16 (1) (2009) 49-58.

[10] R.A. Zanette, A.S. Silva, F. Lunardi, J.M. Santurio, S.G. Monteiro, Occurrence of gastrointestinal protozoa in *Didelphis albiventris* (opossum) in the central region of Rio Grande do Sul state. Parasitol. Int. 57 (2) (2008) 217-218.

[11] E. Vaumourin, G. Vourc'h, P. Gasqui, M. Vayssier-Taussat, The importance of multiparasitism: examining the consequences of coinfections for human and animal health. Parasite. Vector. 8 (2015) 545.

[12] D.S. Morrant, S. Petit, R. Schumann, Floral nectar sugar composition and flowering phenology of the food plants used by the western pygmy possum, *Cercartetus concinnus*, at Innes National Park, South Australia. Ecol. Res. 25 (2010) 579-589.

[13] H.H. Willis, A simple levitation method for the detection of hookworm ova. Med. J. Aust. 8 (1921) 375-378.

[14] A. Elliot, U.M. Morgan, R.C. Aworew Thompson, Improved staining method for detecting *Cryptosporidium* oocysts in stools using malachite green. J. Gen. Appl. Microbiol. 45 (1999) 139-142.

[15] D. Duszynski, P.G. Wilber, A Guideline for the Preparation of Species Descriptions in the Eimeriidae. J. Parasitol. 83 (2) (1997) 333-336.

[16] M. Teixeira, P.D. Rauta, G.R. Albuquerque, C.W.G. Lopes, *Eimeria auritanensis* n. sp. and *E. gambai* Carini, 1938 (Apicomplexa: Eimeriidae) from the opossum *Didelphis aurita* Wied-newied, 1826 (Marsupialia: Didelphidae) from southeastern Brazil. Rev. Bras. Parasitol. Vet. 16 (2) (2007) 83-86.

[17] D.D. Bowman, Georgis Parasitologia Veterinaria, Elsevier, Rio de Janeiro, 2010.

[18] R. Lainson, J.J. Shaw, Two new species of *Eimeria* and three new species of *Isospora* Apicomplexa, Eimeriidae) from Brazilian mammals and birds. Bull. Mus. Hist. Nat. Paris. 11 (2) (1989) 349-365.

[19] F.A.O. Adnet, D.H.S. Anjos, A. Menezes-Oliveira, R.M. Lanfredi, Further description of *Cruzia tentaculata* (Rudolphi, 1819) Travassos, 1917 (Nematoda: Cruzidae) by light and scanning electron microscopy. Parasitol. Res. 104 (2009) 1207-1211.

[20] L.W. Zuccherato, L.F. Furtado, C.S. Medeiros, C.S. Pinheiro, É.M. Rabelo, PCR-RFLP screening of polymorphisms associated with benzimidazole resistance in *Necator americanus* and *Ascaris lumbricoides* from different geographical regions in Brazil. PLoS Negl. Trop. Dis. 12 (9) (2018) e0006766.

[21] S.L. Lake, J.B. Matthews, R.M. Kaplan, J.E. Hodgkinson, Determination of genomic DNA sequences for beta-tubulin isotype 1 from multiple species of cyathostomin and detection of resistance alleles in third-stage larvae from horses with naturally acquired infections. Parasit. Vectors. 2 (2) (2009) S6.

[22] N. Sahimin, Y.A.L. Lim, B. Douadi, M.K.N. Mohd Khalid, J.J. Wilson, J.M. Behnke, S.N. Mohd Zain, Hookworm infections among migrant workers in Malaysia: Molecular identification of *Necator americanus* and *Ancylostoma duodenale*. Acta Trop. 173 (2017) 109-115.

[23] L.F. Furtado, A.C. Bello, H.A. dos Santos, M.R. Carvalho, É.M. Rabelo, First identification of the F200Y SNP in the β-tubulin gene linked to benzimidazole resistance in *Ancylostoma caninum*. Vet. Parasitol. 206 (3-4) (2014) 313-316.

[24] N.C. Cáceres, E.L.A. Monteiro-Filho, Food Habits, Home Range and Activity of *Didelphis aurita* (Mammalia, Marsupialia) in a Forest Fragment of Southern Brazil. Stud. Neotrop. Fauna. E. 36 (2) (2001) 85-92.

[25] A. Oryan, S.M. Sadjjadi, D. Mehrabani, M. Kargar, Spirocercosis and its complications in stray dogs in Shiraz, southern Iran. Vet. Med. 53 (11) (2008) 617-624.

[26] T.L. Mateus, A. Castro, J.N. Ribeiro, M. Vieira-Pinto, Multiple Zoonotic Parasites Identified in Dog Feces Collected in Ponte de Lima, Portugal - A Potential Threat to Human Health. Int. J. Environ. Res. Public. Health. 11 (2014) 9050-9067.

[27] E.M. Miranda, Zoonotic Trematodiasis. In: Farm Animals Diseases, Recent Omic Trends and New Strategies of Treatment, In. R.E. Quiroz-Castañeda (ed.), IntechOpen, 2018. DOI: 10.5772/intechopen.72632.

[28] F.D. Uehlinger, S.J. Greenwood, J.T. McClure, G. Conboy, R. O'Handley, H.W. Barkema, Zoonotic potential of *Giardia duodenalis* and *Cryptosporidium* spp. and prevalence of intestinal parasites in young dogs from different populations on Prince Edward Island, Canada. Vet. Parasitol. 196 (2013) 509-514.

[29] S.M. Thamsborg, J. Ketzis, Y. Horii, J.B. Matthews, *Strongyloides* spp. infections of veterinary importance. Parasitol. 144 (3) (2016) 274-284.

[30] H. Youn, Review of Zoonotic Parasites in Medical and Veterinary Fields in the Republic of Korea. Korean. J. Parasitol. 47 (2009) S133-S141.

[31] D.J. Batchelor, S. Tzannes, P.A. Graham, J.M. Wastling, G.L. Pinchbeck, A.J. German, Detection of Endoparasites with Zoonotic Potential in Dogs with Gastrointestinal Disease in the UK. Transbound. Emerg. Dis. 55 (2008) 99-104.

[32] D.S. Lindsay, J.P. Dubey, B.L. Blagburn, Biology of *Isospora* spp. from Humans, Nonhuman Primates, and Domestic Animals. Clin. Microbiol. Rev. 10 (1) (1997) 19-34.

[33] D.J. Richardson, J.D. Campo, Gastrointestinal Helminths of the Virginia Opossum (*Didelphis virginiana*) in South-Central Connecticut, U.S.A. Comp. Parasitol. 72 (2) (2005) 183-185.

[34] H. Feldmeier, A. Schuster, Mini review: hookworm-related cutaneous larva migrans. Eur. J. Clin. Microbiol. Infect. Dis. 31 (2012) 915-918.

[35] S.F. Costa-Neto, T.S. Cardoso, R.G. Boullosa, A. Maldonado Jr., R. Gentile, Metacommunity structure of the helminths of the black-eared opossum *Didelphis aurita* in peri-urban, sylvatic and rural environments in south-eastern Brazil. J Helminthol. 17 (2018) 1-12.

[36] D. Noronha, J.J. Vicente, R.M. Pinto, A survey of new host records for nematodes from mammals deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC). Rev. Bras. Zool. 19 (3) (2002) 945-949.

[37] K.J. Alden, Helminths of the Opossum, *Didelphis virginiana*, in Southern Illinois, with a Compilation of All Helminths Reported from This Host in North America. J. Helminthol. Soc. Wash. 62 (2) (1995) 197-208.

[38] M.E. Viney, J.B. Lok, The biology of Strongyloides spp., The C. elegans Research Community (ed.), WormBook, 2015. doi/10.1895/wormbook.1.141.2, http://www.wormbook.org.

[39] R.C. Anderson, Nematode Parasites of Vertebrates. Their Development and Transmission. CABI Publishing, Wallingford, UK, 2000.

[40] U. Kawazoe, L.C.S. Dias, J.T. Piza, Infecção natural de pequenos mamíferos por *Schistosoma mansoni*, na represa de Americana (São Paulo, Brasil). Rev. Saúde. Publ. 12 (1978) 200-208.

[41] A.R. Butcher, D.I. Grove, Description of the life-cycle stages of *Brachylaima cribbi* n. sp. (Digenea: Brachylaimidae) derived from eggs recovered from human faeces in Australia. Syst. Parasitol. 49 (2001) 211-221.

[42] J.A.T. Morgan, R.J. Dejong, S.D. Snyder, G.M. Mkoji, E.S. Loker, *Schistosoma mansoni* and *Biomphalaria*: past history and future trends. Parasitol. 123 (2001) S211-S228.

[43] T.R. Haverkost, S.L. Gardner, A review of species in the genus *Rhopalias* (Rudolphi, 1819). J. Parasitol. 94 (3) (2008) 716-726.

[44] D.W. Duszynski, The biology and identification of the coccidia (Apicomplexa) of marsupials of the world, Academic Press: Elsevier, London, San Diego and Waltham, 2016.

[45] T. Joseph, *Eimeria indianensis* sp. n. and an *Isospora* sp. from the Opossum *Didelphis virginiana* (Kerr). J. Protozool. 21 (1) (1974) 12-15.

[46] J.V. Ernst, C. Cooper, B. Chobotar, *Isospora boughtoni* Volk, 1938 and *Isospora* sp. (Protozoa: Eimeridae) from an opossum *Didelphis marsupialis*. Bull. Wildlife. Dis. 5 (1969) 406-409.

[47] M. Bouzid, P.R. Hunter, R.M. Chalmers, K.M. Tyler, *Cryptosporidium* Pathogenicity and Virulence. Clin. Microbiol. Rev. 26 (1) (2013) 115-134.

[48] S. Telfer, X. Lambin, R. Birtles, P. Beldomenico, S. Burthe, S. Paterson, M. Begon, Species Interactions in a Parasite Community Drive Infection Risk in a Wildlife Population. Scienc. 330 (2010): 243-246.

[49] S.E. Bermúdez, N. Gottdenker, A. Krishnvajhala, A. Fox, H.K. Wilder, K. González, D. Smith, M. López, M. Perea, C. Rigg, S. Montilla, J.E. Calzada, A. Saldaña, C.M. Caballero, J.E. Lopez, 2017. Synanthropic Mammals as Potential Hosts of Tick-Borne Pathogens in Panama. PLoS. ONE. 12 (1), e0169047.

[50] M. Cantor, L.A. Ferreira, W.R. Silva, E.Z.F. Setz, Potential seed dispersal by *Didelphis albiventris* (Marsupialia, Didelphidae) in highly disturbed environment. Biota Neotrop 10 (2) (2010) 45-51.

[51] A.A.S. Pereira, E.C. Ferreira, A.C.V.M.D.R. Lima, G.B. Tonelli, F.D. Rêgo, A.P. Paglia, J.D. Andrade-Filho, G.F. Paz, C.M.F. Gontijo, 2017. Detection of *Leishmania* spp in silvatic mammals and isolation of *Leishmania* (*Viannia*) *braziliensis* from *Rattus rattus* in an endemic area for leishmaniasis in Minas Gerais State, Brazil. PLoS. ONE. 12 (11), e0187704.

[52] S.B. Lucheis, G.S. Hernandes, D.K. Lenharo, M.E.B. Santiago, L.C. Baldini-Peruca, Are opossums capable of transmitting leptospirosis in urban areas? J. Venom. Anim. Toxins. Incl. Trop. Dis. 15 (3) (2009) 370-373.

# CHAPTER 3

**Article: *Eimeria* spp. (Apicomplexa: Eimeriidae) in *Didelphis aurita* Wied-Newied, 1826 (Didelphimorphia: Didelphidae) and description of a new species infecting this opossum**

Submitted to the Systematic Parasitology Journal

**Abstract**

*Didelphis aurita* is a marsupial well adapted to anthropogenic activity and commonly found in urban areas of Brazil. Among the gastrointestinal parasites found in this opossum, protozoa of the genus *Eimeria* are frequently detected. The aim of this study was to investigate the diversity of *Eimeria* species infecting *D. aurita* in Southeastern Brazil, and to provide morphological data of a new species of *Eimeria*. From January to June 2019, 43 *D. aurita* were captured, and their fecal samples were collected and evaluated by simple flotation; positive samples were allowed to sporulate in 2.5% potassium dichromate ($K_2Cr_2O_7$), and detailed morphometric analyses were performed to determine the species present. 32/43 (74.4%) of the opossums were infected with from one to five *Eimeria* spp. Four of the eimerians were discovered, described and named by others: *E. auritanensis, E. caluromydis, E. gambai,* and *E. philanderi*. Additionally, sporulated oocysts of a species new to science were detected. Oocysts of *Eimeria vicoensis* n. sp. are spheroidal to subspheroidal, 21.7 × 20.7 (20-23 × 19-23), length/width (L/W) ratio 1.05, with a highly refractile polar granule, but lacking a micropyle and oocyst residuum. Sporocysts are ovoidal, 10.6 × 8.0 (9-12 × 7-9), L/W ratio 1.3, with a small, nipple-like Stieda body and a sporocyst residuum of diffuse granules. 5/32 (16%) of the opossums were infected with only one *Eimeria* sp., 6/32 (19%) with two, 15/32 (47%) with three, 5/32 (16%) with four and 1/32 (3%) with five Eimerians.

**Keywords:** Protozoa, Marsupials, Animal Health, Parasites, *Eimeria vicoensis.*

## 1. Introduction

*Didelphis aurita*, the black-eared opossum, is a widely distributed marsupial found within the eastern Neotropical region of Brazil to southeastern Paraguay and northeastern Argentina (Gardner 2008). This species is well adapted to human habitations where they are found on the roof of houses, in the hollows of trees and other shelters within the cities and peripheral areas (Jansen 2002). Thus, the transmission of zoonotic pathogens to humans and domestic animals by *D. aurita* may occur as has been demonstrated in the sylvatic cycle of visceral leishmaniasis between two other *Didelphis* species, domestic dogs, and humans in other areas of southeastern Brazil (Cabrera et al. 2003).

The gastrointestinal parasite fauna in marsupials of the genus *Didelphis* is diverse with a great variety of helminths (Chero et al. 2017; Aragón-Pech et al. 2018; Pinto et al. 2014) and protozoa identified (Zanette et al. 2008). Some of these parasites are potentially zoonotic, such as *Toxocara cati*, detected in *D. albiventris* (Pinto et al. 2014); *Ancylostoma*, *Toxocara*, *Trichuris*, *Ascaris*, and *Capillaria* spp. identified in *D. virginiana* (Aragón-Pech et al. 2018) and protozoa such as *Giardia* sp. and *Cryptosporidium* sp. detected in *D. albiventris* (Zanette et al. 2008).

Among the parasites identified in *Didelphis* spp., *Eimeria* spp. are commonly found (Duszynski 2016; Teixeira et al. 2007). These protists are obligate intracellular coccidia transmitted from host-to-host by the ingestion of sporulated oocysts. In domestic and wild animals, the infection may be asymptomatic or, depending upon the *Eimeria* species, the number of oocysts ingested, and the immune status of the host to that species, the presence of clinical signs such as diarrhea, dehydration, weight loss, and even death may occur (Macedo et al. 2019; Zanette et al. 2008). Most *Eimeria* spp. were once thought to exhibit strict host specificity; however, phylogenetic and morphologic studies have demonstrated the ability of some eimerians to be able to infect different host species in the same genus or even species in different host families (Duszynski 2016; Vrba and Pakandl 2015; Wilber et al. 1998).

Reports on *Eimeria* spp. infecting *D. aurita* have described *E. philanderi* (Fehlberg et al. 2018), *E. gambai* (Carini 1938; Teixeira et al. 2007), *E. auritanensis* (Teixeira et al. 2007), and *E. didelphydis* (Carini 1936). However, data on the frequency of these eimerians in *D. aurita* are not available since those studies focused mostly on the species description. The work reported here documents the diversity of *Eimeria* spp. infecting *D. aurita* in Southeastern Brazil, and provides morphological data of a new species of *Eimeria*.

## 2. Material and Methods

### *2.1 Study area*

This study was performed in urban and peri-urban areas of the municipality of Viçosa (20°45´14” S and 42°52´54” W), state of Minas Gerais, Southeastern Brazil. The area has a Cwa climate (Köppen climate classification), mesothermic, with hot and rainy summers and cold and dry winters. The annual average temperature varies from 20 to 22 °C and the region is 650 m above sea level.

*2.2 Capture of animals and laboratory procedures*

Animals were captured with Tomahawk live traps (0.45 x 0.21 x 0.21 m), which were daily armed (5 p.m.) and checked (7 a.m.) from January to June 2019, for 516 trap-nights. A mix of corn flour, canned fish and banana were used as bait. After capture, the animals were identified by sex, age group (pups, subadults and adults), and marked with a small V cut on the right ear to identify recaptures (Morrant et al. 2010; Pestell and Petit 2007; Hunsaker 1977).

Fresh fecal samples were collected from the cage or directly from the cloaca as soon as the animals defecated. Each sample was evaluated by a simple flotation technique (Willis, 1921) and observed on microscopic slides under 100x and 400x magnification. Positive samples were placed in Petri dishes containing 2.5% potassium dichromate ($K_2Cr_2O_7$) and stored at 24 °C for 7 days to allow oocyst sporulation (Duszynski and Wilber 1997). Afterwards, the material was transferred into plastic tubes, centrifuged twice with distilled water and once with saline solution at 200 g for 15 min. Finally, sporulated oocysts were photographed and measured (~60 of each species) at 1000x with an Olympus CX31 microscope with a camera connected to the ToupView software version 3.7. Identification of oocysts was based on using structural and morphometric features previously described (Fehlberg et al. 2018; Berto et al. 2014; Teixeira et al. 2007; Duszynski and Wilber 1997; Lainson and Shaw 1989). Phototypes of the oocysts are deposited in the Parasitology Collection of the Laboratório de Biologia de Coccídios at Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, Rio de Janeiro, Brazil. Photographs of the symbiotype-host specimen for the new *Eimeria* species are deposited in the same collection with the repository number [nº P-98/2019] and is available at http://r1.ufrrj.br/labicoc/colecao.html.

*2.3 Data analysis*

Descriptive statistics was performed to calculate the relative and absolute frequency of *Eimeria* spp. infections, and the mean and standard deviation for the morphometry of oocysts. The normality of data was checked using the Lilliefors test. Additionally, the Chi-square test with Yates correction ($\chi^2$) was used to compare *Eimeria* spp. infection in male and females, as well as the ages of infected animals, considering a 5% significance level. All analyzes were carried out using the BioEstat 5.3 software.

## 3. Results

Forty-three animals were captured from January to June 2019. Of those, 22/43 (51.2%) were male and 21/43 (48.8%) were female; 1/43 (2.3%) was a pup, 21/43 (48.8%) were subadults and 21/43 (48.8%) were adults. 32/43 (74.4%) animals were found to be infected with from 1 to 5 *Eimeria* species (Tables 1 and 2). Oocysts of *Eimeria gambai* (Figs. 1A, 2A) were found in 27/43 (62.8%) opossums, oocysts of *E. philanderi* (Figs. 1C, 2C) in 25/43 (58.1%), oocysts of *E. auritanensis* (Figs. 1B, 2B) in 13/43 (30.2%), and those of *E. caluromydis* (Figs. 1D, 2D) in 9/43 (20.9%). Photomicrographs of the sporulated oocysts of these four species were deposited in the Parasitology Collection of the Laboratório de Biologia de Coccídios at the Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil, and their repository numbers are: [nº 94/2019] for *Eimeria auritanensis*; [nº 95/2019] for *Eimeria caluromydis*; [nº 96/2019] for *Eimeria gambai*; and [nº 97/2019] for *Eimeria philanderi*. Oocysts of a fifth morphotype with different structural parameters from all other eimerians known from opossums were detected in 13/43 (30.2%) opossums and it is described here as new.

### *3.1 Eimeria vicoensis* n. sp. (Figs. 3A, B)

*Type host: Didelphis aurita* (Wied-Neuwied 1826) (Didelphimorphia: Didelphidae), Black-eared Opossum.

*Type locality:* South America: Brazil: Atlantic Forest, municipality of Viçosa, Minas Gerais (20°45´14” S and 42°52´54” W).

*Other hosts:* Unknown, none to date.

*Description of sporulated oocyst:* Oocyst shape: Spheroidal to Subspheroidal; number of walls: 1; wall characteristics: smooth, colorless, ~1.0 thick; L x W (n = 58): 21.7 × 20.7 (20-23 × 19-23), L/W ratio: 1.05; polar granule present, highly refractile; micropyle and oocyst residuum, both absent.

*Description of sporocyst and sporozoites:* Sporocyst shape: ovoidal; L x W (n = 58): 10.6 × 8.0 (9-12 × 7-9); L/W ratio: 1.3; Stieda body present; substieda and parastieda bodies both absent; sporocyst residuum present as a few scattered granules; refractile bodies in sporozoites were not seen.

*Prevalence:* Oocysts of this morphotype were found in 13/43 (30.2%) *D. aurita.*

*Sporulation:* Exogenous. Oocysts were completely sporulated after a period of 7 days in 2.5% (1/5) potassium dichromate solution ($K_2Cr_2O_7$).

*Prepatent and patent periods*: Unknown.

*Site of infection:* Unknown, oocysts were recovered from feces after the animals defecated.

*Pathogeny:* Unknown.

*Materials deposited:* Photosyntypes (Frey et al. 1992) are deposited in the Parasitology Collection of the Laboratório de Biologia de Coccídios at the Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil, repository number is P-98/2019.

*Etymology:* The specific epithet is derived from the name of the municipality where the study was performed.

*Remarks*: *E. vicoensis* n. sp. differs from other described *Eimeria* spp. of marsupials, regarding its descriptive structural and morphometric parameters. Among the *Eimeria* spp. described in marsupials of the order Didelphimorfia, *E. didelphydis* is the one that most resembles *E. vicoensis* n. sp. due to its smooth oocyst wall. However, distinctive features such as the presence of a highly refractile polar granule, morphometric parameters, and shape, differentiate the new species from the former. The oocysts of *E. didelphydis* are smaller than the ones described for *E. vicoensis* n. sp., L x W 16 x 16 *vs* 21.7 x 20.7, and also present a smaller L/W ratio: 1.0 *vs* 1.05, given to the species described in this study a shape that goes from spherical to subspherical, against the spherical shape of *E. didelphydis*. Additionally, all oocysts of the new species presented a single oocyst wall, whereas, *E. didelphydis* may present 1 or 2 wall layers (Duszynski 2016; Pellérdy 1974; Carini 1936).

**Table 1.** Morphological and morphometric characteristics of *Eimeria* spp. oocysts in *Didelphis aurita* (~ 60 of each measured)

| Species | Oocyst (Diameter in µm) | | | | | | | | Sporocyst (Diameter in µm) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Shape** | **Wall** | **PG** | **M** | **OR** | **Length** | **Width** | **MI** | **Shape** | **SB** | **SR** | **RB** | **Length** | **Width** | **MI** |
| ***E. gambai*** | Ovoid to ellipsoidal | Striated, two layers, light brown-yellow | - | - | - | 26.67 | 23.09 | 1.16 | Ovoid | + | + | - | 12.47 | 9.20 | 1.36 |
| ***E. auritanensis*** | Spherical to Subspherical | Mammillated, two thick brown-yellow layers | + | - | - | 28.55 | 26.81 | 1.07 | Ovoid | + | + | - | 13.65 | 9.81 | 1.39 |
| ***E. philanderi*** | Subspherical | Striated, two layers, outer colorless, and inner brown | + | - | - | 23.87 | 22.38 | 1.07 | Subspherical | + | + | - | 11.47 | 8.58 | 1.34 |
| ***E. caluromydis*** | Subspherical | mammillated, thick Brown-yellow layer | - | - | - | 29.22 | 27.69 | 1.06 | Ovoid | + | + | - | 14.11 | 9.99 | 1.24 |
| ***E. vicoensis* n. sp.** | Spherical to Subspherical | Smooth, colorless layer | + | - | - | 21.69 | 20.69 | 1.05 | Ovoid | + | + | - | 10.61 | 8.04 | 1.32 |

PG – Polar Granule; M – Micropyle; OR – Oocyst Residuum; MI – Morphometric Index; SB – Stieda Body; SR – Sporocyst Residuum; RB – Refractile Body; (+) – present; (-) – absent.

**Table 2.** Single and multiple infections by *Eimeria* spp. in *Didelphis aurita*.

| **Parasites** | **RF % (AF/n)** | | **RF % (AF/n)** |
|---|---|---|---|
| | **Male** | **Female** | **Total** |
| *E. gambai* | 12.50 (2/16) | 6.25 (1/16) | 9.38 (3/32) |
| *E. philanderi* | 6.25 (1/16) | 6.25 (1/16) | 6.25 (2/32) |
| *E. gambai* + *E. philanderi* | 0.00 (0/16) | 25.00 (4/16) | 12.5 (4/32) |
| *E. philanderi* + *E. vicoensis* n. sp. | 12.50 (2/16) | 0.00 (0/16) | 6.25 (2/32) |
| *E. gambai* + *E. philanderi* + *E. vicoensis* n. sp. | 25.00 (4/16) | 18.75 (3/16) | 21.88 (7/32) |
| *E. gambai* + *E. auritanensis* + *E. philanderi* | 12.50 (2/16) | 12.50 (2/16) | 12.5 (4/32) |
| *E. gambai* + *E. auritanensis* + *E. caluromydis* | 0.00 (0/16) | 12.50 (2/16) | 6.25 (2/32) |
| *E. gambai* + *E. caluromydis* + *E. vicoensis* n. sp. | 6.25 (1/16) | 0.00 (0/16) | 3.13 (1/32) |
| *E. auritanensis* + *E. philanderi* + *E. caluromydis* | 6.25 (1/16) | 0.00 (0/16) | 3.13 (1/32) |
| *E. gambai* + *E. auritanensis* + *E. philanderi* + *E. caluromydis* | 6.25 (1/16) | 12.50 (2/16) | 9.38 (3/32) |
| *E. gambai* + *E. auritanensis* + *E. philanderi* + *E. vicoensis* n. sp. | 0.00 (0/16) | 6.25 (1/16) | 3.13 (1/32) |
| *E. gambai* + *E. auritanensis* + *E. caluromydis* + *E. vicoensis* n. sp. | 6.25 (1/16) | 0.00 (0/16) | 3.13 (1/32) |
| *E. gambai* + *E. auritanensis* + *E. philanderi* + *E. caluromydis* + *E. vicoensis* n. sp. | 6.25 (1/16) | 0.00 (0/16) | 3.13 (1/32) |
| **Total %** | 100 | 100 | 100 |

RF – Relative frequency; AF – Absolute frequency; n – positivite samples

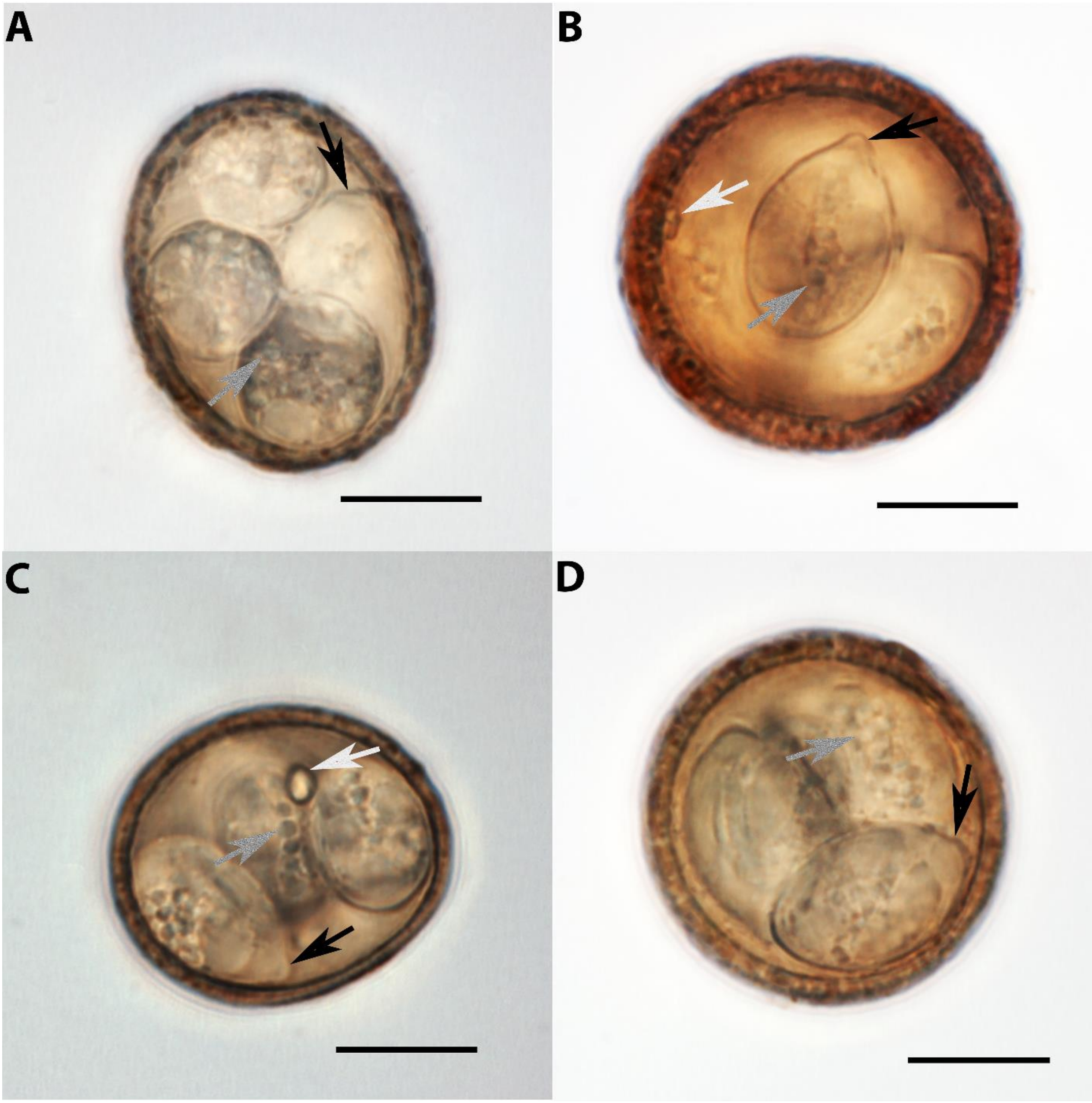


**Figure 1.** A. *E. gambai*; B. *E. auritanensis*; C. *E. philanderi*; D. *E. caluromydis* (Scale bar 10µm). Black arrow – Stieda body; Grey arrow – Sporocyst residuum; White arrow – Polar granule

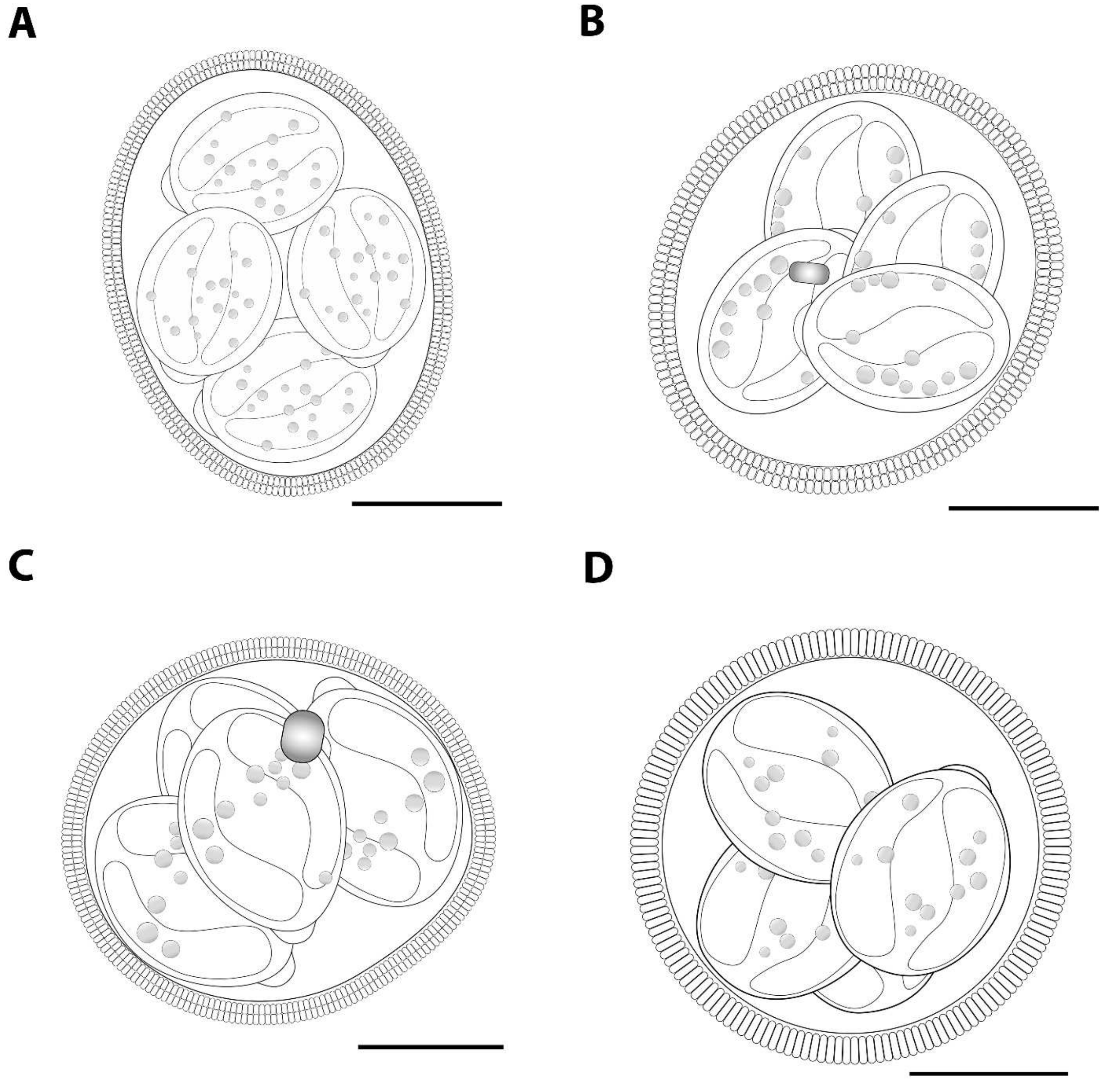


**Figure 2.** Line drawings of A. *E. gambai*; B. *E. auritanensis*; C. *E. philanderi*; D. *E. caluromydis* (Scale bar 10µm)

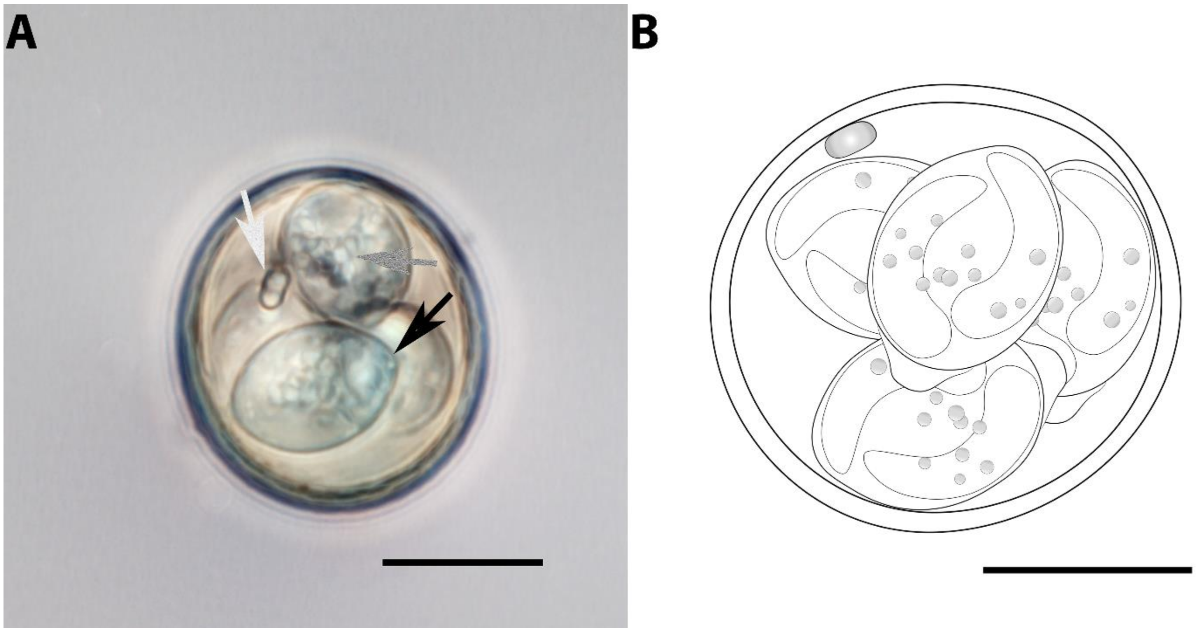


**Figure 3.** A. Sporulated oocyst of *E. vicoensis* n. sp. isolated from the opossum *Dildelphis aurita* in Brazil. Notice the presence of a highly refractile polar granule and the smooth oocyst wall. B. Line drawing of *E. vicoensis* n. sp. (Scale bar 10μm). Black arrow – Stieda body; Grey arrow – Sporocyst residuum; White arrow – Polar granule.

## 4. Discussion

This study assessed the occurrence of *Eimeria* spp. in *D. aurita* opossums from Atlantic forest remnants in Brazil. Five species were detected in the feces of these animals, including the first record of *E. caluromydis*, originally described in *Caluromys philander* (Lainson and Shaw 1989), and the description of a new species. The diversity of *Eimeria* spp. in the black-eared opossum highlights the importance of studies on the incidence and epidemiology of eimerians in these animals, as two of the species we found were first reported in other marsupial species; *E. philanderi* in the *Philander opossum* and *E. caluromydis* in the *Caluromys philander* (Lainson and Shaw 1989). In fact, there is so little known about the coccidia in didelphid marsupials that it is not yet clear whether they are generalists or host specific parasites (Duszynski 2016; Valerio-Campos et al. 2015; Heckscher et al. 1999).

Lainson and Shaw (1989) originally described *E. caluromydis* from the bare-tailed woolly opossum, *Caluromys p. philander* in Brazil and *E. philanderi* from the gray four-eyed opossum, *Philander opossum*, also in Brazil. Fehlbert et al. (2018) recently reported *E. philanderi* from *D. aurita* and the present study confirms that oocysts resembling this species were present in 25 of the opossums we examined. In addition, oocysts of *E.*

*caluromydis* were found in 9 *D. aurita*, being this, the first report of this parasite in another host genus/species. If we are correct, eimerians from these opossums seem to be less host-specific than previously expected.

*Eimeria vicoensis* n. sp. was described in 13/43 animals analyzed in our study. This eimeriid presents distinctive characteristics that allowed us to classify it as a new species. To our knowledge, most *Eimeria* spp. described infecting marsupials of the order Didelphimorfia are characterized by having striated single or double layered oocyst wall, except for *E. didelphydis*, in which this structure is smooth. Due to this parameter, this species is the one that most resemble *E. vicoensis* n. sp. However, individual features such as the presence of a highly refractile polar granule, morphometric parameters (*E. vicoensis* n. sp. is larger than the former), and the spheroidal to subspheroidal shape, differentiate *E. vicoensis* n. sp. from *E. didelphydis*. Additionally, all oocysts of the new species analyzed in our study presented a single oocyst wall, whereas, the former may present 1 or 2 wall layers (Duszynski 2016; Pellérdy 1974; Carini 1936).

All the *Eimeria* spp. herein reported, except for *E. auritanensis* and *E. vicoensis* n. sp., were described in more than one host species in Brazil (Fehlberg et al. 2018; Teixeira et al. 2007; Lainson and Shaw 1989; Carini 1938). These findings suggest a possible lack of host specificity for some components of this genus in South American marsupials. In fact, the coexistence among various marsupial species in wildlife may facilitate the transmission and adaptation of such protozoa to a higher range of individuals. In contrast, it is also believed that in nature, individuals may accidentally become infected by oocysts from feces of natural hosts and develop a “pseudoparasitism” (Barreto et al. 2017). However, further studies are required to elucidate these hypotheses.

## 5. Conclusion

This research provides data on the frequency of *Eimeria* spp. in *Didelphis aurita* as well as the description of a new species infecting this opossum. Results herein obtained demonstrated high infection rates, typically by multiple *Eimeria* species in the population studied. Although none of the animals presented clinical signs caused by *Eimeria* infections, further studies are needed to determine whether or not eimeriid coccidia can damage and cause pathology in the intestines of these opossums.

**Acknowledgements**

The authors acknowledge Professor Frederico Falcão Sales for drawing the scientific ilustrations of oocysts, Professor José Lino Neto who provided support on photomicrography and image measurements, and Professor Gisele Mendes Lessa del Giúdice for providing traps to capture the animals used in our study. Finally, authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), and the Graduate Program in Veterinary Medicine of the Universidade Federal de Viçosa (UFV) for the financial and technical support.

**Compliance with ethical standards**

**Conflict of interest:** The authors declare that they have no conflict of interest.

**Ethical approval:** The use of animals in this study was approved by the Ethics Committee for Animal Experimentation (ECAE) of the Universidade Federal de Viçosa (license number: 80/2018) and by the Biodiversity Information and Authorization System (SISBIO) of the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) under the license number 64930-1.

**References**

Aragón-Pech, R. A., Ruiz-Piña, H. A., Rodríguez-Vivas, R. I., Cuxim-Koyoc, A. D. , Reyes-Novelo, E. (2018). Prevalence, abundance and intensity of eggs and oocysts of gastrointestinal parasites in the opossum *Didelphis virginiana* Kerr, 1792 in Yucatan, Mexico. *Helminthology*, 55 (2): 119-126.

Barreto, W. T. G. B., Viana, L. A., Santos, F. M., Porfírio, G. E. O., Perdomo, A. C., Silva, A. R., Sousa, K. C. M., Oliveira, M. A. C., Herrera, H. M., Andrade, G. B. (2017). New species of *Eimeria* (Apicomplexa: Eimeriidae) from *Thrichomys fosteri* and *Clyomys laticeps* (Rodentia: Echimyidae) of the Brazilian Pantanal. *Parasitology Research*, 116 (11): 2941-2956.

Berto, B. P., McIntosh, D., Lopes, C. W. G. (2014). Studies on coccidian oocysts (Apicomplexa: Eucoccidiorida). *Brazilian Journal of Veterinary Parasitology*, 23 (1): 1-15.

Cabrera, A. A. C., Paula, A. A., Camacho, L. A. B., Marzochi, M. C. A., Xavier, S. C., Silva, V. M., Jansen, A. M. (2003). Canine visceral leishmaniasis in Barra de Guaratiba, Rio de Janeiro, Brazil: assessment of risk factors. *Revista do Instituto de Medicina Tropical de São Paulo*, 45 (2): 79-83.

Carini, A. (1936). *Eimeria didelphydis* n. sp. dell'intestino de *Didelphis aurita. Archivio Italiano di Scienze Medicina Tropical e di Parassitologia (Colon)*, 17 (2): 332-333.

Carini, A. (1938). Mais uma *Eimeria* parasita do intestino do *Didelphis aurita. Arquivos de Biologia*, 22 (1): 61-62.

Chero, J. D., Sáez, G., Mendoza-Vidaurre, C., Iannacone, J., Cruces, C. L. (2017). Helminths of the common opossum *Didelphis marsupialis* (Didelphimorphia: Didelphidae), with a checklist of helminths parasitizing marsupials from Peru. *Revista Mexicana de Biodiversidad*, 88: 560-571.

Duszynski, D. W., Wilber, P. G. (1997). A Guideline for the Preparation of Species Descriptions in the Eimeriidae. *Journal of Parasitology*, 83 (2): 333-336.

Duszynski, D. W. (2016). *The biology and identification of the coccidia (Apicomplexa) of marsupials of the world*. London: Elsevier/Academic Press Inc.

Fehlberg, H. F., Junior, P. A. B., Alvarez, M. R. V., Berto, B. P., Albuquerque, G. R. (2018). *Eimeria* spp. (Apicomplexa: Eimeriidae) of marsupials (Mammalia: Didelphimorphia) in southern Bahia, Brazil. *Brazilian Journal of Veterinary Parasitology*, 27 (4): 604-608.

Frey, J. K., Yates, T. L., Duszynski, D. W., Gannon, W. L., Gardner, S. L. (1992). Designation and curatorial management of type host specimens (symbiotypes) for new parasite species. *Journal of Parasitology*, 78 (5): 930-932.

Gardner, A. L. (2008). *Mammals of South America, Volume I. Marsupials, xenarthrans, shrews, and bats*. Chicago and London: The University of Chicago Press.

Heckscher, S. K., Wickesberg, B. A., Duszynski, D.W., Gardner, S. L. (1999). Three new species of *Eimeria* from Bolivian marsupials. *International Journal for Parasitology*, 29: 275-284.

Hunsaker, D. (1977). Ecology of new world marsupials. In: D. Hunsaker (Ed.), *The biology of marsupials. 3. ed.* (pp. 95-153). New York: Academic Press.

Jansen, A. M. (2002). Marsupiais Didelfídeos: gambás e cuícas. In: A. Andrade, S. C. Pinto, R. S. Oliveira (Ed.) *Animais de Laboratório: criação e experimentação [online].* (pp. 167-173). Rio de Janeiro: FIOCRUZ.

Lainson, R., Shaw, J. J. (1989). Two new species of *Eimeria* and three new species of *Isospora* (Apicomplexa, Eimeriidae) from Brazilian mammals and birds. *Bulletin du Muséum National d'Histoire Naturelle Paris*, 11 (2): 349-365.

Macedo, L. O., Santos, M. A. B., Silva, N. M. M., Barros, G. M. M. R., Alves, L. C., Giannelli, A., Ramos, R. A. N., Carvalho, G. A. (2019). Morphological and epidemiological data on *Eimeria* species infecting small ruminants in Brazil. *Small Ruminant Research*, 171: 37-41.

Morrant, D. S., Petit, S., Schumann, R. (2010). Floral nectar sugar composition and flowering phenology of the food plants used by the western pygmy possum, *Cercartetus concinnus*, at Innes National Park, South Australia. *Ecological Research*, 25: 579-589.

Pellérdy, L. P. (1974). *Coccidia and Coccidiosis, second ed*. Budapest: Akademia Kiado, Berlin, Verlag Paul Parey.

Pestell, A. J. L., Petit, S. (2007). Methods and ethical considerations of pitfall trapping for the western pygmy possum *Cercartetus concinnus* Gould (Marsupialia: Burramyidae), with observations on capture patterns and nest sites. *Wildlife Research*, 34: 296-305.

Pinto, H. A., Mari, V. L. T., Melo, A. L. (2014). *Toxocara cati* (Nematoda: Ascarididae) in *Didelphis albiventris* (Marsupialia: Didelphidae) from Brazil: a case of pseudoparasitism. *Brazilian Journal of Veterinary Parasitology*, 23 (4): 522-525.

Teixeira, M., Rauta, P. D., Albuquerque, G. R., Lopes, C. W. G. (2007). *Eimeria auritanensis* n. sp. and *E. gambai* Carini, 1938 (Apicomplexa: Eimeriidae) from the opossum *Didelphis aurita* Wied-newied, 1826 (Marsupialia: Didelphidae) from southeastern Brazil. *Brazilian Journal of Veterinary Parasitology*, 16 (2): 83-86.

Valerio-Campos, I., Chinchilla-Carmona, M., Duszynski, D. W. (2015). *Eimeria marmosopos* (Coccidia: Eimeriidae) from the opossum, *Didelphis marsupialis*, L., 1758 in Costa Rica. *Comparative Parasitology*, 82: 148-150.

Wilber, P. G., Duszynski, D. W., Upton, S. J., Seville, R. S., Corliss, J. O. (1998). A revision of the taxonomy and nomenclature of the *Eimeria* spp. (Apicomplexa: Eimeriidae) from rodents in the Tribe Marmotini (Sci-uridae). *Systematic Parasitology*, 39: 113-135.

Willis, H. H. (1921). A simple levitation method for the detection of hookworm ova. *The Medical Journal of Australia*, 8: 375-378.

Vrba, V., Pakandl, M. (2015). Host specificity of turkey and chicken Eimeria: Controlled cross-transmission studies and a phylogenetic view. *Veterinary Parasitology*, 208: 118-124.

Zanette, R. A., Silva, A.S., Lunardi, F., Santurio, J.M., Monteiro, S.G. (2008). Occurrence of gastrointestinal protozoa in *Didelphis albiventris* (opossum) in the central region of Rio Grande do Sul state. *Parasitology International*, 57(2): 217-218.

# CHAPTER 4

## Article: Molecular detection of *Toxoplasma gondii* in *Didelphis aurita* Wied-Newied, 1826 opossums (Didelphimorphia: Didelphidae) using real-time PCR

Submitted to the Acta Tropica Journal

**Abstract**

Toxoplasmosis is a worldwide-distributed zoonotic disease of great relevance to public health. The etiological agent, the protozoan *Toxoplasma gondii*, infects a wide variety of vertebrate hosts. Many wildlife species, including marsupials of the genus *Didelphis,* are considered reservoirs of *T. gondii*, which makes them to have important role in the dispersion and maintenance of this parasite in nature. *Didelphis* spp. are synanthropic animals commonly found in urban centers of some regions of Brazil, and serological evidence has demonstrated that these opossums may harbor *T. gondii.* Therefore, the aim of this study is to provide data on the molecular detection of *T. gondii* in *D. aurita* opossums from urban areas of Southeastern Brazil. For this, 57 opossums (48 alive and 9 dead), were captured from January to June 2019. Blood samples and spleen fragments were collected in the animals, and Real Time PCR was performed for the detection of *T. gondii*. 26.32% (15/57) of the animals scored positive, with a frequency of 21.57% (n = 11/51) in blood, and 66.67% (n = 6/9) in spleen samples. BLAST analysis demonstrated 100% identity and 100% cover query with sequences of *T. gondii* available in GenBank database. Data herein reported present great public health importance, since *Didelphis* spp. are usually observed inhabiting close to human dwellings, which facilitates their contact with people and domestic animals, and consequently, the transmission of zoonotic agents, which may include *T. gondii*. However, further studies are needed to elucidate whether or not these opossums play important role in the zoonotic cycle of *T. gondii* in urban areas of Brazil.

**Keywords:** Public health; wildlife; synanthropic animals; protozoa; zoonosis.

## 1. Introduction

*Toxoplasma gondii* is a protozoan that causes a zoonotic disease known as toxoplasmosis. This agent is worldwide distributed and is able to infect a large variety of vertebrate hosts. Mammals of the family Felidae are the definitive hosts, in which infective oocysts are produced and eliminated through feces (Sukthana, 2006). Human infection by this parasite occurs mainly by consumption of uncooked meat containing infective tissue cysts, or by the ingestion of food and water contaminated by oocysts (Elmore et al., 2010; Sukthana, 2006). In addition, the transmission may be congenital from an infected mother to offspring, usually when the infection with *T. gondii* occurs for the first time during pregnancy (Liu et al., 2012; Moncada and Montoya, 2012).

In Brazil, prevalence of toxoplasmosis is high in humans and animals, and varies depending on the host species, epidemic area, socio-cultural habits, and geographical and climate factors (Dubey et al., 2012; Santos et al., 2009). Brazilian children and pregnant women have the highest seroprevalences when compared to other countries worldwide, fluctuating from 19.5% to 84.5% for youngsters and from 36% to 92% for pregnant females (Dubey et al., 2012). Just as humans, Brazilian wild and domestic animals, such as pigs, sheep, goats, cattle, horses, rodents, birds and marsupials, are also reported with high seroprevalences of up to 90% (Gennari et al., 2015; Dubey et al., 2012).

In wildlife, small rodents and birds are commonly infected by *T. gondii*, with prevalences of up to 73% and 71%, respectively (Sukthana, 2006; Tenter et al., 2000). However, serological studies have reported a great variety of wild animal species exposed to this protozoan (Elmore et al., 2012; Dubey et al., 2012), including marsupials of the genus *Didelphis* (Gennari et al., 2015; Siqueira et al., 2013; Yai et al., 2003). These opossums may have important epidemiological role in the cycle of many pathogenic agents due to the anthropogenic behavior they present, which facilitates their participation, along with various species of wild rodents, in the sylvatic cycle of *T. gondii* (Torres-Castro et al., 2016; Dubey et al., 2009).

*Didelphis aurita* is a synanthropic animal commonly found in urban centers of some regions of Brazil, and serological evidence has demonstrated that this opossum may harbor *T. gondii*; however, its role in the transmission cycle has not been stablished (Gennari et al., 2015; Siqueira et al., 2013; Pena et al., 2011). Since in some regions of Brazil *Didelphis* spp. are used as food source and medicine, the risk of acquiring zoonotic diseases through the consumption of their meat should be considered (Carneiro et al., 2019; Barros and Azevedo, 2014; Hanazaki et al., 2009). In this sense, the aim of this study is to provide data on the molecular detection of *T. gondii* in *D. aurita* opossums from urban areas of Southeastern Brazil.

## 2. Material and Methods

*2.1 Study area*

The study was conducted in urban and periurban areas of the municipality of Viçosa (Minas Gerais, Southeastern Brazil, 20°45´14”S and 42°52´54”W). The area is located 650 meters above sea level with annual average temperature varying from 20 to 22 °C. The climate is classified as Cwa (Köppen climate classification), mesothermic, with hot and rainy summers and cold and dry winters.

*2.2 Capture of animals and laboratorial procedures*

Tomahawk live traps (0.45x0.21x0.21m) were armed and checked daily (5PM and 7AM, respectively), in backyards of houses, urban environmental parks and public buildings, from January to June 2019, with a total sample effort of 516 trap-nights. Corn flour, canned fish and banana were used as bait. After capture, the opossums were mechanically contained, classified by sex, age group (pups, subadults and adults), and marked with a small V cut at the right ear to identify recaptures (Morrant et al., 2010; Pestell and Petit, 2007).

Blood samples were collected with a 22G needle coupled in a 3ml syringe, via puncture of the caudal ventral or jugular vein. Blood collection tubes containing EDTA were used to store the collected material. In addition, animals found dead were necropsied to collect spleen samples. All the materials were stored in plastic vials (1.5 ml Eppendorf) and frozen at – 20 ºC until molecular processing.

Genomic DNA from blood and spleen was extracted using a commercial kit (illustra tissue and cells genomicPrep Mini Spin Kit, GE Healthcare Life Sciences) following manufacturer’s recommendations. Real Time Polymerase Chain Reaction (qPCR) was performed using SYBR Green assay (GoTaq® Green Master Mix, Promega, USA), 200 nMol of each primer (5’CACAGAAGGGACAGAAGT3’ and 5’TCGCCTTCATCTACAGTC3’), 4μL of DNA samples and nuclease-free water to complete 20μL of volume. The conditions of amplification for all samples followed initial denaturation of 95 ºC for 2 minutes and 50 cycles of amplification with 95 ºC for 15 seconds for denaturation, and 60 ºC for 1 minute for annealing and extension. The dissociation curve was associated to compare the melting temperature. DNA extracted from a culture cell of *T. gondii* was used as positive control, which was kindly donated by the Biological Science Institute, Parasitology Department of the Federal University of Minas Gerais. For the negative control, nuclease-free water was used.

Positive samples were further tested by conventional PCR, and the amplicons were then purified using PCR Purification Kit (Cellco Biotec) according to manufacturer's instructions. Sanger's method was performed for sequencing amplicons in both directions (Sanger et al., 1977) in an automated sequencer AB 3500 Genetic Analyzer. Sequences were aligned using Mega7 (Kumar et al., 2016) and compared to other sequences available in GenBank Database via Basic Local Alignment Search Tool analysis (BLAST).

*2.3 Data analysis*

Descriptive statistics was performed to calculate relative and absolute frequency of *T. gondii* infections in the animals evaluated. The normality of data was checked using the Lilliefors test. Additionally, the Chi-square test with Yates correction ($\chi^2$) was used to compare *T. gondii* infection in male and females, as well as the ages of infected animals, considering a 5% significance level. All analyzes were carried out using the BioEstat 5.3 software.

**3. Results**

Animals (n = 57) were captured in urban spots of the study area (Figure 1). Out of the 57 opossums (48 captured alive and 9 found dead), 15 (26.32%) scored positive for *T. gondii*. The frequency detected in blood samples was 21.57% (n = 11/51), whereas for spleen samples it was 66.67% (n = 6/9). For three animals found dead, it was possible to collect spleen fragments and blood directly from the heart, two of them scored positive in both spleen and blood samples, and for the other one, only the spleen sample presented *T. gondii* DNA (Table 1). Statistical analysis revealed no significant difference in positivity between male and female (p = 0.3014), neither among the ages of the animals (p = 0.4682).

The BLAST analysis showed 100% identity and 100% cover query with sequences of *T. gondii* available in GenBank database (accession numbers: MH560583.1, MH884741.1, AF146527.1, DQ779195.1).

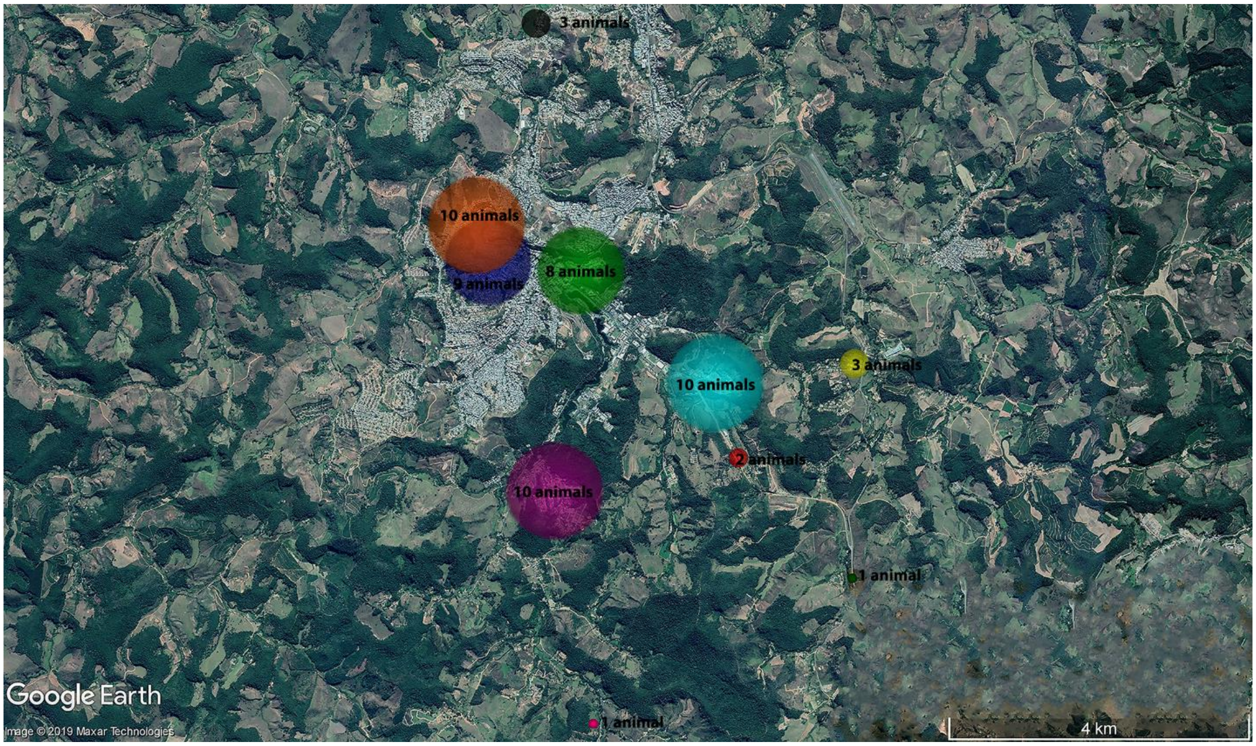


**Figure 1.** Map of the study area where *Didelphis aurita* opossums were captured. Spot size is proportional to the amount of individuals captured in each locality. Notice that most animals were captured in central areas of the municipality of Viçosa, Minas Gerais, Brazil.

**Table 1.** Absolute and relative frequencies of *Toxoplasma gondii* in *Didelphis aurita* according to sex, age and kind of sample analized.

| Age/sex | Captured Animals | + Blood samples RF % (AF/n) | + Spleen samples RF % (AF/n) |
|---|---|---|---|
| Adult male | 12 | 27.27 (3/11) | 50.00 (1/2) |
| Adult female | 17 | 21.43 (3/14) | 33.33 (1/3) |
| Subadult male | 15 | 26.67 (4/15) | 100.00 (2/2) |
| Subadult female | 11 | 10.00 (1/10) | 100.00 (1/1) |
| Pup male | 2 | 0.00 (0/1) | 100.00 (1/1) |
| Pup female | 0 | 0.00 (0/0) | 0.00 (0/0) |
| Total | 57 | 21.57 (11/51) | 66.67 (6/9) |

RF – Relative frequency; AF – Absolute frequency; n – Number of animals

## 4. Discussion

This study assessed the occurrence of *T. gondii* in *D. aurita* opossums captured in urban areas of Southeastern, Brazil, demonstrating that an important percentage of the animals evaluated scored positive for this protozoan. This is an interesting finding to public health, since these animals are commonly found in urban centers and periphery of cities in South America, which imply that they may somehow be involved in the cycle of this agent. Studies involving molecular detection of *T. gondii* in terrestrial and aquatic wildlife have been performed worldwide in a great variety of species demonstrating different prevalence rates that reaches up to 90% (Bachand et al., 2019; Nardoni et al., 2019; Skorpikova et al., 2018; Silva et al., 2018; Lukasova et al., 2018; Hong et al., 2017; Zheng et al., 2016; Calero-Bernal et al., 2015; Fournier et al., 2014; Vitalino et al., 2014). In *D. aurita*, few serological studies have demonstrated prevalences ranging from 3.8% to 20.7% (Horta et al., 2016; Gennari et al., 2015; Siqueira et al., 2013).

Molecular detection of *T. gondii* in *Didelphis* spp. in America was previously reported in liver, brain and heart DNA samples for *D. albiventris*, *D. virginiana* and *D. aurita* opossums, respectively (Richini-Pereira et al., 2016; Torres-Castro et al., 2016; Pena et al., 2011). In our study, a frequency of 26.32% was found in the evaluated animals, which was lower than that found for *D. virginiana* (76.9%) in Mexico (Torres-Castro et al., 2016), and higher than that found for *D. albiventris* (10.0%) in Brazil (Richini-Pereira et al., 2016). Studies performed in the State of São Paulo, Brazil, found seroprevalences for *T. gondii* of 12.5% and 20.7% in *D. aurita* (Horta et al., 2016; Gennari et al., 2015). A similar study performed in the State of Pernambuco, also in Brazil reported a seroprevalence of 3.8% in the same opossum species (Siqueira et al., 2013). Comparing the results of the qPCR analysis of the present study to those of the serological studies, the frequency data herein reported is higher (26.32%). However, factors such as site of capture of the animals, climate conditions of the studied regions and presence of definitive hosts should be considered (Horta et al., 2018; Tenter et al., 2000). In our study, all opossums were captured in urban environments, whereas in previous studies the animals were mostly captured in the Atlantic Forest fragments, except for the work performed by Horta et al. (2016) in which the animals were also from urban environments. Interestingly, in that study a percentage of positive opossums close to that of the current study was detected.

Overall, in American marsupials clinical toxoplasmosis is not observed (Canfield et al., 1990). In contrast, Australian marsupials are highly susceptible to *T. gondii,* and commonly develop the acute form of the disease leading to sudden death (Tenter, 2009). In this study, all animals were clinically healthy when physically evaluated, and the detection of this parasite in a high percentage of the opossums tested reinforces that they harbor the agent but probably do not develop clinical disease. This finding is epidemiologically important, since the presence of this marsupial in human dwellings may favor the zoonotic cycle of *T. gondii* involving *D. aurita*, the domestic cat and people.

The most common form of human infection by *T. gondii* is the consumption of food contaminated with oocysts or ingestion of undercooked meat containing tissue cysts (Jones and Dubey, 2012). In some regions of Brazil, marsupials of the genus *Didelphis* are considered food source for humans, as occur for *D. marsupialis* in the Brazilian Amazon (Barros and Azevedo, 2014), for *D. aurita* in the Southeastern Atlantic Forest coast of Brazil (Hanazaki et al., 2009), and for *D. albiventris* and *D. aurita* in Northeastern, Brazil (Carneiro et al., 2019). In some of these regions, illegal commerce of these marsupial meat products may be observed (Junior et al., 2010). This situation may be a driver to the infection of people with the protozoan, since *Didelphis* spp. are reported to harbor this parasite (Horta et al., 2016; Gennari et al., 2015; Siqueira et al., 2013; Yai et al., 2003).

**5. Conclusion**

The present study demonstrated *T. gondii* infections in *D. aurita* opossums in Southeastern Brazil. Data herein reported present great public health importance, as *Didelphis* spp. are commonly found close to human dwellings, which facilitates their contact with people and domestic animals, and consequently, the transmission of zoonotic agents, including *T. gondii*. Since epidemiological information about the participation of these animals in the zoonotic cycle of this agent is limited, further studies are required to elucidate whether or not they play important role in the transmission of *T. gondii* to humans and domestic animals.

**Acknowledgements**

The authors acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), and the Graduate Program in Veterinary Medicine of the Universidade Federal de Viçosa (UFV) for the support provided. Finally, authors would like to thank

Professor Ricardo Wagner de Almeida Vitor who kindly donate the positive control for *T. gondii*, and Professor Gisele Mendes Lessa del Giúdice for providing traps to capture the animals used in our study. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Declaration of interest**

**Conflict of interest statement**

Authors declare they have no competing interests.

**Animal welfare statement**

This study was approved by the Ethics Committee for Animal Experimentation (ECAE) of the Universidade Federal de Viçosa (license number: 80/2018) and by the Biodiversity Information and Authorization System (SISBIO) of the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA), license number 64930-1.

**References**

Bachand, N., Ravel, A., Leighton, P., Stephen, C., Ndao, M., Avard, E., Jenkins, E., 2019. Serological and molecular detection of *Toxoplasma gondii* in terrestrial and marine wildlife harvested for food in Nunavik, Canada. Parasite. Vector. 12, 155.

Barros, F.B., Azevedo, P.A., 2014. Common opossum (*Didelphis marsupialis* Linnaeus, 1758): food and medicine for people in the Amazon. J. Ethnobiol. Ethnomed. 10, 65.

Calero-Bernal, R., Saugar, J.M., Frontera, E., Pérez-Martín, J.E., Habela, M.A., Serrano, F.J., Reina, D., Fuentes, I., 2015. Prevalence and Genotype Identification of *Toxoplasma gondii* in Wild Animals from Southwestern Spain. J. Wildl. Dis. 51, 233-238.

Canfield, P.J., Hartley, W.J., Dubey, J.P., 1990. Lesions of Toxoplasmosis in Australian Marsupials. J. Comp. Path. 103, 159-167.

Carneiro, I.O., Santos, N.J., Silva, N.S., Lima, P.C., Meyer, R., Netto, E.M., Franke, C.R., 2019. Knowledge, practice and perception of human-marsupial interactions in health promotion. J. Infect. Dev. Ctries. 13, 342-347.

Dubey, J.P., Lago, E.G., Gennari, S.M., Su, C., Jones, J.L., 2012. Toxoplasmosis in humans and animals in Brazil: high prevalence, high burden of disease, and epidemiology. Parasitol. 139, 1375-1424.

Dubey, J.P., Velmurugan, G.V., Alvarado-Esquivel, C., Alvarado-Esquivel, D., Rodriguez-Peña, S., Martinez-Garcia, S., González-Herrera, A., Ferreira, L.R., Kwok, O.C.H., Su, C., 2009. Isolation of *Toxoplasma gondii* from Animals in Durango, Mexico. J. Parasitol. 95, 319-322.

Elmore, S.A., Jenkins, E.J., Huyvaert, K.P., Polley, L., Root, J.J., Moore, C.G., 2012. *Toxoplasma gondii* in Circumpolar People and Wildlife. Vector. Borne. Zoonotic. Dis. 12, DOI: 10.1089/vbz.2011.0705

Elmore, S.A., Jones, J.L., Conrad, P.A., Patton, S., Lindsay, D.S., Dubey, J.P., 2010. *Toxoplasma gondii*: epidemiology, feline clinical aspects, and prevention. Trends. Parasitol. 26, 190-196.

Fournier, G.F.S.R., Lopes, M.G., Marcili, A., Ramirez, D.G., Acosta, I.C.L., Ferreira, J.I.G.S., Cabral, A.D., Lima, J.T.R., Pena, H.F.J., Dias, R.A., Gennari, S.M., 2014. *Toxoplasma gondii* in domestic and wild animals from forest fragments of the municipality of Natal, northeastern Brazil. Braz. J. Vet. Parasitol. 23, 501-508.

Gennari, S.M., Ogrzewalska, M.H., Soares, H.S., Saraiva, D.G., Pinter, A., Nieri-Bastos, F.A., Labruna, M.B., Szabó, M.P.J., Dubey, J.P., 2015. *Toxoplasma gondii* antibodies in wild rodents and marsupials from the Atlantic Forest, state of São Paulo, Brazil. Braz. J. Vet. Parasitol. 24, 379-382.

Hanazaki, N., Alves, R.R.N., Begossi, A., 2009. Hunting and use of terrestrial fauna used by Caiçaras from the Atlantic Forest coast (Brazil). J. Ethnobiol. Ethnomed. 5, 36.

Hong, S., Kim, H., Jeong, Y., Cho, S., Lee, W., Kim, J., Lee, S., 2017. Serological and Molecular Detection of *Toxoplasma gondii* and *Babesia microti* in the Blood of Rescued Wild Animals in Gangwon-do (Province), Korea. Korean. J. Parasitol. 55, 207-212.

Horta, M.C., Guimarães, M.F., Arraes-Santos, A., Araujo, A.C., Dubey, J.P., Labruna, M.B., Gennari, S.M., Pena, H.F.J., 2018. Detection of anti-*Toxoplasma gondii* antibodies in small wild mammals from preserved and non-preserved areas in the Caatinga biome, a semi-arid region of Northeast Brazil. Vet. Parasitol. Reg. Stud. Reports. 14, 75-78.

Horta, M.C., Ragozo, A.M.A., Casagrande, R.A., Matushima, E.R., Souza, G.O., Morais, Z.M., Vasconcelos, A.S., Gennari, S.M., 2016. Occurrence of anti-*Toxoplasma gondii*, *Neospora caninum* and *Leptospira* spp. antibodies in opossums (*Didelphis* spp.) in São Paulo State, Brazil. Braz. J. Vet. Res. Anim. Sci. 53, 1-9.

Junior, P.C.B., Guimarães, D.A., Pendu, Y.L., 2010. Non-legalized commerce in game meat in the Brazilian Amazon: a case study. Rev. Biol. Trop. 58 (3): 1079-1088.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol. 33, 1870-1874.

Liu, Q.L., Singla, L.D., Zhou, H., 2012. Vaccines against *Toxoplasma gondii*. Hum. Vaccin. Immunother. 8, 1305-1308.

Lukasova, R., Marková, J., Bartova, E., Murat, J., Sedlak, K., 2018. Molecular Evidence of *Toxoplasma gondii*, *Neospora caninum*, and *Encephalitozoon cuniculi* in Red Foxes (*Vulpes vulpes*). J. Wildl. Dis. 54, 825-828.

Moncada, P.A., Montoya, J.G., 2012. Toxoplasmosis in the fetus and newborn: an update on prevalence, diagnosis and treatment. Expert. Rev. Anti. Infe. Ther. 10, 815-828.

Morrant, D.S., Petit, S., Schumann, R., 2010. Floral nectar sugar composition and flowering phenology of the food plants used by the western pygmy possum, *Cercartetus concinnus*, at Innes National Park, South Australia. Ecol. Res. 25, 579-589.

Nardoni, S., Rocchigiani, G., Varvaro, I., Altomonte, I., Ceccherelli, R., Mancianti, F., 2019. Serological and Molecular Investigation on *Toxoplasma gondii* Infection in Wild Birds. Pathog. 8, 58.

Pena, H.F.J., Marvulo, M.F.V., Horta, M.C., Silva, M.A., Silva, J.C.R., Siqueira, D.B., Lima, P.A.C.P., Vitaliano, S.N., Gennari, S.M., 2011. Isolation and genetic characterisation of *Toxoplasma gondii* from a red-handed howler monkey (*Alouatta belzebul*), a jaguarundi (*Puma yagouaroundi*), and a black-eared opossum (*Didelphis aurita*) from Brazil. Vet. Parasitol. 175, 377-381.

Pestell, A.J.L., Petit, S., 2007. Methods and ethical considerations of pitfall trapping for the western pygmy possum *Cercartetus concinnus* Gould (Marsupialia: Burramyidae), with observations on capture patterns and nest sites. Wildlife. Res. 34, 296-305.

Richini-Pereira, V.B., Marson, P.M., Silva, R.C., Langoni, H., 2016. Genotyping of *Toxoplasma gondii* and *Sarcocystis* spp. in road-killed wild mammals from the Central Western Region of the State of São Paulo, Brazil. Rev. Soc. Bras. Med. Trop. 49, 602-607.

Sanger, F., Nicklen S., Coulson A.R. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. 74, 5463–5467.

Santos, T.R., Costa, A.J., Toniollo, G.H., Luvizotto, M.C.R., Benetti, A.H., Santos, R.R., Matta, D.H., Lopes, W.D.Z., Oliveira, J.A., Oliveira, G.P., 2009. Prevalence of anti-*Toxoplasma gondii* antibodies in dairy cattle, dogs, and humans from the Jauru micro-region, Mato Grosso state, Brazil. Vet. Parasitol. 161, 324-326.

Silva, M.A., Pena, H.F.J., Soares, H.S., Aizawa, J., Oliveira, S., Alves, B.F., Souza, D.S., Melo, R.P.B., Gennari, S.M., Mota, R.A., Silva, J.C.R., 2018. Isolation and genetic characterization of *Toxoplasma gondii* from free-ranging and captive birds and mammals in Pernambuco state, Brazil. Braz. J. Vet. Parasitol. 27, 481-487.

Siqueira, D.B., Aléssio, F.M., Mauffrey, J.F., Marvulo, M.F., Ribeiro, V.O., Oliveira, R.L., Pena, H.F., Gennari, S.M., Mota, R.A., Faustino, M.A., Alves, L.C., Dubey, J.P., Silva, J.C., 2013. Seroprevalence of *Toxoplasma gondii* in wild marsupials and rodents from the Atlantic forest of Pernambuco state, northeastern region, Brazil. J. Parasitol. 99, 1140-1143.

Skorpikova, L., Reslova, N., Lorencova, A., Plhal, R., Drimaj, J., Kamler, J., Slany, M., 2018. Molecular detection of *Toxoplasma gondii* in feathered game intended for human consumption in the Czech Republic. Int. J. Food. Microbiol. 286, 75-79.

Sukthana, Y., 2006. Toxoplasmosis: beyond animals to humans. Trends. Parasitol. 22, 137-142.

Tenter, A.M., 2009. *Toxoplasma gondii* in animals used for human consumption. Mem. Inst. Oswaldo. Cruz. 104, 364-369.

Tenter, A.M., Heckeroth, A.R., Weiss, L.M., 2000. *Toxoplasma gondii*: from animals to humans. Int. J. Parasitol. 30, 1217-1258.

Torres-Castro, M., Noh-Pech, H., Puerto-Hernández, R.P., Reyes-Hernández, B., Panti-May, A., Hernández-Betancourt, S., Yeh-Gorocica, A., González-Herrera, L., Zavala-Castro, J., Puerto, F.I., 2016. First molecular evidence of *Toxoplasma gondii* in opossums (*Didelphis virginiana*) from Yucatan, Mexico. Open. Vet. J. 6, 57-61.

Vitalino, S.N., Soares, H.S., Minervino, A.H.H., Santos, A.L.Q., Werther, K., Marvulo, M.F.V., Siqueira, D.B., Pena, H.F.J., Soares, R.M., Su, C., Gennari, S.M., 2014. Genetic characterization of *Toxoplasma gondii* from Brazilian wildlife revealed abundant new genotypes. Int. J. Parasitol. Parasites. Wildl. 3, 276-283.

Yai, L.E.O., Cañon-Franco, W.A., Geraldi, V.C., Summa, M.E.L., Camargo, M.C.G.O., Dubey, J.P., Gennari, S.M., 2003. Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* Antibodies in the South American Opossum (*Didelphis marsupialis*) From the City of São Paulo, Brazil. J. Parasitol. 89, 870-871.

Zheng, W., Zhang, X., Ma, J., Li, F., Zhao, Q., Huang, S., Zhu, X., 2016. Molecular Detection and Genetic Characterization of *Toxoplasma gondii* in Farmed Minks (*Neovison vison*) in Northern China by PCR-RFLP. PLOS. One. 11, e0165308.