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Profile of Enteric Parasites of Opossums and Small Wild Rodents in Region of Londrina, Paraná, Brazil

Perfil dos parasitos Entéricos de Gambás e Pequenos Roedores Silvestres em Região de Londrina, Paraná, Brasil

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Rafael Alves Santomauro: Universidade Estadual Paulista "Júlio de Mesquita Filho". SP, Brasil. E-mail: rafaelsantomauro@gmail.com

Mariana da Silva Marques: Universidade Estadual de Londrina. PR, Brasil.

João Gabriel Neves Viana: Universidade Estadual de Londrina, Londrina PR, Brasil.

Tayná Caroline Diniz Esgote: Universidade Estadual de Londrina PR, Brasil.

Gabriel Soares Hengles: Universidade Estadual de Londrina PR, Brasil.

Isabelli Sayuri Kono: Universidade Estadual de Londrina PR, Brasil.

Juliana Izidoro Lucas: Universidade Estadual de Londrina PR, Brasil.

Julian Andrade Santos: Universidade Estadual de Londrina, Londrina, Paraná, Brazil.

Pablo Menegon Castilho: Universidade Estadual de Londrina, Londrina, Paraná, Brazil.

Eloiza Teles Caldart: Universidade Estadual de Londrina PR, Brasil.

Fernanda Pinto-Ferreira: Universidade Estadual de Londrina, PR, Brasil.

Abstract
The parasitological analysis of synanthropic animals is an important tool in studying disease prevalence by region, as it can elucidate key epidemiological links arising from the coexistence of these species with humans. This study aimed to characterize the parasitological profile of opossums' (*Didelphis albiventris*) and small rodents' degradation (*Rattus rattus*) in native forest areas in western Londrina, Paraná, from October 2019 to January 2020. Six animals were captured in valley bottom

areas of the municipality using cage traps, Sherman, and Tomahawk models, with attractive baits. After capture, the animals were euthanized, necropsied, and fecal samples were collected directly from intestinal segments, without distinguishing anatomical portions. For parasitological analysis, spontaneous sedimentation and centrifugal-flotation techniques were applied. The results revealed the presence of enteric parasites, including *Ancylostoma* sp., *Trichuris* sp., *Toxocara* sp., *Toxascaris leonina*, *Taenia* sp., *Capillaria* sp., *Ascaris* sp., *Giardia* sp., *Physaloptera* sp., and *Cruzia tentaculata*. These findings highlight how close interactions between wildlife and urban environments can facilitate the sharing of parasites not only among wild species but also with humans and domestic animals, posing potential risks to public health. This study contributes to the understanding of parasitic transmission dynamics in urban-adjacent ecosystems and underscores the importance of monitoring zoonotic risks associated with wildlife in populated regions.

Keywords: *Didelphis albiventris.* Parasites, *Rattus rattus.* Synanthropy.

Resumo
A análise parasitológica de animais sinantrópicos constitui ferramenta importante no estudo da prevalência de doenças por região, pois pode elucidar importantes vínculos epidemiológicos criados a partir da convivência das espécies com os humanos. Este estudo teve como objetivo caracterizar o perfil parasitológico das fezes de gambás (*Didelphis albiventris*) e pequenos roedores (*Rattus rattus*) em áreas de mata nativa no oeste de Londrina, Paraná, durante o período de outubro de 2019 a janeiro de 2020. Foram capturados seis animais em áreas de fundo de vale do município, utilizando armadilhas do tipo gaiola, modelos Sherman e Tomahawk, com iscas atrativas. Após a captura, os animais foram eutanasiados e necropsiados, e amostras fecais foram coletadas diretamente dos segmentos intestinais, sem distinção de porção anatômica. Para as análises parasitológicas, foram aplicadas as técnicas de sedimentação espontânea e centrífugo-flutuação. Os resultados mostraram a presença de diversos enteroparasitos, como *Ancylostoma* sp., *Trichuris* sp., *Toxocara* sp., *Toxascaris leonina*, *Taenia* sp., *Capillaria* sp., *Ascaris* sp., *Giardia* sp., *Physaloptera* sp. e *Cruzia tentaculata*. Esses achados evidenciam que a proximidade entre fauna silvestre e áreas urbanas pode facilitar o compartilhamento de parasitos, não só entre espécies silvestres, mas também com humanos e animais domésticos, representando um risco potencial para a saúde pública. Este estudo contribui para a compreensão das dinâmicas de transmissão parasitária em ecossistemas próximos a áreas urbanas e reforça a importância de monitorar os riscos zoonóticos associados à fauna silvestre em regiões habitadas.

Palavras-chave: *Didelphis albiventris.* Parasitos *Rattus rattus.* Sinantropia.

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1 Introduction

With the expansion of cities, contact and interaction between humans and wildlife has become increasingly common. This interaction is mainly driven by the destruction of forest fragments, as well as food and water sources, which compels animals to use human resources for their survival, thus creating a synanthropic relationship (Barbosa *et al.*, 2014). However, this coexistence facilitates the transmission of diseases between different animal species and humans.

Opossums of the species *Didelphis albiventris* (white-eared opossum) have generalist feeding habits. As omnivores, they feed on fruits, seeds, small mammals, birds, reptiles, amphibians, and small mollusks. Ecologically, they play an important role as seed dispersers (Lourenço *et al.*, 2024).

As they approach urban environments and adapt to this reality, interactions between human life and animals of this genus may lead to an increase in certain parasites in these animals.

Rodents of the species *Rattus rattus* (roof rat), in turn, have a worldwide distribution, frequently inhabiting human dwellings and adapting to these environments in order to benefit from the resources needed for their survival. They are known to be significant vectors of parasites relevant to public health (Lima *et al*., 2021).

In the state of Paraná, both species are present and may act as disseminators of diseases among wild animals, domestic animals, and humans due to their synanthropic characteristics, which allow them to inhabit both urban areas and more preserved regions (Caldart *et al*., 2017; Dario *et al*., 2022).

Parasitological analysis of these animals is an important tool in studying the regional prevalence of diseases, as it can help elucidate significant epidemiological links resulting from the coexistence of these species with humans. Therefore, this study aimed to characterize the profile of enteric parasites in opossums and small rodents originating from native forest in the western region of Londrina, Paraná.

2 Material and Methods

This study was authorized by the Ethics Committee on Animal Use of Universidade Estadual de Londrina (CEUA/UEL) under protocol number 124/2016, extended by official letter number 73/2019.

Between October 2019 and January 2020, ten animal capture attempts were carried out using Tomahawk and Sherman traps in six different riparian forest areas near human dwellings and watercourses in the western region of the municipality of Londrina. These traps operate through a pressure-activated mechanism triggered when the animal exerts force upon entering.

Captured animals were euthanized according to the protocol recommended by CONCEA (National Council for the Control of Animal Experimentation): standardized anesthetics Xylazine and Ketamine were administered for *Rattus rattus* (40–90 mg/kg of Ketamine Hydrochloride and 5 mg/kg of Xylazine) and *Didelphis albiventris* (20–30 mg/kg of Ketamine Hydrochloride and 5 mg/kg of Xylazine). After anesthesia and sedation, euthanasia was performed using the exsanguination method via intracardiac puncture. Subsequently, necropsies were performed, and feces were collected from intestinal segments and stored in polypropylene containers under refrigeration for later parasitological examination.

To detect heavy eggs, the spontaneous sedimentation technique (Hoffman; Pons; Janer, 1934) was used with adaptations: two to five grams of feces were homogenized in 50 mL of distilled water using a glass rod. The solution was filtered through gauze and transferred to a sedimentation glass.

After 40 minutes, the sediment was collected with a Pasteur pipette, placed on a glass slide with a coverslip, and examined under light microscopy (10X magnification).

Light eggs were detected using flotation techniques (Willis, 1921; Faust *et al.*, 1939). For the first method, two grams of feces were added to a glass tube and homogenized in a saturated sodium chloride solution. The tube was filled until a meniscus formed at the surface. A glass slide was placed over the meniscus and left for 15 minutes. After that time, the slide was removed, inverted, and examined under light microscopy (10X magnification). For the second method, two grams of feces were homogenized in 15 mL of distilled water in a plastic cup. The mixture was filtered through gauze and centrifuged at 2,100 rpm for two minutes. The samples were washed, and the pellet was resuspended in a magnesium sulfate ($MgSO_4$) solution and centrifuged again at 2,100 rpm for eight minutes. A meniscus was formed, and a glass slide was placed on top, stained with a drop of Lugol's solution, and observed under light microscopy (40X magnification).

The modified Ziehl-Neelsen technique (Henriksen; Pohlens, 1981) was used to identify *Cryptosporidium* spp. in fecal samples. A fecal smear was prepared on a glass slide and left to air dry at room temperature. Then, carbol fuchsin solution was applied, and the slide was gently heated until vapors formed to allow the oocysts' primary staining. After this period, the excess stain was drained off, and the slide was rinsed with running water. A 5% aqueous sulfuric acid solution was applied for 30 seconds to remove excess fuchsin from the slide background. The slide was then rinsed, drained, and counterstained with 0.3% methylene blue solution for one minute, staining the background blue and facilitating oocyst visualization. Finally, the slide was rinsed again with running water, air-dried at room temperature, and examined under light microscopy using 100X magnification with an oil immersion lens.

3 Results and Discussion

Six animals were captured at four of the six sampling sites: four *Didelphis albiventris* and two *Rattus rattus*.

Using the Willis technique, 83.3% (5/6) of the animals tested positive, with the main parasites identified being *Ancylostoma* sp., *Trichuris* sp., and *Cruzia tentaculata*. In the Faust *et al*. (1939) test, 33.3% (2/6) tested positive for *Giardia* sp. In the Hoffman, Pons and Janer (1934) test, 50.0% (3/6) of the animals showed the presence of parasites: cestode eggs in opossums, and *Physaloptera* sp. in the rodent (Table 1). In the modified Ziehl-Neelsen technique (Henriksen; Pohlens, 1981), none of the animals tested positive for *Cryptosporidium* sp.

Table 1 - Results of coproparasitological tests performed on Didelphis albiventris and Rattus rattus captured in native forest areas in the western region of Londrina, Paraná, from October 2019 to January 2020, using the techniques of Willis (1921), Faust *et al*. (1939), and Hoffman, Pons and Janer (1934)

Species of Animal	**Parasites Identified**		
	Willis	**Faust *et al.***	**Hoffman *et al.***
D. albiventris (1)	*Ancylostoma* sp. *Cruzia tentaculata Trichuris* sp. *Toxascaris leonina* *Toxocara* sp.	Absent	Cestode
D. albiventris (2)	*Ancylostoma* sp. Cestódeo *Cruzia tentaculata Toxocara* sp. *Trichuris* spp.	Absent	Absent
D. albiventris (3)	*Ancylostoma* sp. *Cruzia tentaculata Trichuris* sp.	Absent	Absent
D. albiventris (4)	*Ancylostoma* sp. *Capillaria* sp. *Cruzia tentaculata*	Absent	Absent
R. rattus (1)	*Ancylostoma* sp. *Ascaris* sp.	*Giardia* sp.	*Giardia* sp.
R. rattus (2)	Ausência	*Giardia* sp.	Absent

Source: research data.

Parasites of the genus *Ancylostoma* were found in all opossums (4/4, 100%) and one of the *Rattus rattus* specimens (1/2, 50%) captured. This parasite is a soil-transmitted helminth of zoonotic importance, responsible for cutaneous larva migrans, often acquired in areas frequented by dispersers such as dogs (Rodriguez-Morales *et al*., 2021). In a study conducted with *Didelphis aurita*, a prevalence of 65.3% was found, suggesting that these opossums may act as new hosts in the zoonotic cycle in urban areas (Bitencourt; Bezerra, 2020). The animals in this study were captured in valley-bottom areas bordering human dwellings and shared their habitat with stray domestic animals, supporting the hypothesis that the infection occurred through ingestion of larvae in these locations.

Cruzia tentaculata was found in all the captured opossums (4/4, 100%). A study by Silva and Costa (1999), which aimed to clarify the helminth fauna of *D. albiventris*, reported a prevalence of 91.0% for this parasite, demonstrating a high infection rate. In our study, all four *D. albiventris* individuals tested positive for *C. tentaculata*, supporting the hypothesis that this species may be an important host. However, further studies are needed to better understand the epidemiology and life cycle of this parasite.

The ascarid *Toxocara* sp. was found in two *D. albiventris* individuals (2/4, 50%). In a study conducted in Minas Gerais with the same species, *Toxocara cati* eggs were found in feces, but a repeated analysis three days later showed a significant reduction in egg count, suggesting the possibility of pseudoparasitism (Pinto; Mati; Melo, 2014). The animals in the current study were captured near human dwellings and possibly shared resources with domestic animals. By ingesting feces from these animals, they may have become infected and served only as dispersers of the eggs, unable to support the parasite's development into adulthood. The same applies to *Toxascaris leonina*, found in one opossum (1/4, 25%). This parasite, similar to *Toxocara* sp., is an ascarid commonly found in wild canids and felids, but it can also infect domestic animals (Rostami *et al.*, 2020).

Trichuris sp. was detected in three opossums (3/4, 75%). The animals eliminate the parasite eggs in feces, and water can play a significant role in their dispersal (Camelo *et al.*, 2020). The animals were captured in peri-urban areas near watercourses, which may explain the infection, considering that basic sanitation is not universally available in many regions.

Ascaris sp. is among the most prevalent intestinal helminths infecting humans, especially in areas with poor sanitation, facilitating parasite transmission (Teixeira *et al.,* 2020). Hosts shed parasite eggs in their feces, and infection occurs through the ingestion of contaminated water or food. In our study, this parasite was found in one *R. rattus* individual (1/2, 50%). It is important to highlight that the animals were captured in forested areas bordering urban zones, near watercourses, and during summer - conditions that favor egg maturation and infectivity.

*Physaloptera sp. was found in only one R. rattus specimen (1/2, 50%).** In an experiment aiming to elucidate the life cycle of *Physaloptera hispida*, a parasite of the cotton rat (*Sigmodon hispidus*), infection occurred via ingestion of second-stage larvae, acquired by consuming intermediate hosts, such as insects (Schell, 1952). The generalist feeding habits of the infected *R. rattus* suggest it may consume insects and potential intermediate hosts, contributing to environmental dissemination of the parasite.

Cestode eggs were found in two *D. albiventris* individuals (2/4, 50%). Although the species was not identified, some cestodes have zoonotic potential, such as *Hymenolepis diminuta* and *H. nana*, which have been previously identified in the same species under study, in the northwest region of Paraná (Rodriguez *et al.*, 2017).

Capillaria sp. was identified in one *D. albiventris* individual (1/4, 25%). The main routes of infection are through ingestion of parasite eggs in the environment or consumption of infected host organs (Simões *et al.*, 2014). Given that the studied animals shared space with potential hosts such as domestic dogs and cats, infection could have occurred easily.

Among the coproparasitological tests conducted, *Giardia* spp. cysts were observed only in the test by Faust *et al*. in two *R. rattus* individuals (2/2, 100%). Since this technique involves centrifuge-flotation to detect protozoan cysts and light helminth eggs, it is more likely to diagnose *Giardia* spp. than the other tests used in this study. A study by Cardoso *et al*. (2018), with 41 dog fecal samples collected from Laranjal Beach, in Pelotas (Rio Grande do Sul), compared the techniques by Faust and Hoffmann for detecting *Giardia* spp. cysts. Of the 41 samples, nine tested positive using the Faust method and seven using the Hoffmann method, corresponding to 22% and 17% positivity, respectively. The authors concluded that, overall, the Faust *et al*. (1939) technique is more effective than the Hoffman, Pons and Janer (1934) method for diagnosing this protozoan genus.

Waterborne transmission of parasite cysts is the most important transmission route. Therefore, capturing animals near watercourses and dwellings may indicate a potential source of infection for both animals and humans (Thompson, 2000). In a comparative study conducted with *Rattus* spp. from a zoo in Lima, Peru, it was found that the percentage of infected animals increases with factors related to basic sanitation (Casana *et al*., 2019). This finding highlights the significant role of water in parasite transmission and suggests that the infection in the animals in this study may have occurred via ingestion of environmental cysts, mainly acquired through contact with contaminated surroundings.

All fecal samples from the collected animals tested negative for *Cryptosporidium* spp. Despite the negative results, these animal species are considered wild reservoirs of the parasite and pose a public health concern, especially as they increasingly inhabit areas near human residences (Bitencourt; Bezerra, 2021; Hancke; Suárez, 2022).

4 Conclusion

Enteroparasites of public health importance were identified in opossums and synanthropic rodents in Londrina, Paraná, including *Ancylostoma* sp., *Trichuris* sp., *Toxocara* sp., *Toxascaris leonina*, *Taenia* sp., *Capillaria* sp., *Ascaris* sp., *Giardia* sp., *Physaloptera* sp., and *Cruzia tentaculata*.

Given the proximity of these animals to urban areas, watercourses and soil may serve as important routes for the dissemination of these etiological agents, potentially affecting other regions of the municipality. Therefore, further studies on these animal species are recommended in the north-central region of the state of Paraná.

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