# Gastrointestinal parasites of common opossums (*Didelphis marsupialis*) in urban protected areas of Antioquia, Colombia

***Parásitos gastrointestinales de la zarigüeya común (Didelphis marsupialis) en áreas protegidas urbanas en Antioquia, Colombia***

***Parasitas gastrointestinais do gambá comun (Didelphis marsupialis) em áreas protegidas urbanas em Antioquia, Colômbia***


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**Abstract**

**Background:** The common opossum (*Didelphis marsupialis*) is a generalist species that hosts several parasites. Nevertheless, no information is available on the specific parasites that this species may host when it inhabits urban areas. **Objective:** To evaluate the presence and frequency of gastrointestinal parasites of urban opossums in the Aburrá Valley, Antioquia, Colombia. **Methods:** A total of 70 opossums were captured in six protected urban areas using Tomahawk traps during six months. From these animals we obtained 65 fecal samples which were fixed wit SAF solution and analyzed with direct smear and sedimentation techniques. **Results:** Sixty-one samples were positive for, at least, one parasite (93.9%) and 60 were positive for two or more parasites, averaging five parasites per animal. We observed nematodes in 58 samples (89.2%), protozoa in 46 samples (70.7%), acanthocephalans in 29 samples (44.6%), and cestodes in eight samples (12.3%). The most frequent parasite was *Aspidodera* spp. in 80% of the samples. In addition, this is the first study reporting the presence of eggs of *Toxocara* spp., *Hymenolepis* spp., an Oxyuridae parasite, and coccidians *Eimeria didelphidis*, *E. caluromydis*, and *E. marmosopos* in *D. marsupialis*. The latter is also reported in Colombia for the first time. *Toxacara* spp. is common to dogs and cats, while *Hymenolepis* and Oxiuridae are common to rodents. We found no association between parasites and sex or life stage of opossums, nor to the urban area of origin. **Conclusions:** Urban opossums in the Aburrá Valley host a high frequency and diversity of gastrointestinal parasites compared to other areas in Latin America. Some parasites are reported here for the first time.



**Keywords**: *Coinfection; Didelphis marsupialis; helminthiasis; marsupialia; parasitic diseases; parasites; opossum; protected areas; urban parks; wildlife.*

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**Resumen**

**Antecedentes:** La zarigüeya común (*Didelphis marsupialis*) es una especie generalista que ha sido reportada como hospedador de varios parásitos, pero no existe información sobre los parásitos que infectan esta especie en áreas urbanas. **Objetivo:** Evaluar la presencia y frecuencia de los parásitos gastrointestinales de zarigüeyas urbanas en el Valle de Aburrá, Antioquia, Colombia. **Métodos:** Se capturaron un total de 70 zarigüeyas en seis áreas urbanas protegidas utilizando trampas Tomahawk durante seis meses. De estas zarigüeyas se obtuvieron 65 muestras de materia fecal, las cuales fueron fijadas con solución SAF y posteriormente analizadas con las técnicas de extendido en placa y sedimentación. **Resultados:** Sesenta y una muestras fueron positivas para al menos un parásito (93,9%) y 60 fueron positivas para dos o más parásitos, con un promedio de cinco parásitos por animal. Específicamente, observamos parásitos nematodos en 58 muestras (89,2%), protozoos en 46 muestras (70,7%), acantocéfalos en 29 muestras (44,6%) y céstodos en ocho muestras (12,3%). El parásito más frecuente fue *Aspidodera* spp. en el 80% de las muestras fecales. Se reporta por primera vez en *D. marsupialis*, la presencia de huevos de *Toxocara* spp., *Hymenolepis* spp., un parásito Oxyuridae, y los coccidios *Eimeria didelphidis*, *E. caluromydis* y *E. marmosopos*, este último también reportado por primera vez en Colombia. *Toxacara* spp. es común en perros y gatos, e *Hymenolepis* y Oxiuridae es común en roedores. No encontramos evidencia de asociación entre los parásitos y el sexo o estadio de vida de las zarigüeyas, ni tampoco con el área urbana de origen. **Conclusiones:** Las zarigüeyas urbanas del Valle de Aburrá son portadoras de una alta frecuencia y diversidad de parásitos gastrointestinales en comparación con otras áreas de Latinoamérica. Este estudio reporta algunos parásitos por primera vez.

**Palabras clave:** *Áreas protegidas; coinfección; fauna silvestre; helmintiasis; Didelphis marsupialis; marsupiales; parasitosis; parásitos; parques urbanos; zarigüeya.*

**Resumo**

**Antecedentes:** O gambá comum (*Didelphis marsupialis*) é uma espécie generalista que tem sido relatada como hospedeira de diversos parasitas, mas não há informações sobre os parasitas que esta espécie pode hospedar em áreas urbanas. **Objetivo:** avaliamos a presença e frequência de parasitas gastrointestinais de gambás urbanos no Vale de Aburrá, Antioquia, Colômbia. **Métodos:** Um total de 70 gambás foram capturados em seis áreas urbanas protegidas usando armadilhas Tomahawk durante 6 meses. Destes gambás obtivemos 65 amostras fecais, que foram fixadas com solução SAF e analisadas com técnicas de esfregaço direto e sedimentação. **Resultados:** Das 65 amostras fecais obtidas 61 foram positivas para pelo menos um parasita (93,9%) e 60 foram positivas para dois ou mais parasitas, com média de cinco parasitas por animal. Especificamente, observamos parasitas nematóides em 58 amostras (89,2%), protozoários em 46 amostras (70,7%), acantocéfalos em 29 amostras (44,6%) e cestóides em oito amostras (12,3%). O parasita mais frequente foi *Aspidodera* spp. em 80% das amostras fecais. Relatamos, pela primeira vez em *D. marsupialis*, a presença de ovos de *Toxocara* spp., *Hymenolepis* spp., um parasita Oxyuridae, e dos coccídeos *Eimeria didelphidis*, *E. caluromydis* e *E. marmosopos*, esta última também relatada na Colômbia para a primeira vez. *Toxacara* spp. é comum em cães e gatos, e *Hymenolepis* e Oxiuridae comuns em roedores. Não encontramos evidências de associações entre parasitas e sexo ou estágio de vida dos gambás, nem com área urbana. **Conclusões:** Os gambás urbanos do Vale do Aburrá são portadores de alta frequência e diversidade de parasitas gastrointestinais em comparação com outras áreas do América latina. Este estudo relata alguns parasitas pela primeira vez.

**Palavras-chave:** *Áreas protegidas; coinfecção; Didelphis marsupialis; fauna silvestre; gambá; helmintíase; parasitoses; parasitas; marsupiais; parques urbanos.*

## Introduction

The common opossum (*Didelphis marsupialis*) is a generalist species. It has an omnivorous diet and can adapt to varied food resources including fruits, vegetables, invertebrates, small vertebrates, and carrion (Flórez-Oliveros and Vivas-Serna, 2020). Opossums are also part of the life cycle of a variety of microorganisms. They are tolerant to disturbed areas and can invade urban environments submitted to several perturbation levels (Méndez, 1983). A recent study found that more opossums can be found in urban parks than in peri-urban forests (Rodríguez *et al.*, 2022). Urban parks are visited by people and their pets; however, scarce information is available on the pathogens or parasites opossums may host or potentially transmit to humans and domestic animals (Gamboa-Osorio, 2020). This is important not only for the wellbeing of opossums but also because pathogens can jump the species barrier causing emergent diseases (Hassell *et al.*, 2017; WOAH, 2021).

Opossums can be the definitive, intermedium, or paratenic host for several parasites. They can also serve as reservoir for various infectious agents, some of them with zoonotic potential. For example, opossums serve as reservoir for protozoans *Trypanosoma cruzi* and *Leishmania* sp. (Barr, 1963; Travi *et al.*, 1994; Travi *et al.*, 1998; Bodini *et al.*, 2007; Cantillo-Barraza *et al.*, 2015; Gamboa-Osorio 2020). In addition, gastrointestinal parasites in opossums are abundant and diverse. Reported coinfections include the protozoa *Eimeria* sp.; the nematodes *Aspidodera* spp., *Cruzia* spp., *Gnathostoma* sp., *Trichuris* sp., *Turgida turgida, Viannaia* spp.; the platyhelminthes *Rhopalias* spp.; and the acantochepalans *Macracanthorhynchus* sp., *Gigantorhynchus* sp., and *Oligacanthorhynchus microcephalus* (García-Prieto *et al.*, 2010; Chinchilla-Carmona *et al.*, 2013; Ramírez and Osorio, 2014; Chero *et al.*, 2017; Aragón-Pech *et al.*, 2018).

Given that opossums are very common in urban parks of the Metropolitan Area of Valle de Aburrá, Colombia, the objective of the present study was to identify the parasites that use opossums in their life cycle as host or reservoir, and to test their potential associations with opossum sex, life stage, and urban area. The mean home range of males (122,7 ha) is 10 times larger than that of females (11,3 ha) (Sunquist *et al.*, 1987); thus, we expected that males would have higher parasite prevalence than females (Illia, 2019). On the other hand, depending on the species, parasite prevalence seems to vary with host age. For example, prevalence of *Capillaria* sp. in *Didelphis albiventris* decreases with age, but prevalence of *Aspidodera* sp. increases with age (Illia, 2019). Thus, we expected that parasite prevalence may differ between age classes (preadult vs. adult) in any direction (but not equal). Finally, because all urban areas were located along the same valley, under similar climate conditions, and they all are visited by people and their pets, we expected no differences in parasite composition. Hopefully, this study can be useful to design future programs to prevent potential zoonotic diseases in cities, including adequate management of synanthropic wildlife (Cuartas-Calle and Muñoz-Arango, 2003).

## Materials and Methods

This project was approved by the Ethics Committee for Animal Experimentation (CEEA) of Universidad de Antioquia, Colombia (Act 126 of August 13, 2019, and Act 135 of September 8, 2020). A wildlife collection permit was obtained from the National Authority of Environmental Licenses (ANLA; Resolution 1461 of December 3, 2014).

### *Study area*

The study was conducted in six protected urban areas (parks) of the Metropolitan Area of Valle de Aburrá, in Antioquia, Colombia. From north to south these areas were: Piamonte (14.2 ha, Bello municipality), El Volador hill (107.1 ha; Medellín municipality), Nutibara hill (33.3 ha; Medellín municipality), La Asomadera hill (26.6 Ha; Medellín municipality), La Heliodora wetland (23.3 ha; Envigado municipality), and Ditaires park (12.5 ha; Itagüí municipality). All areas are located along the Aburrá river on the western side of the Colombian central mountain range, with similar climate conditions. The altitude ranges between 1,468 to 1,628 m.a.s.l., with 1,626 mm average annual precipitation, and 67.5% relative humidity. The parks include different proportions of pastures, weeds, trees, recreational infrastructures, and human settlements (Area Metropolitana del Valle de Aburrá, 2018) and are surrounded by dense urban neighborhoods (Figure 1).

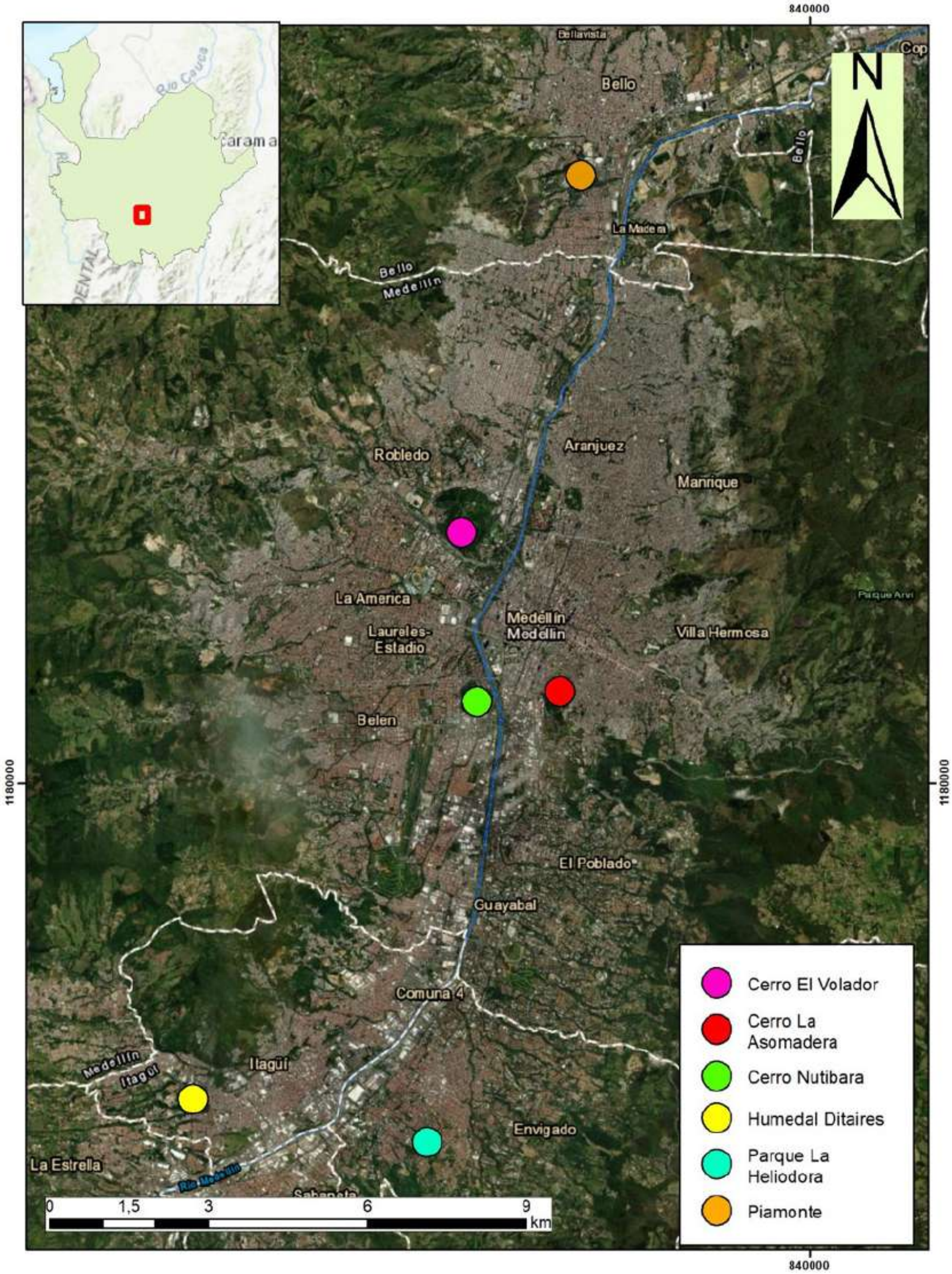


**Figure 1.** Location of the six protected urban areas of the Metropolitan Area of Valle de Aburrá in the Antioquia province (top left), Colombia, where opossums were trapped for this study.

*Field work*

Opossums were trapped with six Tomahawk live traps (82×24×24 cm) set for 20 nights on each protected area. The total capture effort was 120 traps per area. Traps were conveniently located near fruit trees and composting sites used for human organic leftovers, considering they could attract opossums. Each trap was baited with approximately 50 g of fresh pork viscera (spleen and liver) or canned sardines. The traps were set from October 5, 2020 to August 10, 2021 in the following order: Piamonte, Asomadera, Nutibara, Ditaires, Heliodora, and Volador (about five weeks in each area, with no trapping conducted between December 17 and February 2). The traps were checked early each morning. Trapped opossums were sexed and weighed, and fecal samples collected. Opossums under 800 gr were recorded as subadults (including juveniles), and animals heavier than 800 gr were classified as adults (Tyndale-Biscoe and MacKenzie, 1976). Fecal samples were collected directly from the cloaca of the animal at defecation or from the trap floor if feces appeared intact. Samples were stored in plastic containers without preservatives, refrigerated at 4 °C, and delivered to the Parasitology laboratory of the Veterinary School at Universidad de Antioquia (Medellín, Colombia). Then, opossums were released at the same trapping site.

*Laboratory analysis*

Once in the lab, a portion of the fecal sample was preserved on SAF solution (15 g sodium acetate, 20 ml glacial acetic acid, 40 ml 37% formaldehyde, and 925 ml tap water, according to Yang and Scholten, 1977). The other portion was used to prepare a direct smear on a microscope glass slide with a drop of physiological saline on one side of the slide and a drop of Lugol´s iodine stain on the other side. The smear was covered with a coverslip and observed under the microscope (Olympus SZX7) with 10X, 40X, and 100X objective lenses (Rodríguez and Cob, 2005).

A diagnostic test was performed using the sedimentation method with SAF to identify oocysts and eggs of intestinal parasites (Yang and Scholten, 1977). Coccidian oocysts were placed on a 2.5% potassium dichromate solution so they could sporulate to identify the taxa by morphology (Duszynski, 2016; López-Osorio *et al.*, 2020). Eggs were identified to the lowest possible taxonomy level following taxonomic keys (Wolfgang, 1951; Adnet *et al.*, 2009; Chero *et al.*, 2017; Arangón-Pech *et al.*, 2018; Bezerra-Santos *et al.*, 2020). Once the parasites were identified, a frequency analysis was conducted to compare the presence of parasites among areas.

*Data analyses*

Prevalence (P) of each parasite species was calculated as:

*(Number of opossums with parasites / Number of opossums examined) × 100.*

Richness (R) was calculated as the number of species of parasites on each area.

A Fisher´s exact test was used with the function *fisher.test* in R environment (R Core Team 2023) to test for potential associations of parasite species with sex (females vs. males), life stage (subadults vs. adults), and area of origin. For this purpose, we calculated contingency tables for each categorical variable (i.e., we counted the number of males and females with and without the parasite, the number of adults and subadults with and without the parasite, and the number of opossums with and without the parasite at each area).

## Results

*Identified parasites*

A total of 70 opossums were captured between October 5, 2020 and August 10, 2021, and fecal samples from 64 individuals were obtained. From the later, 34 were from males and 30 from females, with 52 recorded as adults and 12 as subadults. From this total, 60 samples were positive to at least one parasite, suggesting 93.75% positivity rate for all areas. On average, opossums were parasitized with 5.6 different taxa (SD=2.86) and a maximum of 11 different parasites.

The identified parasites belong to 19 parasite taxa: 13 nemathelminths (two eggs were not identified at the species level), four protozoans, one acanthocephalan, and one plathelminth (cestode). The parasites that occurred most frequently (>50%) were *Aspidodera* spp. (Prevalence, P=81.3%, 52/64), *Capillaria* spp. (P=75%, 48/64), Coccidias (P=68.8%, 44/64), Spiruroidea (P=57.8%, 37/64), and Trichostrongylidae (P=51.6%, 33/64). Identification of several eggs was possible only to the taxonomic family level because their morphological characteristics did not allow for a more detailed classification (Figure 2 and Table 1).

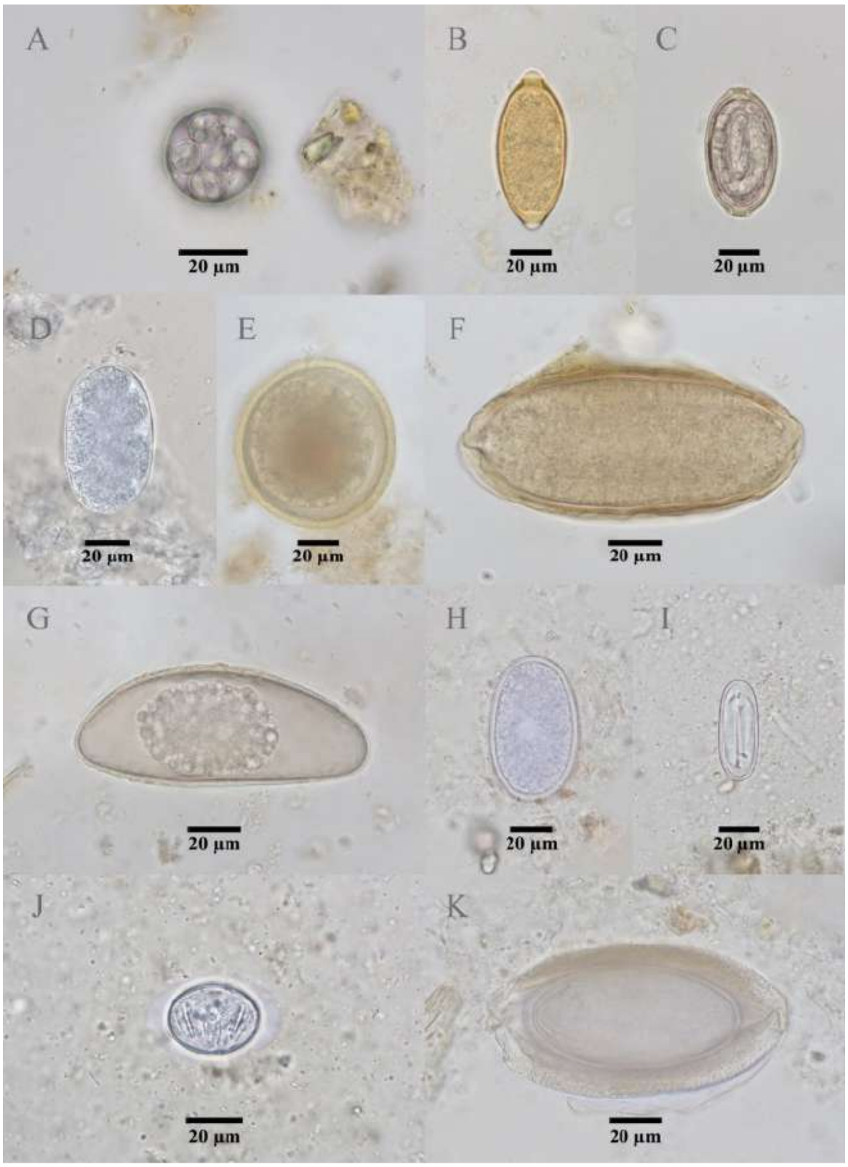


**Figure 2.** Gastrointestinal endoparasites identified in urban *D. marsupialis*. Abbreviations: A. Unidentified oocyst (Apicomplexa, Adeliidae), B. *Trichuris* spp. (Nematoda, Trichuridae), C. Unidentified egg (Nematoda, Singamidae), D. *Ancylostoma* spp. (Nematoda, *Ancylostoma*tidae), E. *Toxocara* spp. (Nematoda, Toxocaridae), F. *Cruzia* spp. (Nematoda, Kathlanidae), G. Unidentified egg (Nematoda, Oxyuridae), H. *Aspidodera* spp. (Nematoda, Aspidoderidae), I. *Turgida turgida* (Nematoda, Physalopteridae), J. *Hymenolepis* spp. (Platyhelminthes, Hymenolepididae), K. *Oligacanthorhynchus* spp. (*Acanthocephala*, Oligacanthorhynchidae).

**Table 1.** Absolute and relative frequency (prevalence) of *Didelphis marsupialis* endoparasites identified in six urban areas of the Aburra Valley in Antioquia, Colombia, discriminated by laboratory method (smear and SAF).

| **Phylum** | **Piamonte n=15** | | **Asomadera n=14** | | **Nutibara n=1** | | **Ditaires n=19** | | **Heliodora n=6** | | **El Volador n=10** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Smear** | **SAF** | **Smear** | **SAF** | **Smear** | **SAF** | **Smear** | **SAF** | **Smear** | **SAF** | **Smear** | **SAF** |
| **Apicomplexa** | | | | | | | | | | | | |
| Coccidia | 7 (47) | 9 (6) | 7 (5) | 11 (79) | 1 (1) | 1 (1) | 12 (63) | 11 (58) | 1 (17) | 2 (33) | 4 (4) | 5 (5) |
| Adelidae | | 1 (7) | 3 (21) | 2 (14) | | | 6 (32) | 6 (32) | 1 (17) | 1 (17) | | |
| **Amoebozoa** | | | | | | | | | | | | |
| *Entamoeba* spp. | 2 (13) | 1 (7) | 2 (14) | 1 (7) | | | | | | | | |
| **Nematoda** | | | | | | | | | | | | |
| *Aspidodera* spp. | 6 (4) | 12 (8) | 6 (43) | 12 (86) | | 1 (1) | 5 (26) | 15 (79) | 3 (5) | 1 (17) | 6 (6) | 7 (7) |
| Aspidoderidae | | 4 (27) | | | | | | 2 (11) | | | | 1 (1) |
| *Ancylostoma* spp. (Z) | 4 (27) | 6 (4) | | | | | | 1 (5) | 2 (33) | | 2 (2) | 4 (4) |
| *Capillaria* spp. | 4 (27) | 1 (67) | 8 (57) | 1 (71) | 1 (1) | 1 (1) | 8 (42) | 13 (68) | | 4 (67) | 5 (5) | 6 (6) |
| *Cruzia* | | 1 (7) | | | | | 1 (5) | 1 (5) | | | | 2 (2) |
| Egg1 not identified | 3 (2) | 3 (2) | 2 (14) | 2 (14) | 1 (1) | 1 (1) | | | | 1 (17) | | |
| Egg2 not identified | | | | | | | | 7 (37) | 1 (17) | 4 (67) | | |
| Oxyuridae* | | 4 (27) | | 1 (7) | | 1 (1) | | | | | | |
| Singamidae* | | 7 (47) | 2 (14) | 2 (14) | | 1 (1) | | 3 (16) | | | 1 (1) | 1 (1) |
| Spiruroidea | 3 (2) | 7 (47) | 9 (64) | 1 (71) | 1 (1) | 1 (1) | 2 (11) | 7 (37) | 1 (17) | 2 (33) | 3 (3) | 8 (8) |
| *Toxocara* spp.* (Z) | | 5 (33) | | 6 (43) | | | | 1 (5) | | | | |
| Trichostrongylidae | 9 (6) | 12 (8) | 1 (7) | 5 (36) | | 1 (1) | 1 (5) | 5 (26) | | | 7 (7) | 8 (8) |
| *Trichuris* spp. | | 5 (33) | 4 (29) | 6 (43) | | 1 (1) | 3 (16) | 4 (21) | | 1 (17) | | 3 (3) |
| Platyhelminthes (Cestoda) | | | | | | | | | | | | |
| *Hymenolepis* spp.* (Z) | 2 (13) | 3 (2) | 4 (29) | 4(29) | | | | | | | | |
| Archiacanthocephala | | | | | | | | | | | | |
| *Oligacanthorhynchus* spp. * | 5 (33) | 8 (53) | 4 (29) | 7 (5) | 1 (1) | 1 (1) | 8 (42) | 8 (42) | 1 (17) | 1 (17) | 3 (3) | 3 (3) |

n = number of stool samples analyzed per site; Z = zoonotic potential; * = first report in *D. marsupialis* in Colombia.

Sporulated oocysts were recovered from three samples and identified as *Eimeria marmosopos* (Duszynski, 2016). Specific features of sporocysts and sporozoites, such as internal structures, could not be identified. Although coccidia oocysts were found in all the areas, the sporulated oocysts corresponded to only two areas (Asomadera and Ditaires). In addition, the latter area presented oocysts, presumably *Eimeria didelphidis*, a smaller coccidia, and *Eimeria caluromydis*, a coccidia larger than *E. marmosopos* (Figure 3 and Table 2).

The number of parasites was not statistically different among parks ($F_{4,58}$ = 1.3124, p = 0.2761, excluding Nutibara because of its low sample size; n=1). Piamonte had the highest number of parasites, with a total of 17 different taxa (Figure 4). Some parasites were present in all six areas, such as *Oligacanthorhynchus* spp., *Aspidodera* spp., Spiruroidea, *Capillaria* spp., *Trichuris* spp., Trichostrongylidae, Singamidae, and coccidia. On the other hand, *Ancylostoma* spp., *Cruzia* spp., and Aspidoderidae were found in only three areas; and *Hymenolepis* spp., *Entamoeba* spp., Oxyuridae, and *Toxocara* spp. in two areas (Table 1).

Note that the number of parasites identified with SAF was higher than with the smear technique (Table 1). Fecal smear was a screening test to determine the relevance of other techniques, such as coccidia sporulation in 2.5% potassium dichromate, but it was through SAF sedimentation that diagnosis of other eggs, such as Oxyuridae and Aspidoderidae, could be made. The SAF technique improved sensitivity by 43% (difference between frequency obtained by smear versus frequency obtained by SAF) allowing a positive diagnosis for Singamidae, Capillarids, *Trichuris* spp., and Oxyuridae parasites.

*Associations*

The parasite species most identified (*Aspidodera* spp., *Capillaria* spp., Coccidias, Spiruroidea, and Trichostrongylidae) were not associated to opossum sex, nor to life stage of the animal (Table 3). Likewise, no associations were observed between parasite species and the park of origin, except for Trichostrongylidae (p<0.0001), which was more common in Volador (P=90%), Piamonte (P=86.7%), and Ditaires (P=73.7%) relative to Asomadera (P=35.7%) and Heliodora park (P=0) (Table 4).

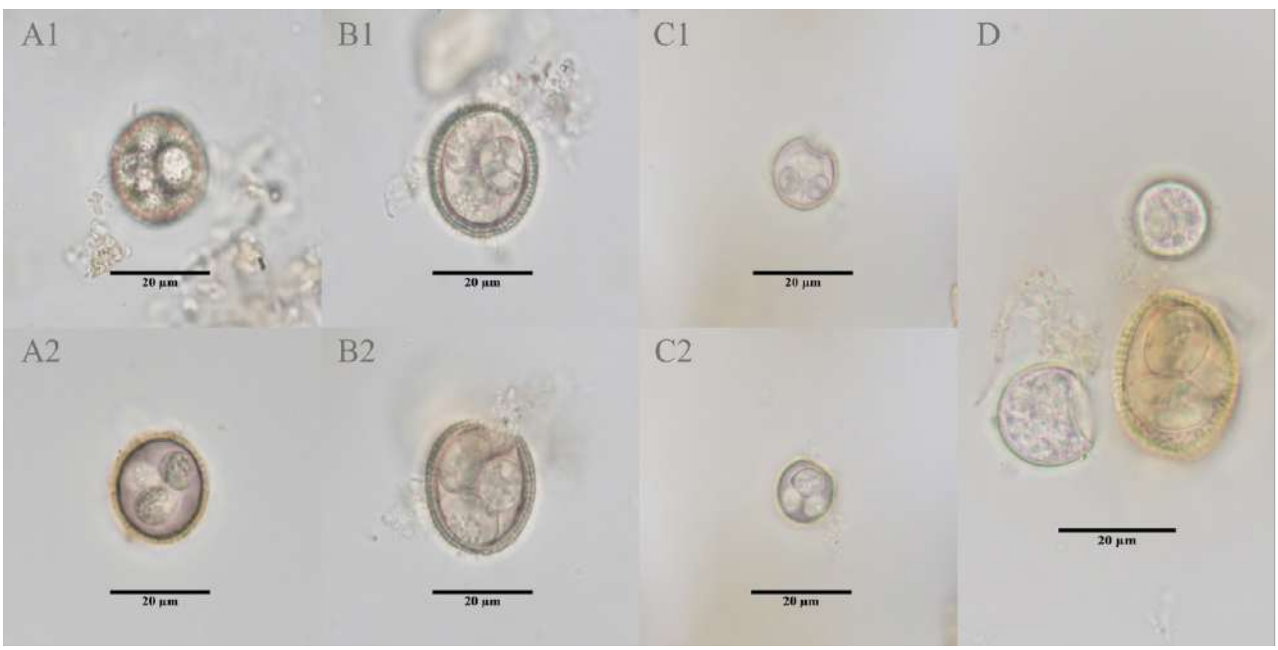


**Figure 3.** Coccidian oocysts found in *D. marsupialis*. Morphological characteristics of oocyst walls and sporocysts: A1-2. *E. marmosopos*, B1-2. *E. caluromydis*, C1-2. *E. didelphidis*. D. Size differences between oocysts.


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**Table 2.** Average measurements (micrometers) and ranges (parenthesis) of oocysts and sporocysts of coccidia found in urban *Didelphis marsupialis*.

| Coccidia (area) | Length oocyst | Width oocyst | Oocyst walls | Oocyst ratio | n | Width sporocyst | Sporocyst ratio | n | Morphological characteristics |
|---|---|---|---|---|---|---|---|---|---|
| *Eimeria caluromydis* (Ditaires) | 27.6 (25.76-31.27) | 24.59 (21.3-27.07) | 2.55 (1.86-3.12) | 1.5 (1.3-1.7) | 18 | 9.9 (8.9-11.98) | 1.1 (1-1.3) | 24 | Spheroidal to subspheroidal oocyst. Prominent wall, mamillated and striated, brown. MP, OR, and PG absent |
| *Eimeria didelphidis* (Ditaires) | 16.63 (15.39-18.7) | 15.37 (14.14-16.37) | 1.35 (1.17-1.51) | 1.1 (1-1.1) | 4 | 5.49 (4.95-6.14) | 1.4 (1.3-1.6) | 7 | Spheroidal oocyst, smooth wall, colorless. MP, OR, and PG absent |
| *Eimeria marmosopos* (Asomadera, Ditaires) | 22.67 (20.18-24) | 21.63 (19.16-23.53) | 1.86 (1.42-2.46) | 1 (1-1.1) | 39 | 7.75 (6.74-9.55) | 1.44 (1.3-1.6) | 31 | Subspheroidal oocyst, rough, and striated wall, MP, and OR absent, PG: highly refractive. SB as a nipple |

MP = micropyle; OR = oocyst residue; PG = polar granule; SB = Stieda body.

**Table 3.** Associations of the most common parasites with sex and life stage of urban *Didelphis marsupialis*.

| Parasite | Sex | Prevalence rate | P-value | Life stage | Prevalence rate | P-value |
|---|---|---|---|---|---|---|
| *Aspidodera* spp. | Females | 25/30 | 0.757 | Subadults | 9/12 | 0.682 |
| | Males | 27/34 | | Adults | 43/52 | |
| *Capillaria* spp. | Females | 24/30 | 0.564 | Subadults | 7/12 | 0.156 |
| | Males | 24/34 | | Adults | 41/52 | |
| Coccidia | Females | 18/30 | 0.185 | Subadults | 7/12 | 0.493 |
| | Males | 26/34 | | Adults | 37/52 | |
| Spiruroidea | Females | 17/30 | 1.0 | Subadults | 6/12 | 0.747 |
| | Males | 20/34 | | Adults | 31/52 | |
| Trichostrongylidae | Females | 16/30 | 0.808 | Subadults | 25/52 | 0.341 |
| | Males | 17/34 | | Adults | 8/12 | |

**Table 4.** Associations of the most common parasites with area of urban *Didelphis marsupialis*.

| Parasite | APU | Prevalence rate | P-value |
|---|---|---|---|
| *Aspidodera* spp. | Asomadera | 12/14 | 0.332 |
| | Ditaires | 16/19 | |
| | Heliodora | 3/6 | |
| | Piamonte | 13/15 | |
| | Volador | 7/10 | |
| *Capillaria* spp. | Asomadera | 11/14 | 0.984 |
| | Ditaires | 14/19 | |
| | Heliodora | 4/6 | |
| | Piamonte | 11/15 | |
| | Volador | 7/10 | |
| Coccidia | Asomadera | 11/14 | 0.372 |
| | Ditaires | 14/19 | |
| | Heliodora | 2/6 | |
| | Piamonte | 10/15 | |
| | Volador | 6/10 | |
| Spiruroidea | Asomadera | 11/14 | 0.076 |
| | Ditaires | 8/9 | |
| | Heliodora | 2/6 | |
| | Piamonte | 7/15 | |
| | Volador | 8/10 | |
| Trichostrongylidae | Asomadera | 5/14 | 0.00001* |
| | Ditaires | 14/19 | |
| | Heliodora | 0/6 | |
| | Piamonte | 13/15 | |
| | Volador | 9/10 | |

*Indicates significant association.

## Discussion

This study shows that urban opossums may host up to 11 different parasites at the same time. This number could be even higher because we identified 19 different taxa in the digestive system of several individuals. These parasitic coinfections may be commonly associated to animals with generalist habits (Tantaleán *et al.*, 2010; Ramírez and Osorio, 2014; Muñoz-Rodriguez *et al.*, 2017; Aragón-Pech *et al.*, 2018). It may also be an indicative of disturbed environments. The loss of biodiversity favors the proliferation of generalist parasitic species which, in turn, promotes the exchange of pathogens and increases the degree of exposure (McFarlane *et al.*, 2012; Vaumourin *et al.*, 2015).

*Aspidodera* spp. was the most frequent parasite observed, followed by Capillarids, which were much higher than previous reports in other species of opossums showing up to 28.6% prevalence for capillaries and 20.4% for Ascarid parasites (Aragón-Pech *et al.*, 2018; Bezerra-Santos *et al.*, 2020). On the other hand, *Cruzia* spp. was uncommon (only 6%; 4/65) in the present study, while it has been abundant in studies with other opossums, with above 60% prevalence (Aragón-Pech *et al.*, 2018; Bezerra-Santos *et al.*, 2020). The presence of parasites depend on several environmental, biological, and anthropogenic factors, e.g., season of the year, presence of intermediate or temporary hosts, type of vegetation,

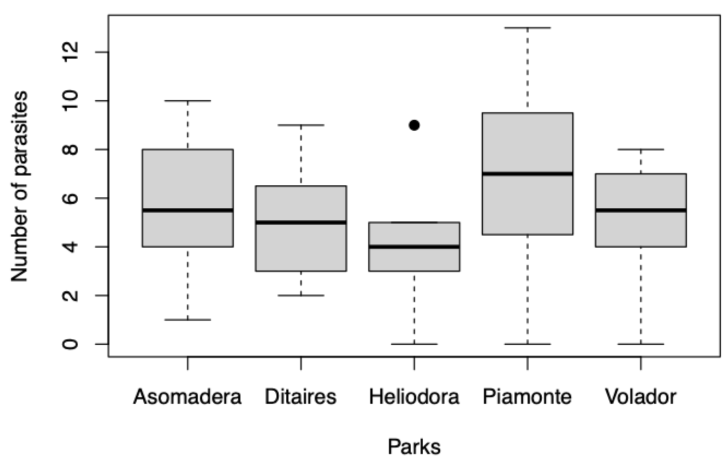


**Figure 4.** Parasite richness in *Didelphis marsupialis* inhabiting five urban areas in Valle de Aburrá, Antioquia, Colombia. Nutibara park is excluded because of its low sample size (n=1).

among others (Vaumourin *et al.*, 2015). These factors were not evaluated in the present study.

We are reporting for the first time the presence of several parasites in *D. marsupialis*: nematodes Singamidae, *Toxocara* spp., and Oxyuridae; coccidias *E. didelphidis* and *E. caluromydis*; acanthocephalic *Oligacanthorynchus* spp.; and cestode *Hymenolepis* spp. In addition, this study adds a new site (Valle de Aburrá Medellín, Colombia) for the distribution of *E. marmosopos*. Importantly, some of these parasites (i.e., *Toxocara* spp., *Hymenolepis* spp., and *Ancylostoma* spp.) have zoonotic potential. Opossum infection with *Toxocara* spp. has been related to cohabitation with dogs and cats in urban environments, and it may be a pseudoparasite, i.e., the parasite egg passes intact through the digestive system of the opossum and is diagnosed in feces (Pinto *et al.*, 2014). Other pseudoparasitisms have been reported in opossums; for example, the genera of Adeleidae when opossums eat infected invertebrates (Hammond and Long, 1973; Teixeira *et al.*, 2003; Berto *et al.*, 2010; Duszynski, 2016).

Regarding infection with cestode *Hymenolepis* spp. in neotropical marsupials, there is only one report on *D. virginiana* (Leigh, 1940) and one report on *Micoureus cinereus* (Campbell *et al.*, 2003). Both researchers reported incomplete morphology of the adult specimen, although it has been widely reported in Australian marsupials (Spratt and Beveridge, 2016). Opossums in the present study inhabit urban areas so there is a possibility that they may be infected by synanthropic rodents (e.g., rats and mice) which are natural hosts of this cestode (Vaucher, 1992; OPS, 2003; Pritchett, 2007). Alternatively, this relationship may be a pseudoparasitism and the opossum may be acting as a marker of contamination (Polo-Terán, 2006; Telfer *et al.*, 2010; Vaumourin *et al.*, 2015).

Preliminary research on the impact of zoonotic agents may help prevent outbreaks and new pandemics (WHO, 2021). Prioritization should be given to long-term research on parasitology and host population/community ecology employing various methods for identifying host species and diagnosing infections, such as preserving vouchers by depositing them in scientific collections (Thompson *et al.*, 2021). According to earlier studies, marsupials are a significant source of zoonotic agents, including infections with helminths, viruses, bacteria, and protozoa (Bitencourt and Bexerra, 2022). Reports of zoonotic parasites parasitizing opossums have been made all over the American continent; some species are thought to be accidental parasitosis while others have high prevalence rates. Further research on the role opossums play in the epidemiology of pathogens of public and veterinary importance is necessary due to the lack of knowledge regarding infectious agents associated with them, despite their significance in the context of One Health (Bezerra-Santos *et al.*, 2021).

In conclusion, urban opossums can simultaneously host up to 11 different species of parasites in the digestive system. Some of these parasites are generalists that can adapt to any condition, others have zoonotic potential, while others may be pseudoparasites given the omnivorous diet of opossums. The present study demonstrates the importance of monitoring wildlife, synanthropic animals, and vectors. Public research institutions, particularly those associated with graduate programs, play a role in providing measures for disease eradication as well as ensuring quality responses to other areas of biodiversity science (Overbeck *et al.*, 2017). In addition, more epidemiological research and public education campaigns regarding the ecological significance of marsupials and the dangers of close contact with them are needed to reduce the risk of pathogen transmission between people, domestic animals, and these mammals. Further research should be conducted in Colombia to characterize parasitofauna of animal species in close contact with human communities.

## Declarations

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*Conflict of interest*

The authors declare they have no conflicts of interest regarding the work presented in this report.

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*Author contributions*

The study was designed by VHM and CPC. Field data were collected by VHM and CPC. Laboratory work was done by VHM, LNR, SLO. The manuscript was written by VHM and CPC. All authors reviewed and approved the final version of the manuscript.

*Use of artificial intelligence (AI)*

No AI or AI-assisted technologies were used during the preparation of this work.

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