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

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

Parasitas gastrointestinais em gambás comuns (Didelphis marsupialis) em áreas protegidas urbanas em Antioquia, Colômbia

Viviana Hernández-Montoya[1*]; Leonardo Navarro-Ruiz[2]; Sara López-Osorio[2]; Jenny J. Chaparro-Gutiérrez[2]; Claudia P. Ceballos[1]

[1]Grupo de investigación GAMMA, Escuela de Medicina Veterinaria, Facultad de Ciencias Agrarias, Universidad de Antioquia, UdeA, Medellín, Colombia.
[2]Grupo de investigación CIBAV, Escuela de Medicina Veterinaria, Facultad de Ciencias Agrarias, Universidad de Antioquia, UdeA, Medellín, Colombia.

[*]**Corresponding autor.** Escuela de Medicina Veterinaria, Universidad de Antioquia, UdeA, Medellín, Colombia. E-mail: viviana.hernandezm@udea.edu.co

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Abstract

Background: The common opossum (*Didelphis marsupialis*) is a generalist species that has been reported as a host of several parasites, but there is no information on the parasites that this species may host in protected urban areas. **Objective:** We evaluated the presence and frequency of gastrointestinal parasites of urban opossums in the Aburrá valley, Antioquia, Colombia. **Methods:** A total of 70 opossums were captured from six urban protected areas using Tomahawk traps for 6 months. From these opossums we obtained 65 fecal samples, which were fixed wit SAF solution, and analyzed with direct smear and sedimentation techniques. **Results:** From the 65 fecal samples 61 were positive for at least one parasite (93.9%), and 60 were positive for two or more parasites, with an average of five parasites per animal. We observed nematode parasites 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 fecal samples. We report, for the first time in *D. marsupialis*, the presence of eggs of *Toxocara* spp., *Hymenolepis* spp., an Oxyuridae parasite, and the coccidians *Eimeria didelphidis*, *E. caluromydis*, and *E. marmosopos*, the latter is also reported in Colombia for the first time. *Toxacara* spp. is common to dogs and cats, and *Hymenolepis* and Oxiuridae are common to rodents. We found no association between the parasites to the sex or the life stage of the opossums, nor to the urban area of origin. **Conclusions:** Urban opossums host a high frequency and diversity of gastrointestinal parasites compared to other studies of *Didelphis* spp. in Latin America. Some of the parasites are reported here for the first time.

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

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 protegidas urbanas. **Objetivo:** Nosotros evaluamos la presencia y frecuencia de 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 6 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:** De las 65 muestras fecales 61 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 cestodos en ocho muestras (12,3%). El parásito más frecuente fue *Aspidodera* spp. en el 80% de las muestras fecales. Reportamos 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 son comunes en roedores. Nosotros no encontramos evidencias de asociaciones entre los parásitos y el sexo o el estadio de vida de las zarigüeyas, ni tampoco con el área urbana. **Conclusiones**: Este estudio confirma que las zarigüeyas urbanas son portadoras de una alta frecuencia y diversidad de parásitos gastrointestinales en comparación con otros estudios de *Didelphis* spp. en Latinoamérica. Algunos parásitos son reportados en este estudio por primera vez.

Palabras clave*: Áreas protegidas; coinfección; helmintiasis; Didelphis marsupialis; marsupiales; parasitosis; parques urbanos; zarigüeyas.*

Resumo

Fundo: 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 naturais. **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**: Este estudo confirma que gambás urbanos são portadores de alta frequência e diversidade de parasitas gastrointestinais em comparação com outros estudos de *Didelphis* spp. na América Latina. Alguns parasitas são relatados neste estudo pela primeira vez.

Palavras-chave*: Áreas protegidas; coinfecção;* <u>Didelphis</u> <u>marsupialis</u>*; gambás; helmintíase; parasitoses; marsupiais; parques urbanos.*

Introduction

The common opossum (*Didelphis marsupialis*) is a generalist species. They have an omnivorous diet and adapt to a diversity of food resources including fruits, vegetables, invertebrates, small vertebrates, and carrion (Flórez-Oliveros and Vivas-Serna, 2020). At the same time, opossums are part of the life cycle of a variety of microorganisms. They are tolerant to disturbed areas and invade urban environments with different perturbation levels (Méndez, 1983). For example, in a recent study it was found that there can be found more opossums in urban parks than in peri-urban forests (Rodríguez *et al.*, 2022). Urban parks are visited by people and their pets, however there are few studies on the pathogens or parasites they may host, or potentially transmit to domestic animals or humans (Gamboa-Osorio, 2020). This is important, not only for the opossum´s wellbeing, but also because pathogens can jump the species barrier and cause emergent diseases (Hassell *et al*., 2017; WOAH, 2021).

Opossums can be definitive, intermedium, or paratenic host of different parasites. They also can serve as reservoir of several infectious agents, some of them with zoonotic potential. For example, opossums serve as reservoir for the 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). Also, 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).

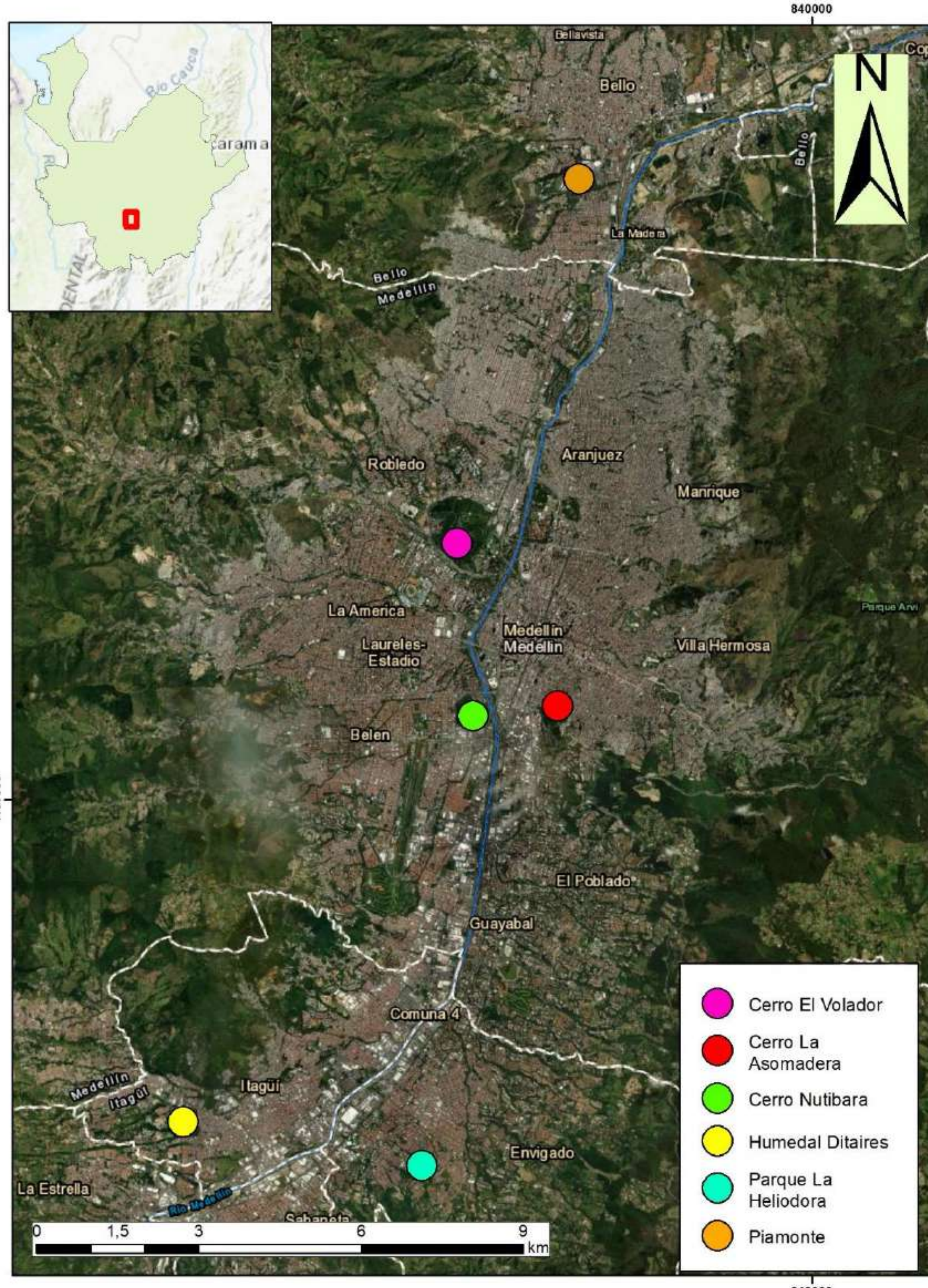
Given that opossums are very common in the urban parks of the Metropolitan Area of Valle de Aburrá, Colombia, the objective of the present study was to identify the parasites that use opossums in their life cycle as host or reservoir, and to test potential associations with the sex and the life stage of the opossums, and with the urban parks. Because the mean home range of males (122,7 ha) has been found 10 times larger than the home range of females (11,3 ha) (Sunquist *et al*., 1987) we expected that males would have a higher prevalence of parasites than females (Illia, 2019). On the other hand, the prevalences of parasites seem to vary with the age of the host, depending on the species of parasite. For example, in *Didelphis albiventris* the prevalence of *Capillaria* sp. decreased with age, but the prevalence of *Aspidodera* sp. increased with age (Illia, 2019). Thus, we expected that the prevalence of parasites may differ between age classes (preadult vs. adult), in any direction (not equal). Finally, because all the urban parks were located along the same valley under the same climate conditions, and they all are visited for people and their pets, we expected no differences in the composition of the parasites. Hopefully this study can be useful to design future programs to prevent potential zoonotic diseases in the cities, including an 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 the University of Antioquia, (act 126 of August 13, 2019, and act 135 of September 8, 2020). The wildlife collection permit was issued by the National Authority of Environmental Licenses (ANLA) to the Universidad de Antioquia, (Resolution 1461 of December 3, 2014).

Study area

We conducted this study in six urban protected areas of the Metropolitan Area of Valle de Aburrá, in Antioquia, Colombia. From north to south these areas were: Piamonte (14.2 ha, municipality of Bello), El Volador hill (107.1 ha; municipality of Medellín), Nutibara hill (33.3 ha; municipality of Medellín), La Asomadera hill (26.6 Ha; municipality of Medellín), La Heliodora wetland (23.3 ha; municipality of Envigado), and Ditaires park (12.5 ha; municipality of Itagüí). All areas are located along the Aburrá river, on the western side of the Colombian central cordillera and had similar climate conditions. They are at an altitude between 1,468 to 1,628 m.a.s.l., with an average annual precipitation of 1626,3 mm, and an average relative humidity of 67.5%. The parks are formed by pastures with trees, weeds, recreational infrastructures, and human settlements, in different proportions (Area Metropolitana del Valle de Aburrá, 2018), and are surrounded by dense urban neighborhoods (Figure 1).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Bellavista
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Villa Hermosa
Parque Arvi
Belen
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0 1,5 3 6 9 km
Cerro El Volador
Cerro La Asomadera
Cerro Nutibara
Humedal Ditaires
Parque La Heliodora
Piamonte

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

Field work

We trapped opossums using six Tomahawk live traps (82×24×24 cm) set for 20 nights on each urban protected area. The total capture effort was 120 traps per area. Traps were conveniently located near fruit trees and compost sites with human organic leftovers that 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 5 weeks each area, and no trapping was done between December 17 and February 2). The traps were checked early each morning, and if there was an opossum it was sexed and weighed, and fecal samples were collected. Opossums under 800 gr were recorded as subadults (includes juveniles), and opossums heavier than 800 gr were classified as adults (Tyndale-Biscoe and MacKenzie, 1976).

Afterwards it was released at the same site of the trap. Fecal samples were collected directly from the cloacae of the opossum if it defecated, or from the floor of the trap if it looked intact. Samples were stored in plastic containers without preservatives, refrigerated at 4 °C, and then taken to the Parasitology laboratory of the Veterinary School of the Universidad de Antioquia (Medellín, Colombia).

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, Yang and Scholten, 1977), and another portion was used to prepare a direct smear on a microscope glass slide with a drop of physiological saline in one side of the slide, and a drop of Lugol´s iodine stain in the other side of the slide. The smear was covered with a coverslip and then 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). The oocysts of coccidian found 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 level of taxonomy possible 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 urban protected areas.

Data analyses

The prevalence (P) of each species of parasite was calculated as the number of opossums with a parasite/number of opossums examined * 100, and the richness (R) was calculated as the number of species of parasites on each natural park. To test for potential associations in the species of parasites with the sex (females vs males), the life stage (subadults vs. adults) of opossums, and the park of origin we used Fisher´s exact test for count data using the function *fisher.test* in R environment. For that purpose, we calculated contingency tables for each categorical variable (i.e., we counted the number of males' and females' opossums 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 park).

Results

Parasites identified

Between October 5, 2020 and August 10, 2021, we captured 70 opossums, but obtained fecal samples from 64 individuals. From the later, 34 were males and 30 were females; and 52 were classified as adults and 12 as subadults. From this total, 60 samples were positive to at least one parasite, which suggests a 93.75% of positivity rate for all urban protected areas. Opossums were parasitized on average with 5,6 different taxa (SD=2.86) and a maximum of 11 different parasites.

The parasites identified belong to 19 parasite taxa: 13 nemathelminths (two eggs were not identified at 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). The identification of some eggs was possible only to the taxonomic family level because their morphological characteristics did not allow a more detailed classification (Figure 2 and Table 1).

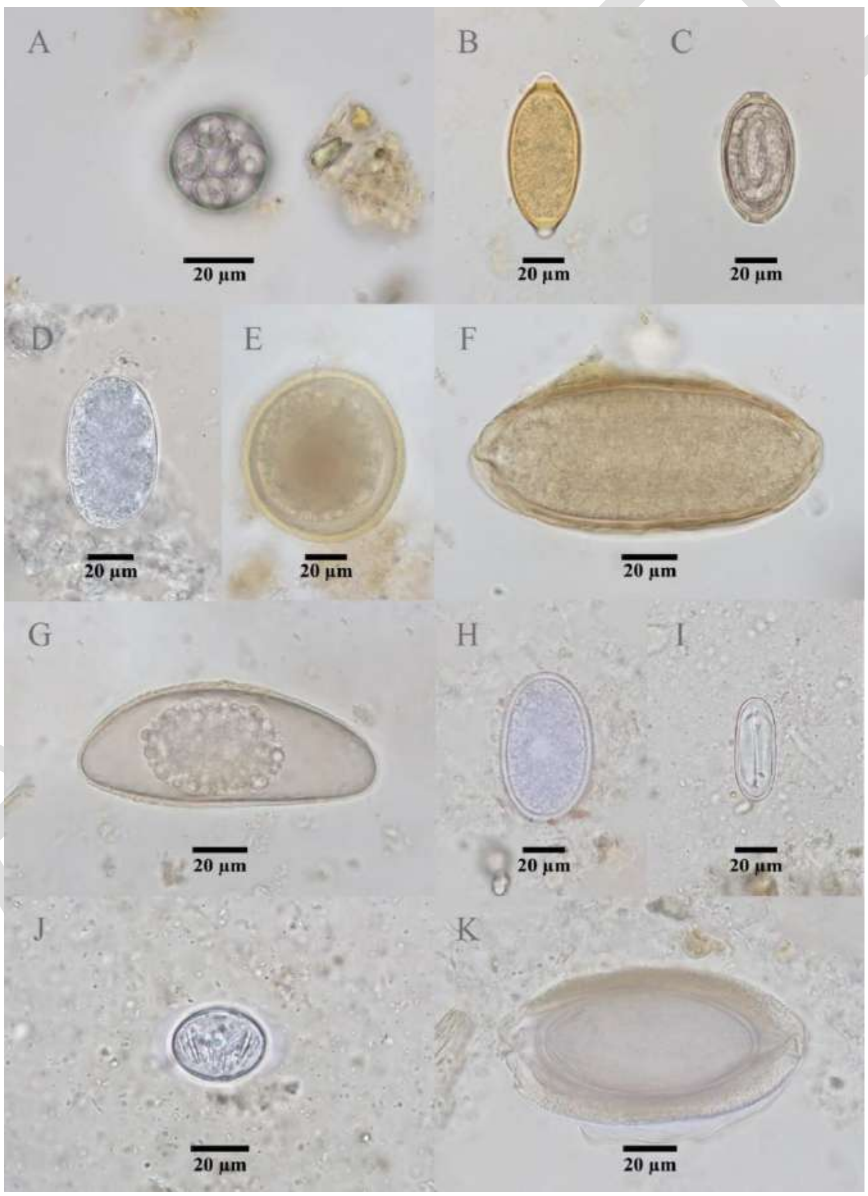

Figure 2. Endoparasites of the digestive system identified in urban *D. marsupialis* in this study. Abbreviations*:* A. Unidentified oocyst (Apicomplexa, Adeliidae), B. *Trichuris* spp. (Nematoda, Trichuridae), C. Unidentified egg (Nematoda, Singamidae), D. *Ancylostoma* spp. (Nematoda, Ancylostomatidae), 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).

Sporulated oocysts were recovered from three samples, and they exhibited characteristics that allowed them to be identified as *Eimeria marmosopos* (Duszynski, 2016). However, more 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 *Eimerai didelphidis*, a smaller coccidia, and *Eimerai caluromydis*, a larger coccidia than *E. marmosopos* (Figure 3 and Table 2).

The number of parasites was not statistically different among the parks ($F_{4,58} = 1.3124$, $p = 0.2761$, excluding Nutibara because of the low sample size; n=1), however Piamonte had the absolute higher 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).

Table 1. Absolute and relative frequency (prevalence) of endoparasites of *Didelphis marsupialis* identified in six urban protected areas discriminated by lab 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)
Adeliidae		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 no identified	3 (2)	3 (2)	2 (14)	2 (14)	1 (1)	1 (1)				1 (17)		
Egg2 no 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.

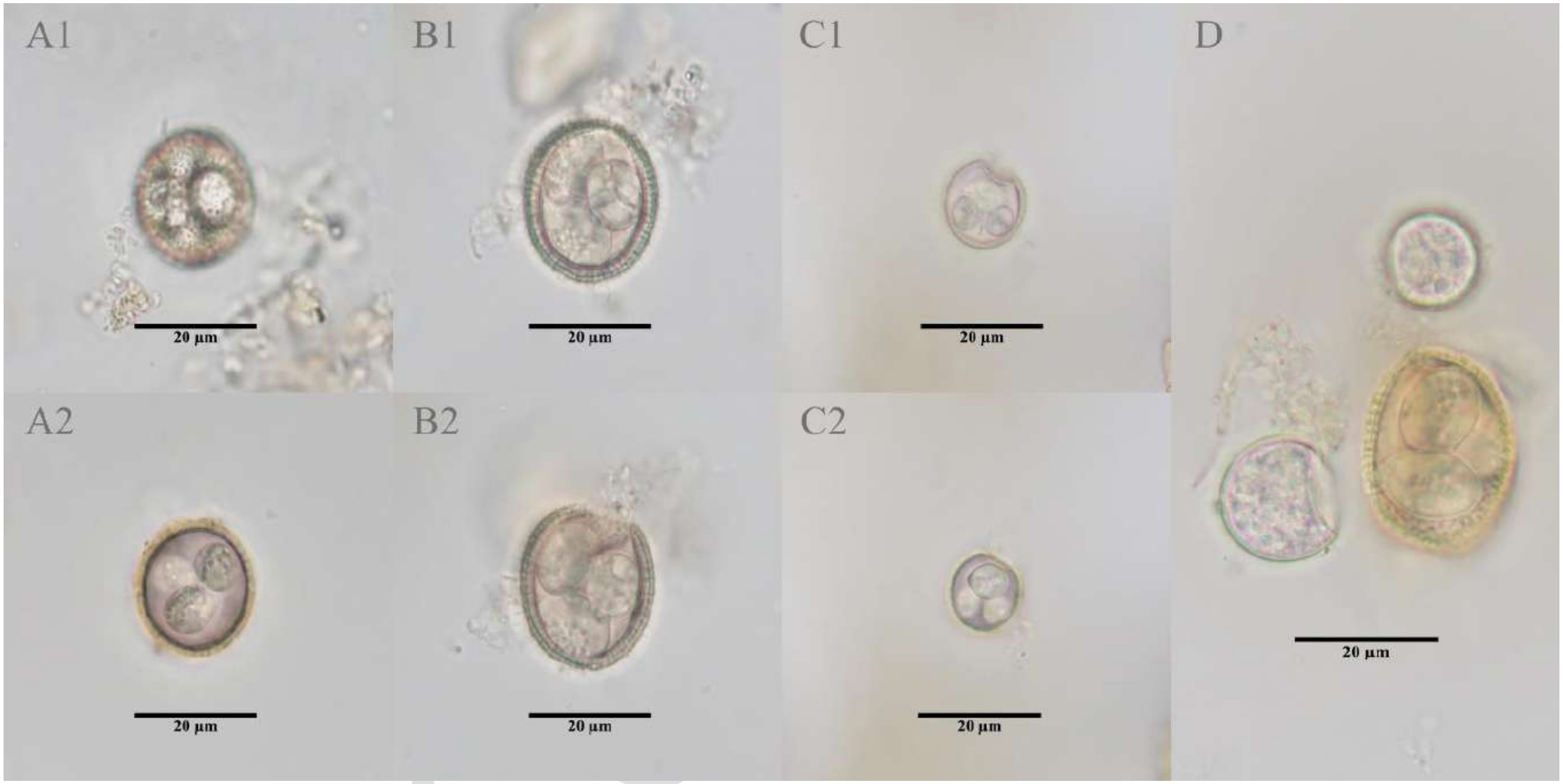

Figure 3. Oocysts of the coccidian found in *D. marsupialis* in this study. Morphological characteristics of the oocyst walls and sporocysts: A1-2. *E. marmosopos*, B1-2. *E. caluromydis*, C1-2. *E. didelphidis*. D. Size differences between the oocysts of the three identified.

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

Coccidia (Protected Area)	**Length oocyst**	**Width oocyst**	**Oocyst walls**	**Oocyst ratio**	**n**	**Length sporocyst**	**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	14.6 (12.18-17.91)	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	7.94 (7.26-9.05)	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	11.17 (9.64-14.55)	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.

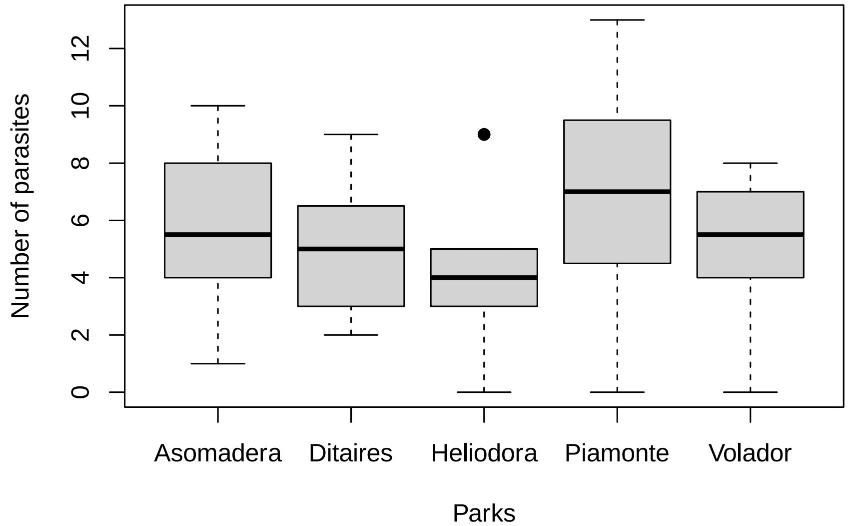

Figure 4. Richness of parasites found in *Didelphis marsupialis* in five urban protected areas in Valle de Aburrá, Antioquia, Colombia. Nutibara park is excluded because of the low sample size (n=1).

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

Associations

The species of parasites most identified in this study (*Aspidodera* spp., *Capillaria* spp., Coccidias, Spiruroidea, and Trichostrongylidae) were not associated to the sex of the opossums, nor to the life stage of the opossum (Table 3). Likewise, there were no associations between the species of parasites with the park or origin, except for Trichostrongylidae ($p<0.0001$) that was found more common in the parks Volador (P=90%), Piamonte (P=86.7%), and Ditaires (P=73.7%), relative to Asomadera (P=35.7%) and Heliodora (P=0) (Table 4).

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

Parasites	Sex	Prevalence rate	Significance	Life stage	Prevalence rate	Significance
Aspidodera spp.	Females	25/30	p=0.7568	Subadults	9/12	p=0.6821
	Males	27/34		Adults	43/52	
Capillaria spp.	Females	24/30	p=0.5639	Subadults	7/12	p=0.1559
	Males	24/34		Adults	41/52	
Coccidia	Females	18/30	p=0.1847	Subadults	7/12	p=0.4925
	Males	26/34		Adults	37/52	
Spiruroidea	Females	17/30	p=1.0	Subadults	6/12	p=0.7469
	Males	20/34		Adults	31/52	
Trichostrongylidae	Females	16/30	p=0.8076	Subadults	25/52	p=0.3407
	Males	17/34		Adults	8/12	

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

Parasite	APU	Prevalence rate	p-value
Aspidodera spp.	Asomadera	12/14	0.3317
	Ditaires	16/19	
	Heliodora	3/6	
	Piamonte	13/15	
	Volador	7/10	
Capillaria spp.	Asomadera	11/14	0.9839
	Ditaires	14/19	
	Heliodora	4/6	
	Piamonte	11/15	
	Volador	7/10	
Coccidia	Asomadera	11/14	0.3723
	Ditaires	14/19	
	Heliodora	2/6	
	Piamonte	10/15	
	Volador	6/10	
Spiruroidea	Asomadera	11/14	0.0755
	Ditaires	8/9	
	Heliodora	2/6	
	Piamonte	7/15	
	Volador	8/10	

Trichostrongylidae	Asomadera	5/14	1.1192E-05*
	Ditaires	14/19	
	Heliodora	0/6	
	Piamonte	13/15	
	Volador	9/10	

*Indicates a significant association.

Discussion

This study shows that urban opossums may host up to 11 different parasites at the same time, and it could be potentially higher because we identified 19 different taxa in the systema digestorium of different 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), but it also may be an indicative of disturbed environments. Biodiversity loss favors the multiplication 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).

In our study *Aspidodera* spp., was the most frequent parasite, followed by the Capillarids, which were much higher than other reports with prevalences up to 28.6% for capillaries and 20.4% for Ascarid parasites in other species of opossums (Aragón-Pech *et al.,* 2018; Bezerra-Santos *et al.*, 2020). On the other hand, in this study *Cruzia* spp. was uncommon (only 6%; 4/65), while in other studies it has been abundant with prevalences of above 60% in other opossums (Aragón-Pech *et al.,* 2018; Bezerra-Santos *et al*., 2020). The presence of parasites can be conditioned by many environmental, biological, and anthropogenic factors, e.g., the season of the year, the presence of intermediate or temporary hosts, the type of vegetation, among others (Vaumourin *et al.,* 2015). These factors were not evaluated in this study.

This study presents the first report of several parasites in *D. marsupialis:* the nematodes Singamidae, *Toxocara* spp., Oxyuridae, the coccidias *E. didelphidis* and *E. caluromydis*, the acanthocephalic *Oligacanthorynchus* spp., and the cestode *Hymenolepis* spp. In addition, this study adds a new site (Valle de Aburrá Medellín, Colombia) in the distribution for *E. marmosopos*. Importantly, some of these parasites (i.e., *Toxocara* spp., *Hymenolepis* spp., and Ancylostoma spp. have zoonotic potential). The infection of opossums with *Toxocara* spp. has been related to cohabitation with dogs and cats in urban environments, and it may be a pseudoparasite, i.e., that the parasite egg passes intact through the systema digestorium of the opossum and is diagnosed in its feces (Pinto *et al.,* 2014). Other pseudoparasitism 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).

In the case of the infection with the 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 authors reported the incomplete morphology of the adult specimen, although it has been widely reported in Australian marsupials (Spratt and Beveridge, 2016). The opossums in this study inhabit urban areas so there is a possibility that opossums 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 to prevent outbreaks and new pandemics (WHO 2021). Prioritization should be given to long-term research on parasitology and host population/community ecology that employ a variety of methods for identifying the 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). All over the American continent, reports of zoonotic parasites parasitizing opossums have been made; some species are thought to be accidental parasitosis, while others have recently been found to 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 host up to 11 different species of parasites in the systema digestorium at the same time. Some of these parasites are generalists that adapt to any condition, others have zoonotic potential, while others may be pseudoparasites given the opossums omnivorous diet. The current study demonstrates the importance of monitoring wildlife and synanthropic animals, as well as vectors in the future. Public research institutions, particularly those associated with graduate programs, play an unequal role in providing, through their research, measures for disease eradication as well as ensuring quality responses for other biodiversity science areas (Overbeck *et al.,* 2017). In addition, more epidemiological research, and public education campaigns regarding the ecological significance of marsupials and the dangers of intimate contact with them are needed to reduce the risk of pathogen transmission between people, domestic animals, and these mammals. Colombia must conduct more studies to characterize the parasitofauna of the species that have the most frequent direct relationship with human communities in towns and large cities.

Declarations

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Conflict of interest

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

Author contributions

The study was designed by VHM and CPC, field work data was collected by VHM and CPC, Lab work was done by VHM, LNR, SLO, the manuscript was written by VHM, CPC, and all authors reviewed and approved the final version.

Use of artificial intelligence (AI)

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

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