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Frequency of gastrointestinal helminths in synanthropic rodents captured in the Barranquilla Zoo

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ABSTRACT

Objective. The aim of this work was to characterize and describe the frequency of the gastrointestinal parasites in synanthropic rodents captured at the Barranquilla Zoo. **Materials and methods.** The rodents were captured using *Tomahawk* traps with the help of non-toxic bait and subsequently sacrificed by isoflurane inhalation. The diagnosis and identification of parasites in the intestinal contents was performed using direct examination in NaCl (0.9%), modified Sheather's flotation, and modified Ritchie's sedimentation techniques. **Results.** A total of 34 rodents (*Rattus rattus*) were captured, with an infection frequency of 82.4% (95% CI = 68.8–95.8%, 28/34). The most prevalent parasites were nematodes (63.2%, 95% CI = 49.2–77.2%, 31/49), followed by protozoans (20.4%, 95% CI = 8.7–32.1%, 10/49) and cestodes (16.3%, 95% CI = 5.6–27%, 8/49), with significant differences between the three groups ($\chi^2 = 20.7$, $p < 0.01$). Among the nematodes, Strongylidae was the most prevalent family (24/31), followed by Ascarididae (4/31), Ancylostomatidae (1/31), Oxyuridae (1/31), and Trichostrongylidae (1/31); for the protozoans, the most prevalent families were Eimeriidae (6/10) and Entamoebidae (4/10), and for the cestodes, the only family found was the Hymenolepididae (8/8) family. **Conclusions.** The *R. rattus* species was the only species of synanthropic rodents caught in the Barranquilla Zoo; because it was infected with various species of gastrointestinal parasites, it should be considered a risk factor for the transmission of these parasites to humans and other animals in the zoo.

Keywords: Infection; epidemiology; parasitology; mammals (*Source: DeCS*).

RESUMEN

Objetivo. El objetivo del presente trabajo fue describir la frecuencia y caracterización de parásitos gastrointestinales en roedores sinantrópicos capturados en el Zoológico de Barranquilla. **Materiales y métodos.** Para la captura de los roedores se utilizaron trampas *Tomahawk* con ayuda de cebos no tóxicos, y posteriormente los individuos capturados fueron sacrificados mediante la inhalación de isoflurano. El diagnóstico e identificación de parásitos en el contenido intestinal fue realizado mediante

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las técnicas de examen directo en NaCl (0.9%), flotación Sheather modificada y sedimentación Ritchie modificada. **Resultados.** Un total de 34 roedores (*Rattus rattus*) fueron capturados, en los cuales la frecuencia de infección fue de 82.4% (IC 95% = 68.8-95.8%, 28/34). Los parásitos descritos con mayor frecuencia fueron los nematodos (63.2%, IC 95% = 49.2-77.2%, 31/49), seguido de los protozoos (20.4%, IC 95% = 8.7-32.1%, 10/49) y cestodos (16.3%, IC 95% = 5.6-27%, 8/49), con diferencias significativas entre los tres grupos ($\chi^2=20.7$, $p<0.01$). Dentro de los nematodos, la familia encontrada con mayor frecuencia fue la Strongylidae (24/31), seguida de la Ascarididae (4/31), Ancylostomatidae (1/31), Oxyuridae (1/31) y Trichostrongylidae (1/31); para el caso de los protozoos, fueron la Eimeriidae (6/10) y Entamoebidae (4/10), mientras para los cestodos fue la Hymenolepididae (8/8). **Conclusiones.** La especie *R. rattus* fue la única especie de roedor sinantrópico capturada en el Zoológico de Barranquilla, la cual al encontrarse infectada con diversas especies de parásitos gastrointestinales, debe ser considerada un factor de riesgo para la transmisión de estos parásitos hacia personas y otros animales en el zoológico.

Palabras clave: Infección; epidemiología; parasitología; mamíferos (*Fuente: DeCS*).

INTRODUCTION

Synanthropic rodents are dominant species, found in most regions of the world because of their high adaptability (1). They are also of importance in the epidemiological chain as they are key in the transmission of various parasites in wild and domestic animals, as well as humans (1). Rodent-borne etiological agents include several species of bacteria: *Leptospira interrogans*, *L. kirschneri*, *Borrelia burgdorferi*, *Rickettsia* sp., *Bartonella* sp.; protozoans: *Entamoeba coli*, *E. muris*, *Trypanosoma cruzi*, *T. lewisi*, *Chilomastix intestinalis*; nematodes: *Calodium hepaticum*, *Syphacia* sp., *Trichuris* sp. *Strongyloides* sp., and cestodes: *Hymenolepis diminuta*, *H. nana*, *Taenia* sp., among others (2,3). Most of these infectious agents are transmitted through contact with feces and urine from infected rodents and/or through their ectoparasites such as *Xenopsylla cheopis* and *Nosopsyllus fasciatus* (2,3).

In Colombia, although rodents have proven to be important in the transmission of zoonotic microorganisms such as *T. cruzi*, *Leptospira* sp., *Rickettsia* sp., *Toxoplasma gondii*, among others (4,5,6,7), studies related to the parasitic fauna of these animals and their role as possible transmitters of gastrointestinal parasites with an impact on public and animal health are scarce (8, 9). Some studies on this topic suggest greater positivity of gastrointestinal parasites in rodents in urban areas with increased food availability and human density, demonstrating the high risk of infection in places with higher urban growth (8).

Recently, during preventive medical examinations at the Barranquilla Zoo, the presence of intestinal parasitism associated mainly with nematode (Strongyloidea, Trichinellidae, Ascarididae, Toxocaridae, Trychostrongyloidea, Ranidae, among others), protozoa (Eimeriidae, Entamoebidae, Balantidiidae, and Sarcocystidae) and cestode families (Taeniidae and Hymenolepididae) was identified in some animals in the collection (unpublished data); however, the role of synanthropic rodents in the transmission of these parasites has yet to be studied. Based on the above, the aim of the present study was to characterize and describe the prevalence of the gastrointestinal parasites in synanthropic rodents captured in the Barranquilla Zoo, Colombia, to explore the epidemiological role of these hosts in the transmission of these infections to animals and humans.

MATERIALS AND METHODS

Description of the study area. This study was carried out at the Barranquilla Zoo (FUNDAZOO), located in the Atlántico department in the urban area of the municipality of Barranquilla (11°00'39" N 74°47'52" W; 30 MAMSL), Colombia. The ecological conditions of this area resemble those of a dry tropical forest with a monthly rainfall between 70 and 178 mm³ and an average temperature of 29°C (24°C–34°C) (10).

Type of study and sampling methods. A cross-sectional study was conducted on the rodents caught during the rodent control plan at the Barranquilla Zoo facility from June 20 to August 09, 2019. Tomahawk traps were used

to capture the rodents (measurements: 45 cm long x 13 cm wide and 13 cm high) with the help of non-toxic bait (a mixture of corn and peanut butter) (2, 8), randomly placed at 55 capture points in sectors A, B, and C for a total of 168 h/trap (Figure 1). This time was distributed in a maximum period of 42 h per week and 14 h a day. For this reason, the traps were placed three times a week (Monday, Wednesday, and Friday) between 5:00 pm and 7:00 am the next day, every other week. The traps were placed 2 weeks before the start of the study to allow the animals to adapt to their presence.

Processing of captured rodents. After capture, the animals were taken alive to the autopsy area for handling as established in the biosecurity standards and processing regulations, in accordance with the protocols of the Center for Disease Control and Prevention in Atlanta, United States of America (11). Initially, the rodents were anesthetized and desensitized in an anesthetic chamber by inhalation of 5% isoflurane (Baxter, Cali, Colombia). During the sedation period, the sex and weight in grams (g) were determined for each animal using a digital scale (Lexus, Nagoya, Japan). For the males and females, age was established according to the live weight of the animals, whereas species classification was performed according to the morphometric parameters: total length, tail length, and ear length (12).



Figure 1. Distribution of the sampling points at the Barranquilla Zoo. The yellow circles (●) and the red crosses (✖) indicate positive and negative trapping points used to capture the rodents, respectively. The animal exhibition areas are represented in dark green (■) and the paths for visitors are represented in beige (■). The diversity of species in the different sectors corresponds to the following: sector A (birds and mammals), sector B (birds, mammals, and reptiles), and sector C (amphibians, birds, mammals, and reptiles) (Source: Taken and modified from FUNDAZOO <https://www.zoobaq.org/info/mapa.php>).

Once the rodents were sedated and desensitized, the animals were euthanized with an overdose (60 mg/kg) of intracardiac sodium pentobarbital (Euthanex, Bogota, Colombia) (11). At the time of autopsy, the gastrointestinal tract was evaluated for adult endoparasites and macroscopic lesions compatible with parasitic cysts in the liver and peritoneum. The largest helminths were collected and preserved in 10% formalin and transferred to the zoo's clinical laboratory for morphological identification. Finally, samples of the intestinal contents were taken from the small and large intestines of each animal and preserved in 10% formalin until parasitological diagnosis.

Parasitological diagnosis. The techniques used for the diagnosis of infection and identification of gastrointestinal parasites in each intestinal contents sample were direct examination with physiological saline solution (NaCl 0.9%), modified Sheather's flotation, and modified Ritchie's sedimentation (13, 14). Each animal was considered positive for infection by gastrointestinal parasites when eggs or larvae were observed by at least one of the above-mentioned techniques. The morphological identification of each class, family, and gender of adult parasite, as well as their immature forms, was carried out according to the morphological keys described in the scientific literature (15,16,17,18).

Data analysis. The frequency of gastrointestinal parasites was expressed in percentages, with their respective confidence interval at 95% (CI 95%). The differences between infection frequencies between parasite types, ages, and rodent capture sector were explored by the chi-squared test (χ^2). The degree of concordance between diagnostic techniques was determined using a Kappa (K) coefficient. All of the analyses were performed using the SPSS v.23.0 statistical package. A $p < 0.05$ was considered significant. All graphs were plotted using GraphPad Prism v.8.1.1 software.

Ethical considerations. All procedures were carried out under the guidelines of good clinical and ethical practices set out in the health code for land animals (19). Bioethics endorsement was granted by the bioethics subcommittee of the Cooperative University of Colombia, Bucaramanga campus, under the ethical principles for the handling of animals, established in the National Statute of animal protection, Law 84 of 1989 and resolution No. 008430 of 1993 of the Ministry of Health (Bogota-Colombia).

RESULTS

Description of sampling. A total of 34 rodents (*Rattus rattus*) were captured during the study period, 53% (18/34) of which were classified as adults and 47% (16/34) as sub-adults. Most of the animals were females (61.8%, 21/34) with an average weight of 181.3 g. The highest capture rates were observed in sectors B (35.2%, 12/34) and C (35.2%, 12/34), followed by sector A (29.4%, 10/34).

Description of the frequency of infection by gastrointestinal parasites. Of the total animals analyzed, 82.4% (95% CI = 68.8%–95.8%, 28/34) were positive for infection with gastrointestinal parasites in at least one diagnostic test. The highest rates of infection were observed in females (85.7%, 95% CI = 69.3%–102%, 18/21), adults (88.9%, 95% CI = 72.8%–104.9%, 16/18) and sector B animals (91.7%, 95% CI = 73.3%–110%, 11/12). No significant differences were observed between the categories analyzed (sex, age, and capture sector) (Table 1).

Table 1. Description of the frequency of gastrointestinal parasites identified in *Rattus rattus* rodents for the different categories analyzed.

Variable	n	Frequency	95% CI	χ^2	p
Sex					
Female	21	85.7% (18/21)	69.3–102%	0.041	0.838
Male	13	76.9% (10/13)	50.4–103.4%		
Age					
Adults	18	88.9% (16/18)	72.8–104.9%	0.109	0.740
Sub-adults	16	75% (12/16)	51.1–98.8%		
Capture sector					
A	10	70% (7/10)	35.4–104.5%	0.175	0.915
B	12	91.7% (11/12)	73.3–110%		
C	12	83.3% (10/12)	58.6–108%		

Regarding the characterization of infections in the positive samples, the most prevalent parasites were nematodes (63.2%, 95% CI = 49.2–77.2%, 31/49), followed by protozoans (20.4%, 95% CI = 8.7–32.1%, 10/49) and cestodes (16.3%, 95% CI = 5.6–27%, 8/49), with significant differences between the three groups ($\chi^2 = 20.7$, $p < 0.01$). Among the nematodes, the most prevalent family was Strongylidae (77.4%, 95% CI = 61.8–93%, 24/31), followed by Ascarididae (12.9%, 95% CI

=0.4–25.4%, 4/31), Ancylostomatidae (3.2%, 95% CI = -3.3% to 9.8%, 1/31), Oxyuridae (3.2%, 95% CI = -3.3% to 9.8%, 1/31), and Trichostrongylidae (3.2%, 95% CI = -3.3% to 9.8%, 1/31), respectively. As for the protozoans, Eimeriidae (60%, 95% CI = 23–96.9%, 6/10) and Entamoebidae were the most prevalent families (40%, 95% CI = 3–76.9%, 4/10), whereas, for cestodes, the only family found was Hymenolepididae (100%, 95% CI = 100–100%, 8/8) (Figure 2).

At the time of autopsy, adult parasites were observed in 20.5% (95% CI = 6.2–34.9%, 7/34) of the animals analyzed, 14.7% (95% CI = 2.1–27.2%, 5/34) of which corresponded to hydatid cysts consisting of immature *Hymenolepis* sp. larvae, and 5.8% (95% CI = -2.4% to 14.2%, 2/34) of *Strongylus* sp. larvae, located in the intestinal lumen.

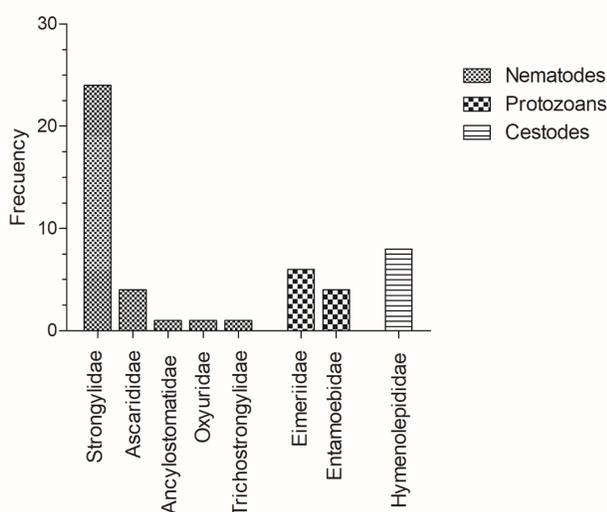


Figure 2. Frequency of families of gastrointestinal parasites identified in synanthropic rodents captured in the Barranquilla Zoo.

Among the techniques used, the sedimentation technique was the most effective at detecting an infection frequency of 76.5% (95% CI = 61.4–91.4%, 26/34), followed by the direct technique (67.6%, 95% CI = 51–84.2%, 23/34) and flotation (32.4%, 95% CI = 15.7–48.9%, 11/34). The highest degree of concordance was observed between the sedimentation and direct techniques with $K = 0.49$ (95% CI = 0.16–0.83) (Table 2).

Table 2. Degree of concordance between coprological tests used in synanthropic rodents captured in the Barranquilla Zoo.

Combination	Kappa Coefficient (K)	95% CI
Sedimentation vs. Flotation	0.26	-0.02 to 0.54
Sedimentation vs. Direct	0.49	0.16 to 0.83
Direct vs. Flotation	0.27	-0.03 to 0.56

DISCUSSION

The Barranquilla Zoo is one of the main recreational centers in the Caribbean region of Colombia, with approximately 300,000 visitors attending each year, and which generates approximately 100 direct and indirect jobs (20). At this institution, synanthropic rodents represent one of the most difficult pests to control as they not only cause large economic losses but also pose a public health risk because of their role in the transmission of zoonotic diseases (1, 2, 8, 9).

In this study, the exclusive presence of synanthropic *R. rattus* rodents was observed with an infection frequency of 82.4% by gastrointestinal parasites. These results are similar to those reported in urban centers in Peru and Cuba (64.1–77.4%) (1, 2, 21) but considerably higher than those observed in urban and rural centers in Mexico, where the prevalence in these same hosts ranged from 42.7% to 52.2% (22,23). Considering that, in America, the frequency of infection with gastrointestinal parasites in synanthropic rodents from zoos has been poorly studied, the high frequency of infection observed here can be explained because of the higher density of definitive hosts and intermediates present in the zoo. In Brazil, some studies in carnivores (24, 25) concluded that captive conditions can increase the prevalence of infection with gastrointestinal parasites, favoring the spread to other animals, as well as humans. In this sense, maintaining gastrointestinal parasite control programs and intensifying rodent control is important to prevent the spread of these parasites to other species. The exclusive presence of *R. rattus* (an arboreal species) and its capture with floor traps may be an indicator of a suitable ecological niche for this species, where the high availability of food, places for shelter, lack of natural predators, and ability to adapt to control strategies can generate a biological advantage over other species (26). However, the effect of the type of trap used in this

study, which reduces the probability of catching other synanthropic species (2,8), and the effect of rodent control strategies in the zoo, which are focused on land rodents, may also explain this result.

In terms of the characterization of gastrointestinal parasites, the nematodes were observed to be the most prevalent group, followed by protozoans and cestodes. These results are similar to those reported in animals in the zoo's collection (unpublished data), suggesting the role of these rodents as possible reservoirs in the study area (1,2,8,9). In the case of nematodes, the Strongylidae family presented a higher frequency of infection; these results are similar to those reported in *R. rattus*, *R. norvegicus*, and *Mus musculus* from the island of Cuba (21). However, they differ from those observed in *R. rattus* and *R. norvegicus* in the metropolitan area of Lima, Peru (1), where the most frequently identified nematodes belonged to the Gongylonematidae family. This indicates that the prevalence of each family varies depending on the ecological conditions of the area and host diversity (26,27). The high presence of parasites from the Strongylidae family may be associated with the various infection pathways, including the fecal-oral, percutaneous, and lactogenic (28) pathways, as well as a possible adaptation of these parasites to the immune system of *R. rattus* (28). This scenario, although common in urban areas, poses a risk of infection for people and animals in the zoo, since this family is considered of zoonotic importance (2). These results encouraged interventions in the rodent control program at the zoo to reduce the risk of infection to humans.

Presence of a high proportion of Hymenolepididae family among the cestodes identified in *R. rattus* is consistent with that observed in other American countries, where *R. norvegicus*, *M. musculus*, and *Sigmodon hirsutus* species have also been found infected with that family (1, 2, 8, 9). Although the direct and indirect transmission of these parasites is common among wild animals, indicating the possible infection of animals in the collection, their zoonotic significance is low, as human infection only occurs through the accidental consumption of intermediate hosts such as beetles and fleas of the genera: *Tenebrio*, *Tribolium*, *Ctenocephalides*, *Pulex*,

and *Xenopsilla*, infected with cysticeroid (9). In contrast, the prevalence of protozoans from the Eimeriidae and Entamoebidae families is higher than that observed in Central American countries where the prevalence of these families does not exceed 5% (21). Given that the consumption of water contaminated with cysts from these parasites is considered the main route of infection in animals and humans (21), it is important that future studies determine if the water used in the zoo is a source of infection.

Finally, although this study determined a high frequency of infection by gastrointestinal parasites, as well as the presence of some families of zoonotic importance, the lack of highly sensitive and specific techniques made it difficult to thoroughly understand the role of these rodents in the transmission of species of significance in public and animal health. Future studies using diagnostic techniques such as polymerase chain reaction (PCR), real time-PCR, among other molecular techniques, are necessary to solve these difficulties. In conclusion, the high prevalence of infection with gastrointestinal parasites in *R. rattus* in the Barranquilla Zoo shows the importance of these animals as sources of infection for other animals and humans. The high frequency of Strongyloidae parasites in rodents captured at the study site suggests a public health risk, that encouraged to intensify control measures to reduce the risk of infection and improve health status.

Conflict of interests

The authors affirm that there are no potential conflicts of interest regarding the research, authorship, or publication of this article.

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