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Hymenolepis diminuta and *Rodentolepis nana* (Hymenolepididae: Cyclophyllidea) in urban rodents of Gran La Plata: association with socio-environmental conditions

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Research Paper

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Abstract

The aim of this survey was to study two Hymenolepididae species in urban rodents, *Rattus rattus* and *Rattus norvegicus*, and to analyse factors that favour their presence in the environment and pose a sanitary risk. *Hymenolepis diminuta* and *Rodentolepis nana* were found in *R. rattus* and *R. norvegicus* in different proportions. Values of prevalence, mean abundance and mean intensity were recorded, and new morphometric characters were described, adding to previously known information. No significant differences were found. However, the results revealed that there is a tendency for these parasites to develop in areas with deficient socio-structural conditions and in water bodies. This study thereby shows that certain areas on the periphery of the Gran La Plata favour the existence of rodents, which act as sentinels of zoonotic diseases, and stresses the need to take action to minimize them in order to avoid putting human and animal health at risk.

Introduction

Helminthiases affect 20% of the Latin-American population and are considered to be neglected diseases, with approximately 3800 million people infected worldwide (Acuña *et al.*, 2003). These infections are frequent in rural populations and in overcrowded areas, which are characterized by having deficient structural conditions and poor sanitation, and by suffering the effects of environmental pollution (Zonta *et al.*, 2007).

Hymenolepiasis is a worldwide parasitosis caused by cestodes of the genera *Hymenolepis* and *Rodentolepis* (Hymenolepipidae: Cyclophyllidea). The life cycles of these parasites involve humans, rats and mice as definitive hosts, and arthropods as intermediate hosts. The disease is more common in warm and humid areas, and causes clinical symptoms mostly in children (Acha & Szyfres, 1986; Llop *et al.*, 2001; Hernández Mazariegos, 2016). Rats and mice act as definitive hosts of *Hymenolepis diminuta* (Rudolphi, 1819) (Mikhail *et al.*, 2009; Martinez-Barbabosa *et al.*, 2012) and it is found in humans only occasionally, since it needs an arthropod to complete its life cycle (Martinez-Barbabosa *et al.*, 2012). *Rodentolepis nana* (Von Siebold, 1852) is a human parasite, even though it can be found in other mammals. It is estimated that 20 million people in the world are parasitized with this cestode species (Incani *et al.*, 2003; Rossomando *et al.*, 2008) mainly due to direct transmission. However, both parasitoses share some epidemiological characteristics, such as higher prevalence in children of marginalized areas with poor hygiene habits and sanitary conditions, and overcrowding (Martinez-Barbabosa *et al.*, 2012).

Argentina possesses a wide variety of climate types. Buenos Aires is a province characterized by having a temperate climate and the Gran La Plata is particularly humid due to its proximity to three streams (El Gato, Pérez and Maldonado) running east to west and north to south (Auge *et al.*, 1995). It is estimated that there are 118 shanty towns with 18,500 homes on the periphery of the Gran La Plata (pers. comm.). This seems to be a favourable scene for urban rodents, which are mostly the house mouse (*Mus musculus* Linnaeus, 1758), the black rat (*Rattus rattus* Linnaeus, 1758) and the Norway rat (*Rattus norvegicus* Berkenhput, 1769). Both *R. rattus* and *R. norvegicus* are considered to be hosts of parasite species that pose a sanitary risk (Stojcevic *et al.*, 2004; Waugh *et al.*, 2006; Easterbrook *et al.*, 2007; Gómez Villafañe *et al.*, 2008; Hancke *et al.*, 2011). Moreover, these species of *Rattus* are the main definitive hosts of *H. diminuta* (Mafiana *et al.*, 1997; Battersby *et al.*, 2002; Zain *et al.*, 2012; Hancke & Suárez, 2016) and sometimes of *R. nana* (Hernández Mazariegos, 2016; Tresnani *et al.*, 2016).

Morphological studies of *H. diminuta* and *R. nana* are scarce, especially considering that these parasite species are cosmopolitan and widely distributed in anthropogenic environments.

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The aim of this study was to analyse Hymenolepididae species found in *R. rattus* and *R. norvegicus*, to evaluate the factors that favour their presence in the environment and to detect possible sanitary risks.

Materials and methods

The Gran La Plata is formed by three departments: La Plata, Ensenada and Berisso, and has approximately 799,523 inhabitants. The study was conducted in five peripheral areas of La Plata: Malvinas (34°56′43″S, 58°00′36″W), El Retiro (34°57′51″S, 58°00′17″W), La Latita (34°58′31″S, 57°58′30″W), La Isla (34°53′28″S, 57°59′25″W) and Abasto (34°58′05″S, 58°01′47″W). Part of the City of La Plata (34°55′16″S, 57°57′16″W) and the area comprising the neighbourhood El Carmen (34°55′33″S, 57°53′09″W) of Berisso were also included. All studied areas registered high levels of garbage concentration, except for the City of La Plata where there is an organized rubbish collection system. Moreover, most households lack fresh water, having instead an elementary water supply system called 'black hose', and have no sewage system, with the exception of the City of La Plata and El Carmen. In addition, El Retiro, La Isla and El Carmen are crossed by highly contaminated streams.

Samples were collected between September 2014 and August 2015, and divided into two periods: spring–summer, with monthly temperatures equal to or above 16°C and precipitation averaging 100 mm; and autumn–winter, with monthly temperatures below 16°C and precipitation below 100 mm.

The specimens were obtained using methods for live capture and were sacrificed. They were studied in the Centro de Estudios Parasitológicos y de Vectores (CEPAVE) of La Plata. Both viscera and faecal samples were fixed in 10% formalin. Cestodes were removed from their infection site and were preserved in 70% alcohol. Specimens were overstained with acid carmine, dehydrated through an alcohol series, diaphanized in eugenol and mounted in Canada balsam. The identification of Hymenolepididae was based on specific bibliography. Faecal samples were processed using a sedimentation technique which included homogenization, filtration and microcentrifugation in 1.5-ml Eppendorf tubes for 2 min at 3000 rpm, in order to concentrate the largest amount of eggs in the minimum possible volume for their identification.

Prevalence (P), mean intensity (MI) and mean abundance (MA) indices were calculated (Bush *et al.*, 1997) for each host species in relation to sex, area and sampling period. A test of difference of proportions and a Fisher test were used to evaluate P differences, while a Bootstrap test (97.5% confidence limits) was performed to compare MI and MA differences (Quantitative Parasitology 3.0 software; Rózsa *et al.*, 2000).

Parasites were deposited in the Colección Helmintológica del Museo de La Plata (He-MLP 7397, 7398, 7399, 7400), La Plata, Buenos Aires, and hosts in the Colección de Mastozoología del Centro Nacional Patagónico, Puerto Madryn, Chubut (*R. rattus* CNP 5919, CNP 5925; *R. norvegicus* CNP 5906, CNP 5908, CNP 5917 – a total of 79 (analysed) specimens were deposited).

Results and discussion

A total of 49 rodents were analysed for this study: 21 *R. rattus* and 28 *R. norvegicus*. Two species of Hymenolepididae highly related to urban rodents and with associated sanitary risk were identified: *H. diminuta* and *R. nana*. Specimens were mostly found in the anterior portion of the small intestine. Morphological and biometric characters of both parasite species were compared to support their identification (fig. 1). Measurements are given in table 1.

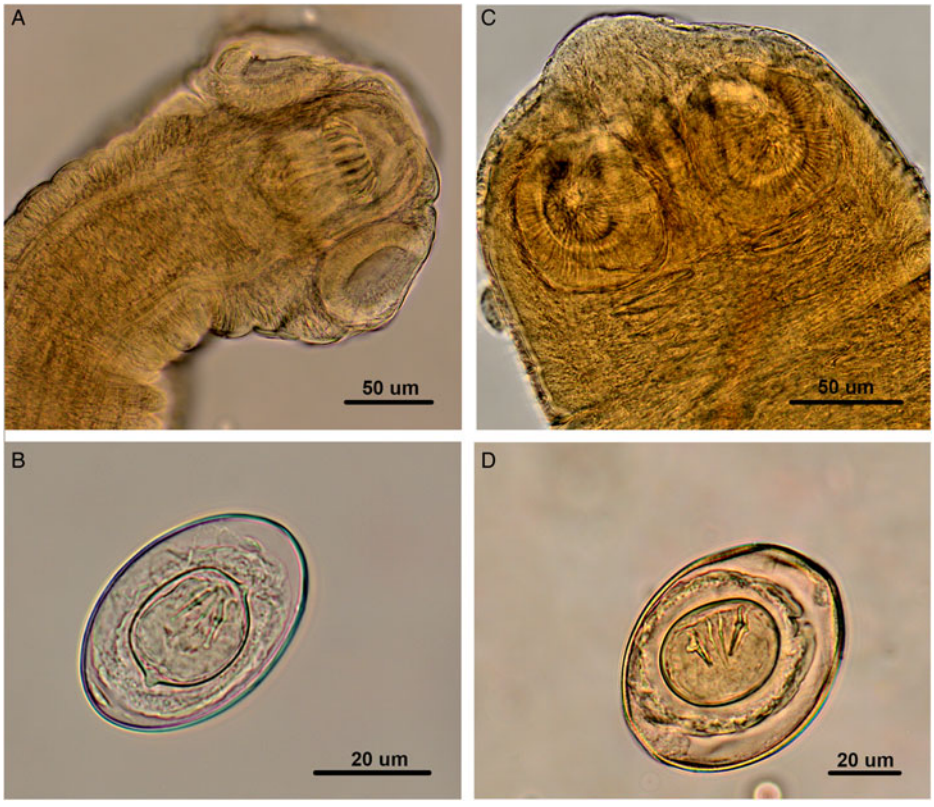

Fig. 1. *Rodentolepis nana*: (A) scolex of a specimen from *R. norvegicus*, (B) detailed view of an egg with polar thickenings. *Hymenolepis diminuta*: (C) scolex of a specimen from *R. rattus*, (D) detailed view of an egg without polar thickenings.

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Table 1. Morphometric description of *H. diminuta* and *R. nana*. *N*, number of host specimens captured.

Species	*H. diminuta*	*R. nana*
Host species	*R. norvegicus*	*R. norvegicus*
	R. rattus	*R. rattus*
N	8	5
Total length (mm)	37.58 (18.1–57.9)	7.25 (4.8–9.7)
Maximum width (mm)	1.10 (0.7–1.3)	0.32 (0.1–0.9)
Scolex width	0.22 × 0.2	0.21 × 0.15
Suckers width	0.11 × 0.09	0.06 × 0.05
Rostellum	–	0.065 × 0.052
Number of hooks	–	23
Hooks length	–	0.017–0.019
Eggs	0.08 × 0.07	0.05–0.058 × 0.03

Hymenolepis diminuta has a scolex with four rounded suckers and an unarmed rostellum (fig. 1A). Moreover, it shows the typical characteristics of the reproductive organs and gravid proglottids, containing a large number of eggs with a diameter of 0.08 × 0.07 mm. With six hooks, the oncosphere is covered with a striated outer membrane and a thin inner membrane. No polar filaments extend between the oncosphere and the outer shell (fig. 1B).

Rodentolepis nana has a scolex with four small, rounded suckers and a rostellum armed with a crown of 20–30 hooks (fig. 1C). Moreover, it shows the typical characteristics of the reproductive organs and gravid proglottids, with a large number of eggs with a diameter of 0.05–0.058 × 0.03 mm. With six hooks, the oncosphere is covered with a thin, hyaline, outer membrane and an inner, thick membrane with polar thickenings that bear several filaments (fig. 1D).

Of all analysed rodents, ten individuals were parasitized with at least one cestode, representing 20.4% total prevalence. Total values of prevalence (P), mean intensity (MI) and mean abundance (MA) were: *H. diminuta* P = 12.2%, MI = 2.3, MA = 0.3; and *R. nana* P = 8.2%, MI = 2.8, MA = 0.2 (table 2). In either case, both parasite species were found in the same host individual.

In relation to host species, *R. norvegicus* revealed the highest infection indices with P, MI and MA values of 21.4%, 2.5 and 0.5, respectively; while the values for *R. rattus* were 19.1%, 2.5 and 0.5, respectively. Total values of P, MI and MA were 28.6%, 2.25 and 0.6 for females; and 17.1%, 2.7 and 0.5 for males.

Concerning the sampling locations, Hymenolepididae specimens were found in four of the seven surveyed areas with the following P, MI and MA values: 33.3%, 3 and 1 in La Isla; 20%, 2.5 and 0.5 in El Carmen; 14.29%, 3.5 and 0.5 in El Retiro, and 40%, 1 and 0.4 in Abasto.

As to the sampling periods, the highest value of total prevalence was recorded in autumn–winter (24.1%), while in spring–summer it was 15%. MI and MA values for autumn–winter were 2.4 and 0.6; while for spring-summer they reached 2.7 and 0.4, respectively.

The values of these indices for each parasite species are given in table 2. No significant differences between host species, sex, area or sampling period were found for P, MI or MA.

Although there have been other studies concerning these parasite species (e.g. Llop *et al.*, 2001; Incani *et al.*, 2003; Hernández Mazariegos, 2016), only a few of them provide morphological and biometric descriptions. It was observed in the present study that the diagnostic measurements of *H. diminuta* and *R. nana* agree with those of Wardle & McLeod (1952) and Guerreiro Martins *et al.* (2014).

Table 2. Total values of prevalence (P), mean intensity (MI) and mean abundance (MA) for each parasite species. *N*, Number of host specimens captured.

		H. diminuta	*R. nana*
Host			
R. rattus (*N* = 21)	P (%)	14.3	4.8
	MI	3	1
	MA	0.4	0.1
R.norvegicus (*N* = 28)	P (%)	10.7	10.7
	MI	1.7	3.3
	MA	0.2	0.3
Total	P (%)	12.2	8.2
	MI	2.3	2.8
	MA	0.3	0.2
Sex			
Male (*N* = 35)	P (%)	11.4	5.7
	MI	1.8	4.5
	MA	0.2	0.3
Female (*N* = 14)	P (%)	14.3	14.3
	MI	3.5	1
	MA	0.5	0.1
Area			
La Isla (*N* = 6)	P (%)	16.7	16.7
	MI	3	3
	MA	0.5	0.5
El Carmen (*N* = 20)	P (%)	15	5
	MI	3	1
	MA	0.5	0.1
El Retiro (*N* = 14)	P (%)	0	14.3
	MI	0	3.5
	MA	0	0.5
Abasto (*N* = 5)	P (%)	40	0
	MI	3	0
	MA	0.4	0
Sampling period			
Spring/summer (*N* = 20)	P (%)	5	10
	MI	6	1
	MA	0.3	0.1
Autumn/winter (*N* = 29)	P (%)	17.2	6.9
	MI	1.6	4.5
	MA	0.3	0.3

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Both species of cestodes are cosmopolitan parasites that have a wide range of prevalence values, ranging from 80% to close to zero. These species have been recorded in *R. rattus* and *R. norvegicus* in different environments, but mostly in urban areas (Mafiana *et al.*, 1997; Battersby *et al.*, 2002; Iannacone Oliver & Alvariño Flores, 2002; Abu-Madi *et al.*, 2005; Easterbrook *et al.*, 2007; Kataranovski *et al.*, 2011; Zain *et al.*, 2012, Hancke & Suárez, 2016).

Hymenolepiasis is more frequent in areas where structural and socio-environmental conditions are poor and where there is close contact between rodents and humans. High prevalence values of *R. nana* in *R. norvegicus* are therefore indicators of this coexistence between this host and humans, who are the principal definitive hosts. A 10.7% prevalence of this parasite species was observed in *R. norvegicus* and 4.8% in *R. rattus*. In contrast, the prevalence of *H. diminuta* was higher in *R. rattus* than in *R. norvegicus* (14.3% vs. 10.7%), in agreement with the results provided by other studies (Mafiana *et al.*, 1997; Battersby *et al.*, 2002; Zain *et al.*, 2012; Hancke & Suárez, 2016).

Concerning the sampling locations, the number of rodents captured was higher in areas adjacent to water bodies, such as La Isla, El Retiro and El Carmen. Both *H. diminuta* and *R. nana* were found in La Isla (P = 16.7% for both) and El Carmen (P = 15% and 5%, respectively) in significant proportions and, therefore, represent possible foci of infection, whereas only *R. nana* was recorded in El Retiro (P = 14.3%). On the other hand, no parasites were registered in sites with no nearby associated water bodies, with the exception of Abasto where two out of the five host specimens captured (40%) were infected with *H. diminuta*. This could indicate the presence of other conditions that favour the life cycle of this parasite.

Although these tendencies were not reflected in the statistical analyses performed, the results allow us to advance in the study of factors related to hosts (e.g. sex and reproductive period, among others) and to the environment (e.g. seasons and relation with water bodies, among others), to evaluate the effects of actions tending to reduce the population density of rodents and, as a consequence, the parasites that might pose a sanitary risk.

This study shows that structural and environmental conditions of certain areas on the periphery of the Gran La Plata favour the existence of rodents, inside and outside houses. In this context, synanthropic rodents act as sentinels of zoonotic diseases and this reveals the need to take action to minimize their presence by, for example, installing appropriate water and sewage systems, adopting new waste management practices and outlining different educational programmes, with the aim of reducing the risk they entail to human and animal health.

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Conflict of interest. None.

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