

Nota



Taenia taeniformis LARVAE (*Strobilocercus fasciolaris*) (CESTODA: CYCLOPHYLLIDEA) FROM COMMENSAL RODENTS IN ARGENTINA: POTENTIAL SANITARY RISK

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ABSTRACT. The main purpose of this study was to characterize the helminthological fauna of three commensal rodents from Argentina, with the final goal of finding *Strobilocercus fasciolaris* (Cestoda: Cyclophyllidea), discussing possible reasons of the maintenance of the cycle in the study areas. *Strobilocercus fasciolaris* was observed in all the studied rodent species, including *Rattus rattus* and *Mus musculus*, which constitute new host records in Argentina. Estimates of prevalence, mean abundance and mean intensity were provided, and the existing morphometric characterization of the parasite was supplemented. Significant differences of prevalence between *R. norvegicus* and *M. musculus* and among seasons were observed. The potential risk of zoonotic transmission of *S. fasciolaris* is suggested.

RESUMEN. Larvas de *Taenia taeniformis* (*Strobilocercus fasciolaris*) (Cestoda: Cyclophyllidea) en roedores comensales de Argentina: potencial riesgo sanitario. El objetivo de este estudio fue identificar la fauna helmintológica de las tres especies de roedores comensales de Argentina, con el fin de hallar *Strobilocercus fasciolaris*, y discutir las posibles razones del mantenimiento del ciclo de vida en las áreas involucradas. *Strobilocercus fasciolaris* fue observado en todas las especies de roedores, incluyendo *Rattus rattus* y *Mus musculus*, que constituyen nuevos registros hospedatorios en Argentina. Se brindan los valores de prevalencia, abundancia media e intensidad media y se complementa la caracterización morfométrica de la especie. Fueron observadas diferencias significativas entre la prevalencia y estaciones del año de captura de *Rattus norvegicus* y *M. musculus*. Se sugiere un riesgo potencial de transmisión zoonótica de *S. fasciolaris*.

Key words: Argentina. Commensal rodents. *Strobilocercus fasciolaris*. Zoonotic parasites.

Palabras claves: Argentina. Parásitos zoonóticos. Roedores comensales. *Strobilocercus fasciolaris*.

In Latin America, more than half of the population lives in urban areas, characterized by a strong social and economic fragmentation (Rodgers et al., 2011; Hancke and Suárez, 2014). In Argentina, the levels of poverty and inequality have grown noticeably, originating multiples shantytowns consolidated mostly in the periphery of the bigger cities (Zonta et al., 2007). The urban environment is particularly problematic with regard to rat-associated health risks since zoonotic disease transmissions are frequent. The main commensal species are the house mouse (*Mus musculus* Linnaeus, 1758), the black rat (*Rattus rattus* Linnaeus, 1758) and the Norway rat (*R. norvegicus* Berkenhput, 1769). Several studies have shown that certain gastrointestinal helminths of these rodents represent a risk to public health (Waugh et al., 2006; Hancke et al., 2011). The helminths *Hymenolepis nana* Von Siebold, 1852, *Hymenolepis diminuta* Rudolphi, 1819 and *Capillaria hepatica* Bancroft, 1893 have been reported as zoonotic, and *Moniliformis moniliformis* Bremser, 1811 and *Raillietina* sp. as potentially zoonotic species (Stojcevic et al., 2004; Waugh et al., 2006; Easterbrook et al., 2007; Gómez Villafañe et al., 2008; Hancke et al., 2011). Moreover, rodents are the intermediate hosts for *Echinococcus multilocularis* Leuckart, 1863, *Toxocara* spp. and *Toxoplasma gondii* Nicolle and Manceaux, 1908, and may serve as indicators for assessing the occurrence and level of environmental contamination and infection pressure with free-living stages of these zoonotic parasites (Reperant et al., 2009). Other parasite that uses rodents as intermediate hosts is the cosmopolitan cestode *Taenia taeniaeformis* Batsch, 1786 (Cestoda: Cyclophyllidae). This Cyclophyllidae, completes its life cycle using predator-prey interactions. Adult stages parasitize the small intestine of felids, viverrids and canids, including domestic dogs and mainly domestic cats (Loos-Frank, 2000; Miño et al., 2013). Besides, rodents, lagomorphs, insectivores, and, sporadically, even humans also act as intermediate hosts (Loos-Frank, 2000; Bowman et al., 2002). The intermediate hosts become infected by ingesting eggs from the contaminated environment, and they develop a characteristic strobilocercus-type larva, called *Strobilocercus fasciolaris*, encysted

in the liver, where they continue their development (Al Salihi et al., 2009; Duque et al., 2012). This larva was recorded around the world in commensal rodents including *M. musculus*, *R. norvegicus* and *R. rattus* (Bowman et al., 2002; Panti May et al., 2013). In Argentina, however, strobilocerci have been found only in *R. norvegicus* (Hancke et al., 2011) and in the sigmodontine rodent *Akodon azarae* (Fischer, 1829) (Miño et al., 2013). The purpose of this survey was to study the helminthological fauna of *M. musculus*, *R. norvegicus* and *R. rattus*, to find *S. fasciolaris* in their livers. The possible reasons of the maintenance of the cycle of this larva in the areas involved, considering the sanitary risk that they represent, are discussed.

Six areas of La Plata and one of Berisso were involved in this study, representing different levels of urbanization, inadequate supply of basic urban services, such as garbage removal, sanitation networks, potable water, pavement, areas susceptible to flooding and domestic animals without sufficient care. Five of the neighborhoods were situated on the outskirts 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), Abasto (34°58'05" S, 58°01'47" W) and the city of La Plata (34°55'16" S, 57°57'16" W); and one in a peripheral area of Berisso: El Carmen (34°55'33" S, 57°53'09" W). Seasonal samplings were carried out between September 2014 and September 2015. For this study, the time of the year was divided in spring-summer (mean monthly temperatures 16 °C or more and monthly precipitation above 100 ml) and autumn-winter (mean monthly temperatures below 16 °C and monthly precipitation 100 ml or less). Sampling was carried out under official permits granted by Dirección de Flora y Fauna, Ministerio de Asuntos Agrarios de Provincia de Buenos Aires (expedient N° 22500-7981/10). The specimens were obtained following the procedures and protocols approved by national laws (Animal Protection National law 14.346 and references in the provincial permits) and Ethics Committee for Research on Laboratory Animals, Farm and Obtained from Nature of National Council of Scientific and Technical

Research (CONICET). Eighty-four traps were set inside and in the backyard of houses of each area. The rodents were sexed. The digestive track and annexed glands were fixed in 10% formalin. Cysts collected from livers were dissected under stereoscopic microscopy, dehydrated and stained for the further observation under optic microscopy. Some cysts were measured and soaked in paraffin, cut in 5µm-thick slides and stained using the hematoxylin-eosin technique. The measurements of specimens are given in mm or µm unless otherwise stated. Conventional studies were realized for the taxonomic identification of the specimens found (Pritchard and Kruse, 1982). Voucher specimens of strobilocercus larva were deposited in the Helminthological Collection of Museo de La Plata (He-MLP), La Plata, Buenos Aires; and hosts in the Mastozoological Collections of Centro Nacional Patagónico (CNP), Puerto Madryn, Chubut, Argentina. Prevalence (P), mean abundance (MA) and mean intensity (MI) indexes were calculated for hosts, areas, seasons and sexes, according to Bush et al. (1997). To evaluate the statistical differences of proportions between host species and seasons a test of difference in proportions and a Fischer's exact test were

used; and to compare the differences of infection (MA and MI) the Bootstrap test was applied (97.5% confidence limits) (Quantitative Parasitology 3.0 software, Rózsa et al., 2000).

The rodents trapped in the present study (n=136) correspond to the following species: *M. musculus* (n=87), *R. norvegicus* (n=28) and *R. rattus* (n=21). The study of the helminthological fauna revealed the presence of *S. fasciolaris* in the livers of the three host species. Forty-six cysts (16 in *M. musculus*, 19 in *R. norvegicus* and 11 in *R. rattus*) were found in 26 rodents (12 *M. musculus*, 10 *R. norvegicus* and 4 *R. rattus*).

Cysts were located uniformly within the liver tissue (Fig. 1A, B). The size varied depending on the larvae development stage, but was similar between among species: 5 ± 2.14 (2-10) mm long by 4 ± 2.43 (2-9) mm wide (n=21). Inside each cyst, there was a strobilocercus (Fig. 1C-F) of 31.67 ± 20.04 (9-85) mm long by 2.67 ± 0.52 (1.5-3.5) mm wide, including a

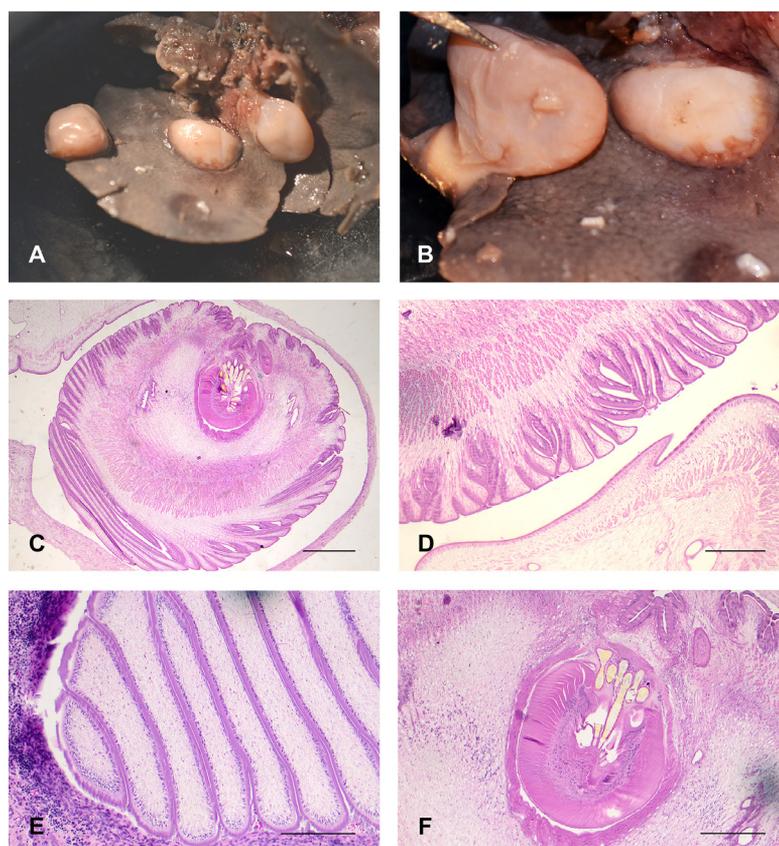


Fig. 1. Macroscopic and histological view of liver and cysts of *Strobilocercus fasciolaris*. (A) Macroscopic view of cysts into the liver of a specimen of *R. norvegicus*. (B) Macroscopic view of cysts into the liver, detail. (C) Histological view of larva inside the cyst, transversal cut (200 µm). (D) Detailed view of limit of larva (200 µm). (E): Detailed view of the segmented body of larva (200 µm). (F) Histological view of a scolex of the larva, transversal cut (200 µm).

well-developed scolex (**Fig. 1F**) of 1.53 ± 0.19 (1.3-1.8) mm diameter, and a long segmented body ended in a bladder. The scolex had 4 prominent round suckers (**Fig. 2A**) of 383 ± 44.21 (291-473) μm of diameter, and a rostellum of 748 ± 99.93 (652-890) μm of diameter, armed with 2 alternating crowns of 17-19 hooks each, with the larger hooks measuring 313 ± 64.18 (211-430) μm and the smaller ones 200 ± 41.29 (124-293) μm (**Fig. 2B**).

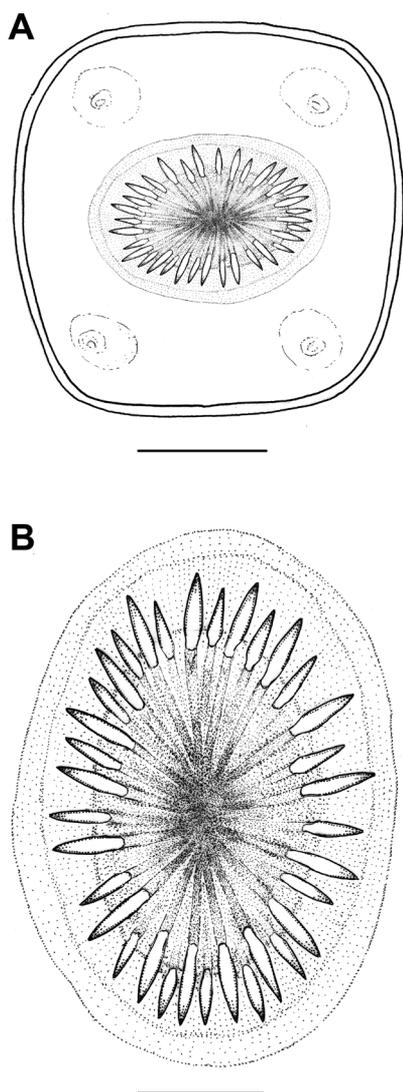


Fig. 2. Illustrations of the scolex of *S. fasciolaris*. (A) Scolex containing four round suckers and a rostellum armed with two alternating crowns of hooks (400 μm). (B) Detail of rostellum, with crowns of large and small hooks (200 μm).

The host species with the highest percentage of infection was *R. norvegicus*. The values of P, MA and MI obtained were: 35.71%, 0.68 and 1.90 for *R. norvegicus*; 19.04%, 0.52 and 2.75 for *R. rattus*, and 13.79%, 0.18 and 1.33 for *M. musculus*, respectively. Regarding seasons, P, MA and MI were: 43.24%, 0.73 and 1.69 for autumn-winter, and 10.10%, 0.18 and 1.8 for spring-summer, respectively. In relation to the different sampling areas, the values of P, MA and MI were: 30.77%, 0.69 and 2.25 for La Isla; 29.17%, 0.63 and 2.14 for El Retiro; 20%, 0.20 and 1.00 for Abasto; 14.29%, 0.18 and 1.25 for La Latita; 13.04%, 0.17 and 1.33 for Malvinas Argentinas; 11.11%, 0.11 and 1.00 for center of La Plata, and 9.52%, 0.10 and 1.00 for El Carmen, respectively. In the case of the different areas, La Isla showed the highest values of P, while concerning sexes, females presented higher values than males. Total values of P, MA and MI regarding seasons, areas and sexes for each host species are shown in **Table 1**, **2** and **3**, respectively. According to estimates of P, significant differences were observed among host species: *R. norvegicus* vs. *M. musculus* ($z = -2.56$; $p = 0.0103$); and among both seasons (considering the distribution of *S. fasciolaris* in

Table 1

Total values of prevalences (P), mean abundance (MA) and mean intensity (MI) from three host species of different seasons. N=Number of hosts specimens captured.

	Season	
	Autumn/ Winter	Spring/ Summer
<i>M. musculus</i>	N=14 P=28.57% MA=0.36 MI=1.25	N=73 P=10.96% MA=0.14 MI=1.25
<i>R. rattus</i>	N=9 P=33.33% MA=0.67 MI=1.33	N=12 P=6.67% MA=0.46 MI=7.00
<i>R. norvegicus</i>	N=17 P=54.92% MA=1.06 MI=2.00	N=11 P=9.09% MA=0.09 MI=1.00

Table 2

Total values of prevalences (P), mean abundance (MA) and mean intensity (MI) from three host species of different areas in Gran La Plata. N = Number of hosts specimens captured.

	AREA						
	La Isla	La Latitia	El Carmen	El Retiro	Casco Urbano	Abasto	Malvinas Argentinas
<i>M. musculus</i>	N = 20 P = 20.00% MA = 0.30 MI = 1.50	N = 25 P = 12.00% MA = 0.16 MI = 1.33	N = 1 P = 0% MA = 0 MI = 0	N = 10 P = 10.00% MA = 0.10 MI = 1.00	N = 9 P = 11.11% MA = 0.11 MI = 1.00	N = 0	N = 22 P = 13.64% MA = 0.14 MI = 1.00
<i>R. rattus</i>	N = 1 P = 100% MA = 7.00 MI = 7.00	N = 3 P = 33.33% MA = 0.33 MI = 1.00	N = 14 P = 7.15% MA = 0.07 MI = 1.00	N = 3 P = 33.33% MA = 0.67 MI = 2.00	N = 0	N = 0	N = 0
<i>R. norvegicus</i>	N = 5 P = 60.00% MA = 1.00 MI = 1.67	N = 0	N = 6 P = 16.67% MA = 0.17 MI = 1.00	N = 11 P = 45.45% MA = 1.09 MI = 2.40	N = 0	N = 5 P = 20.00% MA = 0.20 MI = 1.00	N = 1 P = 0% MA = 0 MI = 0

Table 3

Total values of prevalences (P), mean abundance (MA) and mean intensity (MI) of both sexes from three host species. N = Number of hosts specimens captured.

	Sex	
	Male	Female
<i>M. musculus</i>	N = 40 P = 10.00% MA = 0.13 MI = 1.25	N = 47 P = 17.02% MA = 0.21 MI = 1.25
<i>R. rattus</i>	N = 15 P = 6.67% MA = 0.13 MI = 2.00	N = 6 P = 50% MA = 1.50 MI = 0.30
<i>R. norvegicus</i>	N = 20 P = 35.00% MA = 0.80 MI = 2.29	N = 8 P = 37.50% MA = 0.38 MI = 1.00

the three host species) ($z = 4.37$; $p = 0.000006$). There were no significant differences in AM and IM concerning host species and seasons. In addition, the relationship between the P, AM and MI of infection relative to areas and sex was not tested, due to the small sample size.

Rodents are the cause of extensive economic damage to cultivated fields and, in relation to public health, can transmit, maintain and spread zoonotic agents such as viruses, bacteria and helminths through their faeces, urine, aerosols or ectoparasites (Easterbrook et al., 2007). Particularly, *Taenia taeniaeformis*, and consequently its larval stage *Strobilocercus fasciolaris*, have been reported in different regions of the world (Esterre and Maitre, 1985; Hasslinger et al., 1988; Alcaino et al., 1992). Rodents, birds, insectivores and even human have been reported as intermediate host (Sterba et al., 1976; Loos-Frank, 2000; Bowman et al., 2002; Miño et al., 2013). In Argentina, *S. fasciolaris* has been previously recorded in *R. norvegicus* and the wild rodent *Akodon azarae* (Hancke et al., 2011; Miño et al., 2013); however, the finding reported here in *Rattus rattus* and *Mus musculus* constitute new host records for the country.

Although many studies mention parasites of commensal rodents, only a few of them analyze the distribution of species that represent sanitary risk, and provide morphometric comparisons between host species. In this study a detailed description and measurements of the larvae were provided. The general morphology of cysts examined here agrees with data presented by Loos-Frank (2000); Martínez et al. (2013) and Miño et al. (2013). However, the specimens studied by Voge (1954) parasitizing rodents from Perú (*Ctenomys peruanus*, *Phyllotis osilae* and *Chinchillula sahamae*) have greater number of hooks (44-48 vs. 34-38) and shorter hooks than the ones observed in this study (small hooks: 120-140. vs. 124- 293 µm and large hooks: 190-220 vs. 211 -430 µm). *Mus musculus* was the host species with the highest prevalence (12), followed by *R. norvegicus* (10), and *R. rattus* (4). According to other studies (Traweger et al., 2006; Hancke and Suarez, 2016) the highest number of host specimens (*R. norvegicus* and *R. rattus*) were captured during the spring-summer period. However, the distribution of *S. fasciolaris* indicated a higher percentage in autumn-winter. This is consistent with the results of Panti-May et al. (2013) in México, in which dry season (January to March) presented a higher number of this larva for *M. musculus* (Panti-May et al., 2013). This situation could be related to the life cycle of the parasite. Another coincidence with Panti-May et al. (2013) is related to the distribution of cysts between sexes in *M. musculus*. In both studies, although not statistically significant, females resulted to have a higher prevalence. In the present study, females of *R. rattus* and *R. norvegicus* also showed higher prevalences of *S. fasciolaris*. This contrasts with other studies that consider males more susceptible to infections than females (Klein, 2000; Hancke et al., 2011). Areas involved in this survey present high levels of poverty and inequality, characterized by partial or total lack of sanitation services, and the presence of precarious and critical overcrowding homes (Oyhenart et al., 2007; Zonta et al., 2007). At the same time, the number of dogs and cats without responsible care is high. Approximately 30-40% of the total of households sampled presented

dogs or cats without veterinary control (Pers. com.). Thus, it becomes clear that the conditions for *T. taeniformis* for completing its life cycle benefit the establishment of this parasite in the areas involved. Moreover, the close contact with rodents is evident, demonstrating the importance of studying parasites that represent sanitary risk. In spite of the number of human cases reported up to now, the difficulty in detecting positive cases favors the lack of register. Moreover, in general people who live in the studied areas do not have an easy access to the Health System. The rise of emergent illnesses in different areas of Latin America reflects the lack of studies focused in local issues, being helminthiasis the least attended.

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