

Parasitological and histological aspects of *Holochilus sciureus* naturally infected by *Schistosoma mansoni*

Aspectos parasitológicos e histológicos de *Holochilus sciureus* naturalmente infectados por *Schistosoma mansoni*

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Abstract

Schistosomiasis is a neglected disease that affects millions of people around the world, being common in the state of Maranhão. A total of 225 rodents of the *Holochilus sciureus* species from the Western Lowland Maranhão were studied, of which 144 animals (64%) exhibited *Schistosoma* eggs in their feces samples. Macroscopic lesions characterized as well-defined whitish areas on the liver and spleen surfaces were observed. Histopathological examination revealed multifocal granulomas in the esophagus, liver, spleen, pancreas and duodenum, with structures compatible with *Schistosoma mansoni* eggs, as well as severe hepatic micro-vacuolar degeneration, multifocal and coalescent, with proliferation of random bile ducts and associated epithelial hyperplasia to areas of fibrosis. Adult forms of the parasite were observed in the blood vessels of the portal space. The lungs exhibited moderate and diffuse interstitial pneumonia with intralesional *S. mansoni* eggs. In the kidneys, hyaline cylinders were observed in the pelvis and diffuse hemorrhage. In conclusion, *H. sciureus* displays a pathological picture similar to human being. This rodent plays a role as sentinel in Baixada Maranhense.

Keywords: Rodent, schistosomiasis, histopathology, host.

Resumo

A esquistossomose é uma doença negligenciada que afeta milhões de pessoas em todo o mundo, sendo comum no estado do Maranhão. Um total de 225 roedores da espécie *Holochilus sciureus* da Planície Ocidental do Maranhão foram estudados, dos quais 144 animais (64%) apresentaram ovos de *Schistosoma* em suas fezes. Lesões macroscópicas caracterizadas como áreas esbranquiçadas bem definidas nas superfícies do fígado e baço foram observadas. O exame histopatológico revelou granulomas multifocais no esôfago, fígado, baço, pâncreas e duodeno, com estruturas compatíveis com ovos de *Schistosoma mansoni*, degeneração micro-vacuolar hepática grave, multifocal e coalescente, com proliferação de ductos biliares aleatórios e hiperplasia epitelial associada a áreas de fibrose. Formas adultas do parasito foram observadas nos vasos sanguíneos do espaço portal. Os pulmões exibiram pneumonia intersticial moderada e difusa com ovos de *S. mansoni* intralesionais. Nos rins, foram observados cilindros hialinos na pelve e hemorragia difusa. Em conclusão, *H. sciureus* apresenta um quadro patológico semelhante ao ser humano. Este roedor desempenha um papel de sentinela na Baixada Maranhense.

Palavras-chave: Roedor, esquistossomose, histopatologia, hospedeiro.

Schistosomiasis is the second most important parasitic disease worldwide, in terms of impact on public health. It is estimated that it affects approximately 200 million people around the world and is responsible for 200,000 deaths per year (COLLEY et al., 2014). *Schistosoma mansoni* is the causative agent of schistosomiasis

in Brazil and the intermediate host is a fresh water snail of the genus *Biomphalaria* (KATZ et al., 1972; MIRANDA et al., 2015).

In the Brazilian state of Maranhão, schistosomiasis remains a public health problem (MENDES et al., 2017), predominantly in the western lowlands, which are one of the poorest regions in the state. This micro-region contains vegetations formed by immense flooded fields, which facilitates the incorporation of routine activities. Thus, schistosomiasis is characterized as an occupational disease

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in this region, once people who live there have several reasons for maintaining contact with contaminated water (FERREIRA et al., 1998). Additionally, other authors (BASTOS et al., 1982, 1985; MIRANDA et al., 2015; LIRA et al., 2016) have reported the participation of a wild rodent named *Holochilus sciureus* in the epidemiology of schistosomiasis.

All these aspects, combined with the existence of a climate that favors the spread of *S. mansoni* and the presence of natural vectors (CANTANHEDE et al., 2014) seem to favor the expansion and establishment of schistosomiasis in the western lowlands. The aim of the present study was to describe natural infection in *H. sciureus*, an alternative host, addressing its parasitological and histological aspects.

The present study was performed in the municipality of São Bento (02°41'45"S and 44°49'17"W, 2 m above sea level), in the micro-region of West Lowland (Brazil), an endemic area for schistosomiasis. During one year, from August 2013 to July 2014, twenty Tomahawk live traps were placed near a swamp area at 10-m spacing for one night per month. Fecal samples were collected from each of the 225 rodents caught and analyzed using the Kato-Katz methods (KATZ & ALMEIDA, 2003).

Among 144 positive rodents, 126 animals were released back to the nature, and 18 were euthanized in a CO₂ chamber. This experiment was approved by the Ethics Committee on Animal Experimentation of the Universidade Estadual do Maranhão, under protocol number 05/2014. All experiments strictly followed the institutional guidelines for the care and use of animals (IBAMA license number 14345-3 and registration number 543545).

Fragments of liver, spleen, esophagus, small intestine and lung of euthanized animals were collected and fixed in formalin (10%) for 2 hours. Afterwards, organs samples were processed into a paraffin block. Paraffin-embedded tissues were sectioned at 5 µm and stained with Hematoxylin and Eosin (H&E).

The result showed that among 144 animals out 225 rodents (64%) exhibited *Schistosoma* eggs in their feces samples. Despite the fact that the capture process was performed over 11 months, animals parasitized by *S. mansoni* were only found in the dry season (from August to November). Schistosomiasis is a neglected disease and its prevalence in Maranhão State ranges 9 to 12% (CANTANHEDE et al., 2011). However, in Baixada Maranhense (western lowlands) that is composed by 21 municipalities the true prevalence of this disease is unknown.

Studies performed in one of these municipalities, São Bento, showed that *H. sciureus*, previously classified as *Holochilus brasiliensis* can play a role as a reservoir for *S. mansoni* in this region, given that it has been found naturally infected and expels eggs worms in the feces and it reproduces a chronic disease when experimentally infected (BASTOS et al., 1982; BASTOS et al., 1985; SILVA-SOUZA & VASCONCELOS, 2005; LIRA et al., 2016). Since, the genus *Holochilus* can be considered as an important alternative definite host in the maintenance of the life cycle of *Schistosoma* (KAWAZOE & PINTO, 1983; MIRANDA et al., 2015; MARTINS et al., 2015). The high frequency of schistosomiasis in Maranhão is also related to occupational activities such as fishing and hunting.

The occurrence of natural infection in wild animal such as African monkey and -several species of rodents as *Oxymycterus*

sp., *Necomys lasiurus*, *Akodon* spp., *Sooretamys* spp., *Calomys* spp., *Proechimys* sp., *Cavia aperea*, *Rattus rattus* and *Rattus norvegicus* was reported by Gentile et al. (2012). These authors also describe that genera *Necomys* and *Holochilus* are involved in wild cycle of *S. mansoni*.

Besides that, in this area has been reported the occurrence of a wild strain of *S. mansoni* named S strain (MIRANDA et al., 2017) isolated from *Biomphalaria glabrata*, which in experimental condition showed three times more virulence to the snails than H strain isolated from miracidium obtained from human stools (BASTOS et al., 1984).

Natural infected rodent presented in the macroscopic exam, well-defined whitish areas were observed on the liver, spleen surfaces and free in the abdominal cavity (Figure 1), measuring about 1 mm. Histopathology showed in the liver multifocal periportal granulomas around ovoid structures, measuring approximately 35µm (diameter), with a thick, hyaline, yellow wall, containing laterally projected spicules and miracidium, compatible with *S. mansoni* eggs (Figure 2A). The following alterations were also observed: severe, multifocal to coalescent, micro-vacuolar degeneration; random bile duct proliferation; and epithelial hyperplasia associated with fibrosis areas. Adult forms of the parasite were frequently observed in the blood vessels of the portal space.

Macro and microscopic analysis revealed changes on the liver surface (whitish areas, fibrosis, and granulomas) similar to the results of experimental studies with *Mus musculus* (CARVALHO et al., 1986; AMARAL et al., 2017) and *Mus spretus* (PÉREZ DEL VILLAR et al., 2013). The liver is the most affected organ by the infection and presence of *S. mansoni* eggs (SILVA-SOUZA & VASCONCELOS, 2005; LOPES et al., 2006; SILVA et al., 2012; PÉREZ DEL VILLAR et al., 2013).

The histological analysis of the lungs exhibited moderate and diffuse interstitial pneumonia with intralesional *S. mansoni* eggs (Figure 2B). In the lamina propria of the duodenum and outer longitudinal muscle tunica was observed granulomas with rounded eggs, miracidium and epithelioid macrophages (Figure 2C).



Figure 1. (A) Photomicrography of free adult worm of *Schistosoma mansoni* in abdominal region of *Holochilus brasiliensis*. (B) In the upper right corner adult worm in histological section stained with H&E, Bars = 400 µm.

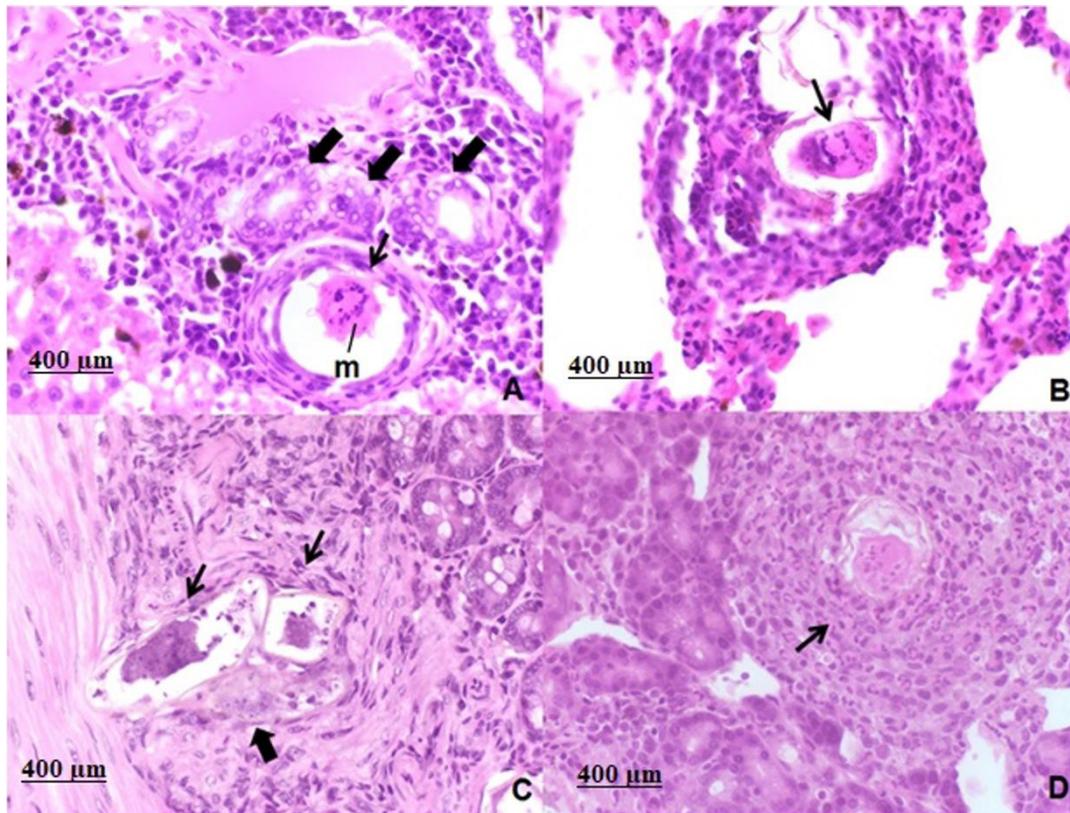


Figure 2. (A) Granuloma around *Schistosoma mansoni* eggs (thin arrows), containing miracidium (m) and bile duct proliferation (thick arrows) in the liver. (B) Inter alveolar septal thickening with histiocyte and lymphocyte infiltrate containing *S. mansoni* egg (thin arrow). (C) Granuloma with *S. mansoni* eggs (thin arrows) and epithelioid macrophages (thick arrow) in large intestine submucosa and (D) granuloma in exocrine portion of pancreas (thin arrow) containing *S. mansoni* egg. Hematoxylin and Eosin. Bars = 400 µm.

In the spleen, *S. mansoni* eggs were also observed both in white and red pulp.

The exocrine region of the pancreas exhibited multiple granulomas, containing neutrophils and eosinophils, as well as rounded eggs with miracidium (Figure 2D). There was an intense and diffuse infiltration of lymphocytes, histiocytes, plasma cells and eosinophils in the lamina propria of the esophagus, involving eggs. In the stomach, *S. mansoni* eggs were found in the crypts and, in the kidneys, hyaline cylinders in the pelvis and diffuse hemorrhage were observed. As demonstrated by our results *Holochilus* causes lesions in several organs, leading a severe picture of schistosomiasis. What could explain this severity? The S strain is also more pathogenic to the rodents as described by Bastos et al. (1984) to the snail? Or *S. mansoni* was recently introduced in this environment and the rodents did not adapt to the parasite infection?

In the present study, there was a high frequency of rodents with positive results in the stool examination and all euthanatized rodents developed a severe disease, characterized by the presence of granulomas in the liver, lungs, intestine and the exocrine part of the pancreas. In humans, the occurrence of hepatic granuloma, which are responsible for portal hypertension, is very common (GRYSEELS et al., 2006).

A high frequency of positive animals in stool examinations during the dry season has been reported (BASTOS et al., 1985).

Flooding occurs in this area in the rainy season. In the beginning of this season, rodents that survived the drought probably deposit *S. mansoni* eggs in small water collections, promoting the infection of snails that subsequently infect humans or other rodents (LEAL et al., 2013; OLIVEIRA et al., 2013).

The control of schistosomiasis is very difficult in the studied area, due to the absence of adequate sanitary conditions, the use of water from the fields in activities like fishing, recreation and laundry, as well as the abundance of snails. In addition, the existence of wild rodents plays an important role in the maintenance of the life cycle of parasites. According to model-based-maps, socioeconomic, environmental and climatic factors contribute to the distribution of schistosomiasis in Brazil. Thus, a governmental intervention is necessary in order to control the disease and the presence of *H. sciureus*, an alternative host for *S. mansoni* in the epidemiological chain of schistosomiasis, in this area. In conclusion, *H. sciureus* reproduce a schistosomiasis picture that resembles human disease.

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