
HISTOPATHOLOGY OF *Holochilus brasiliensis*
(RODENTIA: CRICETIDAE)
INFECTED WITH *Schistosoma mansoni*
(SCHISTOSOMATIDA: SCHISTOSOMATIDAE)

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ABSTRACT

Schistosomiasis prevalence in swamplands of North-eastern Brazil is complicated by the presence of alternative hosts, such as the marsh rat *Holochilus brasiliensis*. However, the effects of *Schistosoma mansoni* on this host – at both individual and population levels - are unknown. We investigated the pathological effects of *S. mansoni* invasion in the liver and spleen of *H. brasiliensis*. Ten infected (5 males; 5 females) and 6 healthy (3 males; 3 females) animals had their liver and spleen extracted 42 days post-treatment. Histological slides were prepared and examined under light microscope. The livers of infected males were seen to contain parasite eggs with moderate or intense granulomas, whereas females showed slight or moderated tissue damage. Parasite eggs were also observed in the spleen of both male and female rodents. There appeared to be a different response to parasite invasion according to host sex.

KEYWORDS: Schistosomiasis. Marsh rats. Granuloma. Liver. Spleen. Alternative host.

INTRODUCTION

Schistosomiasis is a common disease in the swamps of Northeastern Brazil, where economic activities (e.g. fishing) imply frequent contact between man and water. The infection dynamics in those areas is complicated by the presence of alternative hosts, such as wild rodents, in which the trematode is believed to complete its lifecycle in the absence of human hosts. One of these species, the marsh rat *Holochilus brasiliensis* (Rodentia: Cricetidae) (Desmarest, 1819) is relatively

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abundant in northeastern Brazil and also in other South American countries, like the east of the Colombian Andes, the south of Venezuela and northern Argentina and Uruguay (Moojen, 1952; Emmons, 1997). Adult *H. brasiliensis* reaches 23 cm in length from head to tail, with a variety of colors from brown to pale yellow (Emmons, 1997). They are sexually mature at three months of age, with a higher reproductive activity in the rainy season (Veiga-Borgeaud, 1986).

Commonly found in swamps, sugarcane plantations and flooded areas in Maranhão State, *H. brasiliensis* appears to carry a large amount of viable *Schistosoma* eggs throughout the year (Veiga-Borgeaud, 1986). The parasite can complete its cycle in the absence of human hosts, using *H. brasiliensis* as a definitive host (Silva-Souza, 1995), so that the rodent could contribute to the expansion of the disease cycle in endemic areas. Despite its significance, the pathological and behavioural effects of *Schistosoma mansoni* (Schistosomatida: Schistosomatidae) on *H. brasiliensis* are unknown. In humans, *S. mansoni* histopathology is characterized by the presence of granuloma with parasitic eggs, with an increase in the size of the liver and spleen. In the liver, soluble antigens produced by the eggs can cause irreversible tissue damage (Jones et al., 1997; Silva et al., 2000). Susceptibility may be influenced by factors such as parasite load and host age and sex (Kloos et al., 1998). In fact, in endemic areas of Brazil a higher proportion of infection among males has been reported (Dias et al., 1952).

Considering that the infection by *S. mansoni* may alter *H. brasiliensis* behavior – which in turn might expand parasite dissemination, we conducted a study to evaluate the role of *H. brasiliensis* as schistosomiasis reservoirs. As a starting point, we aimed at describing the preliminary aspects of the histopathology in the liver and spleen of *H. brasiliensis* infected with *S. mansoni*, investigating sex-related differences in the response to parasite invasion.

MATERIAL AND METHODS

Sixteen 3 month-old *H. brasiliensis* were obtained from the Universidade Federal do Maranhão where the species have been reared for at least 5 generations. The lineage was started in captivity in 1996, from specimens collected in the municipality of Sao Bento, State of Maranhão. All animals were submitted to prior fecal analysis to corroborate the absence of parasites. Ten rodents, five males and five females, were individually infected with 200 *S. mansoni* cercariae (Brener, 1956), after immersion in warm water for 10 min, to facilitate larval penetration. The *S. mansoni* lineage was originally obtained from infected *H. brasiliensis* collected in the nearby fields, and maintained at the University Estadual do Maranhão (UEMA). As controls, three male and three female rodents were immersed in warm water but not inoculated. Thereafter, both groups were treated identically. Animals were assigned randomly to each treatment.

Following manipulation, animals were kept individually in polypropylene cages in rooms at $28 \pm 2^\circ\text{C}$ and about 70% relative humidity. Water and mouse food were given *ad libitum*. After 42 days of treatment, all rodents were killed after anaesthesia by ether and their liver and spleen were immediately removed. The organs were washed with 0.9% sodium chloride and individually fixed in Bouin's solution. After immersion in acetic alcohol, slides were prepared, then stained with haematoxiline-eosine and observed under a light microscope. The experiment was approved by the Ethic's Committee at UEMA.

RESULTS AND DISCUSSION

No tissue damage or parasite eggs were detected in the liver and spleen of control rodents. The tissue damage observed in the livers and spleens of infected animals was classified as slight, moderate or intense, according to the degree of inflammatory reactions observed and the quantity of eggs present. In livers of males, moderate to intense injuries were observed in all samples, with a large quantity of widespread granuloma with adjacent necrotic areas of tissue and, in some cases, abundant eosinophilic proliferation. The presence of various histiocytes, fibroblasts and a considerable increase in the number of Kupffer cells was also registered. Occasionally, the adult parasite was also found in the liver.

According to their stage of deterioration, three types of eggs were found in the livers: type I – eggs morphologically intact, with clear visualization of their structure, including the miracidia; type II – eggs partially deteriorated showing the presence of eosinophilic granulations, and type III – eggs almost totally destroyed, apparently lacking miracidium. The hepatic injuries observed in females were slight to moderate in all specimens, with periovular necrosis, egg eosinophilia, evidence of a lymphocyte-histiocyte reaction and an increase of the quantity of Kupffer cells. In all female animals, immunological responses appeared to be less intense than those found in males.

The analysis of spleens revealed the presence of *S. mansoni* eggs in all rodents, males and females. In males, the quantity of eggs was moderate, while in females this number was small, being at least half of the amount observed in their male counterparts. Immunological reactions appeared to have occurred to a lesser degree or were seen only occasionally in the spleen of both males and females, when compared to those present in the liver.

Although the presence of *S. mansoni* eggs in the liver was evident in all infected rodents, typical granuloma pathology was found only in males. Inflammatory reactions, eosinophilia and periovular necrosis observed in the liver of males were similar to that reported in rabbits and mice at 35 days post-infection (Dias et al., 1952) and in humans (Hirata et al., 1999). The degree of egg destruction observed may be, partially, a consequence of the time of egg arrival to the liver, as a single animal may show the three types of eggs simultaneously. The pattern of hepatic damage observed suggests that *H. brasiliensis* males and females may exhibit different intensities of

response to *S. mansoni* invasion. In contrast, Duplantier & Sene (2000) reported similar worm burden and prevalence of disease in male and female wild rodents (*Arvicanthis niloticus* and *Mastomys huberti*) in Senegal.

In humans, the higher prevalence of infection detected in men (Pessôa & Martins, 1998) has been commonly explained by behavioral factors, since men are under greater risk of infection than women due to a higher exposure to contaminated water (e.g. by fishing or hunting in marshlands). This may not be sufficient to explain gender-related differences in infection, as women in many endemic regions are involved in similarly risky activities such as washing clothes in rivers (Fulford et al., 1996). For *H. brasiliensis*, there is no report, so far, of sex-related differences in the adult behavior in the wild. An alternative explanation of the observed data is that there may be gender differences in cercarial infectability such that, if females were less susceptible, this would lead to fewer eggs registered in their organs.

The frequency and quantity of eggs found in *H. brasiliensis* spleen is surprising, and the periovular reaction was often discrete or absent. This contrasts with the work of Freitas et al. (1999), who described hyperplasia of the splenic pulp and congestion as the most frequent symptoms associated with the presence of *S. mansoni* in that organ. Reports of *S. mansoni* eggs in the spleen are virtually non-existent. In a study carried out in Puerto Rico, using 147 post-mortem samples from humans, only one individual had *S. mansoni* eggs in the spleen (Dias et al., 1952). It is possible that infection of the spleen represents a type of equilibrium established between *S. mansoni* and *H. brasiliensis* leading to varied egg localization and a lower immunological resistance by the host.

The role of *H. brasiliensis* as *S. mansoni* reservoirs is still ignored; hence ecological and pathological studies must converge to decipher disease development in the field. Also behavioural aspects of host-parasite interaction must be investigated. Many variables (e.g., animal age, nutritional state, and sex-associated susceptibility) could influence susceptibility and maintenance of infection in alternative hosts. Knowledge of these factors will help to predict the risk of local communities to infection and the actual parasite's effect on rodent's populations.

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RESUMO

Histopatologia de *Holochilus brasiliensis* (Rodentia: Cricetidae) infectados com *Schistosoma mansoni* (Schistosomatida: Schistosomatidae)

A prevalência da esquistossomose em áreas alagadas do Nordeste brasileiro é agravada pela presença de hospedeiros alternativos, como o roedor *Holochilus brasiliensis*. Entretanto, os efeitos de *Schistosoma mansoni* nesse hospedeiro, tanto no indivíduo como na população, são desconhecidos. Em investigação dos efeitos patológicos da invasão por *S. mansoni* no fígado e baço de *H. brasiliensis*, dez animais infectados (cinco machos e cinco fêmeas) e seis sadios (três machos e três fêmeas) tiveram seus fígados e baços extraídos 42 dias após o tratamento. Lâminas histológicas foram preparadas e examinadas sob microscópio de luz. Os fígados de machos infectados continham ovos do parasito com granulomas intensos ou moderados, enquanto as fêmeas mostraram dano tissular leve ou moderado. Os ovos do parasito também foram observados no baço de machos e fêmeas. Observou-se uma leve diferença entre machos e fêmeas quanto à resposta à invasão por *S. mansoni*.

DESCRITORES: Esquistossomose. Roedores. Granuloma. Fígado. Baço. Hospedeiro alternativo.

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