

# Susceptibility of *Biomphalaria straminea* from Peixe Angical dam, Tocantins, Brazil to infection with three strains of *Schistosoma mansoni*

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*Environmental changes from water resource developmental projects affect the epidemiology of water-associated diseases, as well as malaria and schistosomiasis. Aiming to investigate the occurrence and distribution of freshwater snails of medical and veterinary importance in the area of influence of the Peixe Angical hydroelectric dam, a survey has been conducted over four years (2004-2008). The study has revealed the occurrence of populations of Biomphalaria straminea (Dunker) in all municipalities surrounding the lake. Studies on parasite-mollusc compatibility were undertaken using 35 populations of B. straminea, descendants of specimens obtained from that area and three strains of Schistosoma mansoni (Sambon) (BH, CM and CMO). The main results are as follows: (i) among the 1,314 specimens used, eight had been infected (infection index of 0.6%) with only the BH strain, (ii) for B. straminea populations, the mortality index was 6.8% and, depending on the strain used, the indexes were 4.6%, 8.49% and 19% with BH, CM and CMO strains, respectively, (iii) the infection indexes varied according to the B. straminea populations, ranging from 0-12.5% and (iv) the duration of the precercarial period varied from 25-49 days. These results, in addition to environmental and social changes that took place in the Peixe Angical dam region, indicate the possibility of B. straminea emerging as a schistosomiasis vector in this area.*

Key words: *Biomphalaria straminea* - susceptibility - *Schistosoma mansoni* - dam - Tocantins - Brazil

Water resource projects in natural ecosystems resulting in environmental disturbances modify established niches and create conditions for new ecological aspects. According to Oomen et al. (1990), the development and management of water resources in tropical and subtropical climate zones have resulted in transmission intensification or the introduction of diseases into previously non-endemic areas. More recently, Steinmann et al. (2006) estimated that 537,000 people at risk for schistosomiasis were living in irrigated areas in the Americas and that 1.22 million individuals were in areas next to large dam reservoirs, most of them in Brazil.

In our previously published reports (Fernandez & Thiengo 2002, 2006) we emphasized the possibility for the introduction of schistosomiasis to non-endemic areas in Brazil as a consequence of hydroelectric power station construction in two reservoirs localised in the Upper Tocantins River Basin, state of Goiás (GO) and in Paraguai River Basin in the state of Mato Grosso. We reported different degrees of susceptibility of *Biomphalaria straminea* (Dunker, 1848) and *Biomphalaria amazonica* Paraense, 1966 when submitted to experimental infection by *Schistosoma mansoni* Sambon, 1907. Studies on snail-trematode interactions were required due to

the wide variation of susceptibility previously reported for snail vectors (from extremely compatible populations to resistant ones) depending mainly on the *S. mansoni* strain and the mollusc origin.

Among other environmental programs developed in hydroelectric power station Peixe Angical (AHE Peixe Angical) and surrounding areas, a survey of freshwater snails was conducted over a four year period (2004-2008), aiming to investigate the occurrence and distribution of species of medical and veterinary importance, as well as to indicate measures to prevent schistosomiasis and other waterborne diseases. The Peixe Angical reservoir is located in the Tocantins River and covers an area of 294 km<sup>2</sup> in the state of Tocantins, in the municipalities of Palmeirópolis, Paranã, Peixe and São Salvador do Tocantins. Considering the marked ecological and social changes that have taken place in that area and the presence of well established populations of *B. straminea* at different collection stations in that reservoir, studies on parasite-mollusc compatibility were undertaken in this region.

The *B. straminea* populations were obtained from 35 collection stations in the area of AHE Peixe Angical. Specifically, they were collected from six stations in the Paranã River, 27 stations in the Tocantins River and only two areas outside of the reservoir (in the municipalities of Paranã and Peixe). For breeding the test specimens, live snails were kept in the laboratory in aquaria containing dechlorinated water and a thin layer of a 2:1 mixture of screened soil and ground oyster shells as a source of mineral nutrients. Snails were fed on fresh lettuce leaves and floating styrofoam tablets were put in each aquarium to facilitate egg collection.

The snails, descendants of specimens obtained in the area of the AHE Peixe Angical, were individually exposed to five *S. mansoni* miracidia of the following

Financial support: Enerpeixe SA

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Received 9 January 2009

Accepted 8 October 2009

strains: (i) BH strain, isolated from infected specimens of *Biomphalaria glabrata* (Say, 1818) from Belo Horizonte, Minas Gerais (MG), (ii) CM strain, isolated from a patient born and raised in Paud'algo, Pernambuco (PE) and (iii) CMO strain, isolated from the naturally infected wild rodent *Oryzomys subflavus* Wagner, 1842, from Ceará-Mirim, Rio Grande do Norte (RN). The strains have been kept in *B. glabrata* snails from Belo Horizonte (BH strain), Pontezinha, PE (CM strain) and Touros, RN (CMO strain) and female Swiss albino mice. All procedures followed the guidelines established by the Fundação Oswaldo Cruz-Fiocruz Committee for the Ethical Use of Animals by license CEUA PO 0143-02. The CM and CMO strains were isolated from areas of Northeast Brazil where *B. straminea* has been reported as a vector.

As a control, *B. glabrata* populations from Belo Horizonte, Pontezinha and Touros were infected with BH, CM and CMO strains, respectively. The procedures for collecting the faeces of infected mice and for later exposure of snails to miracidia were those described by Fernandez et al. (2008).

On the 25th day after exposure to miracidia and then every 5th day thereafter, the snails were exposed to the light of electric lamps to characterise the pre-cercarial period and the infection index. The dead specimens, as well as those that survived for 60 days without shedding cercariae, were examined under a stereomicroscope after crushing their shells. In all experiments, the autolysed specimens that could not be examined were discarded.

The results of the *B. straminea* infection index study, as well as the diameter of molluscs when exposed to *S. mansoni* miracidia, are in the Table. Eight out of 1,314 exposed specimens, of 2-8 mm in shell diameter, became infected (infection index of 0.6%) and, from the molluscs that remained negative, 90 specimens died (a mortality index of 6.8%). Depending on the strain, the results were as follows: of the 955 snails exposed to BH miracidia, eight became infected (0.84%); of the 212 specimens exposed to CM miracidia and the 147 specimens exposed to CMO miracidia, no molluscs became infected. For the BH *S. mansoni* strain, 44 specimens died during the experiment (mortality index of 4.6%) between the 18th-57th day (mean and standard deviation of

28.0 ± 9.15 days), for the CM *S. mansoni* strain, 18 died (8.49%) between the 27th-51st day (41.7 ± 7.96 days) and for the CMO *S. mansoni* strain, 28 specimens died (19%) between the 26th-41st day (34.3 ± 5.86 days).

For the controls, 80.3% of 71 *B. glabrata* snails (6-8 mm in shell diameter) from Belo Horizonte, 52.5% of 47 *B. glabrata* snails (6-10 mm) from Pontezinha and 72.3% of 40 *B. glabrata* snails (5.5-8 mm) from Touros, exposed to the BH, CM and CMO *S. mansoni* strains, respectively, became infected. The following mortality indexes were obtained: BH strain 0%, CM strain 10% and CMO strain 4.25%.

The infection index varied between 0-12.5%, according to the *B. straminea* populations. Of the 23 Paranã collection stations (geographical coordinates: 12°15'51.1"S 48°17'08.3"W; 12°18'02.5"S 48°14'09.2"W; 12°23'57.8"S 48°12'30.5"W; 12°27'26.7"S 48°13'34.4"W; 12°30'55.1"S 48°06'28.1"W; 12°31'39.7"S 48°13'18.9"W; 12°34'17.2"S 48°06'36.6"W; 12°35'11.4"S 48°00'45.5"W; 12°35'20.3"S 47°59'54.5"W; 12°35'27.0"S 47°59'34.4"W; 12°35'40.4"S 47°59'08.6"W; 12°37'06.8"S 48°15'39.4"W; 12°37'11.3"S 47°53'00.2"W; 12°44'21.7"S 48°14'21.8"W; 12°44'34.1"S 48°13'53.3"W; 12°45'29.5"S 48°13'19.8"W; 12°46'52.5"S 48°14'11.7"W; 12°52'26.0"S 48°11'36.7"W; 13°02'33.6"S 48°08'47.4"W; 13°04'34.6"S 48°08'24.6"W; 13°05'08.4"S 48°07'55.7"W; 13°10'40.7"S 48°09'18.1"W; 13°13'16.9"S 48°09'10.7"W), five presented molluscs susceptible to the BH strain (Table). Six snails eliminated cercariae and the pre-cercarial period varied from 25-49 days (mean and standard deviation of 33 ± 8.22 days). As for the municipality of São Salvador do Tocantins, of the seven studied collection stations (12°22'42.9"S 48°15'34.2"W; 12°33'14.6"S 48°17'14.1"W; 12°34'51.6"S 48°16'45.2"W; 12°44'22.3"S 48°16'18.3"W; 12°44'36.6"S 48°14'14.9"W; 12°45'17.4"S 48°13'31.9"W; 12°48'48.9"S 48°14'22.2"W), two presented specimens that were susceptible to the BH strain (only one specimen from each locality, with pre-cercarial periods of 26 and 33 days). In contrast, no snail presented with an infection by *S. mansoni* in the municipalities of Peixe (2 stations: 12°13'24.4"S 48°26'01.4"W and 12°14'55.2"S 48°17'47.9"W) and Palmeirópolis (3 stations: 12°54'41.5"S 48°11'18.1"W, 12°57'04.1"S 48°10'11.5"W and 13°07'10.5"S 48°08'24.5"W).

TABLE

Infection index of *Biomphalaria straminea* from seven localities in the area of influence of the Peixe Angical dam to the BH strain of *Schistosoma mansoni*

Municipality	Geographical coordinates	Snails exposed n	Shell diameter mm	Index infection %
Paraná	12°45'29.5"S 48°13'19.8"W	34	3.0-5.5	5.9
	12°46'52.5"S 48°14'11.7"W	8	2.5-7.0	12.5
	13°02'33.6"S 48°08'47.4"W	17	2.0-5.0	5.9
	13°04'34.6"S 48°08'24.6"W	11	4.0-6.0	9
	13°13'16.9"S 48°09'10.7"W	14	2.0-5.5	7.1
São Salvador	12°34'51.6"S 48°16'45.2"W	10	2.0-4.0	10
	12°44'36.6"S 48°14'14.9"W	18	4.0-5.0	5.5

The increasing numbers of dams in Brazil during the last two decades and the consequent possibility of the introduction of schistosomiasis into new areas are a matter of great concern to schistosomiasis experts (Rozendaal 1997, Gazin et al. 2000, Fernandez & Thiengo 2002, Thiengo & Fernandez 2008).

The low rates of infection reported in the present study are in accordance with those of most previous studies on experimental infection of *B. straminea*. Infection indexes up 10%, such as those found in the population from the Tocantins River (12°46'52.5"S 48°14'11.7"W), have been reported for *B. straminea* specimens from other states: Pará (Paraense 1967), MG (Souza et al. 1996), Piauí (Fernandez 1997), Alagoas, Ceará, Paraíba, PE and RN (Barbosa & Figueiredo 1970). According to Wright et al. (1973), *B. straminea* is the most successful species and is a much more important host than *B. glabrata* in Northeastern Brazil because it is well adapted to all climatic varieties, inhabiting both permanent and temporary bodies of water.

The present study reinforces the existence of intra-population differences in *Biomphalaria* species between susceptible and resistant snails. Paraense and Corrêa (1963) obtained infection indexes between 0-100% using 23 *B. glabrata* populations exposed to a single Brazilian strain of *S. mansoni*. As for *Biomphalaria tenagophila* (d'Orbigny, 1835), the infection index varied from 0-91.5% (Paraense & Corrêa 1978).

Differences in susceptibility of the snail populations to *S. mansoni* strains were found in other studies on experimental infection to be the result of physiological adjustment between the snail and the parasite. The intraspecific variations of *S. mansoni* were also observed in the morphological characteristics of adult worms and in the morphometric analysis of cercariae of the BH, SJ and CMO strains (Machado-Silva et al. 1995, 2000). Although *B. straminea* snails proved unsusceptible to infection with the CM strain in the present study, Fernandez and Thiengo (2002) obtained an infection index of 1.2% for the same mollusc-parasite combination with specimens from Serra da Mesa dam (GO). These results point out that the CM and CMO strains of *S. mansoni* have not reached as high of a degree of compatibility with *B. straminea* populations as that shown by the BH strain.

The present paper infers that the reservoir of the AHE Peixe Angical dam is an unharmed area with potential for schistosomiasis transmission and that actions to prevent it are needed, such as a quantitative survey of *B. straminea* populations and a search for the associated larval helminths in those collection sites where *B. straminea* populations were found to be susceptible and also where close and frequent human contact with the water was observed. Such areas are the São Salvador do Tocantins port side area (12°44'36.6"S 48°14'14.9"W), the river near São Salvador do Tocantins beach (12°45'29.5"S 48°13'19.8"W) and the Paranã beach (12°46'52.5"S 48°14'11.7"W). Also of concern is the construction of the São Salvador hydroelectric power station approximately 70 km from the AHE Peixe Angical dam and encompassing three col-

lection sites (13°13'16.9"S 48°09'10.7"W, 13°04'34.6"S 48°08'24.6"W and 13°02'33.6"S 48°08'47.4"W) in the municipality of Paranã, in which there were susceptible populations of *B. straminea*. Therefore, taking the present data into account as well as the environmental and social changes observed in the AHE Peixe Angical and AHE São Salvador regions, we emphasise the possibility of schistosomiasis introduction in that region if no preventative measures are implemented.

#### ACKNOWLEDGEMENTS

To Enerpeixe SA, for the facilities provided during the field work, and to Dr Lygia dos Reis Corrêa, for supplying *Schistosoma mansoni* strains.

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