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FURTHER EXPERIMENTS ON SUSCEPTIBILITY OF *BIOMPHALARIA AMAZONICA* TO *SCHISTOSOMA MANSONI*

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A sample of Biomphalaria amazonica *from Porto Velho, Rondônia state, was exposed to miracidia of* Schistosoma mansoni *(SJ2 strain) from São José dos Campos, São Paulo state (five miracidia per snail). Water freshly taken from the snails' breeding place was used to make sure that its quality was compatible with hatching of miracidia and their penetration into the snails. The resulting infection rate was 3.5%, as against 45% in* B. tenagophila *controls.*

In comparison with the controls, B. amazonica, *besides a lower infection rate, showed a longer prepatent period and a lower cercarial production. These characteristics seem to indicate that it is a poor host of* S. mansoni, *like* B. straminea, *but it should be considered that, this notwithstanding, the latter is admittedly a good vector of the parasite in hyperendemic areas of northeastern Brazil.*

These results point to the possibility of introduction of schistosomiasis mansoni into the western Amazonian region, where B. amazonica *is widespread.*

Biomphalaria amazonica Paraense, 1966, initially recorded from the outskirts of Manaus and the Careiro island, Amazonas state, was subsequently found at a number of localities along the Solimões river (the Brazilian section of the Amazon between the Peruvian border and the mouth of the Negro river), on the basins of the Juruá and Madeira rivers, and at a single locality (Barão de Melgaço) on the Cuiabá valley, Paraguay basin (Paraense, 1983).

Susceptibility experiments with specimens from Careiro island, exposed each to ten miracidia of *Schistosoma mansoni* from Belo Horizonte (BH strain) and from São José dos Campos (SJ strain), showed that *B. amazonica* is a potential vector of that trematode, producing as high infection rates as 48% and 73%, respectively (Corrêa & Paraense, 1971).

Additional susceptibility experiments, carried out under somewhat different conditions with another population of *B. amazonica* from another environment about 800 km apart, are described below.

MATERIAL AND METHODS

The snails were collected from a drainage ditch running along the Duque de Caxias avenue at Porto Velho, Rondônia state. A section of the ditch, between Joaquim Nabuco and Brasília streets, about 2 m wide and 20 cm deep, with a predominating growth of *Pistia stratiotes*, was selected as the source of snails. On the day of collection the snails proved negative for cercariae after exposure to sunlight for 2 h. They showed no apparent signs of infection in the exposed parts and, as far as possible, on inspection through the shell under the stereomicroscope.

Four hundred and fifty specimens, 3 to 5 mm in shell diameter, were individually exposed to five miracidia of a strain of *S. mansoni* isolated from specimens of *Biomphalaria tenagophila* collected at São José dos Campos, São Paulo state, in April 1980, and kept by passages through Swiss albino mice and *B. tenagophila* descended from specimens of the original breeding place. Although originated from the same spot as a former strain, isolated in 1962 and referred to as SJC by Paraense & Corrêa (1963), or SJ in subsequent papers, the present strain shows some differences in comparison with the preceding one, and therefore is named SJ2.

Exposure to miracidia and subsequent dealing with the snails followed the procedure described in preceding papers (e.g., Paraense & Corrêa, 1978). In this case hatching of miracidia and exposure of snails took place in water of the snails' breeding place (pH 6.8; the water of the nearby Madeira river had a pH of 7.0). The work was performed at the local laboratory of SUCAM (Superintendência de Campanhas de Saúde Pública) on July 28-29, 1981. After exposure, the snails were placed in aquarium with the same water for two days. Then we brought them, under high

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humidity in plastic bags, to our laboratory in Rio de Janeiro, where they were put in aquaria with water of the original breeding place about 10 h later. Thenceforth they were kept under controlled temperature of 24 - 26°C, and once a week one-fifth of the water of each aquarium was replaced by the same amount of water from a balanced artificial pool.

One hundred specimens of *B. tenagophila* (SJ2 strain), dealt with under the same conditions, were used as controls.

The exposed snails were observed at least three times daily. If any specimen happened to die, it was dissected and examined for developing stages of the schistosome, so that not a single dead snail was left undissected. On the 5th day after exposure, and then every 5th day, the snails were singly placed in vials with water and exposed to the light of electric lamps (28- 30°C) to induce shedding of cercariae. The specimens that survived for 70 days without shedding cercariae (50 days in controls) were dissected and examined.

The specimens of *B. amazonica* that shed cercariae (and ten of the controls) were exposed to light for 2 h on alternate days, and the number of cercariae was recorded.

RESULTS

Of the 450 *B. amazonica* (Table I) 15 became infected (3.5%), and only four of the latter (nos. 1, 2, 3 and 4) shed cercariae. The earliest shedding was recorded on the 30th day (nos. 1 and

TABLE I

Results of exposure of 450 *Biomphalaria amazonica* from Porto Velho and 100 *B. tenagophila* (controls) from São José dos Campos to the SJ2 strain of *Schistosoma mansoni* (5 miracidia per snail).

Days after exposure	Exposed snails*	Results +
*B. amazonica*Δ		
5 – 20	11	Negative
21	4	3 negative; 1 with ISS in PW
22	6	5 negative; 1 with ISS in MC
23 – 29	12	11 negative; 1 with ISS in RR, DG
30	3	1 negative; 2 shed first cercariae (nos. 1, 2) §
31 – 34	2	Negative
35	2	1 negative; 1 shed first cercariae (no. 3) §
36 – 39	6	Negative
40	3	1 negative; 1 with ISS in HE, MC; 1 shed first cercariae (no. 4)§
42	3	2 negative; 1 with SS with cercariae in MC
43 – 47	8	5 negative; 3 with ISS in DG
48 – 49	7	6 negative ¶; 1 with ISS in PW, RR, DG, OT
50	10	9 negative; 1 with ISS in HE, DG
51 – 59	40	39 negative; 1 with ISS in DG
62	11	10 negative; 1 with ISS in PW
64 – 69	31	Negative
70	291	Killed, negative
	450	
B. tenagophila∇		
25	10	Shed first cercariae (nos. 1 to 10) §
	7	Shed first cercariae
29	1	Negative
30	24	Shed first cercariae
35	3	Shed first cercariae
45	1	Shed first cercariae
50	54	Killed, negative
	100	

* Dead specimens, except those shedding first cercariae or killed at end of experiment.
+ ISS = immature secondary sporocysts, SS = secondary sporocysts; DG = digestive gland, HE = head (including all cephalopedal organs), MC = mantle collar, OT = ovotestis, PW = pulmonary wall, RR = rectal ridge.
Δ Infection rate 3.5%.
§ See Table II.
¶ 1 with echinostome metacercariae in pericardium.
∇ Infection rate 45%.

2), there following snail no. 3 on the 35th day and no. 4 on the 40th. A specimen that died on the 42nd day had a cluster of secondary sporocysts with nearly mature cercariae in the mantle collar, and seven that died between the 46th and 62nd days had slender and comparatively underdeveloped secondary sporocysts scattered through the internal organs. In a specimen that died on the 49th day without *S. mansoni* infection there was a number of echinostome metacercariae in the pericardium.

Of the 100 *B. tenagophila* from São José dos Campos used as controls (Table I), 45 became infected and shed the first cercariae between 25 and 45 days after exposure. With the single exception of a snail that died on the 29th day, all remaining negative specimens survived up to the 50th day, when they were dissected.

The numbers of cercariae shed by *B. amazonica* and *B. tenagophila* are shown in Table II.

TABLE II

Shedding of cercariae in *Biomphalaria amazonica* from Porto Velho and *B. tenagophila* (controls) from São José dos Campos infected with the SJ2 strain of *Schistosoma mansoni* (counts on alternate days).

Snail species	No.	Period of cercarial output (days)	Total no. of cercariae	Mean daily no. of cercariae
B. amazonica	1	30	164	10.9
	2	20	1,143	114.3
	3	18	22	2.4
	4	8	21	5.2
B. tenagophila	1	82	5,002	122.0
	2	18	1,748	194.2
	3	30	2,245	149.7
	4	44	10,073	457.9
	5	56	4,898	174.9
	6	57	5,254	184.3
	7	38	8,749	460.5
	8	9	1,462	324.9
	9	12	605	100.8
	10	50	2,862	114.5

DISCUSSION

In a previous study of the susceptibility to *S. mansoni* of *B. amazonica* from the Careiro island, infection rates of 48% and 73% resulted from exposure to miracidia of the BH and SJ strains respectively. With the present experiment we aimed to investigate the degree of susceptibility of another population of that species from a different environment. A snail population of Porto Velho was selected owing to the facts that *B. amazonica* is abundant in many breeding places at that city and that Porto Velho is the capital of the Brazilian state of greatest immigration rate not only from southern states but also from schistosomiasis endemic areas of Minas Gerais and northeastern states. Water freshly taken from the original breeding place was used to make sure that its quality was compatible with hatching of miracidia and their penetration into the snails.

The infection rate in Porto Velho snails (3.5%) was much lower than in the preceding experiment (Corrêa & Paraense, 1971) with Careiro ones (73%). So great disagreement may be rather ascribed to different degrees of susceptibility between the two snail populations than to different degrees of infectivity between the SJ and SJ2 *S. mansoni* strains. In fact, although the latter were isolated on different occasions from the same breeding place, as regards infectivity of each to its associate *B. tenagophila* strain (SJ and SJ2) they have shown no significant difference in the protocols of our routine work.

The infection rate in *B. tenagophila* controls was 45% (compare with 48.4% in Paraense & Corrêa, 1978, using SJ *B. tenagophila* and SJ *S. mansoni*).

The 16 infected *B. amazonica*, including four that shed cercariae, died within about 60 days after exposure to miracidia. This relatively short survival cannot be only attributed to infection, but also to poor adjustment to artificial environment, since 112 uninfected specimens died in the same period.

A lower degree of compatibility between the studied population of *B. amazonica* and the parasite strain, as compared with the control snails, is evidenced by the longer prepatent period of the infection in the former: whereas all infected *B. tenagophila* shed cercariae until the 45th day after exposure, only one-fourth of the infected *B. amazonica* did shed, and between the 43th and 62nd days the seven surviving infected snails died with still immature sporocysts in their tissues. Another fact that, in our opinion, hints at an appreciable degree of resistance to the parasite, is the development of the miracidium to the cercarial stage at the site of penetration, first noticed by Brumpt (1941) in *B. glabrata* and now observed in the mantle collar of a specimen of *B. amazonica* which died on the 42nd day (Table I).

The mean number of cercariae shed by *B. amazonica* (337) was much smaller than by *B. tenagophila* (4,290). Even making allowance for the fact that we were dealing with a sample directly transferred from its natural environment to laboratory conditions, *B. amazonica* seems to be a poor host of *S. mansoni*, since infected specimens withstand full development of the parasite, shed few cercariae and die prematurely. In these characteristics *B. amazonica* resembles *B. straminea* (see Coelho & Barbosa, 1956) which, this notwithstanding, is admittedly a good vector of *S. mansoni* in hyperendemic areas of northeastern Brazil.

Although no trematode infection was found on previous examination of the exposed *B. amazonica*, one specimen which died on the 49th day showed echinostome metacercariae in the pericardial sac, indicating the presence of at least an echinostomatid species in the breeding place. Although the probability of some snail with prepatent *S. mansoni* infection having been used in that experiment seems remote, it cannot be discarded at all. In this case, our search for the potentiality of *S. mansoni* transmission in the area would have led us into an established focus.

Household wastes are discharged into the ditches where snails are more or less easily found, but there seems to be no fecal pollution since waste pipes apparently carry off only sink drainage.

RESUMO

Uma amostra de *Biomphalaria amazonica* de Porto Velho, Estado de Rondônia, foi exposta a miracídios de *Schistosoma mansoni* (cepa SJ2) de São José dos Campos, Estado de São Paulo (cinco miracídios para cada molusco). Foi utilizada água recém-colhida do criadouro dos moluscos para verificar se sua qualidade era compatível com a eclosão dos miracídios e a penetração destes nos caramujos. O índice de infecção resultante foi de 3,5%, em comparação com 45% nos controles *(B. tenagophila)*.

Em relação à cepa de *B. tenagophila*, mostrou a *B. amazonica*, além de menor índice de infecção, um período prepatente mais longo e menor produção de cercárias. Estas características parecem indicar que a *B. amazonica* é má hospedeira do *S. mansoni*, como a *B. straminea*, mas deve-se levar em conta que, apesar disso, esta última é reconhecidamente boa vetora do parasito em áreas hiperendêmicas do nordeste do Brasil.

Estes resultados indicam a possibilidade de introdução da xistosomose mansoni na Amazônia Ocidental, onde é comum a ocorrência da *B. amazonica*.

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