

SUSCEPTIBILITY OF *BIOMPHALARIA AMAZONICA* TO INFECTION WITH TWO STRAINS OF *SCHISTOSOMA MANSONI*

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SUMMARY

The planorbid snail *Biomphalaria amazonica* Paraense, 1966, from the lower Negro river, State of Amazonas, a region where schistosomiasis has not so far been recorded, is highly susceptible to infection with two Brazilian strains of *Schistosoma mansoni*. Of 40 snails exposed to miracidia of both strains, 23 became infected and, of these, 6 survived up to the stage of cercarial release. There was massive invasion of the digestive gland and ovotestis, and in most cases developing stages of the parasite were also found in the pulmonary wall, renal tube, rectal ridge, albumen gland and cephalopodal mass.

These results strongly suggest the possibility of *B. amazonica* coming to act as a good vector of schistosomiasis in its geographic range.

INTRODUCTION

Biomphalaria amazonica Paraense, 1966, is a planorbid mollusc occurring in the lower Negro river, so far recorded from the suburbs of Manaus and the island of Careiro, State of Amazonas³.

Experiments on the susceptibility of this species to infection with *Schistosoma mansoni* were carried out in this laboratory, using the offspring of wild specimens from the island of Careiro.

MATERIAL AND METHODS

Two strains of *S. mansoni* were used in these experiments: the BH strain, isolated in 1959 from a patient with a chronic infection contracted from a single exposure to cercariae at Belo Horizonte, State of Minas Gerais, and the SJ strain, isolated in 1962 from naturally infected *Biomphalaria tenagophila* from São José dos Campos, State of São Paulo.

Snails 4 to 7 mm in diameter (8 mm is the largest diameter so far recorded for *B. amazonica*) were individually exposed to 10 miracidia of *S. mansoni* collected, as described by CHAIA¹, from feces of infected mice. The water containing the concentrate of miracidia was taken with a pipette and dropped, under a dissecting microscope, into a small Petri dish 4 cm in diameter, until the required number of miracidia was gathered. Only freshly hatched, fast moving miracidia were used. A snail was then placed in the dish and just enough spring water was added to cover it. The dish was covered and the snail left in it overnight. The next morning the exposed snails were removed to small aquaria, where they were observed at least four times daily. If any specimen happened to die, it was dissected and examined for developing stages of the schistosome. On the 28th and 30th days after exposure, and then every fifth day, the snails were placed singly in vials with spring water and exposed to the light of electric lamps. Those that shed cercariae were isolated for

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TABLE I

Results of exposure of 25 *Biomphalaria amazonica* to the BH strain of *Schistosoma mansoni* (10 miracidia per snail)

| Days after exposure | Exposed snails | Results * |
|---------------------|----------------|--|
| 6 | 3 | Negative |
| 9 | 2 | Negative |
| 13 | 3 | 1 negative; 1 with PS in FH; 1 with PS in MC |
| 22 | 1 | Negative |
| 28 | 2 | 1 with SS in OT, DG, AG, RR, PW, RT, FH; 1 with SS in OT, DG, remaining parts autolysed |
| 30 | 1 | SS in OT, DG, RR, PW, RT, MC, FH |
| 33 | 1 | SS in OT, DG, remaining parts autolysed |
| 35 | 4 | 1 with SS in OT, DG, RR, PW, RT, MC; 1 with SS in OT, DG, remaining parts autolysed; 2 shed cercariae (nos. 1, 2) ** |
| 40 | 1 | SS in OT, DG, RR, PW, MC |
| 45 | 2 | 1 with SS in OT, DG, RT, MC; 1 negative |
| 50 | 1 | Negative |
| 60 | 4 | Dissected, negative |

* Infection rate 48%. AG albumen gland, DG digestive gland, FH foot-head, MC mantle collar, OT ovotestis, PS primary sporocyst, PW pulmonary wall, RR rectal ridge, RT renal tube, SS secondary sporocyst.

** See Table III

TABLE II

Results of exposure of 15 *Biomphalaria amazonica* to the SJ strain of *Schistosoma mansoni* (10 miracidia per snail)

| Days after exposure | Exposed snails | Results * |
|---------------------|----------------|--|
| 13 | 3 | PS in FH, MC, young SS in OT, DG, RR, PW, RT |
| 16 | 1 | PS in FH, MC, young SS in PW, remaining parts autolysed |
| 30 | 3 | 1 with SS in OT, DG, PW, RT, MC, FH; 2 shed cercariae (nos. 3, 4) ** |
| 33 | 1 | SS in OT, DG, remaining parts autolysed |
| 35 | 1 | Shed cercariae (no. 5) ** |
| 40 | 1 | Shed cercariae (no. 6) ** |
| 46 | 1 | SS in OT, DG, AG, RR, RT |
| 60 | 4 | Dissected, negative |

* Infection rate 73%. AG albumen gland, DG digestive gland, FH foot-head, MC mantle collar, OT ovotestis, PS primary sporocyst, PW pulmonary wall, RR rectal ridge, RT renal tube, SS secondary sporocyst.

** See Table III

subsequent observation. On the 60th day after exposure the snails still alive were killed and examined by dissection.

The emission of cercariae was followed until death in surviving infected specimens. Each snail was kept separately in a small aquarium and placed every other day, for 24 hours, in a vial with clean spring water, at a window diffusely illuminated during the day. After that period the snail was removed from the vial, some drops of formalin were added to the water to kill the cercariae, and they were counted.

In the above-mentioned way, 25 snails were exposed to the BH strain and 15 to the SJ strain.

RESULTS

Of the 25 snails exposed to the BH strain (Table I), 12 became infected. With only two exceptions, the infected specimens died before beginning to release cercariae, showing massive invasion of the internal organs by developing stages of the schistosome.

A higher infection rate was observed among the 15 snails exposed to the SJ strain (Table II), 11 of which became heavily infected. Four of the latter reached the stage of cercarial release.

The emission of cercariae was followed in the 6 above-mentioned specimens (Table III). The daily output was widely fluctuating, following no regular pattern. As is not infrequently the case, sometimes the cercarial counts dropped to 0 for one or two days in the course of a productive period. The period of release of cercariae varied from 1 to 20 days and was stopped by death of the snails, all of which showed intense infections in the internal organs on dissection. No recovery from infection was observed.

DISCUSSION

The BH strain, previously used in susceptibility experiments, is highly infective to most populations of *Biomphalaria glabrata* which have been tested⁴. The SJ strain has not proved infective to *B. glabrata*⁵, but easily infects the closely related *B. tenagophila*^{5,6}. The present experiments show that *B. amazonica* is highly susceptible to infection with both strains. In addition, the results presented in Table III show that *B. amazonica* was able, under those conditions, to shed cercariae for an average period of about 10 days.

The short lifetime of the snails after exposure to miracidia of both strains should be ascribed chiefly to the pathogenic action of

TABLE III

Shedding of cercariae and post-mortem findings in *Biomphalaria amazonica* infected with the BH and SJ strains of *Schistosoma mansoni*

| Snail no. * | Schistosome strain | Period of cercarial output | Total no. cercariae ** | Mean daily no. cercariae | Post-mortem findings *** |
|-------------|--------------------|----------------------------|------------------------|--------------------------|----------------------------------|
| 1 | BH | 8 | 721 | 158 | SS in OT, DG, AG, RR, PW, RT |
| 2 | BH | 5 | 192 | 64 | SS in OT, DG, RR, PW, MC |
| 3 | SJ | 20 | 448 | 40 | SS in OT, DG, AG, RR, PW, RT, FH |
| 4 | SJ | 20 | 1172 | 106 | SS in OT, DG, FH |
| 5 | SJ | 1 | 12 | 12 | SS in OT, DG, AG, RR, PW, RT |
| 6 | SJ | 11 | 270 | 45 | SS in OT, DG, AG, RR, RT, FH |

* See Tables I and II

** Counts on alternate days

*** AG albumen gland, DG digestive gland, FH foot-head, MC mantle collar, OT ovotestis, PW pulmonary wall, RR rectal ridge, RT renal tube, SS secondary sporocyst.

the developing schistosomes, and also, to some extent, to unavoidable troubles resulting from frequent handling of the animals.

As stated above, each snail was exposed to 10 miracidia. This is a standard number used in our susceptibility experiments to facilitate comparison of results. Under natural conditions, however, a single snail is not expected to be exposed to 10 miracidia at the same time. If a susceptible species like *B. amazonica* is exposed experimentally to that number of miracidia, a majority of them will develop practically without restraint in susceptible individual snails. In comparatively small organisms as *B. amazonica*, the growth and multiplication of so many parasites will produce changes significant enough to kill most snails before the parasites reach their final stage of development. A direct correlation between infection and mortality has been often observed in snails infected with schistosomes and other trematodes. In the present experiments the tendency to an early mortality is expected to have been increased by the unusually large number of challenging parasites.

From the above results it cannot, of course, be concluded that *B. amazonica* would behave as a good vector of schistosomiasis under natural conditions. Nevertheless, there is some indication that this might be the case. Even considering the exposures to comparatively large numbers of miracidia, the infection rates point to an appreciable degree of susceptibility. The fact that only a few snails survived the incubation period is not incompatible with the idea of a good vector, since the same fact was observed in experimental infections of *Biomphalaria straminea*², the only intermediate host of schistosomiasis in several hyperendemic areas of northeastern Brazil. The numbers of cercariae shed by our snails would certainly be much greater if the latter had been exposed to direct sunlight. This was not done to avoid the risk of death from overheating.

RESUMO

Suscetibilidade da Biomphalaria amazonica à infecção com duas cepas de Schistosoma mansoni

O molusco planorbídeo *Biomphalaria amazonica* Paraense, 1966, da região do baixo

rio Negro, Estado do Amazonas, onde a ocorrência de esquistossomose não foi ainda registrada, é altamente suscetível à infecção por duas cepas brasileiras de *Schistosoma mansoni*. De 40 moluscos expostos a miracidios de ambas as cepas, 23 infetaram-se e, entre eles, 6 sobreviveram até o estágio de eliminação de cercárias. Observou-se a invasão maciça da glândula digestiva e do ôvo-teste e, na maioria dos casos, encontraram-se também formas evolutivas do parasita na parede pulmonar, tubo renal, crista retal, glândula do albúmen e massa cefalopodal.

Estes resultados sugerem enfaticamente a possibilidade de vir a funcionar a *B. amazonica* como bom hospedeiro intermediário da esquistossomose em sua área de distribuição geográfica.

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