

RESEARCH NOTE

Schistosoma mansoni Infections in the First Three Months of Life of Sympatric Intermediate Hosts from Brazil

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One of the factors influencing the intermediate host susceptibility to *Schistosoma mansoni* is the mollusc developmental stage. WL Newton (1953 *Exp Parasitol* 2: 242-257) demonstrated that in the case of *Biomphalaria glabrata* older snails are less susceptible than young ones to infection by miracidia. CA Wright and GC Ross (1963 *Ann Trop Med Parasitol* 57: 47-51) attributed this to the greater variety of proteins circulating in the blood of young individuals, thus providing a better nutrient medium for the mother sporocysts than the protein-depleted blood of sexually mature snails. Yet CS Richards (1984 *Malacologia* 25: 493-502) observed four patterns of susceptibility in *B. glabrata*: (1) non susceptible at any age; (2) juvenile susceptible/adult non susceptible; (3) susceptible at any age; (4) juvenile susceptible/adult variable.

It is known that snails have their life expectancy reduced to a few months in seasonally drying habitats or in areas subjected to control campaigns (OS Pieri et al. 1995 *Mem Inst Oswaldo Cruz* 90: 535-536). Thus, this study investigates comparatively the parasite infectivity for the first three months of life of the three natural vector species of *S. mansoni* in Brazil.

All snails used were from colonies of *B. glabrata* (Belo Horizonte, State of Minas Gerais), *B. tenagophila* (São José dos Campos, State of São Paulo) and *B. straminea* (Picos, State of Piauí)

maintained at the Department of Malacology, Oswaldo Cruz Institute, Rio de Janeiro, for over 10 years. For breeding the test specimens, 20 adult snails of each species were put into aquaria with 2 l of dechlorinated water, and substratum according to B Rozemberg et al. (1992 *Mem Inst Oswaldo Cruz* 87: 223-232). The snails were fed *ad libitum* with fresh lettuce. To facilitate egg collection, floating Styrofoam tablets were left overnight as an oviposition surface. On the following morning the tablets with egg masses were identified and transferred to aquaria similar to those described above. When the snails were one, two or three months old, they were individually measured and exposed to five *S. mansoni* sympatric miracidia of the BH2 strain (*B. glabrata*), of the SJ2 strain (*B. tenagophila*) and of the EC strain (*B. straminea*). The BH2 and SJ2 *S. mansoni* strains were isolated as described by WL Paraense and LR Corrêa (1989 *Mem Inst Oswaldo Cruz* 84: 281-288). The EC *S. mansoni* strain was isolated on June 25, 1980 from the feces of EC Silva, 9 years, born and grown up in Picos, a *B. straminea* exclusive area.

The procedures for collecting feces of infected mice and later exposition of snails to miracidia are as described by Paraense and Corrêa (*loc. cit.*). The onset of egg laying was carefully observed on each aquarium to distinguish the molluscs exposed before the first oviposition (juveniles) from those exposed after having reached sexual maturity (adults). The aquaria were kept at a room temperature of 24-26°C throughout the experiment. Snails were observed daily and, if any specimen happened to die, it was dissected and examined for developing stages of the schistosome. The screening techniques for detecting the positive snails were as described by Paraense and Corrêa (*loc. cit.*), and the specimens that survived for 60 days after exposition without shedding cercariae, were fixed, dissected and examined. In order to ascertain whether there were significant differences between the infection rates in the three age groups a χ^2 test using 2 x 2 contingency tables was carried out (S Siegel 1956 *Nonparametric statistics for the behavioral sciences*, New York: McGraw-Hill, 312 pp.).

For *B. glabrata* the infection rate varied from 82.2% (in snails exposed 1 month old) to 59.5% (those exposed 3 months old). For *B. tenagophila* this rate varied from 28.4% (1 month old) to 53.7% (3 months old). For *B. straminea* the rate varied from 13.4% (1 month old molluscs) to 6.5% (2 months old) (Table). Grouping the infection data by the sexual maturation of molluscs when exposed to *S. mansoni* the following infection rates were obtained for juveniles and adults, respectively: *B. glabrata*, 81.7% and 61.9%; *B. tenagophila*, 40.9%

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and 44.0%; *B. straminea*, 9.9% and 8.1%.

Statistical analysis of the Table data showed that: (1) the percentage of *B. glabrata* snails infected when they were 1 month old is not significantly different from that found in snails exposed when they were 2 months old ($\chi^2=1.983$; $p>0.05$), but it is significantly higher than that was the case with molluscs exposed when they were 3 months old ($\chi^2=17.488$; $p<0.001$); (2) the percentage of *B. glabrata* snails infected when 2 months old was significantly higher than was the case with snails exposed when 3 months old ($\chi^2=5.342$; $p<0.05$); (3) the percentage of *B. tenagophila* snails infected when 1 month old was significantly less than those which were 2 months old ($\chi^2=6.65$; $p<0.01$), as well as those 3 months old ($\chi^2=16.448$; $p<0.001$). There was no significant difference between the percentage of snails infected when they were 2 or 3 months old ($\chi^2=1.898$; $p>0.05$); (4) there was no significant difference in the percentages of *B. straminea* infected at different ages: 1 month old x 2 months old ($\chi^2=3.404$; $p>0.05$), 1 month old x 3 months old ($\chi^2=1.316$; $p>0.05$) and 2 months old x 3 months old ($\chi^2=0.369$; $p>0.05$); (5) the percentage of juvenile *B. glabrata* infected was significantly higher than was the case with adults ($\chi^2=18.636$; $p<0.001$); (6) there was no significant difference ($p>0.05$) between the percentages of juvenile and adult *B. tenagophila* ($\chi^2=0.132$) and *B. straminea* ($\chi^2=0.079$) infected.

As shown in the Table, out of 407 exposed *B. glabrata* snails, 273 (67.1%) released cercariae and 18 (4.4%) died carrying sporocysts in their tissue. From 393 *B. tenagophila* snails exposed to the SJ2 strain, 161 (41%) released cercariae and 2 (2.2%) died carrying sporocysts in their tissue. Out of 496 *B. straminea* snails, 48 (9.7%) released cercariae but none those that infected with sporocysts died during experimentation. The present results concerning infection rates in *B. glabrata* and *B. tenagophila* are similar to those of Paraense and Corrêa (*loc. cit.*) using the same mollusc-parasite combinations. The present infection rates for *B. straminea* exceed most of those found by FS Barbosa and T Figueiredo (1970 *Rev Inst Med Trop S Paulo* 12: 198-206) on snail-parasite combinations from northeastern Brazil. The present study shows that the susceptibility of *B. glabrata* and *B. tenagophila* to *S. mansoni* infections varied significantly between the first month and the third month of mollusc life, but that this was not the case for *B. straminea*. The susceptibility declined during that period of life in *B. glabrata*, while it increased in *B. tenagophila*. During the course of development from juvenile to the adult stage, a significant increase in susceptibility of *S. mansoni* was only observed in *B. glabrata*.

The results with *B. glabrata* confirm and amplify the observations from other investigators, showing that susceptibility to infection by *S. mansoni*, as well as by other trematode parasites, falls with the increasing maturation or age of the molluscan host (Newton *loc. cit.*, JC Boray 1966 *Ann Trop Med Parasitol* 60: 114-124, KM Raymond & AJ Probert 1992 *J Med Appl Malacol* 4: 121-123). The increasing susceptibility to infection with age found in the case of *B. tenagophila*, has also been reported for *B. havanensis* (RM McQuay 1953 *Trans R Soc Trop Med Hyg* 47: 56-61). Although E Chernin and VM Antolics (1975 *J Parasitol* 61: 377-378) found that neonate *B. straminea* (<1 mm shell diameter) were more susceptible than juvenile or adult (4-8 mm). Conspecifics their results do not contradict those from the present study, as only snails from 1 to 3 months of age, ranging from 2 to 7 mm diameter, were considered here.

Complementary observations of the present work showed that when infected younger *B. glabrata* suffered greater mortality than adults which were infected. Thus, of 18 infected snails found dead, 14 (77.8%) had been exposed with 1 month of age. These results are in accordance with those described by M Meier and C Meier-Brook (1981 *Z Parasitenkd* 66: 121-131) for *B. glabrata*, and KN De Kock (1993 *Folia Parasitol* 40: 9-12) for *B. pfeifferi*. Similarly, juvenile *B. tenagophila* also suffered higher mortalities when infected than was the case with adult conspecifics. Thus, of the 18 *B. tenagophila* found dead, 17 (94.3%) had been exposed to *S. mansoni* when 1 month old. Of these only 2 (11.8%) had sporocysts in their tissues. In the case of *B. straminea* there was no significant difference between the mortality rates for snails infected when 1 or 3 months of age (46.7% in both cases).

This work reinforces the view that juvenile *B. glabrata* are important in the transmission of schistosomiasis, as pointed out by LA Cooper et al. (1992 *J Parasitol* 78: 441-446). As regards *B. straminea* further work is needed to clarify the role of immature snails on the dynamics of *S. mansoni* transmission in areas where this is the only host species. These studies will help to explain why this species is apparently effective in to transmission of *S. mansoni* in some areas despite being a poor host.

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TABLE
 Infection and mortality of *Biomphalaria* spp. exposed at the ages of 1, 2 or 3 months to sympatric
Schistosoma mansoni

Age of snails (months)	Number exposed	Shell diameter (mm)	Number dead		Number alive		Total	
			infected	not infected	infected	not infected	infected	%infected
<i>B. glabrata</i>								
1	146	2.5 - 6.0	14	1	106	25	120	82.1
2	108	2.5 - 9.0	3	1	77	27	80	74.1
3	153	4.0-14.0	1	1	90	61	91	59.5
Total	407	-	18	3	273	113	291	71.5
<i>B. tenagophila</i>								
1	141	2.0 - 7.0	2	15	38	86	40	28.4
2	129	3.0 - 8.0	-	-	57	72	57	44.2
3	123	3.0 - 8.5	-	1	66	56	66	53.7
Total	393	-	2	16	161	214	163	41.5
<i>B. straminea</i>								
1	164	2.0 - 5.0	-	7	22	135	22	13.4
2	153	3.0 - 6.0	-	1	10	142	10	6.5
3	179	3.0 - 7.0	-	7	16	156	16	8.9
Total	496	-	-	15	48	433	48	9.7

