

DEVELOPMENT OF *SCHISTOSOMA MANSONI* IN *BIOMPHALARIA TENAGOPHILA*, *BIOMPHALARIA STRAMINEA* AND *BIOMPHALARIA GLABRATA*

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SUMMARY

A comparative study of the development of *Schistosoma mansoni* during the intra-molluscan phase was made by means of histological sections of *Biomphalaria tenagophila*, *B. straminea* and *B. glabrata* from Brazil. Two hundred snails of each species were individually exposed to 50 miracidia of the *S. mansoni*, AL line. No larvae were observed in the snails fixed 72 h after exposure. In specimens shedding cercariae, 31 days after exposure tissue reactions encapsulating the larvae were seen in *B. tenagophila* and *B. straminea*, in the head-foot, mantle collar and renal ducts. No tissue reactions occurred in the digestive glands of these two species. In *B. glabrata* the presence of numerous sporocysts and cercariae without tissue reactions was observed in the digestive gland, and other organs. The levels of infection of the snails and the average numbers of cercariae shed per day were 32.6% and 79 ± 90 respectively for *B. tenagophila*, 11.3% and 112 ± 100 for *B. straminea* and 75.3% and 432 ± 436 for *B. glabrata*. The lower levels of infection and average numbers of cercariae shed by *B. tenagophila* and *B. straminea* are thus related to their more potent internal defense systems.

KEYWORDS: . *Schistosoma mansoni*: development; *Biomphalaria tenagophila*; *Biomphalaria straminea*; *Biomphalaria glabrata*.

INTRODUCTION

PAN^{26, 27} studied in detail the host-parasite interaction between germ free *B. glabrata* and *S. mansoni*. The author made observations concerning the histology and histopathology of the snail, sites of parasite development, effect on snail growth and reproduction as well as causes of snail mortality. He showed that only a small percentage of the penetrating miracidia develop to primary sporocysts and that these are usually located in the compact tissue of the foot.

The histogenesis of cercarial formation in *B. glabrata* has been well studied^{12, 19, 27}. Several generations of sporocysts occur during the intra-

molluscan phase of the *S. mansoni* life cycle^{18, 35}. In the normal process of *S. mansoni* development in *B. glabrata*, the young secondary sporocysts migrate directly and rapidly in the direction of the digestive gland, the period of primary sporocyst production being relatively short and limited to the prepatent period³⁶.

Other host-parasite associations between *Bulinus obtusispira* and *Schistosoma haematobium* and *S. bovis*¹⁶ and between *Schistosoma rodhaini* and *Biomphalaria pfeifferi*³⁷ led these investigators to conclude that the process of sporocyst replication

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occurs in a variety of *Schistosoma* species and that there are various complex strategies used by the larvae of these parasites to develop in their molluscan hosts¹⁷.

The resistance of the molluscan host to infection is dependent on factors in the haemolymph and the capacity of haemocytes (amoebocytes, granulocytes) to envelope and destroy the invading organism by encapsulation^{5, 20, 23}. The tegumental surface of *S. mansoni* sporocysts is the site of nutritive and immunological interactions with haemolymph cells and plasma of *B. glabrata*. Within minutes of being placed in host plasma sporocysts acquire plasma antigens. A wide variety of peptides is acquired. Some differences occur in the peptides acquired from plasma of susceptible and resistant strains of snail². According to KNAAP & LOKER²⁰ the systems used by snails to combat invading organisms is called an "internal defense system" rather than an "immune system" because lymphocytes, immunoglobulins and anamnestic responses to specific antigen are lacking. At least four different cell types function in internal defense. Three of these are non-circulating or fixed cells and include antigen trapping endothelial cells, reticulum cells and pore cells. The most prominent role in defense against digenean larvae of trematodes is played by mobile cells collectively termed-haemocytes. Haemocytes are found in the circulating haemolymphs and because snails have open vascular systems, haemocytes also move freely to and from the tissues. Haemocytes exhibit morphological and biochemical heterogeneity and the functional significance of which is still being studied.

The encapsulation of the *S. mansoni* larvae by host tissues and the formation of "granuloma" in *Biomphalaria* were observed in *B. glabrata*,^{21, 22, 24, 26} *B. straminea*⁹ and *B. tenagophila*^{8, 15}.

In Brazil there are some 10 million of individuals parasitised with *S. mansoni* transmitted by three species of snails of the genus *Biomphalaria*. However, there are few studies about the differences between the processes or strategies used by larvae of this trematode to develop in each species of snail^{8, 9, 15}. The epidemiological importance of *B. glabrata* in our country is indisputable^{10, 31}. The others snail hosts as *B. tenagophila* and *B. straminea* although they were less susceptible to *S. mansoni* infection than *B. glabrata* they have epidemiological importance in south, southeast and in northeast of Brazil^{3, 4, 9, 10}.

In the present work we have determined, in a parallel comparative study, the levels of infection and the mean number of cercariae eliminated per day by *B. tenagophila*, *B. straminea* and *B. glabrata* in standard conditions. In parallel, at the histological level we have observed the development of *S. mansoni* in intra-molluscan phase in the three species of *Biomphalaria* which act as hosts of this trematode in Brazil with the aim of to confront the process of parasite development in each species with the number of cercariae shed per snail.

MATERIALS AND METHODS

Three species of laboratory bred snails were used: (a) *B. glabrata* a strain descendant from specimens collected in Barreiro de Cima, Belo Horizonte (MG), and maintained in the laboratory for more than 20 years and measuring 8-10 mm in diameter; (b) *B. tenagophila* descendant from specimens collected in the Lake of Pampulha, Belo Horizonte in 1988 and measuring 8-10 mm in diameter; (c) *B. straminea* descendant from specimens collected in Paracatu (MG), in 1989 with diameter of 4-7 mm.

The AL strain of *S. mansoni* was derived of cercariae shed by *B. glabrata* from the state of Alagoas, isolated in 1980.

In simultaneous experiments, 200 snails of each species were exposed individually to 50 miracidia. The exposed specimens were maintained in separate aquaria under identical laboratory conditions. Fifty specimens of each species were separated for histological study and the other 150 were used for the determination of levels of infection and quantification of cercarial shedding³¹. For the histological study, the snails were anesthetized with menthol crystals for 30 minutes and then removed from their shell, the soft tissues were distended and fixed in Bouin containing 6.6% glacial acetic acid²⁵. Fixation was allowed to continue for 5 to 6 h and then the tissues were transferred to small plastic flasks containing 70° alcohol which were sent to the Laboratório de Patologia do Centro de Pesquisas Gonçalo Moniz, Salvador, Bahia. The material was then mounted in paraffin blocks, and serial sections made at 5 microns intervals which were then stained with hematoxylin and eosin, PAS and picro-sirius red for collagen. Groups of 10 snails of

each species, exposed or not to miracidia were fixed 72 h after exposure. In addition, 3 to 5 specimens of each species shedding cercariae were fixed 31 days after infection together with 5 non-exposed specimens.

Statistical analysis - the numerical differences obtained in the experiments were analyzed using the X^2 test. Quantitative variations were submitted to analysis of variance and the means compared by Student's t test. The variable number of cercariae (x) was submitted to logarithmic transformation $\log(x+1)$ so that standard deviations were proportional to the means²⁹. In all calculations the level of significance was taken as 5% ($p < 0.05$).

RESULTS

The differences observed in the levels of infection of the three species of snail with the AL strain of *S. mansoni* were statistically significant ($p < 0.05$). The lowest level of infection was with *B. straminea* (11.3%), followed by *B. tenagophila* (32.6%) and *B. glabrata* (75.3%). The total number of *B. glabrata* infected was 113 out of 150 specimens exposed to miracidia. Only forty snails were used as control. The mean numbers of cercariae shed per day were similar for *B. straminea* (mean 112 ± 100 SD) and *B. tenagophila* (mean 79 ± 90 SD) and significantly higher for *B. glabrata* (mean 432 ± 436 SD) (Student's t test). The total number of cercariae shed during the life of infected snails were of 78,416 for *B. tenagophila*, 30,763 for *B. straminea* and 309,910 for *B. glabrata*.

The pre-patent period of *B. tenagophila* infected with AL line of *S. mansoni* was of 30-65 days (mean 44.0 ± 15.9 SD), for *B. straminea* was of 30-58 days (mean 37.0 ± 11.7 SD) and for *B. glabrata* was of 30-51 days (mean 34.6 ± 8.5 SD).

Tissue reactions consistent with the destruction of penetrating miracidia or primary sporocysts were not observed in the histological sections made from snails fixed 72 h after being exposed to miracidia. Sporocysts, with or without an encircling reaction, were observed in various organs of snails eliminating cercariae and fixed 31 days after miracidial exposure.

In *B. tenagophila*, although well preserved sporocysts and cercariae were found (Fig. 1a), reactions around sporocysts with nearby accumulations of haemocytes together with formations of concentric

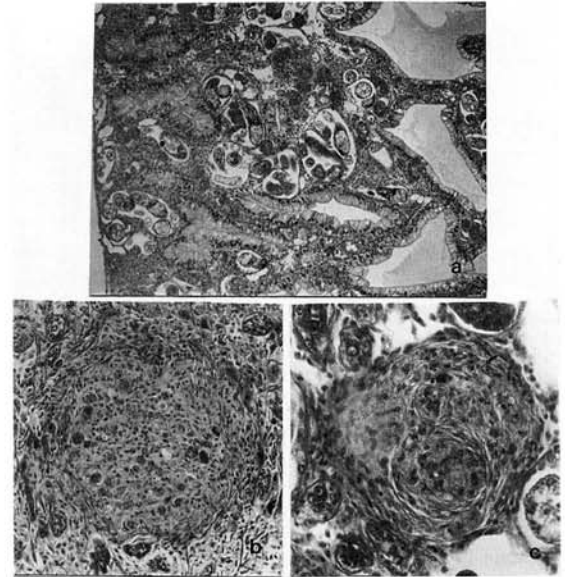


Fig. 1 - Development of *S. mansoni* larvae in *B. tenagophila* 31 days after infection: a) Section of the digestive gland showing secondary sporocysts and cercariae (HE, 125X); b) Head-foot region with intact primary sporocysts and granuloma (350X); c) Mantle collar with normal and encapsulated primary and secondary sporocysts (425X).

layers of fibers encapsulating the parasite elements (granulomas) were frequently observed. Such granuloma and primary sporocysts were seen in the head-foot region (Fig. 1b), in the mantle collar (Fig. 1c) and in various other locations.

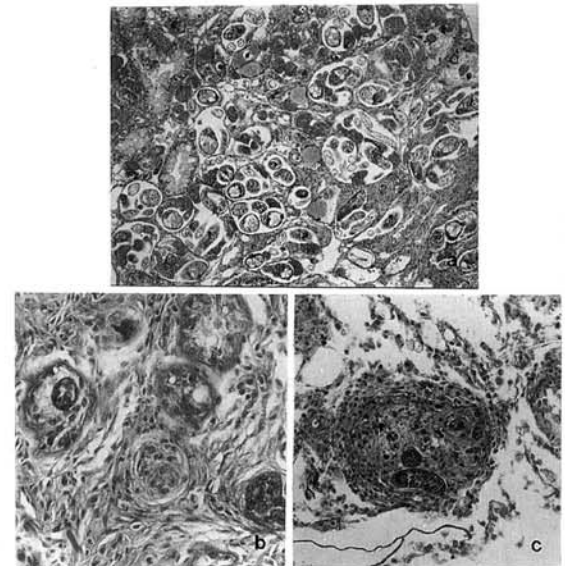


Fig. 2 - Development of *S. mansoni* larvae in *B. straminea* 31 days after infection: a) Digestive gland showing numerous sporocysts and cercariae (HE, 125X); b) Head-foot region with sporocysts and tissue reactions around larvae (425X); c) Renal ducts with sporocysts with tissue reaction around larvae and dense areas around stroma. (350X).

In sections from *B. straminea*, numerous secondary sporocysts and well formed cercariae were observed without tissue reaction principally at the level of the digestive glands (Fig. 2a). Nevertheless, granulomas encircling disintegrating parasite structures, foci of dense areas around stroma and proliferation of haemocytes both in the head-foot region (Fig. 2b) as well as in the renal tubes (Fig. 2c) were also observed. The fibrous layers around the granuloma did not stain with sirius-red showing that they did not contain collagen.

In sections of *B. glabrata*, numerous sporocysts were observed in various phases of growth and differentiation, up to the formation of cercariae, without any indication on reaction by host structures. The parasite was localized principally at the level of the digestive glands (Fig. 3a), in the head-foot region (Fig. 3b) and in the mantle collar (Fig. 3c).

DISCUSSION

The levels of infection of the three species of snail demonstrated the lower level of susceptibility to the *S. mansoni* AL strain of *B. straminea* from Paracatu (11.3%), a result similar to that previously obtained with experimental infections of *B. straminea* from the state of Minas Gerais^{13, 14, 30}.

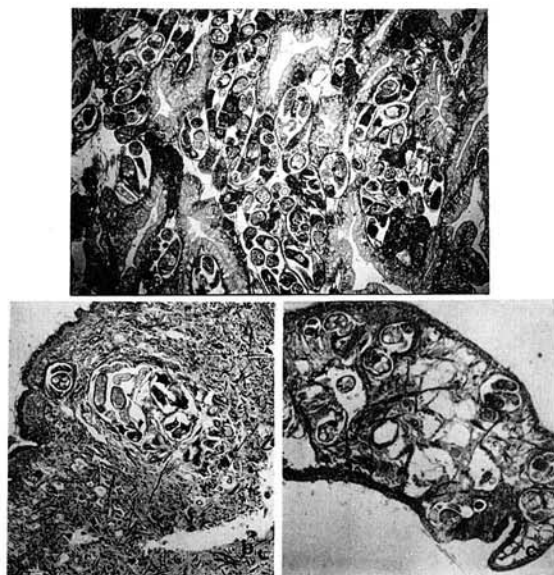


Fig. 3 - Development of *S. mansoni* larvae in *B. glabrata* 31 days after infection: a) Section of the digestive gland with numerous secondary sporocysts and cercariae (HE, 80X); b) Head-foot regions with sporocysts and cercariae (125X); c) Mantle collar with secondary sporocysts (125X).

The level of infection of *B. tenagophila* from Pampulha Lake to the AL strain of *S. mansoni* reported here (32.6%) was similar to that previously obtained (30.0%) with infection of this snail with the same trematode strain³³. Normally the experimental levels of infection of *B. tenagophila* present higher levels of variation than those obtained with *B. straminea*^{11, 28, 30, 33}. However, the natural level of infection of *B. tenagophila* from Pampulha was of 0.03%⁴.

In the histological study of snails fixed 72 h after exposure to miracidia, the presence of larvae was not detected in any of the specimens. This is probably due to the low number of miracidia used in this study (ZANNOTTI-MAGALHÃES, E., personal communication, IV Simpósio Internacional de Esquistossomose, Rio de Janeiro, 1993), because in resistant host individuals a dramatic accumulation of haemocytes encapsulates the sporocyst and, within 24 h, the haemocytes kill the sporocyst and phagocytose its tegument^{2, 5}.

In the sections made from snails shedding cercariae, 31 days after infection, both normal and encapsulated larvae were seen in *B. tenagophila* and *B. straminea* (Figs. 1 and 2 b, c). Tissue reactions were generally not seen in the digestive glands of these snails. Tissue reactions to *S. mansoni* infection were observed for the first time in *B. straminea* and *B. tenagophila* from Minas Gerais. COELHO & BARBOSA⁹ working with *B. straminea* from the northeast and COELHO⁸ working with *B. tenagophila* from São Paulo, Brazil, reported extensive destruction of larvae up to 72 h after miracidial penetration. GUARALDO et al.¹⁵ observed that only 1.6% of the miracidia that penetrate in *B. tenagophila*, from São Paulo develop and that 98.4% were destroyed. The studies of BARRACCO et al.¹, which attempt to morphologically characterize the haemocytes from *B. tenagophila* and define their functional activities, will help elucidate the causes of the severe tissue reactions.

In *B. glabrata*, up to 30% of the miracidia that penetrate are destroyed^{6, 15, 26, 27}. However, with *B. glabrata* population from Belo Horizonte, which is highly susceptible to *S. mansoni* infection and used for life cycle maintenance in our laboratory, we did not observe any tissue reactions in snails shedding cercariae. The levels of infection of this species in our laboratory are approximately 90% under mass infection conditions^{32, 34}.

The results reported here show that miracidia penetrate in the three species of snail hosts but that some or all of the larvae were destroyed by the internal defense systems particularly in *B. straminea* and *B. tenagophila*, revealing that cellular reactions in refractory or partially compatible snails are more severe than in totally compatible snails. Thus, the lower levels of infection and means and totals of cercariae shed by these species, as compared with *B. glabrata*, are not related only to small size of these molluscs⁷. They are related to more potent host defense factors present probably in their haemolymph²⁰. The histopathology of these snails, showing cellular reactions in *B. straminea* and *B. tenagophila* may to explain the lower compatibility of these two species with different Brazilian *S. mansoni* strains³¹ and the retard of development of the trematode in intra-molluscan phase.

Further studies will be necessary in order to elucidate which are the factors of the internal defense systems that determine the development or destruction of the parasite in its intra-molluscan phase and how are the processes of migration of primary sporocysts in these two Brazilian host species.

RESUMO

Desenvolvimento do *Schistosoma mansoni* em *Biomphalaria tenagophila*, *Biomphalaria straminea* e *Biomphalaria glabrata*.

Foi feito estudo comparativo do desenvolvimento do *Schistosoma mansoni* na fase intra-molusco, através de cortes histológicos, em *Biomphalaria tenagophila*, *B. straminea* e *B. glabrata*. Duzentos moluscos de cada espécie foram expostos individualmente a 50 miracidios de *S. mansoni* da linhagem AL. Nenhuma larva foi observada nos exemplares fixados 72 horas após a exposição. Nos exemplares eliminando cercárias, 31 dias após a exposição, foram observadas reações teciduais de encapsulamento de larvas em *B. tenagophila* e *B. straminea*, na região cefalopodal, colar do manto e dutos renais. Nas glândulas digestivas das duas espécies não foram observadas reações. Em *B. glabrata* foi registrada a presença de numerosos esporocistos e cercárias sem reação tecidual na glândula digestiva e outros órgãos. As taxas de infecção desses moluscos com AL e as médias de cercárias eliminadas por dia, foram de 32,6% e 79 ± 90 , respectivamente, para *B. tenagophila* de 11,3% e 112 ± 100 para *B. straminea* e de 75,3% e 432 ± 436 para

B. glabrata. As taxas mais baixas de infecção e as médias menores de cercárias eliminadas por *B. tenagophila* e *B. straminea* foram relacionadas aos sistemas internos de defesa mais eficientes dessas espécies.

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