The effect of early infection with *Echinostoma paraensei* on the interaction of *Schistosoma mansoni* with *Biomphalaria glabrata* and *Biomphalaria tenagophila*

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Infection caused by the trematode Echinostoma paraensei *has been shown to interfere in the natural resistance to infection by* Schistosoma mansoni. Biomphalaria glabrata *is susceptible to infection, while Taim isolate* Biomphalaria tenagophila *is resistant to infection by* S. mansoni. *These two snail species were assessed for infection with* E. paraensei *two days after exposure to* S. mansoni *miracidia. The number of* B. tenagophila *and* B. glabrata *infected with* E. paraensei *was lower in co-infected group, suggesting an antagonistic relationship.* B. glabrata *showed an increase in its susceptibility to* S. mansoni*, whereas* B. tenagophila *maintained its refractoriness to* S. mansoni *infection. Weekly comparisons made between the* E. paraensei *cercariae released from* B. tenagophila *and* B. glabrata *mono-infected snails revealed no quantitative differences. In contrast,* S. mansoni *cercariae released were higher in the* B. glabrata *co-infected group. Mortality rates were significantly greater in both species pertaining to co-infected group and unexpected mortalities were also observed in* B. tenagophila *exposed only to* S. mansoni *miracidia. Our study revealed that the* B. tenagophila *Taim isolate is susceptible to* E. paraensei *infection, although infection did not alter its resistance to* S. mansoni *infection.*

Key words: *Echinostoma paraensei* - *Schistosoma mansoni* - *Biomphalaria tenagophila* - *Biomphalaria glabrata* - resistance

The infectivity of *Biomphalaria* spp by *Schistosoma mansoni* isolates ranges from incompatible levels to a wide range of susceptibility (Basch 1975) and it has been determined to be related to the congruency of the host, the parasitic phenotype (Rosa et al. 2005) and/or the snail innate defence system (Miller et al. 2001).

The snail *Biomphalaria tenagophila* is the second most important intermediate host of *S. mansoni* in Brazil. Studies with the *B. tenagophila* Taim lineage have demonstrated its natural resistance to *S. mansoni* infection and experiments to alter its refractoriness have, until the present, failed (Bezerra et al. 2003, Martins-Souza et al. 2003, 2006, Barbosa et al. 2006).

It is widely known that infection by echinostome larvae may induce a transitory decrease in the resistance of *Biomphalaria glabrata* to *S. mansoni* infection (Lie et al. 1977a). The excretion of soluble proteins from sporocysts and rediae are required to act on hemocytes to maintain their unresponsiveness to the parasite (Loker & Adema 1995).

Echinostoma paraensei is a digenetic trematode that naturally infects *B. glabrata* in Brazil (Lie & Basch 1967) and experiments using the *B. glabrata* lineage have indicated that co-infection with *E. paraensei* interferes with the innate resistance of the snails to *S. mansoni* infection, allowing an increase in both the number of the infected snails and in the number of cercariae released (Lie et al. 1977b).

More recently, the *B. tenagophila* Taim lineage has been suggested as a biological control agent for schistosomiasis through its introduction into specific geographical localities where active transmission is occurring by susceptible *B. tenagophila* snails (Coelho et al. 2004). Nonetheless, there is no information in regards to the *B. tenagophila* Taim isolate concerning its infection by any trematode species and whether natural *E. paraensei* infection could compromise control efforts aimed at schistosomiasis.

The study reported here was designed to determine whether an early infection with *E. paraensei* could alter the innate resistance of the *B. tenagophila* Taim and *B. glabrata* Sumidouro (SU) isolates to *S. mansoni* infection. The pre-patent period, infectivity and mortality rates and the cercariae released for both trematode species were quantified.

MATERIAL AND METHODS

Parasites and snails - *E. paraensei* (SU), which was used in experimental assays, was originally isolated from the Sigmodontinae rodent *Nectomys squamipes* and has been maintained in the laboratory through passage in sympatric *B. glabrata* and golden hamster *Mesocricetus*

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auratus (Maldonado et al. 2001a). *S. mansoni* (São José dos Campos isolate) was obtained from the Malacological Laboratory of Institute Oswaldo Cruz, Brazil and has been maintained through passage in sympatric *B. tenagophila* and Swiss Webster mice.

The snail *B. tenagophila* Taim isolate was obtained from the Research Institute René Rachou-Fiocruz, Brazil. Briefly, the colony was created from specimens collected at the Reserva Biológica do Taim, state of Rio Grande do Sul, Brazil (Bezerra et al. 1997). *B. glabrata* (SU isolate) were obtained from a colony at the Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios-Fiocruz and were employed in this study with the aim of reproducing the interference model according to Lie and Heyneman (1976).

Experimental infection - A total of 167 laboratory raised experimental *B. tenagophila* snails (juvenile Taim isolates) with 6 mm diameter shells were divided into three groups of 49 specimens and one group of 20 snails. Group I consisted of snails singly exposed to 10 miracidia of *E. paraensei*, while group II snails were exposed to 10 miracidia of *S. mansoni* from the same group III hatching. Furthermore, group III snails were initially exposed to 10 miracidia of *E. paraensei* from the same group I miracidia hatching and, after an interval of two days, were exposed to 10 miracidia of group II S. *mansoni*. The uninfected control group was composed of 20 specimens. The experiment was performed in replicate with 25 and 24 snails in groups I, II and III and the replicates were joined to create a total of 49 specimens. The control group, which was unexposed to miracidia, constituted of 10 snails per experiment. The same hatching of miracidia was used to avoid variations due to heterogeneity in the viability of the larvae. All snails were exposed to the miracidia over night in 5 mL of dechlorinated tap water in multi-well plates.

The same experimental design was followed in order to test *B. glabrata*; however, a total of 122 snails were used. In these experiments, 34 specimens (20 and 14 snails from each replicate) were joined within groups I, II and III. One unexposed control group was formed using 10 specimens per replicate. Snails were fed lettuce leaf *ad libitum* and water was changed once a week. Weekly after exposure to miracidia, the snails were individually maintained in 3 mL water for a 1-h period under direct incandescent light (60 watts) to promote cercarial emergence. Infectivity was considered after snails released *S. mansoni* and *E. paraensei* cercariae. Snails releasing cercariae were placed in separate aquaria. The snails were examined for cercariae release for up to seven weeks. At this point, snails that did not release cercariae were considered resistant to infection. Those cercariae that had emerged from the snails were killed with alcohol-iodine, identified and counted under a light stereomicroscope. Infectivity and the mortality of snails were recorded weekly.

Statistical analysis - The number of cercariae released during the weeks of infection and the total number of infected snails were compared among groups by ANOVA. The normality of the data was assessed using the Kolmogorov-Smirnov test. Mortality rates were evaluated through the Kaplan-Meier method (Parmar & Machin 1995). Values less than $p \leq 0.05$ were taken to represent significant differences.

RESULTS

Infectivity and cercarial release - *B. tenagophila* susceptibility to *E. paraensei* infection was significantly lower in the co-infected group III when compared to the control group I ($p < 0.045$). Susceptibility of *B. glabrata* snails previously infected by *E. paraensei* was also reduced in the co-infected group III (Table).

B. glabrata showed an increase in susceptibility to *S. mansoni* infection from 20.5% in the control group II to 50% in the co-infected group III ($p < 0.048$) (Table).

Cercariae of *E. paraensei* were first eliminated from *B. tenagophila* three weeks post-infection and little variation in their numbers was observed onwards in both the group I control and the co-infected group III (Table). In contrast, *B. glabrata* delayed *E. paraensei* cercariae elimination, which was observed only at four weeks post-infection. Moreover, there was no significant variation in the number of cercariae released during the infection. Weekly comparisons between *E. paraensei* cercariae elimination from *B. tenagophila* and *B. glabrata* snails revealed no quantitative differences. In contrast, the *S. mansoni* cercariae shed by the co-infected group III was higher in relation to group II ($p < 0.01$). *S. mansoni* cercariae released from *B. glabrata* progressively increased from weeks 3rd-7th after exposure in the control group II followed by a decrease from week 6th onwards. A similar release of *S. mansoni* cercariae was observed in the group III co-infected snails.

Survival analysis - The percentage of *B. tenagophila* snail survival was 69.5% for snails infected with *E. paraensei* (group I), 65.3% for snails infected with *S. mansoni* (group II) and 57.2% for the co-infected group III snails. When compared with the uninfected group (96%) (Fig. 1), significant differences existed according to the Kaplan-Meier method. The results consisted of $X^2 = 2.88$, $p = 0.003$; $X^2 = 2.87$, $p = 0.003$ and $X^2 = 3.84$, $p = 0.0001$ for group I (*E. paraensei*), group II (*S. mansoni*) and group III (co-infected group), respectively. The *B. glabrata* uninfected group did not show any mortality throughout the assay. The mortality rates for *B. glabrata* infected groups I and II were similar to those of *B. tenagophila*. *B. glabrata* infected with *E. paraensei* (group I) resulted in 70.6% of snails surviving and significant mortality ($X^2 = 4.17$, $p = 0.00003$), while *S. mansoni* infected group II resulted in a 76.5% snail survival and significant mortality values ($X^2 = 3.67$, $p = 0.0002$). The co-infected group III showed increased mortality (44.2%) in relation to the uninfected group, in which no mortality occurred ($X^2 = 6.17$, $p = 0.000001$) (Fig. 2).

DISCUSSION

It has been established that interference by trematode larvae during the course of co-infection may develop due to the ability of echinostome parasitism to act on the snail host's innate defence system in a great variety of *B. glabrata-S. mansoni* models (Lie 1982).

TABLE

Infectivity and mean number of cercariae released from *Biomphalaria tenagophila* Taim isolate and *Biomphalaria glabrata* Sumidouro isolate exposure to 10 miracidia of *Echinostoma paraensei* (Ep) and two days later to 10 miracidia of *Schistosoma mansoni* (Sm)

Species of snails	Experimental groups		Total number/positive snails (%)[a]	Mean ± standard error cercariae released per weeks of infection				
				3	4	5	6	7
B. tenagophila	I	Ep	49 (20.4) (10/49)	12.5 ± 2.1 (2/41)	10 ± 9.8 (7/38)	11.7 ± 9.1 (10/37)	12.8 ± 6.3 (6/36)	25.0 ± 20.2 (8/34)
	II	Sm	49 (0)	-	-	-	-	-
	III	Ep	49 (14.2) (7/49)	7.6 ± 4.6 (3/41)	15.6 ± 11.6 (7/41)	40.1 ± 41.7 (7/36)	26.2 ± 14.9 (5/32)	24.0 ± 34.2 (6/28)
		Sm	49 (0)	-	-	-	-	-
B. glabrata	I	Ep	34 (17.6) (6/34)	-	32.7 ± 0.6 (4/30)	34.0 ± 49.2 (3/29)	11.6 ± 11.4 (5/26)	22.5 ± 17.8 (6/24)
	II	Sm	34 (20.5) (7/34)	-	2875 (1/30)	3040 (1/28)	298.0 ± 230.3 (7/27)	333.6 ± 65.2 (7/27)
	III	Ep	34 (8.8) (3/34)	-	25.0 ± 3.9 (2/26)	19.6 ± 25.7 (3/23)	30.6 ± 18.8 (3/17)	22.0 ± 9.9 (2/15)
		Sm	34 (50.0) (17/34)	-	709.8 ± 68.6 (8/26)	800.9 ± 803.7 (17/23)	2733.8 ± 3124.4 (12/17)	1156.5 ± 1134.0 (12/15)

a: number of snails releasing cercariae/total number of snail alive.

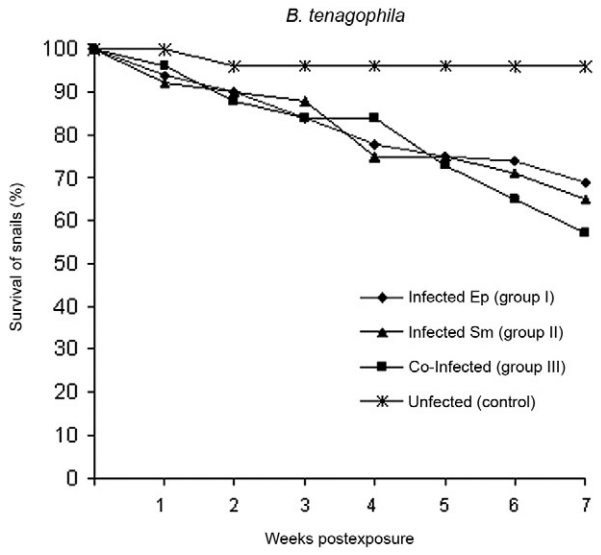

Fig. 1: survival rates of *Biomphalaria tenagophila* Taim lineage (n = 49) after exposure to 10 miracidia of *Echinostoma paraensei* (Ep) (♦), 10 miracidia of *Schistosoma mansoni* (Sm) (▲), 10 miracidia of *E. paraensei* followed by exposure to 10 miracidia of *S. mansoni* 48 h later (■) and uninfected group (*).

A *B. glabrata* SU isolate exposed to single infection develops a *S. mansoni* infection in approximately 20% of snails. Nevertheless, it was observed that there was an increase in the number of co-infected *B. glabrata* that released *S. mansoni* cercariae, which was significantly higher than *E. paraensei* snails releasing cercariae. This suggests that *E. paraensei* exposure may be able to alter the innate resistance of *B. glabrata* to *S. mansoni*. Results herein have confirmed previous studies using this model (Lie et al. 1977a, b).

No significant difference was noticed when comparing the number of *E. paraensei* cercariae released by *B. glabrata* in single and co-infected groups, indicating that *E. paraensei* infection was not affected by the presence of *S. mansoni*. Furthermore, it has been demonstrated that early exposure of *B. glabrata* to *E. paraensei* was able to alter the relationship of the snail during subsequent *S. mansoni* infection, although the mechanism behind this alteration is not entirely clear. These two trematodes provoke distinct humoral responses after their respective infections (Monroy & Loker 1993). Recently, it was verified that a similar expression of fibrinogen-related proteins, involved in non-self-recognition, could make *B. glabrata* susceptible, as well as resistant, to *S. mansoni* infection (Hertel et al. 2005).

There is no record of a natural trematode infection in the *B. tenagophila* Taim isolate, although sporocysts of *S. mansoni* could be seen in the inner tissue after experimental infection (Bezerra et al. 2003). Several attempts to establish *S. mansoni* infection in the *B. tenagophila* Taim isolate have failed, unlike in the *B. glabrata* SU isolate. Since *E. paraensei* was unable to alter the innate resistance of the *B. tenagophila* Taim isolate and permit *S. mansoni* cercariae release in co-infected individuals, it suggested that factors associated with *S. mansoni* resistance had not been affected. However, it has been reported that sporocysts and young daughter rediae of *E. paraensei* induced a rounded morphology in hemocytes from compatible snail *B. glabrata* and that this was associated with an inability to respond to *S. mansoni* larvae (Loker et al. 1986).

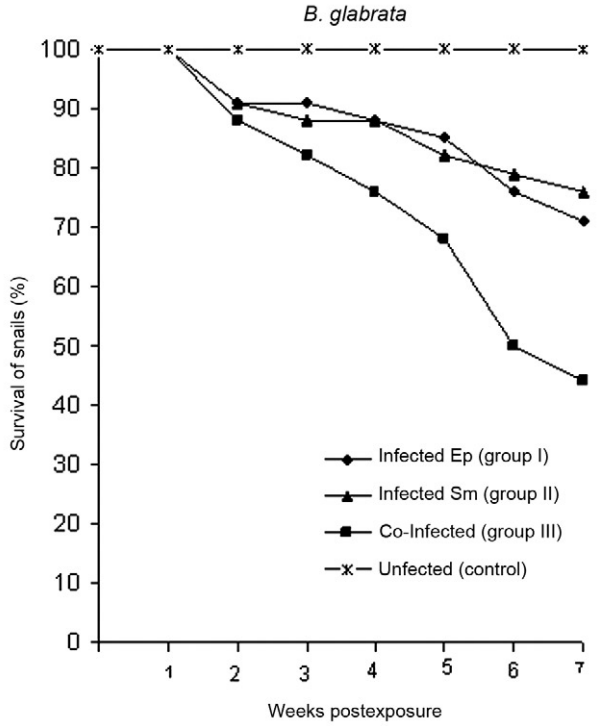

Fig. 2: survival rates of *Biomphalaria glabrata* Sumidouro lineage (n = 34) after exposure to 10 miracidia of *Echinostoma paraensei* (Ep) (♦), 10 miracidia of *Schistosoma mansoni* (Sm) (▲), 10 miracidia of *E. paraensei* followed by exposure to 10 miracidia of *S. mansoni* 48 h later (■) and uninfected group (*).

Recent studies have confirmed that successful transplantation of the amoebocyte-producing organ from the *B. tenagophila* Taim isolate to the susceptible *B. tenagophila* Cabo Frio isolate caused the susceptible organism to be partially refractive to *S. mansoni* infection (Barbosa et al. 2006), indicating that immunological processes play an important role in resistance to infection. In addition, Pereira (2005) and Bezerra and Coelho (2006) showed that inoculation of the hemolymph from *B. tenagophila* Taim to a susceptible isolate of *B. tenagophila* promotes a state of resistance against *S. mansoni* miracidia infection.

Mortality is associated with the infection of snails by different trematode species. As noticed previously, mortality rates are similar in *B. glabrata* and *B. tenagophila* when infected by *E. paraensei*. However, *Physa rivalis* and *Lymnaea columella* presented distinct survival rates (Maldonado et al. 2001b). Mortality among the *B. tenagophila* Taim isolates is high even without parasite pressure (Rosa et al. 2006), unlike *B. glabrata,* which only showed a high mortality rate under pressure in the co-infected system. More recently, Sandland et al. (2007) confirmed that concurrent infection by *Echinostoma caproni* and *S. mansoni* in *B. glabrata* resulted in high rates of mortality.

The inability of *E. paraensei* infection to promote *S. mansoni* infection suggests that different mechanisms of resistance may be involved in the response of snail hosts to trematode parasites.

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