Changes on *Schistosoma mansoni* (Digenea: Schistosomatidae) Worm Load in *Nectomys squamipes* (Rodentia: Sigmodontinae) Concurrently Infected with *Echinostoma paraensei* (Digenea: Echinostomatidae)

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The water rat, Nectomys squamipes, *closely involved in schistosomiasis transmission in Brazil, has been found naturally infected simultaneously by* Schistosoma mansoni *and* Echinostoma paraensei. *Laboratory experiments were conducted to verify parasitic interaction in concurrent infection. It was replicated four times with a total of 42 water rats and essayed two times with 90 mice pre-infected with* E. paraensei. *Rodents were divided into three groups in each replication. A wild strain recently isolated from Sumidouro, RJ, and a laboratory strain of* S. mansoni *from Belo Horizonte (BH) was used. Rats infected with* E. paraensei *were challenged 4 weeks later with* S. mansoni *and mice 2 or 6 weeks after the infection with* S. mansoni. *Necropsy took place 8 weeks following* S. mansoni *infection. The* N. squamipes *treatment groups challenged with* S. mansoni *RJ strain showed a significant decrease (80 and 65%) in the* S. mansoni *parasite load when compared with their respective control groups. There was a significant change or no change in the hosts challenged with the BH strain. The persistence time of* E. paraensei *within host was extended in relation to control groups, with a consequent enhancement of the number of recovered worm. An* E. paraensei *strain-specific influence on* S. mansoni *parasitism is reported. This paper presents some experimental data about this interaction in* N. squamipes *and* Mus musculus.

Key words: *Nectomys squamipes* - *Schistosoma mansoni* - *Echinostoma paraensei* - concurrent infection - heterologous interaction

The co-existence of helminthes in vertebrate hosts has been extensively investigated. Simultaneous infections of two or more helminthes species commonly occur in domestic animals and humans (Christensen et al. 1987). This raises the possibility that one species may influence the transmission patterns or disease features caused by another (Chamome et al. 1990, Chieffi 1992).

The helminth genus *Echinostoma* (Digenea: Echinostomatidae) has a large geographic distribution due to their ability to parasitize a variety of invertebrate and vertebrate hosts (Yamaguti 1971, Huffman & Fried 1990). Some species develop their larval stages in *Biomphalaria glabrata* snails, the most important intermediate host of *Schistosoma mansoni* in Brazil (Loker & Adema 1995).

Some experimental studies describe effects of interactions between *Echinostoma revolutum* (synonym *E. caproni*) and *S. mansoni* in homologous or heterologous infection of definitive hosts. Mice pre-infected by *E. revolutum* showed decrease in natural resistance, with an increase of the *S. mansoni* load in experimental conditions (Christensen et al. 1981, 1987).

The parasitism of *S. mansoni* in a naturally infected population of water rat *Nectomys squamipes* in an endemic area (Sumidouro, RJ) was studied by D'Andrea et al. (2000). This rodent is closely involved in schistosomiasis transmission since it is a definitive host of *S. mansoni* (Rey 1993). *N. squamipes* was also found naturally infected by *E. paraensei* in Sumidouro (Maldonado Jr. et al. 2001), after which this species has been maintained in laboratory conditions with a sympatric snail as the intermediate host (Maldonado Jr. et al. 2001).

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E. paraensei was described by Lie and Basch (1967) from naturally infected *B. glabrata* from Belo Horizonte, Brazil. However, little is known about the relationship with its natural vertebrate host. In addition, no literature is available about the experimental infection by *E. paraensei* on his natural rodent host and its concurrent helminth infection.

This paper presents new information of concurrent infections in *N. squamipes* and *Mus musculus* by *E. paraensei* and *S. mansoni*. Knowledge of this interaction could be useful to schistosomiasis control programs, since the echinostome is able to interfere in the *S. mansoni* ability to establish the parasitism.

MATERIALS AND METHODS

Experimental group - Forty-two *N. squamipes* reared in our laboratory animal house (D'Andrea et al. 1996) representing both sexes and of about three months of age were used. Additionally, we used 90 male albino mice (*M. musculus* - Swiss Webster), weighing about 25 g , obtained from the Central Animal House of Fiocruz. The experiment was repeated four times with *N. squamipes* and twice with the mice. Each replicate contained three treatment groups: two control groups (*S. mansoni* and *E. paraensei*) and one experimental group (*E. paraensei* + *S. mansoni* = concurrent treatment). For *N. squamipes*, three specimens were used per treatment in the 1st, 2nd and 4th replicates, and five specimens per treatment in the 3rd group. Fifteen mice were used for each treatment group.

Experimental infection - For *E. paraensei* infection, animals of experimental and control groups were orally infected by a gastric probe with an inoculums of 50 and 25 metacercariae per rat and mice, respectively. The *E. paraensei* used was isolated from naturally infected *N. squamipes* from Sumidouro, RJ, and passed through sympatric *B. glabrata*. The *S. mansoni* exposure was done four weeks after *E. paraensei* exposure. Each experimental and control animal was inoculated transcutaneously through the tail with about 500 (*N. squamipes*) and 100 (*M. musculus*) cercariae per host. In the 1st and 2nd replicates with *N. squamipes* we used a wild strain of *S. mansoni* from Sumidouro. However, the rats of 3rd and 4th replicates and the mice were infected with a laboratory strain of *S. mansoni* from Belo Horizonte, MG. This strain has been maintained in the laboratory since 1985. Mice were challenged with *S. mansoni* on the 2nd and 6th week after the first infection with *E. paraensei*. The water rats were lethally anesthetized eight weeks after the challenge with *S. mansoni*, a total of 12 weeks after the exposure to *E. paraensei*, while mice were necropsied 10 (1st trial) and 14 weeks (2nd trial). *S. mansoni* adult worms were recovered by perfusion of the hepatic portal system (Pellegrino & Siqueira 1956), followed by collection of worms from the mesenteric veins. As for *E. paraensei*, the worms were collected in pancreas and small intestine. The small intestine was divided in five equal parts to allow evaluating the parasite's distribution (Kaufman & Fried 1994).

Statistic analysis - The data were analyzed by Mann-Withney test; T test and variance analysis showed arithmetic mean. Values less than 0.05 ($P < 0.05$) were statically considered significant.

Experiments were performed according to the laws of the Ethical Commission of Animals Use of Fiocruz.

RESULTS

The *N. squamipes* pre-infected with *E. paraensei* and later challenged by the *S. mansoni* wild strain showed a significant reduction in the establishment of *S. mansoni* worm burdens of 65% in the 1st and 80% in the 2rd replicate ($P < 0.05$). An enlargement of *E. paraensei*'s persistence time within the host occurred, with a consequent greater worm number of 150% and 300% ($P < 0.05$) when compared to *N. squamipes* exposed to *E. paraensei* only.

The *N. squamipes* and mice inoculated with the laboratory strain of *S. mansoni*, showed no significant changes in the *S. mansoni* parasite load. On the contrary, in the 3rd replicate, *N. squamipes* showed an increase of 64% of *S. mansoni* and 9.3% of *E. paraensei*. No change in the 4th replicate, in relation to the *S. mansoni* control was observed (Fig. 1).

An enlargement of 28.5% of *S. mansoni* and 100% of *E. paraensei* parasite burden was observed in the 1st mice trial and a reduction of 92.3% of *E. paraensei* was observed in the 2nd trial (Fig. 2).

In *N. squamipes*, *S. mansoni* had a balanced sex ratio (1:1) for wild and laboratory strains. In mice, a male tendency was observed with a ratio of 2.1/1.0 and 1.2/1.0 in control and experimental treatments groups of the 1st trail, respectively, and 2.2/1.0 and 1.0/1.0 in each treatment of the 2nd trial.

The displacement of *E. paraensei* in the small intestine and pancreatic duct was also evaluated (Table). A tendency for parasites to cluster in the duodenum and jejunum in both hosts was noticed. However, worms were more aggregated in *N. squamipes*. All of them were found in the first part of the small intestine and pancreatic duct. In mice, worms were more dispersed throughout the small intestine and a low percentage was noticed in pancreatic duct (Table).

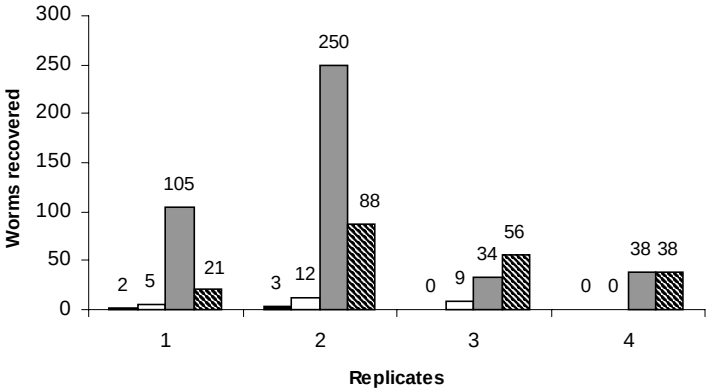

Fig. 1: mean number of adults of RJ strain (1st and 2nd replicates) and BH strain (3rd and 4th replicates) of *Schistosoma mansoni* and adults of *Echinostoma paraensei* (RJ strain) recovered from *Nectomys squamipes*. *E. paraensei* control treatment (black columns), *E. paraenei* concurrent treatment (white columns), *S. mansoni* control treatment (dot columns) and *S. mansoni* concurrent treatment (striped columns). Necropsies occurred 8 weeks after *S. mansoni* infection and/or 12 weeks after *E. paraensei* exposure.

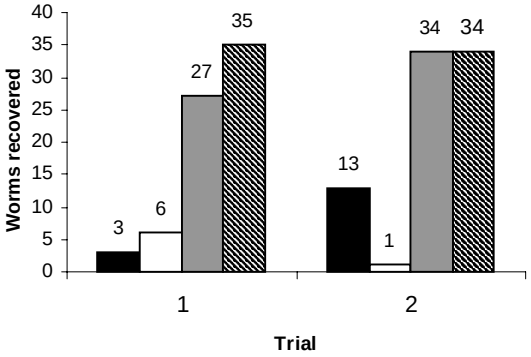

Fig. 2: mean number of adult *Schistosoma mansoni* (BH strain) worms and adults of *Echinostoma paraensei* (RJ strain) control treatment (black column), *E. paraensei* concurrently infected (white column), *S. mansoni* control group (dot column), *S. mansoni* concurrent infected (sriped column). Necropsies occurred 10 weeks (1st trial) or 6 weeks (2nd trial) aafter *S. mansoni* infection or 12 weeks after *E. paraensei* infection.

DISCUSSION

The rodent *N. squamipes* showed different susceptibility to *S. mansoni* strains when pre-infected with *E. paraensei*. The wild strain, that is naturally sympatric to *E. paraensei*, proved to be more susceptible to interference by *E. paraensei* in its life cycle establishment than the laboratory isolate. This can be affirmed from the significant reduction in the sympatric *S. mansoni* (RJ strain) adult worms load recovered from *N. squamipes* pre-infected by *E. paraensei*. On the other hand, parasitism intensity was not affected when a laboratory isolate of *S. mansoni* was the challenger.

The rodent *N. squamipes* has already been tested for resistance development to the BH laboratory strain of *S. mansoni*. This natural vertebrate host, pre-infected with *S. mansoni*, facilitated the establishment of the homologous infection with a

TABLE

Percentage of distribution of *Echinostoma paraensei* worms in the small intestine and pancreas of orally infected *Nectomys squmipes* and *Mus musculus* at the 12th week of infection. Hosts were infected with 50 and 25 metacercariae of *E. paraensei* and concurrently treated with 500 cercariae at 4th week for *N. squamipes* and 100 cercariae of BH strain of *Schistosoma mansoni* for *M. musculus*, respectively

Hosts	Treatment	Week of concurrent infection	Intestine segments					Pancreas
			I	II	III	IV	V	
N. squamipes	Control	-	40.0	0.0	0.0	0.0	0.0	60.0
	Concurrent	4th	35.7	21.4	0.0	0.0	0.0	42.9
M. musculus	Control	-	60.0	10.0	0.0	0.0	0.0	30.0
	Concurrent	4th	91.4	0.0	0.0	0.0	0.0	8.6
	Control	-	94.8	2.6	0.0	2.6	0.0	0.0
	Concurrent	2nd	70.4	21.0	2.5	5.0	1.1	0.0
	Control	-	65.9	20.5	1.0	0.0	0.0	2.8
	Concurrent	6th	88.5	3.5	1.0	0.0	0.0	7.0

consequent increase in worm load when re-infection occurred in the acute phase of the first infection, but it is not observed if the challenge happens in the chronic phase (Maldonado Jr. et al. 1994).

A different pattern of susceptibility to *S. mansoni* infection was noticed within mice trails and between host species, also infected by the laboratory strain. Increase and reduction of *S. mansoni* parasite load occurred in mice, depending on the time of challenge, 2nd or 6th week after the first infection. However, this oscillatory behavior does not present significant levels. Christensen et al. (1981) and Sirag et al. (1980) have also noticed antagonistic data on *S. mansoni* recovery, with no parasite load change in a primary, early and low-level *E. revolutum* (synonim: *E. caproni*) mice infection or with a significant increase in a primary, late and heavy infection.

Both strains of *S. mansoni* have no major differences in adult worms proteins in SDS-poliacrilamide gel electrophoresis profiles (Maldonado Jr. & DeSimone 1991). Therefore, a heterogeneous effect of *E. paraensei* infection to *S. mansoni* susceptibility could be expected as a consequence of the major polymorphism of the wild strain (Simpson 1995) or by diversity among adult schistosomes, including near as well as far species, confirmed through mitochondrial DNA sequence (Le 2000). The wild strain can be upset by environment pressure because of its genetic heterogeneity. In addition, this strain comes from an endemic area where the parasite circulates within human and wild rodents (D'Andrea et al. 2000) and it presents a heterogeneous phenotype in adult worms and cercariae when isolated from the distinct vertebrate hosts (Neves 1998). Experimentally infected sympatric *B. glabrata* presented chronobiological behavior with an early and a later cercarial emergence pattern (Machado-Silva 1981). The laboratory *S. mansoni* strain has been maintained in laboratorial conditions for a long time. Thus, it probably has reduced genetic heterogeneity as that observed in other laboratory maintained parasites (Voltz et al. 1987).

Data showed that *E. paraensei* is able to influence on *S. mansoni* parasitism behavior, although it is a strain-specific action. This fact can be explained by many hypotheses. When compared to echinostomatid, *S. mansoni* has a recent geographic establishment (Rey 1993) and is probably, still adaptting to parasitism in this rodent. Nevertheless, these trematodes are able to co-exist in the same host. These conditions would favour interference by *E. paraensei*, however, these trematodes set themselves in different niches within the host and their interaction mechanisms can be indirect, by induction of immunological factors as shown in *E. caproni* infection (Brunet et al. 1999).

Some studies showed a male bias in the *S. mansoni* sex ratio, in both experimental (Liberatos 1987) and natural infections (Barral et al. 1996). This can be associated to a potential male cercariae infection greater than that of female cercariae (Bossier et al. 1999). In agreement with these finding, our data shows a male bias in mice. This is a phenomenon independent of infection time, yet influenced by concurrent infection. The initial infection by *E. paraensei* seems to favor males more than female.

Usually, *S. mansoni* infections are permanent while *E. paraensei* infections lasts at least 12 weeks in *N. squamipes* and worms are gradually eliminated during this time. The increased persistence of the worm load of *E. paraensei* within hosts harboring *S. mansoni* suggests that the adult schistosome infections are probably down-regulating the immune response of the host (Fallon & Dunne 1999). Fujino et al. (1993) suggested a non-immunological mechanism of *E. caproni* and *E. trivolvis* expulsion that are dependent of mucin secretion increase by intestinal goblet cells. Although it was not possible to establish the exact modulator mechanism yet, data suggested that *S. mansoni* probably delay the mechanisms of *E. paraensei* expulsion, which can be mediated immunologically, either directly or indirectly (Christensen 1981), favouring the echinostomatid survival.

Echinostomatids have specific niches within the small intestine of their definitive hosts. They are typically dispersed in the initial phases of infection (Nollen 1996). *E. paraensei* commonly occurs in the duodenum-jejunal. From our data, the distribution does not change when the host is challenged by *S. mansoni*. However, host species can influence on *E. paraensei* distribution, as shown by the predilection of *E. paraensei* to occupy the pancreatic duct of *N. squamipes* when compared to *M. musculus*.

There are evidences that both adult worms and larvae stages of *E. paraensei* (in *B. glabrata*) can potentially interfere on the survival of *S. mansoni*. Some studies have proposed the use of larvae of *E. paraensei* is schistosomiasis biological control, since *Echinostoma* spp. rediae attack and destroy *S. mansoni* sporocists by cannibalism or induction of host's inflammatory response, when they are in the same molluscan (Lie 1973, Jourdane et al. 1990, Adema et al. 1999).

We conclude that the presence of *E. paraensei* interferes with the development of a wild strain of *S. mansoni* (RJ strain), changing its relationship

with the host. However, the bases of the interaction mechanism are still being evaluated to be considered in schistosomiasis control programs.

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