Susceptibility of *Biomphalaria glabrata* submitted to concomitant infection with *Angiostrongylus costaricensis* and *Schistosoma mansoni*

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Abstract

The easy adaptation of *Angiostrongylus costaricensis*, nematode responsible for abdominal angiostrongyliasis to several species of terrestrial and freshwater molluscs and the differences observed in the interactions of trematodes with their intermediate hosts have induced us to study the concomitant infection of *Biomphalaria glabrata* with *Schistosoma mansoni* and *A. costaricensis*. Prior exposure of *B. glabrata* to *A. costaricensis* (with an interval of 48 hours), favored the development of *S. mansoni*, observing higher infection rate, increased release of cercariae and increased survival of molluscs, when compared to molluscs exposed only to *S. mansoni*. Prior exposure of *B. glabrata* to *A. costaricensis* and then to *S. mansoni* also enabled the development of *A. costaricensis* since in the ninth week of infection, higher amount of *A. costaricensis* L3 larvae was recovered (12 larvae / mollusc) while for molluscs exposed only to *A. costaricensis*, the number of larvae recovered was lower (8 larvae / mollusc). However, pre-exposure of *B. glabrata* to *S. mansoni* (with an interval of 24 hours), and subsequently exposure to *A. costaricensis* proved to be very harmful to *B. glabrata*, causing extensive mortality of molluscs, reduced pre-patent period to release cercariae and greater recovery of L3 *A. costaricensis* larvae.

Keywords: Biomphalaria, Nematoda, Trematoda, concomitant infection.

Suscetibilidade de *Biomphalaria glabrata* submetida à infecção concomitante com *Angiostrongylus costaricensis* e *Schistosoma mansoni*

Resumo

A facilidade de adaptação do *Angiostrongylus costaricensis*, nematódeo responsável pela angiostrongiliase abdominal, a diversas espécies de moluscos terrestres e dulciaquícolas e as divergências observadas nas interações dos trematódeos com seus hospedeiros intermediários nos induziu a estudar a infecção concomitante de *Biomphalaria glabrata* com *Schistosoma mansoni* e *A. costaricensis*. A exposição prévia de *B. glabrata* ao *A. costaricensis* (com intervalo de 48 horas), favoreceu o desenvolvimento do *S. mansoni* observando-se elevação da taxa de infecção, maior liberação de cercárias e maior sobrevivência dos moluscos, quando comparado com os moluscos expostos somente ao *S. mansoni*. A exposição de *B. glabrata* previamente ao *A. costaricensis* e posteriormente ao *S. mansoni* também facilitou o desenvolvimento do *A. costaricensis* uma vez que na nona semana de infecção foi recuperada maior quantidade de larvas L3 de *A. costaricensis*, enquanto nos moluscos expostos somente ao *A. costaricensis,* o número de larvas recuperadas foi menor . Entretanto a pré-exposição de *B. glabrata* ao *S. mansoni* (com intervalo de 24 horas), e posteriormente a exposição ao *A. costaricensis* mostrou-se muito prejudicial à *B. glabrata* provocando grande mortalidade dos moluscos, redução do período pré-patente para liberação de cercárias e maior recuperação de larvas L3 de *A. costaricensis.*

Palavras-chave: Biomphalaria, Nematoda, Trematoda, infecção concomitante.

1. Introduction

The intermediate hosts of *Angiostrongylus costaricensis* are usually slugs of the family Veronicellidae, particularly of genus *Sarasinula* (Thiengo, 1996) and *Phyllocaulis* (Graeff-Teixeira et al., 1989). However, other types of terrestrial molluscs such as *Limax maximus, Limax flavus*, and *Bradybaena similaris* were also found naturally infected (Graeff-Teixeira et al., 1993). Freshwater snails *Biomphalaria tenagophila*, *Biomphalaria straminea* and *Biomphalaria glabrata* are susceptible to *A. costaricensis* in experimental conditions (Lima et al., 1992).

The susceptibility of snails to *Schistosoma mansoni* is genetically controlled as shown by Newton (1953, 1954) and later by Richards and Merritt Junior (1972), Richards (1973) and Santana et al. (1978). Banevicius et al. (2006) reported that genetic screening for susceptibility to *S. mansoni* infection obtained in *B.tenagophila* did not favor the development of *A. costaricensis* larvae, since larger number of nematode larvae was recovered in planorbid not selected genetically. Other factors may influence the susceptibility of molluscs such as trematode strain (Paraense and Correa, 1963a, b), host age (Newton, 1953), planorbid species (Lima et al., 1992) and concomitant infection with other parasites (Machado et al., 1988; Balan et al., 1993). Joe et al. (1977) showed that in concomitant infections, *Biomphalaria glabrata* pre-exposed to *Echinostoma paraensei* became more susceptible to *S. mansoni*. Biochemical and histopathological studies have shown changes in *B.glabrata* in concomitant infection with *Angiostrongylus cantonensis* and *E. paraensei*: the presence of trematode damaged the transmission dynamics of *A. cantonensis* (Bonfim et al., 2014).

The mechanisms involved in mixed infections can be of direct antagonism (Joe et al., 1968; Joe, 1973) by predation by the rediae of larvae of other trematodes (sporocysts, rediae and cercariae) or indirect antagonism through competition for food or space, by immunological mechanisms and toxic substances (Joe et al., 1965; Joe, 1966, 1973; Basch et al., 1969; Lim and Heyneman, 1972). Observations performed in *Biomphalaria tenagophila* naturally infected by other digenean parasite and not by *S. mansoni*, and experimentally superinfected with *S. mansoni* showed resistance to the development of *S. mansoni* sporocysts, with lack of amebocitary reaction around degenerated sporocysts (Machado et al., 1988; Balan et al., 1993). In concomitant infections by *A. cantonensis* and *S. mansoni* in *B. glabrata*, Yousif and Lämmler (1977) found no inhibitory effect of nematode on the development of *S. mansoni*. Pereira et al. (2006) found that prior infection of *B. tenagophila* by *Angiostrongylus vasorum* increased the susceptibility of molluscs to *S. mansoni*.

The antagonism between larvae of different trematode species observed in molluscs can be used in tackling parasites (Joe, 1973). Lim and Heyneman (1972) suggested that these competitive phenomena were used to combat *S. mansoni* in the field. For that, larvae of rival digenean parasites would be used, which would predate or destroy *S. mansoni* sporocysts or cercariae.

This study assessed the development of *S. mansoni* and *A. costaricensis* in concomitant infections in *B. glabrata*.

2. Material and Methods

Melanin *B. glabrata* specimens originated from populations of Belo Horizonte were used (MG, Brazil) and sympatric *S. mansoni* strain was kept in Swiss mice. For infection of *B. glabrata*, 10 BH miracidia obtained from feces of previously infected Swiss mice were used. Snails were exposed to miracidia for a period of 2 hours (Standen, 1951) at temperature of 28 °C and 60-watt incandescent light.

The *A. costaricensis* strain used was Crissiumal (RS, Brazil) maintained in *B. glabrata* and *Sigmodon hispidus* (cotton rat). L1 larvae of *A. costaricensis* were obtained from feces of *S. hispidus* using the method of Rugai et al. (1954). For infection with *B. glabrata*, 120 *A. costaricensis* L1 larvae were used.

Four experimental groups were formed: Group I - 30 *B. glabrata* specimens exposed to *S. mansoni* (Control); Group II - 30 *B. glabrata* specimens exposed to *A. costaricensis* (Control) ; Group III - 30 *B. glabrata* specimens pre-exposed to *A. costaricensis* and then to *S. mansoni*; Group IV - 30 *B. glabrata* specimens pre-exposed to *S. mansoni* and then to *A. costaricensis*.

Snails of groups I and IV were individually exposed to 10 miracidia of *S. mansoni*. Twenty-four hours after exposure to miracidia, snails of group IV were individually exposed to 120 *A. costaricensis* L1 larvae for 12 hours. After this period, surplus L1 larvae were counted.

Snails of groups II and III were individually exposed to 120 *A. costaricensis* L1 larvae for 12 hours. After this period, surplus L1 larvae were counted. Forty-eight hours after exposure to *A. costaricensis*, molluscs of Group III were individually exposed to 10 miracidia of *S. mansoni*.

Thirty days after exposure to *S. mansoni*, snails were observed twice a week to verify the elimination of cercariae and *A. costaricensis* L3 larvae by application of 60-watt incandescent light and temperature of 28 °C. Sixty days after exposure to miracidia, survived snails were sacrificed to verify the presence of secondary sporocysts and for the recovery of L3 larvae by digestion with pepsin and hydrochloric acid (Wallace and Rosen, 1969).

In all groups, snails were individually kept in 250 mL flasks with dechlorinated water. The cleaning of flasks was performed once a week to prevent algae growth and accumulation of debris, adding calcium carbonate every time the water was replaced. The daily feeding of snails was made *ad libitum* with fresh lettuce.

In all groups exposed to *S. mansoni*, cercariae were counted according to technique developed by Paraense and Correa (1989).

The production of cercariae was submitted to an analysis of variance model. The data, their statistical distribution and adequacy of the model were verified by the analysis of the residuals of the fitting. The comparisons among group were based on the least squares means. Tukey-Kramer multiple comparison method was used to correct the p-values for the multiple comparisons effect. Infection is a binary response. The infection rates were compared by Pearson's contingency tables based chi-square statistic. Confidence intervals for the infection rate of each group were produced by exact method. The comparison of positivity rates for *S. mansoni* (Groups IVxI and IIIxI) was performed through odds ratios (OR). If the odds ratio, or (IV, I)> 1, then IV presents higher positivity compared to I. If or (IV, I) <1, IV presents lower positivity compared to I. The odds ratio of IV to I (the same as III to I) is determined by the quotient:

or (IV,I)={p+(IV)/p-(IV)}/{p+(I)/p-(I)}, where p + (IV) is the likelihood of positivity for group IV; + p (I) for group I and similarly, 1-p+(IV)=p-(IV) is the likelihood of negativity for group IV; p-(I) for group I. The same analysis was performed to compare groups III and I. The results of comparisons are shown on 95% confidence intervals. If "1" is out of range, there will be difference in the positivity of both groups. The comparison of the recovery rate of *A. costaricenis* larvae (Groups II, III and IV) was also performed by odds ratio (OR)

The time to death of the mollusks was groupwise compared by Kaplan-Meier product limit estimates.

All computations were performed by SAS 9.4 software (SAS Institute, 2006), on a Linux OS (Fedora 20).

3. Results

Data regarding the susceptibility of *B. glabrata* to *S. mansoni* and *A. costaricensis* are shown in Table 1.

Group III (snails pre-exposed to *A. costaricensis* and then to *S. mansoni*) showed the highest number of molluscs positive to *S. mansoni*, with 96.7%, followed by group I (molluscs exposed only to *S. mansoni*) with 73.3% and Group IV (molluscs pre-exposed to *S. mansoni* and then to *A. costaricensis*) with 63.3% (Table 1). According to Table 2, the infection rate in group III (pre-infected with *A. costaricensis* and subsequently by *S. mansoni*) was higher and significantly different from that shown by group I (infected only with *S. mansoni*). For groups IV and I, no positivity rate difference was observed.

Figure 1 shows the results for the number of cercariae eliminated by snails of groups I, III and IV. Molluscs of group IV (pre-exposed to *S. mansoni* and then to *A. costaricensis*) began eliminating cercariae in the fifth week after exposure to *S. mansoni* miracidia, while the other groups began eliminating cercariae after six weeks. The patent period remained until the ninth week, after which snails were sacrificed. Statistical significance was determined by the Pearson's chi-square test to compare proportions of the first occurrence per group, which resulted 66.2 (8 gl) with significance level p< 0.0001.

The total amount of recovered cercariae was compared among groups. This basic ANOVA showed that there was evidence of differences between means (p = 0.008). Additional tests were performed, showing comparisons of means two by two. Significance levels were corrected for the effect of multiple comparisons by the Tukey test, verifying that there was evidence of difference only between groups III and IV. Group IV produced less cercariae.

As for the number of molluscs positive to *A. costaricensis* (Table 1) where L3 larvae were recovered after the ninth week of infection, snails of group II (exposed only to *A. costaricensis*) were the most susceptible, with 83.3% of infected snails, followed by snails of group III (pre-exposed to *S. mansoni* and then to *A. costaricensis*), with 80.0% of infected snails. Molluscs of group IV (pre-exposed to *S. mansoni* and then to *A. costaricensis*) were the least susceptible, with only 16.7% positive for *A. costaricensis* (Table 1). The total L3 larvae recovered in the 9th week of infection was compared among groups II, III and IV. The Pearson's chi-square statistic test resulted in p <0.0001, which is highly significant. Logistic regression confirmed this result and added comparisons among groups. Table 3 shows the odds ratios for each group compared to group IV. Note that there was no evidence of difference between groups III and IV. There was evidence of difference in the recovery rates between groups II and IV: group II had lower recovery rate of L3 larvae. Only snails of group IV, during exposure to incandescent light, released L3 larvae on the 5th week after infection. When comparing the number of larvae recovered with the number of exposed larvae, it was found that the difference between molluscs of groups III and II was significant. The difference between molluscs of groups IV and II was not clear (Table 4). When the number of recovered larvae was compared with the number of infective larvae, it was found that snails of groups III and IV differ from those of group II (Table 5).

Figure 2 shows the results for the survival rate of snails of groups I, II, III and IV over the nine weeks of experimental period. The test of the hypothesis of equality of survival curves performed by the log-rank statistic was significant (p <0.0001), showing evidence of difference among survival curves; then, it was decided to test the

Table 1. Susceptibility of *Biomphalaria glabrata* exposed to *Schistosoma mansoni* and *Angiostrongylus costaricensis*.

Groups	Number of snails	Number of snails positive to *Schistosoma. mansoni*	Number of snails positive to *Angiostrongylus costaricensis**	Average number of *Angiostrongylus costaricensis* L1 larvae	Average number of recovered *Angiostrongylus costaricensis* L3 larvae
Group I	30	22 (73.3%)	-	-	-
Group II	30	-	25 (83.3%)	97	8
Group III	30	29 (96.7%)	24 (80.0%)	118	12
Group IV	30	19 (63.3%)	5 (16.7%)	98	10

() - Infection rate. * Number of molluscs in which *Angiostrongylus costaricensis* L3 larvae were recovered in the 9th week of infection. Group I: Snails exposed to *Schistosoma mansoni*. Group II: Snails exposed to *Angiostrongylus costaricensis*. Group III: Snails pre-exposed to *Angiostrongylus costaricensis* and then to *Schistosoma mansoni*. Group IV: Snails pre-exposed to *Schistosoma mansoni* and then to *Angiostrongylus costaricensis*.

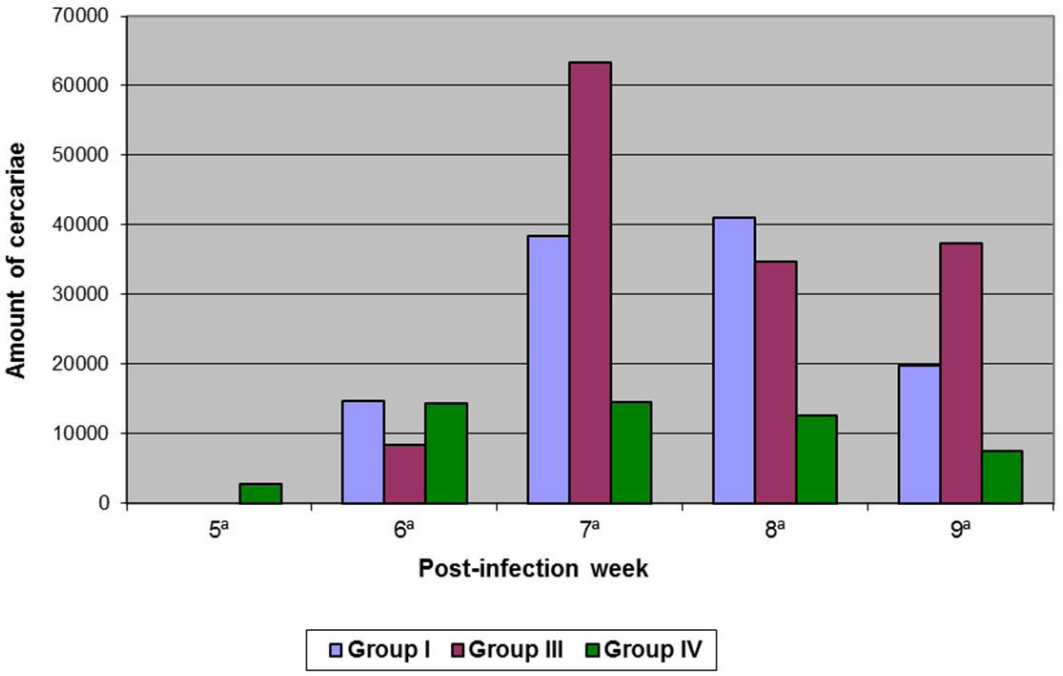

Figure 1. Amount of *Schistosoma mansoni* cercariae eliminated by *Biomphalaria glabrata* exposed or not to *Angiostrongylus costaricensis*. Group I: Snails exposed to *Schistosoma mansoni*. Group III: Snails pre-exposed to *Angiostrongylus costaricensis* and then to *Schistosoma mansoni*. Group IV: Snails pre-exposed to *Schistosoma mansoni* and subsequently to *Angiostrongylus costaricensis*.

Table 2. Comparison of infection rates shown by *Biomphalaria glabrata* exposed to *Schistosoma mansoni* through the odds ratios (OR) with 95% confidence interval. *

Effect	Point estimate	Confidence interval (95%)
Group IV X I	0.628	0.209 1.884
Group III X I	10.545	1.227 90.662

* If or (IVxI) or (IIIxI) >1 then IV or III have higher positivity compared to I; or if (IVxI) or (IIIxI) <1, IV or III have lower positivity compared to I. If "1" is outside the confidence interval, there will be difference in the positivity of both groups (Group IVxI and Group IIIxI). Therefore, group III significantly differed from group I and group IV did not from group I. Group I: Snails exposed to *Schistosoma mansoni*; Group III: Snails pre-exposed to *Angiostrongylus costaricensis* and then to *Schistosoma mansoni*; Group IV: Snails pre-exposed to *Schistosoma mansoni* and subsequently to *Angiostrongylus costaricensis*.

Table 3. Recovery rate of *Angiostrongylus costaricensis* L3 larvae recorded in *Biomphalaria glabrata* exposed or not to *Schistosoma mansoni* miracidia through odds ratios (OR) with a 95% confidence interval. *

Effect	Point estimate	Confidence interval (95%)
Group II x IV	0.679	0.491 0.938
Group III x IV	0.901	0.657 1.235

* If (IIxIV) or (IIIxIV) >1 then II or III have higher recovery rate compared to IV; or if (IIxIV) or (IIIxIV) <1, II or III have lower recovery rate compared to I. If "1" is outside the confidence interval, there will be difference in the recovery rate of both groups (Group IIxIV and Group IIIxIV). Therefore, there was evidence of recovery difference of L3 larvae only between groups III and IV. Group II: Snails exposed to *Angiostrongylus costaricensis*; Group III: Snails pre-exposed to *Angiostrongylus costaricensis* and then to *Schistosoma mansoni*; Group IV: Snails pre-exposed to *Schistosoma mansoni* and subsequently to *Angiostrongylus costaricensis*.

equality among groups I, II and III, concluding that there was only weak evidence of difference among the three curves (p = 0.06). Group IV (snails pre-exposed to *S. mansoni* and then to *A. costaricensis*) had lower survival curves and groups I, II and III had similar survival curves.

4. Discussion

Previous exposure to *B. glabrata* and *A. costaricensis* increased the susceptibility of snails to infection by *S. mansoni*. However, the same did not occur when snails were pre-exposed to *S. mansoni* and then to *A. costaricensis*. Previously, Guerino et al. (2009) had found that *B. glabrata* infected with *A. costaricensis* attracted with less intensity *S. mansoni* miracidia. The increased susceptibility of *B. glabrata* to *S. mansoni* was also reported in snails pre-infected with *E. paraensei* (Joe et al., 1977; Joe, 1982) and *B. tenagophila* pre-infected with *A. vasorum* (Pereira et al., 2006). Several authors, in contrast, have

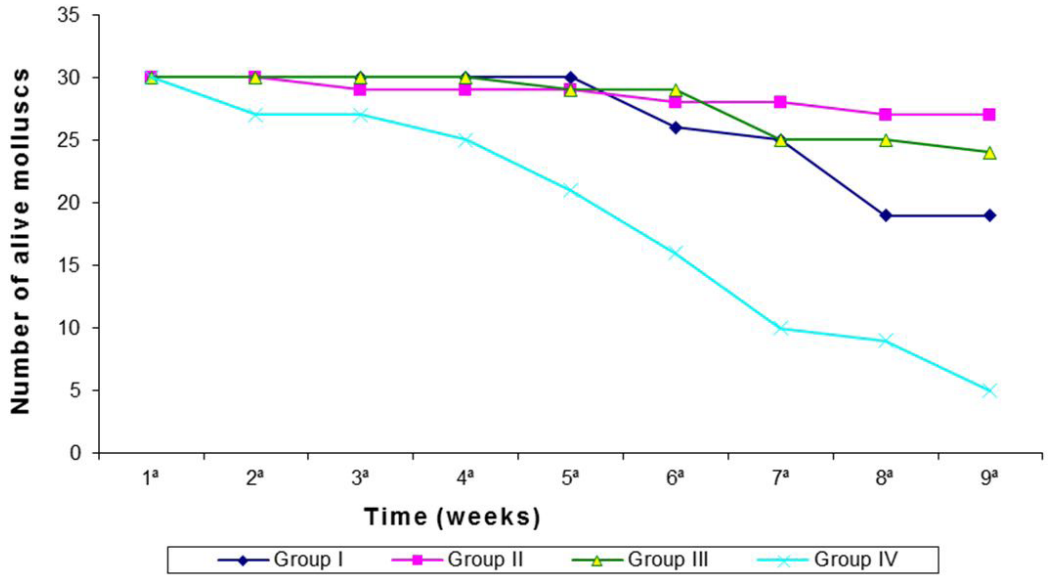

Figure 2. Survival rate of *Biomphalaria glabrata* exposed to *Schistosoma mansoni* and to *Angiostrongylus costaricensis*. Group I: Snails infected with *Schistosoma mansoni*. Group II: Snails infected with *Angiostrongylus costaricensis*. Group III: Snails pre-infected with *Angiostrongylus costaricensis* and then with *Schistosoma mansoni*. Group IV: Snails pre-infected with *Schistosoma mansoni* and later with *Angiostrongylus costaricensis*.

Table 4. Comparison between number of recovered larvae (L3) and number of larvae (L1) of *Angiostrongylus costaricensis* in *Biomphalaria glabrata* exposed or not to *Schistosoma mansoni* miracidia through the odds ratios (OR) considering 95% confidence interval. *

Effect	Point estimate	Confidence interval (95%)
Group IV X II	1.354	0.984 1.864
Group III X II	1.601	1.329 1.929

* If or (IVxII) or (IIIxII) >1 then IV or III have higher recovery rate compared to II; or if (IVxII) or (IIIxII) <1, IV or III have lower recovery rate compared to II. If "1" is outside the confidence interval, there will be difference in the recovery rate of both groups (Group IVxII and Group IIIxII). Therefore, there was evidence of recovery difference of L3 larvae in relation to the number of larvae L1 only between group III and group II. Group II: Snails exposed to *Angiostrongylus costaricensis*. Group III: Snails pre-exposed to *Angiostrongylus costaricensis* and then to *Schistosoma mansoni*. Group IV: Snails pre-exposed to Schistosoma mansoni and subsequently to *Angiostrongylus costaricensis*.

Table 5. Comparison between number of recovered larvae (L3) and number of infective larvae (L1) of *Angiostrongylus costaricensis* in *Biomphalaria glabrata* exposed or not to *Schistosoma mansoni* miracidia through the odds ratios (OR) considering 95% confidence interval. *

Effect	Point estimate	Confidence interval (95%)
Group IV X II	1.474	1.066 2.037
Group III X II	1.327	1.101 1.601

* If or (IVxII) or (IIIxII) >1 then IV or III have higher recovery rate compared to II; or if (IVxII) or (IIIxII) <1, IV or III have lower recovery rate compared to II. If "1" is outside the confidence interval, there will be recovery difference in both groups (Group IVxII and Group IIIxII). Therefore, groups III and IV differed from group II with respect to the number of L3 larvae recovered considering the number of infective L1 larvae. Group II: Snails exposed to *Angiostrongylus costaricensis*. Group III: Snails pre-exposed to *Angiostrongylus costaricensis* and then to *Schistosoma mansoni*. Group IV: Snails pre-exposed to *Schistosoma mansoni* and subsequently to *Angiostrongylus costaricensis*.

verified resistance in molluscs sensitized by previous infections (Joe et al., 1980; Joe, 1982; Sullivan et al., 1982; Machado et al., 1988; Balan et al., 1993; Bonfim et al., 2014). In contrast, Yousif and Lämmler (1977) reported that pre-infection with *B. glabrata* with *A. cantonensis* did not inhibit or slow subsequent infection with *S. mansoni*.

The increased susceptibility of snails pre-exposed to *A. costaricensis* (group III) reflected in the high infection rate by *S. mansoni* (96.7%), considering that in group I, molluscs were exposed only to the trematode, and the infection rate was 73%. Previous infection of *B. glabrata* with *A. costaricensis* and then with *S. mansoni* facilitated the nematode development, since in the ninth week of infection, higher amounts of *A. costaricensis* L3 larvae were recovered (12 larvae / mollusc), while for molluscs exposed only to *A. costaricensis* (group II), the number of larvae recovered was lower (8 larvae / mollusc). Opposite results were observed by Bonfim et al. (2014),

in *B. glabrata* pre-infected with *A.cantonensis* and then with *E. paraensei*: there was a reduction in the L3 larvae recovery rate when compared with molluscs pre-infected with *E. paraensei* and then with *A. cantonensis*.

Survival curves were similar between mollusc from groups I, II and III. Opposite results were obtained by Pereira et al. (2006), who found that *B. tenagophila* pre-infected with *A. vasorum* and then with *S. mansoni* had higher mortality than those infected only with *S. mansoni*, which was also observed by Yousif and Lämmler (1977) in *B. glabrata* previously infected with *A. cantonensis* and then with *S. mansoni*. Our data are similar to those obtained by Basch et al. (1969) on antagonism between *S. mansoni* and trematode *Cotylurus lutzi* in *B. glabrata*, where it was found that when molluscs are pre-infected with *C. lutzi* and subsequently with *S. mansoni*, this infection occurs as easily as in those infected only with *S. mansoni*. Pre-exposure to *S. mansoni* and then to *A. costaricensis* was very harmful to *B. glabrata*, causing high mortality of molluscs. In surviving molluscs, lower production of cercariae was observed, but higher number of *A. costaricensis* larvae was recovered. The higher mortality rate of molluscs from group IV and its early occurrence may have affected the infection rate with *S. mansoni*, given that death may have occurred before parasitism was detected, considering that cercariae elimination was checked once a week. The beginning of the patent period occurred earlier and was followed by a sharp increase in the host mortality rate. These data are consistent with Pan (1965), who observed widespread reaction in the host tissues with the transit of mature cercariae. For Barbosa (1959), the predatory and mechanical action of *S. mansoni* larvae in snail tissues determines the decreased survival rate of hosts.

The number of L3 larvae were recovered per molluscs was higher in those that received double infection (group III and group IV), indicating that the reproduction of *S. mansoni* that occurred inside molluscs did not affect the development of *A. costaricensis* larvae. The increased susceptibility of *B.glabrata* to *S. mansoni* observed in group pre-exposed to *A. costaricensis* group (group III) may be due to a deficiency in the action of host defense mechanisms, since when miracidia newly penetrated the snail tissues, they recruit hemocytes to the site of infection, and a low number of these cells would migrate to the site, as encapsulation of *A. costaricensis* larvae newly penetrated the snail would be occurring (48 hours before *S. mansoni*). Loker et al. (1986) suggested that *E. paraensei* larvae release products that interfere with the ability of *B. glabrata* hemocytes to kill *S. mansoni* sporocysts.

Infection of *B. glabrata* by A. *costaricensis* is accompanied by significant changes in the number of hemocytes, in the calcium and glucose concentrations, alkaline phosphatase, creatine phosphokinase and lactate dehydrogenase in the hemolymph and tissues (Stewart et al., 1985), metabolic changes that were also observed in infections of *B. glabrata* with A. cantonensis (Tunholi-Alves et al., 2012, 2014; Bonfim et al., 2014.). These metabolic disorders, particularly those related to glucose levels in tissues, the main energy source, can damage the host and also the development of the parasites when different species co-inhabit the same animal. This may have occurred in mollusks from group, where despite the lower infection rate with *S. mansoni*, lower recovery of *A. costaricensis* larvae and fewer cercariae produced, increased mortality of molluscs was found, when compared with molluscs from group III. Subsequent infection by *A. costaricensis* in *B. glabrata* previously exposed to *S. mansoni* may have induced higher migration of larval stages of the parasites in search of infection-free tissue with increased availability of nutrients.

Most studies indicate that hemocytes are the main elements involved in the destruction of microorganisms and parasites that penetrate their tissues, including trematode larvae (Van Der Knaap and Loker, 1990). About *A. cantonensis* larvae, Harris and Cheng (1975) observed encapsulation between 24 and 48 hours after *B. glabrata* infection and attributed the development of larvae to this process. In *B. glabrata*, *A. cantonensis* larvae can be found in a period greater than twelve months (Richards and Merritt, 1967). Mendonça et al. (1999) found in *Sarasinula marginata* infected with *A. costaricensis*, perilarval reaction 2 hours after infection in the fibromuscular tissue and 6 hours in larvae located in the gut. As intramolluscan *A. costaricensis* larvae are always surrounded by amebocitary reaction (Mendonça et al., 1999; Bruno, 2005), *S. mansoni* larvae probably developed under lower action of defense mechanisms, thus explaining the higher positivity shown by group III (previously exposed to *A. costaricensis*) and the high mortality rate of molluscs in the pre-patent period shown by group IV (subsequently exposed to *A. costaricensis*).

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