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BEHAVIOR OF *Angiostrongylus costaricensis* IN PLANORBIDS

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ABSTRACT

Some terrestrial mollusks are natural hosts of *Angiostrongylus costaricensis*. In the laboratory, this nematode can be maintained in certain planorbids, which are aquatic mollusks and intermediate hosts of *Schistosoma mansoni*. Mollusks can be infected with *Angiostrongylus costaricensis* by ingestion of or active penetration by the first-stage larvae. In this work we assessed the ability of *Biomphalaria glabrata* to attract first-stage larvae of *A. costaricensis*. Movement of the nematode larvae towards the mollusks was observed after 15 min, 30 min and 1 h. *B. glabrata* did not attract the first-stage larvae of *A. costaricensis* in any of the three intervals. The susceptibility of two populations of *Biomphalaria tenagophila* to infection by *A. costaricensis* was also determined. One population was genetically selected for the susceptibility to *S. mansoni* while the other was not. Third-stage larvae were recovered from the snails 30 days after exposure of the two populations to 120 first-stage larvae. All the mollusks were infected. However, a significantly higher number of third-stage larvae were recovered in mollusks not genetically selected.

Keywords: *Angiostrongylus costaricensis, Biomphalaria glabrata, Biomphalaria tenagophila.*

RESUMO

Comportamento do *Angiostrongylus costaricensis* em planorbídeos

Alguns moluscos terrestres são hospedeiros naturais do *Angiostrongylus costaricensis*. No laboratório, esse nematódeo pode ser mantido em *planorbídeos*, que são moluscos aquáticos e hospedeiros intermediários do *Schistosoma mansoni*. Os moluscos podem ser infectados com *A. costaricensis* por ingestão ou por penetração ativa de larvas de primeiro estágio. Neste trabalho, testamos a habilidade de *Biomphalaria glabrata* em atrair larvas de primeiro estágio de *A. costaricensis*. A movimentação das larvas do nematódeo em direção aos moluscos foi observada após 15 minutos, 30 minutos e 1 hora. *B. glabrata* não atraiu as larvas de primeiro estágio de *A. costaricensis* nos três intervalos de tempo. Verificamos também a suscetibilidade de duas populações de *Biomphalaria tenagophila* à infecção por *A. costaricensis*. Uma população era selecionada geneticamente para a susceptibilidade ao *S. mansoni*, enquanto a outra não o era. Larvas de terceiro estágio foram recuperadas dos moluscos 30 dias após a exposição das duas populações a 120 larvas de primeiro estágio. Todos os moluscos estavam infectados. Entretanto, um número significativamente maior de larvas de terceiro estágio foi recuperado em moluscos não geneticamente selecionados.

Palavras-chave: *Angiostrongylus costaricensis*, *Biomphalaria glabrata*, *Biomphalaria tenagophila.*

INTRODUCTION

The intermediate hosts of *Angiostrongylus costaricensis* are usually terrestrial pulmonate mollusks of the family Veronicellidae (slugs), particularly the genera *Sarasinula* (Thiengo, 1996) and *Phyllocaulis* (Graeff-Teixeira *et al*., 1989). However, other terrestrial mollusks such as *Bradybaena similaris*, *Limax maxinus* and *Limax flavus* have also been found to be naturally infected. A large number of mollusk species can be infected experimentally in the laboratory, thereby confirming the ability of the nematode to parasitize different intermediate hosts (Morera, 1988). The genera *Megalobulinus* (*M. abreviatus*) and *Biomphalaria* (*B. glabrata, B. tenagophila, B. straminea*) are also susceptible to *A. costaricensis* in the laboratory (Graeff-Teixeira *et al*., 1989; Lima *et al*., 1992). The susceptiblity of planorbids to infection by *Schistosoma mansoni* is a genetically controled and hereditary trait (Newton, 1952; Richards, 1970; Santana *et al*., 1978). Thus, populations of *B. tengophila* susceptible to *S. mansoni* have been obtained by selecting the progenies of self-fertilized susceptible mollusks (Santana *et al*., 1978, Zanotti-Magalhães *et al*., 1991).

Kloetzel (1958) reported that miracidia of *S. mansoni* showed chemotropism for *B. glabrata*. Several substances present in snail conditioned water have been considered to be attractants for these miracidia, including fatty acids (MacInns, 1965), H^+ ions (MacInns, 1965), amino acids (Wright & Ronald, 1972; MacInns *et al*., 1974; Prechel *et al*., 1976), serotonin (Etges *et al*., 1975), Mg^{2+} (Stibbs *et al*., 1976), ammonia (Manson, 1979), and peptin (Manson, 1979).

Thiengo (1996) and Mendonça *et al*., (1999) showed that, in addition to infecting *Sarasinula marginata* after ingestion, L1 larvae of *A. costaricensis* can also penetrate the tegument of the host during brief exposure to the parasite (30 min).

We used *B. glabrata*, a planorbid that is more susceptible to *A. costaricensis* (Lima *et al*., 1992), to assess whether larvae L1 of *A. costaricensis* were attracted to the mollusk in a manner similar to that reported for miracidia of *S. mansoni*. Since infection of the mollusks can also occur through penetration of L1 *A. costaricensis*, we considered the possibility that the mollusks may release an attractant or attractants for the larvae. If this proves to be the case, then there will be a greater possibility of finding *B. glabrata* or other freshwater snails infected with *A. costaricensis* in the wild.

In a second experiment, we examined whether *B. tenagophila* genetically selected for susceptibility to *S. mansoni* were also more susceptible to *A. costaricensis* than non-selected snails. This experiment was made to assess whether susceptibility to one species extended to others.

MATERIAL AND METHODS

Melanic specimens of *B. glabrata* from Belo Horizonte (MG, Brazil) and melanic samples of *B. tenagophila* from São José dos Campos (SP, Brazil), genetically selected or not selected for susceptibility to the *S. mansoni*, were kept in the laboratory at the Campinas State University Department of Parasitology and the F_{54} generation was used. The strain of *A. costaricensis* used here was isolated from Crissiumal (RS, Brazil). The L1 *A. costaricensis* were obtained from the feces of the rodent *Sigmodon hispidus* (Rodentia:Cricetidae) (cotton rat), using Rugai *et al*.'s method (1954). In the first experiment, each of three groups of 10 specimens of *B. glabrata* was exposed to 10 L1 for 15 min, 30 min and 1 h. The behavior of the larvae in the presence of mollusks was verified using a glass apparatus, which consisted of two circular chambers 30 mm in diameter attached to a 40 mm x 11 mm channel, as described by Brasio *et al*. (1985) for experiments with *S. mansoni*. The chambers were filled with chlorine-free water. In each experiment, 10 *B. glabrata* were placed in one chamber (A) while the other (B) contained only chlorine-free water. The mollusks were approximately 8 mm in diameter. Using a stereoscopic microscope, 10 L1 were placed with a pipette in the center of the channel (C) connecting the two chambers. The movement of the larvae was observed for 15 min, 30 min and 1 h, and the number of L1 that migrated to each of the two chambers was recorded for each interval. Ten observations using 10 snails each were made for each time period, making a total of 30 snails. After each observation, the apparatus was washed to avoid any possible interference caused by mucus tracks and feces from the mollusks (Brasio *et al*., 1985).

All the experiments were conducted under diffuse illumination with a 60 W incandescent lamp.

In the second experiment, 15 specimens of *B. tenagophila* genetically selected for susceptibility to *S. mansoni* and 20 non-selected snails, all with a diameter of 8-9 mm, were placed individually in glass vials (30 mm x 24 mm) and exposed for 24 h to 120 L1 *A. costaricensis* collected from the feces of *S. hispidus*. After exposure, the L1 remaining in the vials were counted and the mollusks were then placed in individual 250 mL vials filled with chlorine-free water and containing lettuce *ad libitum.* Thirty days after the exposure, the soft parts of each mollusk were artificially digested, as described by Wallace & Rosen (1969). The product of the digestion of each snail was placed in a 50 mL sedimentation cup for 4 h to recover the third-stage larvae (L3) of *A. costaricensis*. After decantation, 5 mL of solution from the bottom of each cup was pipetted onto a petri dish and the L3 *A. costaricensis* were counted with the aid of a stereoscopic microscope. During the 30 days that the mollusks were in the laboratory, two non-selected snails died of natural causes so their data were excluded from the final results.

A statistical analysis was made using the PROC GLM (general linear procedure) option of the SAS® (Statistical Analysis System) statistical package (SAS Institute, 1987). One-way was used to assess the susceptibility of *B. tenagophila* to *A. costaricensis* larvae. The factor in this analysis was the snail population, and the response was the frequency of larvae.

RESULTS

There was no attraction of L1 *A. costaricensis* to *B. glabrata*, regardless of the exposure time (Table 1). Tables 2 to 4 show the susceptibility of *B. tenagophila* to *A. costaricensis*. More L1 *A. costaricensis* were ingested by or penetrated genetically selected *B. tenagophila* than

TABLE 1
Percentage of L1 *Angiostrongylus costaricensis* in compartments A *(B. glabrata)*, C (channel) and B (chlorine-free water).

Time (minutes)	L1 in the compartments (%)		
	A	C	B
15	1	99	0
30	4	95	1
60	1	97	2

TABLE 2
Mean number of infective L1 *Angiostrongylus costaricensis* in *B. tenagophila* genetically selected (S) or non-selected (NS) for susceptibility to *S. mansoni*.

Duncan test*	L1	Number of mollusks	Mollusks
A	112.267	15	S
B	108.833	18	NS

*Mean values with the same letter are not significantly different (overall error rate (α) = 0.05).

TABLE 3
Mean numbers of L3 *Angiostrongylus costaricensis* recovered in selected (S) and non-selected (NS) *B. tenagophila* 30 days after infection.

Duncan test*	L3	Number of mollusks	Mollusks
A	22.133	15	S
B	40.389	18	NS

*Mean values with the same letter are not significantly different (overall error rate (α) = 0.05).

TABLE 4
Difference between the mean numbers of L1 and L3 *Angiostrongylus costaricensis* in selected (S) and non-selected (NS) *B. tenagophila*.

Duncan test*	L1-L3	Number of mollusks	Mollusk
A	90.133	15	S
B	68.444	18	NS

*Mean values with the same letter are not significantly different (overall error rate (α) = 0.05).

non-seleceted snails (F = 4.44; p = 0.0433). The number of L3 *A. costaricensis* that developed after 30 days was greater in non-selected *B. tenagophila* (p = 0.0118) and the difference between the number of L1 ingested or that penetrated and the number of L3 that developed was also significant (p = 0.0049). This difference was greater in the selected mollusks, which were infected by more L1 but which yielded fewer L3. Duncan's multi-range test detected significant differences in L1 and L3, and in the difference between them (L1-L3).

DISCUSSION

The presence of a resistant cuticle, the longitudinal arrangement of the muscle fibers and the presence of celomatic liquid under positive pressure allow nematode larvae to move on solid substrates and to swim in liquid. Considering the short length of L1 *A. costaricensis* (0.26-0.29 mm) and the low frequency of undulating movements, the locomotive power of these larvae is sufficiently small to restrict their movement from one place to another. This behavior is characteristic of larvae living in soil or at the bottom of a liquid mass. In all the attraction experiments conducted here, the larvae remained at the bottom of the apparatus and their movements decreased over time, so that after 60 min they had become practically motionless. The hypothesis that L1 shows tropism towards *B. glabrata* was not confirmed by our results. In the three periods studied (15, 30 and 60 min), most of the *A. costaricensis* larvae remained in channel C, with no movement towards chamber A containing the mollusks (Table 1).

The immunological reactions of mollusks to parasitism by nematodes are poorly understand, although most nematodes that develop in gastropods produce a tissue reaction without actually killing the host (Lie *et al*., 1987). Nematodes are always encapsulated in the body of their hosts, and ultrastructural studies of capsules produced by *A. cantonensis* in *B. glabrata* indicate that they are induced mainly by granulocytes (Harris & Cheng., 1975). These capsules do not have any noticeable harmful effect on the development of the parasites. Stewart *et al*. (1985) observed that in *B. glabrata* the encapsulation of L1 *A. costaricenis* was slower than that of *A. cantonensis*. Harris & Cheng (1975) suggested that the failure of *B. glabrata* to kill the encapsulated larvae of *A. cantonensis* resulted from the insufficient release of lysosomal enzymes by hemocytes. Guaraldo *et al*., (1981) observed that *B. tenagophila* genetically selected for susceptibility to *S. mansoni* showed small reactions around the larvae of the trematode and the sporocysts developed normally.

The balance of the host-parasite relationship depends on the genetic background of the organisms involved, since this background conditions their physiological and metabolic capacity (Zanotti-Magalhães *et al*., 1997). In addition to environmental factors, the development of miracidia in the intermediate host depends on the genetic characteristics that lead to physiological adjustments between the infecting larvae and the mollusk (Machado *et al*., 1988). Although the *B. tenagophila* genetically selected for susceptibility to *S. mansoni* were infected by a larger number of L1 *A. costaricensis*, fewer L3 were recovered when compared with the population of non-selected mollusks. This finding may indicate the presence of factors that affect larval development. Since *B. tenagophila* were genetically selected for susceptibility to the SJ strain of *S. mansoni* through successive self-fertilizations of susceptible progenies, this could have made physiological adjustment more specific for *S. mansoni* relative to other strains of the trematode (Zanotti-Magalhães *et al*., 1997)

and of some nematodes. The genetic selection of the intermediate host could have affected the host-parasite relationship, possibly causing the *A. costaricensis* larvae to become inefficient. In this case, encapsulation and phagocytosis by hemocytes, as well the release of enzymes could have been more efficient against the *A. costaricensis* larvae. This could explain the fewer L3 recovered. In non-selected *B. tenagophila* as well as in *B. glabrata*, the immunological system could have been less efficient, so that the encapsulated larvae did not die. Although the mollusks were infected with a smaller number of L1 *A. costaricensis*, a larger number of L3 was recovered. In conclusion, the genetic selection that occurred in *B. tenagophila* infected with *S. mansoni* SJ was not observed in *B. tenagophila* infected with *A. costaricensis*, since the latter developed better in non-genetically selected *B. tenagophila*.

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