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Research Note

Hemocyte Encapusulation of *Angiostrongylus cantonensis* in M-line *Biomphalaria glabrata* Infected with *Echinostoma paraensei*

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Trematode larvae are capable of interfering with the intermediate host snail's resistance to the same or other trematode species, a phenomenon termed interference (Lie, 1982). In *Biomphalaria glabrata* infected with *Echinostoma paraensei,* hemocytes lose their ability to encapsulate the larvae of trematodes to which the host were previously resistant, but the hemocytes in these snails retain their ability to encapsulate the nematode, *Angiostrongylus malaysiensis (Lie et al.*, 1981).

Angiostrongylus cantonensis is able to develop to the infective stage in *B. glabrata,* even though it is normally encapsulated by hemocytes in this snail (e.g. Harris and Cheng, 1975; Noda and Sato, 1990). The objective of the present study was to investigate the effect of infection with *E. paraensei* on the encapsulation reaction of the host snail *B. glabrata* to *A. cantonensis* larvae.

M-line *B. glabrata* used in this study measured 6–8mm in shell diameter. Snails (E-1) were exposed individually to 10 *E. paraensei* miracidia and 100 first-stage *A. cantonensis* larvae at the same time. At 10 days post-exposure (DPE), snails were checked for the presence of trematode larvae with the aid of a dissecting microscope, and only snails containing unencapsulated *E. paraensei* sporocysts were selected for histological observation. Other snails (E-2) were first exposed to *E. paraensei* miracidia. At 8 DPE, only snails containing unencapsulated sporocysts were exposed to *A. cantonensis* larvae. Control snails were infected with *A. cantonensis* larvae only. Exposed snails were maintained at 26°C in separate 51 aquaria containing deionized water fortified with crushed oyster shell.

At 10 DPE with *A. cantonensis,* a total of 5 snails from control and two experimental groups were fixed in Bouin's solution following removal of shells. Snails were prepared as 7 μm serial histological sections and stained with Mayer's haematoxylin and eosin Y. The outlines of 5 randomly selected hemocyte capsules around *A. cantonensis* larvae from each snails were traced onto paper with the aid of a camera lucida. The center parts of each capsule were chosen for tracing, and the areas were measured using a planimeter.

The size of capsules in control snails (Fig. 1) was significantly larger than that in E-1 snails (Fig. 2): the size of capsules in control snails and E-1 snails was $14,900 \pm 3,100$ μm^2 and $6,900 \pm 1,600$ μm^2, respectively (Student's t-test: $t = 11.49$, $P < 0.001$). In E-2 snails, hemocyte capsules were not observed, and few hemocytes were present around the larvae (Fig. 3).

The results of this study indicate that hemocytes of *B. glabrata* infected with *E. paraensei* lose the ability to encapsulate the larvae

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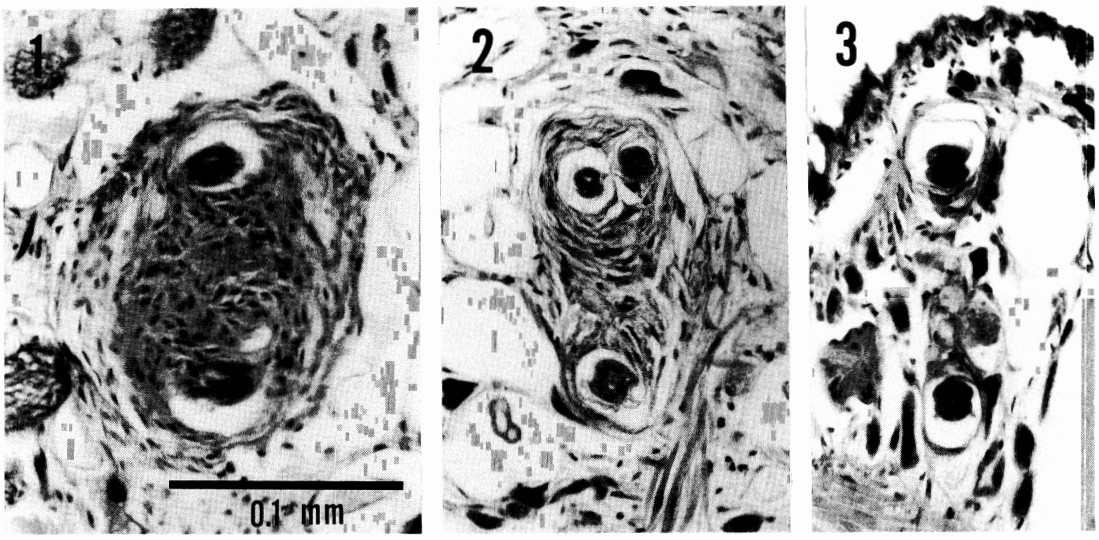

Fig. 1 Hemocyte capsule around *A. cantonensis* larva in snail infected with *A. cantonensis* only (Control), 10 days post-exposure.

Fig. 2 Hemocyte capsule around *A. cantonensis* larva in snail infected with *E. paraensei* and *A. cantonensis* at the same time (E-1), 10 days post-exposure.

Fig. 3 *A. cantonensis* larva in snail 10 days post-exposure (E-2). Snail was infected with *A. cantonensis* 8 days later from first infection with *E. paraensei.*

of nematode, *A. cantonensis.* In E-1 snails, hemocytes retain the ability to encapsulate *A. cantonensis* larvae, though the size of hemocyte capsules was smaller than that in control snails. In E-2 snails, only a few hemocytes were present around the larvae. It is possible that the inhibitory effect of *E. paraensei* on hemocyte function was not fully manifested at early phase of infection. In a similar study, Lie *et al.* (1981) infected *B. glabrata* with *E. paraensei,* and 2 days later exposed them to *A. malaysiensis* larvae. They noted that capsule formation was not suppressed by *E. paraensei.* However, an interval of 2 days may be short for *E. paraensei* to fully manifest the inhibitory effect on hemocyte function. Noda and Loker (1989a, b) examined the effect of infection with *E. paraensei* on the hemocyte function of *B. glabrata.* At 1 DPE, hemocyte populations and phagocytic activity of hemocytes in infected snails were generally similar to unexposed snails. However, at 8 DPE, the infection with *E. paraensei* increased the relative concentration of hemocytes with less ability to adhere to a foreign surface, and also reduced the phagocytic activity of hemocytes.

The results of this study suggest that *E. paraensei* exerts some degree of nonspecific inhibitory effect on hemocyte function of *B. glabrata.*

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