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Research Note

Effects of snail size on encystment of *Echinostoma caproni* in juvenile *Biomphalaria glabrata* (NMRI strain) and observations on the survival of infected snails

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Abstract

The effects of snail size on encystment of *Echinostoma caproni* cercariae in neonatal and juvenile *Biomphalaria glabrata* (NMRI strain) snails were studied. Encystment in neonatal (0.7–1.1 mm shell diameter) and juvenile (2–3 mm shell diameter) snails was compared 24 h post-infection (PI) following individual exposure of snails of each size to 1, 5, 10, 25 and 50 cercariae. Significantly more cysts were recovered from juveniles exposed to 1, 5, 10 and 50 cercariae than from neonatals with comparable exposure. Size of *B. glabrata* was a major factor in determining cyst burden in this planorbid. Survival of infected versus uninfected neonatals and juveniles was also examined for 7 days. Neonatals exposed to 10 cercariae showed a significant decrease in survival at 3, 6 and 7 days PI when compared to the uninfected controls. There was no significant decrease in the survival of juveniles exposed to 10 cercariae compared to uninfected controls at any time point. Snail size was a factor in mortality associated with echinostome cercarial penetration and encystment.

Echinostoma caproni is a 37-collar-spined African species of echinostome that uses the freshwater planorbid snail, *Biomphalaria alexandrina*, as a first intermediate host and various freshwater invertebrates and cold-blooded vertebrates as second intermediate hosts; aquatic mammals and birds serve as definitive hosts (Fried & Huffman, 1996). Although *Biomphalaria glabrata* is not a natural second intermediate host for this echinostome, it is a useful experimental host for studies on cercarial encystment (Frazer & Fried, 1998). A major advantage of using *B. glabrata* as an experimental host is the fact that various strains of this planorbid are readily available from suppliers in the USA. This is not the case for *B. alexandrina*.

Sullivan (1985) examined metacercarial infection of *E. caproni* (referred to as *E. liei* in that study) in neonatal *B. alexandrina* exposed to an undetermined number of cercariae. He found that neonatals (0.8–1.4 mm in shell diameter) were infected with 2–13 *E. caproni* cysts within 48 h post-infection. Kuris & Warren (1980) showed that continuous exposure of *B. glabrata*, 3–8 mm in shell diameter, to about 150 *E. caproni* (referred to as *E. liei* in that study) cercariae per day caused high snail mortality after 4–6 days.

Quantitative information is not available on *E. caproni* encystment in the NMRI strain of *B. glabrata* neonatal (0.7–1.1 mm) and juvenile (2–3 mm) snails. Therefore, we initiated this study to examine quantitative infection in neonatal and juvenile *B. glabrata*. We also repeated the Sullivan (1985) study using *E. caproni* cercariae released from *B. glabrata* and neonatal *B. glabrata* as experimental second intermediate hosts. In addition, we examined the effects of infection of 10 *E. caproni* cercariae on the survival of neonatal and juvenile *B. glabrata* snails.

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Cercariae of *E. caproni* were obtained from *B. glabrata* previously infected with miracidia of this echinostome as described by Idris & Fried (1996). Infected snails were used from 7–12 weeks post-infection (PI). To obtain cercariae, snails were isolated individually at 23 ± 1°C in Stender dishes containing 4 ml of artificial spring water (ASW) prepared as described by Ulmer (1970). About 30 infected snails were used to obtain the several thousand cercariae required for this study. Cercariae were used within 1 h post-emergence.

For studies on the effect of snail size, neonatal (0.7–1.1 mm shell diameter, used within 1 day of hatching and not given exogenous food) and juvenile (2–3 mm juvenile shell diameter, 3–4 weeks old, and maintained on a boiled Romaine leaf lettuce diet) *B. glabrata* snails were used in two experiments; 30 snails were used in each experiment. In both experiments, 6 snails were each exposed to 1, 5, 10, 25 or 50 cercariae in 0.5 ml of ASW for 24 h. After 24 h, snails were crushed between a slide and cover slip and viewed under low power (× 100) of the compound microscope to determine the number of cysts per snail. Exact location of the cysts in the snails was not determined.

To repeat the Sullivan (1985) experiment *B. glabrata* was used instead of *B. alexandrina* as both first and second intermediate hosts. A total of 5 snails shedding *E. caproni* cercariae were placed in a 9 cm fingerbowl containing 150 ml of ASW for 1 h. We estimate that approximately 500 cercariae were released by the snails. After 1 h, these snails were removed, and 25 uninfected neonatals were added to the fingerbowl. At 24 h post exposure, the neonatals were crushed between a slide and cover slip and viewed under low power (× 100) of the compound microscope to determine the number of cysts per snail.

For studies on the survival of infected snails, 24 *B. glabrata* neonatals and 12 juveniles were individually exposed to 10 cercariae in 0.5 ml of ASW for 24 h. Snails were then transferred to 100 × 15 mm Petri dishes (6 snails per dish) containing 15 ml of ASW and a 1-cm^2 piece of Romaine leaf lettuce. An equal number of uninfected neonatals and juveniles were also used, 6 snails per dish, as the uninfected controls. Snail survival was determined every 24 h for 7 days. Dead snails were removed from the cultures and examined for cysts when possible.

The effects of snail size on cercarial encystment in neonatal or juvenile *B. glabrata* are summarized in table 1. Using the Student's t-test (significance considered to be $P < 0.05$), snail size was determined to be statistically significant. The mean number of cysts recovered in the juveniles was significantly greater than that recovered in the neonatals when snails were individually exposed to 1, 5, 10 and 50 cercariae (Student's t-test, $P < 0.05$). In the neonatals, the maximum number of cysts recovered per snail when exposed to 50 cercariae was 26, while the juveniles showed a significant increase in cyst recovery when exposed to 50 cercariae.

The results of exposure of neonatal *B. glabrata* snails to approximately 500 cercariae of *E. caproni* in our study is compared to the Sullivan (1985) work with *B. alexandrina* in table 2. Data from Sullivan (1985) were recalculated in column 5 of table 2. The mean number of cysts recovered from *B. glabrata* was lower than that recovered from *B. alexandrina* in each class, ranging from 0.8–1.1 mm in shell diameter. It appears that neonatal *B. alexandrina* is more susceptible to *E. caproni* cercarial penetration and encystment than is the NMRI strain of *B. glabrata*.

Table 1. Cyst recovery at 24 h post-infection in neonatal or juvenile *Biomphalaria glabrata* snails exposed to cercariae of *Echinostoma caproni*.

	Mean no. of cysts recovered ± S.E. (range)	
No. of cercariae used to expose snails	Neonatal snails	Juvenile snails
1*	0.2 ± 0.2 (0–1)	1.0 ± 0.0 (1)
5*	1.8 ± 0.4 (0–3)	4.0 ± 1.1 (3–5)
10*	3.8 ± 1.5 (0–10)	6.3 ± 1.1 (4–10)
25	8.8 ± 1.7 (3–15)	12.2 ± 1.8 (8–18)
50*	15.7 ± 3.9 (3–26)	22.0 ± 1.5 (16–27)

n = 6 snails for each dose of cercariae for each size class.
*The number of cercariae that encysted in juvenile snails was significantly greater than that in the neonatals (Student's t-test, $P < 0.05$).

Survival of infected versus uninfected neonatals is summarized in fig. 1. The percent survival of uninfected neonatals was significantly higher at 3, 6 and 7 days PI compared to infected neonatals (Student's t-test, $P < 0.05$). Survival of 50% of the infected neonatal snails occurred by day 4, and by day 7, 83% of the uninfected neonatals were still alive. In neonatals that were examined for cysts, 4–9 cysts were recovered per snail. Survival of juvenile infected versus uninfected snails was not significant at any point during the experiment (Student's t-test, $P < 0.05$ considered significant). Only one time point (day 1) showed insignificantly lower survival of infected *B. glabrata* juveniles compared to the uninfected controls. From days 2–7, both infected and uninfected juveniles had survival rates of greater than 80%. In juveniles that were examined for cysts, 5–10 cysts were recovered per snail. Juveniles were less susceptible to the detrimental effects of *E. caproni* cercarial penetration and encystment than the neonatals, at least with a cercarial inoculum of 10 cercariae per snail.

This study shows that neonatal and juvenile *Biomphalaria* snails can serve as second intermediate hosts for *E. caproni* and are important hosts in the transfer of this echinostome to definitive hosts. The susceptibility of *B. alexandrina* to cercarial infection with *E. caproni* in the wild will ensure transfer of this echinostome to aquatic mammals and birds in Africa.

In conclusion, neonatal and juvenile *B. glabrata* snails can become infected with cercariae of *E. caproni*. Size of the snail is a factor in determining the cyst burden per snail. The fact that neonatal survival is effected detrimentally by cercarial infection suggests that echinostomes may play a role in the biological control of *Biomphalaria* species, and may be a means of biological control in geographic areas where *Schistosoma mansoni* and *E. caproni* coexist. In terms of human infection with *Echinostoma* species, the possibility exists of infected neonatal and juvenile snails associated with vegetation being accidentally ingested.

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Table 2. Summary of infectivity data for 25 neonatal *Biomphalaria glabrata* snails and 20 *B. alexandrina* neonatals exposed to an undetermined number of *Echinostoma caproni* cercariae.

	B. glabrata		*B. alexandrina***	
Snail shell diameter (mm)	No. of snails exposed*	Mean ± SE no. of cysts per snail	No. of snails exposed*	Mean ± SE no. of cysts per snail
0.7	2	0.5 ± 0.4	0	–
0.8	4	1.8 ± 0.9	1	5.0
0.9	7	1.3 ± 0.8	5	5.6 ± 1.0
1.0	8	1.6 ± 0.7	5	2.6 ± 0.4
1.1	4	1.8 ± 0.9	1	2.0
1.2	0	–	5	7.0 ± 1.1
1.4	0	–	3	6.7 ± 2.9

* The same as the number of snails examined.
** Data recalculated from Sullivan (1985).

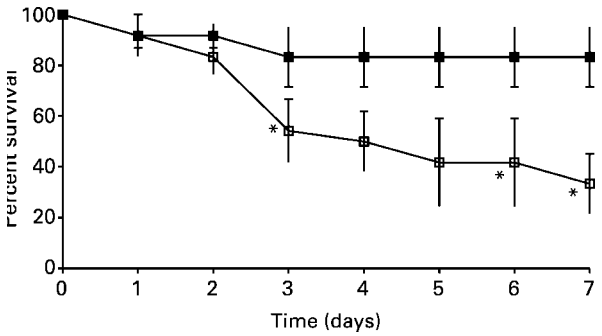

Fig. 1. Survival of infected vs. uninfected neonatal *Biomphalaria glabrata* snails. Four replicates of 6 infected and 6 uninfected snails were used. Bar with open square represents mean ± SE in infected snails; bar with closed square represents mean ± SE in uninfected control. The percent survival of uninfected snails was statistically higher (denoted by *) than infected snails at 3, 6 and 7 days post-infection (two-factor assuming unequal variance, P of interaction of variables >0.05; Student's t-test, $P < 0.05$).

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