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Biomphalaria glabrata (Pulmonata: Planorbidae): A Potential Second Molluscan Intermediate Host of A Human Intestinal Fluke, *Echinostoma cinetorchis* (Trematoda: Echinostomatidae)

Pyung-Rim Chung, Younghun Jung and Chong Yoon Joo[1]

Department of Parasitology, Inha University College of Medicine, Inchon 400-712, Korea
[1]Department of Parasitology, School of Medicine, Keimyung University, Daegu, 700-310, Korea

ABSTRACT

The present study examines the potential involvement of *Biomphalaria glabrata*, a known molluscan intermediate host of *Schistosoma mansoni*, in the lifecycle of *Echinostoma cinetorchis*, one of the echinostomes that are ubiquitous parasites of vertebrates and are of importance in human and veterinary medicine and wildlife diseases. Echinostomes can be maintained easily and inexpensively in the laboratory and provide good models for biological research ranging from the molecular to the organismal. In the present study, no echinostome cercariae were released from the *B. glabrata* experimentally infected with *E. cinetorchis* miracidia, whereas all the *Biomphalaria* snails infected with *E. cinetorchis* cercariae were found to be infected with the metacercariae. This is the first report that *B. glabrata* can experimentally serve as the second intermediate host of *E. cinetorchis*, and that it might be employed as one of the target molluscs for establishing a biological research model with *E. cinetorchis* in the laboratory.

Keywords: *Biomphalaria glabrata, Echinostoma cinetorchis*, Second intermediate host.

INTRODUCTION

A human intestinal fluke, *Echinostoma cinetorchis* (Trematoda: Echinostomatidae), was first described as a new species by Ando and Ozaki (1923), and was later found to occur in Japan, Korea, and several other Asian countries (Miki, 1923; Hirasawa, 1926). This trematode is characterized morphologically by a head crown with 37-38 collar spines and, in particular, six spines on the ventral lobe (Ando and Ozaki, 1923; Seo *et al.*, 1980; Lee *et al.*, 1992).

Life cycle studies on *Echinostoma cinetorchis* were carried out in Korea mostly after Seo and his colleagues reported a human case of echinostomiasis cinetorchis in 1980 (Seo *et al.*, 1984; Lee *et al.*, 1988a; Ahn *et al.*, 1989; Lee *et al.*, 1990; Chung and Jung, 1999; Chung *et al.*, 2001a, b). Six cases of human infection by this trematode have been reported since the initial report (Seo *et al.*, 1980; Ryang *et al.*, 1986; Lee *et al.*, 1988b; Ryang, 1990; Son *et al.*, 1994). Of Korean freshwater snail species, *Hippeutis cantori*, *Segmentina hemisphaerula* and *Austropeplea ollula* have been found to be naturally and experimentally infected with the cercariae of *E. cinetorchis* (Ahn *et al.*, 1989; Chung *et al.*, 2001a, b). Several freshwater snail species, i.e., *H. cantori*, *Radix auricularia coreana*, *Physa acuta*, *Cipangopaludina chinensis malleata*, *S. hemisphaerula*, and *A. ollula* were also reported as the second molluscan intermediate host for this trematode (Lee *et al.*, 1988a; Ahn *et al.*, 1989; Lee *et al.*, 1990; Chung and Jung, 1999; Chung *et al.*, 2001a, b). Other second intermediate hosts include a loach, *Misgurnus anguillicaudatus* (Seo *et al.*, 1984), and tadpole of *Rana nigromaculata* (Chung *et al.*, 2001a).

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Corresponding author: Chung, Pyung-Rim
Tel: (82) 32-890-0981 e-mail: chungpr@inha.ac.kr
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A freshwater snail species, *Biomphalaria glabrata* belonging to the family Planorbidae has been well known as the first intermediate host of *Schistosoma mansoni*. The life cycle studies on *B. glabrata* with other trematode parasites have been previously performed by several investigators. This snail species has also been reported to act as the first intermediate host of *Echinostoma paraensei* and *E. liei* (Lie and Basch, 1967; Jeyarasasingam *et al.*, 1972; Kuris, 1980). Metacercariae of echinostomatid trematodes, such as *E. liei, E. revolutum* and *E. macrorchis*, have been observed in *B. glabrata* (Fried and Bennett, 1979; Kuris and Warren, 1980; Anderson and Fried, 1987; Fried *et al.*, 1987; Lo, 1995).

It was suggested that *Biomphalaria glabrata* may play an important role as a molluscan intermediate host of *Echinostoma cinetorchis*. However, no life-cycle study on cercarial and metacercarial infection to the laboratory-reared *B. glabrata* snails has been conducted to date. Therefore, the present study was designed to determine the susceptibility of Biomphalaria snails to infection with miracidia and cercariae of *E. cinetorchis* in the laboratory.

MATERIALS AND METHODS

1. Cultivation of the snails

The snails of *Biomphalaria glabrata* were originally collected in Puerto Rico, and were maintained in conventional aquaria. These were glass aquaria (20 x 25 x 35 cm) which held seven liters of water. Small plastic trays (7.5 x 20 x 30 cm) were employed as aquaria, especially for the cultivation of medium-sized growing snails. Each tray can contain 1,500 ml of water. Initially, tap water was conditioned before use simply by adding4-5 drops of 5% sodium thiosulfate solution to each aquarium for dechlorination (pH, 6.5-7.0). This water was aerated for one week prior to use. The aquaria were placed on shelves in a shelving unit and exposed alternately to 12 hrs of artificial light provided by a 15-watt cool, white fluorescent tube and 12 hrs of darkness. An automatic timer controlled the cycling of the light. The water of all the glass aquaria, and the plastic trays for the juvenile snails was continuously aerated by bubbling air from a air compressor through the water. The water temperature for the aquaria ranged from 25-27℃. Lettuce and Tetra SML flakes (Tetra SML , Tetra Co., D-452 Melle, West Germany) were supplied to the Biomphalaria snails reared and maintained in the laboratory once a week.

2. Miracidial infection to the laboratory-bred snails

Parasite-free snails from parasites were reared in the laboratory to establish their cycles. The snail eggs were collected and cultured to get laboratory-bred adult snails in a batch which were employed for the susceptibility experiments to the target parasite Echinostoma cinetorchis. The infected rats were used as the source of eggs for the susceptibility studies of snails. For the susceptibility experiments of *E. cinetorchis* miracidia to the laboratory-bred snails, eggs were collected by shredding the worms. The eggs were rinsed three times with distilled water and transferred to the petri dishes containing conditioned water with a few drops of Fungizon solution (Gibco Life Technologies Inc., Grand Island, New York). The eggs were incubated in the conditioned water at 26℃ in a dark incubator with aeration. Some hatched miracidia were used to challenge the *Biomphalaria* snails targeted in this study. Each snail was exposed to a dose of twenty miracidia hatched from the eggs of *E. cinetorchis*. The exposed snails were kept in an aquarium at 26℃.

3. Observation of cercariae from snails

Twenty days after miracidial infection, the release of cercariae was examined from the snails kept under fluorescent illumination (700 Lux) for two hrs. Later, the snails were crushed for further inspection of cercariae inside the snails.

4. Cercarial infection to the laboratory-bred snails

The snails infected with cercariae, were examined in order to obtain metacercariae of *Echinostoma cinetorchis*. Fifty to 200 cercariae shed from *Segmentina hemisphaerula* infected with *E. cinetorchis* miracidia were exposed to each experimental snail of *Biomphalaria*, and the snails infected with experimental doses of cercariae were examined for the

detection of metacercariae. After cracking the snail shell and removing the shell pieces, the animal bodies were dissected. The whole animals of *Biomphalaria* snails were minced and digested in artificial gastric juice for 1 hr at 37℃. After incubation, the digested material in the gastric juice was poured into a strainer to get rid of the large pieces of tissue. The fine tissue suspension was washed several times with 0.85% NaCl solution. The metacercariae were collected from snails every day from two weeks after the cercarial challenge. The number of *Echinostoma* metacercariae per known volume was counted under the low power (x 100) of a light microscope.

5. Metacercarial infection to the final host

Fifty metacercariae obtained from *Biomphalaria* snails were fed orally using a tuberculin syringe connected with a plastic tube to each experimental laboratory rat (Sprague-Dawley strain, 120 g/body weight). Rat feces was examined daily for eggs of Echinostoma cinetorchis. Immediately after finding the eggs, the rats were killed by spinal dislocation and dissected. The small intestines of the rats were removed. The worms were collected under a dissecting microscope and their numbers were determined. Worms were fixed with10% formalin under coverslip pressure and stained with Semichon's acetocarmine. The stained worms were observed for morphological comparisons with the standard parasitic specimens.

RESULTS

Approximately more than 90% of 21-22 day old embryonated eggs (Fig. 1B) hatched under the light sources (17,000 Lux). In the susceptibility experiments with the parasite-free and laboratory-bred *Biomphalaria* snails (Fig. 1A), those infected with miracidia of *Echinostoma cinetorchis* did not release their cercariae (Fig. 1C). However, the snails were found to be completely susceptible to cercariae of *E. cinetorchis* (Fig. 1D) with an infection rate of 100% (Table 1). Each snail, 10-15 mm in shell diameter, was exposed to 50, 100 or 200 cercariae obtained from *Segmentina hemisphaerula* which had been infected with miracidia of *E. cinetorchis*. The infection rates of metacercariae in three experimental groups designed by challenge doses of 50, 100 and 200 cercariae were 11.6%, 52.0% and 70.8%, respectively (Fig. 1E). An average number of metacercariae in the group infected with 200 cercariae was significantly increased as compared with those of metacercariae in the other experimental groups. It is considered that *Biomphalaria* in the group infected with massive numbers of cercariae showed relatively high infectivity of *E. cinetorchis* metacercariae (Table 1).

Fifty metacercariae from *Biomphalaria* taken more than 14 days after cercarial exposure were fed to each rat (S/D strain), and adult worms of *Echinostoma cinetorchis* (Fig. 1F), characterized by 37-38 collar spines on the head crown, were recovered from the ileocecal region of rats 4 weeks after infection with an average recovery rate of 15% (4-12 worms in range, data not shown in Table).

DISCUSSION

Life-cycle studies of an intestinal trematode, *Echinostoma cinetorchis*, in Korea were carried out mainly with the planorbid snails (Ahn *et al.*, 1989; Lee *et al.*, 1990; Chung *et al.*, 2001a). Of three planorbid snail species occurring in Korea, *Hippeutis cantori* and

Table 1. Infection rates of metacercariae in the *Biomphalaria glabrata* infected with *Echinostoma cinetorchis* cercariae.

No. of cercariae exposed/snail	No. of snails examined	No. of snails infected (%)	Total no. of metacercariae	No. of metacercariae infected/snail (%)
50	5	5 (100)	29	5.8 (11.6)
100	5	5 (100)	260	52.0 (52.0)
200	2	2 (100)	283	141.5 (70.8)

Segmentina hemisphaerula were confirmed as the first and second molluscan intermediate hosts. *Gyraulus convexiusculus*, one of the Korean planorbid species, is also a possible candidate for the second intermediate hosts of *E. cinetorchis* based on the experimental results (Lee *et al.*, 1990; Chung *et al.*, 2001a). Although *Biomphalaria glabrata* belonging to the family Planorbidae occurs mostly in South American and African countries, this snail species was experimentally confirmed to serve as the second intermediate host of *E. cinetorchis* in this study.

Biomphalaria glabrata was able to harbor extremely high levels of the metacercariae of *Echinostoma cinetorchis* in this study as shown in the other echinostomatid trematodes, such as *E. liei, E. revolutum* and *E. macrorchis* (Fried and Bennett, 1979; Kuris and Warren, 1980; Fried *et al.*, 1987; Lo, 1995). Authors suggest that *B. glabrata* is highly recommended to be employed as the second intermediate host of *E. cinetorchis* because of higher metacercarial infectivity than the other Korean freshwater snail species and their easy maintenance in the laboratory.

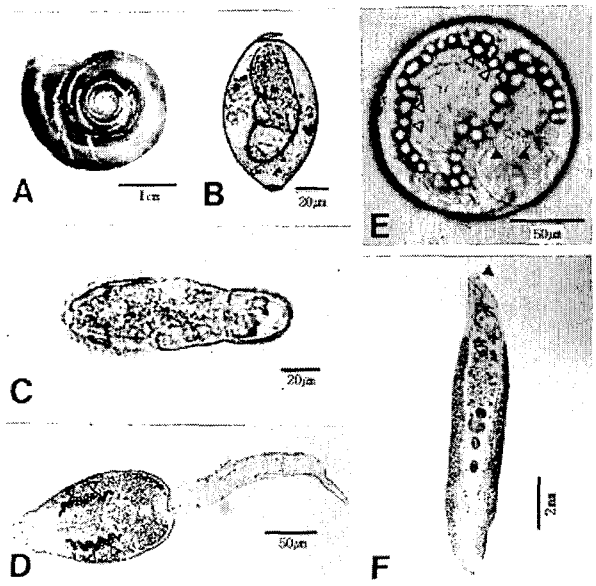

Fig. 1. Experimental subjects employed or observed in this study.
A, A shell of *Biomphalaria glabrata* B. a 21-day-old embryonated egg; C, a miracidium of *Echinostoma cinetorchis* (x 100); D, an echinostome cercaria of *E. cinetorchis* (note the excretory granules); E, a metacercaria of *E. cinetorchis*, characterized by 37-38 spines (black arrowheads) and excretory granules (white arrowheads); F, an adult worm of *E. cinetorchis* (a head crown [arrowhead] is noticeable).

On the other hand, Lo (1995) pointed out the role of host hemocytes for the encapsulation of Echinostoma macrorchis metacercariae in an abnormal snail host, *Biomphalaria glabrata*. In this study, the snails infected with high cercarial doses seem to be with higher metacercarial infections. However, host-parasite relationship was not focussed in this study.

Many authors have mentioned encystment of *Echinostoma revolutum* cercariae in the kidney and pericardial cavity of various physid, lymnaeid, and planorbid snails (Beaver, 1937; Fried and Weaver, 1969; Lo and Cross, 1975; Fried and Bennett, 1979). Anderson and Fried (1987) examined cercarial migration of *E. revolutum* and metacercarial encystment in the kidney of three experimental pulmonate host species, *Biomphalaria glabrata, Helisoma trivolvis*, and *Physa heterostropha*. In *B. glabrata,* 80-90% of the cercariae of *E. macrorchis* entered the kidney via the nephridiopore within 1 hour, moving toward the saccular portion and pericardial cavity, and completely cyst formation within four hours (Lo, 1995). In this study, the cysts of metacercariae of *E. cinetorchis* in the snail specimens examined were mostly observed in the surrounding area of kidney as shown in the other *Echinostoma* species. In general, the similar results were obtained in the Korean planorbid snail species.

With the overall results in this study, this is the first report of *Biomphalaria glabrata* serving as a potential second intermediate host of *Echinostoma cinetorchis,* and the authors recommend that *B. glabrata* is one of the useful candidate snails for establishing biological models with *E. cinetorchis*, if the life cycle of *B. glabrata* has been successfully kept in the laboratory.

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