The Korean Journal of Parasitology
Vol. 39, No. 4, 329-332, December 2001

Brief Communication

Corbicula fluminea (Bivalvia: Corbiculidae): a possible second molluscan intermediate host of *Echinostoma cinetorchis* (Trematoda: Echinostomatidae) in Korea

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Abstract: More than 1,500 clams of *Corbicula fluminea*, the most favorable food source of freshwater bivalves in Korea, were collected from 5 localities to examine cercarial and metacercarial infection with *Echinostoma cinetorchis*. Although 3 clams infected with suspicious *E. cinetorchis* metacercariae out of 200 specimens collected at Kangjin, Chollanam-do were detected, no cercarial and metacercarial infections with *E. cinetorchis* were observed in field-collected *Corbicula* specimens. In the susceptibility experiments with laboratory-reared clams, those infected with miracidia of *E. cinetorchis* did not release their cercariae up to 60 days after infection. To confirm the identity of second intermediate host of *E. cinetorchis* experimentally, a total of 30 clams were exposed to the cercariae from *Segmentina hemisphaerula* that had been infected with miracidia of *E. cinetorchis*. The clams were susceptible to cercariae of *E. cinetorchis* with an infection rate of 93.3%. Metacercariae from clams taken more than 7 days after cercarial exposure were fed to rats (S/D strain), and adult worms of *E. cinetorchis*, characterized by 37-38 collar spines on the head crown, were recovered from the ileocecal regions. This is the first report of *C. fluminea* as a possible second intermediate host of *E. cinetorchis*.
Key words: *Corbicula fluminea*, *Echinostoma cinetorchis*, susceptibility, metacercaria

Human intestinal fluke, *Echinostoma cinetorchis* (Trematoda: Echinostomatidae), was first described as a new species by Ando and Ozaki (1923). This trematode is characterized morphologically by a head crown with 37-38 collar spines and, in particular, 6 spines on the ventral lobe (Ando and Ozaki, 1923; Seo et al., 1980; Lee et al., 1992).

Life cycle studies on *E. cinestorchis* 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). 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., 2001). Several freshwater snail species, i.e., *H. cantori*, *Radix auricularia coreana*, *Physa acuta*, *Cipangopaludina*

• Received 16 October 2001, accepted after revision 16 November 2001.
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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 et al., 2001). Other second intermediate hosts include a loach, *Misgurnus anguillicaudatus* (Seo et al., 1984), and tadpole of *Rana nigromaculata* (Chung et al., 2001). Studies on the involvement of freshwater bivalves in trematode life cycles were previously carried out by many investigators. The cercarial emergence of the bucephalid trematodes *Rhipidocotyle fennica* and *R. campanula* from a freshwater unionid clam *Anodonta piscinalis* kept under natural conditions was reported (Taskinen et al., 1994; Taskinen, 1998). Some bivalves act as the second intermediate hosts for digenean trematodes. Metacercariae of echinostmatid trematodes have been observed in several species of bivalve hosts; those of *E. revolutum* and *E. macrorchis* were experimentally infected in *Corbicula fluminea* from Schukykill River in southeastern Pennsylvania (Fried et al., 1987), and from Taiwan (Lo, 1995), respectively. The zebra mussel *Dreissena polymorpha* collected from the St. Lawrence River between Massena, New York and Cornwall, Ontario acted as the second intermediate host for *Echinoparyphium* sp. (Conn and Conn, 1995). In addition, the metacercariae of gymnophallid trematode *Parvatrema timondavidi* were naturally found from *Tapes philippinarum*, one of the most common marine clam in Korea (Yu et al., 1993; Sohn et al., 1996). It was suggested that *Corbicula* spp. may act as a source of human infection for *E. cinetorchis*. However, neither epidemiological studies on the naturally-infected cercariae and metacercariae in *C. fluminea* clams nor the life-cycle studies with laboratory-reared clams have been conducted to date.

Therefore, the present study was designed to determine the extent of natural infection of *E. cinetorchis* from *C. fluminea* collected at several local sites in Korea as well as the susceptibility of the clams in the laboratory to infection with miracidia and cercariae of *E. cinetorchis*.

Corbicula fluminea Müller 1884, the most common freshwater bivalve in Asian countries and North America (Morton, 1986), is easily found in freshwater systems, and edible as a food source in Korea. More than 1,500 clams collected in 5 local sites of Korea from August to September, 2000 (voucher numbers, IUMC 104-108; Fig. 1A) were first examined for the emergence of trematode cercariae and the presence of encysted metacercariae by the methods of Fried et al. (1987). In field-collected *C. fluminea*, no natural emergence of trematode cercariae was detected. However, 3 of 200 clams collected at Kangjin, Chollanam-do harbored degenerated echinostome-like metacercariae in which typical spines could not be identified except the excretory granules.

For the susceptibility experiments, eggs of *E. cinetorchis* were collected from adult worms and incubated in conditioned water with a few drops of Fungizon solution (Gibco Life Technologies Inc., Grand Island, NY) at 26°C under the illumination of 17,000 lux. About 67% of the eggs hatched 18 days after incubation. Each of 20 laboratory-reared clams was exposed to 20 *E. cinetorchis* miracidia for 20 hours in the laboratory, and the exposed clams were observed for shedding cercariae everyday. However, no cercaria was released up to 60 days after infection. A second experiment was conducted to determine if this corbiculid clams could serve as second intermediate hosts for this echinostome species. Each of 30 laboratory-reared clams, 12-17 mm in shell diameter, was exposed to 100 cercariae obtained from experimental infections of *E. cinetorchis* miracidia in *S. hemisphaerula*. More than 90% of the 30 clams employed were found to be infected with the metacercariae; however, average number of metacercariae in the 14-day old clams after cercarial exposure (5 per infected clam) was significantly decreased as compared with those of metacercariae in the 1-day and 7-day old clams (14 and 15 per infected clams, respectively) (Table 1). The encysted metacercariae were mostly found in the gills and kidneys in this study (Fig. 1B). It is considered that variations of metacercarial numbers in the clams taken more than 14 days postinfection should be observed in the further studies. The usual locations for echinostomatid metacercariae are within the

Table 1. Metacercarial infectivity in the laboratory-reared *Corbicula fluminea* exposed to the cercariae of *Echinostoma cinetorchis* shed from *Segmentina hemisphaerula*[a]

Days after exposure	No. of clams examined	No. of clams infected (%)	Total no. of metacercariae obtained	Average no. ± SD of metacercariae per infected clam
1	10	10 (100)	135	14 ± 6.1
7	10	9 (90)	134	15 ± 11.0
14	10	9 (90)	49	5 ± 3.8[b]
Total	30	28 (93.3)	318	11 ± 8.4

[a]A total of 100 cercariae of *E. cinetorchis* were exposed to each experimental clam.
[b]Average number of metacercariae in the 14-day old clams after cercarial exposure was significantly decreased as compared with the other average numbers (ANOVA test; P = 0.026).

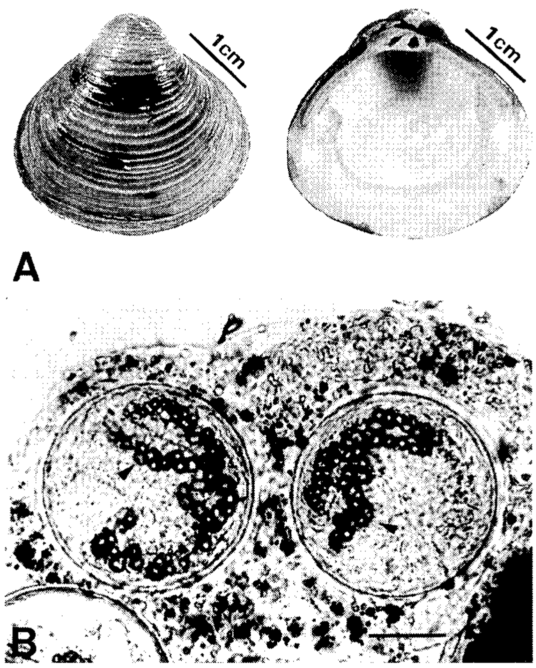

Fig. 1. The shells of *Corbicula fluminea* (A) and two metacercariae of *Echinostoma cinetorchis* in tissue of kidney of *Corbicula fluminea* (B). Note the excretory granules (arrow heads) in the cysts. Collar spines in the cysts are not focused (bar = 50 μm).

metanephridial kidney or pericardial cavity of molluscan hosts (Anderson and Fried, 1987). The cercariae crawl directly up the urinary ducts to encyst at or near the blind end (Anderson and Fried, 1987). Lo (1995) pointed out that the bivalves acquire their food by filtration of water and trapping plankton with the aid of mucus; thus, many cercariae must have been caught by the mucus and ingested. However, authors have not proved how the cercaria of *E. cinetorchis* infected to the tissues of the *C. fluminea* clam. The results obtained in the present study were generally in accord with those of the *E. revolutum* studies on the *C. fluminea* (Fried et al., 1987).

Laboratory rats (Sprague-Dawley strain, 120 g/body weight) were used as the final host of this fluke. Fifty metacercariae from experimentally infected clams were fed per os to each rat. Rat feces were examined for eggs of *E. cinetorchis* daily, starting one week after exposure. Immediately after finding the eggs, rats were killed and examined for adult trematodes. Adult worms, characterized by 37-38 collar spines on the head crown, were recovered from the ileocecal regions of the intestine 4 weeks after being infected, with the average recovery rate of 2%. However, the metacercariae collected from the 1-day old clams after cercarial exposure were not infected to the rats.

In summary, this is the first report of *C. fluminea* serving as the potential second intermediate host of *E. cinetorchis* in Korea.

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