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# Experimental life history of *Echinostoma cinetorchis*†

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**Abstract:** The life history of *Echinostoma cinetorchis* was completed in the laboratory using *Hippeutis cantori* as the first and second intermediate host. The incubation for maturation of eggs and hatching of miracidia took 24 days at 28°C. On the 66th day after miracidial challenge, 16 snails were crushed and examined for the presence of *E. cinetorchis* larvae. The metacercariae were detected in all of the snails, and from three of them were found the rediae and cercariae. The morphological characteristics of the larvae and the experimentally obtained adults were compatible with that of *E. cinetorchis*.

**Key words:** *Echinostoma cinetorchis*, miracidium, redia, cercaria, metacercaria, *Hippeutis* (*Helicorbis*) *cantori*

## INTRODUCTION

*Echinostoma cinetorchis* is one of the intestinal flukes of the family Echinostomatidae. This species is characterized by 37~38 collar spines, and abnormal location or diminished number of the testes(Ando and Ozaki, 1923). The biological studies on this fluke were made mainly in Japan, and its whole life history was described by Takahashi(1927). In Korea, the existence of this fluke was noticed by discovery of adult worms from naturally infected rats(Seo *et al.*, 1964). Thereafter, enzootic studies on the intestinal flukes revealed nationwide prevalence among wild rats and dogs mixed with *E. hortense*(Seo *et al.*, 1981; Cho *et al.*, 1981). Total of 4 cases of human infection have been recorded in Korea(Seo *et al.*, 1980; Ryang *et al.*, 1986; Lee *et al.*, 1988).

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Its metacercariae were detected from the naturally infected loach and *Hippeutis*(*Helicorbis*) *cantori*(Seo *et al.*, 1984; Lee *et al.*, 1988). The loach was supposed to be an infection source of the human cases. Several kinds of frogs and their tadpoles, larvae of salamander, freshwater snails and freshwater fish such as the loach are known to be the second intermediate hosts in Japan and Korea(Takahashi, 1926 & 1927; Hirasawa, 1926; Kurisu, 1930; Yamashita, 1964; Komiya, 1965; Seo *et al.*, 1984; Lee *et al.*, 1988). However, there have been no reports on the first intermediate host except *Segmentina mica* in Japan(Takahashi, 1927).

*Hippeutis* (*Helicorbis*) *cantori* is one of the freshwater snails, frequently found in rice paddies or irrigation ditches in Korea. This snail has drawn medical attentions because of its role as the first intermediate host of *Fasciolopsis buski* and *Fibricola seoulensis*(Pace, 1973; Lee *et al.*, 1986; Seo *et al.*, 1988). Also it was found as the second intermediate host of *E. cinetorchis*(Lee *et al.*, 1988) but the first intermediate host of *E. cinetorchis* is still unknown in Korea. The present study was carried out to

elucidate the role of the freshwater snail, *H. cantori*, as an intermediate host of *E. cinetorchis* in laboratory conditions.

## MATERIALS AND METHODS

### 1. Collection and cultivation of *E. cinetorchis* eggs

The metacercariae of *E. cinetorchis* were collected from the naturally infected freshwater snail, *H. cantori*. They were orally introduced into 4 albino rats to obtain the eggs. The eggs from the adults one month after infection were washed in water several times, and then they were cultured in water at 28°C in an incubator.

### 2. Observation and infection of the larval stages of *E. cinetorchis*

The miracidia hatched from eggs were observed and the actively moving ones were challenged to the snails which were reared in the laboratory for more than 3 generations. The infected snails were kept in an aquarium at 20°C. The cercarial shedding was observed under a stereomicroscope from 20 days after the miracidial challenge. On the 66th day after miracidial challenge, the snails were crushed with a forcep in small petri dishes containing 0.85% saline and were examined for the presence of the larval flukes. Recovered larval worms were measured and observed their morphology under the light microscope. The metacercariae from the snails were infected to albino rats to get adult worms.

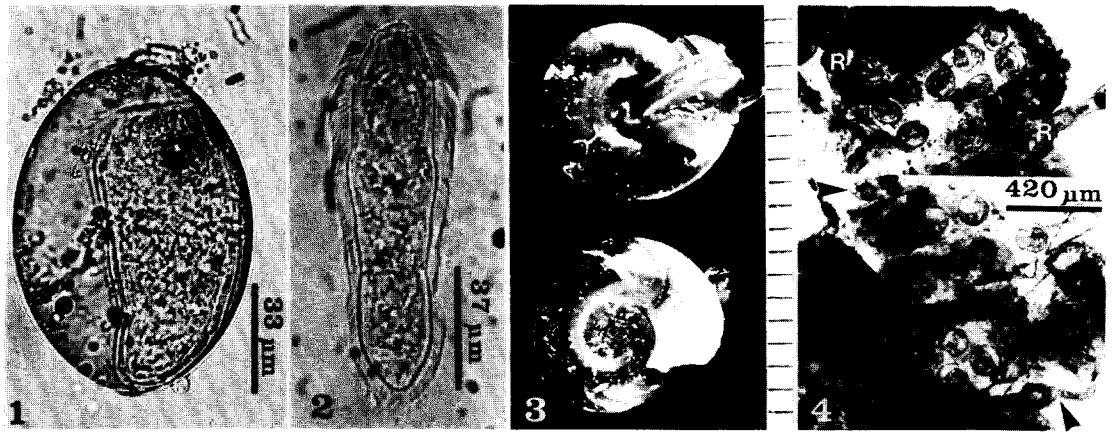
## RESULTS

### 1. Maturation of eggs and hatching of miracidia of *E. cinetorchis*

The eggs of *E. cinetorchis* were fully matured and the miracidia hatched out 24 days after cultivation at 28°C (Fig. 1). The hatched miracidia actively moved with numerous cilia on the body surface(Fig. 2). They were 102.5× 36.8 μm in average size. When they were contacted with the snail, *Hippeutis*(*Helicorbis*) *cantori*(Fig. 3), they actively penetrated into the exposed animal part of the snails such as tentacles or mantles.

### 2. Infection status of snails with *E. cinetorchis* larvae

No cercarial shedding was observed from the snails after miracidial challenge by the 65th day. On the 66th day after miracidial challenge, 16 snails were crushed and examined the presence of *E. cinetorchis* larvae. The metacercariae of




**Figs. 1-4.** The larvae and snail intermediate host of *E. cinetorchis*.

**Fig. 1.** An embryonated egg developed in the 28°C incubator during 24 days.

**Fig. 2.** Miracidium hatched from an embryonated egg, showing eye spots and numerous cilia.

**Fig. 3.** *Hippeutis* (*Helicorbis*) *cantori*, a fresh water snail, identified to be a new intermediate host of *E. cinetorchis*.

**Fig. 4.** Rediae(R), cercariae(arrow heads) and metacercariae(round-shape) detected from an experimental snail in the 66th day after miracidial challenge.

**Table 1.** Detection of *E. cinetorchis* larvae from the experimentally infected snails

| Remarks | No(%) of snails |
|---|---|
| Challenged with miracidia | 20 |
| Examined by crush method* | 16(100) |
| Positive for the redia | 3(18.8) |
| Positive for the cercaria | 3(18.8) |
| Positive for the metacercaria | 16(100) |

*The snails were examined on the 66th day after the challenge.

*E. cinetorchis* were detected in all of the snails in the range of 17~321, three of which harbored the second generation rediae and cercariae of *E. cinetorchis*(Table 1 & Fig. 4).

### 3. Morphology of *E. cinetorchis* larvae from snails

The rediae were elongated and sac-shaped, and were 0.53~2.44 mm long and 0.19~0.28 mm wide. A redia had a muscular pharynx, an intestinal cecum and 7~14 cercariae. The cecum was filled with the brownish materials(Fig. 5). The measurements were summarized in Table 2.

The cercaria was consisted of a snake head-shaped body and a rod-like tail. The body was 150.3~174.4 μm long and 102.0~120.7 μm wide

**Table 2.** Measurements of the second generation rediae of *E. cinetorchis* obtained from the experimental snails

| Item or organ | Measurements*(mm) | |
|---|---|---|
| | Range | Average |
| Body | 0.529~2.442 ×0.186~0.281 | 1.432 ×0.240 |
| Pharynx | 0.033~0.049 ×0.037~0.054 | 0.042 ×0.047 |
| Cecum | 0.143~0.429 ×0.066~0.116 | 0.299 ×0.092 |
| No. of cercariae | 7~14 | 12 |

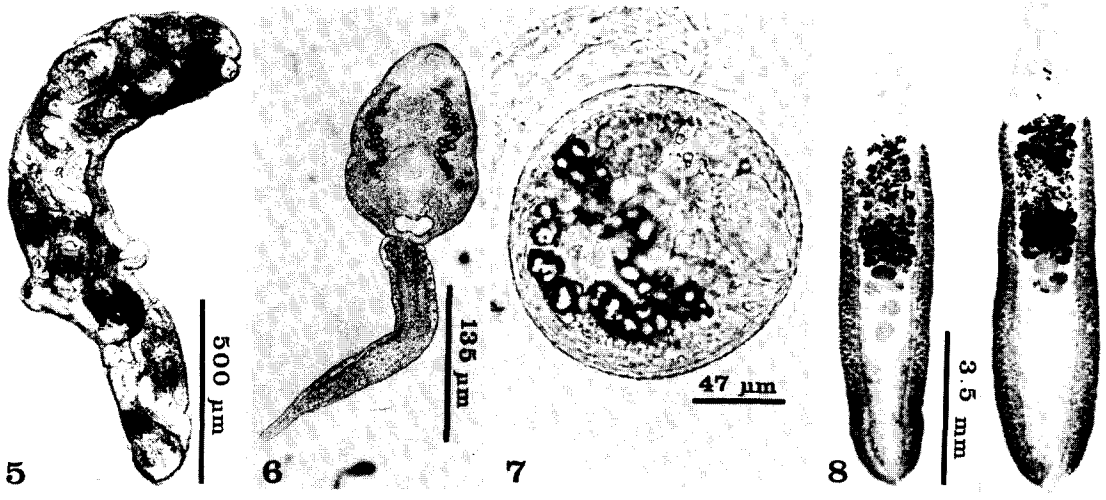
*10 rediae were measured.

and had two muscular suckers, a pharynx, two ceca, refractile excretory granules and a germ cell primordium. The tail was 225.4~254.9 μm long and 34.8~45.6 μm wide without any membrane nor folding(Fig. 6 & Table 3).

The metacercariae were round to elliptical, and 139.0×134.4 μm in average diameter. The refractile excretory granules and two suckers were easily recognized under the light microscope, but it was hard to identify their head crown and collar spines (Fig. 7).

### 4. Adult worms

The adults recovered from experimentally




**Figs.** 5-8. The larvae and adults of *E. cinetorchis*.
**Fig.** 5. The second generation redia obtained from an experimental snail.
**Fig.** 6. Cercaria detected from an experimental snail.
**Fig.** 7. Metacercaria isolated from an experimental snail.
**Fig.** 8. Two adult worms recovered from an experimental rat in 17th day after infection.

**Table 3.** Measurements of *E. cinetorchis* cercariae obtained from the experimentally infected snails

| Item or organ | Measurements*(μm) | |
|---|---|---|
| | Range | Average |
| Body | 150.3~174.4<br>×102.0~120.7 | 163.7<br>×109.1 |
| Tail | 225.4~254.9<br>× 34.8~ 45.6 | 236.6<br>× 39.4 |
| Oral sucker | 34.9~ 42.3<br>× 29.5~ 32.2 | 36.2<br>× 30.4 |
| Pharynx | 18.8~ 21.5<br>× 10.7~ 16.0 | 21.0<br>× 13.9 |
| Ventral sucker | 26.8~ 37.6<br>× 34.9~ 48.3 | 31.8<br>× 42.5 |
| Germ cell primordium | 16.1~ 26.8<br>× 10.7~ 16.1 | 21.0<br>× 13.4 |

*10 cercariae

**Table 4.** Measurements of adult *E. cinetorchis** from experimentally infected rats in 17 days after infection

| Organs | Measurements(μm) | |
|---|---|---|
| | Range | Average |
| Body | 9.32~11.54<br>×2.22~ 2.64 | 10.34<br>× 2.43 |
| Head crown | 0.26~ 0.29<br>×0.51~ 0.58 | 0.27<br>× 0.56 |
| Oral sucker | 0.24~ 0.27<br>×0.26~ 0.29 | 0.25<br>× 0.28 |
| Pharynx | 0.20~ 0.24<br>×0.16~ 0.21 | 0.22<br>× 0.18 |
| Esophagus | 0.48~ 0.70 | 0.61 |
| Ventral sucker | 0.69~ 0.81<br>×0.17~ 0.83 | 0.76<br>× 0.75 |
| Cirrus pouch | 0.33~ 0.47<br>×0.17~ 0.24 | 0.39<br>× 0.21 |
| Ovary | 0.32~ 0.50<br>×0.62~ 0.73 | 0.41<br>× 0.66 |
| Mehlis gland | 0.36~ 0.50<br>×0.78~ 0.96 | 0.40<br>× 0.88 |
| Testis | 0.36~ 0.40<br>×0.44~ 0.60 | 0.37<br>× 0.53 |

*Ten adult worms were measured after staining.

infected rats 17 days after infection were elongated and leaf-like, 9.32~11.54 mm long and 2.22~2.64 mm wide. Their head crown was distinctively beset with 37 collar spines without dorsal interruption. Their testes were irregular in the number and location(Fig. 8). The measurements were summarized in Table 4. All of the recovered worms were identified as *E. cinetorchis*.

## DISCUSSION

The present finding reveals that *H. cantori* is the first and also the second intermediate host of *E. cinetorchis*. Several kinds of freshwater snails, such as *Segmentina mica*, *Planorbis compressus japonicus*, *Lymnaea japonica*, *Viviparus malleatus*, and *Cipangopaludina japonica* are known to serve as the second intermediate host of *E. cinetorchis* in Japan(Takahashi, 1926; Hirasawa, 1926; Kurisu, 1930). The snail, *Hippeutis(Helicorbis) cantori*, is its known second intermediate host in Korea(Lee *et al.*, 1988). Other than this snail, *Radix auricularia*, *Cipangopaludina* sp. and *Physa acuta* have been proved as its experimental second intermediate host(Ahn *et al.*, 1989). The loach was found to harbor its metacercariae naturally(Seo *et al.*, 1984). Various freshwater snails have been commonly found as its first and second intermediate host of other echinostomes(Gulka and Fried, 1979; Vasilev and Kanev, 1981). Usually the snail second intermediate host is not so strict in the host parasite relationship as the first one does because the second one provides only the space for encystment of the cercariae. Therefore, the spectrum of the snail second intermediate hosts is wider than that of the first. When the ecology of the snail in the freshwater is considered, *Lymnaea pervia*, which shares the breeding niche with *H. cantori*, may be another candidate of its second intermediate host in Korea.

The freed cercariae were not found from the experimental snails in the daily observation between the 20th and 65th day after miracidial challenge. But the metacercariae were in all of the snails examined on the 66th day, which suggests the development of cercariae during the period. Ahn *et al.*(1989) found cercarial shedding from experimentally infected snails about 25 days after the challenge. The rediae and cercariae were also found in three of them. It is not clear

whether the cercariae encysted directly without shedding in the snails or we failed to observe the freed cercariae. The cercariae are lophocercous with a long tail, which suggests their capability of very active free swimming just like that of *E. hortense*. The cercariae of *E. hortense* are produced from the snail host, *L. pervia*, into the water with vigorous movement(Roe, 1989). The present result suggests the possibility of encystation of *E. cinetorchis* cercariae into the metacercariae in the same snail host regardless of the occurrence of cercarial shedding. However, when the detection of metacercariae from various snails and from the loach is regarded, the cercaria must be shed from the snail as found by Ahn *et al.*(1989). But the number of freed cercariae seems not high.

The morphology and measurements of the larval stages of *E. cinetorchis* in snails are similar with that of other echinostomes. Therefore, it may not be easy to differentiate their species by only morphological characteristics of the larvae. Only the metacercaria can be identified by the morphology. A better method is needed for easy identification of them.

The snail, *H. cantori*, is common in rice paddies nationwidely in Korea. *H. cantori* must be a natural first and second intermediate host of *E. cinetorchis* as well as an experimental host. The freshwater snails as well as this may be the major source of rat infection by their natural food chain. The present result confirms a new first intermediate host of *E. cinetorchis*, other than *S. mica*. (Takahashi, 1927).

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## =국문초록=

# 이전고환극구흡충의 실험실 내 생활사

서울대학교 의과대학 기생충학교실 및 풍토병연구소, 인제대학교 의과대학 기생충학교실*
이순형 • 채종일 • 홍성태 • 손운목*

실험실 내에서 또아리물달팽이(*Hippeutis cantori*)를 중간숙주로 한 이전고환극구흡충(*E. cinetorchis*)의 생활사를 관찰하였다. 실험감염시킨 흰쥐로부터 회수한 충체에서 충란을 얻어 28°C의 물에서 배양하였던 바, 배양 24일에 탈각 부화하였으며 부화한 미라시디움(miracidium)은 체표면에 밀생해 있는 섬모를 이용하여 매우 빠른 속도로 유영하였고, 크기는 평균 102.5×36.8 μm이었다. 미라시디움을 실험실에서 부화시킨 패류에 접촉 감염시킨 후 유미유충의 유출 여부와 파쇄법에 의한 유충의 감염 상태를 조사하였다. 피낭유충은 검사한 16마리 패류 모두에서 17~321개가 검출되었고, 레디아(redia)와 유미유충(cercaria)이 3마리의 패류에서 다수가 발견되었다. 레디아는 길쭉한 자루 모양이었으며 평균 1.43×0.24 mm의 크기이었고, 근육성의 인두와 길쭉한 맹장 및 성숙한 유미유충을 보유하고 있었다. 유미유충은 타원형의 몸체와 막대 모양의 꼬리로 이루어져 있었고 몸체에는 두개의 흡반과 인두 및 특징적인 광택성의 배설 과립이 존재하였다. 피낭유충은 원형 또는 타원형이었으며 평균 139.0×134.4 μm이었다. 감염 후 17일에 회수한 충체는 크기가 평균 10.3×2.4 mm이었으며, 두극의 수 및 배열, 고환의 수 및 위치 등에 있어서 이전고환극구흡충의 특징적인 소견을 나타내었다. 자연 유출된 유미유충은 전혀 관찰할 수 없었다.

이 연구를 통하여 우리 나라에서 논과 수로에 흔히 서식하는 또아리물달팽이가 이전고환극구흡충의 제1중간숙주이고 동시에 제2중간숙주가 될 수 있음을 실험적으로 확인하였다. 이러한 중간숙주로서의 역할은 자연계에서도 이루어질 것으로 추측된다.

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