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Metacercariae of *Echinostoma cinetorchis* encysted in the fresh water snail, *Hippeutis* (*Helicorbis*) *cantori*, and their development in rats and mice

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Abstract: The fresh water snail, *Hippeutis* (*Helicorbis*) *cantori*, caught from the rice paddies in Namyangju-gun, Gyeonggi-do, was found to be a new second intermediate host of *E. cinetorchis* in Korea. The metacercariae were found from 89 (76.7%) out of 116 snails examined and the average metacercarial burden was 4.7 per infected snail. Some developmental characteristics of *E. cinetorchis* were observed after experimental infection of the metacercariae to rats and mice.

Key words: *Echinostoma cinetorchis*, metacercaria, snails, *Hippeutis* (*Helicorbis*) *cantori*, development in rats and mice

INTRODUCTION

Echinostoma cinetorchis, a member of fluke family Echinostomatidae, was first described by Ando and Ozaki(1923) from the rats, *Rattus norvegicus*. This worm has been discovered from rats in various places of Japan, Korea and Taiwan(Ando and Ozaki, 1923; Ando and Tsuyuki, 1923; Ishii, 1932; Tanabe and Takeishi, 1936; Yamashita, 1964; Seo *et al.*, 1964 & 1981; Rim, 1982). Human infections have been reported in Japan and Korea(Takahashi *et al.*, 1932 a & b; Kawahara and Yamamoto, 1933; Moriyama, 1952; Hyodo and Matsuyama, 1958; Seo *et al.*, 1980; Ryang *et al.*, 1986; Lee *et al.*, 1988).

Several kinds of frogs and their tadpoles, larvae of salamander, fresh water snails and fresh water fishes such as the loaches are known to be the second intermediate hosts in Japan(Takahashi, 1926 & 1927; Hirasawa, 1926; Kurisu, 1930; Yamashita, 1964; Komiya, 1965). However, few reports on intermediate host, other than the loaches, have been available in Korea(Seo *et al.*, 1984).

The development of *E. cinetorchis* in its final host such as the rats has been briefly studied (Seo *et al.*, 1984). However, the developmental characters of this fluke such as organogenesis of genitalia and growth patterns in final host were not observed in detail. In the present study, *Hippeutis* snails were verified to be a new second intermediate host of *E. cinetorchis* and the development of worms was observed in experimental rats and mice.

MATERIALS AND METHODS

1. Detection and identification of *E. cinetorchis* metacercariae

Hundreds of snails, *Hippeutis* (*Helicorbis*)

Fig. 1. *Hippeutis* (*Helicorbis*) *cantori*, a kind of fresh water snail, found to be a new second intermediate host of *E. cinetorchis* (scale : 1 mm).

cantori (Fig. 1) were collected from rice paddies or irrigation ditches in Namyangju-gun, Gyeonggi-do, Korea, from August to October, 1985. They were reared in an aquarium of 18~20°C, and infection status with larval trematodes especially the metacercariae of *E. cinetorchis* was investigated. The snails were crushed with big forceps in small petri dishes containing 0.85% saline, and were examined for the presence of metacercariae under the stereomicroscope. The metacercariae were morphologically observed and some were infected to albino rats to obtain adults for further identification.

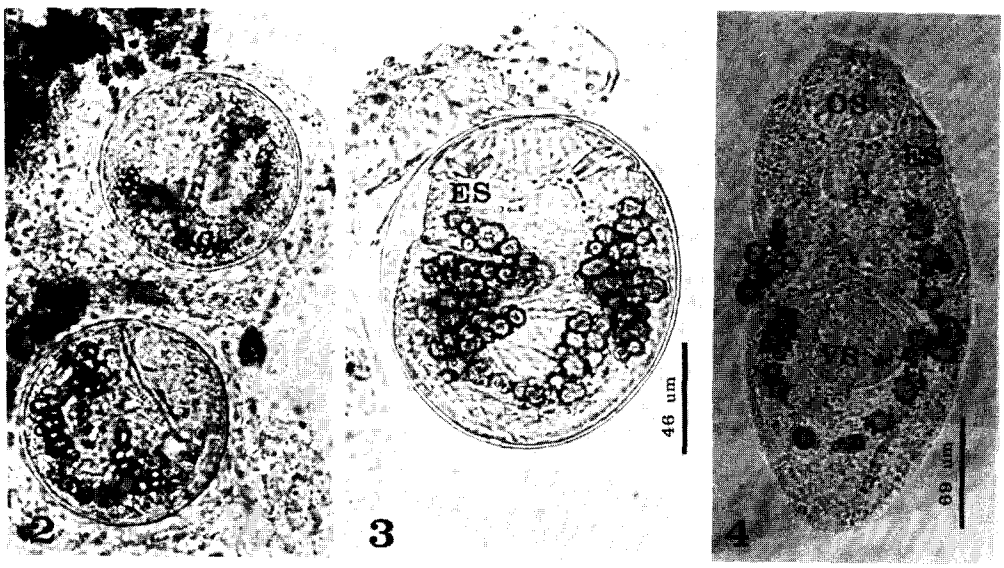
2. Observation of developmental characters in rats and mice

Total 25 albino rats(Sprague-Dawley) and 14 mice(ICR) were given orally 10 or 20 metacercariae each, through the gavage needle. In order to obtain worms of various infection ages, the rats and mice were killed by cervical dislocation after 2, 3, 5, 7, 10, 12, 14, 20, 30, 50 and 88 days. The small intestines of rats and mice were vertically opened by a pair of blunt-tip scissors and dipped in cold normal saline. The flukes were harvested and fixed in 10% neutral buffered formalin. They were stained with Semichon's acetocarmine and observed under light microscope.

RESULTS

1. Infection status of snails with *E. cinetorchis* metacercariae

The metacercariae of *E. cinetorchis* were



Figs. 2~4. *E. cinetorchis* metacercariae.

2. Two metacercariae encysted in the soft tissue of *H. cantori*.
3. A metacercaria isolated from *H. cantori*, showing end group spines(ES).
4. An excysted metacercaria showing oral sucker(OS), pharynx(P) and ventral sucker(VS).

detected from 89(76.7%) out of 116 snails(*H. cantori*) examined. The average metacercarial burden per infected snail was 4.7(Table 1). They were chiefly found from the soft tissue of the snails posterior to the mantle(Fig. 2).

2. Morphology of the metacercariae

The metacercariae were round to elliptical, and 139.2×135.5 μm in average size(Table 2). Many refractile excretory granules and collar spines(especially 5~6 end group ones) were characteristically recognized in the encysted metacercariae(Fig. 3). When excysted, the metacercariae were plump, 279~327 μm long and 69.8~93.8 μm wide(Fig. 4). The measurement data were summarized in Table 2.

3. The recovery rate of worms

The recovery rate of *E. cinetorchis* from the rats, according to the age of infection, was in the range from 40.0% to 85.0%, with overall average value of 55.9%. In case of mice, it ranged from 20.0% to 80.0%, with the average rate of 61.4%(Table 3).

4. Development of *E. cinetorchis* in rats and mice

The measurements of *E. cinetorchis* recovered from rats and mice, by the age of infection, were as summarized in Table 4. The worms obtained from rats were relatively larger than those of same age recovered from mice. They grew slowly for the first 5 days, then rapidly up to 12 days and then slowly thereafter.

The growth pattern curve, in the ratio of posterior body(to ventral sucker)/whole body length was figured to know the general growth pattern in the body length. The pattern was not different in rats and mice, and it was almost a straight line after 10 days postin-

Table 1. Infection status of *E. cinetorchis* metacercariae in *H. cantori*, which were collected from Namyangju-gun, Gyeonggi-do in October, 1985

No. snails examined	116
No. snails positive (%)	89(76.7)
Total number of metacercariae detected	416
Metacercarial density/snail(mean)	1~12(4.7)

Table 2. Measurements of *E. cinetorchis* metacercariae* obtained from *H. cantori*

Organs	length×width (mean) in μm
Cyst	134.0~144.7×126.0~139.4 (139.2×135.4)
Excysted metacercariae	
Body	279.0~327.0×69.8~93.8 (299.0×83.2)
Oral sucker	40.2~48.3×42.9~54.4 (44.7×48.5)
Pharynx	26.8~28.1×21.5~24.1 (27.2×23.2)
Ventral sucker	40.2~53.7×42.9~53.7 (47.5×47.6)
Esophagus	44.0~80.0 (68.0)

* 15 metacercariae were measured.

Table 3. Recovery rate of *E. cinetorchis* from experimentally infected rats and mice

Days after infection	Rats			Mice		
	No. rats infected	No. Mc* given	No. worms recovered(%)	No. mice infected	No. Mc* given	No. worms recovered(%)
2~3	4	60	27(45.0)	2	20	4(20.0)
5	4	40	21(52.5)	2	20	13(65.0)
7	3	30	15(50.0)	2	20	16(80.0)
10~12	2	40	19(47.5)	4	40	28(70.0)
14	4	40	34(85.0)	—	—	—
20~30	4	40	28(70.0)	4	40	25(62.5)
50	2	20	10(50.0)	—	—	—
88	2	20	8(40.0)	—	—	—
Total	25	290	162(55.9)	14	140	86(61.4)

* Metacercariae

Table 4. Measurements of *E. cinetorchis* recovered from experimentally infected rats and mice

Host	Days after infection	No. of specimens measured	Mean length × width (μm)									
			body	head collar	oral sucker	pharynx	esophagus	ventral sucker	cirrus pouch	ovary	Mehlis' gland	testis
Rats	2	10	610 × 171	63 × 117	57 × 61	40 × 40	114	79 × 79	35 × 18	—	—	—
	3	6	815 × 190	71 × 130	64 × 69	53 × 50	147	98 × 98	58 × 34	—	—	—
	5	10	1,369 × 278	83 × 164	75 × 80	72 × 68	151	144 × 140	99 × 52	46 × 41	—	45 × 45
	7	10	2,857 × 486	140 × 278	119 × 131	114 × 112	262	288 × 279	196 × 103	67 × 93	—	68 × 70
	10	9	4,545 × 817	172 × 339	147 × 167	147 × 132	325	358 × 355	228 × 142	91 × 144	144 × 186	187 × 177
	12	5	7,189 × 1,481	210 × 420	171 × 202	173 × 141	424	539 × 524	302 × 169	208 × 372	279 × 460	385 × 348
	14	10	7,429 × 1,758	186 × 411	171 × 191	180 × 137	452	577 × 550	327 × 197	265 × 439	327 × 586	298 × 399
	20	10	9,320 × 2,499	261 × 553	233 × 268	215 × 162	554	707 × 749	389 × 222	400 × 642	397 × 830	—
	30	10	10,605 × 2,620	238 × 507	213 × 260	211 × 167	603	771 × 791	407 × 251	383 × 730	442 × 979	354 × 645
	50	10	12,018 × 3,073	276 × 585	254 × 277	249 × 186	670	909 × 877	534 × 247	433 × 786	522 × 992	517 × 634
	88	8	8,706 × 2,623	247 × 586	222 × 294	276 × 166	541	848 × 902	516 × 264	270 × 724	395 × 907	401 × 645
Mice	2	3	447 × 116	57 × 90	48 × 52	30 × 33	102	58 × 62	21 × 17	—	—	—
	5	10	1,020 × 254	80 × 154	73 × 80	69 × 63	149	124 × 127	56 × 36	41 × 38	—	—
	7	10	2,025 × 383	112 × 219	96 × 110	102 × 84	227	206 × 207	104 × 66	60 × 63	—	—
	10	10	4,605 × 801	158 × 327	140 × 155	144 × 119	295	378 × 366	192 × 111	88 × 157	140 × 185	114 × 109
	12	10	5,464 × 1,307	175 × 412	161 × 189	161 × 128	347	467 × 466	281 × 157	174 × 361	248 × 430	270 × 286
	20	10	7,429 × 1,894	218 × 452	204 × 214	204 × 147	434	619 × 616	305 × 165	230 × 552	329 × 623	352 × 345
	30	9	8,993 × 2,497	221 × 467	206 × 227	210 × 157	525	729 × 765	347 × 221	288 × 727	321 × 829	281 × 451

fection(Fig. 5).

5. Morphological change of worms by the age of infection

There was only a little difference between the developmental patterns of worms in rats and mice. However, full maturation of worms occurred in 12 days in both hosts(Table 5). The 2-day and 3-day juvenile worms were small and plump in shape, and revealed poorly developed genital organs or only their primordial stages(Figs. 6 & 7). The general shape of worms, at 5 to 10 days of age, became more elongated. The ovary, uterine tubule and testis were first recognizable in 5-day old worms(Fig. 8). More convoluted uterine tubules and the cirrus pouch appeared in 7-day old flukes(Fig. 9), and Mehlis' gland and vitellaria were seen in 10-day old ones(Fig. 10). The eggs were firstly observed in the uterus of 12-day old ones(Fig. 11). The worms of 12 days of age or older were quite leaf-like in their shape. Their uteri were compactly filled with eggs, and vitelline follicles were densely distributed in the postero-lateral body of ventral

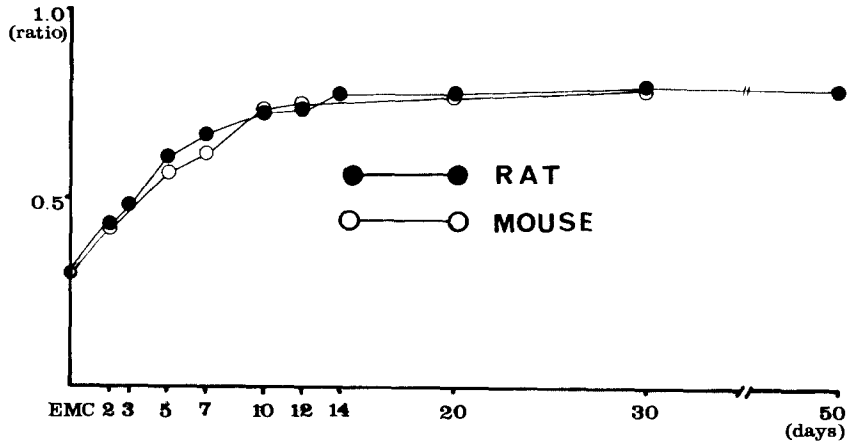


Fig. 5. Growth curves in the ratio of posterior body(posterior to ventral sucker) to whole body length by the age of worms in rats and mice. (EMC : excysted metacercariae)

sucker(Figs. 12, 13 & 14).

Out of 194 worms of various infection ages, 159(82.0%) were with no testis(Fig. 15), 34 (17.5%) had one testis(Fig. 16) and only 1 worm had two testes(Fig. 17). The testis was recognized in 4.2% of 5-day old worms, 6.9%

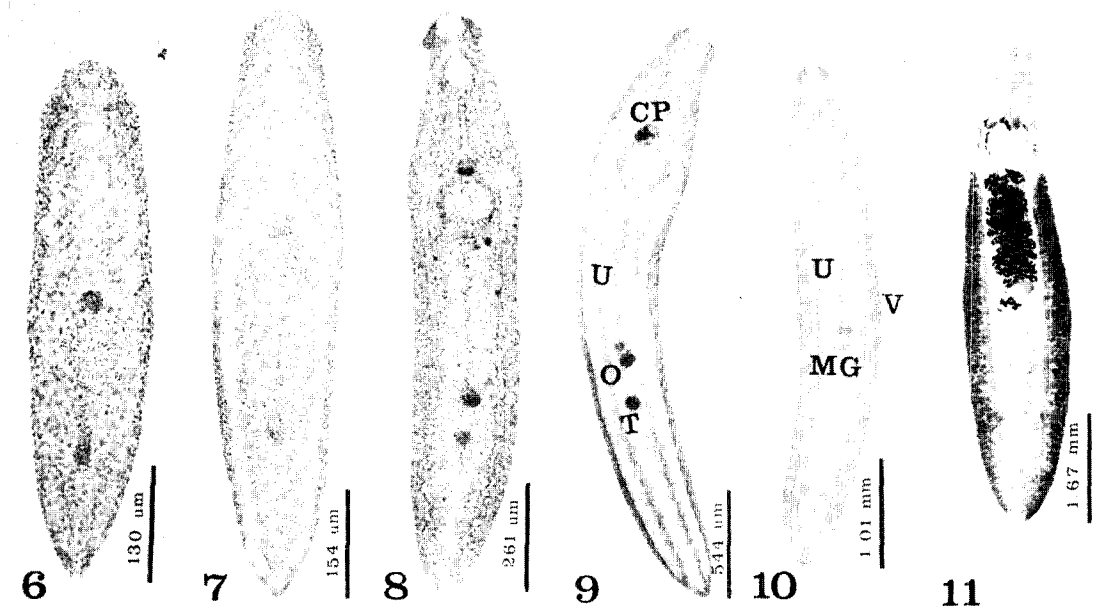


Fig. 6-11. Developmental stages of *E. cinetorchis* recovered from albino rats.

6. A 2-day old worm.
7. A 3-day old worm.
8. A 5-day old worm showing an ovary, testis and uterine tubule.
9. A 7-day old worm showing cirrus pouch(CP), uterus(U), ovary(O) and a testis (T).
10. A 10-day old worm showing more convoluted uterine tubule(U), vitellaria (V) and Mehlis' gland(MG).
11. A 12-day old worm showing many eggs in uterus.

Table 5. Newly appeared organs of *E. cinetorchis* according to the age of infection

Days after infection	Worms recovered from	
	Rats	Mice
2	*	*
3	*	not observed
5	immature ovary, testis, uterine tubules	*
7	ovary, cirrus pouch, convoluted uterine tubules	ovary, uterine tubules
10	Mehlis' gland, vitelline follicles, more convoluted uterine tubules	cirrus pouch, Mehlis' gland, vitelline follicles, convoluted uterine tubules
12	eggs in uterus, enlarged vitelline follicles	eggs in uterus, enlarged vitelline follicles
14	fully matured	fully matured

* All genital organs were at their primordial stages.

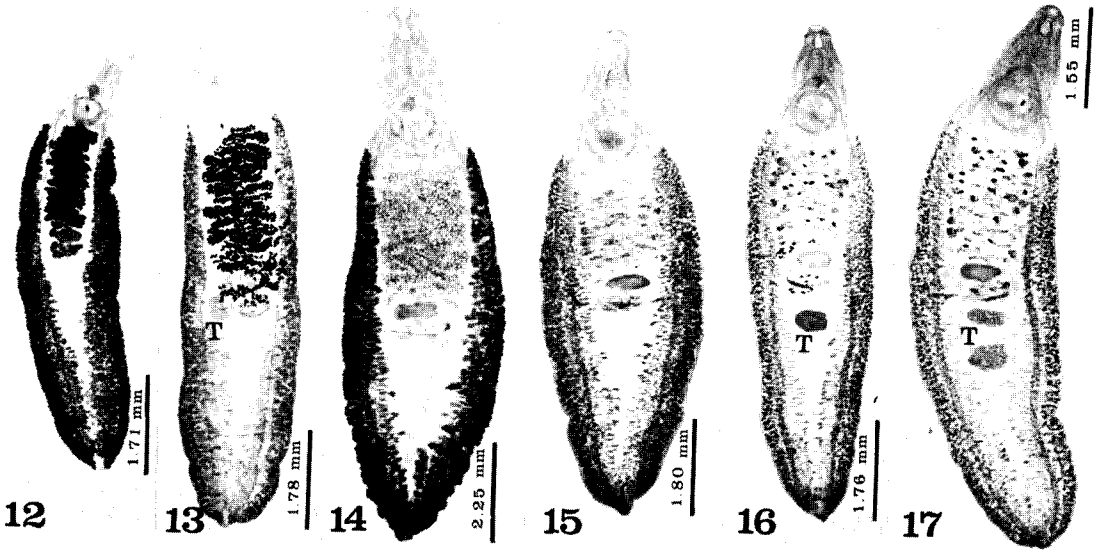


Fig. 12-17. Developmental stages of *E. cinetorchis* recovered from albino rats.
12. A 14-day old worm. **13.** A 20-day old worm showing an abnormally located testis(T).
14. A 50-day old worm. **15.** A 88-day old worm showing no testis.
16. A 88-day old worm showing one testis. **17.** A 88-day old worm showing two testes.

of 7-day old ones, 17.9% of 10-day old, 21.1 % of 12-day old, 20.0% of 14-day old, 25.0% of 17-day old, 16.7% of 20-day old, 26.3% of 30-day old, 30% of 50-day old and in 50% of 88-day old specimens(Table 6), which shows an increasing tendency as the age of worms increased.

DISCUSSION

With the present study, it has been verified that a kind of fresh water snail, *Hippeutis (Helicorbis) cantori*, acts as a second intermediate host of *E. cinetorchis* in Korea. Several

Table 6. Number of testes by the age of *E. cinetorchis*

Days after infection	No. worms studied	No. worms with testis			No. of worms without testis
		One testis	Two testes	Total(%)	
5	24	1	0	1 (4.2)	23
7	29	2	0	2 (6.9)	27
10	28	5	0	5 (17.9)	23
12	19	4	0	4 (21.1)	15
14	15	3	0	3 (20.0)	12
17	12	3	0	3 (25.0)	9
20	30	5*	0	5 (16.7)	25
30	19	5	0	5 (26.3)	14
50	10	3	0	3 (30.0)	7
88	8	3	1	4 (50.0)	4
Total	194	34	1	35 (18.0)	159

* situated in the left side of Mehlis' gland in a worm

kinds of fresh water snails, namely *Segmentina mica*, *Planorbis compressus japonicus*, *Lymnaea japonica*(Takahashi, 1926), *Viviparus malleatus* (Hirasawa, 1926) and *Cipangopaludina japonica* (Kurisu, 1930), are already known to serve as second intermediate hosts of *E. cinetorchis* in Japan. *H. cantori* is taxonomically placed in the subfamily Segmentininae and family Planorbidae, which belong to the subclass Pulmonata and class Gastropoda(Pace, 1973; Beaver *et al.*, 1984). These snails chiefly live in rice paddies and irrigation ditches, and they have drawn medical attentions because of their role as a first intermediate host of *Fasciolopsis buski* or *Fibricola seoulensis* (Pace, 1973; Lee *et al.*, 1986; Seo *et al.*, 1988).

The infection rate of *H. cantori* with *E. cinetorchis* metacercariae was 76.7%, which is a relatively high rate compared with reports in Japan. According to Takahashi(1926), metacercarial infection rates of snails, *S. mica*, *P. compressus japonicus* and *L. japonica*, were 8.3%, 3.5% and 2.5% respectively. Kurisu (1930) reported 4.7%, 6.1%, 2.0% and 5.2% metacercarial infection rates when they examined *C. japonica*. On the other hand, Hirasawa (1926) showed higher infection rates of 65%, 72% and 57% in *V. malleatus* caught from various areas of Japan. The infection rate of snails with trematode metacercariae can be variable not only by the collection date and locality, but also by many kinds of host-parasite factors. It was also a unique feature in this study that the infection rate of snails was high, but the metacercarial burden per infected snail was relatively low.

Takahashi(1923) mentioned that the habitat of *E. cinetorchis* metacercariae in snails was mainly in their liver, mantle and tissue of the head in case of *S. mica*, and mantle and head tissue but not the liver in case of *P. compressus japonicus* and *L. japonica*. In order to know the micro-habitat of trematode larvae in small snails such as *H. cantori*, the subjected animals must be serially sectioned. The snail crushing method was used in this study, and the metacercariae were chiefly found from the soft tissues near the mantle of *H. cantori*. This finding partly agrees to the previous reports.

There are some difficulties in the identification of metacercariae of family Echinostomatidae because they are morphologically very similar. Especially the metacercariae of *E. cinetorchis* have poorly developed collar spines (Saito and Tani, 1982). Therefore, in this study, those metacercariae diagnosed tentatively as *E. cinetorchis* were identified later by adult worms obtained after experimental infection to

rats.

Seo *et al.* (1984) reported that the recovery rate of *E. cinetorchis* from experimentally infected rats was 15.0~40.7% (24.6% in average), and the rate decreased significantly as the duration of infection was prolonged. In the present study, the worm recovery rate from rats or mice was not much affected by the duration of infection, but rather variable by host individuals. The average rates were 55.9% from rats and 61.4% from mice, which were much higher values compared with that of Seo *et al.*(1984).

The growth curves of *E. cinetorchis*, in the ratio of length of posterior body (to ventral sucker)/whole body, in rats and mice, were not different between hosts. The growth curve was very steep during the first 12 days but it became nearly straight thereafter. It may be useful as a criterion for the age of worms younger than 12 days.

One of the characteristic features in the morphology of *E. cinetorchis* is abnormal location and reduction in number of testis (Ando and Ozaki, 1923). Takahashi(1927) and Takahashi *et al.*(1930 a & b) mentioned that the majority of *E. cinetorchis* they collected from human cases or from experimental rats had single testis and sometimes no testis. Seo *et al.* (1980 & 1984) observed total 31 specimens of *E. cinetorchis* which were classified into 1 worm with two testes, 12 with one testis and 18 with no testis. A similar result in the present study was that out of 194 specimens observed only 1 had two testes, 34(17.5%) one testis and 159(82.0%) had no testes. A worm revealed abnormal location of one testis which was situated in the left side of Mehlis' gland. Although it is an intriguing feature of this fluke, it is at present hard to explain what kind of factors operate in this gonad-moving or vanishing phenomenon.

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=국문초록=

담수산 패류 *Hippeutis cantori*에 피낭한 이전고환극구흡충의 피낭유충 및 흰쥐와 마우스에서의 성장발육

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1985년도 8월부터 10월사이에 경기도 남양주군 와부읍에서 채집한 *Hippeutis* (*Helicorbis*) *cantori*가 이전고환극구흡충(*Echinostoma cinetorchis*)의 피낭유충에 감염되어 있음을 발견하였고, 이 피낭유충들을 흰쥐 및 마우스에 10~20개씩 경구 감염시킨 다음 2~88일에 충체를 회수하였으며 회수한 충체를 토대로 충체 발육상황을 관찰하였다. 그 결과를 요약하면 다음과 같다.

1. 조사한 116마리의 *H. cantori*중 89마리(76.7%)에서 416개의 이전고환극구흡충 피낭유충이 검출되어 감염패 마리당 평균 4.7개의 감염량을 나타내었다. *E. cinetorchis*의 피낭유충은 패류의 맨틀 뒷부분에 있는 연부조직에서 검출되었다.

2. 피낭유충은 타원형 또는 원형에 가까운 난원형이었으며 크기는 평균 139.2×135.4μm이었다.

3. 실험감염 흰쥐 및 마우스에서의 충체회수율은 각각 40~85%(평균 55.9%) 및 20~80%(평균 61.4%)이었다. 충체의 성장발육은 흰쥐와 마우스에서 별 차이가 없었고, 충란을 보유한 성충으로 성장하는데 12일이 소요되었다.

4. 조사한 총 194마리의 충체 중 고환이 하나도 없는 것이 159마리(82.0%)이었고, 한 개만 있는 것이 34마리(17.5%)이었으며 두 개가 있는 것은 1마리(0.5%)이었다.

이상의 결과로 *H. cantori*가 우리나라에서 이전고환극구흡충의 제2중간숙주의 하나로 역할을 하고 있음이 확인되었다. 또한 이 흡충이 흰쥐 및 마우스 소장에서 충란을 보유한 성충으로 성장하는데에는 약 12일이 소요됨을 알 수 있었다.