기 생 충 학 잡 지 제22권 제1호
The Korean Journal of Parasitology
Vol. 22, No. 1, 51-60, June, 1984

Studies on Intestinal Trematodes in Korea

XIII. Two Cases of Natural Human Infection by *Heterophyopsis continua* and the Status of Metacercarial Infection in Brackish Water Fishes

Byong-Seol Seo, Soon-Hyung Lee, Jong-Yil Chai and Sung-Jong Hong
Department of Parasitology and Institute of Endemic Diseases,
College of Medicine, Seoul National University

INTRODUCTION

Heterophyopsis continua, one of the fluke family Heterophyidae, is the minute intestinal trematodes of fish-eating birds and mammals. This fluke was firstly described by Onji *et* Nishio(1916) from the cats experimentally fed with the mullets, *Mugil cephalus*, harbouring the metacercariae in Japan. Afterwards the metacercariae were found from more than 10 kinds of fresh and brackish water fishes in the Far Eastern countries (Yamaguti, 1939a; Kanemitsu *et al.*, 1953; Chun, 1960; Komiya, 1965; Kobayasi, 1968). Although these fishes are preferably eaten raw by humans in many countries there is only one paper on the natural human infection with this fluke in Japan (Yamaguti, 1939b).

In southeastern coastal areas of Korea, the brackish water fishes such as the perches, *Lateolabrax japonicus*, and the gobies, *Acanthogobius flavimanus*, were reported to harbour the metacercariae of *H. continua* (Chun, 1960). However, no further study has been performed either on the fishes in other areas or on the definitive hosts including man. The present authors found two natural human cases of *H. continua* infection by obtaining adult worms after treatment with praziquantel. And in order to know the source of this fluke infection a study on fish intermediate hosts was undertaken along the southwestern coastal areas. The metacercariae obtained in this study were experimentally fed to two laboratory rats and a young dog and the adult worms recovered from the dog were identified as *H. continua*.

* This study was supported by the Grant from the Ministry of Education, Republic of Korea(1983).

CASES DESCRIPTION

Case 1: Han, O.O., 24-year old male residing in Seoul but whose native village is a coastal area of Kohŭng-gun, Chollanam-do (Case 1 of Seo *et al.*, 1984). He had the helminthic infections of *Diphyllobothrium latum*, *Heterophyes heterophyes nocens*, *Stellantchasmus falcatus* and *Stictodora* sp. concommitantly with *H. continua* (2 adult worms). He complained of easy fatigability, weakness, indigestion, abdominal pain and cardiac arrhythmia. He said he used to eat raw flesh of brackish water fishes such as the mullets, perches and gobies and treated with 15 mg/kg praziquantel (Distocide) followed by purgation with 30 g magnesium sulfate. This paper only deals with *H. continua* infection.

Case 2: Kang, O.O., 50 year old male residing in Namhae Island, Kyŏngsangnam-do. He visited our Department on November 1983 with the complaints of epigastric pain, weakness, diarrhea, dizziness and palpitation since 10 years ago. He had the history of *Clonorchis sinensis* infection, however, stool examination at the time of his visit to our Department revealed only the eggs of *Ascaris lumbricoides* and *Tri-*

churis trichiura. Oxantel-pyrantel tablet at the dose of 10mg/kg was given to treat these nematode infections, and at the same time, praziquantel 15mg/kg was also given followed by purgation with 30 g magnesium salt to treat any possible coexistent infection of liver or intestinal flukes. Through examination of the successive diarrheal stools 46 specimens of *H. continua* were collected concommitantly with one *A. lumbricoides* and 63 *Enterobius vermicularis* worms. He is the manager of a fishery company and have eaten raw flesh of many kinds of brackish water fishes including the mullets, perches and gobies.

MATERIALS AND METHODS

Fish Examination:

A total of 103 brackish water fish were purchased from the local markets of 8 different areas along the southwestern coasts (Fig. 1). They were 42 mullets (23-32 cm long), 27 perches (13-22cm) and 34 gobies (22-39cm). The fish were transported to laboratory under refrigeration. In order to discriminate the fish which harbour the metacercariae a total of 10-12 pieces of flesh were taken from both lateral sides of each fish. The pieces of muscle were compressed between two slide glasses and examined under stereomicroscopy to find out the metacercariae of *H. continua*.

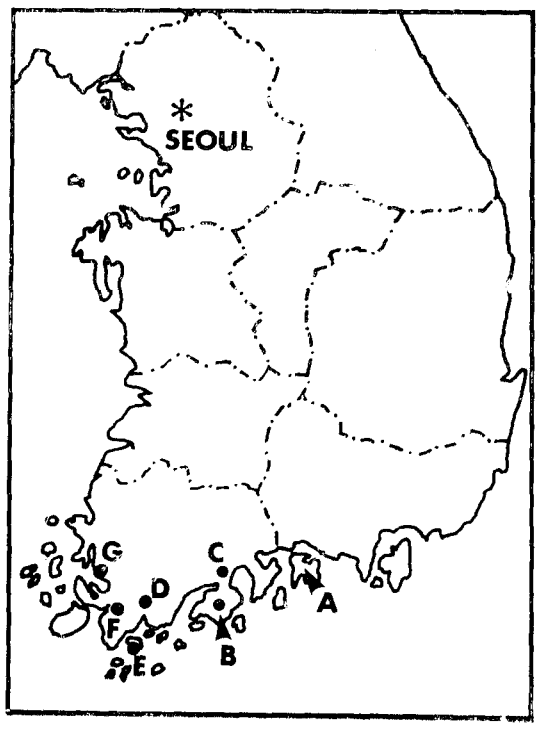

Fig. 1. Areas where the human cases were found (arrows) and the brackish water fishes purchased (black dots). (A: Namhae Island, B: Kohŭng, C: Beolgyo, D: Kangjin, E: Wando, F: Haenam, and G: Mokpo)

Many of the perches and gobies caught at Beolgyo or Wando (Fig. 1) appeared to harbour the metacercariae (Table 1), therefore, the fish were further examined for metacercarial density. The whole bodies of these fish were individually ground in a mortar with pestle, digested with artificial gastric juice in 37°C incubator for 24 hours, and the metacercariae isolated were collected and counted under stereomicroscopy.

For the purpose to figure out the metacercarial distribution in the perches, the body of each fish was divided into 4: head, proximal, middle and caudal parts (Fig. 2). The separated parts were ground, artificially digested, and the metacercariae in each part of each fish were counted.

Animal Experiment:

In order to obtain adult worms from laboratory animals, the metacercariae isolated were experimentally fed to two laboratory rats and a young dog. Actively moving metacercariae, 10 and 12 in numbers, were given to the rats through polyethylene capillary tubes inserted to stomach under ether anesthesia. The dog was infected *per os* with 477 active or less active metacercariae.

The rats and the dog were killed 6 days and 12 days after infection respectively and the whole length of small intestine was resected. The intestinal lumen was opened with a pair of scissors and the mucosal contents examined for heterophyid flukes under stereomicroscopy.

RESULTS

The metacercariae of *H. continua* were found from 17(63.0%) out of 27 perches and 10(29.4%) of 34 gobies but none of 42 mullets examined was found to harbour the metacercariae (Table 1). And all of 10 gobies positive for the metacercariae were those caught from Wando (Fig. 1). Other 22 gobies caught from Beolgyo and Mokpo were free from the metacercariae.

The total number of the metacercariae collected from 9 perches and 10 gobies were 499 and 187 respectively. Therefore, the average metacecarial density per fish were 55.4 in perches and 18.7 in gobies (Table 2). The metacercariae were isolated from both head and body portions of 9 perches examined (Fig. 2 and Table 3). However, only 42 (8.4%) metacercariae among 499 collected were found from the head and remaining 457 (91.6%) from the body portions. The status of metacercarial distribution in body was not much different in the proximal, middle and caudal portions.

Table 1. Infection rate of brackish water fishes with the metacercariae of *H. continua* according to kinds of fish and localities

Fish	Locality*	No. exam.	No. posit. (%)
Mugil cephalus	Kangjin	10	0 (0.0)
	Mokpo	7	0 (0.0)
	Haenam	8	0 (0.0)
	Beolgyo	9	0 (0.0)
	Kohŭng	8	0 (0.0)
	Subtotal	42	0 (0.0)
Lateolabrax japonicus	Beolgyo	27	17(63.0)
Acanthogobius flavimanus	Mokpo	12	0 (0.0)
	Beolgyo	10	0 (0.0)
	Wando	12	10(83.3)
	Subtotal	34	10(29.4)

* The localities are indicated in Fig. 1.

Table 2. Metacercarial density of *H. continua* in brackish water fishes

Fish	Locality	No. fish examined	Total No. metacercariae (/fish)
Lateolabrax japonicus	Beolgyo	9	499(55.4)
Acanthogobius flavimanus	Wando	10	187(18.7)

Table 3. Status of metacercarial distribution in *L. japonicus*

Topography* in fish	Total No. metacercariae collected(% to total)
1	42 (8.4)
2	175(35.1)
3	153(30.7)
4	129(25.9)
Total	499

* Vide Fig. 2.

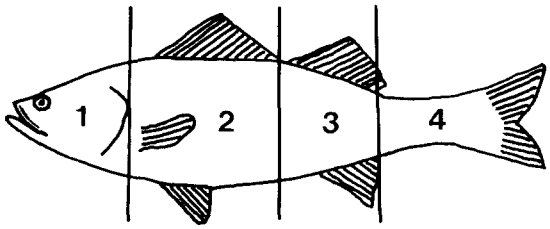

Fig. 2. Topography of a perch to figure out the metacercarial distribution.

Table 4. Worm recovery results from the experimental animals

Animal	No. metacercariae given	No. worms recovered(%)
Rat A	10	0(0.0)
Rat B	12	0(0.0)
Dog	477	2(0.4)

Not a single worm was recovered from the intestine of two rats fed with 10 and 12 metacercariae each (Table 4). From the dog, however, two 12-day old worms of *H. continua* were recovered from the middle part of its small intestine.

PARASITOLOGICAL DESCRIPTIONS

Worms from Human Cases (*Heterophyopsis continua*):

A total of 12 worms (2 from Case 1 and 10 from Case 2) were observed and measured after fixation with 10% formalin. Body elongate and

leaf-like, posteriorly subcylindrical, 2.0～2.8mm long and 0.42～0.53mm wide (Fig. 3 & 4). Culticular spines prominent over anterior half of body, gradually fading to posterior end. Oral sucker subterminal, 0.10～0.13mm in diameter. Prepharynx 0.07～0.30mm long but esophagus very short or nearly absent. Pharynx muscular, 0.10～0.13 by 0.08～0.12mm in size. Intestinal bifurcation about one-fourth or one-fifth portion from the anterior end of body. Ceca extending to posterior end of body. Ventral sucker median, large, muscular, posterior to intestinal bifurcation, 0.16～0.20mm in diameter. Genital sucker nearly round, posterosinistral to but separated from ventral sucker, ventrally protruded and 0.13～0.21mm in diameter, armed with 92～115 sclerotized spines in a single row. Seminal vesicle "L" shape, consisted of two parts junctioned by a constriction, 0.15～0.24 by 0.09～0.13mm in size for longitudinal part and 0.09～0.24 by 0.09～0.12mm for transverse one. Ejaculatory duct opens into genital atrium surrounded by armed gonotyl of genital sucker. Cirrus pouch absent. Ovary globular, median, postequatorial, 0.08～0.18mm in diameter. Vitelline follicles small forming 40～50 groups and distribute lateral fields of greater middle portion of posterior body. Two testes a little obliquely tandem, globular in shape near posterior one third body, 0.12～0.23 and 0.11～0.22mm in diameter respectively. Excretory canal sigmoid and tubular in shape and excretory pore terminal. Intrauterine eggs small but broadly oval in shape with relatively thick shells, 0.025～0.027 by 0.014～0.016mm in size, containing mature miracidia.

Fig. 3. *H. continua*, an adult worm from Case 1 directly after fixation. Note the elongated body form, relationship of ventral and genital suckers, and obliquely tandem testes. (Scale: 0.2mm)

Fig. 4. *H. continua* from Case 2 after acetocarmine staining. (Scale: 0.2 mm)

Metacercariae of *H. continua* from Brackish Water Fishes:

The observation and measurement of the metacercariae were based on 10 encysted and 10 excysted specimens isolated from the muscle of the perches, *L. japonicus*. Metacercarial cyst approximately round (Fig. 5), very large in size compared with other heterophyid metacercariae, 0.31～0.38mm (average 0.35mm) in diameter. Cyst wall about 0.006mm thick. Encysted worm actively moving and bending forward within the cyst. Oral, ventral and genital suckers are well seen.

Excysted metacercariae flat and elongate (Fig. 6), 0.92mm long and 0.35mm wide, beset with small scale-like spines which become more sparsely distributed in posterior body. Oral sucker subterminal, muscular, 0.10mm in diameter. Ventral sucker larger than oral sucker,

median, preequatorial, 0.14mm in diameter. Prepharynx long but esophagus very short. Ceca terminating at posterior end of body. Genital sucker posterosinistral to ventral sucker, round to elliptical in shape, 0.08 by 0.12mm in size, ventrally protruded and armed with small sclerotized spines. Ovary primordia round, median, posterior to genital sucker, 0.09mm in diameter, at anterior margin of excretory bladder. Two testes primordia round to elliptical, 0.09 by 0.04mm for anterior one and 0.10 by 0.06mm for posterior one in size, lying obliquely on both lateral sides of excretory bladder. Excretory bladder "Y" shape containing numerous dark excretory granules and extending to posterior end of body.

Adult Worms Recovered from Experimental Dog

Two adult worms experimentally obtained after feeding the metacercariae to a young dog were identified as *Heterophyopsis continua* (Onji *et* Nishio, 1916). The worms were 2.5 and 2.9mm long and 0.49 and 0.50mm wide at equatorial level. Body elongate with slightly tapering anterior end and bluntly ending posterior body (Fig. 7). Genital sucker prominent and armed with 97 (Fig. 8) and 104 spines in a single row. Intrauterine eggs small, 0.024～0.026 by 0.015～0.016mm in size, broadly oval in shape (Fig. 9), containing mature miracidia. Other morphological characteristics were not different from those recovered from human cases. The measurements of these worms were given in Table 5 in comparison with other authors.

DISCUSSION

When this fluke was firstly discovered in Japan by Onji *et* Nishio (1916) it was named as *Heterophyes continus*. Quite independently in the Philippines Africa *et* Garcia (1935) described *Heterophyes expectans* from dogs. Later Tubangui *et* Africa (1938) created a new genus, *Heterophyopsis*, for the latter fluke. On the other hand, Yamaguti (1939a) also proposed a new genus, *Pseudoheterophyes*, for the former fluke which is morphologically not different from the latter, additionally describing a new subspecies *P. continua major*. The new generic characteristics different from the classical *Heterophyes* Cobbold, 1886 were the elongated body shape, positions of pharynx and testes, post-testicular extension of uterus, *etc.* (Yamaguti, 1939a). In spite of morphological similarity Morosov (1952) accepted both new genera. Later, however, Yamaguti (1958) acknowledged only the genus *Heterophyopsis* and enlisted 2 species and one subspecies belonging to this genus; *H. expectans*, *H. continua* and *H. continua major*.

However, Africa *et al.* (1940) described in their review paper that the former two species are morphologically identical and they (Africa *et* Garcia, 1935) were unaware of the early existence of *H. continua* when they discovered *H. expectans* in Manila. In case of the subspecies *H. continua major*, the matacercariae and adults were found from fishes and birds respectively in Japan (Yamaguti, 1939a). However, the major differences in adult morphology from *H. continua* were only the body size (Table 5) and position of ventral sucker, which are now considered intraspecific variations by the authors. Moreover, according to Komiya (1965), the metacercariae of *H. continua* and *H. continua major* were hardly distinguishable each other. The present authors compared the morphological characteristics and measurements of the adults of three *Heterophyopsis* species (or subspecies) with the present specimens (Table 5) and came to the conclusion that they are not different from one another. And all worms collected in the present study were identified as *Heterophyopsis continua*.

So far as available literature are concerned there is no record on the first intermediate host of *H. continua*. Some kinds of brackish water snails ecologically related with fish hosts described below may be responsible, however, it remains to be elucidated in the future.

The fish intermediate hosts of *H. continua* recorded so far are *M. cephalus*, *Harengula*

Table 5. Comparison of the measurements of *Heterophyopsis* spp. and the present specimens from man and dog (Scale: mm)

Item	*H. continua*		*H. expectans*	*H. continua major*	Present specimens	
	Onji *et* Nishio (1924)	Yoshikawa *et al.* (1940)	Africa *et* Garcia (1935)	Yamaguti (1939)		
Host	bird	dog	dog	bird	man	dog
Body length	2.15	1.84~2.16	1.5~2.2	2.6~3.55	2.0~2.8	2.5~2.9
Body width	0.43	0.43~0.48	0.2~0.3	0.3~0.4	0.42~0.53	0.49~0.5
Oral sucker	0.1	0.09~0.11	0.06~0.09	0.08~0.09	0.1~0.13	0.09~0.1
Pharynx	0.1 ×0.07	0.05~0.08 ×0.08~0.12	0.06~0.09 ×0.05~0.07	0.07~0.1 ×0.05~0.08	0.1~0.13 ×0.08~0.12	0.1 ×0.07~0.09
Ventral sucker	0.17	0.19~0.21	0.08~0.1 ×0.08~0.13	0.16~0.2	0.16~0.2	0.19~0.2
Genital sucker						
Size	0.14	0.15~0.17	?	0.11~0.15	0.13~0.21	0.15~0.18
No. rodlets	89~125*	100~110	86~88**	90~100	92~115	97~104
Ovary	0.1	0.1~0.17	0.09~0.16	0.15~0.17	0.08~0.18	0.15~0.18
Testes	0.16~0.2 ×0.14~0.19	0.13~0.24 ×0.12~0.24	0.12~0.29 ×0.11~0.22	0.2~0.28 ×0.18~0.25	0.11~0.23 ×0.11~0.23	0.24~0.3 ×0.26~0.33
Eggs(Intrauterine)	0.025 ×0.015	0.023~0.027 ×0.016~0.017	0.022~0.026 ×0.014~0.018	?	0.025~0.027 ×0.014~0.016	0.024~0.026 ×0.015~0.016

*Counted in the specimens from cats
**Counted by Velasquez (1973) in the specimens from cats

zunasi, *Dorosoma thrissa*, *Coilia* sp. and *L. japonicus* in Japan (Kanemitsu *et al.*, 1953; Komiya, 1965), *L. japonicus*, *A. flavimanus* and *Clupanodon punctus* in Korea (Chun, 1960) and *Cyprinus carpio*, *Mugil affinis*, *Gobius nebulosus* and *Boleophthalmus pectinirostris* in Southern China (Kobayasi, 1968). In the present study two kinds of brackish water fishes, *L. japonicus* and *A. flavimanus*, were all positive for the metacercariae of *H. continua* while none of *M. cephalus* harboured the metacercariae. But it can not be ruled out that this may be due to much low density of metacercariae of this fluke in *M. cephalus*.

In the southeastern coasts of Korea the metacercarial density in *L. japonicus* and *A. flavimanus* was extremely low, 1~5 metacercariae per the former and only 1 from a total of 10 latter fish examined (Chun, 1960). This is quite comparable to the results of the present study in the southwestern coasts, where average numbers of 55.1 and 18.7 metacercariae per fish were collected respectively from the two kinds of fishes. This probably means some differences in the prevalence of this fluke between two areas.

The natural and/or experimental definitive hosts of *H. continua* are cats (Onji *et* Nishio 1916 & 1924; Kanemitsu *et al.*, 1953), dogs (Yoshikawa *et al.*, 1940; Yamaguti, 1939b; Kanemitsu *et al.*, 1953; Chun, 1960), ducks (Onji *et* Nishio, 1916 & 1924), sea-gulls (Yamaguti, 1939) and man (Yamaguti, 1939b). The rodents seem to be not suitable hosts for this fluke infection considering the unsuccessful results of experimental infection to guinea pigs (Chun, 1960) and to laboratory rats in the present study.

The present paper is the second record on human infection with this fluke in the world, so far as the literature are concerned. The rarity of human cases in spite of frequent raw fish eating by the people in many countries may provide some suggestions. Firstly, this fluke may not be widely prevalent and may exist within some restricted localities. Secondly, the amount of egg production by the fluke may not be sufficient to be detected easily in human stools unless one harbours numerous flukes. Both

of the human cases in the present report did not reveal any eggs of *H. continua* in spite of 46 adult worms infected in Case 2. However, extensive epidemiological study and keen observation on the heterophyid eggs in human feces woud be helpful to detect further cases.

There has been no information on the clincal symptoms due to this fluke infection in man. The Case 2 in the present study had the complaints of epigastric pain, diarrhea and palpitation since 10 years ago. But it is not clear whether such symptoms were directly related with *H. continua* infection or with other concommitant nematode infections (*A. lumbricoides*, *T. trichiura* and *E. vermicularis*) or other unknown reasons. The Case 1 also had the similar complaints but he was also concommitantly infected with *D. latum* and 3 other kinds of heterophyid flukes (*H. heterophyes*, *S. falcatus* and *Stictodora* sp.). Therefore, the symptoms only due to *H. continua* infection are hardly discriminated in the present cases. The clinical problem of heart palpitation in both cases raises a possibility that *H. continua* may as well cause cardiac heterophyidiasis as other heterophyid flukes such as *Haplorchis*, *Stellantchasmus* and *Procerovum* spp. (Africa *et al.*, 1940). However, there is no way to verify it now and further studies are required.

SUMMARY

Two cases of natural human infection by *Heterophyopsis continua* (Heterophyidae) were identified by collection of adult worms in 1983 in Korea. And in order to know the source of infection a study on fish intermediate hosts was performed along the southwestern coastal areas.

The cases were 24 and 50 year old males residing in southern coastal areas. They had the clinical complaints of non-specific gastrointestinal symptoms such as epigastric pain and one of them also complained the discharge of tapeworm segments. Praziquantel in single dose of 10~15 mg/kg was given followed by purgation with magnesium salt and 2 and 46 specimens of *H. continua* were collected from the diarrheal stools. They said to have eaten raw flesh of several kinds of brackish water fishes.

The results of fish examination for metacercarial infection were as follows:

1. Seventeen (63.0%) out of 27 perches (*Lateolabrax japonicus*) and 10 (29.4%) out of 34 gobies (*Acanthogobius flavimanus*) harboured the metacercariae but none of 42 mullets (*Mugil cephalus*) were found to harbour them.

2. The average metacercarial density in perches was 55.1 and 18.7 per fish respectively. And the majority of the metacercariae were collected from the muscle of body portions in these fishes.

3. After the metacercariae were experimentally fed to two rats and one young dog, two adult worms were recovered from the dog while none from the rats. These worms were also identified as *H. continua*.

From the results it was concluded that the brackish water fishes which the human cases said to have eaten were the source of infection.

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=國文抄錄=

韓國의 腸吸蟲에 관한 硏究

XIII. *Heterophyopsis continua*에 의한 人體感染 2例 및 半鹽水産 魚類에 있어서 被囊幼蟲 感染狀況

서울大學校 醫科大學 寄生蟲學敎室 및 風土病硏究所
徐丙喆・李純烱・蔡鍾一・洪性琮

半鹽水産 魚類를 中間宿主로하여 매개되는 *Heterophyopsis continua*에 의한 人體感染 2例가 1983년 praziquantel 치료로 成蟲을 얻음으로써 확인되었다. 또, 정확한 感染源을 알아보기 위하여 全羅南道 海岸地方에서 잡은 半鹽水魚에 있어서의 被囊幼蟲 感染狀況을 調査하였다.

患者는 24세 및 50세된 男子로 南海岸 地方이 고향이며 上腹部痛, 泄瀉 등 일반적인 소화기 증상을 호소하거나 條蟲類의 片節이 排出됨을 호소하여 praziquantel 10-15 mg/kg을 單回 투여하고 下劑를 使用하여 治療하였다. 그 結果 條蟲은 물론 2例에서 각각 2마리 및 46마리의 *H. continua* 吸蟲이 泄瀉便으로부터 수집되었다. 患者들은 몇 가지 종류의 半鹽水魚를 生食한 경력이 있었다.

魚類 中間宿主 調査結果는 다음과 같다.

1. 檢査한 27마리의 농어(*Lateolabrax japonicus*)중 17마리(63.0%), 34마리의 문절망둑(*Acanthogobius flavimanus*)중 10마리(29.4%)에서 *H. continua*의 被囊幼蟲이 檢出되었으나 42마리의 숭어(*Mugil cephalus*)는 모두 음성으로 나타났다.

2. 농어 및 문절망둑에 있어서 마리당 平均感染 被囊幼蟲數는 55.4 및 18.7 個이었고 대부분의 被囊幼蟲은 농어의 경우 몸체부 근육에서 發見되었다.

3. 被囊幼蟲을 實驗的으로 2마리의 흰쥐 및 1마리의 어린 개(犬)에 感染시켜 成蟲을 얻고자 한 바 흰쥐에서는 얻을 수 없었고 개에서 2마리의 *H. continua* 成蟲을 얻을 수 있었다.

이상의 결과로 보아 患者들이 生食한 농어, 문절망둑 등 半鹽水産 魚類가 이들에 있어서 *H. continua*의 感染源이었을 것으로 추정할 수 있었다.

EXPLANATIONS FOR FIGURES

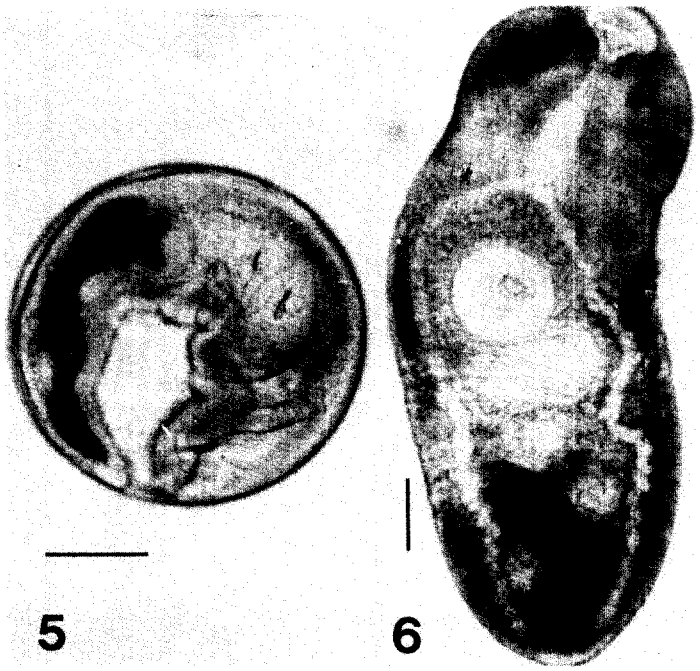
Fig. 5. Metacercaria of *H. continua*, encysted, from *L. japonicus*. Note ventral and genital suckers near equatorial portion of body and dark "Y" shape excretory bladder. (Scale: 0.1mm)

Fig. 6. *Ibid*, excysted, from *L. japonicus*. Note three suckers and ovary and testes primordia adjacent to "Y" shape excretory bladder. (Scale: 0.1mm)

Fig. 7. Adult of *H. continua* from the experimental dog directly after fixation. Note general body feature, suckers and obliquely tandem testes. (Scale: 0.2mm)

Fig. 8. Magnification of genital sucker of the worm in Fig. 7. A total of 97 rodlets (spines) are counted along its outer margin. (Scale: 0.05mm)

Fig. 9. Magnification of distal uterus showing many mature eggs. (Scale: 0.05mm)



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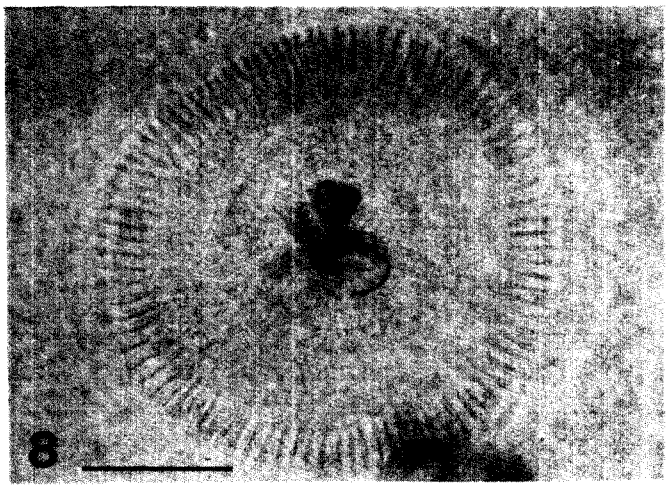

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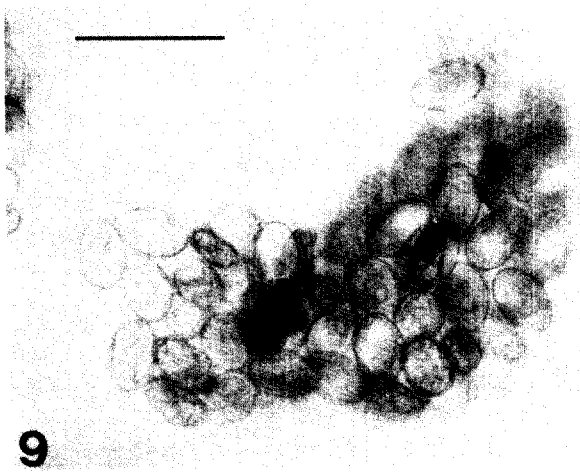

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