기 생 충 학 잡 지 제28권 제1호
The Korean Journal of Parasitology
Vol. 28, No. 1, 53-62, March 1990

Recovery rate, growth and development of *Heterophyopsis continua* in experimental chicks#

Sung-Jong Hong, Soon-Hyung Lee*, Jong-Yil Chai* and Byong-Seol Seo*

Department of Parasitology, College of Medicine, Gyeongsang National University, Chinju 660-280, and Department of Parasitology, College of Medicine, Seoul National University, Seoul 110-460, Korea*

Abstract: The growth and developmental pattern of *H. continua* was observed after experimental infection of their metacercariae to chicks. The recovery rate of worms from the chicks at 1 to 28 days post-infection(PI) was 12.8% in average. The rate remained fairly high for early 4 days of infection but decreased thereafter rapidly till 28 days PI. Most of the flukes, 91.9%, were recovered from the ileum of the chicks. In metacercariae, genital organs such as the ovary, testes, seminal vesicle, seminal receptacle and genital sucker were recognizable. At one day PI Mehlis' gland appeared, and at 2 days follicular vitellaria were observed. At 3 days PI, eggs were formed in the uterine tubule and increased in number as the worm grew old. The worms reached 2,990 μm in length and 525 μm in width at 28 days PI. Genital organs developed rapidly in early stages of infection but slowly thereafter to 28 days PI, whereas non-genital organs developed steadily through the infection period. It was proved by this experiment that chicks should be a moderately suitable final host of *H. continua*.

Key words: *Heterophyopsis continua*, growth and development, organogenesis, chick, recovery rate

INTRODUCTION

Heterophyopsis continua, an intestinal fluke belonging to the family Hetrophyidae, was originally described by Onji and Nishio (1916) from experimental dogs fed the metacercariae collected from mullets. The natural or experimental final hosts recorded were dogs, cats, ducks and sea-gulls (Yamaguti, 1939a; Chun, 1960; Kanemitsu *et al.*, 1953; Onji and Nishio, 1924).

Since the first human infection of *H. continua* had been reported by Yamaguti (1939b) in Japan, this fluke has been watched with keen interests by many parasitologists. In Korea, 4 human infections were recorded recently by Seo *et al.* (1984) and Hong and Han (1989), and naturally infected dogs were found (Eom *et al.*, 1985). Brackish water fishes such as *Lateolabrax japonicus*, *Acanthogobius flavimanus*, *Clupanodon punctatus* and *Plecoglossus altivelis* were reported as the second intermediate hosts (Chun, 1960; Seo *et al.*, 1984; Cho and Kim, 1985). However, there is scarce information on the development and growth of this fluke in definitive hosts.

This study was performed to assess the suitability of chicks as an experimental final host of *H. continua* by observing the recovery rate, growth and development of worms in chicks from 1 to 28 days after infection.

#This study was supported in part by a junior scientist grant from College of Medicine, Seoul National University (1984).

Table 1. Recovery of *H. continua* from the chicks experimentally infected with the metacercariae

Day after infection	No. of chicks	No. of MC* given	No. of worms recovered from					Worm recovery rate (%)
			duodenum	jejunum	ileum	cecum	total	
1	4	400	0	0	58	1	59	14.8
2	3	300	0	0	70**	—	70	23.3
3	4	360	0	0	78	7	85	23.6
4	3	300	0	0	69	11	80	26.7
6	5	430	0	0	48	6	54	12.6
8	4	400	0	0	36**	—	36	9.0
14	7	700	0	1	35	9	45	6.4
28	5	500	0	0	5	0	5	1.0
Total 34		3,390	0	1	399	34	434	12.8

*Metacercaria **Including cecum and rectum

MATERIALS AND METHODS

Perches (*Lateolabrax japonicus*) were collected at a southeastern costal area of Korea Peninsula and digested with artificial gastric juice at 37°C for 1 hour. After several washings with phosphate buffered saline (PBS), the metacercariae of *H. continua* were separated and collected from the sediments under a dissecting microscope. For excystation, some metacercariae were incubated in 1% trypsin solution, pH 8.0 at 38°C. The metacercariae liberated were harvested immediately and washed with PBS 3 times and fixed under a cover glass pressure.

A total of 3,390 metacercariae were grouped into 50~100 in number, and fed orally to 34 chicks, 1~3 week-old hatchery-raised broiler (Table 1). The chicks were sacrificed at 1, 2, 3, 4, 6, 8, 14 and 28 days post infection (PI) and then their whole gut was resected. The small intestine was divided into the duodenum, jejunum and ileum, then opened along the mesenteric border in PBS. After several washings of the intestinal contents, the flukes were recovered from the sediments under a dissecting microscope.

The flukes collected were fixed in 10% neutral formalin under a cover glass pressure and stained with Semichon's acetocarmine. The specimens stained were observed and measured microscopically. Results on the development were described on the basis of morphological findings of these specimens.

RESULTS

Recovery rate of worms:

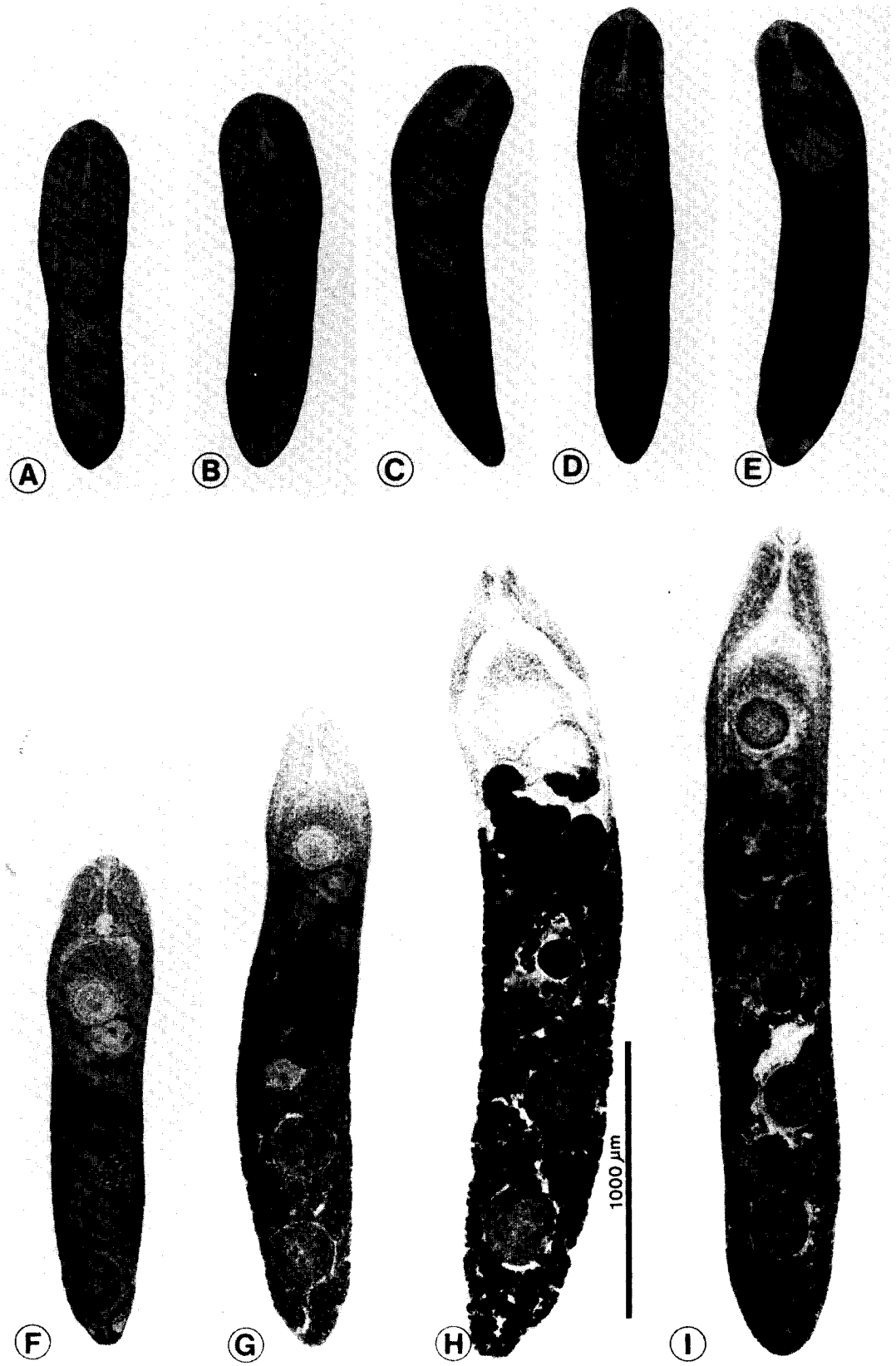
The recovery rate of worms from the experimental chicks was 12.8% in average. The rate remained relatively high for early 4 days of infection but decreased rapidly to 1.0% until 28 days PI (Table 1). Among 434 worms recovered, almost all worms (91.9%) were collected from the ileum and a few (7.8%) were from the cecum.

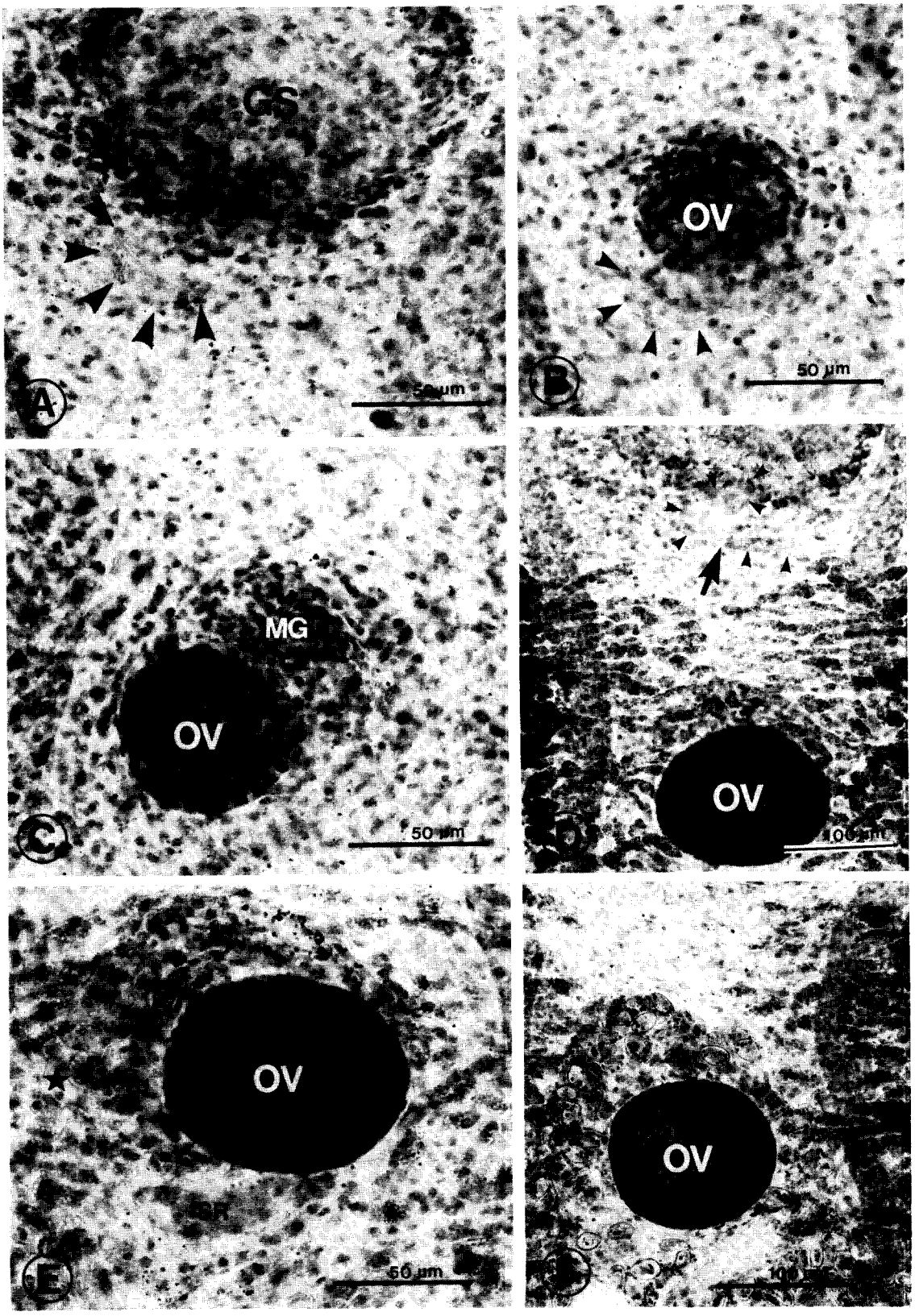
Growth and development:

The metacercariae grew rapidly in their length for 8 days PI, then continued to grow slowly to 28 days PI. However, the growth in body width was not so remarkable through the infection period (Table 2). The length between the anterior end of the body and the ventral sucker was very slightly increased. On the other hand, the length from the ventral sucker to the posterior end of the 28-day-old worms was increased about 3 times that of the excysted

(→)

Fig. 1. (A) Excysted metacercaria of *H. continua*. Developmental stages recovered from experimental chicks at 1 day (B)), 2 days (C), 3 days (D), 4 days (E), 6 days (F), 8 days (G), 14 days (H) and 28 days (I) post-infection (PI). Figures A~I were enlarged at the same scale.


A
B
C
D
E
F
G
H
I
1000 µm


GS
A
50 μm
OV
B
50 μm
MG
OV
C
50 μm
OV
D
100 μm
OV
E
50 μm
OV
F
100

metacercariae. In accordance with the maturation of worms, the maximum width of the body that situated between the pharynx and ventral sucker in juvenile worms moved to the level between the seminal vesicle and ovary in mature worms (Fig. 1A-I).

Excysted metacercariae already had testes lying in tandem position, globular ovary (Fig. 1A), rod or 'C'-shape seminal vesicle without sperm, and retort-form seminal receptacle (Fig. 2A & B). On the first day after the infection ellipsoid Mehlis' gland appeared in front of the ovary and was connected to the seminal receptacle with a tubule along the left border of the ovary (Fig. 2C). Seminal receptacle became balooned to be comma-shaped, of which 45.5% was containing sperms. On the second day the seminal vesicle was sacculated into right and left lobes and the right lobe slightly curved to be 'L'-shape. Vitellaria appeared to be follicular and distributed subtegumentally from the posterior margin of the seminal vesicle to the level between the anterior and posterior testes, and was dense in the region lateral to the intestinal ceca (Fig. 2D). Uterine tubule was formed around the ovary and extended to the genital sucker anteriorly and to the level between the two testes posteriorly (Fig. 2E). On the third day intra-uterine eggs were formed in the uterus of 55.6% of worms and averaged 24.8 (range 2～64) in number, which were spread over the region from the ovary to the posterior extremity of the body. Seminal vesicle was swollen and filled with sperms (Figs. 1D & 2F).

As the worm grew older than 8 days of infection (Figs. 1E-I), the intra-uterine eggs was increased rapidly in number and the uterine tubule extended to the genital sucker and occupied almost all of the space between the genital organs of the worms. Fine elaborate rodlets, 97～107 in number, were observed on the gonotyl of the genital sucker of excysted metacercariae, and became distinct and stout in the worms one day after infection (Fig. 3).

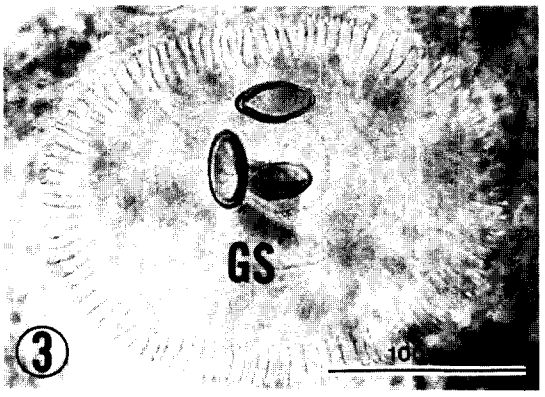


Fig. 3. Rodlets on the gonotyl of the genital sucker (GS), a 28-day-old worm.

The growth curve of the genital organs such as the testes and seminal vesicle was steep up to 6 days PI and became a gentle slope to 14 days and continued to develop slowly until 28 days PI. The growth of the ovary showed a sigmoid curve which was steep between 1 to 4 days PI (Table 2 & Fig. 4A). The growth of non-genital organs such as the oral sucker and pharynx was not so significant, whereas that of the ventral sucker was quite progressive up to 14 days PI and thereafter became a plateau to 28 days (Table 2 & Fig. 4B).

DISCUSSION

In the final hosts infected with intestinal flukes, it is well known that the recovery rate of the fluke is decreased as the infection period goes especially in low susceptible hosts (Mansour *et al.*, 1981; Hong *et al.*, 1983; Kang *et*

(←)

Fig. 2. The organogenesis of *H. continua*. (A) 'C'-shaped seminal vesicle (arrow heads), posterior to the genital sucker (GS), metacercaria. (B) Retort-form seminal receptacle (arrow heads), posterior to the ovary (OV), metacercaria. (C) Mehlis' gland (MG) appeared in front of ovary 1 day PI. (D) 2 days PI seminal vesicle (arrow heads) sacculated into right and left lobes at the middle part (arrow) and follicular vitellaria behind the seminal vesicle to the posterior end of the body. (E) uterine tubule (star) around the ovary and seminal receptale containing sperms at 2 days PI. (F) intra-uterine eggs at 3 days PI.

Table 2. Measurements of *H. continua* recovered from the experimentally infected chicks

Organs	Measurements(mm), day after infection						
	EMC*	1	2	4	8	14	28
No. of worms measured	17	16	15	16	16	20	19
Body length	1,317±168	1,417±157	1,510±175	1,806±182	2,216±270	2,990±418	2,990±309
width	333± 26	371± 30	384± 41	396± 26	456± 32	567± 70	525± 52
Oral sucker							
length	70± 6	66± 6	68± 6	64± 5	64± 5	77± 9	77± 6
width	90± 8	82± 7	85± 6	91± 7	92± 9	94± 12	99± 6
Prepharynx							
length	160± 36	140± 29	141± 52	129± 28	118± 30	154± 64	133± 31
Pharynx							
length	81± 7	78± 4	81± 5	82± 5	83± 13	96± 7	92± 7
width	66± 9	62± 4	62± 4	63± 7	62± 6	74± 6	81± 6
Esophagus							
length	23± 16	26± 22	11± 3	18± 12	19± 20	33± 34	20± 19
Ventral sucker							
length	146± 11	146± 11	151± 10	157± 11	169± 9	200± 13	208± 15
width	157± 12	160± 7	164± 6	167± 14	177± 9	209± 14	218± 12
Genital sucker							
length	94± 10	91± 10	98± 12	110± 9	110± 9	132± 16	144± 15
width	110± 11	114± 13	121± 11	134± 9	141± 17	166± 15	175± 18
Seminal vesicle							
Right lobe							
length	31± 6**	45± 13**	47± 9	87± 20	114± 26	166± 44	204± 37
width	48± 7	75± 26	57± 16	78± 15	117± 27	122± 34	159± 36
Left lobe							
length	—	—	53± 9	88± 17	146± 26	181± 40	184± 39
width	—	—	29± 7	56± 16	80± 21	107± 47	114± 26
Ovary length	49± 6	60± 7	82± 15	116± 8	130± 14	161± 10	167± 7
width	60± 7	70± 5	93± 18	128± 13	148± 13	164± 13	169± 16
Seminal receptacle							
length	26± 4	30± 8	36± 12	73± 24	66± 23	93± 35	99± 34
width	48± 8	60± 17	57± 13	113± 37	122± 26	164± 39	164± 39
Anterior testis							
length	78± 12	118± 14	149± 13	180± 44	219± 18	262± 33	257± 24
width	85± 10	133± 15	148± 15	188± 18	221± 21	255± 30	242± 23
Posterior testis							
length	86± 13	132± 16	166± 24	205± 19	251± 22	286± 45	292± 41
width	90± 11	134± 17	144± 19	187± 20	225± 20	248± 39	250± 32
No. of rodlets	98± 3	101± 3	107± 5	100± 6	99± 6	97± 3	99± 5

*Excysted metacercaria **Not divided into right and left lobes

al., 1983). *Metagonimus yokogawai* are the parasites that inhabit chiefly in the upper part of the small intestine in the final hosts. It is also known, however, that they shift down as the infection period elapses (Hong and Seo, 1969) and they move down more rapidly to the ileum if reinfection occurs (Kang *et al.*, 1983). These tendencies are more significant when the suitability of the host for the intestinal fluke is very low.

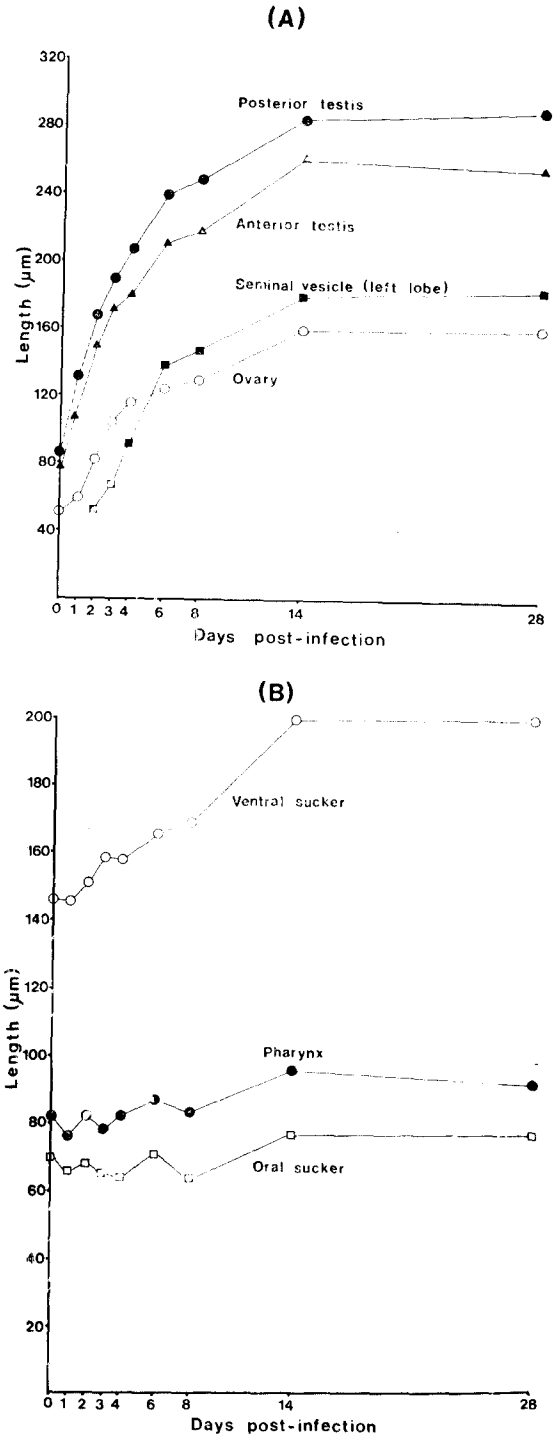


Fig. 4. Growth curves of the genital (A) and non-genital (B) organs of *H. continua* recovered from the experimental chicks.

Although *H. continua* grew to be adults in chicks, as shown in this study the chicks are not ragarded as a highly suitable final host. The recovery rate of this fluke was decreased rapidly in chicks after 14 days of infection and several chicks were found free from infection on 28 days PI. Anyhow, chicks are added to be a fairly good experimental final host of *H. continua*.

Yamaguti (1939a) stated that the habitat of *H. continua* is the small intestine of final hosts. In this experiment, almost all stages of flukes, from juveniles to adults, were collected from ileum of chicks and no shift down of the flukes was observed. It is, therefore, recognized that the ileum is the main habitat of *H. continua* in the chick host.

The body length of *H. continua* showed a steep curve for the first 8 days of infection and continued to grow up to 28 days PI. The flukes older than 4 days should be regarded as adult worms because intra-uterine eggs were formed at 3 days PI and their posterior portions behind the seminal vesicle were filled with eggs and the eggs were markedly increased in number up to 14 or 28 days PI.

The distance between the anterior end of the body and the ventral sucker of *H. continua* was relatively constant throughout the experimental period, so the increase of the body length was considered resulted mainly from the growth of the posterior portion. The posterior body growth was considered chiefly due to the development of genital organs contained there. In *Echinostoma hortense* recovered from experimental albino rats, the equator of the fluke was at the anterior end of the ventral sucker at metacercarial stage but it moved gradually backward as the fluke age advanced, to settle near the posterior testis level after 15 days of infection (Saito, 1984; Seo *et al.*, 1985). It is, therefore, recognizable that the growth of intestinal flukes is mainly due to the increase of the length of the posterior portion where genital organs develop.

The growth curve of the ventral sucker of *H. continua* in this experiment, which was similar

to that of the body length, was expressed as a prominent ascending curve during the early infection period and slowed down later than 14 days PI. Similar features were observed in *E. hortense* (Saito, 1984; Seo *et al.*, 1985) and *E. cinetorchis* (Lee *et al.*, 1988). However, the development of other non-genital organs such as the oral sucker, pharynx and esophagus was so insignificant that their size became only slightly larger than those of matacercariae. The remarkable development of the ventral sucker is considered to meet the increasing requirement of its sucking power according to the growth of the fluke.

Genital organs of *H. continua*, except for Mehlis' gland, were already formed in metacercariae and grew rapidly up to 4 days PI in chicks so that their growth curves appeared as the latter part of a sigmoid curve. However in *E. hortense* and *E. cinetorchis*, only primordial stages as germ cell masses of genitalia were present in matacercariae and they differentiated and developed after infection in the final host (albino rats), so that their growth shows a complete sigmoid curve (Saito, 1984; Seo *et al.*, 1985; Lee *et al.*, 1988).

Seo *et al.* (1984) compared, in taxonomic point of view, the morphologies of the previously reported species which were related to the genus *Heterophyopsis*, that is, *Heterophyes continus* (Onji and Nishio, 1916), *Heterophyopsis expectans* (Tubangui and Africa, 1938) and *Pseudoheterophyes continua major* (Yamaguti, 1939a). They concluded that these three species were not different from one another. By the present study, the differences in body size and position of organs of the flukes, which were adopted by those authors as significant criteria of the speciation of the genus *Heterophyopsis*, are now regarded intra-specific varitions ascribed to many factors such as the age of worms, period after the worm death and pressure at fixation, *etc.* In this connection it is suggested that there should be only one species in the genus *Heterophyopsis*, *H. continua*, as available literatures are concerned.

REFERENCES

Africa, C.M., de Leon, W. and Garcia, E.Y. (1940) Visceral complications in intestinal heterophyidiasis of man. *Acta Medica Philippina*(Monographic Series), No. 1:1-22.

Cho, S.Y. and Kim, S.I. (1985) *Plecoglossus altivelis* as a new fish intermediate host of *Heterophyopsis continua*. *Korean J. Parasit.*, **23**(1):173-174.

Chun, S.K. (1960) A study on some trematodes whose intermediate hosts are brackish water fish, (1) The life history of *Heterophyes continus* the intermediate host of which is *Laterolabrax japonicus*. *Bull. Pusan Fish. Coll.*, 3(1 & 2):40-42 (in Korean).

Eom, K.S., Son, S.Y., Lee, J.S. and Rim, H.J. (1985) Heterophyid trematodes (*Heterophyopsis continua*, *Pygidiopsis summa* and *Heterophyes heterophyes nocens*) from domestic cats. *Korean J. Parasit.*, **23**(2):107-202.

Hong, N.T. and Seo, B.S. (1969) Study on *Metagonimus yokogawai* (Katsurada, 1912) in Korea. I. On the matacercaria, its distribution in the second intermediate host and the development in the final host. *Korean J. Parasit.*, **7**(3):129-142.

Hong, S.J. and Han, J.H. (1989) Two human infections by *Heterophyopsis continua* and *Stictodora* sp. *Korean J. Parasit.*, **27**(4):344.

Hong, S.J., Lee, S.H., Seo, B.S., Hong, S.T. and Chai, J.Y. (1983) Studies on intestinal trematodes in Korea, IX. Recovery rate and development of *Fibricola seoulensis* in exprimental animals. *Korean J. Parasit.*, **21**(2):224-233.

Hong, S.J., Woo, H.C., Chai, J.Y., Chung, S.W., Lee, S.H. and Seo, B.S. (1989) Study on *Centrocestus armatus* in Korea. II. Recovery rate, growth and development of worms in albino rats. *Korean J. Parasit.*, **27**(1):47-56.

Kanemitsu, T., Akagi, T., Otagaki, H. and Kaji, F. (1953) Studies on the trematodes of the genus *Metagonimus*, of which intermediate hosts are brackish water fishes; with additional notes on *Heterophyes continus*, of which intermediate host is *Lateolabrax japonicus*. *Jap. J. Parasitol.*, **4**:296-304 (in Japanese).

Kang, S.Y., Cho, S.Y., Chai, J.Y., Lee, J.B. and Jang, D.H. (1983) A study on intestinal lesions of experimentally reinfected dogs with *Metagonimus yokogawai*. *Korean J. Parasit.*, 1983, **21**(1):58-73.

Lee, S.H., Lee, J.K., Sohn, W.M., Hong, S.T., Hong, S.J. and Chai, J.Y. (1988) Metacercariae of *Echinostoma cinetorchis* encysted in the fresh water snail, *Hippeutis* (*Helicorbis*) *cantori*, and their development in rats and mice. *Korean J. Parasit.*, **26**(3):189-197.

Mansour, N.S., Youssef, M. and Awadalla, H.N. (1981) Susceptibility of small laboratory animals to *Pygidiopsis genata* (Trematoda: Heterophydae). *J. Egyptian Soc. Parasit.*, **11**(1):225-234.

Onji, Y. and Nishio, T. (1916) A review on new intestinal parasites. *Igaku Chuo Zasshi*, **14**(8):439-442 (in Japanese).

Onji, Y. and Nishio, T. (1924) A monograph of intestinal trematodes. *Chiba Igakkai Zasshi*, **2**(3): 113-161 (in Japanese).

Saito, S. (1984) Development of *Echinostoma hortense* in rats, with special reference to the genital organs. *Jpn. J. Parasitol.*, **33**(3):191-201.

Seo, B.S., Chun, K.S., Chai, J.Y., Hong, S.J. and Lee, S.H. (1985) Studies on intestinal trematodes in Korea, XVII. Development and egg laying capacity of *Echinostoma hortense* in albino rats and human experimental infection. *Korean J. Parasit.*, **23**(1):24-32.

Seo, B.S., Lee, S.H. and Chai, J.Y. (1984) Studies on intestinal trematodes in Korea. XIII. Two cases of natural human infection by *Heterophyopsis continua* and the status on metacercarial infection in brackish water fishes. *Korean J. Parasit.*, **22** (1):51-60.

Yamaguti, S. (1939a) Studies on the helminth fauna of Japan. Part 25. Trematodes of birds, IV. *Jap. J. Zool.*, **8**(2):162-165.

Yamaguti, S. (1939b) Studies on the helminth fauna of Japan. Part 27. Trematodes of mammals, II. *Jap. J. Med. Sci.* (VI), **1**(3):131-151.

Yamaguti, S. (1958) Digenetic trematodes of vertebrates. *Systema Helminthum*, Vol. **1**, pp. 699-701.

=국문초록=

병아리에서 *Heterophyopsis continua*의 충체 회수율 및 성장 발육

경상대학교 의과대학 기생충학교실 및 서울대학교 의과대학 기생충학교실*
홍성종 • 이순형* • 채종일* • 서병설*

*H. continua*는 조류 및 포유동물의 소장에 기생하는 이형흡충과의 장흡충이며 농어, 숭어 등 반염수어가 제2중간숙주이다. 우리 나라에서는 4례의 인체 감염례가 보고되었다. 이 연구는 *H. continua*의 실험적 종숙주로서 병아리의 적합성을 알기 위하여 시행하였다.

농어에서 인공소화법으로 수집한 *H. continua*의 피낭유충을 병아리에 경구 감염시키고 1, 2, 3, 4, 6, 8, 14 및 28일 후에 도살하여 소장과 맹장에서 충체를 회수하였다. 회수된 충체를 10% neutral formalin으로 고정하고 Semichon's acetone으로 염색하여 현미경으로 관찰, 계측하였다.

충체 회수율은 평균 12.8%이었으며 감염 제4일까지는 높게 유지되었으나 그 이후 28일까지 빠른 속도로 감소하였다. 회수된 충체의 91.9%는 회장에서 수집되어 병아리에서 회장이 주 기생 부위임을 알게 되었다.

피낭유충에는 비생식기관인 구 • 복흡반, 인두, 식도 등이 상당히 발달되어 있었으며, 생식기관인 난소, 저정낭, 수정낭, 전 • 후고환 및 생식흡반 등도 계측할 수 있을 정도로 윤곽을 나타내고 있었다. 감염 제1일에는 Mehlis 선이 출현하였으며, 제2일에는 저정낭 후방의 표피하 조직에 여포상의 난황소가, 난소 둘레에는 자궁이 나타났다. 감염 제3일에 자궁 내에 충란이 형성되었으며 제28일까지 계속해서 그 수가 증가하였다. 피낭유충은 실험기간동안 계속 성장하여 감염 제28일에는 체장 2,990 μm, 체폭 525 μm이 되었다. 생식기관들의 발육은 감염초기에 빠르게 진행되고 감염 후기에는 완만하게 28일까지 계속되어 일반적인 성장곡선의 후반부를 보여 주었다. 비생식기관들은 전 실험기간에 걸쳐 매우 완만하게 서서히 크기가 커졌다. 본 실험을 통하여 병아리를 비교적 적합한 실험적 종숙주로 추가하게 되었다.

〔기생충학잡지, 28(1):53-62, 1990년 3월〕