Studies on Intestinal Trematodes in Korea

VII. Growth, Development and Recovery of *Fibricola seoulensis* from Experimentally Infected Rats and Mice

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INTRODUCTION

Fibricola seoulensis is an intestinal fluke of family Diplostomatidae. It was originally described by Seo *et al.* (1964), and the prevalence among house rats were found in several inland localities in Korea (Seo *et al.*, 1981). Recently a human case infected by this fluke was experienced (Seo *et al.*, 1982), and the infection source and the second intermediate host was studied (Hong *et al.*, 1982).

Summarizing the records on *F. seoulensis*, this is widely prevalent in Korea, definitive host of which is the house rat, *Rattus norvegicus*. And the frog, *Rana nigromaculata*, is considered as the second intermediate host, and the snake was observed as a paratenic host.

However, there is no available information on the worms in definitive hosts. Present study aimed to observe the growth, development and recovery rate in definitive hosts using experimentally infected albino rats and mice. Also the adult worms were identified of the species by the morphology and dimensions.

MATERIALS AND METHODS

The metacercariae (diplostomula) were obtained

by peptic digestion of the snake, *Natrix tigrina lateralis* (Hong *et al.*, 1982). The snakes were collected in Yeosu Gun, Gyeonggi Do, Hoengseong Gun, Gangwon Do and Jungwon Gun, Chungcheongbug Do in August, 1982.

Each of 18 rats and 14 mice was infected with 200 metacercariae to observe the growth and development of the worms in definitive hosts. Two rats and two mice each were killed by spinal shock every day for a week, and at the 2nd and 4th week after infection. The small intestines were removed and divided into duodenum, jejunum and ileum. The lumen was opened along the mesenteric border. The intestines were stored in cold saline for a night. The worms were collected from the intestinal contents under a dissecting microscope. The worms were fixed with 10% formalin under a cover slip pressure and stained with Semichon's acetocarmine. The stained worms were observed and measured.

The rats were infected with 200, 1,000 and 2,000 metacercariae and the worms were recovered from the intestines at 1, 2 and 4 weeks after infection to observe the recovery rates in experimentally infected albino rats. And the first appearance of eggs was checked daily during the first 10 days after infection by fecal examination. The number of rats in a group was 2 to 6. The small intestines of the rats

were divided into 4 parts: proximal duodenum, from pylorus to bile duct opening (2.3cm long in average); distal duodenum, from bile duct opening to the end of duodenal loop (7.7cm long in average); jejunum, proximal 30cm of the remained small intestine; ileum, the rest of small intestine to cecal junction (47cm long in average). The worms were collected under a dissecting microscope and their numbers were counted by the intestinal segments.

RESULTS

1. Morphology of adult *F. seoulensis*

The worms were distinctively bisegmented into anterior and posterior bodies. Anterior body was ventrally concave with ventrally curved lateral and posterior margins to form spoon-shape, and tapering anteriorly. And posterior body was cylindrical. Oral sucker terminal and pharynx well developed. Esophagus was variable in length, but it was hard to measure the length of esophagus in most of the worms. Ventral sucker small in midline of body overlapped by tribocytic organ or anterior to it. Tribocytic organ was round to elliptical with compact gland cells and with a median ventral slit. Vitelline follicles densely distributed from the midportion between ventral sucker and intestinal bifurcation to the end of posterior testis, but especially abundant in junctional portion of both bodies and decreased remarkably in the density in posterior body. The triangular ovary was in just behind the junction of anterior and posterior bodies in submedian. Two tandem testes were transversely elongated in the posterior body, both were symmetric and butterfly-shaped with median constriction. A sac-like vitelline reservoir was in the submedian in the space between anterior and posterior testes. Seminal vesicle was rectangular and median posterior to the posterior testis. The genital atrium was elliptical

in dorsal median.

2. Growth and development in albino rats and mice

Body growth and sexual maturity of *F. seoulensis* attained within the first 6 to 7 days after infection in rats and mice. There was no distinct difference in growth or development between the two hosts. After 24 hours, the mass of germ cells became deeply stained, and the posterior end body began to protrude grossly. The mass outgrew like a satellite mass posterior to tribocytic organ, which was identified as primitive testes on the second day after infection (Plate, Fig. 1). Posterior body end protruded further on the 3rd day and it was possible to figure the ovary, anterior and posterior testes (Plate, Fig. 2). On the 4th day, posterior body was well segmented from anterior body by a junctional constriction (Plate, Fig. 3). Also vitelline follicles and genital atrium were observed. On the 5th day, the reproductive organs formed as those of mature worms, and a few eggs began to appear in uterus. Almost all worms harboured the eggs on the 6th day after infection and seminal vesicle was noted just behind the posterior testis (Plate, Fig. 4).

Body length grew rapidly during the first 5 days after infection up to 1.05mm from 0.243 mm in metacercarial stage. The worms measured 1.32mm after 6 days, 1.43mm at 7 days and

Fig. 1. Growth curves of length of forebody and hindbody.

Table 1. Measurements of *Fibricola seoulensis* of various ages from 1 day to 28 days in albino rats, mean of 10 acetocarmine stained specimens in millimeter

Organs	Adult worms by day									
	Metacercaria	1	2	3	4	5	6	7	14	28(range)
Body length	0.243	0.344	0.542	0.701	0.838	1.05	1.32	1.43	1.39	1.57 (1.10 ~1.92)
width	0.180	0.216	0.322	0.387	0.514	0.558	0.626	0.680	0.616	0.625(0.511~0.696)
Forebody length	—	—	0.462	0.526	0.529	0.612	0.759	0.761	0.720	0.738(0.481~0.962)
width	—	—	0.322	0.387	0.514	0.558	0.626	0.680	0.616	0.625(0.511~0.696)
Hindbody length	—	—	0.081	0.172	0.309	0.398	0.571	0.666	0.679	0.811(0.570~0.962)
width	—	—	0.159	0.220	0.357	0.434	0.450	0.481	0.524	0.489(0.398~0.583)
Oral sucker length	0.033	0.036	0.046	0.059	0.065	0.064	0.071	0.063	0.069	0.060(0.044~0.081)
width	0.030	0.035	0.054	0.059	0.059	0.059	0.083	0.066	0.070	0.076(0.057~0.095)
Pharynx length	0.030	0.030	0.038	0.051	0.055	0.060	0.070	0.059	0.068	0.063(0.055~0.071)
width	0.015	0.023	0.030	0.035	0.047	0.042	0.057	0.049	0.053	0.045(0.035~0.055)
Esophagus length	—	—	0.033	0.032	0.016	0	0.007	0.017	0.004	0.014(0 ~0.047)
Ventral sucker length	0.028	0.032	0.040	0.050	0.055	0.061	0.068	0.066	0.070	0.077(0.47 ~0.092)
width	0.037	0.039	0.048	0.060	0.076	0.077	0.084	0.077	0.086	0.091(0.087~0.098)
Tribocytic organ length	0.051	0.070	0.132	0.166	0.192	0.223	0.247	0.293	0.304	0.296(0.252~0.365)
width	0.055	0.081	0.125	0.178	0.218	0.211	0.262	0.328	0.299	0.286(0.192~0.398)
Ovary length	—	—	—	0.034	0.049	0.080	0.090	0.107	0.100	0.088(0.066~0.100)
width	—	—	—	0.053	0.117	0.162	0.177	0.026	0.198	0.189(0.133~0.252)
Anterior testis length	—	—	0.046	0.041	0.089	0.123	0.135	0.191	0.195	0.177(0.126~0.219)
width	—	—	0.075	0.139	0.288	0.358	0.371	0.450	0.485	0.407(0.298~0.497)
Posterior testis length	—	—	0.031	0.043	0.097	0.135	0.161	0.209	0.237	0.208(0.159~0.279)
width	—	—	0.056	0.113	0.277	0.344	0.364	0.431	0.462	0.375(0.272~0.431)
Genital atrium length	—	—	—	—	0.024	0.042	0.046	0.040	0.043	0.046(0.033~0.055)
width	—	—	—	—	0.030	0.051	0.062	0.053	0.066	0.065(0.055~0.079)
Seminal vesicle length	—	—	—	—	—	—	0.059	0.087	0.164	0.189(0.166~0.232)
width	—	—	—	—	—	—	0.103	0.113	0.167	0.226(0.166~0.298)
Vitelline reservoir length	—	—	—	—	—	0.036	0.062	0.055	0.068	0.063(0.051~0.087)
width	—	—	—	—	—	0.074	0.094	0.090	0.109	0.116(0.087~0.158)
No. of eggs in uterus	—	—	—	—	—	2.3	11.0	19.2	19.1	15.1(10~22)

Table 2. The ratio in length of hindbody to that of forebody and organogenesis by age of *F. seoulensis* in rats

Age by day	Newly appeared organs	Length(mm) of**		Ratio(B/A)
		forebody(A)	hindbody(B)	
2	Testis	0.462	0.081	0.175
3	Ovary	0.526	0.172	0.326
4	Genital atrium Vitelline follicles	0.529	0.309	0.584
5	Eggs in uterus Vitelline reservoir	0.612	0.398	0.650
6	Seminal vesicle	0.759	0.571	0.753
7		0.761	0.666	0.876
14		0.720	0.679	0.943
28		0.738	0.811	1.099

** $P < 0.01$: highly significant difference between growth of forebody and hindbody

Fig. 2. Schematic drawing of growth of forebody and hindbody by day. **Fig. 3.** The increasing pattern of body ratio, hindbody to forebody by day.

1.57mm after 4 weeks. The measurements of the body and the organs of worms in each day after infection in rats were summarized in Table

1. The growth of posterior body was more remarkable than that of anterior body as shown in Fig. 1 and Fig. 2. The ratio of posterior body length to anterior body length increased from 0.175 on the 2nd day to 1.099 at the 4th week after infection (Table 2 and Fig. 3).

The eggs were detected from feces of rats and mice from the 6th day after infection. The eggs discharged from albino rats measured 0.087mm to 0.094mm (mean 0.094mm out of 30 eggs) in length and 0.052mm to 0.059mm (mean 0.055mm) in width. The eggs were ovoid to elliptical and slightly asymmetric, golden colored, operculated, immature and thin-shelled. Opercular depth was 0.002 to 0.007mm (mean 0.005mm) and width 0.008 to 0.025mm (mean 0.019mm).

3. Recovery and distribution of adult *F. seoulensis* in albino rats and mice

Table 3. Recovery of adult *F. seoulensis* from the rats after infection with 200 metacercariae

Day after infection	No. of worms recovered from				Recovery rate(%)
	Duodenum	Jejunum	Ileum	Total	
1	15	10	0	25	12.5
2	71	1	1	73	36.5
3	75	10	0	85	42.5
4	115	3	0	118	59.0
5	86	0	0	86	43.0
6	42	0	0	42	21.0
7	88	0	0	88	44.0

Table 4. Recovery of adult *F. seoulensis* from mice after infection with 200 metacercariae

Day after infection	No. of worms recovered from					Recov. rate(%)
	*Duodenum 1	*Duodenum 2	Jejunum	Ileum	Total	
3	44	3	0	0	47	23.5
4	60	61	0	0	121	60.5
5	48	57	8	0	113	56.5
6	32	73	2	0	127	63.5
7	38	51	3	0	92	46.0
10	34	66	3	1	104	52.0

* Duodenum 1: proximal duodenum to bile duct opening.

Duodenum 2: distal duodenum to bile duct opening.

Table 3 summarized the recovery of *F. seoulensis* during the first week after infection from albino rats. The rate ranged from 12.5% to 59.0%. The early recovery rate from mice was 23.5% to 63.5% from 3 days to 10 days after infection (Table 4). The recovery rate from rats challenged with various number of metacercariae was summarized by the week after infection in Table 5. One week after infection, 42.6% out of 800 introduced worms were recovered from 4 rats in the 200 metacercariae challenged group. And 60.9% of worms were recovered in 1,000 metacercariae group and 43.9% were recovered from rats of 2,000 metacercariae group. The recovery rate decreased to 16.2%, 29.1% and 3.1% respectively by the group of 200, 1,000 and 2,000 metacercarial challenge at the 4th week after infection. Almost all worms were recovered from both proximal and distal duo-

denum of rats infected with 200 metacercariae. And 25.8% of worms were recovered from

Table 5. Recovery rate of *F. seoulensis* from experimentally infected albino rats after 1, 2 and 4 weeks

No. of metacercariae challenged	No. of rats	No. of worms collected	Recovery rate(%)
200 metacercariae			
1 week	4	341	42.6
2 weeks	5	434	43.4
4 weeks	2	65	16.2
1,000 metacercariae			
1 week	3	1,828	60.9
2 weeks	2	646	32.3
4 weeks	2	581	29.1
2,000 metacercariae			
1 week	6	5,268	43.9
2 weeks	5	2,829	28.3
4 weeks	2	123	3.1

Table 6. Distribution of *F. seoulensis* in small intestine of albino rats

Rat group by No. of metacercariae	Total No. of worms	No. (%) of worms collected from			
		*Duodenum 1	*Duodenum 2	Jejunum	Ileum
200 metacercariae					
1 week	341	247(72.4)	94(27.6)	0	0
2 weeks	434	150(34.6)	279(64.3)	5(1.1)	0
4 weeks	65	55(84.6)	10(15.4)	0	0
1,000 metacercariae					
1 week	1,828	435(23.8)	922(50.4)	471(25.8)	0
2 weeks	646	343(53.1)	240(37.2)	63(9.8)	0
4 weeks	581	345(59.4)	221(38.0)	6(1.0)	9(1.5)
2,000 metacercariae					
1 week	5,268	985(18.7)	2,499(47.4)	1,767(33.5)	17(0.3)
2 weeks	2,829	760(26.9)	894(31.6)	598(21.1)	577(20.4)
4 weeks	123	** 95(77.2)	—	28(22.8)	0

* Duodenum 1: proximal duodenum from pylorus to bile duct opening

Duodenum 2: distal duodenum from bile duct opening to the end of retroperitoneal loop

Jejunum: from duodenal junction to the portion of 30cm long

Ileum: from jejunal junction to cecal junction

** Duodenum was not divided into two parts.

*** $p < 0.01$: highly significant difference in the distribution by the metacercarial group

jejunal portion in 1,000 metacercarial group, 1 week after infection. In 2,000 metacercariae group, 61.1%, 58.5% and 77.2% were recovered from duodenal parts after 1, 2 and 4 weeks respectively. And 33.5%, 21.1% and 22.8% of worms were recovered from jejunal part, and 0.3%, 20.4% and 0% were recovered from ileal part respectively by 1, 2 and 4 weeks after infection in 2,000 metacercariae group. These were summarized in Table 6.

DISCUSSION

The worms obtained from albino rats after experimental infection were identified as *Fibricola seoulensis* Seo, Rim and Lee, 1964. The morphological characters of 4 week old worms coincided very well with the original description. Only the dimensions of 4 week old worms varied more than those of the original record, but the variation of original record was included in the range of the present measurement.

Morphological variation other than dimensional variation was observed in the specimens of *F. seoulensis*. Ventral sucker located anterior to tribocytic organ in some specimens, but overlapped with the organ partially or as a whole in some specimens. This variation was caused by the status of extension or shrinkage of worms as they were fixed. The location of ventral sucker is hardly acceptable as a differential key in speciation of *Fibricola*. In this connection, Palmieri(1979) described the intraspecific variation of *F. ramachandrani*, probably related with the host. Also he added the variation produced by manipulation from fixation to staining.

Although the shape and size of anterior testis varied by the worm, it can be accepted as a characteristic of *F. seoulensis* as Seo *et al.*(1982) described in the human case report. All of the worms older than 7 days in this study had symmetric anterior and posterior testes without exception. However the butterfly-shape of testes varied slightly to be not so typical. These

variations in morphology and measurements should be regarded as a normal range of variation of *F. seoulensis*. The small size of the worms from the human infection case (Seo *et al.*, 1982) was also recognized in the range of variation.

Body growth and sexual maturity of adult *F. seoulensis* needed 6 to 7 days in experimentally infected rats and mice. Cho *et al.* (1982) observed same result for growth of *F. seoulensis* in rats, mice and guinea pigs. They identified the larval trematodes from snakes as *F. seoulensis* too. In case of *F. cratera*, sexual maturity was attained 8 to 10 days after infection in experimentally infected rats, mice and cats (Ulmer, 1955; Sudarikov, 1960).

As for the early development of *F. seoulensis*, the conical primordium of posterior body of the metacercaria began to protrude and to be thickened from the 2nd day after infection. On the 6th day, almost all worms contained eggs in uterus and there was no increase in the morphometries including the number of eggs after the 7th day until the 4th week.

The body length grew almost linearly during the first week after infection. By linear regression analysis, the regression equation for growth of anterior body from the 2nd day to the 7th day was $Y=0.32+0.065X$, $n=6$, $r=0.96$, and that of posterior body was $Y=-0.18+0.121X$, $n=6$, $r=0.99$. The increasing slope of posterior body growth was significantly higher than that of anterior body growth ($p<0.01$). After a week, the length of posterior body grew further, and the body ratio of posterior body to anterior body length reached 1.099 after 4 weeks from infection.

The main habitat of *F. seoulensis* in rats or mice was duodenum. Only in intensively infected rats, the worms were recovered from jejunal or ileal parts of intestine. The duodenum was characterized by wide range of pH fluctuation

and less digested food particles. But some parasites are known to prefer this parasitic location for relatively calm peristalsis and rich nutrient source of detached mucosal epithelial cells (Smyth, 1976). And it is uncertain whether this fluke is influenced by the bile flow or not.

The rat is regarded as a suitable definitive host of *F. seoulensis* in nature by Seo *et al.* (1964 and 1981) and by experimental infection through present study. Most of the species of *Fibricola* parasitized preferably in rodent hosts (Sudarikov, 1960). However, Sudarikov (1960) suggested the possibility of extraintestinal migration of larval *F. cratera* by observing the worms in intercostal muscle and in the lung of intensively infected rats. The worms were described to remain as larval stage and encapsulated in the tissues of the definitive host. The possibility of extraintestinal migration of young worms is a very important information in medical point of view. Further study should elucidate this point in *F. seoulensis* infection, using various experimental animals.

SUMMARY

This study was carried out to observe the growth and development of *Fibricola seoulensis* in experimentally infected rats and mice. The metacercariae were obtained from naturally infected *Natrix tigrina lateralis* which were collected in Hoengseong Gun, Gangweon Do, in August, 1982.

The results were summarized as follows.

1. Growth and development of worms were not different between the rat and mouse. Body growth was attained within a week in both hosts. The testes differentiated first on the 2nd day after infection, and then ovary on the 3rd day, vitelline follicles on the 4th day, vitelline reservoir on the 5th day. A few eggs appeared in uterus on the 5th day and in feces on the

6th day. The body length grew almost linearly for the first 7 days after infection. The growth of posterior body was more remarkable, and the ratio of posterior body length to that of anterior body increased to 1.099 until 4 weeks after infection.

2. The worms of 4 weeks age in rats measured 1.10~1.92mm long and 0.511~0.696mm wide. The morphological characteristics of adult worms were identical to the original description of *F. seoulensis*, though dimensions varied in wider range.

3. The worms were recovered from the rats 12.5% on the first day after infection, and the rate ranged 21.0~59.0% up to 6th day. One week after infection, the rates were 42.6%, 60.9% and 43.9% respectively by the metacercarial group of 200, 1,000 and 2,000 and the rates decreased to 16.2%, 29.1% and 3.1% respectively by the group on the 4th week after infection. Most of the worms were recovered from duodenum, and a part of worms were found in jejunum or ileum in heavily infected rats.

4. The worms of 23.5~63.5% were recovered from mice from 3 to 10 days after infection. A great majority of them was found in duodenum, too.

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韓國의 腸吸蟲에 관한 研究

VII. 실험 감염된 흰쥐와 마우스에서 *Fibricola seoulensis*의 成長 發育 및 回收率

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洪 性 台

*Fibricola seoulensis*는 우리나라에서 집쥐가 終宿主로서 내륙지방에서 그 감염이 流行하고 있음이, 1964년 徐등 (1964)이 新種으로 기록한 이래 알려져 왔다. 최근에 *Fibricola seoulensis*의 人體 感染例가 확인되었고, 그 被囊幼蟲이 뱀과 개구리에서 發見되어 이들이 우리나라에서 각각 운반숙주와 제 2 중간숙주의 역할을 하고 있음이 밝혀졌다(洪등, 1982). 이 연구에서는 실험적으로 감염시킨 쥐와 마우스에서 이 吸蟲의 成長, 發育, 回收率, 腸內 分布 등을 관찰하였다.

그 결과는 다음과 같다.

1. 쥐와 마우스 腸內에서 이 吸蟲의 성장에서 차이가 없었고, 1주 이내에 成蟲으로 성장이 완료되었다. 감염후 2일 제부터 고환이 분화하기 시작하여 5일에는 蟲卵이 자궁내에 생겨나며 6일에는 宿主의 大便에서 虫란이 발견되는 정도로 蟲의 성장과 발육이 빨랐다. 감염후 7일까지는 蟲體의 前·後半部가 거의 일직선적으로 지나나며, 후반부의 成長이 有意하게 빠른 속도이었다.

2. 감염후 4주에 회수한 성충은 길이 1.10~1.92mm, 폭 0.511~0.696mm로 계측치의 변동 폭이 넓었으나, *Fibricola seoulensis*의 原 記載와 蟲體의 모양과 各 器官의 소견이 일치하여 *F. seoulensis*로 同定할 수 있었다.

3. 흰 쥐에 감염 후 처음 1일부터 6일까지는 12.5%~59.0%의 蟲이 회수되었고, 1주에는 평균 회수율이 200, 1,000, 2,000개의 피낭유충 투여 群에서 각각 42.6%, 60.9% 및 43.9%이고, 4주에는 각각 16.2%, 29.1% 및 3.1%로 감소하였다. 대부분의 成蟲은 십이지장에서 회수되었고, 1,000개와 2,000개의 피낭유충 감염 群에서 단 空腸 또는 廻腸 부위에서도 일부 成蟲이 회수되었다.

4. 마우스에서는 감염 후 3일~10일에 투여한 피낭유충의 23.5~63.5%가 회수되었고 거의 대부분이 십이지장에서 회수되었다.

EXPLANATIONS FOR PLATE

Fig. 1. 2-day old *F. seoulensis* in albino rats, acetocarmine stained, $\times 140$.

Fig. 2. 3-day old *F. seoulensis* in albino rats, acetocarmine stained, $\times 100$.

Fig. 3. 4-day old *F. seoulensis* in albino rats, acetocarmine stained, $\times 76$.

Fig. 4. 7-day old adult *F. seoulensis* in albino rat, acetocarmine stained, $\times 48$.