기 생 충 학 잡 지 제24권 제2호
The Korean Journal of Parasitology
Vol. 24, No. 2, 109~114, December, 1986

Growth and development of *Fibricola seoulensis* metacercariae in tadpoles

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INTRODUCTION

Since *Fibricola seoulensis* Seo, Rim and Lee, 1964 (Trematoda: Diplostomatidae) was proposed as a new species from house rats in Seoul, human cases of its infection have been successively recorded in Korea (Seo *et al.*, 1982; Hong *et al.*, 1984 & 1986; Hong *et al.*, 1985). Therefore, its medical importance is increasingly recognized.

Through parasitological surveys, *Rattus norvegicus* and *Apodemus* sp. were proved as its natural definitive host (Seo *et al.*, 1964 &1981; Seo *et* Hong, 1985). *Rana nigromaculata* and its tadpoles and several terrestrial snakes are known as the second intermediate hosts of *F. seoulensis* (Hong *et al.*, 1982 & 1985; Cho *et al.*, 1983; Seo *et* Hong, 1985). As the first intermediate host of *F. seoulensis*, *Hippeutis* (*Helicorbis*) *cantori* was proved, which is one of common aquatic snails in rice paddies throughout the country (Seo *et* Hong, 1985).

According to Chandler(1942) and Cook(1978), the cercariae of *F. texensis* or *F. cratera* shed from the snails penetrate the tadpoles, and become metacercariae (diplostomula). The cercariae are known not to invade adult frogs or snakes (Sudarikov, 1960). However, the metacercariae of *F. seoulensis* or other *Fibricola* spp. were found in frogs or snakes (Sudarikov, 1960; Hong *et al.*, 1982). Therefore, the metacercariae in a tadpole should remain in their host as it metamorphoses to an adult frog, and be transferred to snakes without any morphological change when they engulf infected frogs.

Although the metacercariae of *F. seoulensis* are found in tadpoles, frogs and snakes, the parasitic location of the larvae in these hosts differ each other. In tadpoles, the larvae were observed in abdominal cavity (Hong *et al.*, 1985) while they were recovered from skeletal muscle in adult frogs, and from stomach wall or perigastric soft tissues in snakes (Hong *et al.*, 1982). This indicates a difference in niche of the metacercariae by host.

In this context, the tadpole is considered as an indispensible host which connects the life cycle of *F. seoulensis* between snails and adult frogs. This study was undertaken to observe the susceptibility of tadpoles to the cercariae, developmental pattern of the larvae in this pivotal host and the acquisition of infectivity of the metacercariae to mice.

MATERIALS AND METHODS

1. Collection of the cercariae

The metacercariae (diplostomula) of *F. seoulensis* were obtained by peptic digestion of snakes, *Natrix tigrina lateralis*. The metacercariae were infected to rats, and adult worms were collected after 2 weeks. The eggs laid by the worms were incubated for maturation of miracidia as Lee *et*

** This study was supported by The Clinical Research Grant from Seoul National University Hospital (1986).

al. (1986) described. The miracidia were challenged to *Hippeutis* (*Helicorbis*) *cantori* in laboratory, and the cercariae shed from the snails were used in this study.

2. Rearing of the tadpoles and experimental infection

Hundred eggs of the frog, *Rana nigromaculata*, were collected from rice paddies in Yangju-gun, Kyonggi-do, Korea, were incubated in an aquarium at 18-20°C. One month old tadpoles were experimentally exposed to the cercariae of *F. seoulensis*. After the exposure, the infected tadpoles were kept separately in other aquaria.

3. Growth and development of the metacercariae

Three tadpoles each were killed at 2, 3, 5, 8, 10, 14, 20, 30, 45 and 65 days after the infection respectively. The metacercariae of *F. seoulensis* at various developmental stages recovered from experimentally infected tadpoles were fixed in 70°C neutral buffered formalin and stained with Semichon's acetocarmine for morphological observation and measurement.

4. The infectivity of metacercariae to mice

Each of 2-4 mice was infected with 50 larvae which were recovered from the tadpoles on 8, 10, 14, 18, 20, 25, 30 and 40 days after infection respectively. On 7-18 days after the infection, the mice were killed for recovery of adult worms.

RESULTS

1. Cercarial infection of tadpoles

All of tadpoles exposed to the cercariae in each group were observed to be infected by the larvae of *F. seoulensis*. They were all recovered from abdominal cavity of tadpoles.

2. Growth and development of metacercariae

On 2 days after infection, the metacercariae were 121.2 μm long and 63.3 μm wide in average. They grew 262.0 μm long and 166.4 μm wide during the first 14 days in abdominal cavity of tadpoles (Table 1 & Fig. 1). Thereafter, the average size of worms were 224.7×

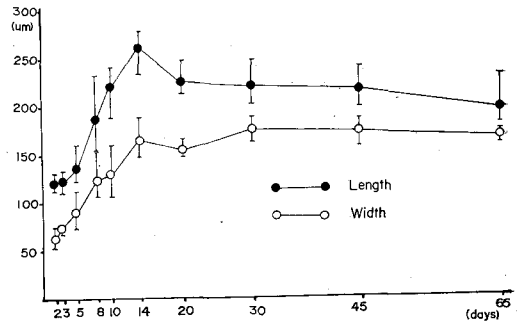

Fig. 1. The growth curve of the metacercariae of *Fibricola seoulensis* recovered from the experimentally infected tadpoles by age.

Table 1. Measurements of acetocarmine stained metacercariae of *Fibricola seoulensis* recovered from experimentally infected tadpoles (average of 10 metacercariae)

Age of metacercariae* (days)	Mean (μm)					
	Body	Oral sucker	Pharynx	Ventral sucker	Tribocytic organ	Germ cell mass
2	121.1× 63.3	20.4×22.2	8.0×11.8	17.7×17.4	—	15.0×13.4
3	123.0× 74.5	21.4×24.7	9.4×12.6	19.3×19.3	—	13.7×13.4
5	136.7× 90.6	22.0×24.4	10.5×12.3	19.0×19.0	—	15.5×12.9
8	187.6×126.3	24.1×26.5	12.1×15.4	21.2×23.5	44.4×43.8	18.4×16.4
10	221.3×129.5	27.5×26.8	13.9×15.6	21.4×24.8	43.8×43.3	21.2×15.1
14	262.0×116.4	31.3×27.5	24.1×18.6	26.1×28.4	48.2×48.7	20.3×21.2
20	224.7×156.1	30.4×26.6	16.5×14.5	21.0×29.3	46.2×45.1	15.0×19.6
30	220.3×176.1	25.2×27.9	18.0×16.1	23.9×27.6	45.0×48.5	17.7×18.2
45	214.9×171.5	26.0×28.7	20.1×15.0	22.2×26.8	47.4×46.1	17.4×18.2
65	193.3×164.8	26.1×23.8	19.8×15.8	22.1×27.8	39.5×45.6	19.2×21.4
from snake**	291.9×166.4	27.6×32.2	28.4×16.1	26.0×30.8	53.9×43.7	21.2×20.1

* From the day of cercarial exposure ** Naturally infected in *Natrix tigrina lateralis*

156.1 μm at age of 20 days, 220.3×176.1 μm at 30 days, 214.9×171.5 μm at 45 days and 193.3×164.8 μm at 65 days (Table 1 & Fig. 1). The measurements of organs were summarized in Table 1.

3. Morphology of the metacercariae

All of the metacercariae recovered from tadpoles moved actively. General shape of two days old metacercariae were not differed from that of cercarial body (Fig. 2). Oral sucker subterminal, round and well distinguished. Pharynx muscular and connected to posterior margin of oral sucker. A short esophagus followed and two ceca extended to posterior end of body. Ventral sucker round at posterior one third on midventral surface. A primordium of germ cells was observed dark red by acetocarmine staining posterior to ventral sucker. A few glandular cells were stained near pharynx and esophagus. Posterior body end was notched slightly as a mark of

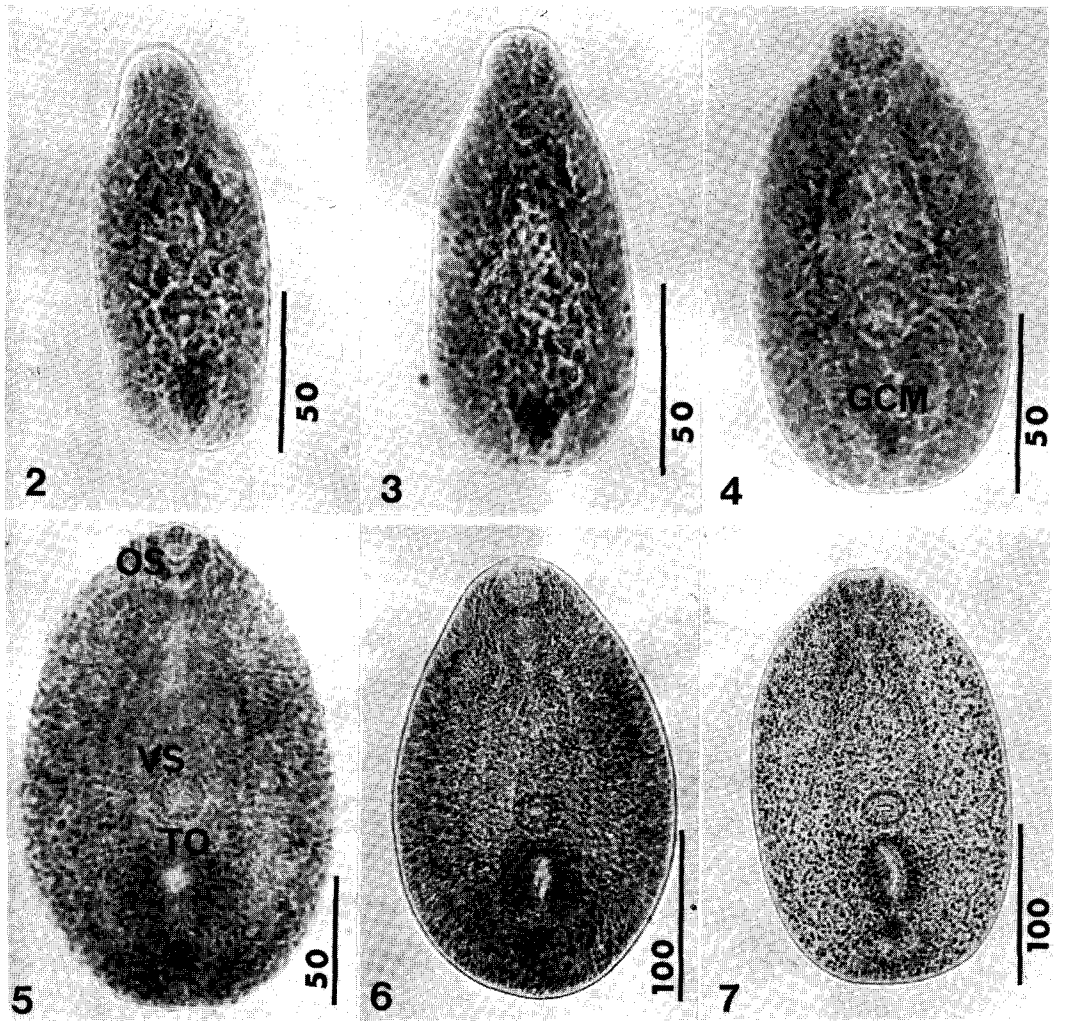

Fig. 2. A 2-day old metacercaria of *F. seoulensis* recovered from tadpoles, acetocarmine stained (scale; 50μm).

Fig. 3. A 3-day old metacercaria of *F. seoulensis*, acetocarmine stained (scale; 50μm).

Fig. 4. A 5-day old metacercaria of *F. seoulensis*, acetocarmine stained (scale; 50μm, GCM; germ cell mass).

Fig. 5. A 8-day old metacercaria of *F. seoulensis*, acetocarmine stained (scale; 50μm, OS; oral sucker, VS; ventral sucker, TO; tribocytic organ).

Fig. 6. A 14-day old metacercaria of *F. seoulensis*, acetocarmine stained (scale; 100μm).

Fig. 7. A metacercaria of *F. seoulensis* from the naturally infected snake, acetocarmine stained (scale; 100μm).

Table 2. The infectivity of *Fibricola seoulensis* metacercariae by age

Age of metacercariae* (days)	No. of challenged mice	No. of infected mice	Total No. of metacercariae given	Total No. of recovered adults	Recovery rates(%)	
					mean	range
8	3	0	150	0	0	—
10	3	0	150	0	0	—
14	4	4	200	38	19	10~32
18	2	2	100	33	33	28~38
20	2	2	100	42	42	36~48
25	3	3	150	91	60.7	40~86
30	4	4	200	119	59.5	28~82
40	4	4	200	140	70	54~78

* From cercarial infection to tadpoles

cercarial tail.

The larvae of 3 days age grew a little, posterior body became wider (Fig. 3). Oral and ventral suckers were same as those of 2 days old. Also the primordium was not changed, but the distance between it and ventral sucker lengthened.

The general body shape of five days old larvae still resembled that of cercarial body, but became wider (Fig. 4). Between ventral sucker and the primordium, a mass of carmine-stained cells appeared.

In 8 days old larvae, body contour became similar with that of mature metacercariae although their size was still smaller (Fig. 5). A round tribocytic organ became evident between ventral sucker and primordium. The organ was stained red with a ventral slit. The morphology of 8 day old larvae resembled that of mature metacercariae in stained specimens.

The larvae older than 8 days showed little difference in morphology from mature ones in snakes, however they were still smaller. They became darker in body color as their age increased (Figs. 6 & 7).

4. Infectivity of the metacercariae to mice

The metacercariae younger than 10 days did not infect the mice. Only the larvae older than 14 days grew to adult flukes in mice (Table 2). The recovery rates of adult worms from mice by the age of metacercariae were 19.0% on 14 days, 33.0% on 18 days, 42.0% on 20 days, 60.7% on 25 days, 59.5% on 30 days and 70.0% on 40 days. The recovery rates increased by the sojourn period in tadpoles as shown in Table 2.

DISCUSSION

The cercariae of *F. seoulensis* showed great affinity to the tadpoles in this study. All of the experimentally exposed tadpoles were infected by the metacercariae, and the tadpoles in natural environment were also found to be infected in high rates throughout the country (Hong *et al.*, 1985). Therefore, it is evident that tadpoles are highly susceptible for cercariae of *F. seoulensis*. Actually snails and tadpoles are found together in rice paddies in spring and summer in Korea. Such coexistance might play an important role in maintaining its high prevalence.

The cercariae appeared to penetrate a tadpole directly through skin. After their penetration, they develop exclusively in abdominal cavity until their host metamorphoses to a frog in which they are detected in skeletal muscle. The abdominal cavity of tadpoles may be important for growth and maturation or latency of *Fibricola* metacercariae.

The larvae grew linearly during the first 14 days after infection, and their size did not increase thereafter (Fig. 1). By linear regression analysis, the regression equation for growth of length from the 2nd day to the 14th day was $y=32.48+4.27x$, $n=6$, $r=0.96$, and that of width was $y=18.72+3.05x$, $n=6$, $r=0.95$.

The larvae of 2 days age in tadpoles had

morphological characteristics of cercarial body which had no tribocytic organ. On 8th day, definite tribocytic organ was observed in the larvae, and their morphology was similar with that of mature metacercariae still small in size.

This study showed also that it took at least 14 days for the larvae in tadpoles to be infective to the definitive host. The infectivity is one of important indices of larval maturation. It means, therefore, the larvae of *F. seoulensis* in tadpoles, need at least 14 days to be mature metacercariae. In *F. cratera*, the metacercariae began to be infective to albino rats after 35 days in tadpoles (Hoffman, 1955). Compared with *F. cratera*, the metacercariae of *F. seoulensis* became mature earlier.

As the sojourn period in the tadpoles went on, the infectivity of metacercariae to mice increased. This may be due to increased proportion of fully matured metacercariae by age. After 25 days, the recovery rates remained in high rates of 60~70%. Consequently, it is indicated that the metacercariae of *F. seoulensis* become fully mature between 14 and 25 days after infection, which is an enough duration for development of the metacercariae in tadpoles.

SUMMARY

In order to observe the growth and development of *Fibricola seoulensis* metacercariae, the tadpoles of *Rana nigromaculata* were experimentally infected with the cercariae.

The metacercariae of various developmental stages were recovered from the tadpoles after 2 to 65 days of infection. They were prepared for morphological observation, and were given orally to mice to observe their infectivity. The following results were obtained.

1. All of the tadpoles exposed to the cercariae were observed to harbour the larvae in their abdominal cavity.

2. The young metacercariae of 2 days after infection were 121.1 μm long and 63.3 μm wide. They grew linearly for the first 14 days to be 262.0 μm long and 166.4 μm wide. Thereafter, no more growth recognized until 65 days.

3. The larvae of 2 days old were similar with cercarial body and had 2 suckers, a pharynx, 2 ceca and a primordium of germ cells but no tribocytic organ. On the 8th day, they had tribocytic organ, and their morphology resembled that of mature metacercariae.

4. The metacercariae younger than 10 days could not infect the mice. Only the metacercariae older than 14 days had infectivity. The recovery rates increased by the age of metacercariae from 19.0% in 14 days old to 70.0% in 40 days old.

Above findings indicate that the tadpole is indispensable for metacercarial development and it needs at least 2 weeks for maturation. The tadpole is a pivotal host in the life cycle of *F. seoulensis* for connection between the snail and the frog.

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=국문초록=

Fibricola seoulensis 피낭유충의 실험감염 올챙이내 성장 및 발육

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이순형 · 신손문 · 홍성태 · 손운목 · 채종일 · *서병설

Fibricola seoulensis 피낭유충의 올챙이내 성장, 발육 및 마우스에 대한 감염력을 알아보고자 이 실험을 실시하였다.

실험실 내에서 *Fibricola*의 생활사를 유지하면서 유미유충(cercaria)을 얻어 이것을 실험실에서 키운 참개구리(*Rana nigromaculata*)의 올챙이에 실험감염 시킨 후 2일, 3일, 5일, 8일, 10일, 14일, 18일, 20일, 25일, 30일, 40일, 45일 및 65일에 올챙이로부터 피낭유충을 회수하였다.

회수한 피낭 유충의 일부는 뜨거운 포르말린(70°C, neutral buffered formalin)으로 고정한 후 Semichon's aceto-carmine으로 염색하여 관찰, 계측하였고, 일부는 성장 및 발육에 따른 감염력을 알아보기 위해 2~4마리의 마우스에 각각 50개씩 경구 감염시킨 후 7~18일에 마우스를 도살하여 성충을 회수하였다.

그 결과는 다음과 같다.

1. 실험적으로 유미유충에 노출된 올챙이는 100%가 피낭유충에 감염되어 있었다. 또한 피낭유충은 모두 올챙이의 복강 내에서만 관찰되었다.

2. 감염 2일 후 평균 길이 121.1μm, 폭 63.3μm이던 유충이 14일 후에 길이 262.0μm, 폭 166.4μm로 급격히 성장하여 최대 성장치에 이르렀고 그후 20일부터 65일까지 평균 길이가 224.7—193.μm, 폭이 176.1—156.1μm 범위의 크기에 있었다.

3. 감염 2일후부터 전관찰 기간에서 피낭유충의 후단에 원생식세포(germ cell primordium)가 관찰되었고 8일 이후부터 tribocytic organ이 나타났다.

4. 감염후 8일, 10일에 회수된 피낭유충은 마우스 내에서 성충으로 자라지 못하였고, 올챙이에서 14일 동안 성장한 피낭유충이 마우스에서 감염력을 나타내기 시작하였다. 올챙이체내 체류기간이 길수록 마우스에서의 성충 회수율도 높아져서 14일에 19.0%이던 것이 40일에는 70.0%로 증가하였다.

이상의 결과로 올챙이가 *F. seoulensis*의 유미유충이 피낭유충으로 발육하는 데에 필수적인 역할을 하는 것으로 생각되며, 올챙이 체내로 침입한 유미유충이 성숙한 피낭유충으로 발육하는 데에 약 2주의 기간이 소요됨을 알 수 있었다.