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Metacercariae of *Pharyngostomum cordatum* found from the European grass snake, *Rhabdophis tigrina*, and its experimental infection to cats

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Abstract: The metacercariae of *Pharyngostomum cordatum* were found naturally infected in the European grass snake, *Rhabdophis tigrina*, purchased from a local snake collector in Jinju, Kyongsangnam-do. They were experimentally fed to several kinds of animals such as mice, rats, hamsters, ducklings, a dog, and cats. The adult worms were recovered from the cats 5 weeks after the infection, but none from other animals. The measurements and other morphological characters of the metacercariae and adults were both compatible with those of *P. cordatum* described by previous authors. The present study confirmed that the snake, *Rhabdophis tigrina*, serves as a second intermediate (or paratenic) host of *P. cordatum* in Korea.

Key words: *Pharyngostomum cordatum*, metacercariae, intermediate host, snake (*Rhabdophis tigrina*), cat

INTRODUCTION

Pharyngostomum cordatum, a member of the fluke family Diplostomidae, was originally described by Diesing(1850) under the name, *Hemistomum cordatum*. It was renamed later by Ciurea(1922) as *P. cordatum*, and its detailed morphology was described by La Rue(1926). This fluke has been discovered worldwidely from carnivorous mammals such as wild cats in Germany (Diesing, 1850) and Romania (Ciurea, 1922), domestic cats in China (Faust, 1927 & 1930; Chen, 1934; Tang, 1935; Wallace, 1937), Japan (Kifune *et al.*, 1967; Machida, 1970; Kondo *et al.*, 1974), India (Dubey, 1970) and Korea (Cho and Lee, 1981; Huh *et al.*, 1988), a tiger in India (Rao, 1943), and a cheetah in Tanzania (Baer and Dubois, 1951).

In China, the life cycle of this fluke was successfully studied in the laboratory, using *Segmentina calathus* as the first intermediate host, and tadpoles of *Bufo* or *Rana* spp. as the second intermediate host (Wallace, 1939). After then it was reported also in the field that the frogs and tadpoles serve as the second intermediate host, and frog-eating animals such as toads, snakes or shrews play the role of a transport or paratenic host (Wallace, 1939; Yamaguti, 1958; Kurimoto, 1976).

In Korea, adult worms of *P. cordatum* were discovered by Cho and Lee(1981) from the cats purchased from Namdaemoon Market in Seoul. However, intermediate hosts have not yet been reported. Recently we found the metacercariae of *P. cordatum* from the peritoneal membrane

of the European grass snake, *Rhabdophis tigrina*, and identified them by obtaining adult worms after experimental infection to cats.

MATERIALS AND METHODS

A total of 30 European grass snakes, *Rhabdophis tigrina*, were purchased from a local snake collector in Jinju, Kyongsangnam-do (Province), Korea. The snakes were stripped off their skins, and their peritoneum and other visceral membranes were carefully removed, if any whitish spots (*i.e.*, diplostomula) were found on the membrane. The membranes were digested by artificial gastric juice, filtered through a mesh to remove large tissue debris, and the precipitates were examined for the metacercariae under a stereomicroscope.

The diplostomid larvae were classified largely into two groups based on their size and internal morphology. The smaller sized larvae have already been identified to be those of *Fibricola seoulensis*, a commonly found species from this snake (Cho *et al.*, 1982; Hong *et al.*, 1982). The larger larvae collected under stereomicroscopy, were morphologically observed and measured in life and after stain. The metacercariae were orally fed to 6 mice, 5 rats, 5 hamsters, 2 ducklings, 1 dog and 3 cats through a gavage needle each animal with 200~500 metacercariae. The infected animals were killed between 2 and 7 weeks after the infection, and their intestine was removed for recovery of the adult worms. The adults recovered were fixed, stained with Semichon's acetocarmine, and observed.

RESULTS

1. Metacercariae

Numerous diplostomid metacercariae(diplostomula) were found attached to the peritoneal membrane of the snake, *Rhabdophis tigrina*. The infection rate of the snakes was very high and up to hundred per cent. The larvae collected by peptic digestion were actively moving, and revealed their large, ventrally concave anterior body (Fig. 1), with very small conical posterior body. Their anterior body was somewhat attenuated apically and rounded caudally, and 0.595 ~0.845 mm long and 0.417~0.595 mm wide. Pseudosucker was absent. Oral sucker was located at the anterior end, and 0.044~0.059 mm long and 0.046~0.064 mm wide. Muscular pharynx just behind the oral sucker was 0.049 ~0.061 mm long and 0.026~0.046 mm wide. Acetabulum was a little smaller than the oral sucker, 0.033~0.049 mm long and 0.038~ 0.056 mm wide, and located along the median line of the body. Tribocytic organ, a sucker-like structure with a slit open cavity, was distinctly seen in the posterior third of anterior body, and 0.115~0.205 mm long and 0.077~0.128 mm wide (Table 1 and Fig. 2). Excretory system was highly developed, Y-shaped, and containing numerous spherical or oval lime bodies(0.015~ 0.025 mm long) (*i.e.*, excretory corpuscles) (Fig. 1).

2. Adults

A total of 90 adult *P. cordatum*(6.0% of the infected) were recovered from 3 experimentally infected cats at 5 weeks after the infection (Table 2). They were firmly attached to the wall of the anterior part of the small intestine, but were with no much difficulty isolated by a pin. Except from the cats, no worms were recovered from other animals experimentally infected with the metacercariae.

The worms were in general very stout and fleshed, therefore, it was not always easy to flat the worms under a cover glass pressure. The measurements and observations of adult worms were performed on 20 well flattened specimens (Fig. 3). The worm dimensions were as presented in Table 3. Both the measurements and other morphological characters were compatible with those of *P. cordatum* described by Ciurea (1922) or Cho and Lee(1981) (Table 3 and Fig. 3). The eggs were first detected from the feces of an experimentally infected cat on day 27 after the infection. They were golden-yellow in color, thin-shelled and operculated,

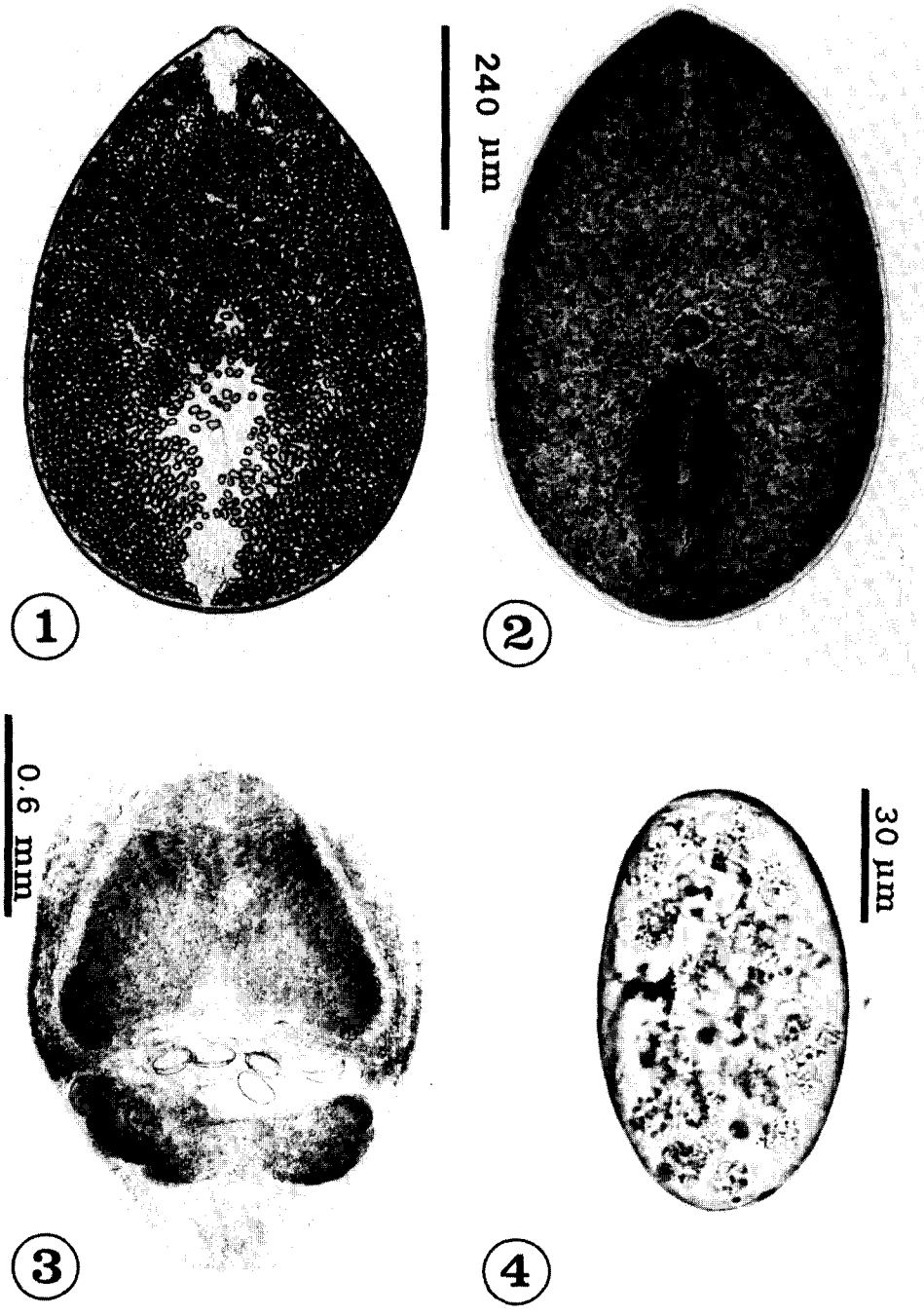

Fig. 1. A metacercaria of *P. cordatum* in fresh preparation, which is characteristically retaining its numerous round or elliptical excretory corpuscles.
Fig. 2. A metacercaria after stain with Semichon's acetocarmine, showing more obvious contour of each organ.
Fig. 3. Ventral view of a flattened adult *P. cordatum* after stain with Semichon's acetocarmine.
Fig. 4. An egg of *P. cordatum* isolated from the feces of an experimentally infected cat.

Table 1. Measurements* of *P. cordatum* metacercariae in comparison with those of previous authors

Organs	Present study		Wallace(1939)	Kurimoto(1976)	
	Range	Average	Range	Range	Average
Body					
Length (L)	0.595~0.845	0.708	0.350~1.000	0.364~0.565	0.463
Width (W)	0.417~0.595	0.492	0.175~0.325	0.273~0.410	0.346
Oral sucker					
(L)	0.044~0.059	0.052	0.043~0.047	0.039~0.052	0.047
(W)	0.046~0.064	0.056	0.048~0.054	0.039~0.046	0.041
Pharynx					
(L)	0.049~0.061	0.054	0.046~0.050	0.046~0.059	0.053
(W)	0.026~0.046	0.035	0.038~0.042	0.026~0.033	0.028
Acetabulum					
(L)	0.033~0.049	0.042	0.039~0.044	0.033~0.046	0.035
(W)	0.038~0.056	0.048	0.037~0.049	0.033~0.046	0.040
Tribocytic organ					
(L)	0.115~0.205	0.155	0.121~0.130	0.104~0.137	0.115
(W)	0.077~0.128	0.102	0.073~0.078	0.052~0.078	0.064

*25 metacercariae were measured (unit : mm).

Table 2. Results of experimental infection of the metacercariae to animals

Animals used	No. animals infected	No. metacercariae given	No. worms recovered(%)
Mouse	6	1,200	0
Albino rat	5	1,000	0
Golden hamster	5	1,000	0
Duckling	2	600	0
Dog	1	500	0
Cat	3	1,500	90(6.0)

and 0.102~0.125 mm by 0.059~0.072 mm in size(Fig. 4).

DISCUSSION

In the present study, it was verified that the European grass snake, *Rhabdophis tigrina*, harbours the metacercarial stage of *P. cordatum* and serves as a second intermediate host in Korea. In a strict sense, however, the snake should be a paratenic or transport host, and although not known yet in Korea, some kinds of frogs and their tadpoles are suspected as true second intermediate hosts. In other countries such as China and Japan, several species of frogs and their tadpoles, for example, *Rana limnocharis*, *R. rugosa*, *R. guentheri*, *R. nigromaculata*, *R. brevipoda*, *Microhyla ornata*, *M. pulchra*, *Bufo melanostictus*, *Ooeidozyga lima* or *Polypedates leucomystax megacephalus*, were reported as the second intermediate hosts (Wallace, 1937 & 1939; Kurimoto, 1976; Nakamoto, 1986).

As for the paratenic hosts, several kinds of snakes such as *Rhabdophis*(=*Natrix*) *piscator*, *R. stolata*, *Enhydris chinensis* and *Elaphe radiata*, toads, tortoises and shrews were recorded in China (Wallace, 1937 & 1939). However, *Rhabdophis tigrina*, had never been reported to harbour the metacercariae of *P. cordatum*. Here, *R. tigrina* is added as a new paratenic host of *P. cordatum* in the literature.

Table 3. Measurements of *P. cordatum** adults in comparison with those described by previous authors

Organs	Present study		Previous study by	
	range	average	Ciurea(1922)	Cho & Lee(1981)
Body length	1.414~2.194	1.744	2.60 ~3.82	1.58
Body width(F)**	1.071~1.684	1.359	1.58 ~1.98	1.25
Body width(H)***	0.956~1.466	1.161	—	0.44
Oral sucker				
Length	0.100~0.135	0.116	0.19	0.148
Width	0.120~0.185	0.164		0.102
Pharynx				
Length	0.120~0.150	0.135	0.21 ~0.23	0.145
Width	0.120~0.150	0.137	0.21 ~0.22	0.140
Ventral sucker				
Length	0.040~0.060	0.047	0.066	—
Width	0.045~0.055	0.049		
Tribocytic organ				
Length	0.500~0.750	0.590	0.99 ~1.32	0.77
Width	0.800~1.225	0.983	1.15 ~1.78	0.76
Ovary				
Length	0.360~0.495	0.417	—	0.26
Width	0.100~0.175	0.141		0.12
Egg				
Long diameter	0.102~0.125	0.113	0.118~0.132	0.107~0.120 (0.114)
Short diameter	0.059~0.072	0.065	0.074~0.088	0.063~0.065 (0.064)

*20 worms were measured(unit : mm)
**Forebody
***Hindbody.

Wallace(1937) mentioned that in the above snake hosts *P. cordatum* metacercariae were found usually in the muscles between the ribs or just below the vertebrae in the anterior part of the snake body. However, in the present study with *R. tigrina*, the metacercariae were found chiefly from the peritoneal membranes, and only a few were collected from intercostal muscles, by unknown reason.

The metacercariae of *P. cordatum* were at first thought to be some variant of the metacercariae of *F. seoulensis*, the only known diplostomid trematode whose adult stage is parasitic in the human host (Seo, 1990), since the morphology of these two kinds of metacercariae was very similar to each other. Important differential characters were in their body size and the distribution of excretory granules. The metacercariae of *F. seoulensis* were relatively small, 0.199~0.312 mm long and 0.153~0.252 mm wide(Hong *et al.*, 1982), whereas those of *P. cordatum* in this study were much larger, 0.595~0.845 mm long and 0.417~0.595 mm wide. The excretory system was very well developed in *P. cordatum* containing numerous, large and prominent excretory corpuscles, whereas it was less markedly developed in *F. seoulensis*.

In the infection experiment of cats with *P. cordatum*, it seems worth while to note that the present result was obtained from a second trial, after a first trial to infect cats was failed (not described in this paper). Concerned with this point, an interesting comment by Wallace(1939) was that, in his experiment, not all of the metacercariae developed in the definitive host and some, as in the reservoir host(rats), migrated

to the intercostal muscles and remained there without visible changes. Although not certain and has to be confirmed, the failure of our first trial might have been due to extraintestinal migration of worms. Such extraintestinal migration of larval diplostomes(=mesocercariasis), as reported also in *Alaria* spp., is important since it can occur in the human host (Beaver *et al.*, 1977), sometimes with fatal outcome (Fernandes *et al.*, 1976).

No human infection with *P. cordatum*, intestinal or extraintestinal, has been reported so far, and thus susceptibility of humans to *P. cordatum* infection is unknown. However, we cannot exclude a possibility of human infection with *P. cordatum*, considering that natural human infections(intestinal) were reported in a kind of diplostomid fluke, *F. seoulensis* (Seo, 1990), and an experimental human infection(intestinal) was successful in *F. cratera* (Shoop, 1989). More attention will be helpful to make it clear in the future.

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=국문초록=

유혈목이(*Rhabdophis tigrina*)에서 검출된 *Pharyngostomum cordatum*의 피낭유충 및 고양이 실험 감염

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경남 진주시의 밭집에서 구입한 유혈목이(*Rhabdophis tigrina*)에서 *Pharyngostomum cordatum*의 피낭유충을 검출하였으며, 수집된 피낭유충을 마우스, 흰쥐, 햄스터, 오리, 개 및 고양이에 경구 감염시켰던 바, 고양이에서만 감염 후 5주에 성충이 회수되었다.

피낭유충은 전단이 후단에 비해 조금 뾰족하였으며 복측으로 만곡되어 있었고 체내에는 원형 또는 타원형의 배설과립이 충만되어 있었다. 체장은 0.60~0.85(평균 0.71)mm, 체폭은 0.42~0.60(평균 0.49)mm이었으며 각 기관의 계측치 및 형태학적 특징이 *P. cordatum*과 일치하였다.

고양이로부터 회수한 성충은 충체가 매우 육질성이었으며 앞, 뒤 두 부분으로 나뉘어지 있었고 체장은 1.41~2.19 mm(평균 1.74 mm)이었다. 각 기관의 계측치 및 형태학적 특징이 기 보고된 Ciurea(1922), Cho and Lee (1981) 등의 결과와 일치하여 *P. cordatum*(Diesing, 1850) Ciurea, 1922으로 동정하였다.

이상의 결과로 우리 나라에서 유혈목이가 *P. cordatum*의 제 2 중간숙주(또는 paratenic host)로 작용하고 있음을 확인하였다.

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