The Korean Journal of Parasitology
Vol. 32, No. 4, 277-280, December 1994

☐ **Brief Communication** ☐

Infectivity of *Paragonimus westermani* developing in a final host to another final host

Yoon Kong[1], Hyun Jong Yang[2] and Seung-Yull Cho[1]*

Department of Parasitology[1], College of Medicine, Chung-Ang University, Seoul 156-756 and Department of Parasitology[2], College of Medicine, Kyung Hee University, Seoul 131-702, Korea

Abstract: In the definitive hosts, metacercariae of *Paragonimus westermani* excyst in host duodenum, penetrate intestinal wall, migrate peritoneal and thoracic cavities, and develop to sexual maturity in 8 weeks. This study was undertaken to examine the age of the maturing *P. westermani* when their infectivity to the other definitive hosts was retained. On 3, 7, 10, 14, 21 and 28 days after feeding the metacercariae to cats through a gastric tube, the developing worms were harvested. The juveniles of different age were fed again to other experimental cats. One to 12 weeks after the oral-transfer infections, the experimental cats were examined for establishment of infections. In the cats to which 3-day and 7-day old juveniles (grown up to 1.4 mm long) were fed, 31.4% and 22.6% of the transferred worms were found infected. The worms of 10-28 days old were not infective. Early maturing stages grown up to 7 days maintained their infectivity to the other definitive hosts.

Key words: *Paragonimus westermani*, metacercariae, juvenile worm, transfer infectivity

Paragonimus westermani, which is the most important species causing human paragonimiasis in East and Southeast Asia, is contracted mostly by raw eating of freshwater crayfish or crab. In addition, human infections can be contracted by raw eating of wild boar meat (Miyazaki and Habe, 1976). Rats, mice, rabbits and hens were also proved to play roles of the paratenic hosts of *P. westermani*. Excysted metacercariae infected in muscle of the paratenic hosts were found infective to maturation in the carnivorous mammals (Miyazaki and Hirose, 1976; Fan *et al.*, 1993).

To infect definitive hosts, the juvenile worms, in muscle of the paratenic hosts should survive in the acidic conditions of stomach, arrive duodenum without bodily damage possibly caused by host digestive enzymes, and retain their ability to penetrate intestinal wall. Therefore, juvenile worms in the paratenic hosts can be speculated to retain the most of their ability of survival and penetration as much as newly excysted metacercariae have. In this connection, the age of *P. westermani* developing in a definitive host are not known when the worms retain their infectivity to another definitive host. In order to solve this uncertainty, we undertook oral transfer experiments of *P. westermani* which are maturing in the final hosts.

Metacercariae of *P. westermani* were harvested from naturally infected freshwater crayfish (*Cambaroides similis*), which were collected in an endemic focus (Soh *et al.*, 1986). Oral transfer experiment was undertaken twice as shown in Table 1. The

• Received Oct. 12 1994, accepted after revision Nov. 8 1994.

• This study was supported by a Research Grant for Basic Medical Science, Ministry of Education, Republic of Korea Government (1994).

* Corresponding author

Table 1. Results of oral-transfer experiment of *P. westermani* of different ages which were reared in cats

Experiment/ Cat No.	Age of worms fed (days)	No. of worms fed	No. of infected (%)	Interval between oral infection and examination (days)	Examined cat organ[a]
First experiment					
1	3	15	6 (40)	30	P[b], T
2	7	20	5 (25)	21	P, T
3	14	22	Not-examined	Not-examined	Not-examined
4	21	26	0	12	P, T
5	28	40	0	7	P, T
Second experiment					
6	3	20	5 (25)	8	P
7	7	21	2 (9.5)	9	P
8	10	5	0	23	P, T
9	14	7	0	12	P, T
10	21	21	0	7	P, T
11	28	21	0	85	T, L

[a]Based on the data of Im *et al.* (1993), [b]P: peritoneal cavity, T: thoracic cavity, L: lung

metacercariae were fed to cats in doses of 50-300 to secure juvenile worms of different ages. In the first experiment, the cats were killed on 3, 7, 14, 21 and 28 days after the infection. Peritoneum was opened, and exposed to physiological saline for 3 hours by placing the cats in a prone position. Juveniles were collected in the saline, and fed to other cats as shown in Table 1. The experimental cats were killed to examine whether the infection is established. Only the cats transfer-infected with 3- and 7-day old juveniles were found infected. Number of the recovered worms were 6 out of 15 fed (40%) in the 3-day old juveniles and 5 of 20 (25%) in the 7-day old worms, respectively (cat No. 1-5 in Table 1). They were all recovered at peritoneum. Because a cat infected with 14-day old juveniles was not examined and because infections may be established in some juveniles of 7- and 21-day old, the experiment was repeated (cat No. 6-11 in Table 1). In the second oral-transfer experiment, 3- and 7-day old juveniles were found also infected as much as 25% and 9.5%, respectively. The juveniles, grown for more than 10 days in the definitive hosts, were not infected by the transfer infection.

A part of recovered worms were fixed under cover glass pressure in 10% neutral formalin, dehydrated in serial alcohols, and stained with Semichon's acetocarmine. The length of the worms of different ages were measured (Fig. 1). Mean length was: 435 μm in excysted metacercariae, 657 μm in the 3-day old, 1,087 μm in the 7-day old, and 1,500 μm in a 10-day old worm. The worm grew linearly until the observation period of 28 days up to 4,075 μm. As far as the mean length is concerned, 10 day-old *P. westermani* corresponded to the so-called excysted metacercariae (1-1.36 mm long, Miyazaki and Hirose, 1976) found in muscle of the paratenic hosts. Orally transferred worms grew in similar rates with those grown in cats continuously although their length was a little shorter (Fig. 1).

The worms of the different ages were observed for their sex organ development. In the 3-day and 7-day old worms, only cell masses of ovary were seen. In the 10-day old worm, primordia of testes appeared additionally. In 14-day old worms, ovary was lobulated. In 21-day old worms, testes were also branched while vitellarian ducts and Mehlis' gland were developed.

This experiment exhibited that *P. westermani*, which were grown and developed for a week in their final hosts, retained their infectivity to the final hosts. *P. westermani* infection by raw eating definitive hosts is therefore proved feasible. In the nature,

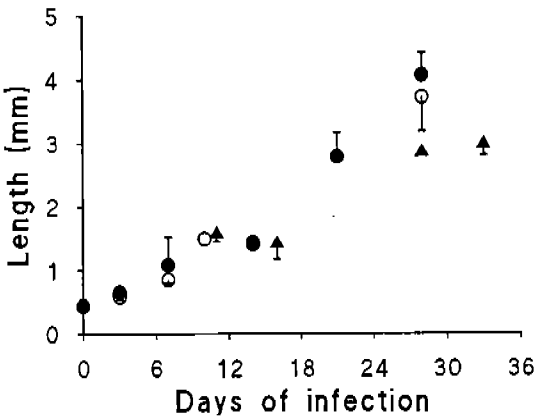

Fig. 1. Growth of length in early stages of *Paragonimus westermani* when infected in cats. (●); excysted metacercariae and juvenile worms recovered from cats (the first experiment), (○); juvenile worms from the second experiment, (▲); juveniles recovered from experimental cats which were infected by oral-transfer experiment (days in a cat + days in transferred infections). Vertical bars indicate one standard deviation.

however, this mode of infection can occur rarely because the infectivity is maintained only for a short period. Rather than its ecologic implication, the present experimental results may be related with functional transformations occurred in the early maturing stages of *P. westermani*. An interesting experiment of Nishimura (1966) demonstrated that adult *P. westermani* maintained their infectivity, migrated hemisphere and caused intracranial lesions when transplanted to subdural space of experimental dogs. Fan *et al.* (1994) showed also that the juveniles in paratenic hosts were infective to other paratenic hosts when transferred by subcutaneous or intraperitoneal routes. Lee *et al.* (1994) showed that intracranially inoculated juveniles and adults of *P. westermani* were infective to experimental cats. These results suggest that, once penetrating the intestinal wall, migration of *P. westermani* through tissue is facilitated by mechanisms different from those used in penetration of intestinal wall. At present, we can not explain exactly why juveniles older than 10 days are not infective when orally transferred. In this respect, intestinal wall seems to be a barrier to establish the infection. An explanation is that the older worms are degenerated when passed through acidic conditions of stomach whereas the younger worms passed undamaged. Another plausible mechanism is that the juveniles of 7-10 days undergo massive functional transformation which are related with the proteolysis. In this connection, proteases, secreted by newly excysted juvenile *P. westermani* have been partially purified and characterized as cysteine proteases (Yamakami and Hamajima, 1989; Song and Dresden, 1990; Chung *et al.*, 1994). Song and Dresden (1990) also reported that late maturing stages revealed far decreased activities of cysteine protease. Further studies on proteases in developing stages seems necessary in this regard.

REFERENCES

Chung YB, Kong Y, Joo IJ, Cho SY, Kang SY (1994) Excystment of *Paragonimus westermani* metacercariae by endogenous cysteine protease. *J Parasitol* (in press).

Fan PC, Lu H, Lin LH (1993) Experimental infection of *Paragonimus westermani* in mice and rats. *Korean J Parasitol* **31:** 91-97.

Fan PC, Lu H, Lin LH (1994) Experimental transfer of *Paragonimus westermani* from rodents to rodents following subcutaneous and intraperitoneal route. *J Helminthol* **68:** 41-44.

Im JG, Kong Y, Shin YM, *et al.* (1993) Pulmonary paragonimiasis: Clinical and experimental studies. *RadioGraphics* **13:** 575-586.

Lee SK, Chang KH, Goo JM, *et al.* (1994) An experimental study on cerebral paragonimiasis using cats. *J Korean Radiol Soc* **30:** 1003-1012.

Miyazaki I, Habe S (1976) A newly recognized mode of human infection with the lung fluke, *Paragonimus westermani* (Kerbert 1878). *J Parasitol* **62:** 646-648.

Miyazaki I, Hirose H (1976) Immature lung flukes first found in the muscle of the wild boar in Japan. *J Parasitol* **62:** 836-837.

Nishimura K (1966) Experimental studies on cerebral paragonimiasis. *Jpn J Parasitol* **15:** 429-446 (in Japanese with English abstract).

Soh CT, Lee KT, Min DY, *et al.* (1986) Field operational research on the control of paragonimiasis in Korea. *Yonsei Rep Trop Med* **17:** 30-41.

Song CY, Dresden MH (1990) Partial purification

and characterization of cysteine proteinases from various developmental stages of *Paragonimus westermani*. *Comp Biochem Physiol* **95B:** 473-476.

Yamakami K, Hamajima F (1989) Purification of a neutral thiol protease from *Paragonimus westermani* metacercariae by single-step chromatography and preparation of antibodies *Intern J Parasitol* **19:** 9-12.

=국문초록=

종숙주에서 발육중인 폐흡충의 종숙주에 대한 감염력

중앙대학교 의과대학 기생충학교실[1] 및 경희대학교 의과대학 기생충학교실[2]

공윤[1], 양현종[2], 조승열[1]

폐흡충의 피낭유충이 종숙주에 도입되면 십이지장에서 탈낭하여 장벽을 통과하고, 복강과 흉강을 돌아다닌 후 8주일이면 성충으로 발육한다. 이 연구에서는 종숙주에서 발육중인 폐흡충 발육단계의 어느 시기까지 종숙주에 대한 감염력을 유지하는지를 관찰하였다. 가재에서 분리한 폐흡충 피낭유충을 고양이에 경구감염(經口感染) 시키고 3일, 7일, 10일, 14일, 21일 및 28일 후 각 발육단계의 충체를 얻었다. 그리고 각 단계를 고양이에 다시 경구감염시킨 다음 1-12주일 후 고양이를 해부하여 감염 여부를 조사하였다. 종숙주에서 3일간 발육한 충체와 7일간 발육한 충체를 경구감염시킨 고양이에서 각각 투여 충체수의 31.4% 및 22.6%를 검출하였다. 종숙주에서 10일, 14일, 21일, 28일간 성장 발육한 충체를 경구감염시킨 고양이에서는 감염충체를 발견할 수 없었다. 이상의 결과, 종숙주에서 최소한 7일간 발육한 폐흡충의 유충은 다시 종숙주를 감염시킬 수 있는 능력을 지니고 있었다.

〔기생충학잡지 32(4): 277-280, 1994년 12월〕