

Studies on the Lung Fluke, *Paragonimus westermani*-Diploid Type,  
in the Northern Part of Hyogo Prefecture, Japan  
VII. Experimental Oral Infection of a Red Fox with the Metacercariae

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**Key words:** Lung fluke, *Paragonimus westermani*, diploid type, red fox, host adaptation

The red fox, *Vulpes vulpes*, is a wild mammal which is widely distributed throughout the Northern hemisphere and its food habit varies greatly from carnivorous to herbivorous. In Japan, it has been regarded as a suitable host for the lung fluke, which adopts crabs as intermediate hosts. However, there have been few reported cases of natural infection; by Onji, 1918; Nagase *et al.*, 1977; Matsuno, 1978 and Ashizawa *et al.*, 1980. The report by Onji (1918) was made when it was thought that the only one species of the lung fluke in Japan was *Paragonimus westermani* (Kerbert, 1878) Braun, 1899. Since the species discussed in the report was not fully investigated, it is not clear to which species it belongs. On the other hand, the report by Matsuno (1978) was on *P. miyazakii* Kamo, Nishida, Hatsushika *et Tomimura*, 1961. Also, it has become clear that both the species shown by Nagase *et al.* (1977) and that shown by Ashizawa *et al.* (1980) are the diploid type of *P. westermani*. In the present research, as a part of a series of

ecological studies of *P. westermani*-diploid type in the northern part of Hyogo Prefecture, the susceptibility of a red fox (*Vulpes vulpes japonica*) and the growth and development of the worms obtained are presented.

#### Materials and Methods

*Parasite and experimental host:* Metacercariae were collected by the digestive method in artificial gastric juice from freshwater crab, *Geothelphusa dehaani*, captured in a mountain stream of Tanto-cho, Izushi-gun, upstream of the Maruyama River in the northern part of Hyogo Prefecture, Japan. It is known that Tanto-cho is a densely endemic area for the diploid type of *P. westermani* (Shibahara, 1982). An adult male of the red fox, *Vulpes vulpes japonica* (5.1 kg in body weight, bought from a fur farm), was used. It was checked to be negative for *Paragonimus* infection by fecal examination prior to the experiment.

*Method of infection:* The fox was anesthetized with ketamine hydrochloride (Ketalar<sup>®</sup> 50; Parke-Davis), and a gelatin capsule containing 20 metacercariae was orally administered to the fox.

*Care of the animal:* It was given a commercial diet (DS & DK; Oriental Yeast Co., Ltd.) and tap water on occasion. The feces were collected every other day from day 50 after infection and were examined by the

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formalin-ether sedimentation technique (MGL-method) for determination of prepatent periods.

*Recovery of parasite:* The fox was put under general anesthesia using sodium pentobarbital (Somnopentyl; Pitman-Moore) on day 100 after infection with metacercariae. After anesthesia

was induced, it was bled from a carotid artery to induce death and then was autopsied. The investigation for worms was made in the pleural cavity, abdominal cavity and visceral organs by gross examination only.

*Worms and eggs:* The recovered worms were

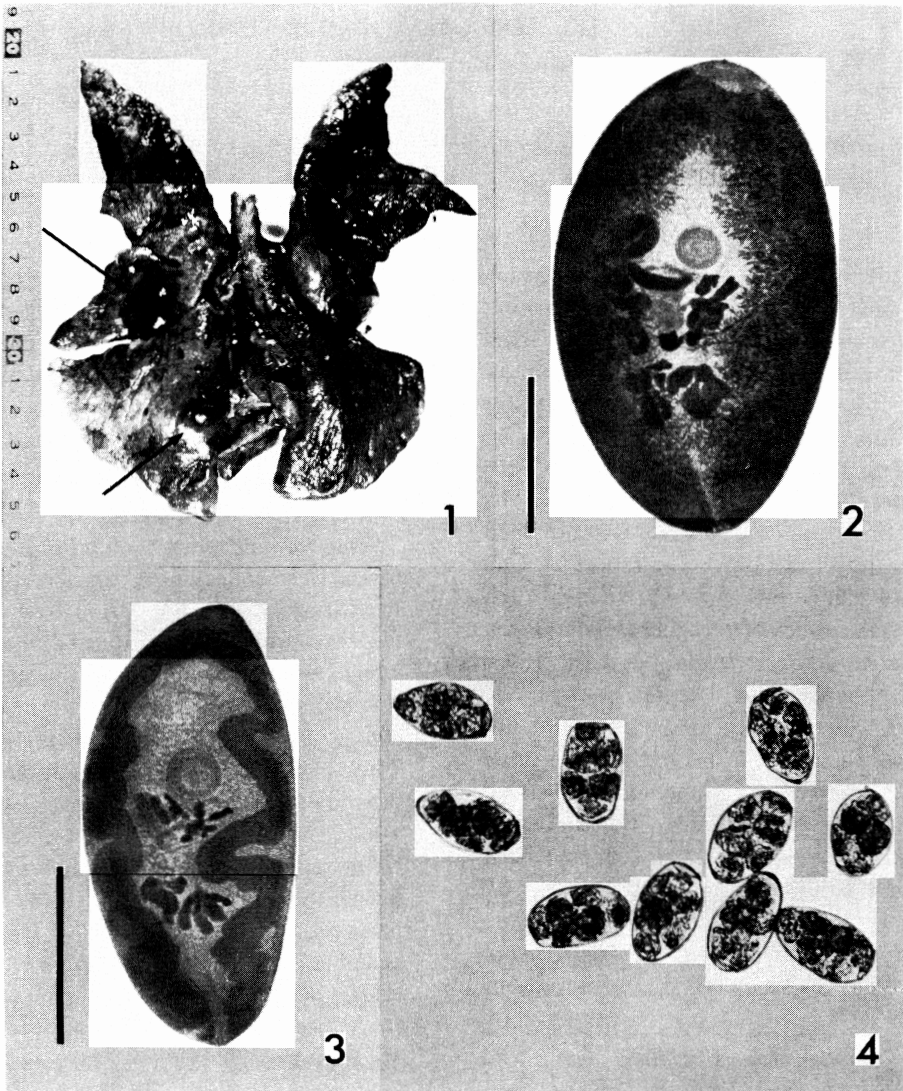


Fig. 1 The lung of the red fox infected with metacercariae of *Paragonimus westermani*-diploid type. Arrows indicate worm cysts.

Fig. 2 Adult worm recovered from a lung cyst (balsam-mounted specimen, dorsal view). Scale = 2 mm

Fig. 3 Pre-adult worm recovered from the pleural cavity (balsam-mounted specimen, dorsal view). Scale = 2 mm

Fig. 4 Eggs removed from a lung cyst.

flattened, fixed, stained, dehydrated, cleared and mounted in much the same way as the previous paper (Shibahara, 1985). Morphological observations and measurements were made on these mounted specimens. The worms were classified as follows by the developmental stages: 1) adult worms with eggs in the uterus, 2) pre-adult worms with spermatozoa in the seminal vesicle or with vitelline cells in the vitelline duct instead of eggs in the uterus. Eggs recovered from the worm cysts in the lungs were preserved in 10% formalin and were then used for morphological observations and measurements.

### Results

Gross examination showed four worm cysts in the lungs (Fig. 1). A total of 11 worms were recovered from the lungs and pleural cavity at the recovery rate of 55.0%, of which 2 were from the pleural cavity, 2 were from the lung tissue and the remaining 7 were from the cysts in the lungs. Of the 11 worms, 6 from cysts in lungs were adults with eggs in their uteri, as shown in Fig. 2, and the remaining 5 were pre-adults, as shown in Fig. 3. The morphology of the worms, especially of the adults, showed the characteristics typical of the diploid type of *P. westermani*, i.e., the singly spaced spines, the ovary divided into 6 lobes, the testes divided into 5 to 6 lobes and the seminal receptacle

and seminal vesicle filled with large amounts of spermatozoa. However, only 2 adult worms contained spermatozoa in the seminal receptacle. The sizes of the worms recovered from various sites of the body were as indicated in Table 1. The average body size of pre-adult worms obtained from the pleural cavity, lung tissue and cysts in the lungs measured  $4.24 \pm 0.40$  (3.66~4.78) mm in length and  $2.19 \pm 0.17$  (1.93~2.37) mm in width. Those of 5 adult worms measured  $6.31 \pm 0.82$  (5.47~7.42) mm in length and  $3.69 \pm 0.52$  (2.93~4.16) mm in width.

Observation of 50 eggs from the worm cysts in the lungs morphologically showed some variation, as shown in Fig. 4. Of the 50 eggs, 9(18%) were symmetrical, but 41(82%) were asymmetrical. The maximum width was located in the anterior half for 10(20%), in the middle for 34(68%) and in the posterior half for 6(12%). Thickening of the shell at the abopercular end was clearly recognized in 18(36%), but was slight or not observable in the remaining 32(64%). The average size of the eggs was  $73.1 \pm 3.2$  (64.4~80.9)  $\mu\text{m}$  in length and  $43.8 \pm 2.2$  (38.8~48.7)  $\mu\text{m}$  in width. The average thickness of the eggshells was  $1.3 \pm 0.2$  (1.1~1.8)  $\mu\text{m}$ .

Examination of the feces for eggs was carried out every other day from day 50 to day 100 after infection with metacercariae, but no eggs were detected.

Table 1 Measurements of the worms of *Paragonimus westermani*-diploid type recovered from various tissues of a red fox, *Vulpes vulpes japonica*

Recovery site	Type* of worms	No. of worms measured	Body		Oral sucker	Ventral sucker	
			length (mm)	width (mm)	width ( $\mu\text{m}$ )	length ( $\mu\text{m}$ )	width ( $\mu\text{m}$ )
Pleural cavity	Pre-A	2	4.29	2.09	491	538	524
Lung tissue	Pre-A	2	4.47	2.24	506	532	542
Cyst in lung	Pre-A	1	3.66	2.30	548	502	528
Cyst in lung	A	5	$6.31 \pm 0.82^\dagger$	$3.69 \pm 0.52$	$753 \pm 62$	$606 \pm 50$	$691 \pm 56$

\*A) adult worm with eggs in uterus, Pre-A) pre-adult worm with sperm in seminal vesicle or with vitelline cells in vitelline duct.

†Mean  $\pm$  SD.

### Discussion

In the past in Japan, most workers have thought that there were only a few cases of natural infection of red fox with lung flukes, though many foxes inhabit the endemic area of the lung flukes. To this date, only 4 cases of natural infection have been reported in Japan. Two of them have been determined to be due to *P. westermani*-diploid type. Only a small number of cases of natural infection of the fox with lung flukes have been observed abroad as well, except those with *P. kelliottii* in North America, where a comparatively large number of cases have been reported. To our knowledge, there have been only 3 cases of natural infection with *P. westermani* on the Korean Peninsula and the far eastern part of the USSR (Kobayashi, 1926; Posokhov and Shabanova, 1977; Dovgalev *et al.*, 1980). Ashizawa *et al.* (1980) stated that the reason for this low incidence rate may be due to the fact that few foxes have been captured. In fact, in surveys of wild mammals by previous Japanese workers, the number of the foxes captured and examined is extremely small as compared with that in North America.

The present authors, aware of this point, have tried to obtain as many foxes as possible from the densely endemic areas of the diploid type of *P. westermani* in the northern part of Hyogo Prefecture. However, only 31 foxes were obtained in 5 years and the results were all negative (Shibahara and Nishida, 1985). Further investigation may reveal the natural infections in this area, too.

The cases of natural infection in foreign countries, especially, the 3 cases caused by *P. westermani* described above, are all those in areas where crawfishes (*Cambaroides* spp.) are reported as the intermediate hosts, as with those of *P. kelliottii* in North America. In Japan, however, the second intermediate hosts are the mitten crabs and/or freshwater crabs, the latter mainly carrying the diploid type. The freshwater crabs are smaller and often leave the water to search for food, thus being easier

prey of the fox. The distribution of the diploid of *P. westermani* is rather restricted according to the location (Shibahara, 1986). Therefore, it seems that a survey should be conducted where the freshwater crabs positive for the metacercariae are found at a high rate in order to clarify the actual conditions of natural infection in foxes.

In the present experimental infection with the metacercariae, it was demonstrated that the metacercariae easily infected the fox and was able to reach sexual maturity in it. Hence, it became clear that the fox, as well as raccoon dogs and wild boars, also may serve as a natural definitive host of the diploid type of *P. westermani*.

### Summary

As a part of a series of ecological studies of *P. westermani*-diploid type in the northern part of Hyogo Prefecture, Japan, an experimental infection of a red fox (*Vulpes vulpes japonica*) with the metacercariae was performed. As a result, it was demonstrated that the metacercariae easily infected the fox and reached sexual maturity in it. Hence, it became clear that the fox, as well as raccoon dogs and wild boars, also may serve as a natural definitive host of the diploid type of *P. westermani*.

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## 兵庫県北部におけるウエステルマン肺吸虫(二倍体型)に関する研究

### VII. キツネへの感染実験

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兵庫県北部産ウエステルマン肺吸虫(二倍体型)に関する生態学的研究の一環として、ホンドギツネ1頭を用いて本虫メタセルカリアの感染実験を行い、その感受性および虫体の発育状況を観察した。その結果、従来日本では、キツネが肺吸虫の自然終宿主として報告された例は少ないが一本虫によるものであ

ることが明らかなのは2例に過ぎない。本虫メタセルカリアはキツネに容易に感染して性成熟に至ることが証明された。このことから、タヌキやイノシシと同様に、キツネもまた本虫の自然界における終宿主としての役割を演じているものと思われる。