

## Experimental Infection of Raccoon Dogs with Two Types (Diploid and Triploid) of the Lung Fluke, *Paragonimus westermani*

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**key words:** lung fluke, *Paragonimus westermani*, diploid type, triploid type, raccoon dog, host adaptation

### Introduction

Recently, it is revealed that there are two chromosomal types in *Paragonimus westermani* (Kerbert, 1878), a diploid type ( $2n=22$ ) and a triploid type ( $3n=33$ ) (Sakaguchi and Tada, 1976; Terasaki, 1977). Miyazaki (1978, 1983) proposed to call the former as *P. w. japonicus*, and the latter as a different species, *P. pulmonalis*.

The raccoon dog, *Nyctereutes procyonoides viverrinus*, is one of common wild mammals in Japan, and is known as the definitive host of *P. ohirai* Miyazaki, 1939 and *P. miyazakii* Kamo *et al.*, 1961 (Yokogawa *et al.*, 1957a; Ashizawa *et al.*, 1974; Hirai *et al.*, 1978). However, the raccoon dog has been regarded as an unsuitable host for *P. westermani* (Yokogawa *et al.*, 1957b; Yokogawa, *et al.*, 1960; Yokogawa, 1964). According to Yokogawa (1964) the raccoon dog was not easily infected with *P. westermani* and was therefore not considered to be a proper definitive host. The reason for this is that Yokogawa *et al.* (1957b) could find neither fully matured adult worms nor cyst formation in the lungs in an experiment with a raccoon dog infected orally with metacercariae of *P. westermani*.

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However, recently, Shibahara (1981) and Yoshimura *et al.* (1983) reported that they found wild raccoon dogs naturally infected with *P. westermani*, discovering fully matured adult worms in cysts in the lungs. Therefore, it became necessary to reconsider the susceptibility of the raccoon dog to *P. westermani*. The present study was undertaken to obtain fundamental data for a comparative study of the susceptibility of raccoon dogs to both types of *P. westermani*.

### Materials and Methods

#### *Parasite and experimental hosts:*

Metacercariae of the diploid type were collected by the digestive method in artificial gastric juice from freshwater crab, *Geothelphusa dehaani*, captured upstream of the Maruyama River in the northern part of Hyogo Prefecture, Japan. Metacercariae of the triploid type were also collected by the same method from *Eriocheir japonicus* captured in the central section of the Shimanto River in the western part of Kochi Prefecture, Japan.

The four adult raccoon dogs used weighed about 3kg and were captured by a hunter in the non-endemic areas of paragonimiasis in Ehime Prefecture and/or were bought from a skinner (Fig. 1). They were found to be negative for *Paragonimus* infection by fecal examinations prior to the experiment.

#### *Method of infection:*

Raccoon dogs were divided into two groups and put under general anesthesia. Each rac-

coon dog in both the two groups was orally administered a gelatin capsule containing 20 metacercariae of the diploid type or of the triploid type of *P. westermani*.

#### Recovery of parasite :

One raccoon dog from each group was autopsied 100 days after infection with metacercariae and rest of them 150 days after infection. First, their visceral organs, and the peritoneal and pleural cavities were searched for lung fluke infection and the worms were recovered. Then, the lungs, liver and muscle of the whole body were cut into 4-5mm thick slices and kept in physiological saline solution at about 37°C. After 6-7 hours, the worms emerged and were collected.

#### Worms :

The worms recovered were flattened to about 0.3mm in thickness between two slide glasses, fixed in 70% alcohol, stained with borax-carmin, dehydrated, cleared in xylene and mounted in balsam. The worms were classified into three types by the developmental stages: 1) adult worms with eggs in their uterus, 2) pre-adult worms with sperm in their seminal vesicle or with vitelline cells in their vitelline ducts instead of eggs in their uteri and 3) immature worms with no eggs, sperm, vitelline cells and with no granules in their excretory bladder.

#### Statistical analysis :

Statistical analysis was made by the Welch method.

## Results

#### Experimental infection of raccoon dogs :

The worm recovery rate of the diploid type was at the high level of 95.0% on day 100 after infection, but 50.0% on day 150 (Table 1). The worms were recovered from the lungs, pleural and peritoneal cavities, but not from the muscle or other visceral organs. The number and the recovery rates of adult worms were 9 (47.4%) on day 100 after infection, and 4 (40.0%) on day 150. Although the worm recovery rate of the triploid type was 35.0% on day 100 after infection, neither worms nor cysts were found except for

Table 1 Results of experimental oral infection of the raccoon dog, *N. p. viverrinus*, with 20 metacercariae of two types of *P. westermani*

Type	Experimental host No.	Sex	Body weight at autopsy (kg)	Days after infection	No. and (%) of worms recovered	No. of worms recovered from			No.* and (%) of worms with eggs in uterus
						peritoneal cavity	pleural cavity	cyst in lungs	
diploid	1	♀	3.7	100	19 (95.0)	5	2	12	9/19 (47.4)
	2	♀	3.4	150	10 (50.0)	1	3	6	4/10 (40.0)
triploid	1	♂	3.8	100	7 (35.0)	2	5		0/7 ( 0.0)
	2	♂	3.6	150	13 (65.0)		1	12	9/13 (69.2)

\* No. of worms having eggs in uterus/No. of worms recovered

Table 2 Comparison of the sizes of the worms of two types obtained from raccoon dogs infected with metacercariae of each type of *P. westermani*

Type	Days after infection	Body size (mm)					
		Immature		Pre-adult		Adult	
		length	width	length	width	length	width
diploid	100	—	—	4.9±0.8* (3.5~6.4)	2.8±0.6 (2.3~4.1) [10]†	6.9±0.7‡ (5.9~8.0)	3.8±0.1 (3.6±4.0) [ 9]
	150	—	—	5.1±0.7 (3.7~5.7)	2.9±0.4 (2.3~3.2) [ 6]	7.4±0.8 (6.4~8.3)	4.4±0.5 (3.9~4.9) [ 4]
triploid	100	4.7±0.6 (4.1~5.8)	2.6±0.3 (2.3~3.1) [ 7]	—	—	—	—
	150	—	—	6.8±0.8§ (6.0~7.6)	4.6±0.2‡ (4.4~4.8) [ 4]	9.0±1.2§ (7.0~11.1)	5.9±0.9§ (4.8~7.3) [ 9]

\* All values are the means and the standard deviations.

† Figures in brackets are the numbers of worms measured.

‡, § Significantly different from values of the diploid type on day 150 after infection (‡ :  $P < 0.005$ , § :  $p < 0.05$ ).

some migrating immature worms in the pleural and peritoneal cavities. On the contrary, the recovery rate of the worms was 65.0% on day 150 after infection, mostly from cysts in the lungs (Fig. 2) and 9 of 13 (69.2%) were fully matured adult worms. No worms were recovered from the muscle. No eggs were detected in the feces of any experimental host, though the feces were examined every day from day 30 to day 100 after infection.

#### Morphology of adult worms and eggs :

Both types of *P. westermani* obtained were morphologically characterized by singly spaced spines, the division of the ovary into 6 lobes and the division of the testis into 5-6 lobes. The worms mounted in balsam were generally roundish in shape. The average ratio of length/width was 1.76 in the diploid type and 1.63 in the triploid type. Of the worms recovered in this experiment, all the worms of the triploid type on day 100 after infection were immature (Fig. 3), but the others were pre-adult worms (Figs. 4 and 8) and adult worms (Figs. 5 and 9), as shown in Table 2. The adult worms of the diploid type recovered on day 150 after infection were significantly larger in body size ( $p < 0.005$ ) than those on day 100. The body size of the triploid type was considerably larger and

more mature than that of the diploid type. A significant statistical difference was also observed between the two types 150 days after infection ( $p < 0.005$ , 0.05). Moreover, as shown in Table 3, the adult worms obtained from raccoon dogs on day 150 after infection were apparently smaller than those obtained from dogs and cats which are considered more suitable hosts for both types than raccoon dogs ( $p < 0.005$ , 0.01). The sizes of the worms recovered decreased in descending order from cats, dogs, raccoon dogs. A great amount of sperm was found in the seminal vesicle of most of the worms of the diploid type, and also in the seminal receptacle. However, no sperm was found in either the seminal vesicle or seminal receptacle in the triploid type.

On the other hand, although the eggs of the diploid type 150 days after infection varied in shape in comparison with those of the triploid type, the latter (Figs. 6 and 7) was apparently larger in size than the former (Figs. 10 and 11), as shown in Table 3 ( $p < 0.005$ ). The thickening of the egg-shell at the abopercular end was also remarkable in the latter.

#### Discussion

In this study, it was clarified that the raccoon dog was a suitable host for the two types

Table 3 Comparison of the sizes of the adult worms and eggs of two types obtained from various animals on day 150 after infection with metacercariae of each type of *P. westermani*

Type	Experimental host	Body size (mm)		Egg size† (μm)		Authors
		length	width	length	width	
diploid	cat	10.3±0.8*§ (9.0~11.4)	5.6±0.5 <sup>  </sup> (4.6~6.4) (18)†	78±5.0 (67~88)	43±2.7 (37~50)	Shibahara (1983)
	dog	9.2±1.1 <sup>  </sup> (7.1~11.4)	4.8±0.7 (3.6~5.9) [26]	76±3.5 (68~84)	43±3.0 (34~51)	Shibahara (1983)
	raccoon dog	7.4±0.8 (6.4~8.3)	4.4±0.5 (3.9~4.9) [4]	75±4.7 (64~83)	43±2.3 (38~49)	present author
triploid	cat	14.6±0.8§ (13.1~16.0)	8.6±0.6§ (6.6~9.1) [18]	91±4.4 (81~100)	49±2.8 (43~56)	present author
	dog	11.4±0.4 <sup>  </sup> (11.1~11.7)	7.9±0.8§ (6.9~8.5) [4]	93±3.6 (87~101)	53±2.8 (47~58)	present author
	raccoon dog	9.0±1.2 (7.0~11.1)	5.9±0.9 (4.8~7.3) [9]	88±4.9 <sup>  </sup> (77~100)	50±2.8 <sup>  </sup> (43~56)	present author

\* All values are the means and the standard deviations.

† Figures in brackets are the numbers of adult worms measured.

‡ Fifty eggs were measured.

§ <sup>||</sup> Significantly different from values in the raccoon dog (§ :  $p < 0.005$ , <sup>||</sup>  $p < 0.01$ ).

<sup>||</sup> Significantly different from values of the diploid type in the raccoon dog ( $p < 0.005$ ).

of *P. westermani* because the worms reached sexual maturity. The triploid type seems to take a longer time to reach sexual maturity than the diploid type in raccoon dogs, because, in the triploid type infection, no worm-cyst formation was found in the lungs and no worms that developed to sexual maturity were found in the pleural cavity on day 100 after infection. Therefore, it seems that raccoon dogs are less suitable for the triploid type.

Yokogawa *et al.* (1957b) reported the ex-

perimental infection of a raccoon dog with 100 metacercariae of *P. westermani*. In their experiment, a raccoon dog was autopsied on day 104 after infection and was examined for the worms. Although seven worms were recovered from the pleural and peritoneal cavities, neither worm-cyst formation nor matured adult worms were found in the lungs. As is clear from the results of this experiment, the triploid type seems to require more time to reach maturity. The metacercariae

Fig. 1 Raccoon dog.

Fig. 2 The lung of the raccoon dog (150 days after infection) infected with metacercariae of *Paragonimus westermani*-triploid type. Arrows indicate worm cysts. Ventral view.

Figs. 3-7 *P. westermani*-triploid type. Scale: 3 mm.

3: Immature worm recovered from the raccoon dog 100 days after infection with metacercariae.

4: Pre-adult worm recovered from the raccoon dog 150 days after infection with metacercariae.

5: Adult worm from the raccoon dog 150 days after infection with metacercariae. 6 and 7: Eggs removed from the cyst of the lung of the raccoon dog 150 days after infection with metacercariae, preserved in 10% formalin.

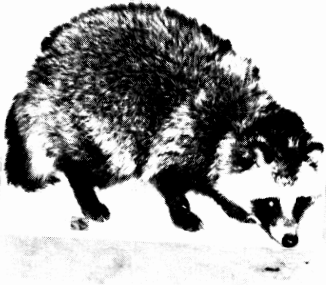
Figs. 8-11 *P. westermani*-diploid type.

8: Pre-adult worm recovered from the raccoon dog 150 days after infection with metacercariae.

9: Adult worm recovered from the raccoon dog 150 days after infection with metacercariae. 10

and 11: Eggs removed from the cyst of the lung of the raccoon dog 150 days after infection with metacercariae.

Same magnifications in Figs. 3-5, 8 and 9, and in Figs. 6, 7, 10 and 11. All worms shown in Figs. 3-5, 8 and 9 are balsam-mounted specimens.



1



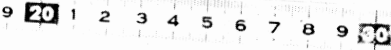
3



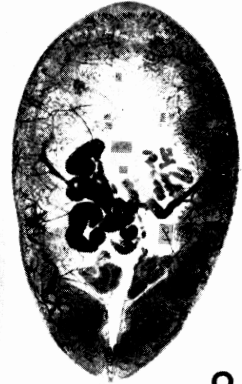
8



2



4



9



5



6



10



7



11

which Yokogawa *et al.* (1957b) used in their experiment were perhaps those of the triploid, which might explain why they could find neither worm-cyst formation nor matured adult worms.

There are only three previous reports of natural infection of raccoon dogs with *P. westermani* in Japan; Onji (1918), Shibahara (1981) and Yoshimura *et al.* (1983). Of these three, the report by Onji (1918) lacks a morphological description of the worms, therefore the flukes described in the paper cannot be identified as either the diploid type or the triploid type of *P. westermani* or as either *P. westermani* or a different species of *Paragonimus*. The flukes reported by Shibahara (1981) are the diploid type of *P. westermani*, and as are the flukes reported by Yoshimura *et al.* (1983) according to their morphological descriptions. No definite reports of natural infection of raccoon dogs with the triploid type of *P. westermani* have been heretofore made in Japan. However, as proved in this study, the triploid type can become fully matured adult worms in raccoon dogs. Therefore, it is possible that raccoon dogs may be natural final hosts of the triploid type.

The adult worms recovered from the raccoon dogs were apparently smaller in size than those from such suitable hosts as cats and/or dogs in both types, as shown in Table 3 ( $p < 0.005, 0.01$ ). Although the development in size of *P. westermani* seems to depend on various factors, such as the kind of the host, its physiological conditions, the number of parasites and the like, it may be possible to say that the degree of susceptibility for the raccoon dog is somewhat inferior to that for cats and/or dogs in both types.

### Summary

Raccoon dog, *Nyctereutes procyonoides viverrinus*, a common wild mammal in Japan, was experimentally infected with metacercariae of two types (diploid and triploid) of *Paragonimus westermani*, to investigate its susceptibility. The results revealed that the raccoon dog was, in fact, a suitable host for two types of *P. westermani* although it

has been heretofore regarded as unsuitable. Raccoon dogs, however, seem to be not as good hosts as cats or dogs, judging from the period needed to reach sexual maturity and the degree of development. Of the two types, the triploid type grew much slower and took a longer time to reach sexual maturity than the diploid type.

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## ウエステルマン肺吸虫の2型（二倍体型および三倍体型） のタヌキへの感染実験

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日本に普通に見られる野生哺乳動物であるタヌキに、ウエステルマン肺吸虫 *Paragonimus westermani* の2型（二倍体型および三倍体型）のメタセルカリアを感染させ、その宿主適合性について比較検討した。その結果、従来より本虫の非好適宿主であると考えられてきたタヌキが、実際には、本虫の両型にとって好適な宿主で

あることが明らかとなった。しかし、その好適度は成熟に要する期間および発育の程度からネコおよびイヌに対する程は良くないものと思われる。また、三倍体型は二倍体型に比べてさらに発育が悪く、成虫に至るまでにはより長時日を要した。