

GROWTH OF MALAYSIAN *PARAGONIMUS WESTERMANI* IN MAMMALS AND THE MODE OF TRANSMISSION OF THE FLUKE AMONG MAMMALS

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ABSTRACT: The host susceptibility of Malaysian *Paragonimus westermani* was observed in cats, dogs and rats, infected with metacercariae. In rats, worms were harboured in the muscles and the flukes were morphologically similar to the excysted metacercariae except for their slightly larger body size. In cats, about a half number of the flukes were recovered from muscles and 40% from cysts found in the lungs. Majority of the flukes from these cysts were mature. In dogs, few mature flukes were obtained from cysts in the lungs and about 90% of flukes were recovered from muscles. The flukes from the muscles of cats and dogs were of the same juvenile stage as those from rats. The juvenile flukes recovered from muscles of the animals were orally given to dogs and cats. Majority of the flukes were found in cysts in the lungs and most of them were fully mature. However, some juvenile flukes still remained in the muscles of cats and dogs. Therefore, the Malaysian *P. westermani* has a higher final host susceptibility than those from other localities. The present study suggests that many species of animals living in Malaysian jungle serve as paratenic hosts and may play an important role in the completion of the life cycle of *P. westermani* in Malaysia.

INTRODUCTION

Japanese *Paragonimus westermani* (Kerbert, 1878) usually remains in the muscles of rat, mouse, rabbit, pig and wild boar and for long period without development, and such animals could play a role as paratenic hosts of the fluke (Habe, 1978, 1983; Miyazaki and Habe, 1976; Shibahara and Nishida, 1986). Ingestion of raw wild boar meat is an important source of human infection of *P. westermani* in Japan (Miyazaki *et al.*, 1978; Norimatu *et al.* 1975; Tokudome *et al.*, 1977). In contrast *P. westermani* in Philippines does not remain in the muscles of mammals as a juvenile fluke (Miyazaki and Habe, 1979; Yokogawa *et al.*, 1979). The study of lung flukes in mammals provide knowledge on the life cycle and these information would be useful for prevention and control of paragonimiasis. In Malaysia, only one species of lung fluke, *P. westermani* has been recorded from various animals belonging to the family Felidae and from the crab-eating monkey, and a few experimental infections of Malaysian *P. westermani* in cats have been

done only to get adult worms specifically for identification. The growth of Malaysian *P. westermani* was not clear in the mammalian hosts. The present paper reports the sites and development of Malaysian *P. westermani* in cat, dog and rat and discusses the role of mammals as paratenic hosts, referring to the presumptive life cycle of this fluke.

MATERIALS AND METHODS

1) Experimental Animals and *P. westermani* Metacercariae

Seven adult dogs and 11 cats were used for investigation of site and growth of Malaysian *P. westermani* in mammalian hosts. Before experimental infection, the dogs and cats were shown to be free from lung fluke and intestinal parasites by stool examination. Thirty-two female albino rats, *Rattus norvegicus*, SD strain about 9 weeks old were also used.

Malaysian *P. westermani* metacercariae were recovered from fresh water crabs, *Parathelphusa maculata* and

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Parathelphusa malaysiana which were collected from Kampong Langkap near Kuala Pilah and Sungai Wa at Taman Negara, Peninsular Malaysia, respectively. They were inoculated into dogs, cats and rats. The metacercariae from *P. maculata* collected at Ulu Langat near Kuala Lumpur were inoculated into cats.

2) Inoculation of Metacercariae

The experimental animals were infected with 25 to 150 metacercariae (Tables 1-3). Five cats and a dog were orally infected using a pipette and 29 rats were also orally infected with metacercariae in a little water using a syringe connected to a slender tube inserted into the stomach. Other animals, 6 cats, 6 dogs and 3 rats, were injected with the metacercariae into the peritoneal cavity with a little Ringer's solution using a syringe connected to a slender vinyl tube.

3) Recovery of the Flukes

Dogs, cats and rats were bled to death under anesthesia on days 208 - 258, 155 - 249 and 10 - 150 after infections, respectively, and flukes were recovered. Postmortem examinations were carried out on each animal immediately. Gross lesions which might be

attributable to parasitic invasion were recorded. Visceral organs and cavities were examined for lung fluke infection and worms were recovered. Subsequently, the lungs, liver and muscles of the whole body were cut into slices, 3 to 4 mm thick, and kept in Ringer's solution at 36 - 38 °C for 6 - 8 hours to release flukes from the tissues. The sediment was poured into a petri dish and examined for flukes under a stereomicroscope. Most of the juvenile flukes recovered were inoculated into dogs and cats again. The remaining worms were used for morphological observations; they were pressed between two slide glasses in 70% alcohol and mounted with Canada balsam after staining with carmine.

4) Transfer of Juvenile *P. westermani* to Cats and Dogs

Most of the juvenile worms recovered from the muscles of cats, dogs and rats were fed again to 5 cats and 2 dogs. Numbers and ages of the juvenile flukes and animals used are shown in Table 4. The animals were autopsied at 161-212 days after inoculation. The same procedures mentioned above for fluke recovery and examination were employed.

Table 1 Results of experimental infection of cats with Malaysian *Paragonimus westermani* metacercariae (Mc.)

Cat No.	Body weight at necropsy (kg)	Sex	No. of Mc. given	Method of infection	Autopsy days after infection	No. of worms recovered (%)	No. of worms recovered from			
							cyst in lungs	lungs	pleural cavity	muscles
1	2.0	F	30	oral infection	220	14 (46.7)	10*	0	3†	1‡
2	2.9	F	30	oral infection	229	3 (10.0)	2*	0	0	1‡
3	2.6	F	30	oral infection	234	5 (16.7)	4*	0	0	1‡
4	2.0	M	30	oral infection	242	4 (13.3)	2*	0	1†	1‡
5	2.7	M	30	oral infection	249	5 (16.7)	4*	0	0	1‡
6	2.8	F	50	peritoneal inoculation	155	26 (52.0)	4*	14†	0	8‡
7	3.7	M	50	peritoneal inoculation	192	32 (64.0)	18*	0	1†	13‡
8	3.2	F	50	peritoneal inoculation	192	37 (74.0)	16*	0	2†	19‡
9	2.9	M	40	peritoneal inoculation	208	26 (65.0)	14*	0	0	12‡
10	2.9	F	47	peritoneal inoculation	237	30 (63.8)	10 (8*) 2†	0	2†	18‡
11	3.2	M	30	peritoneal inoculation	242	25 (83.3)	12*	0	1†	12 (1† 11‡)

F: female, M: male, * Adult worm (with eggs in uterus), † Immature worm (without eggs in uterus),

‡ Juvenile worm (similar to excysted metacercariae),

Cat Nos. 2, 4, 5, 7 and 8 were infected with metacercariae from Kuala Pilah.

Cat Nos. 1, 3, 6 and 9 from Sungai Wa and Cat Nos. 10 and 11 from Ulu Langet.

RESULTS

Experimental Infection of Cats with Metacercariae
Distribution of flukes:

The results of the experimental infection in cats with the metacercariae are shown in Table 1. The average recovery rate of five cats (Nos. 1-5), orally infected with metacercariae, was 20.7% ranging from 10.0 to 46.7%. Out of 31 flukes recovered, 22 were found in cysts in the lungs, 5 in the muscles and 4 in the pleural cavity. A total of 176 worms was recovered from the cats (Nos. 6 - 11) inoculated with metacercariae intraperitoneally and the average recovery rate of worms was 57.0% (52.0 - 83.3%). Eighty-two worms were recovered in the muscles and 88 in lungs or cysts in the lungs and a small number of flukes were found in the pleural cavity.

Postmortem examinations:

On gross examination of the infected cats, in which 1 to 9 cyst formations were recognized in the pulmonary parenchyma, membranous rust coloured fibrinous exudate was present in the cavity, and adhesions between the lung lobes were present. In the cats, in which migrating flukes were found in the pleural cavity or lungs, collections of stagnant thoracic fluid were present and small number of haemorrhagic spots, 4 - 6 mm in diameter, were recognized in the lungs. In cat No.6, a large amount of stagnant thoracic fluid with blood cells was present, and about 30 haemorrhagic spots or nodules, 1-16 mm in diameter, were present in the lungs.

Development of the flukes:

In cat No. 6, autopsied 155 days after infection, fourteen flukes recovered from nodules of the lungs were immature, while four flukes from cysts in the lungs were mature (Table 1). The sizes of the mature flukes were variable, 5.8 - 7.4 mm long and 3.1 - 4.8mm wide, with a few eggs in the uteri. In cats autopsied 192 days later, all flukes recovered from cysts in the lung were fully mature. The sizes were 8.2 - 9.1 mm in length and 5.1 - 6.0 mm in width and the worms had numerous eggs in the uteri. Flukes recovered from muscles were morphologically similar to excysted metacercariae except for their slightly larger body size (0.7 - 1.4 mm in length and 0.4 - 0.7 mm in width). The worms had numerous granules in their excretory bladder. The flukes from the pleural cavity were immature and their sizes were about 3.5 mm in length and 2.1 - 3.2 mm in width. Their ovary and testes were divided into 5 or 6 lobes but vitelline glands were less developed. No remarkable differences were observed in the development of the flukes from the three different localities in Malaysia.

Experimental Infection of Dogs with Metacercariae
Distribution of flukes:

Table 2 shows the results of experimental infection of 7 dogs with metacercariae from Kuala Pilah and Sungai wa. In dog No.1, orally infected with 150 metacercariae and necropsied 234 days after infection, only 3 (2%) worms were recovered from muscles. The remaining six dogs were infected intraperitoneally with 80 or 100 metacercariae and were necropsied 208 to 258

Table 2 Results of experimental infection of dogs with Malaysian *Paragonimus westermani* metacercariae (Mc.)

Dog No.	Body weight at necropsy (kg)	Sex	No. of Mc. given	Method of infection	Autopsy days after infection	No. of worms recovered (%)	No. of worms recovered from				
							cyst in lungs	lungs	pleural cavity	peritoneal cavity	muscles
1	17.7	F	150	oral infection	234	3 (2.0)	0	0	0	0	3‡
2	14.7	F	80	peritoneal inoculation	208	52 (65.0)	2*	0	1†	0	49‡
3	12.0	F	100	peritoneal inoculation	227	29 (29.0)	0	2†	1†	0	26 (3†, 23‡)
4	12.2	F	100	peritoneal inoculation	240	9 (9.0)	0	0	0	0	9‡
5	10.3	M	100	peritoneal inoculation	245	35 (35.0)	4 (2*, 2†)	0	0	1†	30‡
6	11.2	M	80	peritoneal inoculation	252	23 (28.8)	0	0	2†	0	21‡
7	15.6	F	80	peritoneal inoculation	258	23 (28.8)	2 (1*, 1†)	0	2†	0	19‡

F: female, M: male, *, †, ‡: Cf. the foot in Table 1,
Dog Nos. 1, 3 and 4 were infected with metacercariae from Kuala Pilah.
Dog Nos. 2, 5, 6 and 7 were infected with metacercariae from Sungai Wa.

days after inoculation. The average recovery rates of worms was 32.6% (9.0 - 65.0%) and a total of 171 worms were recovered. Majority of the flukes (154 out of 171; 90.1%) were recovered from muscles and 6 and 8 flukes were found in cavities and cysts in lungs, respectively.

Postmortem examinations:

On gross examination, no abnormality was observed in any organs of 2 infected dogs (Nos. 1 and 4), from which flukes were only recovered from muscles. In the other dogs, some haemorrhagic lesions, 1 - 10 mm in diameter, were present in the lungs and flukes were found in the pleural cavity or in the lungs. In these dogs, stagnant haemorrhagic exudates were present in the pleural cavity, and adhesions of the lungs to the thoracic wall and between lung lobes were also observed. One or 2 typical worm-cysts were present in the lungs of dogs Nos. 2, 5 and 7; and 2 flukes were found in each cyst.

Development of flukes:

A total of 157 flukes were recovered from muscles of the dogs. Three of them measured 1.9 - 3.1 mm in length, and primordial cells of ovary, testis and uterus were recognized. The remaining 154 flukes were very stunted and they had granules in the excretory bladder, and a small stylet was still visible on the oral sucker. Their sizes ranged from 0.7 to 1.2mm in length and 0.4 to 0.6 mm in width. Five out of 8 flukes recovered from cysts of lung were 4.5 - 6.7 mm in length and 2.8 - 3.4 mm in width, and eggs were present in their uteri. Three other flukes from cysts in lungs and all the flukes from cavities and lungs were immature. Their sizes were 1.9 - 4.2mm in length and 1.4 - 3.3 mm in width, though their ovary and testes were developed slightly.

Experimental Infection of Rats with Metacercariae Distribution of flukes:

Table 3 shows the results of oral infection of 29 albino rats with metacercariae from Kuala Pilah. The

average recovery rate of flukes from the rats was 42.8%. Almost all of the worms, 307 out of 310, were recovered from the muscles and only a few worms were found in the lungs or abdominal cavity. The 3 intraperitoneally inoculated rats with 50 metacercariae each from Taman Negara were necropsied 100 days after infection. The recovery rate of the flukes from these rats was 48.7% (46.0 - 52.0%) and all of them were recovered from muscles.

Postmortem examination:

On the gross examination of the infected rats, no pathological changes were generally observed except those examined 10 to 20 days after infection. In these rats, haemorrhagic tracks were found in the muscles of abdominal walls and legs, presumably due to migration of juvenile flukes.

Development of flukes in rats:

All the flukes recovered from rats were juvenile and morphologically similar to the excysted metacercariae with the exception that they were slightly larger in size. They were 0.7 - 0.9 mm in length and 0.3 to 0.5 mm in width.

Transfer of Juvenile *P. westermani* to Cats and Dogs

The results of oral infection in cats and dogs with juvenile worms recovered from muscles of cats, dogs and rats of the previous experiments are shown in Table 4. The average recovery rate of worms in five infected cats was 68.2%, ranging from 48.0 to 84.0%. Out of 99 flukes obtained, 77, 4, 11 and 7 flukes were recovered from cysts in the lungs, tissues of lungs, pleural cavity and muscles, respectively. Sixty-four out of 77 flukes recovered from cysts in the lungs were mature while 1 out of 11 from pleural cavity and five out of 7 flukes from muscles were still juvenile worms with granules in the excretory bladder.

In dog No. 8, 2 and 3 flukes were recovered from the pleural cavity and muscles, respectively. The recovery

Table 3 Results of experimental oral infection of albino rats with 25 *Paragonimus westermani* metacercariae from Kuala Pilah

Days after infection	No. of rats used	No. of worms* recovered (%)	No. of worms recovered from		
			lungs	peritoneal cavity	muscles
10	5	48 (38.4)	0	0	48
20	5	54 (43.2)	0	1	53
30	5	55 (44.0)	0	0	55
60	5	62 (49.6)	0	1	61
100	4	57 (57.0)	1	0	56
150	5	34 (27.2)	0	0	34

* All worms recovered were juvenile.

Table 4 Results of experimental infection of dogs and cats with juvenile *Paragonimus westermani* recovered from some paratenic host animals

Host animal	Body weight at autopsy (kg)	Sex	Juvenile worms recovered from (days after postinfection)	No. of juvenile worms fed	Autopsy days after infection	No. of worms recovered (%)	No. of worms recovered from			
							cyst in lungs	lungs	pleural cavity	muscles
Dog No.8	14.5	F	Rat (150)	40	185	5 (12.5)	0	0	2†	3($\frac{2†}{1†}$)
Dog No.9	16.4	F	Rat (100)	35	209	10 (28.6)	8*	0	2†	not examined
Cat No.12	2.8	F	Cat (192)	25	200	12 (48.0)	4*	0	5†	3($\frac{2†}{1†}$)
Cat No.13	4.3	M	Cat (237, 242)	23(12.11)	166, 161	19 (82.6)	16($\frac{11*}{5†}$)	0	2†	1†
Cat No.14	3.6	M	Dog (227)	17	172	12 (70.6)	12*	0	0	0
Cat No.15	4.3	M	Dog (245, 252)	50(30, 20)	195, 202	42 (84.0)	33($\frac{25*}{8†}$)	4†	2†	3‡
Cat No.16	3.9	M	Rat (100)	25	212	14 (56.0)	12*	0	2($\frac{1†}{1†}$)	0

F: female, M: male, *, †, ‡: Cf. the foot in Table 1,

rate was 12.5%. All of the flukes recovered from the dog were immature, especially a single fluke from muscle having the same characteristics of the metacercarial stage. In dog No.9, typical cyst formation was recognized in the lungs, 8 fully matured flukes which measured 7.9 - 8.5 mm long by 3.8 - 4.4 mm wide were recovered. Two immature flukes were recovered from pleural cavity but the muscles of this dog were not examined.

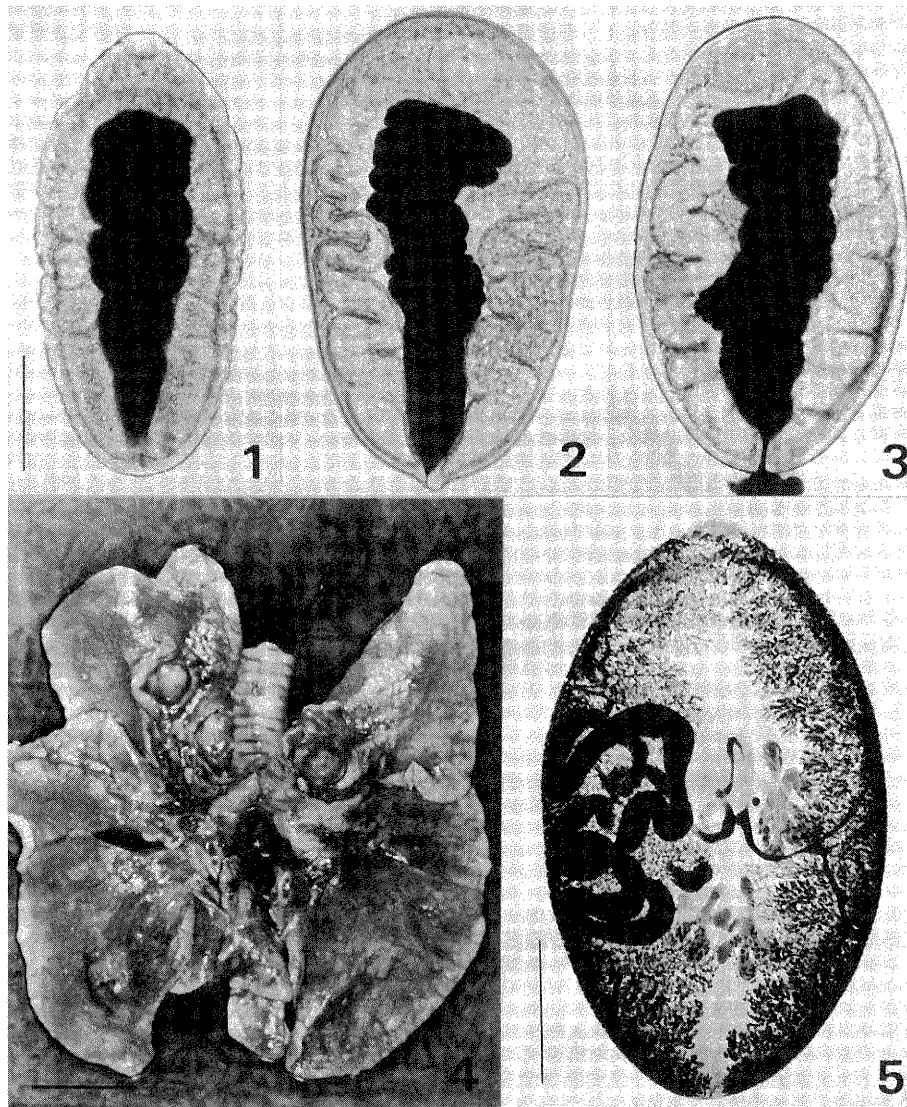
DISCUSSION

It is well known that the distribution and development of *P. westermani* in favorable definitive hosts, like cats and dogs and in unfavorable hosts, such as rodents are quite different. In the former hosts, worms are found in cysts in the lungs and are mature, but in the latter hosts, majority of worms remain in the muscle with little development. In dogs and cats infected with *P. westermani* from Japan, the triploid fluke takes about 2 months to reach maturity, and the diploid fluke takes almost two and a half months (Takizawa, 1964; Habe, 1978; Shibahara, 1983). *P. westermani* from Korea, China and the Philippine also shows similar growth in dogs and cats (Fan, 1966; Miyazaki and Habe, 1979; Unpublish data, S. Habe). In general the worm recovery rates in cats and dogs infected with metacercariae from these countries were about 60 to 80% by oral inoculation and 90% more by peritoneal inoculation.

In our present study the average worm recovery rates of cats and dogs, orally infected with Malaysian *P. westermani*, were 20.7% and 2%, respectively. Kim (1969) infected six cats with 109 metacercariae from

freshwater crabs, *Parathelphusa maculata* and *Johora johorensis*, and recovered 31 worms from 61 to 108 days after infection. These rates are very low compared to those of *P. westermani* from other localities. The results of experimental intraperitoneal inoculation also shows a similar tendency. The Malaysian *P. westermani* has a lower infectivity to mammalian hosts than those from other localities. Furthermore, in spite of keeping the infections for 5 or more months after feeding with metacercariae, about 50% and 90% of the flukes were still recovered from the muscles of the cats and dogs respectively. The worms were very stunted and morphologically similar to the excysted metacercariae with the exception that their body sizes were slightly larger. The rates of mature flukes out of total worms recovered were only 40% from infected cats and 3% from infected dogs.

All the flukes recovered from albino rats were juvenile just like those that parasitized the muscles of dogs and cats. Such stagnated worms have been collected from other animals, such as pig, wild boar, mouse, hamster, guinea pig, rabbit, monkey and hen infected with *P. westermani* from Japan (Habe, 1978, 1982, 1983; Shibahara and Nishida, 1986; Takizawa, 1964). This is the first report where juvenile worms were recovered from cats and dogs infected with *P. westermani*. The development and distribution of Malaysian *P. westermani* in cat and dog are very different from the flukes of other countries but no significant differences are recognized in rat. However, Philippine *P. westermani* does not remain in the muscle of rats and migrates into the lungs and then mature in relatively high rate. This suggests that Malaysian *P. westermani* has a higher



Figs. 1-3. Living juvenile worms. Bar=0.2mm.

1. Worm recovered from muscle of rat 150 days after infection with metacercariae.

2. Worm recovered from muscle of dog 240 days after infection with metacercariae.

3. Worm recovered from muscle of cat 237 days after infection with metacercariae.

Fig. 4 Lungs of dog; 209 days after inoculation with 35 juvenile worms from muscles of rat. Bar=3cm.

Fig. 5 Mounted adult worm from worm cyst in lungs of cat; 172 days after inoculation with juvenile worms from muscles of dog. Bar=2mm.

final host susceptibility than the Japanese, Chinese and Korean flukes while the Philippine *P. westermani* has the lowest.

The final host of *P. westermani* is widely distributed among mammals, especially of the families Felidae and Canidae. In Malaysia, only one species of lung fluke, *P. westermani* has been recorded from tiger, *Felis tigris*, domestic cat, *Felis domestica*, clouded leopard, *Neofelis*

nebulosa diardithe, wild cats, *Felis bengalensis*, *F. planiceps* and *F. temminckii*, black panther, *Felis pardus*, and crab-eating monkey, *Macaca irus irus* (Groves et al., 1967; Lee and Miyazaki, 1965; Lim and Betterton, 1977; Rohde, 1963, 1967; Miyazaki and Kwo, 1969). Most of the natural final hosts belong to Felidae. In spite of the juvenile worms recovered from muscles of experimental cats in the present study, and it is without doubt

that the animals of Felidae are the most important and suitable final hosts for Malaysian *P. westermani*. However, our results showed Canidae to be unsuitable hosts for Malaysian *P. westermani*.

It was experimentally proved that juvenile flukes remaining in the muscles of dogs, cats and albino rats were able to reinfect other carnivores. In the case of cat infections, the recovery rate of flukes was 68.2% on the average, while in dogs, it was 20.7%. In the animals inoculated with juvenile flukes, the worm recovery and maturity rate were also higher than those infected with the metacercariae. Carnivorous animals are more readily infected by the Malaysian *P. westermani* via feeding of juvenile worms from paratenic hosts than metacercariae from crabs. Therefore, the present study suggests that many wild mammals living in jungle must serve as paratenic hosts and they play a very important role in the completion of the life cycle of the flukes in Malaysia and its neighborhood. In experiments the same mode of infection has been shown in the triploid and diploid types of *P. westermani*, *P. miyazakii* Kamo et al., *P. mexicanus* Miyazaki et Ishii and *P. heterotremus* Chen et Hsia (Fan and Khaw, 1964; Fan and Hsu, 1965; Habe, 1978, 1983; Miyazaki and Habe, 1976; Shibahara and Nishida, 1986; Sugiyama et al., 1990). For example, it was proven that the wild boar, *Sus scrofa leucomystax*, plays an important role as the paratenic host for human paragonimiasis *westermani* (triploid type) in the southern part of Kyushu, Japan (Norimatsu et al., 1975; Miyazaki et al., 1978; Tokudome et al., 1977). Kwo and Miyazaki (1968) reported heavy infections of *P. westermani* in all the tigers examined in North Sumatra, Indonesia, and Miyazaki and Habe (1976) postulated that those tigers might have been infected due to feeding on animals harboring immature worms and serving as paratenic hosts. Lim and Betterton (1977) reported that civet cats have the potential of being paratenic hosts from feeding experiments and suggested that wild boar and a number of small mammals were also potential paratenic hosts. Although locally acquired paragonimiasis has not been observed in man in Malaysia, *P. westermani* has been found in a number of animals and freshwater crabs. Lim and Betterton (1977) gave the reason that freshwater crab is not generally used as a source of food and wild boar meat is infrequently eaten. In addition to that, the development of *P. westermani* in man could be considered to be similar to that seen in the present experiments where mature worms may not be found.

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