CRAB HOSTS FOR *PARAGONIMUS WESTERMANI* (KERBERT, 1878) IN MALAYSIA

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Abstract: During July and August 1985, a field survey on the lung fluke was carried out at six localities in the peninsular Malaysia. A total of 1,009 fresh-water crabs belonging to eight species were examined and *Paragonimus westermani* metacercariae were found in four species of the crabs, *Parathelphusa maculata* from Kuala Pilah and Ulu Langat, *Parathelphusa malaysiana*, *Johora tahanensis* and *Irmengardia pilosimana* from Sungai Wa. The three species of crabs except *Parathelphusa maculata* are recorded as new crustacean hosts of *P. westermani*. *Parathelphusa maculata* harboured more than 60% of the total metacercariae in the muscle, 20-25% in the gills and about 10% in the liver, but no metacercariae were found in the heart. In contrast, a substantial number of metacercariae parasitized the heart of the crab, *Parathelphusa malaysiana*.

INTRODUCTION

In Malaysia, three species of fresh-water crabs, *Johora johorensis* (Roux, 1936), *Parathelphusa maculata* de Man, 1879 and *Potamiscus cognatus* (Roux, 1936) have been reported as the second intermediate hosts of *Paragonimus westermani* (Kim, 1967; Lee and Miyazaki, 1965; Miyazaki *et al.*, 1968; Miyazaki and Kwo, 1969).

During July and August 1985, the authors carried out a field survey on the Malaysian lung fluke. In the investigation, three species of the fresh-water crabs were found as new second intermediate hosts of *P. westermani*.

We report here the new crab hosts of *P. westermani* in Malaysia, the prevalence of *Paragonimus* infection in crabs, and the distribution of its metacercariae in the crab tissues.

MATERIALS AND METHODS

A total of 1,009 fresh-water crabs belonging to eight species were collected from the following six different localities: Kampong Langkap near Kuala Pilah (Negeri Sembilan), several streams in Baling District (Kedah), Sungai Wa and Sungai Kuching at Taman Negara (Pahang), Sungai Lui at Ulu Langat (Selangor) and Sungai Lalang (Selangor) in the peninsular Malaysia

(Figure 1 and Table 1). They were brought back to the Institute for Medical Research in Kuala Lumpur and were examined for *Paragonimus* infection after being classified by species, sex and carapace width. The gills of each crab were examined under a stereomicroscope.

Some of crabs were examined in detail for the distribution of metacercariae in the crab tissues. The examination procedure used is as follows. Gills, liver, intestine, heart and gonad were separately pressed between two glass plates and examined under a stereomicroscope. Muscles of the cephalothorax and legs were chopped up into small pieces by scissors and then transferred into a metal sieve of about 5-mesh. The chopped muscle was further ground with a wooden rod in the sieve immersed in a beaker of tap-water. The filtrate was allowed to stand still for a few minutes and then the supernatant was discarded. The sediment was washed several times by repeating this procedure and then strained through a sieve of 30-mesh. The supernatant of the final filtrate was poured off and the sediment was examined for metacercariae under a stereomicroscope. The metacercariae recovered from crabs were measured and used for experimental infection. Identification of the crabs was performed by Dr. M. Takeda of National Science Museum, Tokyo and the results have been published (Ng and Takeda, 1992).

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Figure 1 A map showing the localities surveyed in Malaysia ●: positive for metacercariae of *P. westermani* ○: negative

RESULTS

The results of the crab examination for *Paragonimus* infection are shown in Table 1. *Paragonimus* metacercariae were recovered from *Parathelphusa maculata* collected from Kuala Pilah and Ulu Langat, *Parathelphusa malaysiana* Ng and Takeda, 1992, *Irmengardia pilosimana* (Roux, 1936) and *Johora tahanensis* (Bott, 1966) from Sungai Wa (Photos. 1-4). The table

Table 2 Relationship between carapace size of crabs, *Parathelphusa maculata*, and number of *Paragonimus westermani* metacercariae in crab gills

Carapace size (in mm)	No. of crabs		Ave. No. of metacercariae in a positive crab
	examined	infected	
25.0-29.9	17	6 (35.3%)	1.7
30.0-34.9	49	12 (24.5%)	2.3
35.0-39.9	79	29 (36.7%)	1.5
40.0-44.9	57	31 (54.4%)	2.5
45.0-49.9	60	45 (75.0%)	2.9
50.0-54.9	21	18 (85.7%)	2.6
55.0-	4	4 (100.0%)	5.8

The crabs were collected from Kuala Pilah

also indicates the prevalence rate and the number of metacercariae in the gills.

145 (50.0%) of 287 *Parathelphusa maculata* (carapace width 25-57 mm) from Kuala Pilah, were positive. Although the infection rate was relatively high, the number of metacercariae per positive crab was low. One or two metacercariae per crab were found in many of the positive crabs, and eleven was the maximum number in the gills. The prevalence rate and number of metacercariae in gills increased with carapace size, therefore they are proportional to the age of the crab (Table 2).

In Ulu Langat, 6 (24.0%) out of 25 *Parathelphusa maculata* (carapace width 20-36 mm) were positive. The number of metacercariae per positive crab ranged from 1 to 7, and 3.0 on the average.

In Sungai Wa (Taman Negara), 1 (1.0%) of 98 *Johora tahanensis*, 4 (6.2%) of 65 *Irmengardia pilosimana* and 12 (63.2%) of 19 *Parathelphusa malaysiana* were found to be infected with *Paragonimus* metacercariae. The average number of metacercariae

Table 1 Prevalence of *Paragonimus westermani* metacercariae in crab gills in Malaysia

Locality	Crab host	No. of crabs		No. of metacercariae /positive crab
		examined	infected	
Kuala Pilah	<i>Parathelphusa maculata</i>	287	145 (50.0%)	2.5
Ulu Langat	<i>Parathelphusa maculata</i>	25	6 (24.0%)	3.0
Sungai Lalang	<i>Parathelphusa maculata</i>	14	0	
	<i>Johora intermedia</i>	3	0	
	<i>Geosesarma peraccae</i>	12	0	
Baling	<i>Somanniathelphusa sexpunctata</i>	213	0	
	<i>Stoliczia tweedei</i>	52	0	
Taman Negara				
Sungai Kuching	<i>Johora tahanensis</i>	221	0	
Sungad Wa	<i>Parathelphusa malaysiana</i>	19	12 (63.2%)	11.2
	<i>Irmengardia pilosimana</i>	65	4 (6.2%)	1.0
	<i>Johora tahanensis</i>	98	1 (1.0%)	6.0

Photo. 1 *Parathelphusa maculata* de Man, 1879 Photo. 2 *Parathelphusa malaysiana* Ng and Takeda, 1992 Photo. 3 *Johora tahanensis* (Bott, 1966) Photo. 4 *Irmengardia pilosimana* (Roux, 1936)

in the gills per positive crab was 6.0, 1.0 and 11.2, respectively (Table 1).

Table 3 shows the distribution of metacercariae in the crabs hosts. In 21 (13 males and 8 females) *Parathelphusa maculata* from Kuala Pilah, out of 250 metacercariae counted, 156 (62.4%) were found in the muscles, 58 (23.2%) in the gills, 18 (7.2%) in the liver, 4 (1.6%) in the genital organs and 14 (5.6%) in other parts of the body (mostly in the inner membrane of the carapace). The infection rate of each organ or tissue was follows: 20 (95.2%) out of 21 crabs harboured 1-31 metacercariae (7.8 on the average) in the muscles; 14 (66.7%), 1-10 in the gills; 7 (33.3%), 1-5 in the liver and 4 (19.0%), a

single metacercariae each in genital organs. No metacercariae were found in the heart region of the crabs. There was no significant difference between both sexes of crabs in the infection rate of metacercariae in each organ or tissue.

An infected crab, *Parathelphusa maculata* from Ulu Langat, was examined for the distribution of the metacercariae in the host. The crab (carapace width 35.8 mm) harboured 21 metacercariae in the muscles, 7 in the gills and 2 in the liver.

A total number of 437 metacercariae were recovered from 4 *Parathelphusa malaysiana* from Sungai Wa. More than half, 252 (57.7%), were obtained from mus-

Table 3 Distribution of *Paragonimus westermani* metacercariae in the crab hosts

Crab host	No. of crabs examined	No. of Metacercariae obtained	No. of metacercariae recovered from					
			gills	liver	heart	genital organs	muscle	other tissues
<i>Parathelphusa maculata</i>	21 *	250	58 (23.2%)	18 (7.2%)	0	4 (1.6%)	156 (62.3%)	14 (5.6%)
<i>Parathelphusa malaysiana</i>	4 †	437	52 (11.9%)	38 (15.6%)	68 (8.7%)	8 (1.8%)	252 (57.7%)	19 (4.4%)
<i>Irmengardia pilosimana</i>	4 †	18	4 (22.2%)	0	0	0	14 (77.8%)	0
<i>Johora tahanensis</i>	1 †	18	6 (33.3%)	2 (11.1%)	0	0	9 (50.0%)	1 (5.6%)

* Collected from Kuala Pilah. † Collected from Sungai wa, Taman Negara
All crabs harboured metacercariae somewhere in their bodies.

Table 4 Measurements of the inner cyst of metacercariae of Malaysian *Paragonimus westermani* (in μm)

Crab host	Locality	No. cysts measured	Cyst size	
			maximam	minimam
<i>Parathelphusa maculata</i>	Kuala Pilah	56	334.9 ± 17.0	312.2 ± 17.0
<i>Parathelphusa maculata</i>	Ulu Langat	14	352.4 ± 20.8	320.6 ± 20.8
<i>Parathelphusa malaysiana</i>	Sungai Wa	52	342.9 ± 15.1	314.7 ± 15.1
<i>Johora tahanensis</i>	Sungai Wa	13	345.5 ± 23.6	327.0 ± 16.1
<i>Irmengardia pilosimana</i>	Sungai Wa	18	341.0 ± 15.1	321.8 ± 13.2

cles and 68 (15.6%) from heart or pericardium. Only 52 (11.9%) of them were distributed in the gills and the rest in the liver, genital organs, and other part of the body.

Another infected crab, *Johora tahanensis*, from Sungai Wa, harboured 18 metacercariae, 9 of them were

found in the muscles, 6 in the gills, 2 in the liver and 1 elsewhere.

In the examination of 4 infected individuals of *Irmengardia pilosimana* from Sungai Wa, only one crab harboured a single metacercaria in the gills but all four

Photo. 5 Metacercariae in the gill of a host crab, *Parathelphusa malaysiana*. (Scale=100 μm)

Photo. 6 Metacercariae removed from a host crab, *Parathelphusa malaysiana*. (Scale=100 μm)

had 3-4 metacercariae in the muscles.

P. westermanni metacercariae obtained from different crab hosts in Malaysia were oval in shape and had two cyst walls, i.e., outer and inner layers. Thickness of outer layer was 2-3 μm and that of inner layer varied from 5 to 25 μm . The measurements of the encysted metacercariae from different localities are shown in Table 4. Pinkish granules in the metacercariae were rarely seen (Photos. 5 and 6).

On the basis of the morphological features of the metacercariae together with those of the adults obtained from experimentally infected animals and the cytogenetical studies of the flukes, these flukes were confirmed as *P. westermanni* (diploid type).

DISCUSSION

Up to the present, the following 3 species of the fresh-water crabs have been reported as the second intermediate host of *P. westermanni* in Malaysia: *Potamon johorensis* from Ulu Langat (Lee and Miyazaki, 1965), *Parathelphusa maculata* from Ulu Langat (Miyazaki *et al.*, 1968) and *Potamiscus cognatus* from Baling (Miyazaki and Kwo, 1969). Kin (1967) also reported two species of crabs, *Potamon johorensis* and *Parathelphusa maculata* as second intermediate host. The genus *Johora* (type species: *Potamon (Potamon) johorensis* Roux, 1936) was first established by Bott (1966) as a subgenus of *Stoliczia* Bott, 1966 and Ng (1987) elevated it to a full genus. Nowadays, *Potamon johorensis* comes under the genus *Johora*. In the present investigation, 4 species of the fresh water crabs, *Parathelphusa maculata* from Kuala Pilah and Ulu Langat, *Parathelphusa malaysiana*, *Johora tahanensis* and *Irmengardia pilosimana* from Sungai Wa, were found to be infected with *P. westermanni*. These crab hosts except *Parathelphusa maculata* are recorded as new crustacean hosts of *P. westermanni*.

P. westermanni is widely distributed in Asia, and at each locality the fluke may have many crustaceans as the second intermediate host and some differences have been observed in the distribution of the metacercariae in the crab hosts. Observations on the crab, *Geothelphusa dehaani* White, in Japan showed that 86.5% of *P. westermanni* (diploid type) metacercariae were found in the muscles, 7.8% in the liver, and 4.0% in the gills (Habe and Miyazaki, 1982). In the case of the Japanese *P. westermanni* (triploid type) in *Eriocheir japonicus* (de Haan), 76% of metacercariae were found in the muscles, 16% in the gills, and 5% in the liver, while in *Geothelphusa dehaani*, 82.9% were obtained from the muscles

and the remaining from liver or gills (Habe, 1979., Habe and Terasaki, 1982). As for Philippine *P. westermanni*, namely *P. w. filipinus* Miyazaki 1978, in the crab, *Sundathelphusa philippina* Martens, 70.0% were found in the muscle, 22.3% in the heart and 4.9% in the gills (Miyazaki and Habe, 1979). The above mentioned results were obtained by the same methods as used in the present study.

In the present investigation on the distribution of *P. westermanni* metacercariae in the crab, *Parathelphusa maculata* harboured more than 60% of the total metacercariae in the muscles, 20-25% in the gills and about 10% in the liver. No metacercariae were found in the heart region. The distribution of metacercariae showed no found to be significant difference between the Japanese *P. westermanni* in *Eriocheir japonicus* and the Malaysian *P. westermanni* in *Parathelphusa maculata*. The distribution of metacercariae in *Johora tahanensis* and *Irmengardia pilosimana* was similar to that in *Parathelphusa maculata*. Lee and Miyazaki (1965) also obtained 23 *Paragonimus* metacercariae from 4 infected crabs, *Johora johorensis*, 14 were in the muscle, 6 in the gills and 3 in the liver. On the other hand, *Parathelphusa malaysiana* showed a high percentage of the metacercariae in the heart region. This is similar to the Philippine *P. westermanni* in *Sundathelphusa philippina*.

In the present study, it became clear that the Malaysian *P. westermanni* shows different metacercarial distribution in accordance with the host species. The distribution pattern of metacercariae in the crab hosts is important to know accurate prevalence of *Paragonimus*. For example, Table 1 shows the prevalence rate and the number of metacercariae per crab host by examining only the gill. This value is quite a low rate of infection as compared with the whole body examination.

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