

# STUDIES ON THE MIGRATORY ROUTE OF *PARAGONIMUS SIAMENSIS* IN THE BANDICOOT (*BANDICOTA INDICA*)

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## INTRODUCTION

Paragonimid trematodes, the lung fluke, have been recognized as one of the important and harmful parasites of man (Belding, 1965). New endemic areas have been discovered and many new species of *Paragonimus* have hitherto been reported especially in the South-east Asia region (Vajrasthira, 1969). In 1965, a new species of lung fluke, *Paragonimus siamensis* obtained from cats in Thailand was reported by Miyazaki and Wykoff. The prevalence of this *Paragonimus* infection in cats and crabs were 20.0 % and 2.2 % respectively, in vicinity of Udon province (Miyazaki and Wykoff, 1965). No human cases have yet been reported in these villages although the inhabitants are fond of eating raw crabs. The natural hosts of this *Paragonimus* sp. are bandicoots and rats (*Rattus rattus* and *R. berdmorei*) (Miyazaki and Wykoff, 1965). In 1969, Vajrasthira reported another locality of this species in Sakon-Nakhon, Khon Kaen in the Northeast; Nakhon Nayok, Saraburi, Ayutthaya and Ratchaburi provinces of Central Thailand.

The development and the pattern of migration of *P. siamensis* from the intestine to the lungs of the final hosts has not been well established. The objective of the present paper was to study on the migratory route and the development of *P. siamensis* infection *per os* in the bandicoot (*Bandicota indica*).

## MATERIALS AND METHODS

Rice field crabs (*Somanniathelphusa germaini* and *S. juliae*) were collected monthly from

the Nahinlard district, Nakhon Nayok province. Metacercariae of *P. siamensis* were removed from the heart and blood vessels of these infected crabs and kept in tyrode solution.

Bandicoots (*B. indica*) were caught from the fields around the campus of Kasetsart University. They were kept in the individual cages and examined for the eggs of *Paragonimus* in the faeces for 2 months to exclude the presence of this parasite. They were then anaesthetized with ether and each given 50 metacercariae *per os*. The bandicoots were killed at various intervals from 12 days to 111 days after infection. Each bandicoot received an intravenous injection of 10 to 15 ml of 0.3 to 0.5 % Evans blue solution per kg body weight 15 minutes before necropsy. After opening the abdomen, the body cavity was washed repeatedly with tyrode solution. The digestive organs and the liver were removed from the cavity. The abdominal cavity was again washed repeatedly with tyrode solution. The washed solutions were pooled in a Petri dish and the worms collected were examined under the dissecting microscope. The liver, spleen, gastrointestinal tract, heart and lung were minced with scissors and pressed between two glass plates and the worms found were collected and examined. The presence of worms in the pleural cavity, wall of the cavity and thoracic muscles were examined by the same procedure as described above.

The *Paragonimus* eggs in the bandicoot's faeces were determined qualitatively by a formalin-ether method (Ritchie, 1948) and quantitatively by a Stoll's dilution technique (Stoll, 1923).

RESULTS

A total of 467 *Somaniathelphusa juliae* and 332 *S. germani* were examined. There was no significant difference ( $P>0.05$ ) between the percentage of the infection rate in the first group (range from 1.7 % to 30.4 %) and in the second group (range from 10.2 % to 100.0 %) as shown in Table 1.

The location and number of worms recovered at various intervals, i.e., 12 - 111 days after infection, are shown in Table 2. The results showed that worms were found in the abdominal cavity on day 12 after infection. On 14th to 21st day after infection, all worms were recovered from the pleural cavity and on day 30, most of the worms were found in the lung tissue. The average percentage of worms recovered in the present study was 14.87.

Worms obtained from bandicoots after infection at 14, 65 and 240 days were fixed in 10 % formalin (see Fig. 1). Measurements of

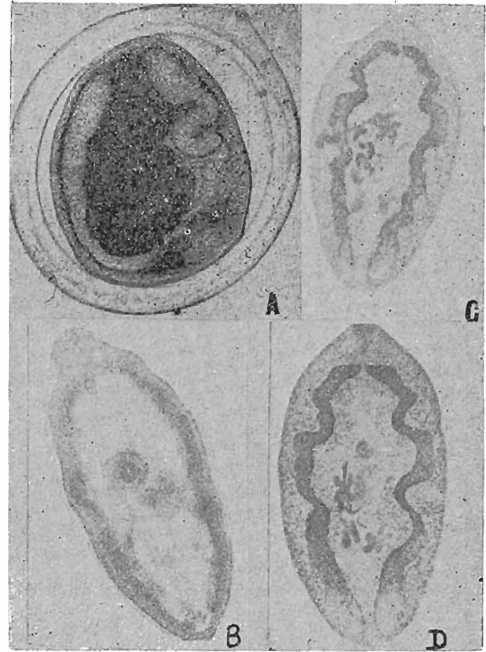


Fig. 1—Showing the metacercaria (a) and worms recovered from bandicoots after infection for (b) 14 days, (c) 65 days and (d) 240 days.

Table 1

The number of crabs examined for the metacercaria of *P. siamensis*.

Month and year	No. exam.	No. posit.	% posit.
<i>Somaniathelphusa juliae</i>			
Aug. 1974	23	7	30.4
Sep. 1974	21	14	66.7
Oct. 1974	90	25	27.7
Nov. 1974	222	37	16.7
Dec. 1974	24	1	4.1
Feb. 1975	30	7	23.3
Apr. 1975	57	1	1.7
Total	467	92	19.7
<i>Somaniathelphusa germani</i>			
Oct. 1974	117	32	27.4
Dec. 1974	4	4	100.0
Mar. 1975	88	9	10.2
Apr. 1975	43	8	18.6
May 1975	60	11	18.3
June 1975	20	7	35.0
Total	332	71	21.4

the length and width of the body, oral and ventral suckers of these worms are shown in Table 3. The results showed that the worms at 14 days were 5 times larger than their metacercariae. The size increased considerably and they had increased nearly twice from the ages of 65 days to 240 days after infection. The comparative sizes of mature worms at 240 days after infection in bandicoots in the present study compared with those obtained from cats are shown in Table 4.

The *Paragonimus* eggs were first detected in the faeces on day 47 after infection. There was a considerable increase of the egg output

from day 105 onwards and then the output fluctuated as shown in Fig. 2. The mean

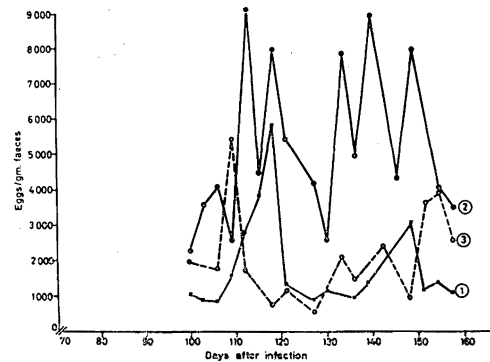


Fig. 2—Showing the number of eggs/gm faeces in 3 experimental bandicoots.

Table 2

Location and number of worms recovered at various interval after infection with 50 metacercariae of *P. siamensis*.

Days after infection	No. of mice	No. of worms recovered*			Total
		Abdominal cavity	Pleural cavity	Lung	
12	2	1	-	-	1 (1.0)
14	3	-	16	-	16 (10.7)
15	1	-	1	-	1 (2.0)
21	3	-	11	-	11 (7.3)
30	3	-	2	15	17 (11.3)
40	3	-	1	-	1 (0.7)
50	3	-	8	34	42 (28.0)
65	4	-	-	67	67 (33.5)
67	1	-	-	15	15 (30.0)
111	2	-	-	24	24 (24.0)
Total	25	1	39	131	195 (15.6)

\* No worms were recovered from the gastrointestinal tract, liver, spleen, heart and diaphragm. Percentage in parenthesis.

value  $\pm$  one standard deviation and ranges of egg output in 3 experimental bandicoots are shown in Table 5. The results showed

that one worm, on average, laid eggs  $145 \pm 105$  per one gm of faeces or  $443 \pm 364$  in one day.

Table 3

Measurements (in mm) of *P. siamensis* obtained from the experimental *B. indica* at various period of infection (average from 25 measurements).

	14 days		65 days		240 days	
	Mean $\pm$ S.D.*	Range	Mean $\pm$ S.D.	Range	Mean $\pm$ S.D.	Range
Body width	0.51 $\pm$ 0.12	0.42 - 0.83	4.39 $\pm$ 0.34	3.5 - 5.0	5.60 $\pm$ 0.41	5.0 - 6.5
Body length	1.96 $\pm$ 0.57	1.04 - 2.92	7.14 $\pm$ 0.73	6.0 - 9.0	9.14 $\pm$ 0.88	7.5 - 10.5
Oral sucker						
Width	0.20 $\pm$ 0.02	0.15 - 0.23	0.55 $\pm$ 0.07	0.43 - 0.73	0.65 $\pm$ 0.07	0.36 - 0.73
Length	-	-	0.49 $\pm$ 0.09	0.33 - 0.69	0.58 $\pm$ 0.06	0.50 - 0.79
Ventral sucker						
Width	0.22 $\pm$ 0.03	0.19 - 0.29	0.52 $\pm$ 0.05	0.40 - 0.66	0.58 $\pm$ 0.06	0.50 - 0.66
Length	-	-	0.45 $\pm$ 0.05	0.36 - 0.53	0.51 $\pm$ 0.06	0.40 - 0.59

\* From 14 measurements.

Table 4

Measurements (in mm) of the mature worms of *P. siamensis* (average from 25 measurements) from bandicoots in comparison with those obtained from cats.

	Miyazaki and Wykoff (1965)	Present study
	Mean (Range)	Mean $\pm$ S.D.(Range)
Host	Cats	bandicoot
Age of worms	-	240 days
Size of egg ( $\mu$ )		
Length	82 (78-88)	80 $\pm$ 6 (70-99)*
Width	46 (39-49)	41 $\pm$ 3 (25-54)*
Body		
Width	4.7 (4.0-5.2)	5.6 $\pm$ 0.4 (5.0-6.5)
Length	8.4 (7.0-10.2)	9.1 $\pm$ 0.9 (7.5-10.5)
Oral sucker		
Width	0.73 (0.63-0.83)	0.65 $\pm$ 0.07 (0.36-0.73)
Length	0.44 (0.32-0.60)	0.58 $\pm$ 0.06 (0.50-0.79)
Ventral sucker		
Width	0.69 (0.63-0.79)	0.58 $\pm$ 0.06 (0.50-0.66)
Length	0.64 (0.58-0.71)	0.51 $\pm$ 0.06 (0.40-0.59)

\* From 100 measurements.

Table 5

Egg output of the adult *P. siamensis* in *B. indica*.

No.	Number of worms	Eggs/gm faeces/worm		Eggs/day/worm	
		Mean $\pm$ S.D.	Range	Mean $\pm$ S.D.	Range
R-1	13	125 $\pm$ 100	65 - 442	318 $\pm$ 260	92 - 922
R-2	26	207 $\pm$ 144	88 - 538	725 $\pm$ 597	108 - 2154
R-3	18	102 $\pm$ 70	33 - 211	288 $\pm$ 234	37 - 679
Mean	-	145 $\pm$ 105	-	443 $\pm$ 364	-

## DISCUSSION

According to Naiyanetra, there are 5 species of *Somnathiophusa* in Thailand, i.e., *S. germaini*, *S. sinensis*, *S. juliae*, *S. dugasti* and *S. branti* (Naiyanetra, 1975 pers. comm.). Findings that 19.7 % and 21.4 % of *S. juliae* and *S. germaini* collected from Nakhon Nayok province were positive for *P. siamensis* were relatively high in comparison with the those of 2.2 % in *S. germaini* in Udon province (Miyazaki and Wykoff, 1965) and 0.6-2.4 % in *S. dugasti* in Nakhon Nayok province (Vajrasthira, 1969). This was probably due to the seasonal variation. The highest percentage of infection were recorded in August and September from *S. juliae* and in June and December from *S. germaini*.

The migration route and the development of various *Paragonimus* sp. in different final hosts have been studied extensively. According to Yokogawa, recovery of excysted larvae of *P. westermani* in their early stages was as low as 10 % of metacercaria given to the cats (Yokogawa, 1919). Later Yokogawa *et al.*, (1962) using the Evans blue technique found that more than 50 % of metacercariae of this *Paragonimus* sp. given were recovered during the period from 60 minutes up to 14 days after infection. In the present study, the Evans blue technique was also employed and the mean recovery rate was 15 %.

Results in the present study showed that the worms were found in the pleural cavities and in the lungs of *B. indica* on day 14 and day 30 respectively. These findings were in accordance with the results obtained by other authors. Okura (1963) experimentally infected rats with metacercariae of *P. ohirai* and found worms in the abdominal cavity within 1-6 hours after infection. Worms were found in the liver on day 10 and in the pleural cavity on day 14 and matured worms were found 35 days after infection (Okura, 1963). Yokogawa *et al.*, (1962) infected cats with *P. westermani* and found worms in the abdominal cavity within 3-6 hours, in the pleural cavity and in the lungs on day 4-10 and 10-14 respectively after infection. However, the pattern of migration and development of *P. westermani* in rats, an unfavorable host, differed from that of cats since no worms were found to reach maturity in the body of rats (Yokogawa *et al.*, 1962).

The results in the present study showed that *P. siamensis* were in mature stage and laid eggs on day 47 after infection. These observations agreed with those of Ishii (1966) and Ameel (1934) who reported *P. kellicotti* became sexually mature and eggs appeared in the faeces of the experimental cats at about 2 months and 6 weeks of infection respectively. However, *P. siamensis* still increased in size and produced more eggs on day 105 onwards.

The size on day 240 was nearly twice that of day 65. The worms at 240 days after infection in bandicoot were of a similar size with those obtained from cats reported by Miyazaki and Wykoff (1965). The average egg output of *P. siamensis* in experimental *B. indica* were 144 eggs/gm faeces/worm or 443 eggs/day/worm. No data on the egg output of *Paragonimus* species in experimental animals have been reported, so comparative results were therefore not possible.

### SUMMARY

The migration route and the development of *P. siamensis* obtained from *S. juliae* and *S. germaini* were studied in *B. indica*. The infection rates of *P. siamensis* in these 2 species of crabs were 19.7% and 21.4% respectively. Worms were recovered in the abdominal cavity, pleural cavity and lungs on day 12, 16 and 30 respectively after oral infection. Worms became mature and laid eggs on day 47 after infection, but their size still increased considerably. The average egg output was found to be 144 eggs/gm faeces/worm or 443 eggs/day/worm. The average recovery rate of *P. siamensis* in the experimental *B. indica* was 15%.

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