ECOLOGICAL STUDIES ON THE LUNG FLUKE, *PARAGONIMUS OHIRAI* MIYAZAKI, 1939

1. INFECTION RATE WITH *P. OHIRAI* METACERCARIAE OF BRACKISH WATER CRABS COLLECTED FROM THE SIX RIVERS IN THE TOKAI DISTRICT, CENTRAL JAPAN

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Abstract: A survey for the detection of *Paragonimus ohirai* metacercariae in brackish water crabs was carried out during the period from October 1982 to September 1985 in the six rivers in the Tokai district, central Japan. These rivers are the Ibi, Nagara, Kiso, Nikkou, Shin and Shounai river which flow into the Ise Bay between Aichi and Mie Prefectures. The brackish water crabs collected were of the species *Sesarma dehaani*, *S. intermedia*, *Helice tridens* and *Chasmagnathus convexus*. *Paragonimus ohirai* metacercariae were detected in the first two species. Metacercariae were detected in crabs collected at 17 out of 19 study sites which are situated from about 5.5 to 13.0 km from the estuaries of the Ibi, Nagara and Kiso rivers. The infection rate was 2.7–100 per cent for *S. dehaani* and 0.9–100 per cent for *S. intermedia*, while the average number of metacercariae per positive crab was 1.0–96.0 and 1.0–30.2, respectively. The infection rate was higher among the crabs collected from the twelve study sites situated about 6–11 km up from the estuaries of the three rivers. Although a survey done in 1958 reported that only two sites in the Nagara river were positive for crabs, the present study revealed that the infected areas were distributed widely along the three rivers. In the present survey, a new *P. ohirai*-infected area was found along the Shin river for the first time, despite only limited investigations in the other three rivers. No crabs with metacercariae were observed along the Nikkou river, where *P. ohirai* had been found in the past.

INTRODUCTION

It has already been reported by Miyazaki (1944) and Yoshida *et al.* (1958) that the crabs with *P. ohirai* were distributed in some areas of the estuary of the Nagara river, Mie Prefecture, central Japan. However, no intensive survey has been conducted in the Tokai district.

The present authors started ecological studies on *P. ohirai* in 1982 in the Tokai district, where six main rivers flow into the Ise Bay at the boundary between the north-eastern part of Mie Prefecture and the western part of Aichi Prefecture. This report deals with the infection rate with *P. ohirai* metacercariae of the brackish water crabs in the study area.

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SURVEY AREAS AND METHODS

The survey was made during the period from October 1982 to September 1985 in the estuary areas of six rivers, the Ibi, Nagara, Kiso, Nikkou, Shin and the Shounai (Figure 1). Brackish water crabs from these areas were examined for *P. ohirai* metacercariae.

The Ibi, Nagara and Kiso rivers flow into the Ise Bay in the border area between Mie and Aichi Prefectures, after forming dry riverbeds of various sizes. The first two are about 500 m and the third about 750 m wide, at their estuary. Long breakwaters are constructed on the right side of the Ibi river, on the left side of the Nagara and on both sides of the Kiso river. There are many dry riverbeds of various sizes along their waterways, which are covered with reed communities (*Phragmites communis*) and flooded partially or entirely at high tide.

The Nikkou river, about 700 m wide, flows into the Ise Bay about 8 km east of the Kiso. The Shin and the Shounai between 100–200 m wide, are close to the Nikkou. Sandbanks with reed vegetation develop between the waterway and the long breakwaters on both sides of the Nikkou, on the right side of the Shin and the left side of the Shounai. Between the Kiso and the Shounai are wide stretches of reclaimed land which are utilized for farming, industrial areas and

Figure 1 Sketch map showing the six rivers where the surveys were carried out. A-S: Collection sites on the Ibi, Nagara and Kiso rivers.

seaports. In September 1959, the survey areas were hit by a big typhoon and flooded.

After measuring the maximum carapace width of the collected crabs, their livers and gills were removed and separately compressed between two glass plates. The specimens were checked for metacercariae under a stereo-microscope. The metacercariae detected were identified as those of *Paragonimus ohirai* Miyazaki, 1939 based on their morphological characteristics. In some cases this was corroborated by checking the morphology of adult worms obtained from experimentally infected animals (Figure 2). Although Iwata and Nagayoshi (1985) are of the opinion that *P. ohirai* is not an independent species, the authors here adopt the current theory that *P. ohirai* is an independent species within the genus *Paragonimus*.

Figure 2 a: Metacercaria of *P. ohirai* obtained from *Sesarma dehaani* collected from Kiso river.
b: Adult worm of *P. ohirai* recovered from experimentally infected rat (59 days after infection, balsam-mounted specimen).

RESULTS AND DISCUSSION

1. Infection rate of crabs with *P. ohirai*: In our preliminary experiments for elucidating the relative abundance of metacercariae in different organs of crabs, the infection rates for liver, gills, muscles and reproductive organs were 90, 10, 5 and 0 per cent, respectively, in 20 specimens of *Sesarma dehaani* collected from the Kiso river. According to Yoshida *et al.* (1959), the rates for 29 specimens of *S. dehaani* from the Yura river, Kyoto Prefecture, were 93.0, 17.2, 20.7 and 6.9 per cent, respectively. Only one crab was observed to have negative liver and positive reproductive organs, while another had a negative liver but positive muscles and gills. Based on the present results and the previous report, the examination for metacercariae was carried out on the whole liver and gills which could easily be removed for this survey.

Four species of brackish water crabs were collected; *Sesarma dehaani*, *S. intermedia*, *Helice tridens* and *Chasmagnathus convexus*, in order of abundance. The majority of the crabs collected were *S. dehaani* and *S. intermedia*, in which *P. ohirai* metacercariae were detected as shown in Table 1. A total of 985 crabs was examined and metacercariae were recovered from 454. The

Table 1 Infection rate of crabs (*Sesarma dehaani* and *Sesarma intermedia*) with *Paragonimus ohirai* metacercaria (mc) collected from five rivers in the Tokai district

Locality	Site*	Date of survey	<i>Sesarma dehaani</i>				<i>Sesarma intermedia</i>			
			No. examined	No. infected (%)	Average mc per positive crab (range)	No. examined	No. infected (%)	Average mc per positive crab (range)	No. examined	Average mc per positive crab (range)
Ibi river	A	May 16 1984 Jul. 10 1984 Aug. 30 1984	16	0		108	1 (0.9)	1.0 (1)		
	B	Oct. 17 1982 Nov. 14 1982 Feb. 20 1984	42	38 (90.5)	7.8 (1-39)	6	5 (83.3)	5.0 (1-19)		
	C	May 16 1984	3	3 (100)	19.7 (14-23)	14	11 (78.6)	6.6 (1-22)		
	D	Apr. 17 1984	17	15 (88.2)	31.2 (4-167)	1	1 (100)	10.0 (10)		
	E	Jul. 10 1984	20	18 (90.0)	28.2 (1-95)	9	8 (88.9)	12.4 (1-30)		
	F	Apr. 17 1984	55	31 (56.4)	13.4 (1-156)	2	2 (100)	5.0 (2-8)		
Nagara river	G	Jul. 10 1983	12	5 (41.7)	28.4 (1-81)	1	1 (100)	5.0 (5)		
	H	Jul. 10 1984	24	17 (70.8)	12.2 (1-27)	7	4 (57.1)	9.5 (5-14)		
	I	Sep. 22 1983	7	1 (14.3)	20.0 (20)					
	J	Oct. 13 1982	10	0						
Kiso river	K	Jul. 23 1985	37	1 (2.7)	1.0 (1)	19	1 (5.3)	1.0 (1)		
	L	Jul. 23 1985	54	18 (33.3)	1.7 (1-4)	11	1 (9.1)	1.0 (1)		
	M	Nov. 14 1982	24	21 (87.5)	11.3 (1-44)	13	10 (76.9)	7.6 (1-17)		
	N	Nov. 7 1983 May 5 1985	54	34 (63.0)	9.6 (1-57)	6	4 (66.7)	21.3 (7-46)		
	O	Sep. 4 1985	19	11 (57.9)	6.7 (1-23)	25	11 (44.0)	6.9 (1-24)		
	P	Oct. 17 1982 -Jul. 23 1985	179	124 (69.3)	23.1 (1-255)	35	30 (85.7)	30.2 (1-114)		
	Q	Oct. 18 1983 -Sep. 4 1985	39	13 (33.3)	2.6 (1-12)	17	5 (29.4)	1.8 (1-4)		
	R	Feb. 2 1983	10	8 (80.0)	96.0 (20-219)	1	0			
	S	Sep. 4 1985	59	0						
	T	Aug. 10 1983	18	0		4	1 (25.0)	1.0 (1)		
Nikkou river	U	Sep. 6 1983	7	0						

* Collection sites A-S are indicated in the sketch map in Figure 1.

infection rate for *S. dehaani* and *S. intermedia* was 50.7 and 34.4 per cent, respectively (overall infection rate=46.1%). The infection rate for liver and gills was 50.1 and 4.5 per cent in *S. dehaani* and 33.7 and 3.9 per cent in *S. intermedia*, respectively. A total of 7,866 metacercariae was recovered from the two species of crabs (average number of metacercariae per positive crab =17.3), of which 7,752 were obtained from the liver (98.6%). The number of crabs with positive gills and negative liver for *P. ohirai* was four in *S. dehaani* and two in *S. intermedia*.

The relation between metacercaria infection and crab size was analyzed for *S. dehaani* at Site P of the Kiso river, where many crabs were collected. The infection rate increased with the size of the crabs; 0 per cent in crabs of less than 9 mm carapace width, 62.9 per cent in those of 10–19 mm, 78.1 per cent in those of 20–29 mm and 100 per cent in those of over 30 mm. The corresponding values for the average number of metacercariae per positive crab were 0, 20.1, 29.5 and 31.3. A similar relation was observed in *S. dehaani* from the Maruyama river by Miyamoto (1961); 2.0–14.5 per cent in the group of less than 10 mm carapace width, 46.2–60.4 per cent in the 11–20 mm, 75.7–84.1 per cent in the 21–30 mm and 87.5–87.9 per cent in the 31–40 mm group, respectively. The average number of metacercariae per positive crab increased with the size of the crabs. This indicates that larger crabs with a longer life span and more chance of being infected, have a higher infection rate and heavier worm burden.

2. Distribution of the infected crabs in the survey areas: As shown in Table 1, *S. dehaani* and *S. intermedia* with *P. ohirai* metacercariae were collected from 17 (A-I and K-R) of the 19 study sites of the three rivers, Ibi, Nagara and Kiso, and one site on the Shin river.

Along the Ibi, a high infection rate was observed at Sites B-F on both sides of the river (56.4–100%), and the maximum number of metacercariae per crab was 167 in *S. dehaani* at Site D. The average number of metacercariae per positive crab was 7.8–31.2 for *S. dehaani* and 5.0–12.4 for *S. intermedia*, respectively. The infection rate was lowest at Site A, about 2.5 km up from Site B, where only one metacercaria was detected among 16 *S. dehaani* and 108 *S. intermedia* (infection rate: 0% for *S. dehaani* and 0.9% for *S. intermedia*).

Along the Nagara, a high infection rate was observed at Sites G and H (41.7–100%). The average number of metacercariae per positive crab was 12.2–28.4 in *S. dehaani* and 5.0–9.5 in *S. intermedia*, respectively. A low infection rate was observed at Site I (about 1.5 km down from Site H), where the rate was 14.3 per cent for *S. dehaani*, while no *S. intermedia* was collected. Metacercariae were not found at Site J, about 4 km from the estuary.

Along the Kiso, a high infection rate was observed at Sites M-P and R; 57.9–87.5 per cent for *S. dehaani* and 44.0–85.7 per cent for *S. intermedia*. The maximum number of metacercariae was 255 at Site P, and the average number of metacercariae per positive crab was 6.7–96.0 in *S. dehaani* and 6.9–30.2 in *S. intermedia*, respectively. The infection rate was not so high at Sites L and Q; 33.3 per cent for *S. dehaani* and 9.1–29.4 per cent for *S. intermedia*. The minimum infection rate was 2.7 per cent for *S. dehaani* and 5.3 per cent for *S. intermedia* at Site K (about 5 km up from Site L). There were no metacercariae at Site S, which is about 4 km from the estuary.

Some of the areas in the present study had already been surveyed 27 years ago for *P. ohirai* infection in crabs by Yoshida *et al.* (1958). During this quarter-century the estuary area has been greatly altered by various factors, such as flooding caused by a typhoon (1959), expansion of harbour facilities following land reclamation, development of industrial and farming areas, and construction of breakwaters along the rivers. One area of the Nagara river where crabs with *P. ohirai* metacercariae were collected 27 years ago is close to Site J of the present survey.

The infection rates in 1958 were 11.1–25.9, 5.6 and 7.0–33.3 per cent for *S. dehaani*, *S. intermedia* and *C. convexus*, respectively. At present, the riverbed is markedly narrower than before and is submerged at high tide. The population density of the crabs was very low, and the few crabs collected were negative for metacercariae. However, the present study revealed that *S. dehaani* and *S. intermedia* with *P. ohirai* metacercariae are distributed in wide areas along the riverbeds 5.5 to 13.0 km from the estuaries of the Ibi, Nagara and Kiso rivers, and that the infection rate for *P. ohirai* was highest among crabs at a distance of 6–11 km from the estuaries. There are two endemic areas of *P. ohirai* near the present study area, the Shimoda area in Shizuoka Prefecture and the Maruyama river area in Hyogo Prefecture. The former is small, and the infection rate for crabs was 2.4 per cent (Sano *et al.*, 1981). In the Maruyama river area, a high percentage of *P. ohirai* metacercaria infection was observed on the wide riverbed along 0–2.5 km to 7.5–10 km from the estuary by Miyamoto (1961). But these endemic areas are narrower than those in the present study.

Along the Nikkou river, the eastern part of the present survey area, a limited number of crabs collected were negative for metacercariae of *P. ohirai*, although this area was positive in *S. dehaani* and *S. intermedia* in 1958. In the whole Shin and Shounai river area, a single *P. ohirai* metacercaria was detected in one specimen of *S. intermedia* from the Shin. This result reveals that *P. ohirai* is distributed in this river area for the first time, though the infection rate is low.

CONCLUSION

The examination of *Paragonimus ohirai* in crabs was carried out for three years from October 1982 in the rivers, Ibi, Nagara, Kiso, Nikkou, Shin and Shounai in the Tokai district of Japan. The majority of crabs collected were *Sesarma dehaani* and *S. intermedia*, in which *P. ohirai* metacercariae were detected. This study revealed that crabs with *P. ohirai* are distributed in wide stretches along the Ibi, Nagara and Kiso rivers, and that the infection rate was higher in crabs collected in areas 6–11 km distant from the estuary. Despite a limited number of surveys on the other three rivers, a new *P. ohirai*-infected area was found in a stretch of the Shin river for the first time in the present survey.

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大平肺吸虫の生態学的研究

1. 東海地方の6河川産カニにおける 本種メタセルカリア検出成績

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1982年10月から1985年9月の3年間に、伊勢湾に注ぐ三重、愛知県下の揖斐川、長良川、木曾川、日光川、新川、庄内川の河口周辺に生息するカニを採集し、大平肺吸虫メタセルカリアの検出を行った。採集されたカニの大部分はクロベンケイ、ベンケイガニの2種で、他に少数のアシハラガニ、ハマガニが採集された。揖斐川、長良川、木曾川では河口から約5.5～13km間の17地点のクロベンケイ、ベンケイガニから本種メタセルカリアが検出された。地点別感染率はクロベンケイ2.7～100%、ベンケイガニ0.9～100%であった。特に河口から約6～11km間のカニに高い感染率(41.7～100%)が認められ、陽性カニ1個体当たりのメタセルカリア最大寄生数は、クロベンケイの255虫体であった。1958年のこの地域における調査では、調査範囲が小さく、長良川河口付近の2地点産のカニにメタセルカリア寄生を認めたに過ぎなかったが、今回の調査により、揖斐川、長良川、木曾川流域に広大な大平肺吸虫分布地のあることが初めて明らかになった。他の3河川においては調査地点が非常に少なかったが、新川では初めて本種の分布が認められた。なお、過去に分布を認めた日光川では陰性であった。