

## Geographical Distribution of Diploid *Paragonimus westermani* in the Inaba District, Tottori, Japan

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### Abstract

In order to clarify the geographical distribution of diploid *Paragonimus westermani* in the Inaba district, eastern part of Tottori Prefecture, Japan, a total of 2,156 freshwater crabs, *Geothelphusa dehaani* were collected at 48 locations in mountainous areas between September 1989 and September 1991, and examined for *Paragonimus* metacercariae. Among these, 34 crabs (positive rate, 1.6%) collected in 6 different locations were positive for *Paragonimus* metacercariae, and a total of 145 metacercariae were obtained from the crabs. These metacercariae were inoculated into experimental final hosts (one cat and two dogs). All adult flukes recovered from the final hosts were identified as the diploid type of *P. westermani* on the basis of their morphological features. Based on the present findings, Iwami-gun has been confirmed to be a new habitat of diploid *P. westermani*. This result also indicates the possibility of the existence of human paragonimiasis caused by diploid *P. westermani* in Iwami-gun and its vicinity, Tottori Prefecture.

**Key words:** lung fluke, *Paragonimus westermani*, diploid type, geographical distribution, Tottori

### Introduction

*Paragonimus westermani* is well known as one of the important causative agents of human paragonimiasis and it is widely distributed not only in Japan but also throughout the Southeast and East Asian countries. It is also known that there are two chromosomal types (diploid and triploid) of *P. westermani* (Sakaguchi and Tada, 1976; Terasaki, 1977, 1980). Although human paragonimiasis have widely prevailed in Japan, it seems that they have been caused by triploid *P. westermani* because its incidence has rapidly decreased with the accumulation of knowledge on prevention. The most important factor in its

effective prevention was that individuals avoid eating uncooked or half-cooked mitten crab, *Eriocheir japonicus*, which is the second intermediate host and main source of human paragonimiasis. In recent years, however, some cases of paragonimiasis caused by eating freshwater crab, *Geothelphusa dehaani*, have been reported (Suzuki *et al.*, 1978; Kanzaki *et al.*, 1983, 1985; Yazaki *et al.*, 1990). Yazaki *et al.* (1990) found one case of human paragonimiasis caused by the diploid type and in the subsequent survey they found the metacercariae in freshwater crabs from the middle part of Tottori Prefecture. In the northern part of Hyogo Prefecture, some infected areas of diploid *P. westermani* have been reported (Shibahara, 1982; 1984, 1986). Therefore, the present survey was carried out in order to clarify the geographical distribution of diploid *P. westermani* in the eastern part of Tottori Prefecture, which is the adjacent area to the infected area of Hyogo Prefecture.

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**Materials and Methods**

*Detection of parasite*

A total of 2,156 freshwater crabs, *G. dehaani* were collected from 48 different locations (18 locations in Iwami-gun, 21 locations in Yazu-gun, 5 locations in Tottori City and 4 locations in Kedaka-gun) in the upstream areas of mountainous terrains in Tottori Prefecture, between September 1989 and September 1991 (Table 1,

Fig. 1). These crabs were crushed, digested for 6 to 12 hours at 37°C in artificial gastric juice and examined for *Paragonimus metacercariae*.

*Experimental infection in the animal hosts and recovery of the parasites*

The metacercariae obtained were orally administered to a cat (female, 2.5kg) and two dogs (male, 7.1kg and female, 6.2kg), giving the metacercariae from one location to one animal.

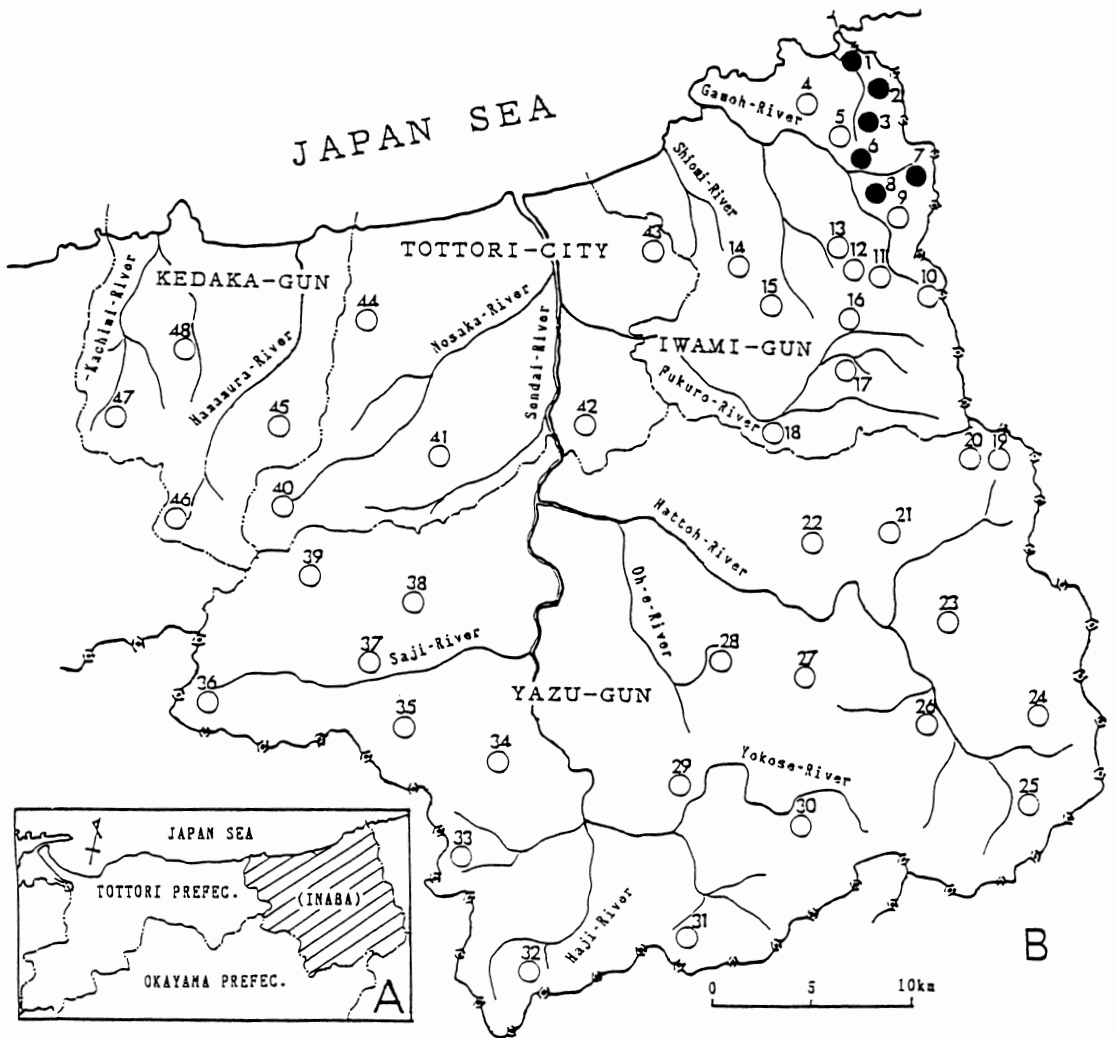


Fig. 1 A) Map showing the locations where the present survey was carried out.  
 B) Map showing 48 locations in the Inaba district, Tottori Prefecture where the freshwater crabs, *Geothelphusa dehaani*, were collected for the present survey.  
 ● : positive for *Paragonimus metacercariae*. ○ : negative

After a general anesthesia using pentobarbital sodium, the animals were bled to death from the carotid arteries and autopsied between 142 and 250 days after the administration of the metacercariae. The investigation for these was made in the pleural and peritoneal cavities and in visceral organs by visual examination only.

#### *Worms and eggs*

The worms removed were compressed and flattened, fixed, stained with carmine and mounted in balsam according to the method used by Shibahara (1986). The worms were then observed and measured. The eggs removed from the lung cysts of the cat which was fed with metacercariae from Tanokohji B area were preserved in 10% formalin and used in morphological observations and measurements.

### Results

#### *Prevalence of Paragonimus metacercariae in crabs*

As shown in Table 1 and Fig. 1, a total of 2,156 freshwater crabs were collected from 48 different locations in Iwami-, Yazu-, Kedaka-gun and Tottori City, all in the Inaba district of Tottori Prefecture. Among them, 34 crabs from 6 locations in the northeastern part of Iwami-gun bordering with Hyogo Prefecture, were positive for *Paragonimus metacercariae* and the infection rate for each location varied from 1.9% to 12.7%. A total of 145 metacercariae were obtained and the average number of metacercariae in a single crab ranged from 1.0 to 5.9. The maximum number of metacercariae in one crab was 48 in Tanokohji B area.

#### *Morphology of the metacercariae*

The metacercariae obtained were mostly spherical in shape as shown in Fig. 2 but sometimes spheroidal. The capsule enveloping the larvae consisted of two layers, a thin outer layer and a thick inner cyst wall. The larvae had an I-shaped excretory bladder, which was almost the same in size and thickness as the inner cyst wall. The average size of the inner cyst wall was  $439.0 \pm 20.4 \mu\text{m}$  in length and  $425.7 \pm 19.9 \mu\text{m}$  in

width. The average thickness of the inner cyst wall was  $14.5 \pm 1.9 \mu\text{m}$  and that of the outer cyst wall was  $1.5 \pm 0.4 \mu\text{m}$ .

#### *Morphology of the adult worms and their eggs recovered from animal hosts*

The results of the experimental infection were that a total of 17 worms (recovery rate, 85.0%) were recovered from the cysts in the lungs and/or pleural cavity of the infected cat with 20 encysted metacercariae obtained from Tanokohji B area. These were recovered on the 250th day of administration. On the other hand, on day 142 of administration, a total of 12 worms (average recovery rate, 60.0%) were recovered from the two dogs which were fed with 19 encysted metacercariae that were collected in Nagatani B area and one from Shiraji, respectively. Most of the flukes recovered from these animals were in the adult stage and had eggs in their uteri. The adult flukes obtained were morphologically ellipsoidal in shape (Fig. 3). The surface of the body was covered with singly spaced cuticular spines. They had six lobed ovaries and 5 to 6 lobed testes. A large number of sperms were observed both in the seminal receptacle and the seminal vesicle. The average size of the seven adult flukes from the cat was  $8.06 \pm 1.04\text{mm}$  in length and  $4.64 \pm 0.63\text{mm}$  in width. The average ratio of length/width was 1.74:1.

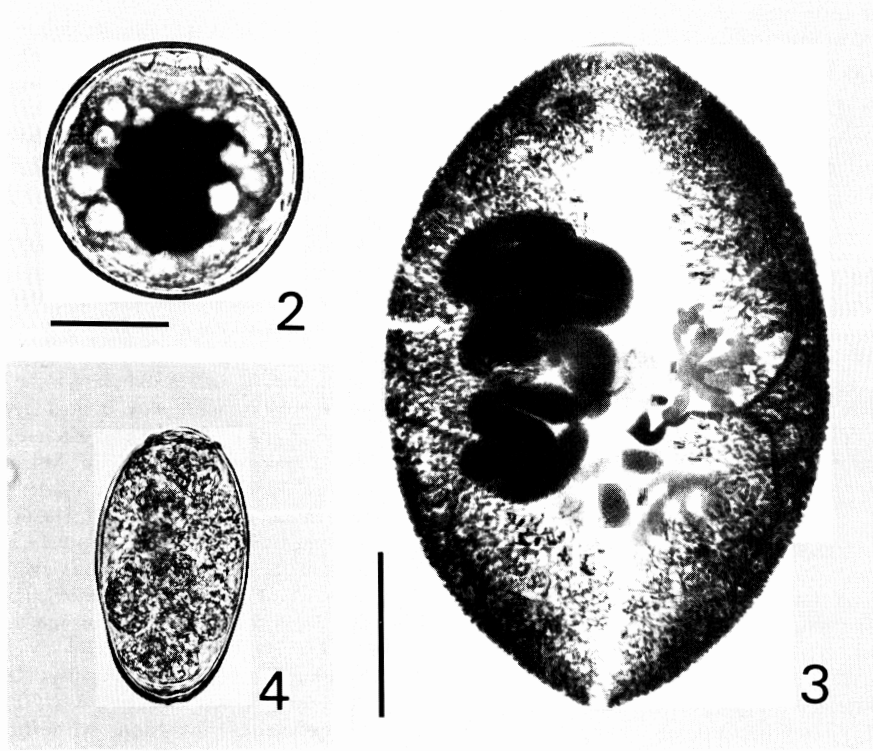
The eggs were generally inverted oval or oval in shape (Fig. 4). Out of 50 eggs observed, 18 (36%) were symmetrical and the remaining 32 (64%) were asymmetrical. As for the location of the maximum width, in 26 (52%) eggs it was in the anterior half, in 18 (36%) in the middle and in 6 (12%) in the posterior half. The average size of the eggs was  $73.7 \pm 2.8 \mu\text{m}$  in length and  $42.6 \pm 1.5 \mu\text{m}$  in width. The average thickness of the eggshell was  $1.4 \pm 0.2 \mu\text{m}$ . The thickening of the shell at the abopercular end was markedly observed in 38 (76%) eggs, slightly in 9 (18%) eggs and not at all in the remaining 3 (6%) eggs.

According to the morphological features of the larvae, adult and eggs mentioned above, all the metacercariae recorded from the freshwater crabs, *G. dehaani* in Iwami-gun were identified as the diploid type of *P. westermani*.

Table 1 Prevalence of infection of crabs, *Geothelphusa dehaani*, with *Paragonimus* Metacercariae (Mc) in the Inaba district, Tottori Prefecture, Japan

Locality	[No.]*	Date of survey	No. of crabs		Infection rate (%)	Total No. of Mc recovered	Average No. of Mc in a positive crab (Min.-Max.)
			examined	infected			
Iwami-gun							
Kugami	[ 1]	Oct. 1990	52	1	1.9	1	1.0
Tanokohji A	[ 2]	Sept. 1989	49	2	4.1	3	1.5 (1- 2)
Tanokohji B	[ 3]	Sept. 1989 ~Sept. 1991	189	19	10.1	113	5.9 (1-48)
Aidani	[ 4]	Aug. 1990	70	0			
Uji	[ 5]	July 1991	51	0			
Nagatani A	[ 6]	Sept. 1989	49	4	8.2	8	2.0 (1- 4)
Nagatani B	[ 7]	Aug. 1990	55	7	12.7	19	2.7 (1- 7)
Shiraji	[ 8]	Aug. 1990	32	1	3.1	1	1.0
Shiotani	[ 9]	Oct. 1990	62	0			
Torigoe	[10]	Oct. 1990	42	0			
Kaburashima	[11]	Sept. 1989	16	0			
Arai	[12]	Oct. 1990	20	0			
Aragane	[13]	Oct. 1989	32	0			
Sako	[14]	Nov. 1989	48	0			
Karakawa	[15]	Nov. 1989	25	0			
Kiwara	[16]	Sept. 1989	15	0			
Oh-ishi	[17]	Sept. 1989	8	0			
Matuo	[18]	Oct. 1989	39	0			
Yazu-gun							
Hirodomeno	[19]	May 1990	9	0			
Takidani	[20]	May 1990	21	0			
Megano	[21]	May 1990	16	0			
Yamashitani	[22]	May 1990	18	0			
Yadohra	[23]	May 1990	47	0			
Ochiori	[24]	Oct. 1990	32	0			
Ohno	[25]	Oct. 1990	61	0			
Susumi	[26]	May 1990	33	0			
Motani	[27]	May 1990	40	0			
Oh-e	[28]	May 1990	62	0			
Nishino	[29]	Oct. 1990	83	0			
Ashizu	[30]	Oct. 1990	33	0			
Nishidani	[31]	Oct. 1990	16	0			
Wasano	[32]	Oct. 1990	31	0			
Unami	[33]	Oct. 1990	78	0			
Yamaguchi	[34]	Oct. 1990	72	0			
Enami	[35]	Oct. 1990	25	0			
Tochiwara	[36]	Oct. 1990	18	0			
Ohmizu	[37]	Oct. 1990	35	0			
Kanba	[38]	Oct. 1990	24	0			
Somagoya	[39]	Oct. 1990	27	0			
Tottori City							
Azoh	[40]	Nov. 1989	40	0			
Kohro	[41]	Nov. 1989	79	0			
Koeji	[42]	Nov. 1989	55	0			
Momodani	[43]	Nov. 1989	39	0			
Tsutsumi	[44]	Nov. 1989	51	0			
Kedaka-gun							
Ohata	[45]	Nov. 1989	86	0			
Kohchi	[46]	Nov. 1989	79	0			
Kuwabara	[47]	Nov. 1989	37	0			
Hayauji	[48]	Nov. 1989	55	0			
Total			2,156	34	1.6	145	4.3 (1-48)

\*Nos. in parentheses are indicated in Fig. 1.



Figs. 2-4 Diploid *Paragonimus westermani* - 2: A metacercaria obtained from the freshwater crab, *G. dehaani*, collected in Iwami-cho, Iwami-gun. Bar: 200  $\mu$ m. 3: A mounted adult specimen from the lung cyst of the cat 250 days after administration of metacercariae. Bar: 2mm. 4: Egg from the lung cyst of the cat, preserved in 10% formalin.

### Discussion

In the past, several cases of human paragonimiasis have been reported in the San-in district (Toda, 1950; Fujito and Ishihara, 1952; Yoshida *et al.*, 1955; Nagahana *et al.*, 1959). As for Tottori Prefecture, only a few cases of paragonimiasis have been reported by Nagahana *et al.* (1959). They reported four paragonimiasis patients, so diagnosed by the examination of their stools, and detected one metacercaria in one mitten crab, *E. japonicus* caught in the river flowing through the endemic area, but not from the freshwater crabs, *G. dehaani*. On the other hand, Nishida (1958) reported a high frequency of *Paragonimus* metacercariae in mitten crabs collected in different places in Tottori Prefecture. Therefore, the frequency of paragonimiasis might actually be high in this prefecture. Since then,

however, no one has reported any paragonimiasis in Tottori Prefecture.

There have been several reports on the existence of diploid *P. westermani* in the San-in district (Shibahara, 1982, 1984, 1986; Yazaki *et al.*, 1990). However, there is only one report to date on the prevalence of the diploid type of the organism in Tottori Prefecture (Yazaki *et al.*, 1990). The present study has revealed that diploid *P. westermani* might be found not only in Tottori Prefecture, but also in other part of the San-in district and its vicinity.

It is known that human paragonimiasis by triploid *P. westermani* can occur not only when man eats uncooked or half-cooked crabs, but also by eating raw or half-cooked wild boar meat which harbors the metacercariae (Norimatsu *et al.*, 1975; Miyazaki and Hirose, 1976; Tashiro *et al.*, 1984). On the other hand, it has been ex-

perimentally demonstrated that diploid *P. westermani* can also infect the human beings in the same way (Shibahara and Nishida, 1986). Therefore, it is necessary to pay special attention to the possibility of human paragonimiasis caused by these sources.

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