

*Paragonimus westermani*, Diploid Type,  
from Otaki-machi in Chiba Prefecture, Japan

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(Received for publication; May 28, 1986)

**Key words:** *Paragonimus westermani*, diploid type, *Geothelphusa dehaani*, paragonimiasis

### Introduction

Four cases of paragonimiasis found in Chiba Prefecture have already been reported (Kanzaki *et al.*, 1985). The patients ate raw freshwater crabs, *Geothelphusa dehaani*, which they caught at Yourou Valley in Otaki-machi in Chiba Prefecture, Japan. Our preliminary results of experimental infections using metacercariae obtained from the crabs of the valley indicated that the metacercariae were those of the diploid type of *Paragonimus westermani*. The purpose of this paper is to describe in detail the results of surveys of the crabs in this area. In addition, results of morphological and chromosomal studies on the lung fluke and clinical features due to the infection with *P. westermani*, diploid type, will be presented.

### Materials and Methods

*Geothelphusa dehaani* were collected at 12 localities, that is, Yokoyama, Kamishiki, Komyo, Ohto, Kotadai, Monomizuka, Yokoze, Awamata, Unobe, Kaisho, Mamembara and Umigi in Otaki-machi, Chiba Prefecture, Japan, from August 1983 to November 1984 (Fig. 1).

The size of freshwater crabs collected was

first measured. Then, all crabs were individually examined for metacercariae of *Paragonimus* sp. by Tsuda's method (1959). The metacercariae were collected in Oshima's solution (Oshima *et al.*, 1958) and kept at 4°C.

These metacercariae were orally administered to dogs. The animals were autopsied 4 months later. Lung flukes recovered from worm cysts were pressed and fixed in Carnoy's solution. They were stained with carmine for morphological observations. The number of chromosomes of the worm was examined by the air dry technique (Terasaki, 1977; Terasaki and Nakamura, 1978). Measurement of eggs recovered from the worm cysts was also carried out.

The correlations between the size of the crabs and both of infection rates and numbers of metacercariae were analyzed by the regression method.

### Results

The results of the examination of *G. dehaani* are summarized in Table 1, indicating that a total of 168 crabs (27.2%) among 383 males and 234 females were infected with metacercariae of *Paragonimus* sp. The mean number of metacercariae recovered from a single infected crab was 20.1. The infection rate was lower in the northern part of Otaki-machi than in the southern part. Especially, it was 98.3% (60 crabs examined) in a stream in Awamata.

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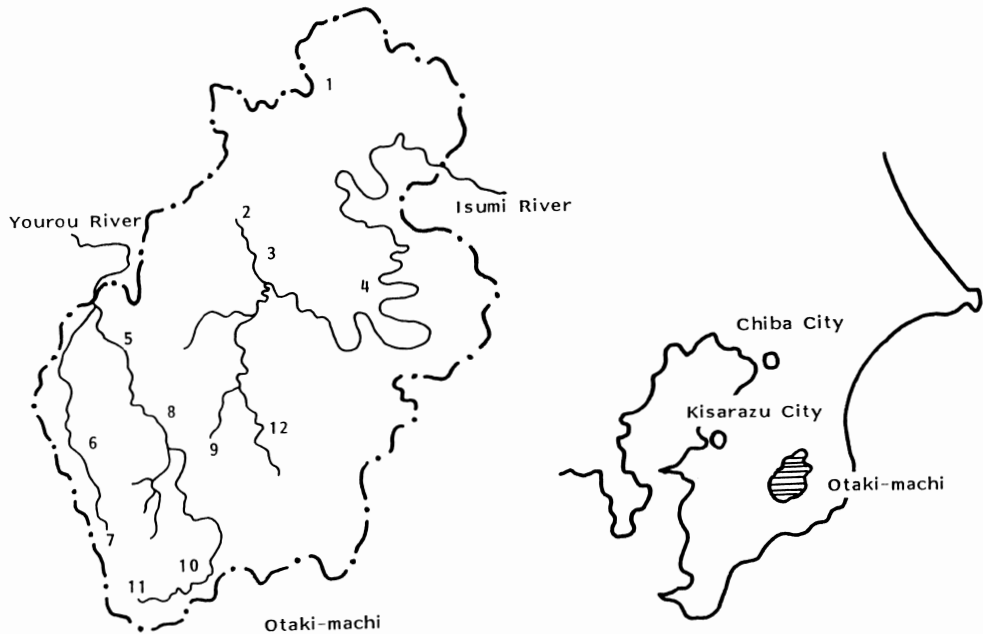


Fig. 1 Map of Otaki-machi in Chiba Prefecture showing the survey areas.

1: Yokoyama, 2: Kamishiki, 3: Komyo, 4: Ohto, 5: Kotadai, 6: Monomizuka, 7: Yokoze, 8: Awamata, 9: Unobe, 10: Kaisho, 11: Mamenbara, 12: Umigi.

The mean number of infected metacercariae per positive crab was 31.2.

The distribution of metacercariae in the body of the crab was examined for all crabs collected. They were found as follows; 4.0% in gills, 15.5% in the liver and 80.2% in muscles. No correlations between the size of crabs and both the metacercarial infection rate and their number were observed.

These metacercariae were administered to dogs and adult lung flukes were recovered. The measurements of adult worms were 7.05–9.76 mm in length and 2.91–4.84 mm in width (8.22 × 3.71 mm). The diameter of oral sucker and acetabulum was 0.67–0.47 mm and 0.64–0.59 mm, respectively. The cuticular spines were single. The ovary was branched into 6 lobes in most of the specimens and testis into 5 to 6 lobes. Spermatozoa were observed in the seminal receptacle and in the seminal vesicle (Photos. 1–3). The number of chromosomes was  $n = 11$  and  $2n = 22$  (Photos. 4–5).

We measured 30 metacercariae and the size

of the metacercariae was 365–470 × 395–500  $\mu\text{m}$  (mean, 430 × 449  $\mu\text{m}$ ) (Photo. 6). No pinkish granules were found in metacercariae.

The size of eggs was 62.5–82.5 × 45.0–62.5  $\mu\text{m}$  (mean, 74.8 × 52.8  $\mu\text{m}$ ) (30 eggs examined (Photo. 7)). The shape was asymmetrical and thickening of the egg shell on the nonoperculated end was recognized in some of them. The maximum width of the eggs was often observed on the side of the operculate from the mid-position.

On the basis of these observations, the lung fluke from Otaki-machi was identified to be *P. westermani*, diploid type.

### Discussion

The diploid type of *P. westermani* has been found in Akita Prefecture (Suzuki *et al.*, 1978), Ishikawa Prefecture (Yoshimura *et al.*, 1983), Gifu Prefecture (Shiwaku *et al.*, 1986), Fukui Prefecture (Nishida *et al.*, 1980), Shiga Prefec-

Table 1 Results of examinations of *Paragonimus westermani* metacercariae among freshwater crabs, *Geothelphusa dehaani*, collected from various localities in Otaki-machi, Chiba Prefecture

Locality	Sex and No. of crabs examined	No. of crabs infected	Infection rate (%)
1 Yokoyama	M 39	0	0
	F 23	0	
2 Kamishiki	M 29	1	1.4
	F 42	0	
3 Komyo	M 54	1	1.4
	F 18	0	
4 Ohto	M 5	5	85.7
	F 9	7	
5 Kotadai	M 4	4	77.8
	F 5	3	
6 Monomizuka	M 16	2	11.8
	F 1	0	
7 Yokoze	M 7	3	34.8
	F 16	5	
8 Awamata	M 97	58	53.1
	F 82	37	
9 Unobe	M 62	17	22.7
	F 13	0	
10 Kaisho	M 37	9	21.4
	F 5	0	
11 Mamenbara	M 20	2	11.1
	F 16	2	
12 Umigi	M 13	10	70.6
	F 4	2	
Subtotal	M 383	112	29.2
	F 234	56	23.9
Total	617	168	27.2

M: male, F: female.

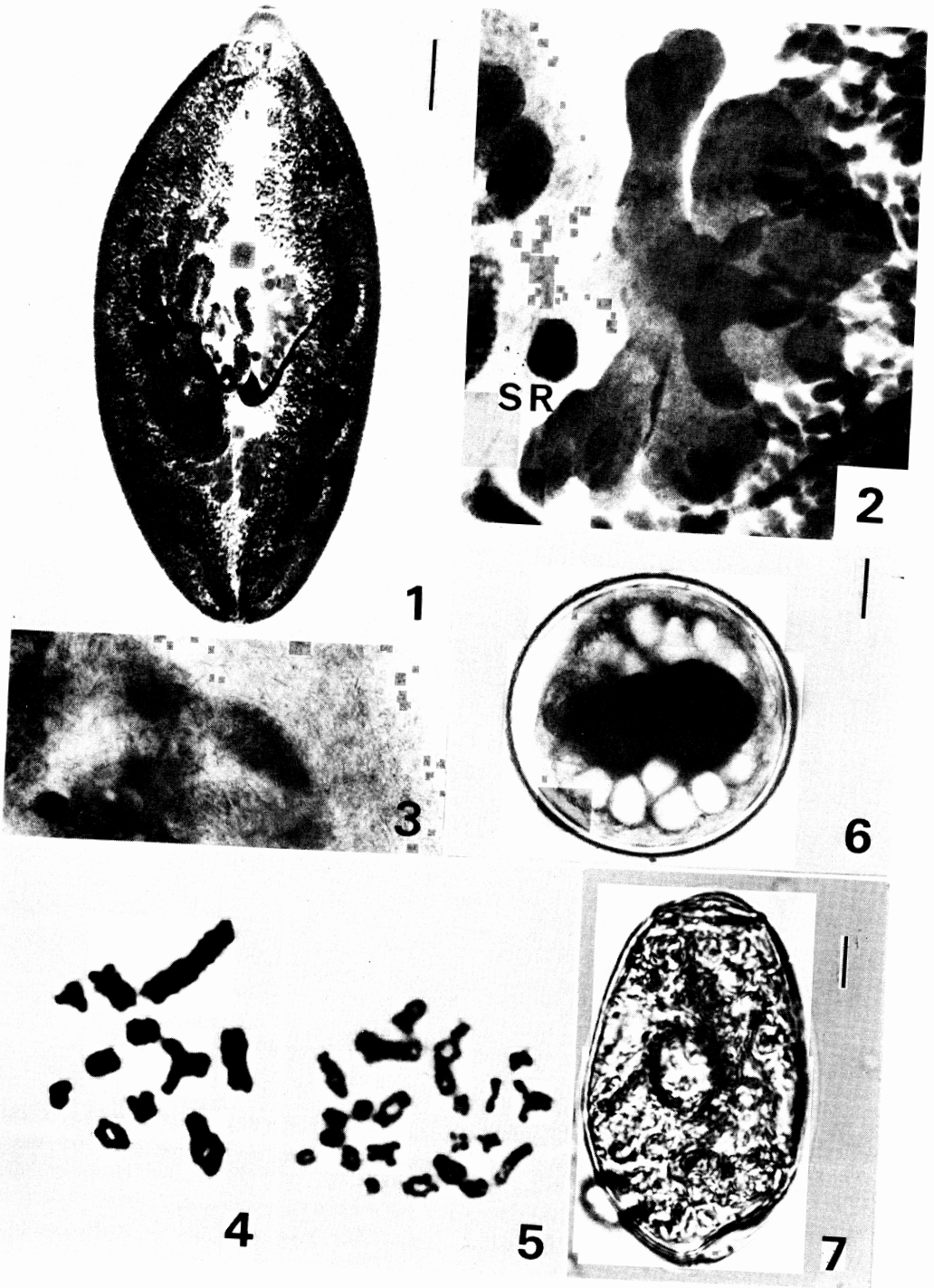
No. of the locality corresponds to the one in Fig. 1, respectively.

ture (Nishida *et al.*, 1981), Nara Prefecture (Sugiyama *et al.*, 1983a, 1984), Mie Prefecture (Sugiyama *et al.*, 1983b), Hyogo Prefecture (Shibahara, 1982, 1986), Oita Prefecture (Habe and Miyazaki, 1982) and Okinawa Island (Yokogawa *et al.*, 1986) in Japan. In this paper, we add a new habitat of the diploid type which is first in the Kanto district.

Yokogawa *et al.* (1958) reported that meta-

cercaria of *P. westermani* was found in *Eriocheir japonicus* caught in the Ichinomiya river in Chiba Prefecture. However, no survey has been carried out since then.

The Yourou Valley is in the southern part of Otaki-machi. The infection rate of metacercariae among freshwater crabs was higher in this mountainous area than in the northern part where there are many paddy fields. There-



fore, the final hosts of *P. westermani* in Otaki-machi are probably wild animals. However, this problem is remained to be solved, because there has been little chance to examine such wild animals.

There was no correlation between the crab size and both of the infection rate and number of metacercariae, in the case of *G. dehaani*. This is in accordance with a previous observation by Shibahara (1982).

No distinct differences were observed in the morphological characteristics of the adult worm, metacercaria and egg of the diploid type of *P. westermani* in Chiba Prefecture, as compared to the parasites collected in the other prefectures, although considerable variations are found in size among the flukes from each prefecture. However, it was observed that the branches of the ovary were thick and the surface of them were smooth in some specimens (Photo. 2).

It is possible that *P. westermani*, diploid type, is distributed in areas in Chiba Prefecture other than Otaki-machi. Further surveys are necessary for determining the distribution of *P. westermani* in this Prefecture.

Some cases of paragonimiasis *westermani* with atypical clinical signs or symptoms such as pneumothorax or pleural effusion have been recently reported. Concerning this matter, Miyazaki (1982) suggested that the pathogenesis of the two types of *P. westermani* was different from each other. According to him, such cases with atypical symptoms were probably infected with a single or a few worms of the diploid type. However, 4 cases of paragonimiasis *westermani* which we previously reported (Kanzaki *et al.*, 1985) were thought to be infected with many metacercariae of the diploid type, not with only one or a few,

because the patients ate over 10 uncooked freshwater crabs collected from that little valley where the infection rate of the crabs was found to be 98.3% and the mean number of infected metacercariae was 31.2 per crab. On the other hand, the triploid type has not been found on Okinawa Island as yet (Yokogawa *et al.*, 1986), despite the fact that some patients showing clinical signs or symptoms of typical paragonimiasis *westermani* were reported there. Therefore, further studies will be needed to clarify whether there may be any difference of pathogenesis between these two types.

In any case, this disease seems to require more public attention, because many people go hiking to Yourou Valley where they enjoy fishing or catching freshwater crabs to eat.

### Summary

Among 617 freshwater crabs, *Geothelphusa dehaani*, collected from Otaki-machi in Chiba Prefecture, 168 crabs (27.2%) were found to be infected with metacercariae of *Paragonimus* sp. Adult lung flukes were recovered from dogs 4 months after the oral administration of the metacercariae. On the basis of morphological and chromosomal examinations, the parasite was identified as the diploid type of *Paragonimus westermani*.

Since previously reported patients with paragonimiasis got infection after eating in raw the crabs obtained in this area, their atypical clinical signs and symptoms were discussed from the viewpoint of the pathogenesis of this type of *P. westermani*.

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Photo. 1 Adult worm (scale = 10 mm). Recovered from the lung of the dog, 4 months after infection.

Photo. 2 Ovary and Seminal receptacle (SR).

Photo. 3 Seminal vesicle.

Photo. 4 Primary spermatocyte in diplotene to diakinesis with 11 bivalents.

Photo. 5 Mitotic metaphase with 22 chromosomes from the gonad.

Photo. 6 Metacercaria (scale = 0.1 mm).

Photo. 7 Egg (scale = 10  $\mu$ m).

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## 千葉県大多喜町におけるウエステルマン肺吸虫 2 倍体型

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千葉県大多喜町で採取した 617 匹のサワガニを検索し, 168 匹 (27.2%) のカニに肺吸虫のメタセルカリアの寄生を認めた。これらのメタセルカリアを犬に感染させ, 肺に形成された虫嚢より成虫を回収することができた。成虫の形態および染色体数より, この肺吸虫はウエステルマン肺吸虫 2 倍体型であることが明らかとなった。

我々が以前報告した 4 例の肺吸虫症患者はこの地

区のある沢で採取したサワガニを生食して感染したものであり, 同沢の調査の結果, これらの患者は, 単数あるいは少数のメタセルカリアを摂取したのではなく, 大量の感染を受けたものと考えられた。しかし, これらの患者は典型的なウエステルマン肺吸虫症の臨床像を呈さなかったことから, 2 倍体型の病原性について若干の考察を行った。