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Studies on *Paragonimus westermani* in Taiwan: Experimental Infection of the Diploid Type in Rats and Mice

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Abstract

Recent studies on chromosomes have shown the presence of diploid type and triploid type of *Paragonimus westermani*. Metacercariae originated from potamonid crabs in Taiwan were proved to be the diploid type.

In this study, the migration route and development of the diploid type of *P. westermani* in rats and mice were examined. The worms in both of the animals were retrieved mainly from muscles, and most of the worms were retarded in growth. A few pre-adult and/or mature worms were recovered from body cavities, thoracic wall muscles and cysts of lungs of some rats between 6–14 weeks after infections. Worms well developed and cysts in lungs were not observed in rats after the infection was longer than 15 weeks.

A few pre-adult worms were recovered from body cavities of mice between 3–7 weeks after infection, but cyst in lungs were never found in mice by 20th week of infection.

From these results, rats and mice are considered unsuitable final hosts for the diploid type of *P. westermani*.

Key words: *Paragonimus westermani*, diploid type, rats, mice, potamonid crabs, experimental infection

Introduction

The migration route and development of *Paragonimus westermani* in cats and rats have been studied by Yokogawa *et al.* (1962). They concluded that the pattern of the migration and development of the larvae of *P. westermani* in rats markedly differed from those in cats, the definitive host of *P. westermani*. In the case of rats, most of the larvae which penetrated the abdominal wall did not return to the abdominal cavity. Most of them migrated into deep muscles and remained there for a long time without further development. Some of the larvae made their way to the lungs and formed worm cysts there. However, no worms reached sexual maturity in rats.

Recent studies on chromosomes of *P. westermani* by Sakaguchi and Tada (1976) and Terasaki (1977) revealed that there are two types of *P. westermani*, triploid and diploid. Sperms are not observed in the seminal receptacle of the former.

Miyazaki (1977, 1978) confirmed these findings and proposed that the diploid, bisexual type be called *P. westermani* and the triploid, parthenogenetic type *P. pulmonalis*. However, whether *P. westermani* can actually be divided into two species is now in a controversy (Yokogawa, 1982).

Miyazaki (1979) mentioned that there is a significant difference between these two types of *P. westermani* regarding their infectivity to rats. The larvae of the diploid type rapidly

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disappear from muscles of rats and pass to the lungs, some of them becoming fully mature about 2 months after infection. On the other hand, the larvae of the triploid type rarely mature in lungs and most of them remain in muscles for a long time without any development.

Kanazawa *et al.* (1987) reported that in rats, some orally administered metacercariae of the diploid type from potamonid crabs in Japan formed cysts in lungs and laid eggs whereas the larvae recovered from muscles were retarded in growth. On the other hand, the larvae of triploid type from *Eriocheir japonicus* never formed cysts and most of the larvae were found in muscles without any development even by 20 weeks after infection. From these results, they concluded that there were some differences in the migration route and development in rats between these two types, however, it seemed likely that they were closely related.

Miyazaki and Chiu (1980) reported that both of the diploid and triploid types existed in Taiwan and the diploid type utilizes potamonid crabs as its second intermediate host whereas the triploid type uses *E. japonicus*. With regard to infection in rats, however, Fan and Khaw (1963) obtained different results from those of Yokogawa *et al.* (1962), using the metacercariae from *E. japonicus* in Taiwan. According to them, some of the larvae formed cysts in the lungs and reached sexual maturity. The metacercariae used by Yokogawa *et al.* (1962) in Japan and by Fan and Khaw (1963) in Taiwan were supposed to be triploid type.

The study on the migration route and development in rats using the diploid type in Taiwan has never been conducted.

This study was undertaken to examine the distribution and development of the diploid type of *P. westermani* in rats and mice using the metacercariae from potamonid crabs in Taiwan.

Materials and Methods

Parasites:

Metacercariae of the diploid type of *P. westermani* were obtained from *Geothelphusa miyazakii* or *G. candidiensis* (formerly classified as *Potamon dehaani*) from Taipin, Taipei County and *Candidiopotamon rathbuni* from Ponlai, Miaoli County by the method of Huang *et al.* (1966). Metacercariae from different localities were kept separately and stored in isotonic solution for crayfish (Tsuda, 1959) at 8–10°C until used.

Experimental Animals:

Female Sprague-Dawley (SD) and Long-Evans (LE) rats weighing about 150 g and ICR mice weighing about 20 g were purchased from the Animal Center, National Taiwan University Hospital.

Infection of Animals:

Rats and mice were infected orally with 20 and 5 metacercariae, respectively. Most of the infection experiments were done using metacercariae from *G. miyazakii* from Taipin, Taipei County.

In Experiment 1, 65 SD rats were infected, 5 rats were examined weekly between 1–10 weeks after infection, and then at 15, 20 and 30 weeks after infection.

In Experiment 2, 10 LE rats were infected to determine whether the distribution of the worms varied with the strain of rats. In this group, 2 rats were examined weekly between 6–10 weeks after infection.

In Experiment 3, metacercariae from *C. rathbuni* of Ponlai, Miaoli County, were used to infect 10 LE rats and 3 SD rats to see if the distribution and development of the worms in rats varied with the localities of the metacercariae. Two or 3 LE rats were examined at 3, 5, 7 and 10 weeks after infection and 3 SD rats were also examined at 10 weeks after infection.

In Experiment 4, 50 mice were each infected with 5 metacercariae. In this group, 3 mice were examined at 1, 3, 5, 6, 7, 8, 9, 10, 15 and 2 mice at 20 weeks after infection.

Recovery of Worms:

Worms were recovered by the method of Yokogawa *et al.* (1962) with some modification. Briefly, rats or mice were first anes-

thetized with ether, then killed by cutting the jugular veins and arteries, and then skinned. The abdominal and pleural cavities were opened and washed several times with physiological saline. Washing fluids were placed in Petri dishes and examined under a dissecting microscope.

If worm cysts were found in lungs or any other organs, the cyst content was examined first for worms and eggs. Internal organs and muscles, abdominal wall muscles, diaphragm and deep muscles (muscles of the extremities, lumbar and paravertebral muscles) were minced with a pair of scissors in a 100 ml beaker, except the deep muscles which were chopped with a cutting knife on a board, and incubated separately in physiological saline at 37°C for 3–4 hours before examination under a dissecting microscope. The worms recovered from different parts of each animal were enumerated, fixed in Bouin's solution and then stained with Semichon's carmine for morphological observation.

Results

Distribution and Development of Worms in Rats:

Results of Experiment 1 are summarized in Table 1. Total worm recovery rate averaged 53%, with a range of 42% to 66%. On an average, about 95% of the recovered worms were found in the muscles including abdominal wall muscles, thoracic wall muscles, diaphragm, and deep muscles throughout the course of the experiment.

In the first 3 weeks after infection, more than half of the retrieved larvae were found in the abdominal wall and thoracic wall muscles, however, the number of the larvae recovered in deep muscles increased sharply at 3 weeks after infection. Thereafter, the majority of the larvae recovered resided in deep muscles. At 30 weeks after infection, the larvae were recovered only in muscles of the abdominal wall, thoracic wall and deep muscles.

Formation of worm cysts was observed in lungs of some rats at 8 and 9 weeks, one cyst in one rat at 8 weeks and 2 cysts, one cyst each, in

Table 1 Average number of worms recovered from 5 Sprague-Dawley rats at various intervals after infection with 20 metacercariae of *P. westermani* from *Geothelphusa* crabs from Taipin, Taipei County

Weeks after infection	Average number of worms recovered from								Total No. of worms (%) recovered per rat
	Abdominal cavity	Abdominal wall	Liver	Diaphragm	Pleural cavity	Thoracic wall	Lung worm cyst	Deep muscles	
1	0.6	8.0	0.2	0.6	0.6	3.2		ND	13.2(66)
2		3.8	0.2	1.0	0.6	2.4		2.8	10.8(54)
3	0.4	1.8		0.2		3.6		4.8	10.8(54)
4	0.4	2.8		0.8	0.2	1.6		5.0	10.8(54)
5	0.2	1.2		0.4		2.0		4.6	8.4(42)
6	0.2	2.0		0.2	0.2	2.2		5.4	10.2(51)
7	0.2	1.4		0.2	0.4	2.4		7.0	11.6(58)
8		0.4		0.2	0.2	0.6	0.4*	8.6	10.4(52)
9		1.2			0.2	1.0	0.4†	7.6	10.4(52)
10		2.0		0.2	0.6	2.0		6.4	11.2(56)
15		1.0		0.2	0.4	1.4		6.0	9.0(45)
20		0.8			0.2	2.2		6.6	10.0(50)
30	0.2	0.8				1.4		8.8	11.0(55)

ND not done

* 2 pre-adult worms were found in one worm cyst

† 2 worm cysts were found from 2 rats, one each. One cyst was empty and the other cyst had 2 mature worms and eggs in cyst content

Table 2 Average number of worms recovered from different locations of 2 Long Evans rats at various intervals after infection with 20 metacercariae of diploid type of *P. westermani*

Weeks after infection	Average number of worms recovered from					Total No. (%) of worms recovered per rat
	Abdominal cavity	Pleural cavity	Thoracic wall	Lung worm cyst	Deep muscles	
6		1.0	3.0		7.5	11.5(58)
7		1.0	3.5		3.5	8.0(40)
8	0.5	0.5	1.5	1.0*	8.0	11.5(58)
9	0.5	0.5	1.0	1.0*	8.5	11.0(55)
10			1.5		11.5	13.0(65)

* 2 pre-adult worms were found in one worm cyst

2 rats at 9 weeks after infection, respectively.

Most of the larvae recovered in the muscles showed almost no growth even till the infection was 30 weeks old, and their average dimensions were 0.8 x 0.4 mm. The worms recovered in the cysts in lungs and, occasionally, in the pleural cavity at 8–9 weeks after infection were well developed in size (4–6 mm in length). They were either mature worms with eggs in uterus or pre-adult worms whose gonads were well differentiated but the vitellaria were still developing and the uterus contained no eggs.

The results of Experiments 2 were listed in Table 2. Total worm recovery rate in LE rats averaged 55%, with a range of 40–65% and most of the larvae were recovered in the muscles. Formation of worm cyst in lungs was also observed in one rat each at 8 and 9 weeks after infection as seen in SD rats. On both occasions, 2 pre-adult worms were found in each worm cyst. These results were quite similar to those of Experiment 1 using SD rats.

In Experiment 3, rats were infected with the metacercariae obtained from *C. rathbuni* of Ponlai, Miaoli County and 2 or 3 rats were examined at 3, 5, 7 and 10 weeks after infection.

The total worm recovery rate was 48%, with a range of 35–75%. The muscles were also the main habitat for the larvae. One worm cyst each was observed in 2 rats, one LE and one SD rats, at 10 weeks after infection. Both cysts contained 2 pre-adult worms each.

From the above experiments, it seemed that the results were similar in experiments using the metacercariae from different localities in Taiwan to infect different strains of rats.

In order to examine the duration of survival of the pre-adult and mature worms in rats, more infections were carried out and all the data on the distribution and the average size of larger worms were pooled and summarized in Table 3.

Pre-adult and mature worms were most frequently recovered in worm cysts in lungs, followed in sequence by the pleural cavity, thoracic wall muscles and abdominal cavity. The average ratio of larger worms to juvenile larvae recovered between 6–14 weeks after infection was only about 4%.

A total of 11 worm cysts in the lungs were observed in 11 rats during the period from 7 to 14 weeks after infection and most of the cysts in lungs contained 2 worms each. An empty cyst or a cyst containing only one worm was also observed.

The average size of the larger worms ranged between 3.8 x 1.5 mm at 6 weeks after infection and 5.9 x 3.8 mm at 14 weeks after infection, and it showed a tendency to increase in size with the age of infection between 6 and 9 weeks. The larger worms and cysts in lungs were no longer observed when the infection was for greater than 15 weeks.

Distribution and Development in Mice:

The worm recovery rate in mice ranged from 20 to 100%, with an average of 61%. As shown in Table 4, the recovery rate was generally higher when the infection was less than 8 weeks

Table 3 Number and size of larger worms recovered from different locations of rats at various intervals after infection with 20 metacercariae of diploid type of *P. westermani*

Weeks after infection	No. of rats examined	No. of larger worms recovered in					No. of worm cysts in lung	Average size in mm. (n)
		Abdominal cavity	Pleural cavity	Thoracic wall	Lung worm cyst	Total		
6	7		2			2		3.8×1.5(2)
7	20		4(1*)	1*	5	10	3	3.8×2.0(5)
8	7				4	4	2	4.3×2.5(4)
9	10		3(1*)		4(2*)	7	3(1†)	5.7×2.4(5)
10	14			1	4	5	2	5.0×2.4(5)
11	6		1*			1		5.0×2.9(1)
12	3							—
13	6	1				1		4.4×2.5(1)
14	4				2(1*)	2	1	5.9×3.8(2)
15	9							—
16	5							—

* Mature worm with eggs in uterus
† Empty cyst
n Number of measurements

Table 4 Average number of worms recovered from different locations of ICR mice at various intervals after infection with 5 metacercariae of diploid type of *P. westermani*

Weeks after infection	No. of mice examined	Average number of worms recovered from					Total No. (%) of worms recovered per mouse
		Abdominal cavity	Abdominal wall	Pleural cavity	Thoracic wall	Deep muscles	
1	3	1.0	1.0	0.7		0.3	3.0 (60)
3	3	0.7	0.3	0.3*	1.0	2.7	5.0(100)
5	3	0.7	0.7	0.7	0.7	1.0	3.8 (74)
6	3	1.0	0.3	0.3*	1.0	1.0	3.7 (73)
7	3	0.7(0.3*)		0.3*	0.7	1.3	3.0 (60)
8	3	0.3	1.3		0.3	1.0	3.0 (60)
9	3			0.3		0.7	1.0 (20)
10	3	0.3	0.7	1.0		0.7	2.7 (54)
15	3	0.3	0.7				1.0 (20)
20	2	0.5				0.5	1.0 (20)

* Larger worm

in duration. From then on till the experiment ended at 20 weeks after infection, the recovery rate was usually around 20% except 54% at the 10th week. The larvae were never found in liver, diaphragm and lungs. Similar to the infection in rats, larvae were also found more often in muscles than in the body cavities except for the 1st week of the infection.

Most of the worms recovered were greatly retarded in growth, with average dimensions of 0.8 x 0.4 mm. At 3 and 6 weeks after infection, a larger worm each was found in the pleural cavity at the 7th week, a larger worm each was found in the pleural cavity and abdominal cavity of another mouse. The 3-week-old worm was 2.6 x 1.1 mm in dimension and the gonads had not developed yet. Average size of the 6- and 7-week-old worms was 3.5 x 1.8 mm. The

testes, ovary and uterus were visible, but no vitellarial development occurred. No larger worms had been recovered after the infection was over 8 weeks old.

Discussion

The results of our experiments revealed that rats and mice were unsuitable hosts for the diploid type of *P. westermani* in Taiwan. This is quite similar to those obtained with the diploid type in Japan in rats and mice by Shibahara (1984) and Kanazawa *et al.* (1987). Most of the larvae recovered form muscles were retarded in growth but some were able to reach sexual maturity in worm cysts of lungs and in pleural cavity. At 30 weeks after infection all the worms were recovered only from the muscles.

However, the following differences were noted: 1) In our results, formation of worm cyst in lungs was always single per rat and the worm cysts were recovered only in the period of time from 7 to 14 weeks after infection and thereafter no worm cyst was observed. Whereas in the Japanese studies, multiple cysts in lungs were not uncommon and worm cysts in the lungs, although empty, were still observed at 30 weeks after infection; 2) In the study by Kanazawa *et al.* (1987), average recovery rates ranged from 43.5% to 56.5% until 7 weeks and decreased remarkably thereafter to 8.3% at 30 weeks after infection. In our study the worm recovery rate remained fairly constant and the figure was still high as 55% at 30 weeks; 3) Over 90% of the recovered larvae in our work were from muscles throughout the course of experiments and only a small number of worms, less than 5% were discovered in the pleural cavity and lungs. Kanazawa *et al.* (1987) noted that a greater fluctuation in worm recovery rates from muscles were observed, and that greater number of worms, 38% on an average, were found in the pleural cavity or lungs.

Whether these differences were caused by geographic differences of the parasites or variation in strains of rats used need further investigation. In our studies, the results were similar in experiments using metacercariae from different localities in Taiwan to infect different strains as SD and LE rats.

It is known that the diploid types of *P. westermani* from different localities in Japan behave differently in rats. Those from Hyogo Prefecture (Shibahara, 1984) and Mie Prefecture (Sugiyama *et al.*, 1985) were able to form worm cysts and produce eggs in rats whereas those from Akita Prefecture (Habe, 1983) and Oita Prefecture (Hata, 1986 personal communication) neither form worm cyst nor produce eggs in rats. Habe (1983) examined a total of 5 rats at about 18 and 21 weeks after infection, and noted that worms were retarded in growth and found only in muscles. According to Hata, 30 rats were examined during the period from 72 hours to 30 weeks after infection, and larvae retarded in growth were found only in the abdominal wall muscles, thoracic wall muscles and deep muscles. No worms were recovered in lungs. It would be interesting to find out whether the diploid type from Akita and Oita Prefectures never entered the lungs of rats.

According to Miyazaki and Chiu (1980), two types of *P. westermani* also existed in Taiwan. The worms originated from *E. japonicus* were all identified as the triploid type while those derived from the metacercariae in the potamonid crabs such as *G. miyazakii, G. candidiensis* and *C. rathbuni,* belong to the diploid type.

It is interesting to note that the results of Fan and Khaw (1963), using metacercariae from *E. japonicus* to infect LE rats, were quite similar to those obtained with the diploid type of Japan. According to them sexually mature worms were observed in the pleural cavity and in the worm cysts in lungs of some rats. Multiple worm cysts were still seen 24 weeks after infection. The triploid type of Japan rarely mature in lungs of rats and most of the larvae remained in muscles for a long time without any development (Yokogawa *et al.*, 1962; Miyazaki, 1977; Kanazawa *et al.*, 1987).

It might be possible that *P. westermani* used by Fan and Khaw (1963) was the diploid type.

Recently, Yokogawa *et al.* (1986) found that the metacercariae obtained from *E. japonicus* in Okinawa Prefecture, Japan were the diploid type. This controversy would be solved by chromosomal studies on adult worms derived from the metacercariae from *E. japonicus* in Taiwan. Unfortunately, we were unable to find a single infected *E. japonicus* in the examination of more than 1000 crabs from the previously highly endemic area of paragonimiasis.

Miyazaki and Chiu (1980) noted that the diploid type in Taiwan may be the same with the diploid type in the Philippines, however, in our study, the pattern of migration and development of the diploid type in Taiwan was quite different from those of the diploid type of the Philippines.

It is well known that rat is a favorable final host for the diploid type in the Philippines (Cabrera and Vajrasthira, 1973; Yokogawa *et al.*, 1979; Miyazaki and Habe, 1979).

According to Yokogawa *et al.* (1979), most of the larvae of the diploid type in the Philippines rapidly disappear within 2–3 weeks after infection from the abdominal wall muscles and enter the lungs of rats. The worm cysts in lungs were observed in almost all rats and the worms reached sexual maturity at 8–10 weeks after infection.

On the other hand, in our study it has been proved that the larvae of the diploid type in Taiwan rarely mature in rats and the larvae remained in muscles for a long time without any development just like the diploid type of Japan.

In the experiment using mice, the average recovery rate of the diploid type of *P. westermani* in mice was slightly higher than those in rats as 61% vs. 53%. However, the range of recovery rate in mice was greater than those in rats as 20–100% vs. 42–66%. As in rats, most of the larvae recovered from mice were retarded in growth except for a few worms recovered in the pleural or abdominal cavity and worms were never found in lungs. Our results in mice agreed with that of Kanazawa *et al.* (1987) except that the worm recovery rate remained lower throughout the experiments.

From the results of this study and those of the studies by Yokogawa *et al.* (1962), Miyazaki (1977), Habe (1983), Shibahara (1984) and Kanazawa *et al.* (1987), it may be considered that rats and mice are unsuitable final hosts for the diploid type in Japan and Taiwan and the triploid type of Japan.

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References

1) Cabrera, B. D. and Vajrasthira, S. (1973): Endemicity of *Paragonimus siamensis* Miyazaki and Wykoff, 1965. The second species of lung fluke found in the Republic of the Philippines. Southeast Asian J. Trop. Med. Pub. Hlth., 4, 509–518.
2) Fan, P. C. and Khaw, O. K. (1963): Comparative studies on larval migration of *Paragonimus westermani* in various experimental animals. Part II. Migration of larval *Paragonimus westermani* in rats (Long-Evans). Chinese Med. J., 10, 284–296.
3) Habe, S. (1983): A newly recognized paratenic host of *Paragonimus* spp. Jpn. J. Trop. Med. Hyg., 11, 1–6.
4) Huang, W. H., Chiu, J. K., Lu, S. C. and Chen, H. H. (1966): Epidemiologic studies on *Paragonimus westermani* and related species in Taiwan. I. Distribution patterns of *P.westermani* metacercariae in *Eriocheir* crabs. J. Formosan Med. Assoc., 65, 141–152.
5) Kanazawa, T., Hata, H., Kojima, S. and Yokogawa, M. (1987): *Paragonimus westermani*: A comparative study on the migration route of the diploid and triploid types in the final hosts. Parasitol. Res., 73, 140–145.
6) Miyazaki, I. (1977): A newly introduced question on *Paragonimus westermani* (Kerbert, 1878). Nihon Iji Shinpo. 2788, 43–46. (in Japanese)
7) Miyazaki, I. (1978): Two types of the lung fluke which has been called *Paragonimus westermani* (Kerbert, 1878). Med. Bull. Fukuoka Univ., 5, 251–256.
8) Miyazaki, I. (1979): On the newly proposed lung fluke, *Paragonimus pulmonalis* (Baelz, 1880). Med. Bull. Fukuoka Univ., 6, 267–276.
9) Miyazaki, I. and Habe, S. (1979): *Paragonimus westermani filipinus* Miyazaki, 1978, stat. n. occurring at Jaro, Leyte, the Philippines. Med. Bull. Fukuoka Univ., 6, 447–462.

10) Miyazaki, I. and Chiu, J.K. (1980): Examination of the so-called *Paragonimus westermani* in Taiwan. Med. Bull. Fukuoka Univ., 7, 277–279.
11) Sakaguchi, Y. and Tada, I. (1976): Chromosome of lung fluke, *Paragonimus westermani*. Chrom. Inf. Serv., 20, 23–24.
12) Shibahara, T. (1984): Studies on the lung fluke, *Paragonimus westermani* diploid type in the northern part of Hyogo Prefecture, Japan. III. Experimental oral infection with metacercariae of rats, with reference to juvenile worms removed from muscle. Jpn. J. Parasitol., 33, 119–132.
13) Sugiyama, H., Kikuchi, T., Matsumoto, M. and Tomimura, T. (1985): The albino rat as an experimental paratenic host for *Paragonimus westermani* (Kerbert, 1878). (II). Jpn. J. Parasitol., 34 (Suppl.), 10.
14) Terasaki, K. (1977): Studies on chromosomes of the lung flukes in Japan. Jpn. J. Parasitol., 26, 333–339.
15) Tsuda, M. (1959): Biological studies on *Paragonimus westermani*. (2). On the resistance of the metacercariae of *Paragonimus westermani*. Jpn. J. Parasitol., 8, 812–821.
16) Yokogawa, M. (1982): Newly introduced questions concerning *Paragonimus westermani*. J. Formosan Med. Assoc., 81, 774–780.
17) Yokogawa, M., Yoshimura, H., Sano, M., Okura, T. and Tsuji, M. (1962): The route of migration of the larvae of *Paragonimus westermani* in the final hosts. J. Parasitol., 48, 525–531.
18) Yokogawa, M., Cabrera, B. D., Kobayashi, M., Hata, H., Tazaki, T. and Araki, K. (1979): Comparative studies on Philippine *Paragonimus westermani* and Japanese *Paragonimus westermani*. Acta Medica Philippina, 15, 119–131.
19) Yokogawa, M., Sato, Y., Hasegawa, H., Takai, A., Otsuru, M., Asato, R., Kanazawa, T. and Hata, H. (1986): Studies on *Paragonimus* in Okinawa, Japan. Jpn. J. Parasitol., 35, 331–338.