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Experimental infection of *Paragonimus westermani* in mice and rats

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Abstract: To determine the infectivity and maturity of metacercariae of *Paragonimus westermani* after keeping at low temperature for a long period, 45 mice and 45 rats were each infected with 20 metacercariae which were kept at 4°C for 8 to 234 days. The worm recovery in mice increased with age of worm and reached a peak of 32% at 41-50 days and then decreased with age. The rate in rats first decreased to a lowest point of 6% at 71-100 days and then increased with age. In 42 infected mice and 41 infected rats, 187 immature worms (183 tiny and 4 juvenile ones) and 190 worms (164 tiny, 19 juvenile and 7 mature ones) were recovered respectively. Two wormcysts with eggs only and 8 empty wormcysts were also found in the rats. In addition, the frozen metacercariae can still develop to mature worms in SD rats.

Key words: *Paragonimus westermani*, infectivity, albino mice, Sprague-Dawley rats

INTRODUCTION

In experimental infection with metacercariae of *Paragonimus westermani* to white rats (Long-Evans), Fan and Khaw (1963) found mature worms in the cysts of the lung 75 days post-infection. However, Ando (1917) and Miyazaki (1946) obtained a negative result in infecting Japanese rats using metacercariae of *P. westermani*. The main reason for this disagreement might be explained that the biological and physiological factors inherent in the various strains of Taiwan and Japanese animals.

Shimono and Hirono (1957) and Tsuda (1959) investigated the period spent by the metacercariae of *P. westermani* in water and the life span of the metacercariae in the second intermediate host, *E. japonicus*, after its death. Most of the metacercariae removed from the hosts in water at high temperature in summer died within 3-5 days. However, in winter, when the temperature of water decreased to 4-5°C, they survived for 10-20 days. They reported also that the metacercariae in *E. japonicus* continued to live for 3-4 weeks even after its death, as long as the crab was kept at 2-5°C.

Fan *et al.* (1990) reported that metacercariae of *P. westermani* can survive and remain infective after keeping at 4°C up to 560 days and even develop to the mature stage in cat. This duration is much longer than those reported by Shimono and Hirono (1957) and Tsuda (1959).

In order to further determine the infectivity of metacercariae of *P. westermani* keep at low temperature for a long period, we infected mice and rats experimentally. In addition, we also observed whether the larval *P. westermani* can

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develop to the mature stage in the rodents.

MATERIALS AND METHODS

1. Preparation of metacercariae of *Paragonimus westermani* and infection to the animals

The metacercariae of *P. westermani* were collected from *Cambaroides similis*, caught from streams in Kanghwa-gun, Kyonggi-do, Korea in June 1979. After removing from the crayfish, the metacercariae were placed into the screw-cap small vials which were half-filled with physiological saline. These vials were kept in a refrigerator at 4°C up to 234 days. Between the day 8 and day 234 after collection, the metacercariae were examined with stereoscopic microscope to determine whether they were alive or not. The metacercariae were then fed to Albino mice and Sprague-Dawley (SD) rats by a plastic stomach tube connected with a 10 ml-syringe.

2. Recovery of the worm

Between 21 to 201 days after infection, the rats and mice were sacrificed by capital dislocation. The pleural and abdominal cavities were first washed separately with physiological saline. Then the lungs, liver, kidneys, spleen, heart, brain, lymph nodes, diaphragm, esophagus, stomach, small and large intestines, and testes/ovaries were removed from the carcass. In addition, muscles of fore and hind legs, thoracic muscles and abdominal muscles were also isolated. These specimens were pressed with two glass plates and examined by steroscopic microscope for worms. From positive specimens, worms were removed. Their size, number, development and distribution were examined. Infection rates and worm recovery rates were also calculated.

RESULTS

1. Susceptibility and worm recovery of *P. westermani*

Each of 19 mice inoculated with 20 metacercariae 8 days after storage at 4°C, all of them were found to be infected. The infection rate was 100%. These mice were killed between 34 and 83 days post-infection and 101 immature worms were recovered (Worm recovery rate: 27%) (Table 1).

Of 26 mice each inoculated with 20 metacercariae 55 days after storage at 4°C, 23 were found to be infected (Infection rate: 89%). These mice were killed between 21 and 137 days post-infection and 86 immature worms were recovered (Worm recovery rate: 17%) (Table 1).

Each of 34 SD rats fed with 20 metacercariae 10 days after storage at 4°C, 31

Table 1. Susceptibility and worm recovery of *Paragonimus westermani* in rodents

	Matacerceriae		Rodent			Worm recovery		
Rodent	Days after storage	No. fed/ rodent	No. inocul.	No. (%) inf.	Age of worms (days)	No. imm.	No. mat.	Total (%)
Mice	8	20	19	19 (100)	34-83	101	0	101 (27)
	55	20	26	23 (89)	21-137	86	0	86 (17)
Rat	10	20	34	31 (91)	32-201	151	3(1)[a] (7)[e]	154 (23)
	234	20	11 (3)[b]	10 (91)	56-129	32	4(1)[c] (1)[d] (1)[e]	36 (16)

[a]One mature worm was found in the pleural cavity. [b]Three SD rats were fed with a total number of 60 metacercariae, which were accidentally frozen in a refrigerator (GE) 117 and 199 days after removal from crayfishes (*Cambaroides similis*) respectively, but the period of freezing time in each of two badly occurrings was uncertain. [c]One dead mature worm. [d]No. of wormcysts with eggs only. [e]No. of empty wormcysts.

were found to be infected (Infection rate: 91%). These rats were killed between 32 and 201 days after inoculation and 154 (151 immature and 3 mature) worms were recovered (Worm recovery rate: 23%) and seven empty wormcysts were also found (Table 1).

Of 11 SD rats each fed with 20 metacercariae 234 days after storage at 4°C, 10 were found to be infected (Infection rate: 91%). The 60 metacercariae inoculated to three rats were accidentally frozen twice between 117 and 199 days after removal from the crayfishes. However, the period of freezing time was uncertain. These rats were killed between 56 and 129 days after inoculation and 36 (32 immature and 4 mature) worms were recovered (Worm recovery rate: 16%) while one wormcyst with eggs only and one empty wormcyst were also found (Table 1).

2. Relationship between worm recovery rate and age of worms in mice

The infection rate of *P. westermani* to 45 albino mice were 93%. Except those sacrificed between 31 and 40 days after inoculation (75%), the infection rates were 100%. The worm recovery rate was 21%. The worm recovery rate increased from 10% in mice killed between 21 and 30 days post-infection to a peak of 32% in those killed between 41 and 50 days post-infection. It then decreased to 8% and 15% in the 71-100-day and 101-137-day after the infection respectively. All worms collected were immature (Table 2).

3. Relationship between worm recovery rate and age of worms in rats

The infection rate of *P. westermani* to 45 rats were 91%. Except those sacrificed between 41 and 50 days (60%) and between 51 to 70 days (50%) after inoculation, the infection rates in the remaining groups were 100%. The worm recovery rate was 21%. This rate decreased from 16% in rats killed between 41 and 50 days post-infection to 6% in those killed between 71 and 100 days post-infection and then increased to 33% in the 171-201-day group (Table 3).

4. Distribution pattern of *P. westermani* in mice

In 42 infected mice, 187 immature worms were recovered. The highest percentage of worms was recovered from the pleural cavity (29%). The percentages recovered from thoracic muscle, abdominal muscle, abdominal cavity, liver, fore leg muscle, hind leg muscle, lung, spleen and kidney were 24%, 16%, 12%, 6%, 4%, 4%, 3%, 1% and 1% respectively. Eighty-seven (47%) worms were found in the right side of mice. In addition, 112 (60%) worms located in the upper part (Table 4).

5. Distribution pattern of *P. westermani* in rats

In 41 infected rats, 190 worms were recovered. The worms were recovered from abdominal muscle (35%), thoracic muscle

Table 2. Worm recovery rate of *Paragonimus westermani* in albino mice by age of worms

Age of worms (days)	Mice		Metacercariae		No. of recovered worms (%)
	No. inocul.	No. (%) infect.	No. fed/ mouse	Total no.	
21-30	6	6 (100)	20	120	12 (10)
31-40	12	9 (75)	20	240	43 (18)
41-50	10	10 (100)	20	200	63 (32)
51-60	8	8 (100)	20	160	40 (25)
61-70	5	5 (100)	20	100	21 (21)
71-100	3	3 (100)	20	60	5 (8)
101-137	1	1 (100)	20	20	3 (15)
Total	45	42 (93)	20	900	187[a](21)

[a]All immature worms.

Table 3. Worm recovery rate of *Paragonimus westermani* in SD rats by age of worm

Age of worms (days)	SD rats		Metacercariae		
	No. inocul.	No. (%) infect.	No. fed/ mouse	Total no.	No. of recovered worms (%)
41-50	5	3 (60)	20	100	16 (16)
51-70	4	2 (50)	20	80	8 (10)
71-100	5	5 (100)	20	100	6 (6)
101-120	7	7 (100)	20	140	28 (3)[a](20)
121-140	7	7 (100)	20	140	30 (2)[a](21)
141-170	4	4 (100)	20	80	15 (19)
171-201	13	13 (100)	20	260	87 (2)[a](33)
Total	45	41 (91)	20	900	190 (7)[a](21)

[a]No. of mature worms in the parenthesis.

Table 4. Comparison of distribution pattern of *Paragonimus westermani* in albino mice and SD rats

Location	42 mice (187)[a]	41 rats (190)[b]	
	No. (%) of worms	No. of worms	%
Pleural cavity	55 (29)	27 (1)[d]	14
Abdominal cavity	22 (12)	0	0
Liver	12 (6)	0	0
Lung	5 (3)	16 (10[c]5[d],1[g]) (8)[f] (2)[e]	8
Spleen	2 (1)	0	0
Kidney	2 (1)	0	0
Fore leg muscle	8 (4)	17	9
Hind leg muscle	8 (4)	27	14
Thoracic muscle	44 (24)	37	19
Abdominal muscle	29 (16)	66	35
Right side	87 (47)	98	52
Left side	100 (53)	92	48
Upper part	112 (60)	97	51
Lower part	75 (40)	93	49

[a]All immature worms. [b]Total number of worms. [c]Immature worms. [d]No. of mature worms (each of two wormcysts with two worms, one wormcyst with one worm). [e]No. of wormcysts with eggs only. [f]No. of empty wormcysts. [g]One dead mature worm was found in a wormcyst.

(19%), pleural cavity (14%), hind leg muscle (14%), fore leg muscle (9%) and lung (8%). No worms were collected from abdominal cavity, liver, spleen and kidney. One of 27 worms recovered from the pleural cavity was mature. In the lung, 6 of 16 worms recovered were mature (each of 2 cysts with 2 worms and 2 cysts each with 1 worm), 2 wormcysts with eggs only and 8 empty wormcysts were also found. All of 147 worms recovered from the remaining parts were immature. In addition, 98 worms were found in the right side and 97 worms were located in the upper part (Table 4).

6. Comparison of size of *P. westermani* in mice and rats

Most (183) worms recovered from mice were under 1.0-2.0 mm x 0.2-0.5 mm. Only four (or 2%) worms were 2.1-4.0 mm × 0.5-1.0 mm. All worms were immature (Table 5).

Most (166) worms collected from rats were under 1.0-2.0 mm × 0.2-0.5 mm. Ten worms were 2.1-4.0 mm × 0.5-1.0 mm, seven worms were 4.1-6.0 mm × 1.0-2.0 mm and five mature worms were 6.1-8.0 mm × 2.0-3.0 mm. Two mature worms (one dead and one alive) each in a wormcyst were 8.1-10.0 mm × 3.0-5.0 mm (Fig. 1).

DISCUSSION

In 1963, we infected rats (Long-Evans) with *P. westermani* and obtained an infection rate of 62.6% and a worm recovery rate of 13.6% (Fan and Khaw, 1963). In the present study, we obtained an infection rate of 100% among 19 albino mice infected with metacercariae 8 days after keeping at 4°C and a rate of 89% among 26 with metacercariae 55 days after keeping at 4°C. These findings did not agree with the results of Miyazaki (1946) who reported that it was difficult to infect mice with metacercariae of *P. westermani*. However, our finding that no mature worms were recovered is agreed partly with those reported by Ando (1917 & 1920) and Miyazaki (1946).

P. westermani does not become mature in the mouse and most worms in the rat are also immature. However, the rodents can act as paratenic hosts for this parasite. The parasite can be transferred to large carnivorous hosts and becomes mature. We have reported the results of a preliminary experimental infection

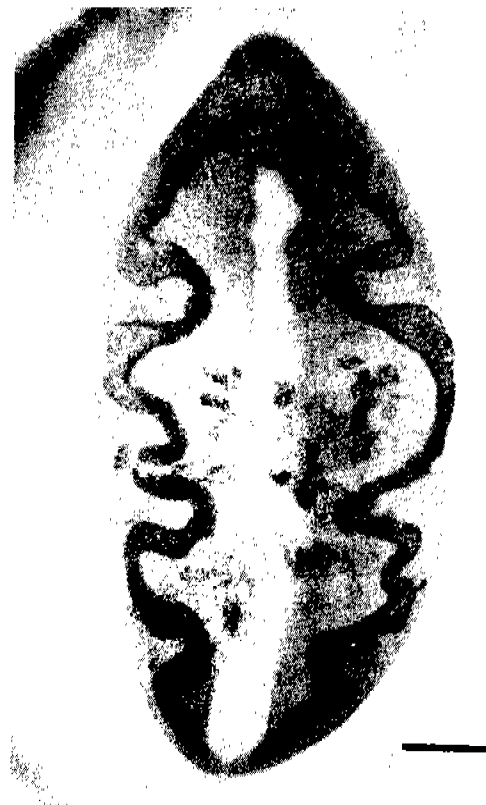

Fig. 1. A 123 days old adult worm of *P. westermani* in a lungcyst of the left upper lobe of a SD rat (Bar = 1 mm).

Table 5. Comparison of size of *Paragonimus westermani* in albino mice and SD rats

Size (LxW, mm)	Mice			Rats		
	No. imm.	No. mat.	Total no.	No. imm.	No. mat.	Total no.
1.0-2.0 × 0.2-0.5	183	0	183	166	0	166
2.1-4.0 × 0.5-1.0	4	0	4	10	0	10
4.1-6.0 × 1.0-2.0				7	0	7
6.1-8.0 × 2.0-3.0					5[a]	5
8.1-10.0 × 3.0-5.0					2(1)[b]	2
Total	187	0	187	183	7	190

[a]Each of 2 wormcysts with 2 worms and 1 wormcyst with 1 worm. [b]One dead mature worm in a wormcyst, the other in pleural cavity.

on 8 cats and 2 dogs with 191 metacercariae of *P. westermani* and later with 47 carcasses of infected rats (with 30 metacercariae each) without the lungs. We recovered 201 more worms than we fed to the domestic carnivorous animals (Fan and Khaw, 1964). Later, we confirmed our preliminary findings and obtained an infection rate of 53.9% among 26 cats fed with carcasses of infected rats containing 3-week-old-worms (Fan and Khaw, 1965a). In addition, rat-harbored *P. westermani* juvenile worms of all ages were infective to cats. This findings give evidence to the fact that large carnivorous can acquire *Paragonimus* infection by devouring smaller paratenic hosts of *P. westermani* (Fan and Khaw, 1965b).

Yokogawa *et al.* (1958 & 1959) reported that on autopsy 1-3 hours after feeding rats with metacercariae of *P. westermani*, 50-60% of the excysted larvae were found in the abdominal cavity. But, the number of the parasite collected two weeks after infection was reported to be decreased tremendously. Our finding agrees with Yokogawa *et al.* (1958 & 1959), since we obtained low worm recovery rates both in mice (17% and 27%) and in rats (23% and 15%). In addition, we found that the pattern in the variation of the worm recovery rates in mice and rats were different. In mice, the worm recovery increased with age of worm and reached a peak at 41-50 days and then decrease with age while the rate in rats decreased to a lowest point at 71-100 days and then increased with age.

We have demonstrated previously that different parts of carcasses of rats had different infection rates to cats (abdominal muscles 41.6%, thoracic muscles 40.6%, hind leg muscles 13.9%, lung and liver 2.0%) (Fan and Khaw, 1965a). In the present study, we also found that the distribution of the juvenile worms in mice was nearly throughout the body and that in rats was many parts of the body.

Although the development in size of *P. westermani* in the definite host varies depending upon the kind of the host, physiological conditions and the number of parasites, even the age of host (Yokogawa, 1965), we found that all mature worms in rats were over 7.0 mm in length. Since the worms in mice were not growing to 5.0 mm long, therefore, no mature worms were found in mice. Instead, 7 mature worms and 2 wormcysts with eggs only were found in 6 rats. In the former, one of them was observed in the pleural cavity. Each of 2 wormcysts with 2 worms and 2 worms each in a wormcyst were also found. These results also confirmed our previous reports that *P. westermani* could develop to mature stage either in outside of lung or a single worm in a wormcyst (Fan and Chiang, 1970).

In the present study three SD rats were fed with 20 metacercariae each, which were accidentally frozen in a refrigerator 117 and 199 days after removal. However, two mature worms (one in pleural cavity and one in a wormcyst) and several immature worms were found in the two infected rats. This result suggested strongly that the metacercariae of *P. westermani* either remaining in the dead crabs or outside the crabs which frozen during winter in the endemic areas of northeastern part of China, some of the metacercariae are still alive and matured if eaten by wild or domestic mammals.

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