

EXPERIMENTAL STUDIES ON MIXED INFECTION WITH
PARAGONIMUS MIYAZAKII KAMO ET AL., 1961 AND
OTHER SPECIES OF *PARAGONIMUS* IN AN ANIMAL

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Abstract

The mixed infection with *Paragonimus miyazakii* and *P. ohirai* and/or *P. westermani* to an experimental animal was designed to study the mutual relationship on the subjects of the susceptibility and the biological affinity as well as the host adaptation between different species of *Paragonimus*. The experimental animals of 3 cats, an albino rat and 13 dogs were fed on the metacercariae of these 2 or 3 species of *Paragonimus* simultaneously or at intervals of 5 to 20 days. The cohabitation of two species (*P. miyazakii* and *P. ohirai*) in a worm cyst was revealed in 5 animals of 2 cats, an albino rat and 2 dogs, whereas that of the other cohabitation with *P. miyazakii* and *P. westermani* was revealed only with one dog in spite of experiment on 9 animals. The cohabitation with *P. ohirai* and *P. westermani* was also shown, though only in one trial. The worm cyst revealing the cohabitation of two species amounted to 1 or 2 in number regardless of the number of metacercariae given in each experimental animal. No delay in development and maturity was recognized in 2 worms of different species or 3 worms of the same species of *P. miyazakii* and *P. ohirai* cohabiting in a cyst of each animal. In the case of 3 worms of different species cohabitation in a cyst the size of a worm belonging to one species was somewhat smaller than that of the other two worms belonging to the other species. The solitary worm of *P. miyazakii*, *P. ohirai* or *P. westermani* in a cyst also developed and matured as full as the cohabitants.

INTRODUCTION

The lung flukes of the genus *Paragonimus* are parasitic in the lung of man and mammals, and causing paragonimiasis among them. Although the genus *Paragonimus* hitherto reported in literature amounts about forty species in the world, of which five species of *Paragonimus* are distributed in Japan

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such as *Paragonimus westermani* (Kerbert, 1878), *P. ohirai* Miyazaki, 1939, *P. iloktsuenensis* Chen, 1940, *P. miyazakii* Kamo, Nishida, Hatsushika et Tomimura, 1961 and *P. sadoensis* Miyazaki, Kawashima, Hamajima et Otsuru, 1968. Recently, the taxonomical revival of the species *Paragonimus pulmonalis* (Baelz, 1880) has become a theme of some researchers in Japan, and the biological or ecological problems of *P. pulmonalis* are being cautiously discussed.

In the course of the investigations on the biological aspects of *P. miyazakii*, mixed infection of different species of *Paragonimus* in an experimental animal have been attempted to make clear the biological affinity among several species of *Paragonimus* and the host adaptation as well as the host-*Paragonimus* relations by the present author. Previously, the authors¹⁾ reported that a frequent occurrence of the cohabitation of two species, *P. miyazakii* and *P. ohirai* in a worm cyst of the lung of experimental animals, but such a cohabitation was not observed in other species of *P. miyazakii* and *P. westermani*. Later, the additional experiments on mixed infection with *P. miyazakii* and *P. westermani* were conducted for the same purposes. The present author has conducted the studies on mixed infection of *Paragonimus* in the past several years, and reports here the results obtained so far, though they do not yet give definitive answer.

MATERIALS AND METHODS

The metacercariae of three species of *P. miyazakii*, *P. ohirai* and *P. westermani* were used for the experimental infections. The metacercariae of *P. miyazakii* were removed from the fresh water crabs, *Potamon dehaani* collected at Rokuroshi, Iwakuni City, Yamaguchi Prefecture. The metacercariae of *P. ohirai* and *P. westermani* were also removed from the fresh and the brackish water crabs, *Sesarma dehaani* and *Eriocheir japonicus* respectively, and the former were collected at the Maruyama River, Hyogo Prefecture, and the latter were collected at the Shimanto River, Ehime Prefecture.

Three cats, an albino rat and 13 dogs were used for the experiments, and these animals received orally 8 to 20 metacercariae which were mixed with *P. miyazakii* and *P. westermani* and/or *P. ohirai*. Each of the animals was fed on the mixed metacercariae simultaneously or at 5 to 20 days intervals, and all the animals were sacrificed for autopsy within the period of 67 to 468 days after ingestion. The fecal examinations were made every two days by means of MGL sedimentation until the eggs began to appear in the feces after ingestion. The worms recovered from the animals were fixed in 70% alcohol and stained with Delafield's hematoxylin, and examined microscopically for the morphological and developmental observations.

RESULTS

1. Mixed infection of *P. miyazakii* and *P. westermani*

The experiments of mixed infection with *P. miyazakii* and *P. westermani* were carried out on 9 animals (Cat No. 1, Dog Nos. 1 and 7 to 13) as shown in Table I. The cohabitation of two species in a cyst of the lung was revealed only in one animal of Dog No. 9 which was fed on 7 metacercariae each of these two species simultaneously, and the typical eggs in feces were found on the 55th day after ingestion. The animal was sacrificed for autopsy at 112th day after ingestion and 12 mature worms were recovered from 6 cysts of the lung (Table II). The recovery rate of the worms in Dog No. 9 was 100% in *P. miyazakii* and 72% in *P. westermani* respectively (Table I). The occurrence of the cohabitation of two species in a cyst of Dog No. 9 was revealed in the right lower lobe of the lung (Fig. 13).

In the rest of 8 animals (Cat No. 1, Dog Nos. 1, 7, 8, 10, 11, 12 and 13) the cohabitation of two species in a cyst did not occur.

2. Mixed infection of *P. miyazakii* and *P. ohirai*

The experiments of mixed infection with *P. miyazakii* and *P. ohirai* were carried out on 7 animals (Cat Nos. 2 and 3, an Albino rat and Dog Nos. 2 to 5) as shown in Table I. The cohabitation of two species in a cyst was revealed in 5 animals (Cat Nos. 2 and 3, Albino rat and Dog Nos. 2 and 3). The recovery rates and the state of cohabitations of two species in a cyst of these animals were shown in Table I and Figures.

Cat No. 2 was fed on 6 metacercariae of *P. miyazakii* and 2 metacercariae of *P. ohirai* simultaneously, and the cohabitation of two species in a cyst was revealed in the left lower lobe of the lung (Fig. 2). The recovery rate of the worms in Cat No. 2 was 50% in *P. miyazakii* and 100% in *P. ohirai* including a worm recovered from the pleural cavity (Table I).

Cat No. 3 was fed on 15 metacercariae of *P. ohirai* exactly 5 days after 15 metacercariae of *P. miyazakii* were given, and the cohabitation of two species in a cyst was revealed in the left lower lobe and in the right middle lobe of the lung (Fig. 3). The worms recovered from the cyst of the left lower lobe revealed the cohabitation with 2 worms of *P. ohirai* and a worm of *P. miyazakii*. The recovery rate of the worms in Cat No. 3 was 74% in *P. miyazakii* and 47% in *P. ohirai* (Table I).

Albino rat was fed on 6 metacercariae each of *P. miyazakii* and *P. ohirai* exactly at 15-day intervals in much the same way as Cat No. 3, and the cohabitation of two species in a cyst was revealed in the cardiac lobe of the lung (Fig. 4). The recovery rate of the worms in Albino rat was 17% in *P. miyazakii* and 84% in *P. ohirai* including a worm recovered from the pleural

TABLE I. Mixed infection in an experimental animal with *Paragonimus miyazakii* and other species of *Paragonimus*

Animals	Species and No. of metacercariae given	Time lag in meta-cercariae given	Appearance of eggs in feces (days after ingestion)	Cohabitation of different species in a cyst	No. of worm recovered(%)
Cat No. 1	{P. m 5 P. w 5}	simultaneously	87	-	P. m 4 (80) P. w 4 (80)
Cat No. 2	{P. m 6 P. o 2}	simultaneously	43	+	P. m 3 (50) P. o 2 (100)*
Cat No. 3	{P. m 15 P. o 15}	5 days later, P. o given	49	+	P. m 11 (74) P. o 7 (47)
Albino rat	{P. m 6 P. o 6}	15 days later, P. o given	51	+	P. m 1 (17) P. o 5 (84)*
Dog No. 1	{P. m 16 P. w 14}	simultaneously	52	-	P. m 4 (25) P. w 10 (72)
Dog No. 2	{P. m 10 P. o 10}	simultaneously	46	+	P. m 10 (100) P. o 7 (70)
Dog No. 3	{P. m 10 P. o 10}	5 days later, P. o given	50	+	P. m 3 (30) P. o 5 (50)
Dog No. 4	{P. m 10 P. o 10}	10 days later, P. o given	58	-	P. m 4 (40) P. o 3 (30)
Dog No. 5	{P. m 10 P. o 10}	15 days later, P. o given	63	-	P. m 5 (50) P. o 10 (100)
Dog No. 6**	{P. o 12 P. m 12 P. w 12}	15 days later, P. m given		+	P. o 4 (33) P. m 3 (25) P. w 3 (25)
Dog No. 7	{P. w 8 P. m 24}	10 days later, P. m given	59	-	P. w 8 (100) P. m 9 (38)
Dog No. 8	{P. m 6 P. w 6}	simultaneously	65	-	P. m 4 (67) P. w 6 (100)
Dog No. 9	{P. m 7 P. w 7}	simultaneously	55	+	P. m 7 (100) P. w 5 (72)
Dog No. 10	{P. m 8 P. w 8}	simultaneously	61	-	P. m 3 (38)* P. w 6 (75)
Dog No. 11	{P. m 6 P. m 6}	20 days later, P. m given	63	-	P. m 5 (83) P. m 3 (50)*
Dog No. 12	{P. m 6 P. w 6}	simultaneously	71	-	P. m 4 (67) P. w 4 (67)
Dog No. 13	{P. m 6 P. w 6}	simultaneously	57	-	P. m 4 (67) P. w 6 (100)

* An adult worm recovered from pleural cavity.

** The dog had been infected with *P. westermani* for 395 days before other two species were given.P. m = *Paragonimus miyazakii*, P. o = *Paragonimus ohnari*, P. w = *Paragonimus westermani*.

cavity (Table I).

TABLE II. Measurements of the worms recovered from the experimental animals

Animals	No. of worms examined	Days from infection to autopsy	Size of worms (in mm)		
			length	× width	average
Cat No. 1	$\begin{cases} \text{P. m} & 4 \\ \text{P. w} & 4 \end{cases}$	115	6.5-11.0	× 3.0-5.0	8.3 × 3.9
			11.0-13.0	× 6.0-7.0	11.5 × 6.8
Cat No. 2	$\begin{cases} \text{P. m} & 3 \\ \text{P. o} & 2 \end{cases}$	103	9.0-10.5	× 4.0-5.0	10.1 × 4.7
			8.0-11.0	× 4.0-5.5	9.5 × 4.8
Cat No. 3	$\begin{cases} \text{P. m} & 11 \\ \text{P. o} & 7 \end{cases}$	72	6.5-9.5	× 2.5-4.5	8.3 × 3.5
			7.0-16.8	× 2.0-6.0	11.2 × 4.8
Albino rat	$\begin{cases} \text{P. m} & 1 \\ \text{P. o} & 5 \end{cases}$	67			7.0 × 2.5
			7.5-11.0	× 4.0-5.0	8.9 × 4.4
Dog No. 1	$\begin{cases} \text{P. m} & 4 \\ \text{P. w} & 10 \end{cases}$	468	14.5-16.5	× 6.7-7.0	15.4 × 6.9
			15.0-16.3	× 8.2-9.5	15.7 × 8.8
Dog No. 2	$\begin{cases} \text{P. m} & 10 \\ \text{P. o} & 7 \end{cases}$	133	7.6-13.0	× 3.5-6.8	11.2 × 5.3
			7.8-12.2	× 4.5-7.0	10.8 × 6.3
Dog No. 3	$\begin{cases} \text{P. m} & 3 \\ \text{P. o} & 5 \end{cases}$	203	12.0-14.8	× 5.3-7.2	13.3 × 6.4
			10.0-13.0	× 4.8-6.4	11.5 × 5.5
Dog No. 4	$\begin{cases} \text{P. m} & 4 \\ \text{P. o} & 3 \end{cases}$	203	12.6-15.2	× 5.2-5.8	13.7 × 5.5
			10.0-13.8	× 5.7-6.1	12.3 × 5.9
Dog No. 5	$\begin{cases} \text{P. m} & 5 \\ \text{P. o} & 10 \end{cases}$	222	11.6-14.2	× 5.2-6.3	13.2 × 5.8
			11.0-13.5	× 5.7-6.2	12.6 × 5.9
Dog No. 6	$\begin{cases} \text{P. o} & 4 \\ \text{P. m} & 3 \\ \text{P. w} & 3 \end{cases}$	205	10.2-12.0	× 4.8-5.8	11.1 × 5.3
			11.0-12.5	× 5.5-5.7	12.0 × 5.7
			12.3-15.8	× 6.8-8.8	14.5 × 7.7
Dog No. 7	$\begin{cases} \text{P. w} & 8 \\ \text{P. m} & 9 \end{cases}$	113	8.0-10.0	× 4.2-5.5	9.3 × 4.9
			6.5-15.5	× 3.5-6.5	11.4 × 5.1
Dog No. 8	$\begin{cases} \text{P. m} & 4 \\ \text{P. w} & 6 \end{cases}$	112	10.5-11.5	× 4.8-5.5	11.0 × 5.1
			9.0-11.2	× 4.0-6.7	10.0 × 5.2
Dog No. 9	$\begin{cases} \text{P. m} & 7 \\ \text{P. w} & 5 \end{cases}$	112	10.0-13.0	× 4.2-5.4	11.2 × 4.8
			9.3-13.0	× 5.0-6.7	11.2 × 5.8
Dog No. 10	$\begin{cases} \text{P. m} & 3 \\ \text{P. w} & 6 \end{cases}$	109	5.0-11.3	× 2.7-5.8	9.1 × 4.4
			9.0-12.0	× 4.8-6.5	10.6 × 5.5
Dog No. 11	$\begin{cases} \text{P. w} & 5 \\ \text{P. m} & 3 \end{cases}$	112	10.0-12.0	× 5.0-6.5	10.8 × 5.7
			8.0-10.0	× 3.7-5.0	9.3 × 4.5
Dog No. 12	$\begin{cases} \text{P. m} & 4 \\ \text{P. w} & 4 \end{cases}$	111	11.0-14.0	× 4.5-6.0	12.3 × 5.3
			11.0-13.0	× 5.7-6.5	12.1 × 6.1
Dog No. 13	$\begin{cases} \text{P. m} & 4 \\ \text{P. w} & 4 \end{cases}$	112	11.5-12.0	× 4.5-5.7	11.6 × 5.0
			9.0-12.5	× 4.8-6.3	10.8 × 5.6

P. m=*Paragonimus miyazakii*, P. w=*Paragonimus westermani*, P. o=*Paragonimus ohirai*.

Dog Nos. 2 and 3 were fed on 10 metacercariae each of *P. miyazakii* and *P. ohirai* simultaneously or intervals of at 5 days. In Dog No. 2, the cohabitation of two species in a cyst was revealed in two cysts of the right middle and lower lobes of the lung (Fig. 6). The worms recovered from the right middle lobe revealed the cohabitation of a worm each of *P. miyazakii* and *P. ohirai*, and 2 worms of *P. ohirai* and a worm of *P. miyazakii* were

also recovered from a cyst of the right lower lobe. The recovery rate of the worms in Dog No. 2 was 100% in *P. miyazakii* and 70% in *P. ohirai* respectively (Table I). In Dog No. 3, on the other hand, the cohabitation of two species in a cyst was revealed in the right middle lobe of the lung, and 2 worms of *P. miyazakii* and a worm of *P. ohirai* were recovered from the cyst (Fig. 7). The recovery rate of the worms in Dog No. 3 was 30% in *P. miyazakii* and 50% in *P. ohirai* (Table I).

TABLE III. Measurements of worms cohabited in a worm cyst of each experimental animal

Animals	Species	Size of worms (in mm)	
		length	width
Cat No. 2	{P. m	8.5	4.0
	{P. o	11.0	5.5
Cat No. 3	{P. m	6.5	2.5
	{P. o	7.0	2.0
Albino rat	{P. m	9.0	4.0
	{P. o	16.8	5.5
	{P. o	13.5	6.0
	{P. m	7.0	2.5
	{P. m	8.0	3.0
	{P. m	7.0	2.5
Albino rat	{P. m	7.0	2.5
	{P. o	7.5	4.0
Dog No. 2	{P. m	7.6	3.5
	{P. o	11.0	7.0
	{P. o	11.0	6.5
Dog No. 3	{P. m	11.0	5.0
	{P. o	11.0	5.5
	{P. m	14.8	7.0
	{P. m	13.2	5.5
	{P. o	10.0	4.8
	{P. o	12.5	6.4
Dog No. 4	{P. o	11.0	5.5
	{P. o	11.0	4.8
	{P. o	11.0	4.8
Dog No. 4	{P. o	13.8	6.1
	{P. o	13.3	5.7
	{P. o	10.0	5.7
Dog No. 6	{P. m	12.5	5.5
	{P. o	12.0	5.0
Dog No. 6	{P. w	12.3	6.8
	{P. o	10.2	5.5
	{P. o	10.2	5.5
Dog No. 9	{P. m	13.0	5.4
	{P. w	11.0	5.8

P. m = *Paragonimus miyazakii*.

P. o = *Paragonimus ohirai*.

P. w = *Paragonimus westermani*.

TABLE IV. Measurements of the solitary worms in a worm cyst recovered from each experimental animal

Animals	Species of worms	Distribution of cysts in the lung	Size of worms (in mm)	
			length	width
Albino rat	P. o	right lower lobe	9.0	4.5
Dog No. 3	P. m	right upper lobe	12.7	5.4
	P. o	left lower lobe	11.6	5.3
Dog No. 5	P. o	left lower lobe	12.0	5.7
Dog No. 7	P. m	cardiac lobe	6.7	3.7
Dog No. 11	P. w	left lower lobe	11.2	5.8

P. o = *Paragonimus ohirai*.

P. m = *Paragonimus miyazakii*.

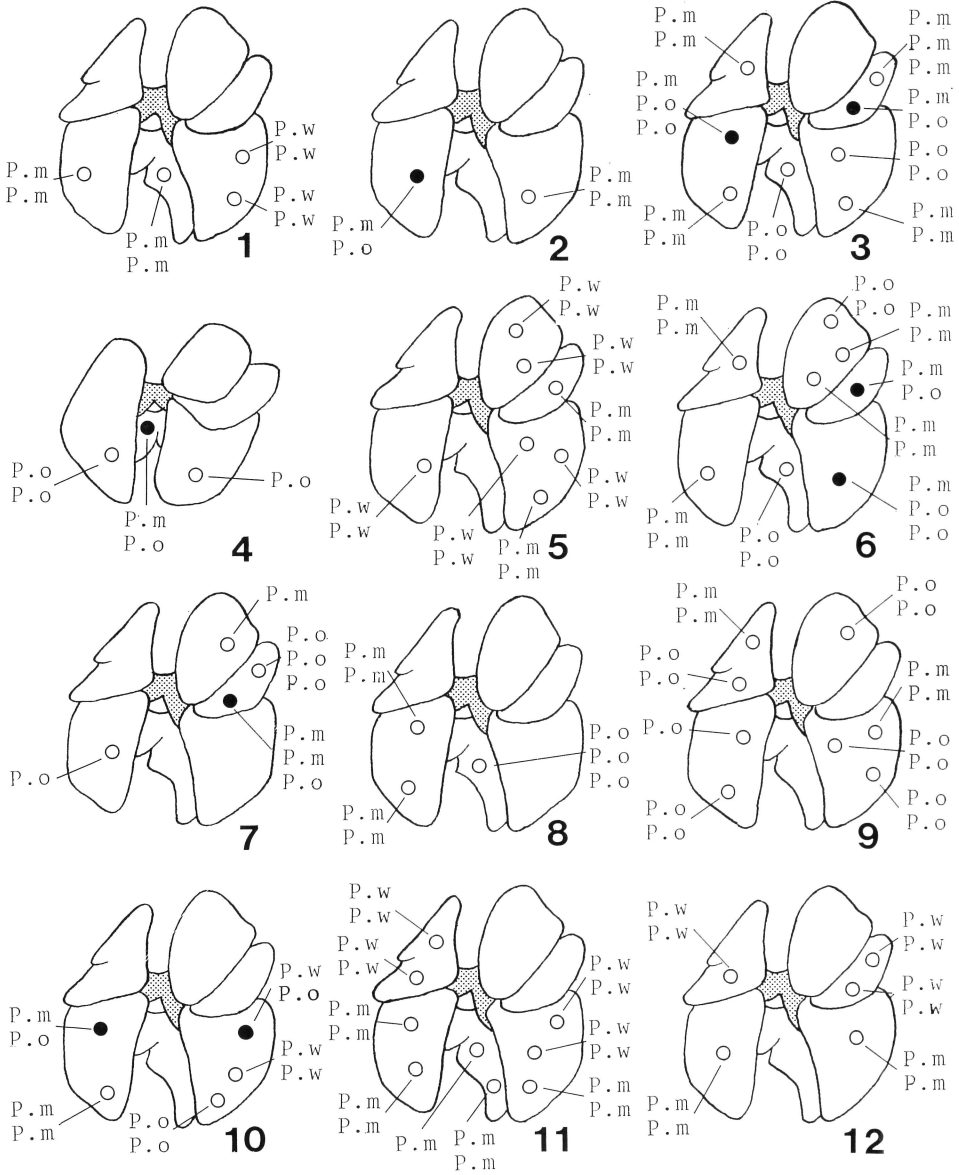
P. w = *Paragonimus westermani*.

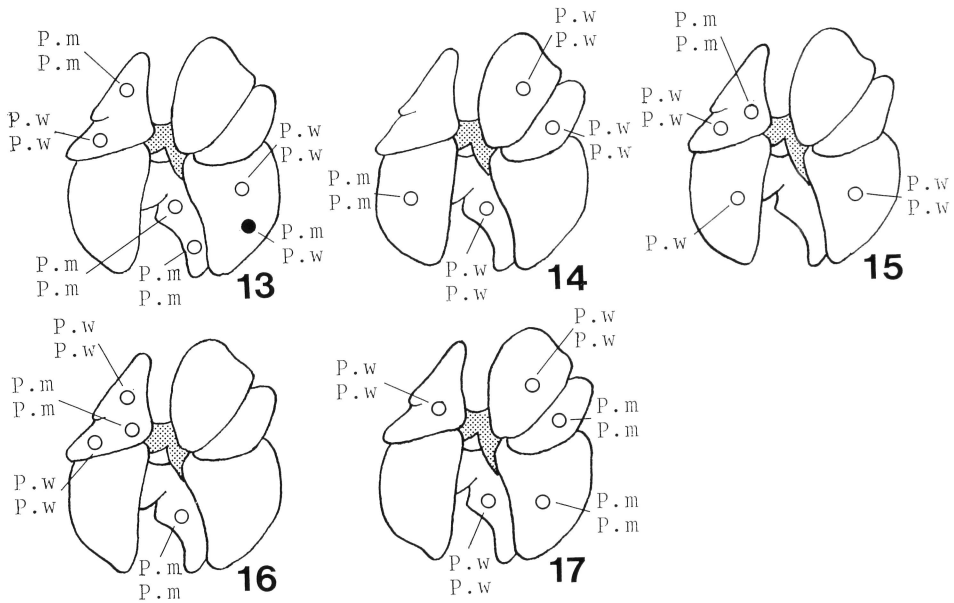
3. Mixed infection of three species of *P. miyazakii*, *P. ohirai* and *P. westermani*

The experiment of mixed infection of three species of *P. miyazakii*, *P. ohirai* and *P. westermani* was carried out only with one animal of Dog No. 6. Dog No. 6 had been infected with *P. westermani* for 395 days before the animal was used for the present experiment. The animal was fed on 12 metacercariae of *P. miyazakii* exactly 15 days after 12 metacercariae of *P. ohirai* were given (Table I). The cohabitation of different species in a cyst was revealed in the left lower lobe and in the right lower lobe of the lung. The worms recovered from the cyst of the left lower lobe revealed the cohabitation of a worm each of *P. miyazakii* and *P. ohirai*, and a worm each of *P. ohirai* and *P. westermani* were also recovered from the cyst of the right lower lobe (Fig. 10). The recovery rate of the worms in Dog No. 6 was 25% in *P. miyazakii* and 33% in *P. ohirai* though the recovery rate of the worm of *P. westermani* could not be obtained on account of the full number of the ingested metacercariae being not known.

4. Development and maturity of the worm cohabitation in a cyst

Measurements of the worms which cohabited in a cyst of each experimental animal were shown in Table III. Two worms of the cohabitation of different species in a cyst show little difference in size except for 2 worms of the cohabitation with *P. miyazakii* and *P. ohirai* in Cat No. 2, *P. westermani* and *P. ohirai* in Dog No. 6, or *P. miyazakii* and *P. westermani* in Dog No. 9. However, 3 worms of the cohabitation of different species in a cyst show marked differences in the development and maturity of worms. In the cases of 3 worms belonging to the same species in a cyst, no developmental delay in the individual worms was recognized (Cat No. 3 and Dog Nos. 3 and 4), but





Figs. 1-17. Diagram of lungs, showing distribution of worm cysts in each experimental animal (dorsal view)

Fig. 1. Cat No. 1 ; Fig. 2. Cat No. 2 ; Fig. 3. Cat No. 3 ; Fig. 4. Albino rat ;
 Fig. 5. Dog No. 1 ; Fig. 6. Dog No. 2 ; Fig. 7. Dog No. 3 ; Fig. 8. Dog No. 4 ;
 Fig. 9. Dog No. 5 ; Fig. 10. Dog No. 6 ; Fig. 11. Dog No. 7 ; Fig. 12. Dog No. 8 ;
 Fig. 13. Dog No. 9 ; Fig. 14. Dog No. 10 ; Fig. 15. Dog No. 11 ; Fig. 16. Dog No. 12 ;
 Fig. 17. Dog No. 13.

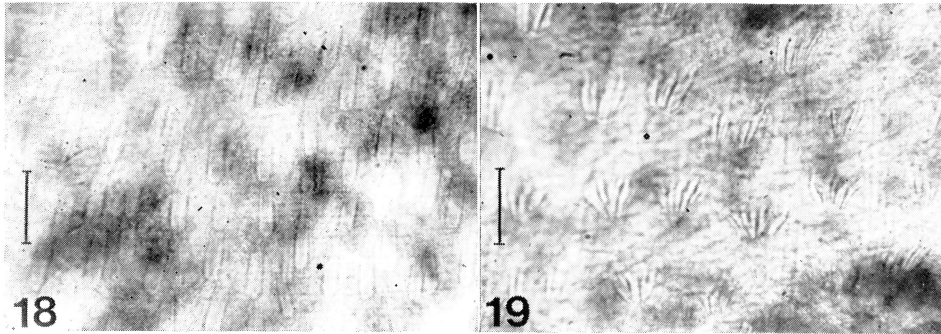
P. m = *Paragonimus miyazakii*.

P. o = *Paragonimus ohirai*.

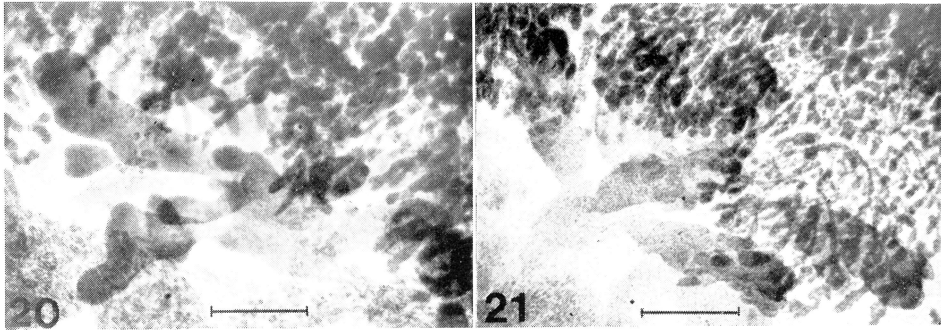
P. w = *Paragonimus westermani*.

in the other cohabitation with 2 worms belonging to one species and a worm belonging to the other species the size of 2 worms of one species was conspicuously larger than that of a worm of the other species (Cat No. 3 and Dog Nos. 2 and 3). Especially, in the case of the cohabitation with 2 worms of *P. ohirai* and a worm of *P. miyazakii* in a cyst (Cat No. 3 and Dog No. 2), the developmental delay of the worms was clearly noticed in *P. miyazakii* as is evident from Table III.

The solitary worm found in a cyst of each experimental animal totaled 6 (Figs. 4, 7, 9, 11 and 15), and there are little difference in size between the solitary worm and the other worms recovered from the cysts of the same animal. As shown in Table IV, the size of the solitary worms in a cyst from each experimental animal showed results approximately similar to these of the other



Figs. 18-19. Cuticular spines of *P. miyazakii* (18) and *P. ohirai* (19), cohabitants in a worm cyst of Cat No. 2 (Scales=0.05mm).



Figs. 20-21. Ovaries of *P. miyazakii* (20) and *P. westermani* (21) from a worm cyst of Dog No. 9 (Scales=0.5mm).

worms from the same animal except for a worm of *P. miyazakii* from Dog No. 7. The morphological features of the cuticular spines and ovary in each worm from the present experimental animals maintained the typical characteristics, as manifested by the adult worms of each species of *Paragonimus* (Figs. 18 - 21). Four worms from the pleural cavity of 4 animals (Cat No. 2, Albino rat and Dog Nos. 10 and 11) in the present experiments, however, did not reach its full sexual development.

DISCUSSION

The present investigations aroused a great interest in a phenomenon of the cohabitation with *P. miyazakii* and the other species of *Paragonimus* in a cyst of an experimental animal. At the instances observed the cohabitation with *P. miyazakii* and *P. ohirai* in a cyst of the lung occurred frequently in cats and dogs, but it is predicted that such a cohabitation with *P. miyazakii* and *P. westermani* in a cyst is very low. Habe *et al.*²⁾ reported a case of

mixed infection with *P. miyazakii* and *P. westermanni* in a naturally infected cat captured at Amakusa, Kumamoto Prefecture, but the cohabitation of these two species in a cyst was not found.

The cohabitation with *P. miyazakii* and *P. ohirai* in a cyst is frequent phenomena in rats as reported by Araki *et al.*³⁾ and Hashiguchi⁴⁾, but the aggregate number of cysts which cohabited these two species per animal is one or a few irrespective of the number of metacercarial ingestion.

The time lag of the metacercarial ingestion to the experimental animals was designed on the models of the time required for the larval migration of *Paragonimus* in each animal. Yokogawa *et al.*⁵⁾ have reported that the worms of *P. miyazakii* in infected kittens and rats penetrate into the lung about 35 days after ingestion. Miyazaki⁶⁾ has also reported that the worms of *P. ohirai* in such animals as dogs, cats, and rats penetrate into the lung about 30 days after ingestion. Therefore, in ingesting of the metacercariae to each animal in the present experiments, a consideration was given to the time of the larval migration of three species of *Paragonimus* in each animal.

The cohabitation with *P. miyazakii* and *P. ohirai* in a cyst was observed in 6 animals such as 2 cats, an albino rat and 3 dogs (Table III), but for the occurrence the cohabitation of these two species in an animal a consideration must be given to the fact that there is no effect of the time lag of the metacercarial ingestion. The facts obtained from the experimental animals (Cat Nos. 2 and 3, Albino rat and Dog Nos. 2 and 3) indicate decidedly that the meeting between a worm of one species and one of the other species before they have penetrated into the lung is not necessary for the cohabitation of two species in a cyst. It has been suggested by Yokogawa *et al.*⁷⁾ that a young worm of *Paragonimus* migrating in the definitive host seems to penetrate into the lung together with another young one, and in which a pair of worms seem to undergo a rapid sexual development after a cyst formation. Although this statement by Yokogawa *et al.*⁷⁾ seems to indicate a part of the inevitable processes for the sexual maturity of the worms of *Paragonimus*, it can be considered that the worm of belated arrival at the lung of host may penetrate into the cyst where a single worm or two worms had already inhabited as shown in the results of Dog No. 6.

The cohabitation with *P. miyazakii* and *P. westermanni* in a cyst seems to hardly occur as compared with the cohabitation of *P. ohirai* and *P. miyazakii*. As reported by Yokogawa *et al.*^{5,8,9)} and Okura¹⁰⁾, there are considerable differences in the route of worm migration arising from the combination with the animal host and the species of *Paragonimus* in mixed infection. From all considerations, more detailed examinations on the cohabitation with

P. miyazakii and *P. westermani* seem to be necessary.

As shown in Table III, a definite delay in development of the worms of *P. miyazakii* in Cat No. 3 and Albino rat seems to be quite all reasonable to consider that each experimental animal was sacrificed for autopsy in a brief period of time such as 72 days in Cat No. 3 and 67 days in Albino rat after ingestion, though a large majority of the worms from the experimental animals reached its similar developmental stage without apparent connection with the species of *Paragonimus*. In the cases of the cohabitation of a pair of the worms of *P. miyazakii* and *P. ohirai* in a cyst (Cat No. 2, Albino rat and Dog Nos. 2 and 6), the worms of two species were roughly equal in size except that the size of the worm of *P. miyazakii* which cohabited with *P. ohirai* in a cyst of Cat No. 2 was much smaller than that of the worm of *P. ohirai* as is evident from Table III. In the case of 3-worm cohabitation in a cyst, on the other hand, the size of the worms was marked by a noticeable difference. Three worms of the same species cohabitation in a cyst (Cat No. 3 and Dog Nos. 3 and 4) were substantially the same in size, but in the case of 3 worms of different species cohabitation in a cyst (Cat No. 3 and Dog Nos. 2 and 3) the size of a worm of one species was somewhat smaller than that of 2 worms of the other species as shown in Table III. Yokogawa *et al.*⁷⁾ reported that in 3 worms of the same species cohabitation in a cyst, one of them was remarkably smaller in size as compared with other two worms, and they also observed the defective development of the reproductive organs and the growth of the worms. In the present case of Cat No. 3 and Dog Nos. 3 and 4, no notable differences were found in the size of 3 worms of the same species cohabitation in a cyst though the results of the present investigation are not to be directly comparable with those of yokogawa *et al.*⁷⁾

The measured values of the solitary worm in a cyst were similar to those of the other worms recovered from the cysts of the same experimental animal, and a large number of eggs were recognized in the uterus of each worm. Even if it is the case of a single worm, the worm of *P. miyazakii* as well as *P. ohirai* might have settled in the cyst after a cyst formation and reached its sexual maturity, though the worm of *P. westermani* might have completed the sexual development after a mutual copulation by the plural number of worms as described by Yokogawa *et al.*⁷⁾ Yokogawa *et al.*¹¹⁾ and Yoshimura *et al.*¹²⁾ indicated that each worm of *P. miyazakii* and *P. ohirai* in rats infected with a single metacercaria reaches its full development without a cyst formation in the lung. Although the developmental states of each worm from the present experimental animals have aroused much interest in the subject of host parasite relationships under the condition of mixed infection, these important problems

are left for further investigation because of insufficient data. The continual development of the larvae hatched from the eggs produced by the worms of different species cohabitation in a cyst offers an important subject for the further study.

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