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Studies on the Lung Fluke, *Paragonimus westermani*-Diploid Type in the Northern Part of Hyogo Prefecture, Japan
VI. Experimental Oral Infection of Wild Boars and Pigs with the Metacercariae

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Key words: Lung fluke, *Paragonimus westermani,* diploid type, wild boar, pig, host adaptation

Introduction

Some cases of natural infection of wild boars and pigs with *Paragonimus westermani* in Japan have been reported (Miyagawa, 1935; Oishi and Saito, 1952; Isshiki and Tomimura, 1953; Morita *et al.,* 1954; Suzuki, 1958; Miyazaki and Hirose, 1976; Miyazaki *et al.,* 1978). Some of them, however, had been reported before the other four species of *Paragonimus* were discovered in Japan. Therefore, there might be some confusion as to the species of *Paragonimus* described in those previous reports. Moreover, it was clarified that there are two chromosomal types, diploid and triploid in "*P. westermani*" (Sakaguchi and Tada, 1976; Terasaki, 1980). Since Miyazaki (1979) proposed to call these two types *P. westermani* and *P. pulmonalis* as different species, respectively, the necessity arose to differentiate these two types in their natural definitive hosts. For the time being, the present authors call them *P. westermani*-diploid type and *P. westermani*-triploid type. Recently, the present authors (1985) found some cases of natural infection with adult worms of the diploid type in wild boars and therefore suggested that wild boars sometimes serve as natural definitive hosts. However, many researchers have previously reported that no mature worms of *P. westermani* had been obtained from wild boars or pigs in their experiments and those animals could not become experimental definitive hosts for *P. westermani* (Miyagawa, 1935; Isshiki, 1952; Miyazaki and Habe, 1975; Habe, 1978). The reason for this is presumed to be that the metacercariae used in their experiments were perhaps those of the triploid type. Therefore, they might be able to get no mature worms. In the present research, experimental infection of wild boars and pigs with the metacercariae of the diploid type was performed, and the growth and development of the worms obtained and the infectivity to those animals were investigated to compare with the results for the triploid type.

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Materials and Methods

Parasite and experimental hosts:

Metacercariae were collected by the digestive method in artificial gastric juice from freshwater crab, *Geothelphusa dehaani,* captured

upstream of the Maruyama River in the northern part of Hyogo Prefecture, Japan.

Three wild boars (four months old, two males and one female), three pigs (Sangen, 3 to 6 weeks old, female), two dogs (adult, female) and a cat (adult, male) were used for the experiment. The wild boars and pigs were purchased from a swine farm. The dogs and the cat were checked to be negative for *Paragonimus* infection by fecal examination prior to the experiment.

Method of infection:

The animals were anesthetized with ketamine hydrochloride (Ketalar® 50–Parke–Davis) or sodium pentobarbital (Somnopentyl–Pitman-Moore). After anesthesia was induced, gelatin capsules containing 200 to 500 metacercariae or 10 to 25 juvenile worms recovered from the muscle of the wild boar or pigs were orally administered to each animal. Each one of the wild boars and pigs was orally administered the metacercariae several times at intervals of several days.

Recovery of parasite:

The wild boars and pigs were autopsied on day 100 to 170 and 110 to 120 after infection with metacercariae. First, their visceral organs and cavities were searched for the lung fluke infection. The cavities were repeatedly washed by physiological saline solution, and the search for the worms from the sediments was carried out. Then, according to Habe's method (Habe, 1978) the lungs, liver and muscle of the whole body were cut into 4 to 5 mm thick slices and kept in physiological saline solution at about 37°C. After 12 to 15 hours, the worms emerged from the collected fragments. The dogs and the cat were autopsied on day 56 to 300 after infection with juvenile worms, and the visceral organs, peritoneal and pleural cavities were searched for lung fluke infection and the worms were recovered.

Worms:

The larger worms recovered were flattened between two slide glasses and were fixed in 70% alcohol, and smaller ones were flattened between a cover glass and a slide glass and were fixed in Bouin's solution. Then, they were stained with borax carmine, dehydrated, cleared in xylene and mounted in balsam. Morphological observations and measurements were made on these mounted specimens. The worms were classified into four types by the developmental stages: 1) adult worms with eggs in the uterus, 2) pre-adult worms with spermatozoa in the seminal vesicle or with vitelline cells in the vitelline duct instead of eggs in the uterus, 3) immature worms without eggs, spermatozoa or vitelline cells and without granules in the excretory bladder, and 4) juvenile worms with granules in the excretory bladder.

Statistical analysis:

Statistical analysis was made by the Welch method or Chi-square (x^2) test.

Results

1. *Experimental infection of wild boars and the degree of the growth and development of the worms obtained*

Three wild boars were used for the experiment and were orally administered 200 metacercariae, as shown in Table 1. Wild boar No. 2 was given 30 to 50 metacercariae in six times at intervals of 10 days. Wild boar No. 1 was autopsied on day 100 after infection, No. 2 on day 150 after first infection and No. 3 on day 170 after infection. The worm recovery rates in these wild boars were at the low level of 7.0 to 17.0% as shown in Table 1 and those worms were mostly of the juvenile type. Most of the worms recovered were from the muscle and the recovery rate ranged from 71.4 to 80.0%, with an average of 76.8%. Some worms were recovered from the pleural cavity or lungs. Worm-cyst formation was also recognized in the lungs. Of the worms recovered, the number of worms removed from the cysts in lungs of wild boars Nos. 1, 2 and 3 were 4(20.0%),

Table 1 Results of experimental oral infection of wild boar, *Sus scrofa leucomystax*, and pig*, *Sus scrofa* var. *domesticus* with metacercariae (Mc) of *P. westermani*-diploid type

Experimental host (No.)	Body weight at autopsy (kg)	No. of Mc fed	Days after infection	No. and (%) of worms recovered	No. of worms recovered from					No. of worms with eggs in uterus
					muscle	abdominal cavity	pleural cavity	lung tissue	cyst in lungs	
Wild boar (No. 1)	11.7	200	100	20 (10.0)	15(1)‖	0	1	0	4	0/20#
Wild boar (No. 2)	18.0	200 (30,30,30,30,30,50)†	(100-)§150	14 (7.0)	10(1)	0	1	0	3	0/14
Wild boar (No. 3)	30.5	200	170	35 (17.5)	28(1)	0	2	1	4	0/35
Total		600		69 (11.5)	53(3)	0	4	1	11	0/69
Pig (No. 1)	55	500	110	66 (13.2)	62(1)	3	0	0	1	0/66
Pig (No. 2)	75	500	110	13 (2.6)	13	0	0	0	0	0/13
Pig (No. 3)	68	489 (250,239)‡	(100-)120	59 (12.1)	59	0	0	0	0	0/59
Total		1489		138 (9.3)	134(1)	3	0	0	1	0/138

* Breed: Sangen.
† Given 30 to 50 Mc in six times at intervals of 10 days.
‡ Gigen 250 and 239 Mc in two administrations at an interval of 20 days.
§ Days after last infection.
‖ Figures in parentheses are the number of worms from the diaphragm.
No. of worms with eggs in uterus/No. of worms recovered.

3(21.4%) and 4(11.4%), respectively. However, all were immature or pre-adult type, and there was no worm which had grown to adulthood (Figs. 2, 3).

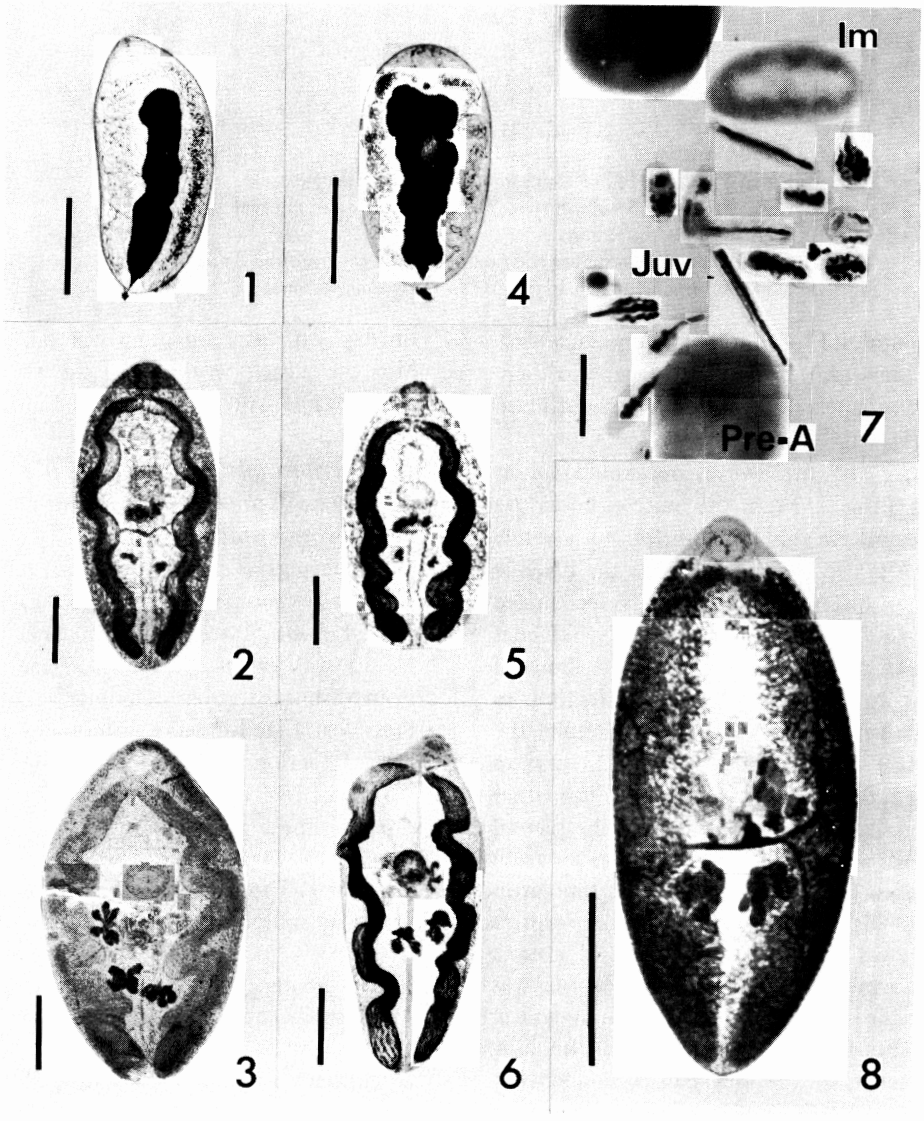
The sizes of the worms recovered are as shown in Table 2. From the muscle, immature and pre-adult worms in addition to juvenile worms were also recovered. As to the juvenile worms, no difference in size was recognized between those from the muscle of wild boar No. 1 100 days after infection and those of wild boar No. 3 170 days after infection, as shown in Table 2 (Fig. 1). For example, the average size of the former was 1.23 mm in length and 0.54 mm in width. On the other hand, the sizes of the worms from the pleural cavity had a tendency to be larger – from immature to pre-adult type – with time after infection, although the number of worms observed was small. But, the size of even a pre-adult worm 170 days after infection, was 3.88 mm in length and 2.06 mm in width. Contrary to this, the worms from the lung tissue or cysts in the lung had a tendency to gradually become smaller as the days after infection increased, and the worms obtained on day 170 after infection were all immature. Moreover, each worm from the two cysts in the lungs of wild boar No. 3 had already died.

2. *Experimental infection of pigs and the degree of the growth and development of the worms obtained*

Three pigs as well as wild boars were used for the experiment and were orally administered 500, 500 and 489 metacercariae. Pig No. 3 was given 250 and 239 metacercariae in two administrations at an interval of 20 days. Pigs Nos. 1 and 2 were autopsied on day 110 after infection and No. 3 on day 120 after first infection, respectively. The worm recovery rates of these pigs, as well as of wild boars, were at the low level of 2.6 to 13.2%, as shown in Table 1. Those worms were mostly recovered from the muscle and the recovery rate ranged from 93.9 to 100.0%, with an average of 97.1%. In pig No. 1, one cyst was recognized in the lungs and one worm was recovered from it. But, the worm, which appeared to be an immature type, had already died. Also, one of the worms from the abdominal cavity was a less developed pre-adult worm with a small number

of spermatozoa in the seminal vesicle (Fig. 6). Although no worms were recovered from the liver of each pig, some white small foci of necrosis which seemed to be caused by penetration of the worms were observed on the surface. Especially, although two or three whitish button-like cysts of 10 to 15 mm in diameters were recognized on the liver of pig No. 1, no worms were detected, and they were only filled with a yellow-white substance.

The sizes of the worms recovered were as shown in Table 2. From the muscle, both juvenile and immature worms were recovered (Figs. 4, 5). As to the size of the juvenile worms, differences based on the days after infection were not clearly apparent. The average sizes of the juvenile worms from pigs Nos. 1 and 2 110 days after infection were 1.12 ± 0.15 mm in length and 0.51 ± 0.06 mm in width and 1.12 ± 0.13 mm in length and 0.44 ± 0.07 mm in width, respectively. No significant difference in size between the two was



recognized. But, that from pig No. 3 was 1.31 ± 0.21 mm in length and 0.56 ± 0.08 mm in width and was slightly larger than those from pigs Nos. 1 and 2 ($P < 0.005$). On the other hand, as to the size of the immature worms, no significant difference among the three was recognized. The worm from the abdominal cavity of pig No. 1, which was the only pre-

Table 2 Measurements of *P. westermani*-diploid type recovered from various tissues of wild boars and pigs

Experimental host (No.)	Recovery site	Type* of worms	No. of worms measured	Body length (mm)	Body width (mm)	Oral sucker width (μm)	Ventral sucker length (μm)	Ventral sucker width (μm)
Wild boar (No. 1)	Muscle	Juv	4	1.23±0.10†	0.54±0.03	139± 6	145± 9	166±11
	Muscle	Im	4	1.88±0.31	0.90±0.18	213±48	226±37	244±45
	Pleural cavity	Im	1	2.53	1.69	345	328	383
	Cyst in lungs	Pre-A	3	3.95±0.30	2.16±0.34	472±60	427±52	480±56
Wild boar (No. 2)	Muscle	Im	3	2.19±0.11	0.97±0.24	227±31	273±18	236±90
	Pleural cavity	Im	1	3.44	1.63	329	353	403
	Cyst in lungs	Im	2	3.34	1.50	356	382	416
	Cyst in lungs	Pre-A	1	3.47	1.97	349	419	480
Wild boar (No. 3)	Muscle	Juv	14	1.23±0.29	0.53±0.18	120±10	134±12	154±14
	Muscle	Im	3	1.68±0.99	1.02±0.81	256±86	274±132	300±135
	Muscle	Pre-A	1	3.88	2.44	412	513	516
	Pleural cavity	Im	1	3.44	1.91	467	474	524
	Pleural cavity	Pre-A	1	3.88	2.06	455	472	517
	Lung tissue	Im	1	2.32	1.09	244	274	301
	Cyst in lungs	Im	2	2.69	1.75	416	426	451
Pig (No. 1)	Muscle	Juv	19	1.12±0.15	0.51±0.06	131±12	140±12	162±13
	Muscle	Im	3	2.86±0.87	1.18±0.12	301±84	300±82	307±21
	Abdominal cavity	Im	1	3.19	1.31	255	241	314
	Abdominal cavity	Pre-A	1	4.47	1.86	431	491	451
Pig (No. 2)	Muscle	Juv	6	1.12±0.13	0.44±0.07	114±13	140±15	146±14
	Muscle	Im	1	2.94	1.19	–	275	313
Pig (No. 3)	Muscle	Juv	18	1.31±0.21	0.56±0.08	141±21	146±22	166±15
	Muscle	Im	4	2.78±0.86	1.27±0.20	298±65	314±70	336±78

* Juv) juvenile worm with granules in excretory bladder, Im) immature worm without granules in excretory bladder, Pre-A) pre-adult worm with sperm in seminal vesicle or with vitelline cells in vitelline duct.

† Mean ± SD

Fig. 1 Juvenile worm recovered from the muscle of the wild boar 100 days after infection with metacercariae (living specimen, dorsal view). Scale = 500 μm.

Fig. 2 Immature worm recovered from the lung tissue of the wild boar 170 days after infection with metacarcariae (balsam-mounted specimen, dorsal view). Scale = 500 μm.

Fig. 3 Pre-adult worm recovered from the cyst in lungs of the wild boar 100 days after infection with metacercariae (balsam-mounted specimen, dorsal view). Scale = 1 mm.

Fig. 4 Juvenile worm recovered from the muscle of the pig 110 days after infection with metacercariae (living specimen, dorsal view). Scale = 500 μm.

Fig. 5 Immature worm recovered from the muscle of the pig 110 days after infection with metacercariae (balsam-mounted specimen, dorsal view). Scale = 500 μm.

Fig. 6 Pre-adult worm recovered from the abdominal cavity of the pig 110 days infection with metacercariae (balsam-mounted specimen, dorsal view). Scale = 1 mm.

Fig. 7 Living worms recovered from the muscle of the wild boar 170 days after infection with metacercariae (Juv: Juvenile worm, Im: Immature worm, Pre-A: Pre-adult worm). Scale = 1 mm.

Fig. 8 Adult worm recovered from the cyst in lungs of the dog 61 days after infection with juvenile worms from the muscle of the wild boar 170 days after infection with metacercariae (balsam-mounted specimen, dorsal view). Scale = 2 mm.

Table 3 Results of experimental oral infection of two dogs and a cat with juvenile worms of *P. westermani*-diploid type recovered from muscle of a wild boar and two pigs

Final host	Paratenic host (days of postinfection)	No. of worms fed	Days after infection	No. and (%) of worms recovered	No. of worms recovered from			No. and (%) of worms with eggs in uterus
					pleural cavity	lung tissue	cyst in lungs	
Dog	Wild boar No. 3 (170)	10	61	6 (60.0)	0	0	6	3/6*(50.0)
Cat	Pig No. 1 (110)	20	56	8 (40.0)	0	5	3	0/8 (0.0)
Dog	Pig No. 3 (110-120)	25	300	1 (4.0)	1	0	0	1/1 (100.0)

* No. of worms with eggs in uterus/No. of worms recovered.

adult worm recovered from pigs, was 4.47 mm in length and 1.86 mm in width.

3. *Experimental infection of dogs or a cat with juvenile worms recovered from the muscle of wild boars and pigs*

The juvenile worms recovered from the muscle of wild boar No. 3, pigs Nos. 1 and 2 were orally administered to dogs or a cat (Fig. 7). The results obtained are as shown in Table 3. In an experimental infection of a dog with the worms from wild boar No. 3, six worms (60.0%) were recovered from the cysts in lungs. Of them, three were adult (Fig. 8) and the remaining three were pre-adult worms. On the other hand, in an experimental infection of a cat with the worms from pig No. 1, the cat was autopsied on day 56 after infection as it died during the experiment. Eight worms (40.0%) were recovered. Of them, three were recovered from a cyst in the lung and the remaining five from the lung tissues. All of them were pre-adult worms. In an experimental infection of a dog with the worms from pig No. 3, although two worm cysts were recognized in the lungs, no worms were recovered except one worm which was migrating in the pleural cavity. It was an adult worm with eggs in the uterus. The reason for this is presumed that too much time, 300 days after infection with the worms, had passed.

Discussion

In an experimental infection by Miyazaki and Habe (1975) of wild boars and pigs with meacercariae of the triploid type of *P. westermani,* about half (38.6 to 57.2%) of the metacercariae administered was recovered in both wild boars and pigs, and most of them (89.8 to 99.8%) were from the muscle, and it seemed that there were no distinct differences in size between the worms from each host. On the contrary, in the present survey, in which metacercariae of the diploid type of *P. westermani* were used, the worm recovery rate in wild boars was 11.3% in average, ranging from 7.0 to 17.0%, and that in pigs was 9.3% in average, ranging from 2.6 to 13.2%, as shown in Table 1. Although there was no significant difference in the recovery rates between those hosts, they showed very low values in comparison with those of the triploid type ($P < 0.001$). As for the recovery-site of the worms, in pigs, except for one worm from a cyst in lung, most of the worms were recovered from the muscle at the high level of 97.1% in average, ranging from 93.9 to 100.0%, and the result was similar to that of the triploid type of *P. westermani* by Miyazaki and Habe (1975). In wild boars, however, the worm recovery rate from the muscle was 76.8% in average, ranging from 71.4 to 80.0%, and 16 (23.2%) of the worms detected were recovered from the thorax. Among

them, 12 worms (17.4%) were recovered from the lung tissue or cysts in lungs. Judging from these facts, it is presumed that the worms of the diploid type are more infective to wild boars and are more apt to penetrate into the thorax and form worm cysts than those of the triploid type though no adult worms were obtained.

In the present research, repeated experimental infections of each wild boar and pig with the metacercariae were also performed two or six times as shown in Table 1. Then the growth and development of the worms obtained was compared with those in the case of single infection. However, no distinct difference was recognized between the two.

Most recently, the present authors (1985) performed an epidemiological survey of the lung fluke, *Paragonimus* spp. in wild mammals of the northern part of Hyogo Prefecture and found some worm cysts in the lungs of 8(9.1%) of 88 wild boars inspected in a survey of lungs only. A total of 14 worms were obtained from those cysts, and 5(35.7%) of them were adult worms. Although the adult worms were not well-developed, the detection of those worms proved that wild boars can serve as a natural definitive host. In this experiment, though cyst-formation in the lungs was recognized in all three wild boars, the worms obtained were not adult but immature or pre-adult type. Therefore, the infection of more metacercariae or a longer duration of time after infection might have produced fully matured adult worms. The worms from the muscle of wild boars occupied 76.8% in average of the total worms recovered and were mostly juvenile type. The recovery rate, however, was slightly less than that of the triploid type of *P. westermani* by Miyazaki and Habe (1975). It was demonstrated that when these juvenile worms were orally administered to a dog they easily penetrated the lungs and formed worm cysts, and then fully matured to adult worms. Hence it is presumed that wild boars will also be able serve as a paratenic host as well as in the case of the triploid type. On the other hand, in pigs, not as many worms were recovered from the thorax as in wild boars. Most were from the muscle, as mentioned above. As shown in Table 3, some of these juvenile worms matured to pre-adult or adult in the cat or dog. It is, therefore, presumed that pigs as well as wild boars will also be able to play a role as a paratenic host.

Formerly, many human paragonimiasis had occurred in Kagoshima Prefecture (Norimatsu *et al.*, 1975). Thereafter, it was proven that this was due to the local custom of eating raw wild boar meat and that wild boars play an important role as a paratenic host. More recently, many cases of human paragonimiasis, which seemed to be caused by eating raw wild boar meat, occurred in Oita Prefecture, and once more interest has been shown in wild boars as the source of human paragonimiasis (Araki *et al.*, 1985). It has been proven that the former was caused by the triploid type of *P. westermani* (Miyazaki *et al.*, 1978), however, since the symptoms were similar to those of *P. miyazakii* and the diploid type of *P. westermani* were found in Oita Prefecture by Habe and Miyazaki (1982) it was suspected that the latter was caused by the diploid type of *P. westermani*. Accordingly, it seems to be necessary to pay special attention to the juvenile worms of *P. westermani*-diploid type in the muscle when man eats raw wild boar meat.

Summary

Three wild boars and three pigs were orally infected with the metacercariae of the diploid type of *P. westermani* to observe the growth and development of the worms obtained and compare the infectivity to those hosts. The results were then compared with those of experiments using the metacercariae of the triploid type of *P. westermani* by Miyazaki and Habe (1975) and Habe (1978). In pigs the worms were mostly recovered from the muscle and most of them were juvenile type, and few worms were from the pleural cavity. Contrary to this, in wild boars 16(23.2%) of the

worms detected were recovered from the pleural cavity, among them, 12 worms (17.4%) were from the lung tissue or cysts in lungs. Those worms, however, were less developed and even those from cysts in lungs were immature or pre-adult type. In comparing the diploid type with the triploid type, contrary to the results which showed the worms of the triploid type mostly staying in the muscle of wild boars and pigs for a long time (Miyazaki and Habe, 1975), the worms of the diploid type were recognized to have a stronger tendency to penetrate the pleural cavity of wild boars and form worm cysts than those of the triploid type. Judging from the facts, it is presumed that the diploid type of *P. westermani* is more infective to wild boars than the triploid type. However, it is concluded that wild boars are not very suitable hosts for either the diploid type or the triploid type. In the present research, experimental oral infection of dogs or a cat with the juvenile worms from the muscle of a wild boar and pigs was successful in the recovering of adult or almost matured pre-adult worms. Hence it is presumed that both hosts can serve as paratenic hosts of the diploid type of *P. westermani* as well as of the triploid type.

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兵庫県北部におけるウエステルマン肺吸虫(二倍体型)に関する研究
VI. イノシシとブタへの感染実験

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イノシシおよびブタそれぞれ3頭を用いて兵庫県産ウエステルマン肺吸虫二倍体型メタセルカリアの感染実験を行い，それらに対する感染性の違いと発育状況を観察すると共に，宮崎・波部(1975)および波部(1978)による本虫三倍体型での成績との比較を行った．その結果，ブタの場合大部分が筋肉内から幼若虫として見出され，殆ど胸部から虫体が回収されることがなかったのに対し，イノシシでは得られた虫体の23.2%（16隻）が胸部から回収され，そのうちの17.4%（12隻）は肺あるいは肺に形成された虫嚢内からのものであった．しかし，それら得られた虫体の発育は不十分で，肺に形成された虫嚢内から得られるものでも未熟虫かせいぜい亜成虫であった．二倍体型と三倍体型との比較では，三倍体型は，イノシシおよびブタで胸部へ入る傾向は余り認められず殆どの虫体が長期間筋肉内に留まっているのに対し，二倍体型はイノシシで胸部に入る傾向がより強く，肺に虫嚢を形成しやすい傾向が認められた．このことから，ウエステルマン肺吸虫の二倍体型は，三倍体型と比較して，イノシシに対してはより感染性が強いものと思われる．しかし，イノシシは二倍体型にとってもそれほど好ましい終宿主とは思われない．また今回，イノシシ，ブタ双方の筋肉内から得られた幼若虫の，イヌあるいはネコへの感染実験によっていずれからも成虫が得られたことから，両宿主は三倍体型と同じく待機宿主としての役割を果しうるものと考えられる．