

## Parasitological and Histopathological Studies of Experimental Paragonimiasis\*

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### Introduction

The Oriental lung fluke, *Paragonimus westermani* was one of the medically important endemic diseases in Korea, and was familiarly known as "To-jil" from very early times in the Korean herb medicine.

Kobayashi(1918, 1919) conducted the studies on the life history and morphology of the lung fluke from a variety of both natural and experimental hosts in Korea, and reported that this infection was prevalent not only in man but also in the domestic and wild animals such as dogs, cats, tigers, leopards, foxes, wolves, and wild cats.

Bercovitz(1937) in a clinical studies on 20 patients with lung fluke diseases caused by *P. westermani* infections reported that the most common subjective symptoms were cough and the presence of reddish brown sputum, occasionally accompanied by fever and chill. Miller and Walker(1955) studied 227 infected prisoners of war from a roentgenographic standpoint in an attempt to delineate the radiographic features of *P. westermani* infections. Chien(1955) and Geher(1957) described a typical patten of pulmonary changes due to paragonimiasis from the very early active stages to the late and residual stages after the parasites had died and the disease had disappeared clinically. Sadun and Buck(1960) made an immunodiagnostic epidemiologic, clinical, roentgenologic and therapeutic studies of

paragonimiasis in south Korea and emphasized the clinical and public health importance of *P. westermani* infections in south Korea than in any other country in the world.

After the establishment of the first "Five-year economic development plan" in 1962, the results of comprehensive studies of *P. westermani* on its biology, epidemiology, pathology, clinical symptoms and therapeutic have been reported by many investigators in Korea. As a result, paragonimiasis are found to be distributed widely over the south Korea, however, the infection rates among the residents and intermediate hosts are gradually decreasing in recent years.

In addition, It has been known that histopathological findings and clinical symptoms of human paragonimiasis are found to be differences according to the number of infecting worms, the duration varying from the onset of this disease to autopsy and the susceptibility of the individuals to the worms.

The parasitological and histopathological studies of experimental paragonimiasis in cats and dogs have been done by Yokogawa et al.(1958), Yokogawa and Yoshimura(1960), Chyu(1962), Chyu and Kim(1962), Yoo and Chyu(1966), Chung(1971) Lee et al.(1976), Lee(1979), Choi et al.(1979) and Chi et al.(1982).

It was found that the pathological changes of the lung elicited by worms of *P. westermani* in experimental animals were not constant, because the changes are different in the number of flukes,

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the habitat in the host, the period of infection and the animal host.

After the experimental infection, the flukes grow in the pleural cavity or the pleura, and then enter the lung. The flukes become damage to the lung in varying degrees, producing adhesion to neighboring organs, thickening of the wall, and finally forming worm cysts in the lung.

Comprehensive studies of histopathology of the lungs infected with *P. westermani* in the dog were carried out by Lee(1979). Lee(1979) reported that the lung lesions could be classified into 2 categories, direct mechanical effects by the flukes and eggs, and the changes secondary to the worm infection. The relative importance of these 2 was almost equal. Choi(1990) commented the same pathological findings in the review of *Paragonimus* and paragonimiasis in Korea. Adult flukes reside inside the lumen of the dilatated bronchi in most cases. Only exceptions were secondary abscess formation and subsequent scar where no epithelial lining was detected around the flukes. *P. westermani* eggs were found as isolated and aggregated form, provoking granulomatous inflammation.

It is still debatable whether there is a clear relationship between the different methods of the route of infection and the pathological findings of the lung infected with the fluke.

The present paper describes experiments on the recovery efficiency and distribution of *P. westermani* in the lung of cats by 3 infecting methods e. g. oral administration of the metacercariae, intraperitoneal injection(IP) of the excysted metacercariae and IP of metacercariae, and the histopathological findings in the lung of cats killed 90 days after primary infection and 60 days(150 days) after challenge.

#### Materials and methods

Collection of crayfish: The crayfish, *Cambaroides similis* were collected from Sangdong stream, Buk myun, Ulchin county, Kyungpook Province, which

has been known to be an endemic area of *P. westermani*.

Isolation of *P. westermani* metacercaria: The crayfish were dissected into several pieces, and the metacercariae were isolated from the livers, the gills and the cephalothorax under a stereoscope. The metacercariae isolated were stored in a beaker containing normal physiologic saline until the excystation or experimental infection.

Excystation of the metacercariae: The metacercariae were put into a beaker containing 0.1% sodium taurocholate. The beaker was left to stand for 30 to 40 minutes in a water bath adjusted at 40°C. As soon as the larvae excysted, the larvae were transported and washed 3 times in physiological saline.

Animal used: Mongrel house cats(outbred), weighing 2.5–3.0kg were used.

Preparation of Medium-199: Powered M-199 (Gibco, Grand Island, New York) was dissolved in 500ml of distilled water and the pH of the solution was adjusted to be alkaline by addition of 7% sodium bicarbonate. Penicillin-streptomycin mixture(Whittaker, M. A. Bioproducts, Walkersville, Md.) was added to the medium, to have the final concentration of Pot. penicillin G, 50units/ml and streptomycin sulfate, 50µg/ml. The medium was sterilized by filtration through a membrane pored 0.2µm and stored at 4°C. Before use, the medium was diluted with an equal volume of triple distilled water.

Experimental infection: In order to infect the cats orally with *P. westermani*.

They were left to starve for 8 hours and given a section of beef surrounding the metacercariae. They were divided into 3 group, and consisted of 6 cats each group. A half(3 cat) of each group was used for primary infection.

The remaining 3 cats of each group were used to secondary infection 90 days after primary infection.

The cats of group 1 were infected orally with 5 metarcercariae of *P. westermani*, those of gorup 2 infected per os with 10 metacercariae, and those

of group 3 with 20 metacercariae, and then killed 90 days after primary infection. In order to perform the trials of secondary infection, 12 cats were divided into 4 groups. The cats of all groups were infected secondarily per os with 10 metacercariae of *P. westermani*, and killed 60 days after secondary infection.

In order to determine the recovery efficiency and distribution of *P. westermani* in the lung of cats by 3 infecting methods, 15 cats were divided into 3 groups.

Each cat of group 1 was left to starve for 8 hours and given a section of beef surrounding the encysted larvae.

Each cat of group 2 was injected intraperitoneally with 10 excysted larvae suspended in medium-199 while each of group 3 was injected with metacercariae.

The cats of 3 groups were killed 90 days after infection, and the adult flukes were recovered.

Gross observations: The cats were killed by striking the posterior head with an iron hammer. The lungs were taken out and number and distribution of flukes recovered were observed.

Microscopic observations: The tissue specimens of fluke cysts in the lung were obtained from

killed cats and fixed in 10% neutral formalin for 10 days.

The specimens fixed were embedded in paraffin and cut in 5–6 $\mu$ m of slices, and then stained with hematoxylin–eosin. The adult flukes were stained with acetocarmine.

## Results

### 1. Parasitological study

Table 1 shows the design of experiments to infect mongrel house cats with different numbers of *P. westermani* metacercariae. Three out of 6 cats in each group were killed for gross and pathological examinations on 90 days after primary infection.

Table 2 lists the distribution of the adult *P. westermani* recovered from 3 groups of cats. A total 105 metacercariae of *P. westermani* was given orally.

The number of the adult flukes recovered from the lungs of cats was 90, with an average of 85.7%. The recovery rate of group 2 given 10 metacercariae each was the highest, 93.3% whereas the rate of group 3 given 30 metacercariae each was

Table 1. Experiments of infection with *Paragonimus westermani* in mongrel house cats

Group	No. of cat	Primary infection	Necropsy day
1	6	5 metacercariae given orally	3 cats killed 90 days after primary infection
2	6	10 metacercariae given orally	"
3	6	20 metacercariae given orally	"

\* The remaining 3 cats of each group were used to secondary infection.

Table 2. Distribution of *P. westermani* in lung of cats killed on 90 days after primary infection

Group*	No. of cyst given	Recovery Number and % of flukes										Total fluke recovered	
		Right lung					Left lung						
		Upper	Middle	Lower	No.	%	Upper	Middle	Lower	No.	%	No.	%
1	15( 5 each)	2	2	4	8	53.3	1	1	3	5	33.3	13	86.7
2	30(10 each)	4	2	11	17	56.7	3	2	6	11	36.7	28	93.3
3	60(20 each)	7	4	20	31	51.6	4	3	11	18	30.0	49	81.7
Total	105	13	8	35	56	53.3	8	6	20	34	32.4	90	85.7

the lowest, 81.7%. The rate of group 1 where 5 cysts were administered was intermediate, 86.7%.

In the distribution of the flukes in the lung, 53.5%, 56 out of 105 adult flukes, were found from the right lung and 32.4%, 34 out of 105 flukes, from the left lung. The number of flukes recovered in the right lung was greater than that in the left lung. A large number of flukes, 58.8 to 62.5%, was found in the lower lobes, and the least, 14.3 to 30.0%, in the middle lobes of the both lungs.

Table 3 presents the design of secondary infection of cats with *P. westermani*.

Twelve cats of 4 groups, 3 cats in each group, were infected secondarily per os with 10 metacercariae of the fluke. In general, the recovery rate of the fluke decreased with the increase in the number of metacercariae given (Table 4).

The rate for the fluke in the control, group 4, was the highest 90.0%, followed by group 1 77.8%, group 2

71.7%, and group 3 68.9%, in decreasing order.

The recovery rates of the fluke in the lobes of lung in cats on 60 days after secondary infection was similar to those of cats on 90 days after primary infection.

Table 5 shows the recovery and distribution of the flukes recovered from 3 groups of cats. The recovery rate for the fluke was 90.7% in total. The rate of group 3 injected IP with the metacercariae was the highest, 94.0%, whereas the rate of group 1 infected orally with 10 metacercariae was the lowest, 86.0%.

The rate of group 2 injected IP with 10 excysted metacercariae was intermediate, 92.0%. The recovery rate for the fluke in the right lung was higher than that in the left lung and similar to those of the groups infected primarily and secondarily.

Table 6 lists the comparison of size of flukes from the lung of cats between 90 days after primary

Table 3. Experiments of secondary infection with *P. westermani* in cats

Group	No. of cat	Primary infection	Secondary infection	Necropsy day
1	3	5 metacercariae given orally	10 metacercariae	60 days after secondary infection
2	3	10 metacercariae given orally	"	"
3	3	20 metacercariae given orally	"	"
4	3	control	"	"

Table 4. Distribution of *P. westermani* in lung of cats killed on 60 days after secondary infection

Group*	No. of cyst given	Recovery Number and % of flukes										Total fluke recovered	
		Right lung					Left lung						
		Upper	Middle	Lower	No.	%	Upper	Middle	Lower	No.	%	No.	%
1	45(5+10** each)	4	3	13	20	44.4	4	5	8	15	33.3	35	77.8
2	60(10+10 each)	7	4	15	26	53.4	5	3	9	17	28.3	43	71.7
3	90(20+10 each)	6	7	21	33	36.7	7	5	17	29	32.2	62	68.9
4	30	4	3	10	17	56.7	3	2	5	10	33.3	27	90.0
Total	225	21	17	59	96	42.7	19	13	39	71	31.6	167	74.2

\* Each group consisted of 3 cats

\*\* 5+10 : Each cat was given primarily 5 metacercariae and infected secondarily with 10 metacercariae

Table 5. Recovery and distribution of *P. westermani* in lung of cats by 3 different routes of infection

Group	No. of cats	Route of infection	Total No. of metacercariae	Recovery No. and % of flukes					
				Right lung		Left lung		Total	
				No.	%	No.	%	No.	%
1	5	10 metacercariae infected orally	50	24	48.0	19	38.0	43	86.0
2	5	10 excysted metacercariae injected IP*	50	24	48.0	22	44.0	46	92.0
3	5	10 metacercariae injected IP	50	26	52.0	21	42.0	47	94.0
Total	15		150	74	49.3	62	41.3	136	90.7

\* IP means intraperitoneally.

Table 6. Size of *P. westermani* obtained from lung of cats between 90 days after primary infection and 60 days after secondary infection

Group	No. of fluke	Primary infection size(mm)		No. of fluke	Secondary infection size(mm)	
		Length	Width		Length	Width
1	11	11.3±1.4	6.2±1.1	8	5.7±0.5	3.1±0.2
2	21	11.6±1.8	6.3±0.8	7	5.5±0.7	3.0±0.5
3	25	10.5±1.6	6.1±1.3	8	5.8±0.8	2.9±0.4
4	0	—	—	6	5.7±0.5	3.0±0.6

infection and 60 days after secondary infection.

The adult fluke stained with acetocarmine was somewhat elongated, with tapering anterior and posterior ends(Fig. 1). The size of flukes on 90 days after primary infection ranged from 10.5 to 11.6mm in length by 6.1 to 6.3mm in width, and the ratio of the body length to the body width was 1.7:1. Whereas the size on 60 days after secondary infection ranged from 5.5 to 5.8mm in length by 2.9 to 3.1mm in width, and the ratio was 1.9:1. The difference in size was statistically significant at 5% level(Fig. 2).

## 2. Histopathological study

Gross observations showed that the fluke cysts in the lung of infected cats appeared as round nodules on the surface of the lung(Fig. 3, 4).

The nodules are rough and whitish-gray on the protrudent part, and the other parts revealed

a dark blue and brown. They are located mostly in the superficial part of the lung(Fig. 3, 4, 5, 6).

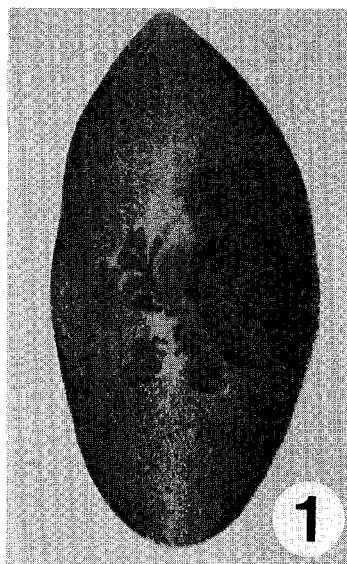
Fig. 1. Adult *Paragonimus westermani*



Fig. 2. The lung flukes resected 150 days after infection, and small one 60 days after secondary infection.

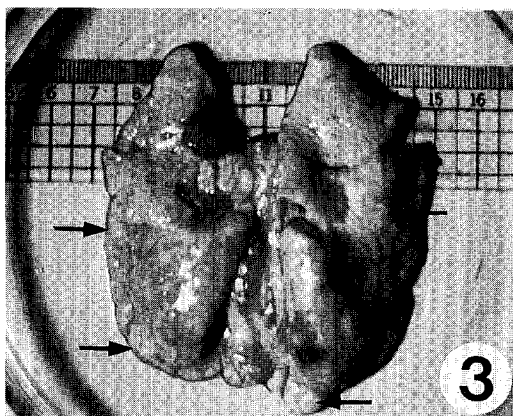


Fig. 3. The lung of cat 60 days after secondary infection. An arrow indicates the nodule.

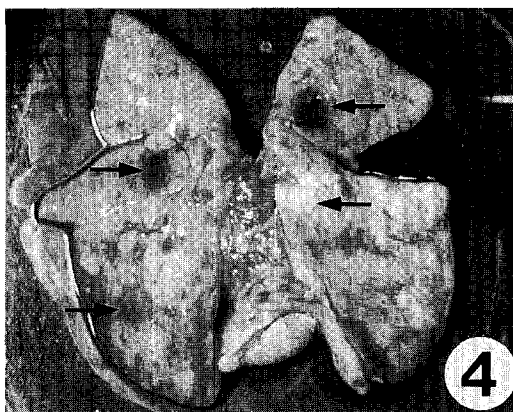


Fig. 4. The lung of cat 60 days after secondary infection.

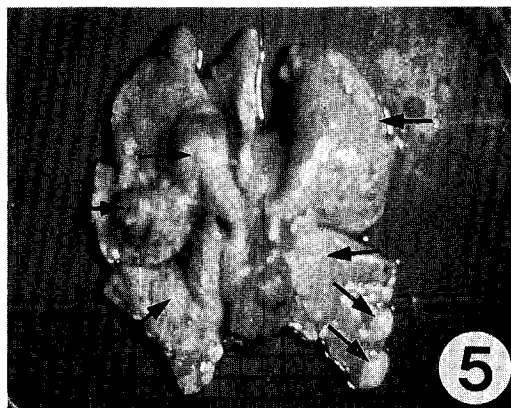


Fig. 5. The lung of cat 150 days after infection. The nodules in the lung are whitish-gray or dark blue and brown.

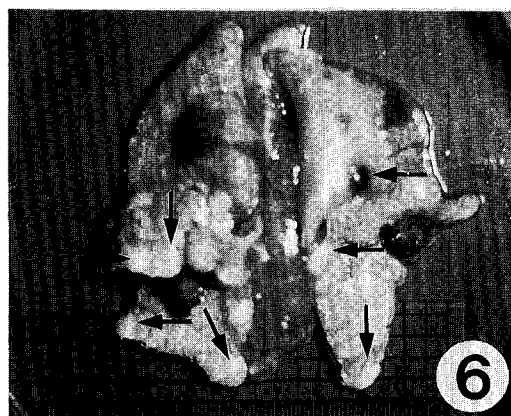


Fig. 6. The lung of cat 150 days after infection.

The fluke cysts were relatively large and harbored only one adult fluke, but no cysts resided 2 or 3 flukes were found.

Microscopic observations: The fluke cysts on 60 days after infection, showed leukocytic infiltration and a thin layer of fibrous tissue, and a thin cystic capsulation around fluke (Fig. 7). There are inflammatory changes, chronic bronchitis and granulomatous proliferation, in the neighbouring tissues of the cyst (Fig. 9, 10). The cyst walls, 150 days after infection, are composed of thick fibrous granulomatous tissues and plasma cells (Fig. 8). There are also eosinophils, neutrophils and histiocytes in the cyst cavity and exudative inflammation and localized fibrination around the eggs (Fig. 11, 12).

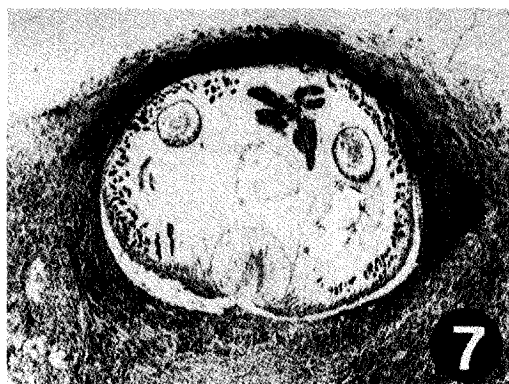


Fig. 7. Granulomatous proliferation of the fluke cyst with eosinophilic infiltration 60 days after infection.



Fig. 10. Chronic bronchitis and granulomatous proliferation in the neighbouring tissue.

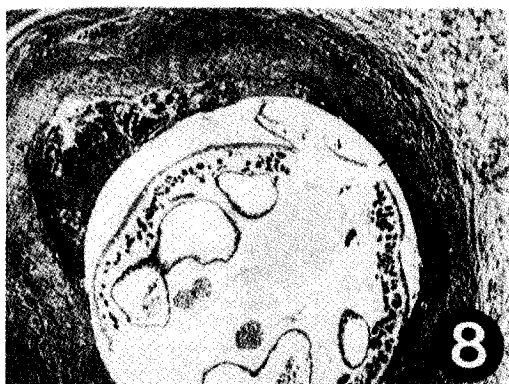


Fig. 8. Thick fibrous cyst surrounded the fluke 150 days after infection.

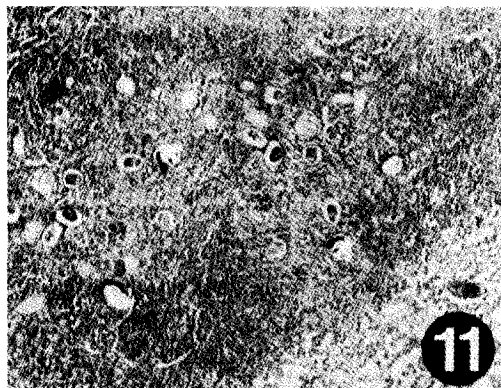


Fig. 11. Formation of granumola and the eggs are found in the bronchiols.

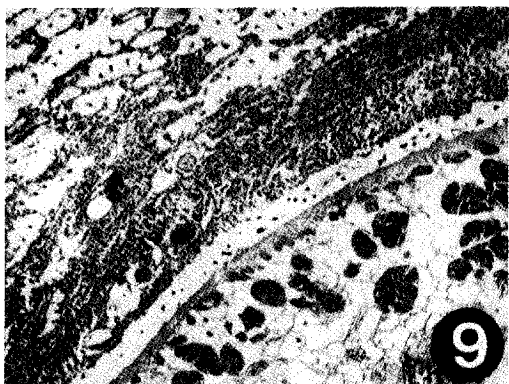


Fig. 9. Fibrination and collagenic changes in the wall of cyst.

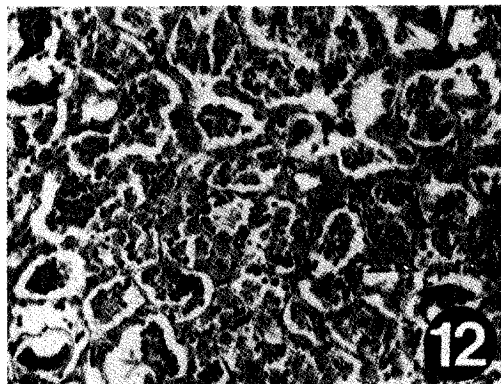


Fig. 12. Exudative inflammation and fibrination around the eggs of *P. westermani*.

## Discussion

The parasitological and histopathological findings of human paragonimiasis had been studied in detail by Otani(1887-1888) and other many investigators, and the knowledges have been piled up mainly on the findings obtained in human cases; but the histopathological findings due to *P. westermani* can not be stated in a generalized form, because they are different depending upon the number of worms, the site of worms, the length of infections, and the species of the host.

Chyu(1962) carried out the parasitological and histopathological study of paragonimiasis in dogs, and reported that the dog infectivity with *P. westermani* was 66.6 to 90.0% and the right lung harbored more flukes than the left lung.

The growth of the flukes was revealed in parallel with the infection period.

Microscopic observations showed that obvious histological changes appeared, such as acute inflammatory processes characterized with hemorrhage, the infiltration of plasma cells and histiocytes. Chronic changes were also marked with the proliferation of fibrous connective tissue. As time progressed, the cell infiltration decreased, cyst walls thickened and the eggs were diffusely spread over the adjacent tissue.

Chyu and Kim(1962) observed X-ray findings of dogs artificially infected with *P. westermani* were compared with the histopathological pictures by necropsy, and reported that pneumothorax was probably initiated by the pleural penetration of larval flukes, the increased lung markings by bronchitis and peri-bronchitis, the infiltrative shadows by inflammatory processes around the cysts and nodules, the nodular shadows by the capsule formation and thickening around the flukes and tubercles, and the ring shadows were caused by air infiltration of the cysts.

The X-ray classification of paragonimiasis is difficult and it requires repeated X-ray taking.

Yoo and Chyu(1966) undertook a study to show the recurrence of the etiological factor, course of migration and pathogenesis of lesion in the extra-pulmonary paragonimiasis in favorable cats and dogs and unfavorable rats, rabbits and guinea pigs hosts.

The animals treated were killed at the different periods up to 10 months, and the location, fate of flukes and the pathogenic changes were observed. It was found that in favorable hosts, either infected with metacercariae or transplanted with larvae or adults, the most flukes migrated to the lung and formed fluke-cysts. A few flukes were found in the ectopic location in younger hosts and in the massive infection. When the unfavorable hosts were given metacercariae orally or young larvae surgically, the flukes could not mature fully, and infrequently formed the ectopic lesion. When the adult flukes were transferred into proper hosts, the characteristic pathological and parasitological changes of ectopic infection were seen. However, the flukes or larvae transplanted into the non-proper hosts were degenerated or were destroyed by necrosis or liquefaction, and many eggs were found in the lesion in some cases. They stressed that young larvae and even adult flukes migrate through various tissues or by the blood stream.

Kim and Lee(1970) reported that immature *P. westermani* recovered from the unfavorable host, develops to adult stage when the immature flukes are ingested by the favorable host.

Chung(1971) stated in the book, Pathology of protozoal and helminthic diseases that a review of the experimental work on dogs, the animal which has long been valuable in the study of lesions caused by *P. westermani*, will undoubtedly help the understanding of the lesions occurring in human hosts.

Lee et al.(1976) conducted a study concerning the susceptibility of some unfavorable host animals, i.e: the hamster, gerbil, mouse, rat, guinea pig, swine, rabbit, hen and duck, to *P. westermani*, morphological development of the immature



flukes in the hosts, and the mode of infection by transport of the immature flukes to the favorable host. They reported that poultry was considered the most unfavorable to *P. westermani*, infection and distribution of the immature flukes in the host being the highest, 76.3% in the muscle, next 12.4% in the pleural cavity, and 5.5% in the peritoneal cavity, 2.5% in the liver, and 3.4% in the lungs in decreasing order.

Some immature flukes recovered from the pleural cavity of the small animals were found to be more developed in morphology, as compared to the common miniature flukes normally found in the muscle.

The immature *P. westermani* parasitized in the unfavorable hosts developed to fully mature adults in the favorable host when ingested by the host.

Ultrastructural observations on the cyst wall by Lee(1979) showed fair numbers of plasma cells and mast cells. Some of the cyst lining epithelial cells showed increased deposit of glycogen granules, suggestive of early metabolic alteration of the respiratory epithelium. Obliterative endobronchiolitis was prominent feature among changes that were not directly associated with flukes or eggs. This finding was more prominent in the distal segments of bronchial trees that harbor the flukes.

Choi et al.(1979) undertook a pathological study to elucidate sequential changes of the lungs in various time intervals following experimental paragonimiasis in dogs and cats. Gross and microscopic examinations of the lung showed that there was no difference in pathological findings between dogs and cats.

Pathological findings were first noticed on 20th day of infection in the thoracic cavity. No fluke was found in the lung parenchyma, but juveniles were seen in the pleural cavity. The juveniles were found for the first time inside the lung parenchyma on 30th day of infection, and the lungs parasitized showed multiple areas of hemorrhage and probably active penetration by

small worms.

*Paragonimus* fluke cyst was essentially composed of fibrous scar and heavy inflammatory cellular infiltrate. Fibrous pleuritis with pleural effusion was very prominent finding and bronchiolitis and focal vascular sclerosis were frequently recognized.

Chi et al.(1982) examined microscopically the tissues of dogs and cats with the main emphasis on changes of the liver and the diaphragm in experimental paragonimiasis.

The liver changes were of 2 folds. The first one was characterized by numerous pin-point or linear tissue defects on the surface of the lobes. Some of these defects were impacted by the larvae. These scratch marks appeared to be of mechanical effect, and the margins were often banal without a significant inflammatory reaction. The second were a large amount of hemosiderin pigment in the Kupffer cells and hemosiderin-laden macrophages in the spleens of dogs and cats. However, no associated degenerative changes were noted in these cells.

The change of diaphragm was quite unique in early phase of infection. Numerous pin-point perforative lesions could be seen, and some of these lesions included migration larval flukes inside the tunnels. The tracts or tunnels appeared to have been made by pressure necrosis and surrounding edema, and subsequently were associated with a massive eosinophilic influx and myocytolysis.

The diaphragmatic changes seemed to be repaired with or without fibrous scar formation.

Park(1986) performed a study to demonstrate acquired immunity against *P. westermani* in cats. Twenty mongrel house cats were divided into 4 equal groups.

The cats of group 1 were infected orally with 10 metacercariae of *P. westermani*, those of group 2 were injected IP with 10 excysted metacercariae, and those of group 3 were injected IP with 1ml of cat immune serum. The group 4 cat

served as controls. 70 days after infection, all cats were challenged orally with 10 metacercariae and killed at 50 days after challenge infection. The flukes were parasitized in the right lung more than those in the left lung, and the acquired immunity to *P. westermani* in the cat model elicited by IP of excysted larvae and not by the oral infection of metacercariae. However, there was no significant difference in the growth of flukes among the 4 groups.

In order to determine the best methods in infecting cats with *P. westermani*, Jeong et al. (1991) conducted a study to compare the efficacy of 3 methods, e. g. oral administration of the cysts, IP of excysted metacercariae and IP of metacercariae, and reported that IP of excysted metacercariae of *P. westermani* is regarded as the best method in infecting cats.

In the present study, the recovery rate of adult flukes in the lung of cats decreased with the increase of the number of metacercariae given. In addition, the adult flukes were found more in the right lung than in the left lung. These results are in general agreement with those observed previously in dogs and cats by Chyn(1962) and Park(1986). Attention is called to fact that IP of the intact metacercariae is found to be more effective available infecting method than oral administration and IP or the excysted metacercariae. Therefore, it seemed that some of the metacercariae excysted in the intestinal canal failed to penetrate the intestinal wall and a few metacercariae given orally failed to infect because of regurgitation or vomiting of a part of beef containing the metacercariae after feeding. The results are similar to the data reported by Jeong et al.(1991).

The results of the histopathological observations paralleled those of the parasitological examinations. A good correlation was observed between the number of flukes recovered at necropsy and the severity of lung damage in each animal. The tissue responses of the lung observed in this series of cats were in agreement with those re-

ported previously in dogs and cats by Chyu (1962), Chyu and Kim(1962), Lee(1979) and Choi et al.(1979), and those observed in unfavorable host animals(Lee et al., 1976).

There are two theories concerning the pathogenesis of the formation of the fluke cyst in the lung. (1) The theory of softening of the lung tissue. (2) The bronchiectatic theory.

The authors recognized the importance of these 2 theories in studying the experimental paragonimiasis in animals. However it is impossible to clarify because the pathological findings were limited 2 periods, 90 days and 150 days after infection. Further pathological studies are needed to evaluate the 2 theories on the pathogenesis.

### Summary

To elucidate the distribution and growth of *Paragonimus westermani* and subsequent histopathological changes in the lung of infected cats, and experimental study was undertaken.

One group of cats was infected orally with different number of the metacercariae and given 10 metacercariae, the other group was infected 3 different methods, fed orally and the intraperitoneal injection(IP) of metacercariae or IP of excysted metacercariae.

The cats were killed 90 days and 150 days after infection, and microscopic observations were made.

The recovery rate of adult *P. westermani* in the lung of cats decreased with the increase in the number of metacercariae given.

The adult flukes were parasitized more in the right lung than in the left lung, and the largest number in the lower lobe of both lungs.

IP of metacercariae was found the best method in infecting the flukes compared with IP of excysted metacercariae or oral administration.

The fluke cysts on 90 days after injection showed leukocytic infiltration, a thin layer of fibrous tissue and a thin cystic capsulation around the fluke.

The neighbouring tissue of the cyst showed inflammatory change.

150 days after infection, the cyst wall revealed thick fibrous granulomatous tissues and plasm cells.

Key words : *Paragonimus westermani*, paragonimiasis, Histopathological change

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=국문초록=

## 실험적 폐흡충증의 기생충학 및 조직병리학적 연구

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고양이 폐흡충증에서 폐흡충의 분포상과 발육에 따른 조직병리학적 변화를 규명하기 위해 실험하였다.

실험군 고양이는 두 군으로 나누어 한 군에는 5, 10, 20개의 각각 다른 수의 피낭유충을 경구감염시킨 후 다시 10개의 피낭유충으로 2차 감염시켰다. 다른 군은 10개의 피낭유충의 경구투여, 복강내 주사 및 10개의 탈낭유충의 복강내 주사 등 3가지 방법으로 감염시켰다. 감염 후 90일과 150일에 고양이를 도살하여 기생충학 및 조직병리학적으로 관찰하였다.

투여한 피낭유충수가 많을수록 폐흡충 성충회수율은 낮았고, 왼쪽 폐보다 오른쪽 폐에서 더 많은 수의 성충이 기생되어 있었다. 양쪽 폐 모두 하엽에서 가장 많은 수의 성충이 회수되었다.

폐흡충을 감염시키는 방법으로는 피낭유충의 복강내 주사가 피낭유충의 경구투여나 탈낭유충의 복강내 주사보다 더 효과적인 것을 알 수 있었다.

감염 후 90일째 폐흡충낭을 둘러싼 얇은 섬유조직층과 침윤된 백혈구를 볼 수 있었고, 주변 조직에도 염증소견을 볼 수 있었다.

감염 후 150일째에는 폐흡충낭이 두꺼운 섬유 육아조직과 형질세포로 싸여 있었다.