

## Studies on the Lungfluke, *Paragonimus iloktsuenensis*

### III. Migration, development and egg-production in albino rats

Byong-Seol Seo and Won-Jae Lee

Department of Parasitology and Institute of Endemic Diseases,  
College of Medicine, Seoul National University

#### INTRODUCTION

Since Chen (1940) gave the first detailed description on the morphological and developmental studies of *Paragonimus iloktsuenensis*, additional recognitions to the life cycle, migration and development in the final hosts and susceptibility of the various hosts have also been well established by many investigators in Japan and Taiwan (Miyazaki, 1944; Tomimura et al., 1958; Yoshida, 1959; Tomimura et al., 1960; Chiu, 1962).

In the previous papers (Seo et Koo, 1971; Seo et Kwak, 1972), senior author reported adult worms in house rats, naturally infected and also their observation on the development of this lungfluke in experimentally infected mice and suggested that mouse was less susceptible for obtaining the full maturity of the fluke and no cystic formation in the lungs was recognized. However, there seemed to be little informations in details on the host-parasite relationships of the fluke in the albino rats. In view of the feasibility of albino rats as the most optimum laboratory hosts for the experimental paragonimiasis, the authors have attempted to know the host-parasite relationships in rats, particularly the development and egg-laying capacity of the worm according to the course of infection.

The present study summarized observations on the route of migration, development and the egg passage pattern of the worms in experimentally infected albino rats in various stages of infection.

#### METHODS AND MATERIALS

The albino rats used in this experiment were all obtained from market through commercial dealers and were healthy adolescents weighing approximately by 120-150g at the time of infection.

Fecal examinations prior to exposure revealed no lungfluke ova. Metacercariae for experimental feeding to the rats were obtained from the crabs; *Sesarma dehaani* which were collected at the basin areas of Sumjin River, South Kyong Sang Do (Province). The technique applied for the isolation of metacercariae has already been described in the previous report (Seo et Kwak, 1972).

The albino rats were fasted previously for a day prior to exposure to infection. The experimental feeding of metacercariae to rats was made on the quantitative basis.

In order to follow the route of migration in earlier period in the host, mice and rats were experimentally fed with a certain number of encysted or excysted metacercariae and sacrificed. The location of worms found at autopsy

was recorded according to the course of infection. The penetration of the intestinal wall of rats by the young worm was verified using Evans-blue technique and also histological finding.

1.0 to 1.5cc per 100g of body weight of 0.3 per cent Evans-blue solution was injected into the femoral vein and 15 minutes after injection, the small intestine of rats was thoroughly examined for the site of the penetration.

Fecal examination was begun at approximately 30 days after infection and continued at 4 to 5 days intervals until the first detection of eggs. Egg counts were entirely based upon an examination of fecal pellets collected during a period of 24 hours for the first period of several weeks after passage of eggs and later the average egg counting was also made from the pellets collected for 2 to 3 day period. After immersion of the collected pellets in saline solution overnight, the suspension was strained out for removing the coarse debris and centrifugalized for washings. After decanting the supernate, the final sediment in the centrifuge tube mixed with 10cc of 4 per cent formaldehyde and allowed to stand for five minutes and then 3cc of ether were added and vigorously shaken. Following centrifugalization at about 1,500 rpm for 2 minutes, the supernate was decanted again. A thin film of the sediment was placed on a slide and the number of eggs was counted under the low power magnification of a compound microscope.

All of rats were sacrificed after a certain period to follow the route of migration as the development. Worms collected at autopsy were stained with Semichon's acetocarmin for the parasitologic examination and were measured. Host tissues and worm cysts in the lesion of the lungs for pathohistological examination were fixed in neutral buffered 10%

formalin and stained with Delafield's hematoxylin and eosin.

## RESULTS

### The route of migration in prepatent period:

As shown in Table 1, the young flukes penetrated into the abdominal cavity were found in experimentally infected mice one hour after feeding of metacercariae with the average recovery rate of 45.0%. Blue spots were also recognized one hour after infection in rats, applied the Evans-blue technique, and the lesions penetrated were verified by histological findings in the upper portion of small intestine. In the course of infection, the penetration into the liver was revealed in rat 20 days after infection (Plate Fig. 4). The young worms were detected by teasing of the liver and pathologic lesions were also recognized in the tissue section. In the thoracic cavity of rats infected, the worms were first found 2 to 3 weeks after infection and the worm cysts seemed to form 3 to 4 weeks after infection in rat hosts.

The results obtained in the present experiment suggested that the migration of the lungfluke in this prepatant period occurred from intestinal lumen into abdominal cavity one hour after infection and was completed from the abdominal cavity to the thoracic cavity through the penetration of the liver from 14 days up-to 21 days after infection. Thereafter, the formation of worm cyst in the pulmonary tissues began three to four weeks after infection (Plate Fig. 7).

### Development of worms in albino rats:

As indicated in Table 2, measurements of worms collected at various developmental stages were made on the specimens stained with acetocarmin.

**Table 1.** Experiments in mice and albino rats infected with metacercariae of *Paragonimus iloktsuenensis*.

No. of experi- ment	No. of animals used	Duration of infection	Total No. of Mc. used	No. of worms found by location						No. of worms found	Rates of worm recovery (%)
				Abd. cavity	Liver	Thorax		Lung			
						Rt.	Lt.	Rt.	Lt.		
Mice-1	1	1hr	20	9						9	45
Mice-2	1	3hrs	20	3						3	15
Rat-1	1	9 "	30	4						4	13.3
" -2	1	12 "	25	3						3	12.0
" -3	1	24 "	20	5						5	25.0
" -4	1	1week	20	2						2	10.0
" -5	1	2 "	20	2						2	10.0
" -6	2	3 "	50		1	3	1			5	10.0
" -7	2	4 "	50			1		4(2)*	1(1)	6	12.0
" -8	1	5 "	30					2(2)	4(2)	6	20.0
" -9	6	23-25 "	65					7(5)	3(3)	10	15.4
" -10	6	Ca 1 year	90					2(2)	4(4)	6	6.7
Total	24		440	28	1	4	1	15(11)	12(10)	61	13.9

\* : No. in bracket means worm cysts in the lungs. Mc: Metacercaria.

Within 24 hours after infection: All worms were found only in the abdominal cavity. The enlargement of the cell masses in genital primordia was clearly observed. The sizes of the worms obtained at 3, 6, 12 and 24 hours after infection were in the range of  $0.19 \pm 0.03$ mm long and  $0.11 \pm 0.01$ mm wide (Plate Fig. 8 & 12).

Within one week after infection: All worms in this stage remained still in the abdominal cavity. The structural outlines of oral sucker, acetabulum, pharynx and esophagus became distinct in 7 day-old worm. The cell number of the genital primordia, such as ovary and testes, increased and particularly two cell masses of testis primordium seemed to separately descend posterior one third of the body length and occupied both side of inner field of third loop of bilateral intestinal ceca (Plate Fig. 12). The excretory bladder extended in the median line with the shape of long straight I-form from posterior excretory opening to the site of the intestinal bifurcation. The uterine tubules and vitelline cells were not appeared

yet. The cuticular spines not serrated, but pointed in shape. The size of this stage worm showed in the range of  $1.19 \pm 0.21$ mm  $\times$   $0.90 \pm 0.10$ mm (Plate Fig. 9).

Within two weeks after infection: The adolescent stage of the worm began to find in the thoracic cavity. All morphological characteristics of oral sucker, acetabulum, pharynx, esophagus and intestine became much distinctly shaped. The blunt branches of ovary or testes were recognizable. The vitelline cells not yet fully developed. The cell masses posterior to the ovary enlarged enough to show the oötype and vague structure of uterine tubules. The seminal vesicle was hardly to recognize. The cuticular spines grouped in to 2 to 6. The measurements of the worms in this period showed  $2.64 \pm 0.42$  mm  $\times$   $1.37 \pm 0.15$ mm.

Within three weeks after infection: All worms were found in the thoracic cavity or in the worm cysts. The ovary and testes became to show close resemblance to those of adult worms. The uterine tubules, seminal

Table 2. Measurements of *Paragonimus iloktsuenensis* collected experimentally infected albino rats.

(Worms stained in acetocarmin. Unit: mm)

Days after infection	No. of worms measured	Body		Oral sucker		Ventral sucker	
		Length	Width	Length	Width	Length	Width
6~24hrs	12	0.19±0.03	0.11±0.01	0.03	0.03	0.03	0.04
1 week	2	1.19±0.21	0.90±0.10	0.18	0.18	0.20	0.23
2 "	2	2.64±0.42	1.37±0.15	0.22	0.26	0.29	0.30
3 "	5	4.33±0.56	2.17±0.45	0.34	0.41	0.45	0.52
4 "	6	6.21±0.56	2.84±0.21	0.39	0.46	0.50	0.54
5 "	6	8.89±0.53	3.99±0.10	0.45	0.61	0.66	0.68
5 over	9	10.04±1.73	6.14±1.15	0.54	0.76	0.76	0.82

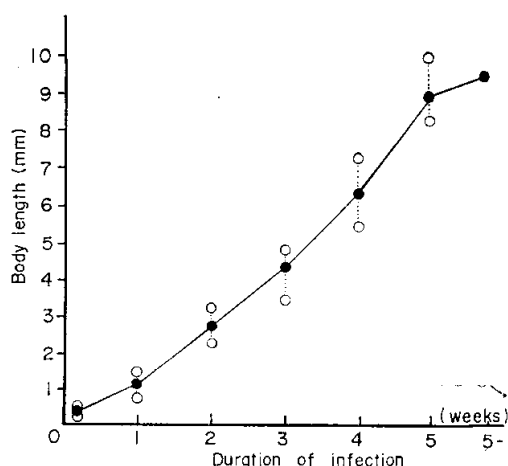


Fig. 1. Growth curve of *P. iloktsuenensis* according to the course of infection in albino rats.

vesicle and oötype also appeared distinctly. The increased distribution of vitelline cells in subcuticular fields and vague outline of vitelline duct were observed. The body size of the worm became  $4.33 \pm 0.56 \text{ mm} \times 2.17 \pm 0.45 \text{ mm}$  in average (Plate Fig. 5).

More than four weeks after infection: The testes showed the full development in shape and the ovary became more branched like characteristic coral form. The vitelline duct clearly appeared. Eggs in uterine tubules were abundantly observed (Plate Fig. 6 & 3). The spermatozoa in seminal vesicle were also recognized. In about five week-old worm, all anatomical structures seemed to reach the

full maturity and abundant mature eggs filled in the uterine tubules were visible in this stage. The vitelline glands were widely distributed in subcuticular areas and particularly densely aggregated in dorsal side (Plate Fig. 1 & 2).

As indicated in Fig. 1, it is suggested that the full growth of the fluke was completed about 4 to 5 weeks after infection. The body length and width of adult worms were in average  $10.04 \pm 1.73 \text{ mm} \times 6.14 \pm 1.15 \text{ mm}$ .

The development of the worm cysts in the lung of albino rats has been observed. The first worm cyst were formed in the lung of the rats 24 days after infection. Six out of 21 worm cysts observed were found in the dorsal surface of the right lung (Fig. 2). The size of worm cyst measured  $18.3 \times 12.4 \text{ mm}$ . One or two fully matured adult worms were found usually collected from a cyst.

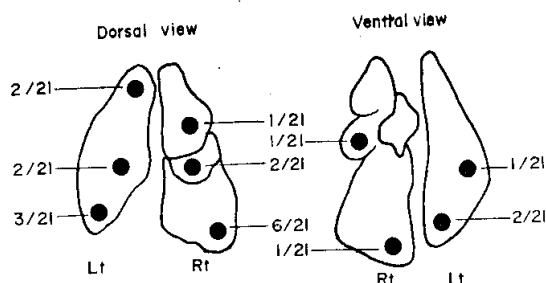


Fig. 2. Locations of 21 worm cysts found in experimentally infected rats.

Table 3.

Egg-passage of *Paragonimus iloktsuenensis* in albino rats.

Rat No.	No. of Mc. fed	First egg appearance	Duration of egg product.	Total No. of eggs passed	Days in peak period	E. P. D. in peak	No. of worm cyst		Days until sacrificed after infection	No. of worms found in cyst
						per worm	Lt. lung	Rt. lung		
9(1)	10	82 day	106 day	14,238	7 day	698	1	0	180	1
9(2)	10	81	29	21,645	7	899	0	1	120	2
9(3)	15	73	106	76,198	16	1,304	0	1	170	2
9(4)	15	73	106	96,158	14	1,082	1	2	170	3
9(5)	10	76	101	19,161	14	795	0	1	170	1
9(6)	5	68	110	15,967	5	971	1	0	160	1
10(1)	20	76	42	109	6	13	0	1	Ca. 1 year	1
10(2)	20	68	144	288	8	15	1	0	"	1
10(3)	20	78	132	1,937	8	163	1	0	"	1
10(4)	20	76	132	5,357	22	212	1	0	"	1
10(5)	5	77	144	1,223	16(10)*	30(32)**	1	0	"	1
10(6)	5	81	152	3,669	10(16)	199(71)	0	1	"	1

Mc: Metacercariae.

\*, \*\*: Days &amp; E. P. D. in the second peak period

**Egg laying capacity in albino rats:** As shown in Fig. 3 & 4, there were marked variations not only in the intervals between time of exposure and first appearance of egg in feces, but also in the periods of peak production of eggs and definite egg reduction, and particularly the number of eggs passed per day.

As indicated in Table 3, although the egg-laying patterns varied considerably from one individual to another, eggs first appeared in the feces 68 to 82 days after infection. The peaks of egg production were of irregular, shorter duration. Usually after the first appearance of eggs in pellets, there were sharp increase and subsequently eggs were passed in decreased quantities until they disappeared. The peaks of egg production occurred roughly between one to three weeks. The durations of egg production were also roughly estimated 18 to 21 weeks after the first passage of eggs. In certain number of rats, the second peaks of egg production were also recognized.

In the group of Rat No. 10(1-6), the autopsies were performed about a year after

infection and the exact number of worms retained in the cysts was hardly estimated because of degeneration of worms and worm-cysts. Therefore, the mean number of eggs per day per worm was counted during a peak period in the course of infection only in the group of Rat No. 9 (1-6), in which the accurate estimation of the worm burden was made possible at autopsy. The E. P. D. per worm in this group was in the ranges of 698 to 1,304 with a mean of  $958 \pm 197$ .

Although no parasites were present in Rat No. 10 (5-6) at autopsy, eggs were usually found in the worm cysts. Particularly in case of Rat No. 10(1-2), it was noted that the duration of egg production was too short to show any peak production of eggs (Table 3). However, large number of eggs were found deposited in the lungs, and it was revealed in the histopathological specimens that cluster of eggs were trapped by host tissue reactions and never released into the intestine. These histological findings gave the reasonable explanation of the exceptionally irregular and poor passage of eggs in the cases, above two Rat No. 10 (1-2) (Plate Fig. 10 and 11).

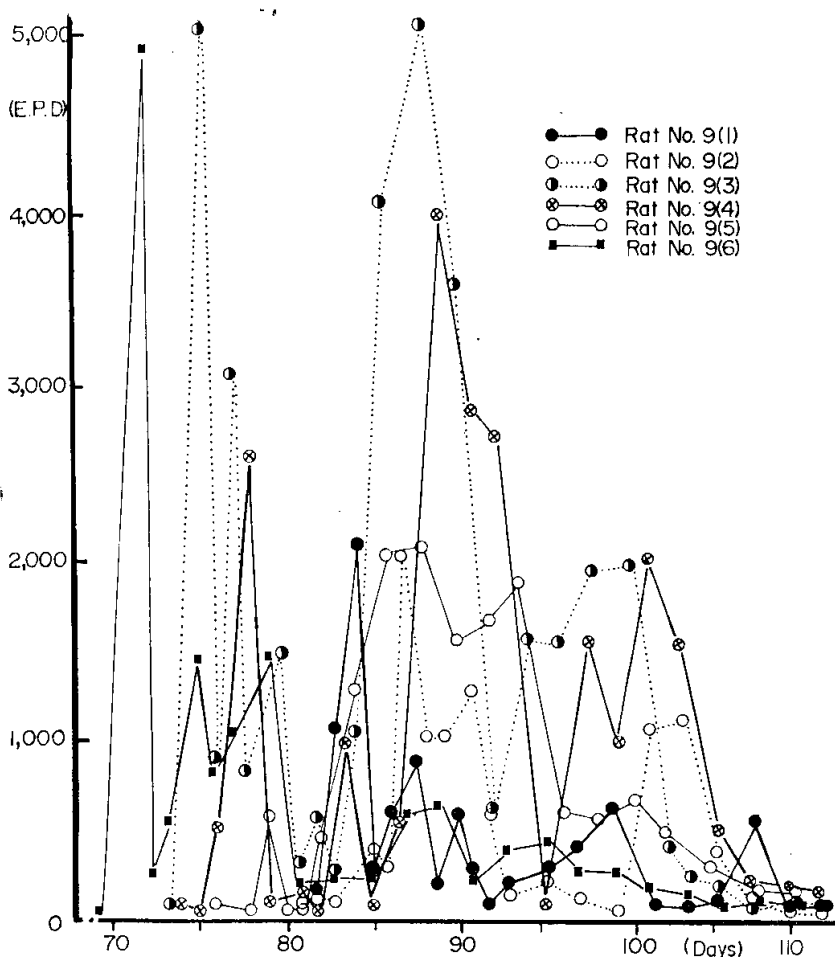


Fig. 3. Fluctuation showing the egg-passage of *Paragonimus iloktsuenensis* in Rat No. 9(1-6).

## DISCUSSION

In the previous paper (Seo et Kwak, 1972), experimental infection in mice with *Paragonimus iloktsuenensis* was reported and it was revealed that the mouse host was easily infected with metacercariae of the fluke with fairly high rate of recovery. However, it was not enough to be susceptible for obtaining the full maturity of the fluke. And the positive appearance of ova in the feces was hardly expected. In the previous experiment, Seo et al. failed to find the formation of the worm cysts in the lungs at autopsy of mouse host.

Since Chen's first description on the morphology of this lungfluke has appeared at 1940, various investigators have described the development of the worm in the final hosts experimentally fed with the metacercariae of this fluke, such as albino rats (Miyazaki, 1944; Tomimura et al., 1958; Chiu, 1962), albino mice (Miyazaki, 1944; Seo et Kwak, 1972), cats (Eto, 1934; Chiu, 1962) and rabbits (Eto, 1934). However, little informations have been known on the development of *P. iloktsuenensis* in albino rats according to the course of infection. Particularly, egg-laying capacity of the fluke in albino rats has never been dealt by workers except Chiu's study (Chiu,

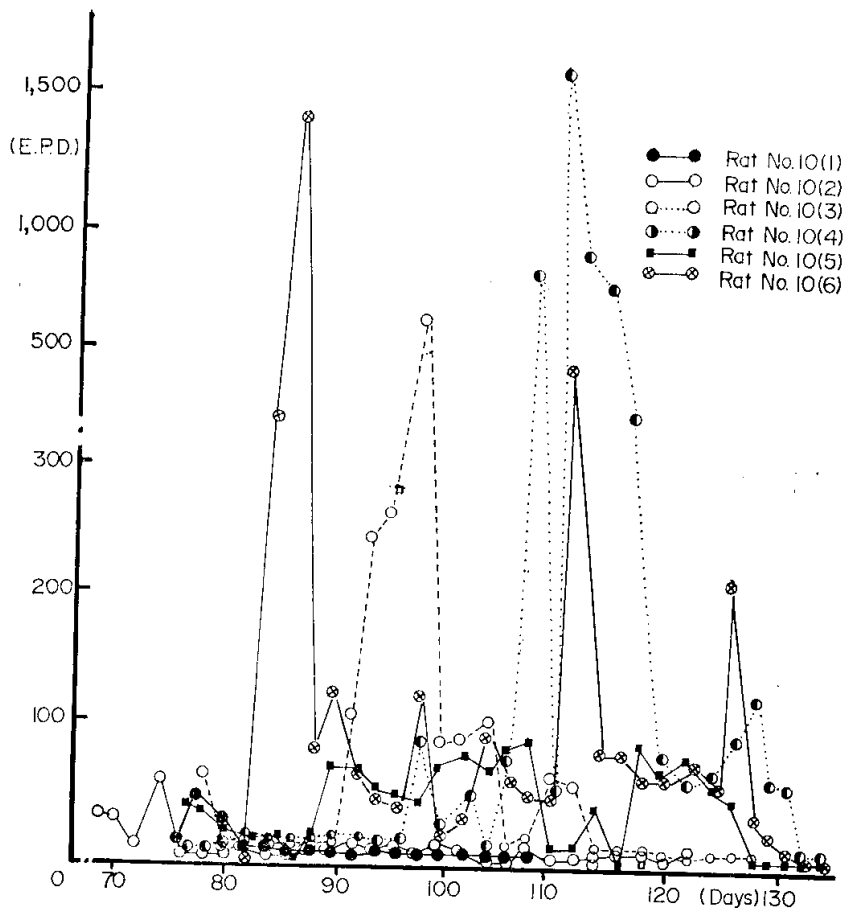


Fig. 4. Fluctuations showing the egg-passage of *Paragonimus iloktsuenensis* in Rat No. 10 (1-6).

1962).

In his experimental studies of *Paragonimus ohirai* in albino rats, using Evans-blue technique to trace out the migration route in the earlier period, Okura (1963 a) reported that the young flukes appeared in the abdominal cavity one to six hours after infection and completed their penetration into the liver 14 days after infection and thereafter migrated into the thoracic cavity and then began to form the worm cyst in the lungs 21 days after feeding.

In the comparative studies on *Paragonimus miyazakii* and *P. ohirai* in the experimentally infected albino rats, Tada (1969) observed the necrotic lesions and abscess formation on

the surface of the liver, caused by penetration of the migrating young flukes into the liver.

Considering the close resemblance of *P. iloktsuenensis* to *P. ohirai* in developmental and morphological characteristics, authors attempted to follow the route of migration of *P. iloktsuenensis* in prepatent period, and observed the almost same pattern of migration. In the present study, the young worms were found penetrated in the abdominal cavity one hour after infection in mice and rats and their penetration into the liver was also ascertained 20 days after feeding in rats.

According to Okura's observation on the development of *P. ohirai* in albino rats, the ovary of this fluke completed its full develop-

pment 25 days after infection with its characteristic coral shape, meanwhile, the rather earlier development of testes was observed 21 days after infection with first appearance of spermatozoa in seminal vesicle. Okura also noted that eggs in uterine tubules were first found 28 days after infection.

These findings were well in accord with those of author's observations except the period of the first appearance of eggs in uterus, which was in the present study on 14th day after infection. The measurements of the body length and width of the two species were compared with each other according to the developmental stages. There were no distinct differences in size.

Chiu (1962) experimentally infected five rats with the unknown number of metacercariae of *P. iloktsuenensis*. Four out of five rats were injected into abdominal cavity with excysted young fluke and the remained one was fed with uncalculated, encysted metacercariae. And then he obtained 8 mature adult worms (2 in pleural cavity, 3 in lungs and 3 in livers). The passage of eggs was also observed by him for about two months in a laboratory rat and a wild rat, both of them were experimentally fed with uncalculated metacercariae. According to his results, metacercariae developed to maturity in rats 31 days after infection and eggs began to appear in feces at 41-45 days.

In a laboratory rat which was fed with encysted, uncalculated metacercariae, he reported that the first appearance of eggs in feces was 44-45 days after feeding and the peak of egg production was shown 26th day and suddenly the egg output, however, decreased at the 28th day and completely disappeared at 34th day and at autopsy of this rat, two decomposed worms were found in the

right lung. There was the EPD of 7th to 28th day ranged from 8,856 to 67,700 with an average of  $23,860 \pm 10,823$ . Because of his observation made on one rat, he presumably never recognized the remarkable individual variations with the irregular daily fluctuations of egg passages of *P. iloktsuenensis* in rats, as confirmed by the present experiment. So far the first appearance of eggs in feces concerned, in the present study, it was in average 76 days after infection, meanwhile, it was 41-45 days in the above experiment. The duration and peak of egg production and mean number of EPD were also quite different from each other.

As verified by pathohistological observation in the present study, the marked individual variations of egg passage patterns may be reflected by the tissue-trapping degree and release activity of eggs at the deposited site in the lungs.

In spite of the irregularity observed in the pattern of egg passage, it was concluded that the passage of eggs may be used as a criterion for the determination of certain effects, such as chemotherapy, at least in a certain period in the course of experimentally known infection.

## SUMMARY

Experimental infection in albino rats with *Paragonimus iloktsuenensis* was carried out to trace the route of migration and development of the fluke in albino rats. The egg-passage pattern of the fluke has also been followed in the rats for periods up to about a year after feeding of metacercariae.

From the present experiment, it was found that all the albino rats and mice became infected. Worm recoveries in rats varied from 6.7 to 25.0 per cent. In prepatent period of



developmental cycle in rats, migration from intestine to abdominal cavity was completed one to six hours after infection. The young fluke appeared in the thoracic cavity via the penetration of liver 14 to 21 days after infection. The worm cyst formation in the lungs was first observed 24 days after infection. It took more than 35 days to reach the full maturity in rats.

The gradual increase of worm size was noticed up to 4 to 5 weeks after infection. The average body size of adult worm was measured  $10.04 \pm 1.73 \text{ mm}$  long  $\times$   $6.14 \pm 1.15 \text{ mm}$  wide. Following observations in albino rats were made on the genital development of the fluke. The genital primordia of the young worm began to develop immediately after infection. Three to four weeks after infection, the ovary showed its characteristic coral shaped branches. The branched testes also appeared two weeks after infection, and spermatozoa in seminal vesicle were visible in stained slides three weeks after infection. The development of vitelline glands and uterus were also completed almost four to five weeks after exposure.

The pattern of egg passage of the fluke by rat hosts showed marked variations. However, it was revealed that the first appearance of eggs in feces was from 68 to 82 days after infection and the peak of egg production appeared usually within one to five weeks after the first detection of eggs in feces. The mean number of egg counts per day per worm was  $958 \pm 197$ .

From the pathohistological observation in connection with the egg output it was noted that a worm cyst, retained usually one or two worms, was most commonly found and the irregularity of egg-release was presumably caused by the tissue-trapped eggs in the

lungs.

—國文抄錄—

# 怡樂村肺吸虫(*Paragonimus iloktsuenensis* Chen 1940)에 關한 研究

## Ⅲ. 白鼠體內에서의 移行 經路, 發育 및 產卵能力

서울大學校 醫科大學 寄生蟲學敎室  
및 風土病研究所

徐 丙 高·李 源 宰

종숙주 내에서의 怡樂村肺吸虫의 初期 移行經路 및 發育狀況을 觀察할 目的으로 白鼠 및 마우스를 本吸虫 被囊幼虫으로 實驗感染을 試圖하였다. 동시에 白鼠體內에서의 本虫의 產卵能力을 觀察하였다.

白鼠 및 마우스는 다같이 높은 感染率로 容易하게 감염시킬 수 있었으나 虫體回收率은 相當한 差가 있었으며 白鼠에서의 回收率은 平均 6.7~25.0%였다.

發育初期에 있어 移行經路를 보면 腸에서 腹腔까지의 移動은 感染後 한 時間에서 볼 수 있었으며 大體로 6時間 以內에 完了된다. 幼若吸虫이 肝을 經由 腹腔에 出現하는 것은 感染後 15日에서 35日까지였으며 完全 成熟虫體가 되기까지 約 35日 以上이 必要한 것 같다.

發育時期에 있는 虫體의 成長은 漸進的이었으며 成虫體의 平均크기는  $10.04 \pm 1.73 \text{ mm} \times 6.14 \pm 1.15 \text{ mm}$ 였다.

白鼠 體內에서의 發育狀況을 보면 發育初期幼虫에서 生殖原基의 細胞數는 感染直後부터 增加하기 시작하였다. 特히 卵巢가 完全成熟하고 典型的인 珊瑚型으로 發育하는데 感染後 3 乃至 4週가 必要하나 辜丸은 約 2週면 相當히 分岐되고 精子가 貯精囊에 보이기 시작하는 것은 約 3週後였다. 卵黃腺 및 子宮의 發育이 完成되기까지 感染後 約 4週 乃至 5週가 所要되는 것 같다.

白鼠 體內에서의 本虫 產卵作用을 觀察하였던 바 甚한 變動이 認定되기는 하였으나 다음과 같은 것을 綜合할 수 있었다.

虫卵 便內 最初 出現 時日은 感染後 68日에서 82日 사이에 있었다. 產卵最高値를 나타내는 時期는 大體로 虫卵 便內出現 후 1週에서 5週사이에 있었으며 平均 E. P. D. (Number of eggs per day : 每日產卵數)는  $958 \pm 197$ 였다.

剖檢所見에서 보면 肺組織內 虫囊은 大體로 한個 乃至 두個 形成된 것이 大部分이었으며 採取虫體數는 한

個 또는 두個였었다. 肺組織의 病理組織學의 所見으로 미루어 볼때 産卵作用의 甚한 不規則性이 肺組織內에 捕捉된 虫卵에 起因한다고 생각되었다.

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## Explanation of Plate

- Fig. 1. Fully matured 120 day-old adult of *Paragonimus iloktsuenensis* in albino rat, stained with Semichon's acetocarmin.
- Fig. 2. High magnification of genital organs of the above adult(Fig.1).
- Fig. 3. High magnification of genital organs of four week-old worm(Fig.6).
- Fig. 4. Twenty day-old worm, collected from liver and stained with acetocarmin.
- Fig. 5. Three week-old worm, collected from thoracic cavity and stained with acetocarmin.
- Fig. 6. Four week-old worm, collected from worm cyst and stained with acetocarmin.
- Fig. 7. Tissue section from the lungs of the rat infected with *P. iloktsuenensis*.
- Fig. 8. and 12. One hour, and 24 hour-old worms, stained with acetocarmin.
- Fig. 9. One week-old worm, stained with acetocarmin.
- Fig.10. and 11. Low power magnification of the tissue trapped egg-mass in the lungs of the rat infected with *P. iloktsuenensis*. And its high magnification of the cluster of eggs.
- Ov:Ovary, Ut:Uterus, Vt:Vitelline duct, Int:Intestine, T:Testes and E:Egg-mass.

