

Studies on the Lungfluke, *Paragonimus iloktsuenensis*

II. On the Metacercaria, the Second Intermediate Hosts and the Development in Mice

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INTRODUCTION

The metacercariae of *Paragonimus iloktsuenensis* have recently been isolated in Korea from the crab host, *Sesarma dehaani* for the first time in 1970 (Yokogawa et al., 1971). And later, house rats, *Rattus norvegicus* have also been ascertained to serve as definitive host of the fluke by Seo et al. (1971) at the Nakdong & Sumjin River Deltas, Kyong-Sang Nam Do (Province). Up to the present time, therefore, two species of *Paragonimus*, such as *Paragonimus westermani* and *Paragonimus iloktsuenensis*, have been reported in Korea. Meanwhile, three more species of *Paragonimus*, except the above two, such as *P. ohirai*, *P. miyazakii* and *P. sadoensis* have been described in Japan.

The possible occurrence of some other species of *Paragonimus* should be carefully taken into consideration. In this connection, the further taxonomic approaches are necessitated. In this sense, authors attempted to better recognize the morphology of metacercaria and to know the development of the adult worm in the experimentally infected mouse host.

MATERIALS AND METHODS

Isolation of metacercariae: All of the metacercariae used in this study were isolated from the crabs mainly *Sesarma dehaani* which was collected at two delta areas of Nakdong and Sumjin Rivers, South Kyong-Sang Do (Province). The collected crabs were kept in an aquarium with shallow water and rocks for the hiding place for over a month. For the collection of metacercariae, the liver of crabs was removed from inside of the carapace taken away from the soft parts of the cephalothorax, and out into several small pieces. Each of these liver pieces was separately pressed between two glass plates and was thoroughly searched for the metacercariae of *Paragonimus* under the binocular dissecting microscope. To isolate metacercariae from the piece of liver tissue, the glass plate on the tissue was taken off carefully and then the metacercaria was put apart from the tissue with the aid of dissecting needles under the microscope. For the detailed observation, the isolated metacercaria was slightly pressed with a cover glass. The encysted metacercariae were stored in the 0.85% NaCl solution, in refrigerator 5°C for

a couple of days.

Survey of the second intermediate hosts: From March, 1971 to August, 1972, the following four species of crabs; 569 *Sesarma dehaani*, 39 *Helice tridens tridens*, 7 *Sesarma intermedia* and *Sesarma haematocheir* were collected from the above mentioned two areas of Hadan and Hadong, where *S. dehaani* were already found infected with metacercariae of *P. iloktsuenensis* (Yokogawa et al., 1971 and Seo et al., 1971). In order to know the rate and intensity of infection, the crabs were separated into groups according to size, sex and the place of collection. And to know the distribution of the metacercariae in the crab hosts and their total number, the liver, gill, muscle and other organs were also separately examined in some crabs. The average number of metacercariae per crab infected was counted.

Experimental infection in mice with metacercariae: Mice were selected as the animal hosts in present study. The mice were fasted previously for a day prior to exposure to infection. They were experimentally fed with certain number of counted metacercariae in liver tissue of crabs. Autopsies of experimentally infected mice were made at various intervals after the exposure. The thoracic and abdominal cavities were thoroughly examined for the detection of worms, particularly the lungs were removed and teased in warm saline solution for the search of the fluke. The number and location of worms found were noted.

RESULTS

The structure of metacercariae: The metacercaria of *P. iloktsuenensis* was oval or elongated elliptical in shape and rather flat, measuring 0.342mm(0.282—0.364)×0.289mm

(0.254—0.312). The outer most layer of cyst wall was 0.0015—0.003mm in thickness and transparent, thin and easily broken. The inner cyst wall was lacking. The cysts was pedunculated with a short fibrous attachment to the liver tissues of the crab host at one end of outer cyst wall. Between larva and cystwall there was a considerable space, in which the larva expanded and contracted it's body length. The characteristic I-formed excretory vesicle and winded ceca were distinctly recognizable. The slightly pinkish pigments were seen. The excysted metacercaria was an elongated oval in shape, tapering to its posterior extremity. It measured 0.339mm (0.299—0.388) long, 0.193mm(0.177—0.218) wide. The surface of the entire body was covered by fairly distinct minute cuticular spines. No pigmented eye-spots were present. The oral sucker was rather elliptical and subterminal in shape, measuring 0.042mm×0.047mm. It was smaller than the acetabulum, lying at or little posteriorly to center of body, measuring 0.047mm×0.050mm. The oral sucker was provided with a stylet (0.015mm×0.002mm) on its dorsal margin. The prepharynx was not seen and the pharynx was round, measuring 0.025mm×0.026mm. The esophagus was relatively short, measuring 0.016mm. The intestinal ceca narrowed after bifurcation proceeded bilaterally to the posterior part of the body with three inwardly formed loops and attained to its posterior extremity of the body. The excretory bladder was long straight I-formed, containing excretory granules, measuring from 0.001mm×0.003mm. The excretory pore opened terminally. The genital primordia were not see in fresh specimen. However, the primordia of two testes and ovary were clearly recognized in the acetocarmin

Table 1. Measurements of metacercariae of *Paragonimus iloktsuenensis*.

| Encysted metacercariae (30) in unstained, non fixed specimens | | (in average) | unit: mm |
|--|---------------------------------------|--------------------------------------|----------|
| Size | 0.342(0.282—0.364)×0.289(0.254—0.312) | | |
| Thickness of cyst wall | 0.003(0.002—0.004) | | |
| Excysted metacercariae (15) | | (in average) | unit: mm |
| | Stained specimen (5) | 5% Formalin fixed specimen (10) | |
| Size (Length×Width) | 0.238×0.129 | 0.339×0.193(0.388—0.299×0.218—0.177) | |
| Oral sucker | 0.019×0.031 | 0.042×0.047(0.045—0.031×0.056—0.042) | |
| Esophagus | | 0.016(0.020—0.012) | |
| Ventral sucker | 0.031×0.033 | 0.047×0.050(0.050—0.042×0.054—0.043) | |
| Pharynx | | 0.025×0.026(0.029—0.019×0.029—0.022) | |
| Testes | | | |
| Right | 0.014×0.009 | | |
| Left | 0.020×0.011 | | |
| Ovary | 0.006×0.009 | | |
| Excretory bladder | | 0.001×0.003 | |
| Stylet | 0.015×0.002 | 0.015×0.002 | |
| Granules in bladder | | 0.001×0.003 | |

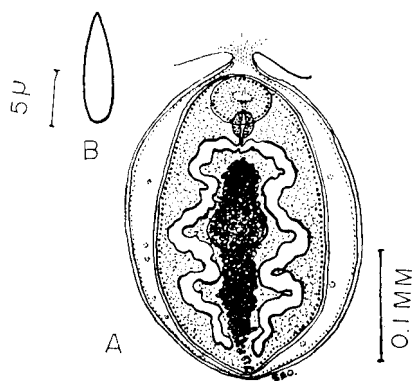


Fig. 1. A: The excysted metacercaria, B: Stylet stained specimen. The testes primordia represented as two compact cell masses, situated bilaterally within concavity inside of the third loop of the intestinal ceca. The ovary primordium as a cell mass was located immediately behind the ventral sucker. The size of the excysted metacercaria in stained specimen was 0.238mm long × 0.129 mm wide. The cell

masses of the testes and ovary measured 0.020 mm×0.011mm(left), 0.014mm×0.009(right) and 0.006mm×0.009mm respectively.

Metacercaria in the crab host: A total of 615 brackish water crabs collected from two areas; Hadan and Hadong at Nakdong and Sumjin River deltas were examined for the metacercariae of *Paragonimus*. The majority of crabs were caught in or near the field of reeds.

The crabs examined two genera and four species, i.e., *Sesarma dehaani*, H. Milne-Edwards, *Sesarma intermedia* (de Haan), *Helice tridens tridens*(de Haan) and *Sesarma haematocheir* de Haan. As shown in Table 2, all species of crabs examined except *S. haematocheir* were found infected with the metacercariae of *P. iloktsuenensis*. The highest rate of infection (40.1% in Hadong) was observed in the crab, *S. dehaani* which

Table 2. The infection rates of *Paragonimus iloktsuenensis* in crab hosts.

A total No. of crabs examined: 615

| Species | | <i>S. dehaani</i> | | | | | | <i>S. intermedia</i> | | |
|--------------------|-----|-------------------|--------------------|-----------------------|--------------|--------------------|---------------------|----------------------|--------------------|---------------------|
| Carapace width(mm) | Sex | Hadong | | | Hadan | | | Hadong | | |
| | | No. of exam. | No. of infected(%) | No. of Mc. ☆ per crab | No. of exam. | No. of infected(%) | No. of Mc. per crab | No. of exam. | No. of infected(%) | No. of Mc. per crab |
| 16-20 | M | 11 | 3(27.3) | 1.0 | 1 | 0(0) | 0 | | | |
| | F | 21 | 1 (4.8) | 3.0 | 1 | 0(0) | 0 | | | |
| 21-25 | M | 104 | 44(42.3) | 2.4 | 2 | 0(0) | 0 | 3 | 1(33.3) | 1.0 |
| | F | 123 | 50(40.7) | 2.3 | 9 | 2(22.2) | 1.5 | 1 | 0(0) | 0 |
| 26-30 | M | 83 | 34(41.0) | 4.9 | 26 | 1(3.8) | 4.0 | 1 | 0(0) | 0 |
| | F | 67 | 31(46.3) | 3.6 | 50 | 0(0) | 0 | 2 | 2(100.0) | 5.5 |
| 31-35 | M | 6 | 2(33.3) | 73.0 | 50 | 0(0) | 0 | | | |
| | F | 6 | 4(66.7) | 8.3 | 9 | 0(0) | 0 | | | |
| Total | | 421 | *169(40.1) | 4.0 | 148 | 3(2.0) | 2.3 | 7 | 3(42.9) | 4.0 |

*: *H. tridens tridens*, 6 out of 39 crabs examined were found infected (15.4%) and none out of 4 *S. haematocheir* were infected.

** : Correlation between the size of crabs and rate of infection; statistically significant ($0.01 < p < 0.02$).

☆ : Mc. means Metacercaria.

Table 3. Development of *Paragonimus iloktsuenensis* in the experimentally infected in mouse host.

| Duration of infection | No. of mouse used | No. of Mc.* fed | No. of infected (%) | Location of worms found | | | No. of worms found (rate of recovery, %) |
|-----------------------|-------------------|-----------------|---------------------|-------------------------|--------------|--------------|--|
| | | | | Abd. cavity | Thoracic Rt. | Thoracic Lt. | |
| 18 hrs | 13 | 95 | 3 (23.1) | 13 | 0 | 0 | 13 (13.7) |
| 2 day | 13 | 95 | 3 (23.1) | 15 | 0 | 0 | 15 (15.8) |
| 3 day | 8 | 35 | 3 (37.5) | 12 | 0 | 0 | 12 (34.3) |
| 2 week | 5 | 25 | 2 (40.0) | 10 | 0 | 0 | 10 (40.0) |
| 3 week | 12 | 30 | 4 (33.3) | 6 | 1 | 0 | 7 (23.3) |
| 4 week | 5 | 25 | 2 (40.0) | 6 | 2 | 1 | 9 (36.0) |
| 5 week | 5 | 27 | 3 (60.0) | 7 | 1 | 1 | 9 (33.3) |
| 6 week | 11 | 55 | 7 (63.6) | 10 | 4 | 2 | 16 (29.1) |
| Total | 72 | 387 | 27 (37.5) | 79 | 8 | 4 | 91 (23.5) |

*: Metacercaria

were the dominant crabs as the second intermediate host of this fluke, six crabs, *H. tridens* out of 39 examined were infected. The metacercariae were also found for the first time in three out of seven crabs, *S. intermedia*, which was therefore, listed as the second intermediate host of *P. iloktsuenensis*. The number of cysts per positive crab, *S.*

dehaani ranged from 1 to 108, average, 4.0. The rate and intensity of infection in crabs from Hadong (40.1%, 4.0) was much higher than those from Hadan (2.0%, 2.3). There was no distinct difference in the rate of infection between male and female crabs. The correlation of the size (carapace width) and the rate of infection was shown in Table 3.

The highest infection rate was found in the crabs of 31 to 35mm in carapace width. There seemed no significant correlation between the size and the infection rate. The distribution of metacercariae in four divided parts of the crabs; such as liver, muscle, gill and other viscera was examined. The incidence was shown only in liver tissues in this study.

Experimental infection in the mouse host:
In determining the susceptibility of the mouse host to the metacercariae of *P. iloktsuenensis*, 72 mice were given from 10 to 40 metacercariae each. A total of 387 metacercariae were given to 72 mice and out of these, 91 flukes (23.5%) were collected. Seventy two out of these worms collected were found in abdominal

cavity, and the remained in thoracic cavity. The observation of the development patterns of the flukes in mice was made according to the course of infection.

Within one day after infection: All worms in this stage were found only in abdominal cavity and the recovery rate (13.7%) was lowest because of difficulty to detect the young flukes, which were still small in size, measuring 0.29mm long and 0.15mm wide in unstained specimen of 18 hour-old worms.

The genital primordia were clearly visible in the stained specimen. The cell mass of the testes situated at bilateral side obliquely between the ceca and the cell mass of the ovary was seen at left or right, immediately

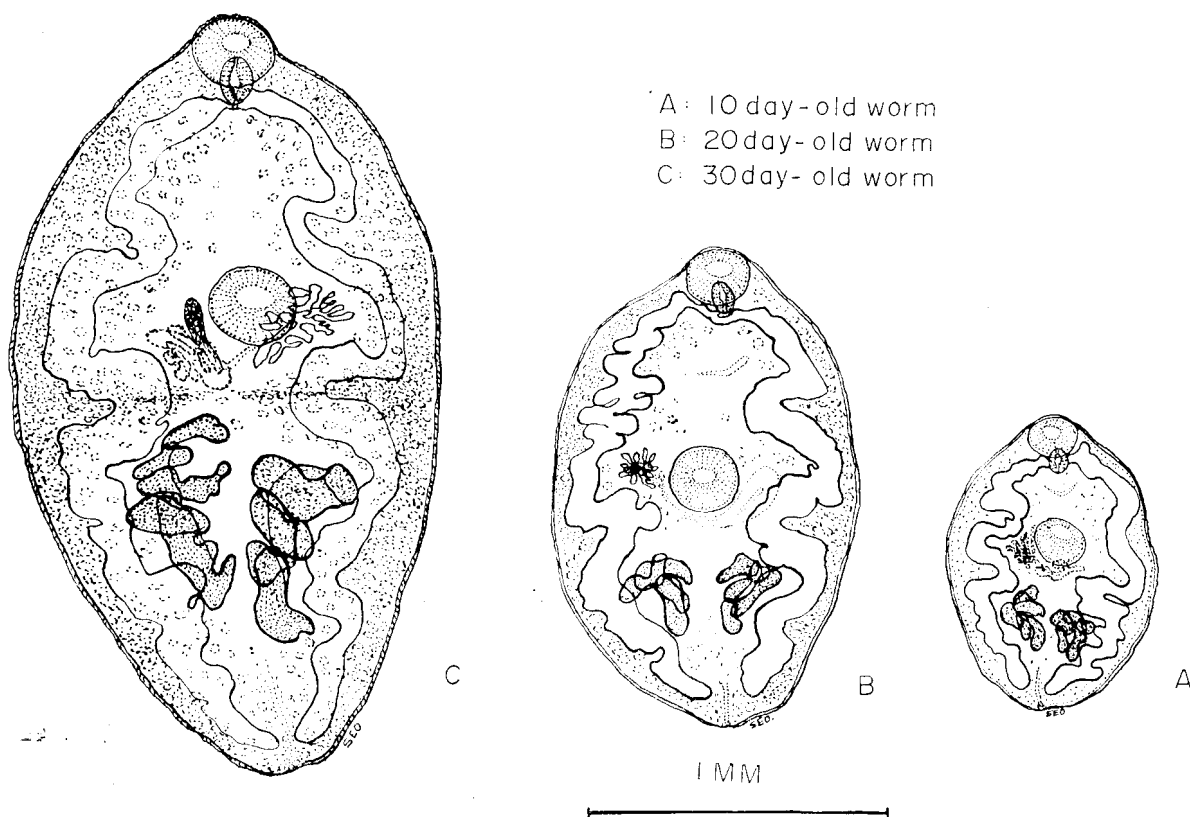


Fig. 2. Development of *Paragonimus iloktsuenensis* in mice during the course of infection.

Table 4. Measurements (in average) of *Paragonimus iloktsuenensis* in mouse host.

(unit: mm)

| Duration of 18 hrs infection (1)* | 2 day (4) | 3 day (12) | 2 week (4) | 3 week (7) | 4 week (7) | 4 week (3) | 5 week (5) |
|-----------------------------------|--|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| Length × width | 0.29 × 0.30 (0.27-0.33) × 0.15 (0.16-0.19) | 0.36 (0.26-0.42) × 0.18 (0.12-0.20) | 1.37 (1.03-1.79) × 1.26 (0.94-1.80) | 2.34 (1.85-2.90) × 1.25 (0.92-1.50) | 2.84 (2.77-3.12) × 1.64 (1.54-1.76) | 5.40 (4.54-6.08) × 2.39 (2.30-2.48) | 5.43 (4.41-6.32) × 2.67 (2.01-3.49) |
| Oral sucker | — | 0.03 × 0.04 | 0.20 × 0.20 | 0.17 × 0.25 | 0.22 × 0.32 | 0.28 × 0.41 | 0.37 × 0.50 |
| Ventral sucker | — | 0.04 × 0.04 | 0.22 × 0.22 | 0.26 × 0.30 | 0.38 × 0.45 | 0.49 × 0.49 | 0.52 × 0.56 |
| Testes (right) | — | 0.02 × 0.01 | 0.35 × 0.23 | 0.43 × 0.27 | 0.76 × 0.51 | 1.11 × 0.62 | 0.94 × 0.87 |
| (left) | — | 0.03 × 0.01 | 0.32 × 0.23 | 0.43 × 0.29 | 0.67 × 0.42 | 0.94 × 0.59 | 1.03 × 0.96 |
| Ovary | — | 0.01 × 0.01 | — | 0.18 × 0.25 | 0.28 × 0.36 | 0.54 × 0.51 | 0.49 × 0.63 |

*: No. in bracket shows the number measured.

All were measured in stained specimen except 18-hrs and 2-day specimens.

behind the ventral sucker. All of these cell masses were enlarged, compared with those in metacercaria. The vitelline follicles, uterine tubules and seminal vesicle were not recognized.

Within one week after infection: In this stage of infection, the size of the 3 day-old worm showed 0.313mm long and 0.136mm wide, when measured in stained specimen. The oral and ventral sucker also enlarged. The cell masses of testes and ovary measured 0.028mm × 0.014mm (left), 0.016mm × 0.006mm (right) and 0.011mm × 0.011mm respectively. The gland cell mass below the pharynx also became visible. The stylet in oral sucker disappeared.

Two weeks after infection: All of the collected worms were also found only in abdominal cavity. The size of the worm was 1.37 mm long 1.26mm wide in average. The oral and ventral sucker became conspicuously larger, measuring 0.20mm × 0.20mm and 0.22 mm × 0.22 mm respectively. The fairly well lobed testes have grown almost twice bigger than that in the one week-old worm. The two testes measured 0.36mm × 0.23mm (left), 0.32 mm × 0.23mm (right). The ovary also became much distinct, although it's branches were still poorly outlined. The stained spots just behind the ventral sucker were too vague to recognize as seminal vesicle or uterine tubules in the carmin-stained specimens.

Three weeks after infection: One out of 7 worms collected in this stage was found in right thoracic cavity, and others were all in abdominal cavity. The body of the worm became 2.34mm long and 1.25mm wide. The outline of seminal vesicle appeared much clearly near the posterior margin of the ventral sucker. The feature of the branched ovary became more conspicuous. The ovary

measured 0.18mm×0.25mm in average. The vitelline follicles in the bilateral sides of the body outside the intestinal ceca were sparsely visible. The two testes became bigger and much distinct in outline. The vitelline duct and uterine tubules were not recognizable.

Four weeks after infection: The size of the worms increased, measured 2.84mm long and 1.64mm wide. The testes became definitely enlarged and typically lobed and the branched ovary showed the characteristic feature, as the ovary of the so-called *ohirai-ilohtsuenensis* group. The seminal vesicle eminently enlarged and appeared to contain sperms. The uterine tubules were visible with poor outline. However, any fertilized ova were not detectable. The vitelline duct seemed to be formed with increasing number of vitelline follicles.

Five to six weeks after infection: In this stage, the body of worm measured in average 5.40mm long and 2.39mm wide in 35 day-old

worms and 5.43mm long and 2.67mm wide in 45 day-old worms. The increase of the body size appeared abruptly slow down. The testes and ovary showed the characteristic features, almost same in adultworm. The seminal vesicle, vitelline follicles and their ducts were well developed. However, the uterine tubules still poorly developed. Some unmaturred ova were sparsely visible in one out of five week-old worms and five out of ten of six week-old worms. The eggs in the uterus increased in number according to the course of infection.

Over six weeks after infection: The body structures were about the same as those observed in five to six weeks after infection, except the more convolution of the uterine tubules and dense aggregation of vitelline follicles. As indicated in Fig. 3, the growth curve was represented with the body length of the worms developed in mice during the course of infection. According to this figure, on the whole, the majority of the five to six week old worms may be considered as shown their maximum growth, although the size of the collected worms showed the great variation even in the same host and at the same age of infection. As mentioned above, only a few worms were found in thoracic cavity without any cystic formation in the lungs. This was why the first positive appearance of eggs in feces was not observed in this study.

DISCUSSION

Since the metacercaria of *P. iloktsuenensis* has been found by Yokogawa et al. (1971) for the first time in the crab host, *Sesarma dehaani* at 1970, in this country, it has brought up for attention to the possible existence of the other species of *Paragonimus* in Korea. The structural differences of the metacerc-

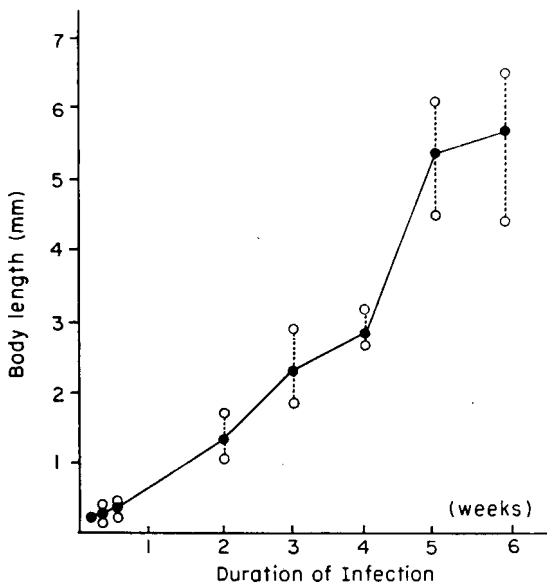


Fig. 3. Growth curve showing of *Paragonimus iloktsuenensis* during the course of infection in mouse host.

cariae among these species of *Paragonimus* may provide rather important criteria for the species identification than those of adult worms. It is generally confirmed that adult worms of *P. iloktsuenensis* and *P. ohirai* are hardly to be differentiated each other, whereas the metacercariae of these two flukes are clearly different. In this study, the metacercaria of *P. iloktsuenensis* was thoroughly reexamined. The size of the unstained encysted metacercaria was, when measured under the coverglass pressure without fixation, 0.342mm×0.289mm in average of 30 cysts. The excysted metacercaria fixed in 5% formalin measured 0.339mm×0.193mm in average of 10 worms. These figures were compared with those of the former investigators. As shown in the Table 1, the measurement of the cyst size was almost the same as Yokogawa et al., and Miyazaki have made. However it was a little larger than that of Chen's original description. The crabs, *Sesarma dehaani* and *S. sinensis* have been firstly described by Chen(1940) to serve as the second intermediate host of *P. iloktsuenensis*. Later *S. dehaani* (Miyazaki, 1945; Tanabe, 1948; Mera, 1951; Miyazaki, 1951; Tomimura, 1957), *Helice tridens tridens* (Mannoji, 1952) in Japan and *Potamon miyazakii*(Miyazaki et Chiu, 1965) in Taiwan have been proved to be the second intermediate host in the above two countries, *Sesarma dehaani*, *Helice tridens tridens* and *S. intermedia* were all found infected with the metacercariae of *P. iloktsuenensis* in this survey. And the latter, *S. intermedia* was newly added to the list of the crab hosts of this fluke. However, as described above, *S. dehaani* was proved to be the most important crab host in both areas of Hadan and Hadong. Chen(1940) reported that 697 out of 2,420 crabs, *S. dehaani* (28%) were found infected in Canton China (From I. Miyazaki, 1961). According to Miyazaki(1945),

Tanabe(1948) and Mera(1951), the rates of infection in *S. dehaani* were from 27.9% to 41.6% in Osaka, Japan. In this survey, Hadong (40.1%) was higher in the rate of infection in crab host than Hadan (2.0%). Mannoji(1952) reported that six out of 96 examined crab, *H. tridens tridens* were found infected (6.2%). In the areas of Hadong and Hadan, 6 out of 39 crabs, *H. tridens tridens* were found infected (15.4%). In case of *S. intermedia*, three out of seven crabs were found infected for the first time. No metacercaria was detected in the present survey as already reported by Yokogawa et al.(1971).

The metacercariae were only isolated in the liver in this experiment and the number of cysts per positive crab was 4.0 in average in Hadong, ranging from one to 108.

Some workers reported that although there was no difference in the rate of infection of the metacercariae between male and female crabs, there was a distinct close relationship between the infection rate of metacercaria and the size of the crab hosts (Chiu, 1962). Yokogawa et al. (1971) mentioned that the highest infection rate was observed in crabs in the range of the carapace size of the crab, *S. dehaani*, from 21 to 25mm and the infection rate was higher in the smaller crabs (less than 25mm carapace width) than in the larger crabs (more than 26mm width). In this survey, it was presumed that the correlation between the size of crabs and the rate of infection may be statistically significant ($0.01 < P < 0.02$).

The development of the fluke in mice during the course of infection has little been known. So far authors are aware, there is one description that Miyazaki(1944) has attempted to infect mice with the metacercaria of *P. iloktsuenensis*. From the present study, it was assumed that mouse host may be easily infected

with the metacercariae of this fluke with fairly high rate of recovery. However, the majority of worms was found in abdominal cavity, not in thoracic cavity. And any invasion into lungs with cystic formation has never been observed. The development of the internal structure of the youth fluke in mice has been shown during the course of the infection. As indicated in Fig. 3, up to five to six weeks after infection, the worm body has enlarged to their maximum size. Some reproductive organs, such as testes, ovary and seminal vesicle have successfully grown almost within 40 days after infection. However, the development of the uterine tubules and vitelline glands seemed to slow down. Up to six weeks after infection, the complete maturity of the worm has never been observed. The eggs were never discharged in feces during the course of infection in this study.

From the above observations, it may be concluded that mice were less susceptible to infection with this fluke and in this host the complete maturity of worm was hardly expected.

SUMMARY

1. The metacercariae of *Paragonimus iloktsuenensis* were isolated from the crab, *Sesarma dehaani*, collected at Hadan and Hadong areas, South Kyong-Sang Do. The rate of infection of crab host in Hadong area (40.1%) was higher than that in Hadan area (2.0%). The structure of the metacercaria of *P. iloksuenensis* was reviewed.

2. A total of 615 crabs, comprised two genera and four species were examined for the metacercariae. Among these crabs, *Sesarma dehaani*, *Helice tridens tridens* and *S. intermedia* were found infected. The latter, *S. interme-*

dia was recorded for the first time as a new host of *P. iloktsuenensis*. The highest rate of infection (40.1%) was observed in the crab, *S. dehaani*, in which an average number of cyst per crab was 4.0 (1 to 108). It was presumed that the brackish water crab, *S. dehaani* may serve as the most important second intermediate host of *P. iloktsuenensis* in these areas.

3. Experimental infection in mice with *P. iloktsuenensis* was carried out. From this experiment, it was found that mouse host was easily infected with the metacercariae. However, it was less susceptible for obtaining the full maturity of the fluke. The development of the metacercaria of *P. iloktsuenensis* in the mouse host was searched during the course of infection up to six weeks after infection. In an earlier stage within one week after infection, the rapid growth of the genital primordia was observed, particularly the enlargement of the cell mass of the testes was distinctly recognized. The vaguely outlined lobed testes were observed ten days after infection, whereas the branches of ovary were scarcely recognized 20 days after infection. The seminal vesicle appeared about three weeks after infection. However, the uterine tubules and vitelline follicles slowed down to appear. Five to six weeks after infection, the testes, ovary and seminal vesicle almost fully developed. In some specimens in this stage, unmaturing eggs were detected in the uterine tubules. The positive appearance of ova in the feces was hardly expected because of no cystic formation in the lungs.

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

II. 被囊幼虫의 形態, 第二中間宿主의 種類, 그 寄生相 및 終宿主內에서의 發育

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徐丙高·郭宗源

釜山市 下端 및 慶南 河東에서 *Sesarma dehaani*(말뚝게)等 2屬 4種의 半鹹水産介 總 615마리를 採取하고 怡樂村肺吸虫(*P. iloktsuenensis*)의 被囊幼虫을 分離하였으며 그 形態를 再檢討하였다. 아울러 兩地域에 있어서의 感染率을 比較하였던바 下端·河東 各 2.0% 및 40.1%로 河東地域이 훨씬 感染率이 높은것을 알 수 있었다. 調査한 介 *S. dehaani*, *S. intermedia* 및 *Helice tridens tridens*에서 被囊幼虫을 發見할 수 있었으나 *S. haematocheir*은 感染된것을 發見하지 못하였다. 따라서 *S. intermedia*를 *P. iloktsuenensis*의 新宿主로 記録하였다.

感染率이 가장 높은 介는 *S. dehaani*로 40.1%였으며 平均保有虫體數는 4.0마리(最小 1.0~最高 108.0마리)였다.

以上 所見으로 미루어 볼때 *S. dehaani*는 위 地域에 있어 *P. iloktsuenensis*의 가장 重要한 第二中間宿主라 할 수 있을것 같다.

本 被囊幼虫에 對한 마우스의 感受性과 마우스宿主內에서의 宿主 寄生虫 相互關係를 把握할 目的으로 마우스에 本被囊幼虫으로 實驗感染을 試圖하였다. 그 結果 마우스는 *P. iloktsuenensis*에 比較的 높은 感染率과 좋은 虫體回收率로 感染시킬수는 있으나 마우스에서 完全 成熟된 成虫體를 얻을 수 있을 程度로 感受性이 높지는 못한것 같다.

感染經過에 따른 마우스宿主內에서의 發育狀態를 觀察하였다. 即 感染後 一週以內에서는 辜丸 및 卵巢原基의 發育이 顯著하여 原基細胞塊가 急速 增大함을 볼 수 있었다. 感染 第 10日後 虫體에서는 辜丸의 輪廓이 漸次 明瞭하게 되었으나 이에 比하면 卵巢의 分枝는 뚜렷하지 못한 便이었다. 卵黃腺의 發育은 거의 認識하기 어려운 程度였다.

感染 第20日後 虫體에서는 虫體의 長徑 및 幅이 增大하였고 同時에 口吸盤 및 腹吸盤도 發育하였으나 特히 生殖器의 發育이 더욱 顯著하게 되었다. 即 辜丸의 輪廓이 成虫의 그것과 같은 狀態로 뚜렷이 分葉化하였고

卵巢의 分枝도 더욱 進展하였음을 알 수 있었다. 貯精囊, 子宮管과 卵黃腺도 出現하기 始作하였다.

感染 第 30日後 虫體에서는 辜丸의 發育狀態는 成虫과 恰似할 程度이고 卵巢의 分枝는 所謂 *ohirai- iloktsuenensis* 群의 特異한 形態를 나타내었고 貯精囊內에는 精子를 볼 수 있었다. 卵黃腺은 急速 增加되었으나 아직도 뚜렷한 卵黃管을 形成하지 못하였다. 子宮管의 發育이 相當히 遲延되는것 같다. 感染 5 乃至 6週後 虫體에서는 不明瞭한 子宮管內에 小數의 未成熟 虫卵을 發見할 수 있었다.

虫體는 大部分이 마우스腹腔內에서 發見되었을 뿐 아니라 胸廓內에서 發見된 虫體에서도 肺組織에 侵入한 例를 볼 수 없었으며 肺內 虫囊을 發見할 수 없었다. 따라서 感染 6週後까지도 便內에서 虫卵을 檢出하지 못하였다.

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EXPLANATION OF PLATE

1. Metacercaria of *P. iloktsuenensis* unstained
2. 10 day-old worm, stained in acetocarmin
3. 20 day-old worm, stained in acetocarmin
4. 30 day-old worm, stained in acetocarmin
5. 37 day-old worm, stained in acetocarmin
6. 45 day-old worm, stained in acetocarmin
7. Stylet of metacercaria, stained in acetocarmin
8. Ova in uterine tubules in 6 week old worm (high magnification)
9. Genital primordia of metacercaria (T: testis, O: ovary, V: ventral sucker)
10. Genital primordia of 3 day-old worm
11. Genitalia of 6. (T: testis, O: ovary, VD: vitelline duct, UT: uterine tubules)

