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STUDY ON *METAGONIMUS YOKOGAWAI* (KATSURADA, 1912) IN KOREA

I. ON THE METACERCARIA, ITS DISTRIBUTION IN THE SECOND INTERMEDIATE HOST AND THE DEVELOPMENT IN THE FINAL HOST

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INTRODUCTION

Katsurada (1917) has reported first that the metacercaria of *Metagonimus yokogawai* was found in *Carassius auratus* in Korea. In the previous survey by the fecal examination among the people all over the country, there are many reports that the eggs like *Metagonimus* were found not rarely (Muta, 1913; Murata, 1914; Kobayashi et Kon, 1917; Kojima, 1919; Yabe et al., 1923 and Hara et Himeno, 1924; etc.).

These early literatures have shown already that human metagonimiasis is widely distributed in Korea, particularly in the southern part of Korea. However it is quite uncertain whether these were to be identified as only the eggs of *Metagonimus*, because it can not be distinguished from that of Heterophyidae only with great difficulty (Furuyama, 1930).

According to recent helminthological survey, the prevalence rate of *Metagonimus yokogawai* in southern Korea due to the detection of the eggs in the stool specimens of 40,581 people was 0.4 per cent. Some endemic foci of this fluke were also noticed in South Kyongsang Do (=Province) and South Cholla Do. (Seo et al., 1969).

However, there was no known published case report of human metagonimiasis with the distinct clinical symptoms and the diagnosis based on the adult worm. Seo et al. (1969) recently noticed a distinct clinical case with heavy worm burden.

From the above point of view, the clinical significance of the human metagonimiasis should be carefully taken into account in Korea. In this sense, author set up the present experiment as a first step of the study in order to obtain some informations on the host-parasite relation in the second and final host of *M. yokogawai*.

MATERIALS AND METHODS

Isolation of Metacercariae:

All of the metacercariae in present study were isolated from the sweetfish, *Plecoglossus altivelis* which was collected in September at Hwa-gae Myon, South Kyongsang Do. For the isolation of metacercariae from the fish, the digestion technique was applied; the flesh of fish was ground in the meat grinder and mixed with artificial gastric juice, glass beads added. The artificial gastric juice is a solution of 0.6% HCl and 1% pepsin distilled water. The above mixture was incubated at 37°C

for 4 to 5 hours with occasional shaking. After digestion the isolated metacercariae can be separated from debris by repeated sedimentation in tapwater. Metacercariae thus obtained were kept in 1.2% hypertonic saline solution in refrigerator at 4 to 5 °C. For obtaining the excysted metacercariae, 0.8 % trypsin-saline solution was used (Seo et al., 1969).

Counting of Metacercariae in the Fish Host:

In order to know the distribution of the metacercariae in the fish host and their total number, the metacercariae found on scales, in muscles and subcutaneous tissues were separately counted. For the convenience of counting, the fish was divided into five parts; head, anterior trunk, posterior trunk, tail and subcutaneous parts and they were separately digested by a solution of 0.1N HCl and 0.1% Difco Pepsin (1 : 10,000) in distilled water for 3 hours at 37°C.

The scales in 1 cm^2 area between lateral lines and dorsal fin were collected and separately the number of metacercariae on these scales was counted on the binocular dissecting microscope.

Infection of the Animal Host with Metacercariae:

Mice were selected as the animal hosts in present experiments. The mice were fasted previously for a day prior to expose to infection. They were lightly anaesthetized with ether and a certain number of metacercariae was administered orally into stomach by means of polyethylene tubing (Intramedic, Clay-Adams Inc., RE-90/s12, I.D., 0.034″, O.D., 0.05″). The counted metacercariae were taken up in 0.2 to 0.5ml of tapwater by a finely drawn bulbed pipette and connected into the polyethylene tubing which was previously catheterized into stomach. Then, after careful inoculation of metacercariae, the pipette and tubing were checked under the microscope.

Autopsy and Worm Counts:

The mice were sacrificed in various intervals after the exposure. The intestine was removed and cut into 6 pieces, placed separately in warm saline, and teased for search of worms. The measurements of the worms were mostly done under 5% hot formalin fixed, slightly pressed materials. Semichon's acetocarmine was used for staining of the worms collected.

EXPERIMENTAL RESULTS

The Structure of Metacercariae:

The metacercaria of *M. yokogawai* was disc-like or elliptical in shape, measuring 0.163mm (0.143 ~0.190) × 0.150mm (0.129~0.163). Thickness of the cyst wall is 0.003mm (Table 1). In press preparation of fish-flesh, the cyst wall was covered by a further thick transparent layer which seemed to be produced by the host reaction. Inside the cyst, the rotatory movement of metacercaria was frequently seen. The body contraction or ventral bending of the anterior body part was also observed with intermittent revolving movements. The cyst wall applied by digestion technique was easily broken by a slight pressure under the cover glass.

Table 1. *Measurements of metacercaria of* M. yokogawai

Encysted Metacercariae* (in average) unit : mm	
Size	0.163(0.143~0.190) × 0.150(0.129~0.163)
Thickness of cyst wall	0.003(0.0026~0.0039)
Excysted Metacercariae+ (in average) unit : mm	
Size: (Length × Width)	0.286(0.252~0.320) × 0.170(0.163~0.190)
Oral sucker	0.050(0.032~0.035) × 0.037(0.034~0.038)
Esophagus	0.031(0.029~0.032)
Ventral sucker	0.034(0.024~0.037) × 0.026(0.023~0.029)
Testes primordia Right	0.018(0.016~0.019) × 0.013(0.011~0.015)
Left	0.016(0.014~0.019) × 0.012(0.009~0.015)
Ovary primordia	0.021(0.018~0.026)
Excertory bladder	0.093(0.089~0.096) × 0.085(0.082~0.088)
Granules in bladder	0.005(0.003~0.009)
Genital atrium	0.012

*No. measured: 20, using non-fixed living materials
+No. measured: 10, using 5% formalin-fixed, slightly cover glass pressed materials

The excysted matacercaria was an elongated oval in shape, tapering to its posterior extremity. It measured 0.286mm(0.252～0.320) long, 0.170mm (0.163～0.190) wide. The surface of the body was covered by fairly distinct spines all over the body. The brownish pigments were scattered throughout the anterior part of the body. No pigmented eyespots were present. The oral sucker was rather round or oval in shape, measuring 0.050mm×0.037mm, it was larger than the acetabulum. The short prepharynx was hardly recognizable measuring 0.0066mm. The pharynx was round in shape, measuring 0.035mm in diameter. The esophagus was relatively long, measuring 0.031mm. The intestine bifurcated and proceeded bilaterally to the posterior part of the body and attained to its extremity. The ventral sucker was transversely elliptical in shape, measuring 0.034mm×0.026mm. It was deviated right from median line innerside the bifurcate intestine with its long axis in a diagonal plane. The genital atrium was closely associated with the acetabulum. The genital primordiá consisted of two testes as two compact cell masses, situated symmetrically on the lateral margins of the excretory bladder. The ovarian primordium as well as testes could be recognized just above the upper margin of the bladder. It's shape was irregular oval form. The excretory bladder was V-form in which many strongly refractile excretory granules were found, measuring from 3 to 6 μ. The excretory pore opened terminally. (Fig. 1, 2, 3, Plate I)

Distribution of Metacercaria in the Fish Host:

As shown in Table 2, ten sweetfishes ranging from 15 to 18cm in length and from 20 to 40 grams in weight were examined for the incidence and distribution of metacercariae. All fishes examined were found infected. A total of 38,511 metacercariae were obtained, giving an average of 3,851

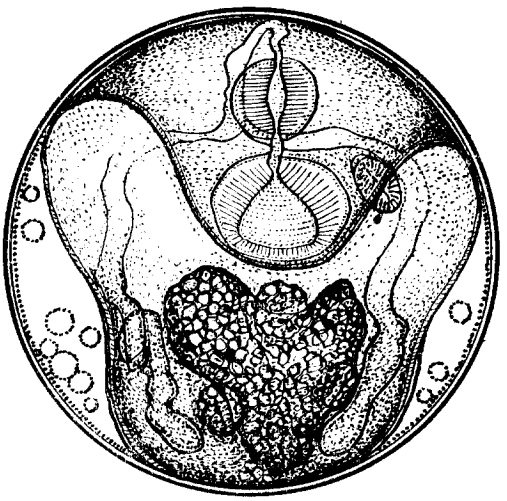

Fig. 1. Metacercaria of *Metagonimus yokogawai*, ventrally bent for revolving movement

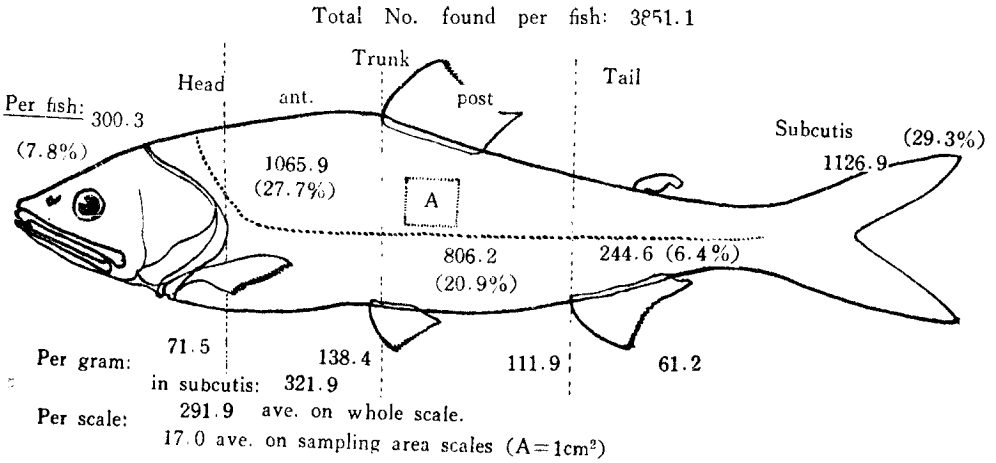

Fig. 2. Distribution of metacercariae of *M. yokogawai* in the sweetfish, *Plecoglossus altivelis*

Table 2. *Distribution of Metacercariae of* Metagonimus yokogawai *in* Plecoglossus altivelis

Fish No.	Weight (gm)	Number of metacercariae found								
		On scales		In subcutaneous tissues	In muscles					Total
		sampling scales	whole scales		head	ant. trunk	post. trunk	tail	subtotal	
1	—	—	95	84(4)˘	80(5)˘	141(5)˘	45(5)˘	17(4)˘	283(22)˘	462
2	40	5(239)*	214	207(4)	103(7)	87(10)	87(10)	47(6)	287(33)	713
3	30	22(318)	389	1,837(4)	407(4)	805(8)	805(8)	119(6)	2,679(26)	4,927
4	29	2(308)	210	1,773(4)	609(4)	1,495(6)	1,495(6)	556(4)	5,369(20)	7,354
5	21	29(335)	365	2,642(3)	834(3)	1,570(6)	1,570(6)	635(3)	4,964(18)	8,000
6	26	60(224)	1,095	4,140(3)	810(4)	3,705(9)	3,705(9)	842(2)	9,192(23)	14,487
7	27	15(352)	129	96(4)	46(4)	46(8)	46(8)	28(3)	268(23)	508
8	25	4(288)	202	18(3)	12(4)	6(7)	6(7)	11(5)	48(24)	272
9	22	9(290)	122	38(3)	7(4)	8(7)	8(7)	37(4)	59(22)	228
10	25	7(237)	98	434(3)	95(4)	295(6)	295(6)	154(3)	1,021(20)	1,560
Total		153(2,591)	2,919	11,269(35)	3,003(42)	10,659(77)	8,062(72)	2,446(40)	24170(231)	38,511
Infection rate (%)			7.7	29.3					62.8	
Average No. per fish			291.9	1,126.9	300.3	1,065.9	806.2	244.6	2,417	3,851.1
Average No. per gram of flesh				321.9	71.5	138.4	111.9	61.2	104.6	

Avg. No. of metacercariae on the scales of sampling area: 17 *: Number of scales in sampling area(1cm²)

˘: Weight (in gram) of muscles in the each part of fish — : not examined.

metacercariae per fish. The number of metacercariae found in each fish varied from the minimum 219 to the maximun 14,487. The average number of total metacercariae found on scales per fish was 291.9. The distribution pattern of metacercariae in the four divided body parts was observed. The highest incidence was shown in muscle of the trunks. The frequency was recorded in descending

Table 3. *Measurements (in average) of Developing Worms in Mouse Host carrying Age of Infection.*

unit : mm

Age		24 Hours	2nd day	3rd day
Length × Width		0.272(0.266~0.286) × 0.204(0.190~0.210)	0.442(0.408~0.612) × 0.230(0.210~0.306)	0.476(0.462~0.482) × 0.257(0.244~0.279)
Oral sucker		0.043(0.042~0.045) × 0.041(0.040~0.043)	0.054(0.042~0.062) × 0.052(0.040~0.063)	0.055(0.043~0.062) × 0.062(0.051~0.064)
Pharynx		0.033(0.029~0.034) × 0.037(0.036~0.039)	0.033(0.030~0.063) × 0.039(0.036~0.066)	0.038(0.037~0.040) × 0.040(0.038~0.042)
Esophagus		0.031(0.030~0.033)	0.051(0.040~0.054)	0.061(0.057~0.064)
Ventral sucker		0.037(0.034~0.040) × 0.029(0.026~0.033)	0.039(0.034~0.043) × 0.034(0.032~0.035)	0.052(0.040~0.054) × 0.049(0.042~0.052)
Ovary		0.026(0.032~0.043)	0.031(0.029~0.034)	—
Seminal vesicle		—	—	—
Seminal receptacle		—	—	0.070(0.066~0.072) × 0.039(0.037~0.040)
Testes	Right	0.031(0.027~0.036) × 0.023(0.020~0.032)	0.093(0.080~0.099) × 0.062(0.057~0.065)	0.124(0.101~0.147) × 0.101(0.097~0.105)
	Left	0.027(0.025~0.031) × 0.019(0.016~0.021)	0.085(0.079~0.086) × 0.062(0.058~0.068)	0.121(0.108~0.124) × 0.085(0.082~0.091)
Vitellaria		—	—	—
A.B.L.*	(P.B.L.*)	0.109 (0.163)	0.139 (0.303)	0.158 (0.318)

order as follows; 24,170 (62.8%) in the muscle, 11,269 (29.3%) in the subcutaneous tissues and 291.9 (7.7%) on the scales. From the result shown in Table 2, it was presumed that there was no close relationship between the size of the fish and the intensity of infection.

The average number of the metacercariae found on the sampling scales in the one cm^2 area just above the lateral lines beneath the dorsal fin was 15.3. On the other hand, it seemed to be most probable that the number of metacercariae found on the above sampling scales was not correlated with the total number of metacercaria found per fish.

Development of the Worm in the Mouse Host:

One day after infection; The size of the worm was 0.27mm long and 0.20mm wide. In the anterior part of the body many yellowish brown pigments were still found scattered. The body surface was covered with spines. The oral sucker and pharynx were about the same as those in metacercaria. The ventral sucker became a little more distinct in shape than that of metacercaria, and it was slightly enlarged. The excretory bladder was completely shrinked in form showing irregular Y-letter. A few of excretory granules was seen inside the bladder. Small corpuscle or granules were also found numerously in the intestinal ceca. In this stage, the most characteristic distinct changes were observed in two testes and ovary. The testes situated at bilateral side of excretory bladder in the metacercaria were enlarged mostly twice in size and fairly well defined. The ovarial primordium seen at the front margin of the excretory bladder in the metacercaria stage became also larger. The cell mass recognized vaguely between the ovary and ventral sucker also developed. However vitellaria, uterine tubules, seminal receptacle were not visible. (Fig. 4, Plate I)

Two days after infection; The size of the worm was clearly enlarged, it measured, 0.44mm long and 0.23mm wide. The posterior body legnth distance from the posterior end of esophagus to the posterior extremity of the worm body was much extended. The ventral sucker became well defined.

* A.B.L.: Anterior Body Length: Distance form the anterior end of the body to the posterior end of esophagus.
* P.B.L.: Posterior Body Length: Distance from the posterior end of esophagus to the posterior end of body.

No. of worms measured: 10 5% *hot formalin fixed slightly cover glass pressed meterials were used.*

4th day	5th day	6th day	7th day
0.517(0.442~0.612)× 0.272(0.257~0.306)	0.523(0.428~0.639)× 0.272(0.245~0.286)	0.694(0.564~0.850)× 0.333(0.279~0.360)	0.707(0.666~0.747)× 0.367(0.340~0.381)
0.064(0.057~0.068)× 0.068(0.064~0.071)	0.062(0.057~0.064)× 0.068(0.064~0.071)	0.064(0.057~0.066)× 0.062(0.051~0.064)	0.064(0.062~0.068)× 0.060(0.041~0.062)
0.034(0.030~0.037)× 0.040(0.038×0.042)	0.037(0.034~0.039)× 0.040(0.038~0.042)	—	—
0.064(0.057~0.066)	0.066(0.062~0.068)	—	—
0.052(0.043~0.062)× 0.042(0.040~0.046)	0.055(0.040~0.062)× 0.042(0.040~0.046)	0.062(0.060~0.065)× 0.056(0.054~0.064)	0.067(0.066~0.076)× 0.062(0.060~0.065)
—	0.046(0.042~0.051)× 0.039(0.038~0.057)	0.059(0.054~0.062)× 0.062(0.060~0.069)	0.078(0.074~0.080)× 0.078(0.074~0.084)
0.093(0.091~0.096)× 0.031(0.027~0.034)	0.093(0.091~0.096)× 0.039(0.038~0.057)	—	0.122(0.116~0.127)× 0.054(0.047~0.062)
0.093(0.091~0.096)× 0.049(0.045~0.053)	0.093(0.091~0.096)× 0.049(0.045~0.053)	—	0.140(0.132~0.155)× 0.108(0.096~0.147)
0.131(0.122~0.145)× 0.109(0.075~0.156)	0.130(0.122~0.150)× 0.116(0.108~0.121)	0.135(0.108~0.141)× 0.129(0.125~0.131)	0.138(0.131~0.140)× 0.107(0.101~0.111)
0.129(0.102~0.163)× 0.102(0.075~0.142)	0.129(0.125~0.132)× 0.114(0.099~0.125)	0.130(0.122~0.150)× 0.122(0.102~0.125)	0.124(0.119~0.131)× 0.108(0.102~0.125)
0.006×0.011	—	—	—
0.164 (0.353)	0.166 (0.357)	0.177 (0.514)	0.177 (0.570)

The sharply outlined testes have grown almost three times bigger than that in the one-day-old worm. The ovary also was enlarged in size with poor outline even in stained preparations.(Fig. 5, Plate I).

Three days after infection; The body of worm became 0.47mm long and 0.25mm wide, the testes and ovary became more clearly seen and in the Semichon's acetocarmine stained preparations, these were stained conspicuously in oval or elliptical shape. The anterior part of the ventral sucker and the genital atrium were not stained and transparently seen. The uterine tubules and vitelline follicle were not distinguishable. The seminal vesicle and seminal receptacle were vaguely seen. However the latter was able to measure (0.070×0.039mm). Although the excretory canal extending forward from the anterior end of the bladder became clearly visible. (Fig. 6, Plate I).

Four days after infection: The body of worm became 0.52mm long 0.27mm wide. In this stage the general aspect of the development was about the same that of the three day-old worm. However the seminal vesicle became clear in measureable shape. It measured 0.049mm in width. The uterine tubules was partly recognized near the acetabulum. However they contained yet no eggs. The number of vitelline follicles was able to clearly count in formalin fixed, nonstained specimens. It was about 22 to 24 in each side. The vitelline duct was also seen. (Fig. 1, Plate II).

Five to Six days after infection; In this stage of the five day-old worms, the outline of uterine tubules appeared much clearly near the genital atrium and the transparent, uncoloured egg shells were sparesely visible at this area if the uterine tubules. The size of the collected worms even in the same host and with the same age of infection showed the great variations. In the six day-old worm, the body length and width became conspicuously larger, measuring 0.69×0.33mm. The ventral sucker eminently enlarged and its size closed up to the oral sucker. In some specimens the ventral sucker was lager than the oral sucker. Two testes situated obliquely, the right testis was slightly longer than the left one. The uterus was found well developed and contained fairly many undeveloped, yellowish coloured eggs. The thickness of the uterine tubule was measured 0.016mm and the number of eggs was about 30 in each worm in average. The extension of the posterior body length was noticed. (Fig. 2 & 3, Plate II).

Seven days after infection; The body of worm became 0.707mm long(0.666~0.747)and 0.37mm (0.340~0.381) wide. The posterior body length was much extended. The over 50 per cent of the collected worms were filled with the yellowish coloured eggs. The majority of eggs remained still undeveloped. The number of eggs in the uterus varied from 50 to 200. (Fig. 5, Plate II). The size of ventral sucker exceeded the oral sucker. In this stage, particularly the eminently enlarged seminal receptacle was characteristic. Meanwhile, in some specimens the size of the testis apparently increased. On the whole, the majority of the seven day-old worms might be considered as matured worms. (Fig. 4 & 6, Plate II).

Eight days after infection; The size of worms increased a little in some specimens. The ventral sucker became clearly larger than the oral sucker. The eggs in the uterus increased in number. However the other body structures were about the same as those observed in seven day-old worms. The first positive appearence of eggs in feces was on the 10th day after infection.

Distribution of Worms in the Intestine of the Mouse Host:

In order to observe the change of habitat in the intestine, twenty mice were selected and inoculated with 200 metacercariae in each worms. Every one or two mice were sacrificed and the worms were collected separately from five divided parts of small intestine and the large intestine. As shown in Fig. 3, in early day after the initial infection, the worms were found mostly at the upper part of the small intestine. However, according to the course

of infection, it was noticed that the sites of the worm collection apparently shifted down toward the lower part of the small intestine or even to the large intestine. The gradual decrease of the recovery rate of the worms was also recognized. The growth of the worms collected at these lower portion of the intestine was poor and it caused the great variations of the size of the worms. At the fifth day after infection, the worms were more obtained in the lower two parts of the small intestine than in the upper two parts. This tendendy was especially noted after the seventh day after infection.

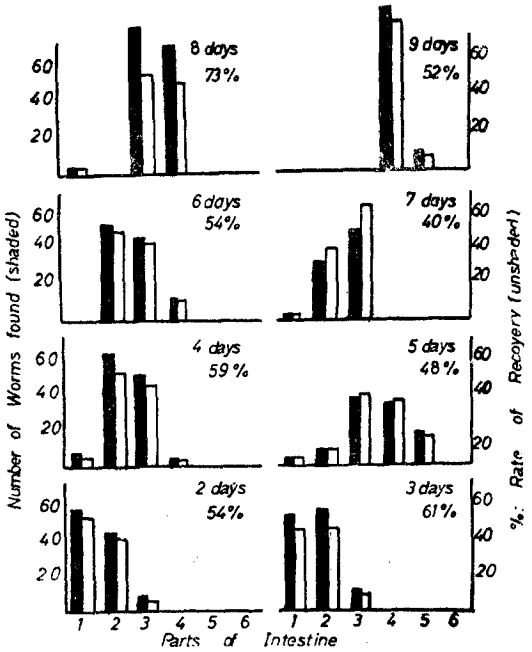

Fig. 3. Distribution of the worms in the intestine and recovery rate by various intervals after infection
1–5: parts of the small intestine, 6: large intestine

DISCUSSION

The metacercaria of *Metagonimus yokogawai* was first found by S. Yokogawa (1912), from *Plecoglossus altivelis* and described in detail. Later, in Japan many additional observations on the morphology of metacercaria have been published. In Korea, Chun (1960) first described his observation of the metacercaria of *M. yokogawai* from *Plecoglossus altivelis* caught at Miryang, South Kyongsang Do. He also described the metacercaria isolated from *Carassius carassius* and identified as *Metagonimus takahashii* (Chun, 1960). Later Choi et al. (1966) also reported the description of the metacercaria from brackish water fish *Tribolodon taezanowskii* collected at North Kyongsang Do and identified as *M. yokogawai*. On the other hand, recently Lee (1968) reported an unidentified metacercaria of *Metagonimus* sp. from several kinds of fresh water fishes. However the complete measurements of the metacercaria that the above authors observed have never given in any of their papers.

In general, our observations on the structure of the metacercaria mostly coincide with those made by the above authors. However, no distinguishable morphological characteristics enough to identify the species of the genus *Metagonimus* in metacercarial stage were found.

Many investigators have ever reported the distribution of metacercaria, the infection rate and the intensity of infection in the second intermediate host of *M. yokogawai*. The relation between the number of metacercaria and the size of the fish was also studies by various workers. Chun(1960) examined 100 sweetfishes ranging from 16 to 25 cm in length and 10 to 52 gram in weight. He stated that out of 100 fishes examined, 54 were found infected, and the infection rate of the metacercaria on scales and fins was 45.3 per cent, in the subcutaneous tissues, 26.3%, in the muscles, 14.3% and in the head part, 14.1% and the average number of metacercaria per fish was 36.9 and the maximum number of metacercaria found was 256.

As shown in the above Table, the result obtained in present study were quite different from those indicated by Chun in the following points; namely, 1) According to our results, the highest frequency was observed in muscles(62.8%) and the distribution in the other parts was recorded in descending order as follows: in sudcutaneous tissues (29.3%) and on scales(7.7%). This observation did not coincide with the above mentioned data by Chun. Concerning the incidence of metacercaria of *M.*

yokogawai in sweetfishes, several Japanese workers have already confirmed that in case of *Plecoglossus altivelis*, *Salangichthys microdon*, the metacercaria were found mostly in the muscle and subcutaneous tissues with over fifty percent incidence, whereas on the scales, there were very poor in number. However in case of *Carassius carassius* and *Leuciscus hakonensis*, it was vice versa, therefore the metacercariae were mostly found on scales (Ito; 1957, Komiya et al.; 1958, Yokogawa et al.; 1962). It has been explained that the lower incidence on scales in sweetfish was caused by the pausity of scales in this kind of fish. 2) In our present survey, the rate of infection was 100 per cent and in addition the average number of metacercariae found per fish was extremely higher than those reported by Chun. It closed up over hundred times. The maximum number found in a fish was also tremendously higher than that of the above result. In our survey it was 14,487, meanwhile the number by Chun was only 256.

It has been well known that the metacercarial prevalence of trematodes within the host are closely correlated with the many factors, namely the size of the host, the sex, the season of the collection, the endemicity of the area etc. In case of metacercaria of *M. yokogawai* in sweetfishes, all of the conditions, such as the the size, the season of collection etc were about the same in the both surveys by Chun and the present authors, except the endemicity in the area where the fishes were caught. In our study, the fishes were collected at Hwagae Myon, Ha-dong Gun, South Kyongsang Do, whereas in the former they were obtained at Miryang, South Kyong-sang Do. Therefore it was presumed that the area where the authors surveyed could be considered more highly endemic than that of the latter.

Kang et al. (1964) reported that 56 out of 62 sweetfishes examined in Cheju Do were found infected.

Oshima et al.(1966) described a simple method of estimation for the intensity of infection with metacercariae of *M. yokogawai* in sweetfishes and proposed the "infection index" of fish which can be easily made from the average number of metacercariae on 200 scales in sampling areas of more than 10 sweetfishes caught in August and September. The sampling area indicated by him is the one cm^2 zone between the just above the lateral line and the below the dorsal fin. He also stated that the total number of metacercariae within the fish can be estimated by multiplying the number of metacercariae on 200 scales in the above specified sampling area with the following numerical coefficient according to the month of collection of fishes; namely they are 10, 30, 50, 40, and 30 in case of June, July, August and September, October and November respectively.

We have attempted to apply his proposal to the present study and the theoretical number was compared with that obtained through the examination; the so-called "infection index" by Oshima et al. was 11.8 in our case. Therefore, according to him, this should belong to the category of heavy infection. On the other hand, the fishes examined in this experiment were all collected at September, therefore the numerical coefficient by month should be 50 according to his proposal. However the actual total number found was not seemingly coincide in general with the theoretical estimation using his index.

Development of worms in the mouse host has first been described by S. Yokogawa (1912). Komiya et al.(1958) also attempted to infect mice with the brackish water fishes infected and traced the growth of the worms. The observations made by S. Yokogawa on the development in mice according to the age of infection were in general about the same as those made by us.

In present study, it took at least 7 days for the full growth as a mature adult, although the rate of growth showed individually the great variation. For one or two days after infection, the most characteristic changes were the rapid growth of the genital primordia, particularly the enlargement of

testes was eminent and the development of ovary, seminal receptacle, seminal vesicle and vitelline follicles followed. The ventral sucker became larger on 7 days after infection than the oral sucker. The size of seminal receptacle also exceeded the testes in some specimens on 6th day after infection. The first appearence of eggs in uterus was recognized on the 6th day after infection. However at least the over 7 day-old worms evenly contained the eggs in uterus, which showed typical shape and yellowish coloured egg shell. The first appearance of eggs in feces seemed to depend upon the worm burden. We observed the first positive appearance on 10th day after infection from the mouse inoculated with 200 metacercariae.

Yokogawa et al. (1968) reported that the recovery rates of the worms in mice were 2.7 per cent, 1.1 per cent and 2.5 per cent in 6, 3 and two weeks after infection respectively. In present observation, the recovery rates within one week after infection varied from 39.5 per cent to 60.5 per cent. Except the rate in one day after infection (18.5%), the rates were all over 39.5%. It is presumed that the lowest rate in the 1st day was probably caused by high difficulty to detect out. Anyhow, these rates obtained in our experiment were much higher than those observed the previous authors. And the size of the worms in average also measured larger than that shown by them.

As shown in Fig. 3, according to the course of infection the sites of parasitism in the mouse intestine seemingly moved downward to the lower portion. And it was also distinctly recognized that the worms collected at the lower parts of the small intestine were all poorly developed and extremely retarded in the growth of their genitalia. Komiya et al. (1958) stated that the number of the worms was found clearly decreased on 16 to 26 days after infection, and on 32 days after infection no worm was found.

From the above observations, it was presumed that this phenomenon may cause the gradual decrease of the recovery rate in the course of infection.

SUMMARY

1) The metacerariae of *Metagonimus yokogawai* were isolated from the sweetfish *Plecoglossus altivelis*, collected at Hwagae, South Kyongsang Do, one of the newly known endemic foci of metagonimiasis in Korea. The body structure of metacercaria of *M yokogawai* was described and the measurements of the excysted metacercaria were also made.

2) In order to know the distribution of metacercariae within the host, the rate of infection and the intensity of infection, a total of 10 sweet fishes was examined and it was found all infected, from which a total of 38,511 metacercariae was isolated. The number of metacercariae in a fish varied from 219 to 14,427. The average number of metacercaria per fish was 3,851.

The distribution of metacercariae in the four divided parts of fish was observed in the following order; number of metacercaria in the muscles; 2,417 (62.8%), in the subcutaneous tissues; 1,126.9 (29.3%), and on the scales; 291.9 (7.7%).

3) The development of the metacercaria of *M. yokogawai* in the mouse host was experimentally traced every day for 10 days after infection. In an earlier period of infection, the growth rate of the genital primordia was distinctly high, particularly in the testes. The seminal reseptable and seminal vesicle became clearly recognized in measureable size at 4 days after infection. The vitelline follicles and their ducts were also first visible in the living specimens at 6 days after infection. The oral sucker was larger in size than the ventral sucker in an early stage of the worms, however after 7 days after infection it reversed. The posterior part of body began to extend since two days after infection. Fully matured worms were able to collect only after 7 days after infection. At this stage, the body of worm became 0.7mm long and 0.3mm wide. The first positive appearance of eggs in the uterine tubule and in feces was on the 6th day and 10th day of infection, respectively.

In an earlier stage of infection, the worms were

found mostly in the upper portion of the small intestine and the recovery rates of the worms were high, however according to the course of infection in later stage they were seen rather in the lower part of the intestine and the recovery rate also decreased.

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EXPLANATION OF PLATES

Plate I:

Fig. 1 and 2: Metacercaria of *M. yokogawai*

Fig. 3: Excysted metacercaria (Formalin fixed, unstained)

Fig. 4: One day-old worm (Formalin fixed, unstained)

Fig. 5: Two day-old worm (Formalin fixed, unstained)

Fig. 6: Three day-old worm (Semichon's acetocarmine stained)

Plate II:

Fig. 1: Four day-old worm (Semichon's acetocarmine stained)

Fig. 2: Five day-old worm (Semichon's acetocarmine stained)

Fig. 3: Six day-old worm (Semichon's acetocarmine stained)

Fig. 4: Seven day-old worm (Semichon's acetocarmine stained)

Fig. 5: Eggs in Uterus of the seven day-old worm (unstained)

Fig. 6: Seven day-old worm (Semichon's aceto-carmine stained)

＝國文抄錄＝

橫川吸蟲(*Metagonimus yokogawai*)에 關한 硏究

Ⅰ. 被囊幼蟲의 形態, 第2中間宿主內의 寄生樣相및 終宿主內에서의 發育

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慶南 河東郡花開面에서 銀魚(*Plecoglossus altivelis*)를 採取하고 요꼬가와 吸蟲의 形態를 再檢討하였다. 한편 第2中間宿主인 銀魚體內서의 被囊幼蟲分布狀態를 調査하였고 被囊幼蟲을 終宿主인 마우스에 試驗感染하여 다음과 같은 成績을 얻었다. 즉 0.1N HCl, 0.1% Difco pepsin (1:10000) 수용액을 使用한 人工消化法에 依하여 meat grinder에 處理한 魚肉을 37°C에서 3時間 充分히 消化시키고 分離한 被囊幼蟲의 形態 및 0.8% trypsin으로 脫囊시킨 幼蟲의 構造를 計測 檢討 記載하였다.

銀魚體內에서의 本幼蟲의 分布樣相을 보기 爲하여 무게 21~40gm, 길이 15~18cm의 銀魚 10마리를 使用하여 頭部, 前後胴體部, 尾部 및 皮下組織等 部分으로 區分하여 分離消化하고 發見된 被囊幼蟲數를 計算하였다. 銀魚 한마리당 平均 幼蟲數를 部位別로 보면 皮下組織에서 1,126.9 (29.3%)個, 前胴體部의 筋肉에서 1,065.9(27.7%)個, 後胴體部의 筋肉에서 806.2(20.9%)個, 頭部筋肉에서 300.3(7.8%)個, 비늘에서 291.9(7.7%)個, 尾部筋肉에서 244.6(6.4%)個였다. 따라서 發見된 幼蟲의 合計는 38,511로 銀魚한마리당 平均 3,851.1個이고 部位別 感染率의 順位는 筋肉(63.0%), 皮下組織(29.3%) 및 비늘 (7.7%)의 順이었다.

銀魚의 側線直上部로서 背鰭의 아랫部分 1cm² 標本區域內의 비늘을 兩側에서 採取하고 그 區域에서 發見된 幼蟲總數와 銀魚全體에서 採取한 幼蟲의 總數를 比較하고 相關關係 有無를 檢討한바 特히 相關關係가 있는 것으로 생각되지 않았다. 따라서 標本區域內 비늘에서의 幼蟲數를 各地域別銀魚의 感染指數(Infection Index)로 하기에는 考慮할 點이 있다고 생각한다.

마우스 體內에서의 本吸蟲의 發育樣相을 보기 爲하여 마우스 20마리에 200~5000個의 被囊幼蟲으로 感染시키고 每日 屠殺하여 蟲體를 採取하고 感染經過에 따르는 發育狀態를 觀察, 計測하였다. 즉 感染 第1日蟲體에서는 睾丸 및 卵巢原基의 發育이 顯著하였으며 第2日에서 睾丸의 輪廓이 明瞭하게, 크기는 約 3倍로까지 增大하였다. 第3日에서는 受精囊이 計測可能할 程度로 增大하였

고 兩側性 排泄管이 뚜렷이 보이게 되었다. 第4日蟲體에서는 貯精囊이 計測되었고 第5日乃至6日에서는 20여개의 卵黃腺이 蟲體後部兩側에 뚜렷이 出現하였고 輸卵管도 蟲體背部에서 明確히 볼 수 있었다. 子宮管內에 蟲卵이 出現하기 始作하였으며 特히 7日 以后蟲體에서는 子宮管內에 蟲卵이 더욱 充滿되었으며 蟲體의 거의 完全한 成熟形態를 認定할 수 있었다. 그러나 大便內에 蟲卵이 처음으로 나타나기 始作한 것은 第10日이었다. 第6日 以后 蟲體後半部의 成長이 뚜렷하고 腹吸盤의 크기는 口吸盤의 크기를 凌駕하였다. 以上의 結果로 미루어 볼때 同一年齡, 同一宿主內에서도 蟲體의 發育이 均等하지는 않았으나 마우스 體內에서 發育하는데 必要한 날자는 最少 7日 以上인 것으로 推測할 수 있었다.

感染經過에 따라 腸管內 寄生狀況을 觀察하였던바 蟲體가 大體로 感染初期에는 小腸의上部에서 多數 發見되었으나 感染日數가 經過함에 따라 漸次 發見部位가 小腸의 上部로부터 下部로 移動되었다. 小腸下部에서 發見된 蟲體는 發育도 不良하였으며 一般으로 蟲體回收率(Recovery rate)도 感染經過에 따라 漸次 低下되는것 같다.

Plate I

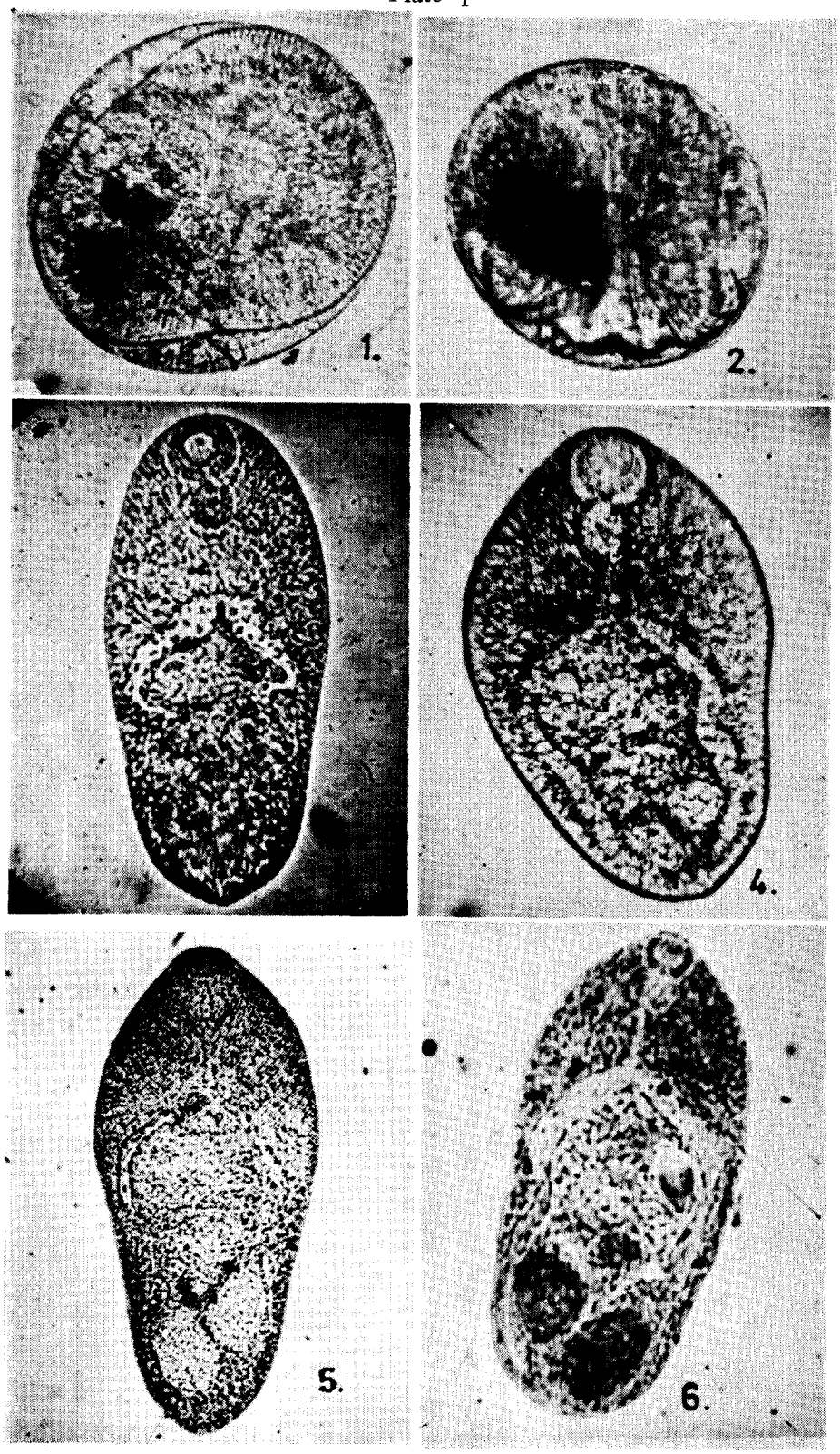

Plate Ⅱ