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Infection Status of Estuarine Fish and Oysters with Intestinal Fluke Metacercariae in Muan-gun, Jeollanam-do, Korea

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Abstract: The source of human infection with intestinal flukes was surveyed in estuarine fishes, including the dotted gizzard shad, common sea bass, common blackish goby, redlip mullet, black sea bream, and oyster collected from Muan-gun, Jeollanam-do, Korea during August and September 2007. Collected fishes and oysters were artificially digested in pepsin-HCl solution and examined under a stereomicroscope. In 36 shads (*Konosirus punctatus*) and 20 basses (*Lateolabrax japonicus*) examined, *Heterophyopsis continua* metacercariae were found in 58.3% and 100%, and their average numbers were 12.0 and 6.3 per infected fish, respectively. In 34 gobies (*Acanthogobius flavimanus*) examined, metacercariae of *H. continua* were detected in 79.4%, *Stictodora lari* in 97.1%, and *Acanthotrema felis* in 92.1%, and their average numbers were 45.8, 189.3, and 235.3 per infected fish, respectively. In 37 redlip mullets (*Chelon haematocheilus*), *Heterophyes nocens* metacercariae were found in 56.8%, *Pygidiopsis summa* in 94.6%, and *Stictodora fuscata* in 45.9%, and the average metacercarial densities were 17.4, 31.3, and 35.1 per infected fish, respectively. In 30 black sea breams (*Acanthopagrus schlegeli*) and 45 oysters (*Crassostrea gigas*) examined, no metacercariae were detected. From the above results, it has been confirmed that the dotted gizzard shad, common sea bass, common blackish goby, and redlip mullet from Muan-gun, Jeollanam-do, Korea are infected with the metacercariae of heterophyid flukes.

Key words: *Heterophyopsis continua, Stictodora lari, Acanthotrema felis, Heterophyes nocens, Pygidiopsis summa, Stictodora fuscata*, metacercaria, fish, oyster, Muan-gun

INTRODUCTION

Foodborne intestinal trematodes affect the health of more than 40 million people around the world. About 70 species belonging to 14 families are known to participate in human infections. Among them, heterophyid trematodes (members of the family Heterophyidae) are minute parasites that are clinically important in both intestinal and extraintestinal infections. More than 30 species in 13 genera have been known to infect human beings all over the world [1-4]. In the Republic of Korea (= Korea), 11 species in 8 genera have been reported as human-infecting species of heterophyid flukes. Among them, 7 species (*Heterophyes nocens, Heterophyopsis continua, Pygidiopsis summa, Stellantchasmus falcatus, Stictodora fuscata, Stictodora lari,* and *Acanthotrema felis*) are infected by consumption of the raw flesh of estuarine fish [2,5-11].

It has been shown that a lot of residents in south and west coastal areas of Korea are infected with several species of intestinal flukes [12-17]. Estuarine fish, such as *Lateolabrax japonicus, Konosirus punctatus, Mugil cephalus, Chelon haematocheilus, Acanthogobius flavimanus, Boleophthalmus pectinirostris,* and *Scartelaos* sp., have been reported as the second intermediate host and/or the source of human infection of heterophyid flukes in endemic areas [5-11,18-24]. These fish hosts are sometimes heavily infected with metacercariae of heterophyid flukes, and are popularly eaten raw by many residents in coastal areas. On the other hand, Muan-gun, Jeollanam-do was recently known as a high endemic area of intestinal flukes [25]. However, the infection source of these flukes were partly reported in the surveyed area. Therfore, we performed an epidemiological survey

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to know the infection status of metacercariae in estuarine fish and oysters collected form 3 coastal areas in Muan-gun, Jeollanam-do, Korea.

MATERIALS AND METHODS

During August and September 2007, the dotted gizzard shad (*K. punctatus*), common sea bass, (*L. japonicus*), common blackish goby (*A. flavimanus*), redlip mullet (*C. haematocheilus*), black sea bream (*Acanthopagrus schlegeli*), and oyster (*Crassostrea gigas*) were collected in 3 administrative regions of Muan-gun, Jeollanam-do, Korea (Fig. 1). All collected fishes and oysters were transferred to our laboratory (Department of Parasitology, Gyeongsang National University School of Medicine, Jinju, Korea) with ice, measured for length and weight, and examined by artificial digestion method (Table 1). Each fish was finely ground with a mortar with a pestle, or grinder, the ground fish meat was mixed with artificial gastric juice, and the mixture was incubated at 36℃ for 2-3 hr. The digested material was filtered with 1×1 mm of mesh, and washed with 0.85% saline untill the supernatant became clear. Metacercariae were collected from the sediment under a stereomicroscope, and categorized according to the measurements and morphological characters. Infection rates and intensities were then calculated.

RESULTS

Heterophyopsis continua metacercariae

Metacercariae of *H. continua* were detected in 21 (58.3%) dotted gizzard shads, *K. punctatus*, 20 (100%) common sea basses, *L. japonicus*, and 27 (79.4%) common blackish gobies, *A. flavimanus*. The average metacercarial desity per infected fish was 11.9 in *K. punctatus*, 6.3 in *L. japonicus*, and 45.8 in *A. flavimanus*, respectively. The infection status of 3 fish species by their collection site is depicted in Table 2.

Stictodora lari and *Acanthotrema felis* metacercariae

Besides the metacercariae of *H. continua*, those of *S. lari* and *A. felis* were also detected in *A. flavimanus*. Metacercariae of *S. lari* were detected in 33 (97.1%) gobies, and *A. felis* metacercariae were found in 31 (91.2%) of 34 gobies examined. The average number of metacercariae per infected fish was 189.3 (*S. lari*) and 235.3 (*A. felis*), respectively. The infection status

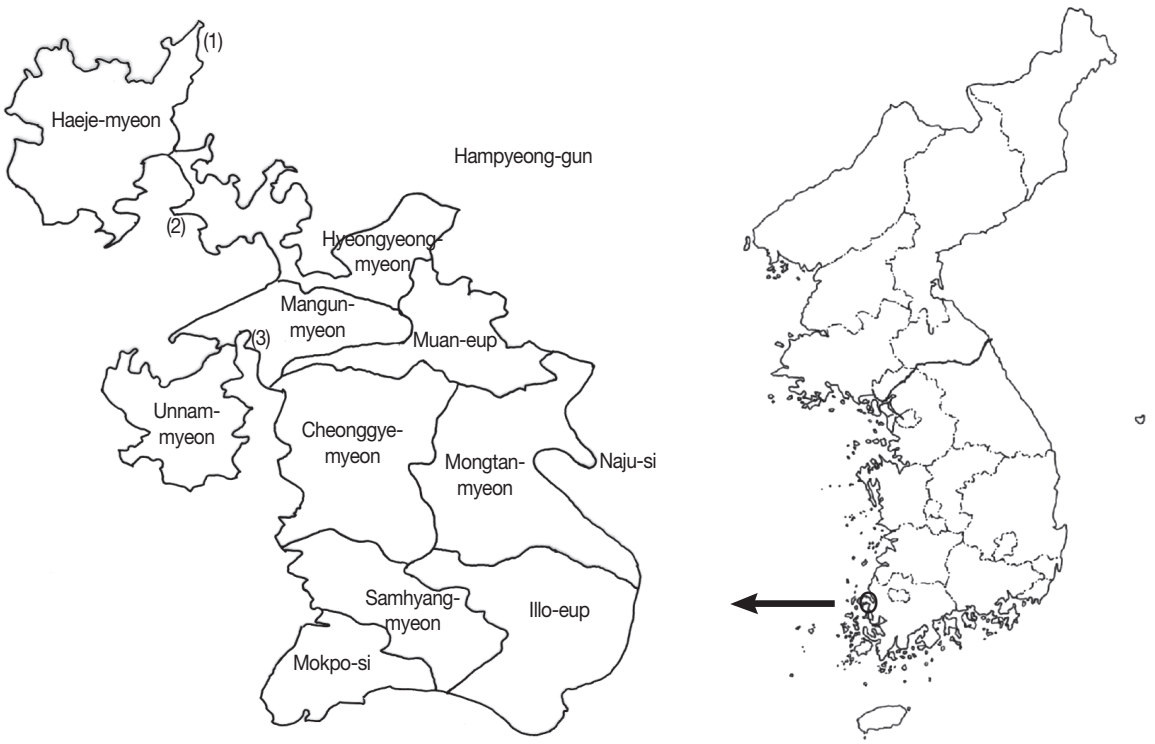

Fig. 1. Surveyed areas (O). 1, Songseok-ri in Haeje-myeon; 2, Oryu-ri in Hyeongyeong-myeon; 3, Piseo-ri in Mangun-myeon, Muan-gun, Jeollanam-do, Korea.

Table 1. Estuarine fish and oysters collected from Muan-gun, Jeollanam-do, Korea

Fish and locality[a]	No. of fish examined	Length (cm)		Weight (g)	
		Range	Average	Range	Average
Konosirus punctatus					
Haeje-myeon	14	17.6-19.9	18.5	41.2-67.4	52.7
Hyeongyeong-myeon	12	17.5-19.0	18.6	42.7-55.7	49.6
Mangun-myeon	10	10.7-18.0	14.3	8.6-52.0	28.8
Lateolabrax japonicus					
Hyeongyeong-myeon	10	18.0-21.0	19.5	61.4-91.7	79.6
Mangun-myeon	10	18.3-21.0	19.4	58.6-100.1	74.0
Acanthogobius flavimanus					
Haeje-myeon	13	11.5-15.3	13.8	10.6-27.0	19.6
Hyeongyeong-myeon	10	13.0-20.7	17.2	13.9-63.8	35.4
Mangun-myeon	11	12.5-18.5	14.7	14.4-43.5	22.4
Chelon haematocheilus					
Haeje-myeon	12	24.5-30.0	27.1	103.3-231.4	157.0
Hyeongyeong-myeon	15	15.0-23.2	18.3	27.9-97.7	50.8
Mangun-myeon	10	14.3-27.5	18.0	20.2-156.9	49.9
Acanthopagrus schlegeli					
Haeje-myeon	10	9.7-14.0	12.2	17.6-48.0	31.8
Hyeongyeong-myeon	10	11.7-20.2	16.5	23.2-68.7	47.8
Mangun-myeon	10	10.2-17.5	14.6	18.8-54.7	40.3
Crassostrea gigas					
Haeje-myeon	15	-	-	17.6-48.0	31.8
Hyeongyeong-myeon	15	-	-	23.2-68.7	47.8
Mangun-myeon	15	-		18.8-54.7	40.3

[a]Surveyed areas: Songseok-ri in Haeje-myeon; Oryu-ri in Hyeongyeong-myeon; Piseo-ri in Mangun-myeon, Muan-gun, Jeollanam-do, Korea.

Table 2. Infection status of *Heterophyopsis continua* metacercariae in fish collected from Muan-gun, Jeollanam-do, Korea

Fish species and locality	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Konosirus punctatus					
Haeje-myeon	14	8 (57.1)	70	1-56	8.8
Hyeongyeong-myeon	12	9 (75.0)	161	1-88	17.9
Mangun-myeon	10	4 (40.0)	19	2-8	4.8
Total	36	21 (58.3)	250	1-88	11.9
Lateolabrax japonicus					
Hyeongyeong-myeon	10	10 (100)	98	3-35	9.8
Mangun-myeon	10	10 (100)	27	1-8	2.7
Total	20	20 (100)	125	1-35	6.3
Acanthogobius flavimanus					
Haeje-myeon	13	13 (100)	501	1-135	38.5
Hyeongyeong-myeon	10	10 (100)	449	4-165	44.9
Mangun-myeon	11	4 (36.4)	11	1-6	2.8
Total	34	27 (79.4)	961	1-165	45.8

by the fish collection site is shown in Table 3.

Heterophyes nocens, Pygidiopsis summa, and *Stictodora fuscata* metacercariae

In the redlip mullet, *C. haematocheilus,* metacercariae of *H. nocens, P. summa,* and *S. fuscata* were detected. *H. nocens* metacercariae were found in 21 (56.8%) mullets, and their average number per infected fish was 17.4. A total of 1,097 *P. summa* metacercariae were collected from 35 (94.6%) mullets. *S. fuscata* metacercariae were detected in 17 (45.9%) of 37 mullets

Table 3. Infection status of *Stictodora lari* and *Acanthotrema felis* metacercariae in *Acanthogobius flavimanus* fish from Muan-gun, Jeollanam-do, Korea

Trematode species and locality	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Stictodora lari					
Haeje-myeon	13	12 (92.3)	1,368	17-377	114.0
Hyeongyeong-myeon	10	10 (100)	2,475	22-572	247.5
Mangun-myeon	11	11 (100)	2,403	5-830	218.5
Total	34	33 (97.1)	6,246	5-830	189.3
Acanthotrema felis					
Haeje-myeon	13	10 (76.9)	509	1-187	50.9
Hyeongyeong-myeon	10	10 (100)	4,322	55-1,156	432.2
Mangun-myeon	11	11 (100)	2,462	8-789	223.8
Total	34	31 (91.2)	7,293	1-1,156	235.3

examined, and their average number per infected fish was 35.1. The infection status by the mullet collection site is shown in Table 4.

Table 4. Infection status of *Heterophyes nocens*, *Pygidiopsis summa*, and *Stictodora fuscata* metacercariae in *Chelon haematocheilus* fish from Muan-gun, Jeollanam-do, Korea

Trematode species and locality	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Heterophyes nocens					
Haeje-myeon	12	11 (91.7)	196	1-71	17.8
Hyeongyeong-myeon	15	2 (13.3)	67	2-65	33.5
Mangun-myeon	10	8 (80.0)	103	1-62	12.9
Total	37	21 (56.8)	366	1-71	17.4
Pygidiopsis summa					
Haeje-myeon	12	11 (91.7)	356	1-126	32.4
Hyeongyeong-myeon	15	14 (93.3)	318	1-257	22.7
Mangun-myeon	10	10 (100)	423	2-240	42.3
Total	37	35 (94.6)	1,097	1-257	31.3
Stictodora fuscata					
Haeje-myeon	12	10 (83.3)	378	9-113	37.8
Hyeongyeong-myeon	15	7 (46.7)	219	1-57	31.3
Mangun-myeon	10	0	-	-	-
Total	37	17 (45.9)	597	1-113	35.1

Other metacercariae

No metacercariae were detected in 30 black sea breams, *A. schlegeli*, and 45 oysters, *C. gigas*, examined. However, metacercariae of *Gymnophalloides seoi* were found in group examinations of oysters, about 100 oysters each from Haeje-myeon (40 metacercariae of *G. seoi*) and Hyeongyeong-myeon (8 metacercariae of *G. seoi*).

DISCUSSION

By the present study, it has been confirmed that intestinal flukes are prevalent not only in the human definitive host [25] but also in fish intermediate host in Muan-gun, Jeollanam-do, Korea. Chai et al. [12] reported a high egg positive rate (75.0%) of heterophyid flukes among 108 residents in a small coastal village of Muan-gun. Cho et al. [25] detected eggs of heterophyids from 62 (4.9%) of 1,257 residents in Muan-gun, and they recovered 6 species of heterophyid flukes (*H. nocens*, *P. summa*, *S. falcatus*, *S. fuscata*, *S. lari*, and *A. felis*), and *G. seoi* from 9 residents after praziquantel treatment and purgation. In the present study, 6 heterophyid species metacercariae (*H. nocens*, *H. continua*, *P. summa*, *S. fuscata*, *S. lari*, and *A. felis*) were detected from the redlip mullet, common blackish goby, dotted gizzard shad, and common sea bass collected in Muan-gun.

H. nocens is known to be the domimant species of heterophyid among the residents of western and southern coastal areas, including Shinan-gun, Gangjin-gun and Muan-gun (Jeollanam-do), Buan-gun (Jeollabuk-do), and Sacheon-si (Gyeongsangnam-do), Korea [12-16,25]. For the second intermediate host of this fluke, several species of estuarine fish (*M. cephalus, C. haematocheilus, A. flavimanus*, *B. pectinirostris*, and *Scartelaos* sp.) have been reported [7,18,22]. In the present study, *H. nocens* metacercariae were detected only in the redlip mullet, *C. haematocheilus*. Their infection rate (56.8%) and intensity (17.4 metacercariae per infected fish) were not so high, compared to high worm burdens in human infection cases [12,25]. This finding suggests that residents in endemic areas habitually eat raw flesh of mullets, and worms may be accumulated by repeated infections.

The common blackish goby, *A. flavimanus*, is a suitable fish host for 6 species of heterophyid flukes (*H. nocens*, *H. continua*, *P. summa*, *S. fuscata*, *S. lari*, and *A. felis*) in Korea [10,11,18,19]. In the present study, 3 species of metacercariae (*H. continua*, *S. lari*, and *A. felis*) were detected; their infection rates (79.4, 97.1, and 91.2%) were relatively high and the intensity was about 46, 189, and 235 metacercariae per infected fish, respectively. Sohn et al. [24] also detected 3 species of metacercariae (*H. continua*, *Stictodora* spp., and *H. nocens*) in 15 gobies from Haeje-myeon in Muan-gun. In the case of *H. continua* metacercariae in the goby from Muan-gun, the infection rate was all 100%, and the intensity was slightly higher in the present study (38.5 metacercariae) than in Sohn et al. [24] (23.3 metacercariae).

In addition, by the present study, it is confirmed that *A. flavimanus* briskly act as a second intermediate host for *A. felis* in Muan-gun, Jeollanam-do, Korea [11].

No metacercariae were detected in 30 black sea breams and 45 oysters examined individually. However, *G. seoi* metacercariae were found in group examination of about 100 oysters from Haeje-myeon and Hyeongyeong-myeon. These findings provide a background for positive adult worm recovery in residents [12,25], and suggest that oysters from Muan-gun, Jeollanam-do act as the source of *G. seoi* infection, although their metacercarial density is very low.

Metacercarial examination in the second intermediate host, in combination with a survey on adult worms in humans and also on larvae in the snail intermediate host, can be a usefull index in the epidemiology of trematodes in a particular area. However, in the case of heterophyid trematodes, fecal examinations are not suscessful to determine the infection status in humans and reservoir hosts, since the egg production amount is too small, and the first intermediate host is not well known. Therefore, we investigated the infection status of estuarine fish with metacercariae in order to provide epidemiological information for heterophyid flukes in Muan-gun, Jeollanam-do. Conclusively, the present study suggested that those who consume raw estuarine fish from Muan-gun are at a high risk of infection with heterophyid flukes.

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