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, Stictodora lari, and Acanthotrema felis) are known to be infected by consumption of the raw flesh of estuarine fish [2,5-11].

In South Korea, 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
to know the infection status of metacercariae in estuarine fish and oysters collected from 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 schlegelii*), 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˚C for 2-3 hr. The digested material was filtered with 1 \times 1 mm of mesh, and washed with 0.85% saline until 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 density 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

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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 | 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 schlegelii |                |             |            |             |            |
| 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       |

*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      |                      | 8 (57.1)     | 70 1-56  | 8.8  |
| 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    |                      | 10 (100)     | 98 3-35  | 9.8  |
| Hyeongyeong-myeon        | 10                   | 10 (100)     | 27 1-8   | 2.7  |
| Mangun-myeon             | 10                   | 10 (100)     | 125 1-35 | 6.3  |
| Total                    | 20                   | 20 (100)     | 250 1-35 | 11.9 |
| Acanthogobius flavimanus |                      | 13 (100)     | 501 1-135| 38.5 |
| 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.
examined, and their average number per infected fish was 35.1. The infection status by the mullet collection site is shown in Table 4.

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. continua, P. summa, 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 dominant 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. haematobheilus, 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. haematobheilus. 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, Sictodora 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).

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  | 216.5  |
| 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  |

Table 4. Infection status of Heterophyes nocens, Pygidiopsis summa, and Stictodora fuscata metacercariae in Chelon haematobheilus 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   |
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 Hyeonggyeong-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 useful index in the epidemiology of trematodes in a particular area. However, in the case of heterophyid trematodes, fecal examinations are not successful 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.

**ACKNOWLEDGMENTS**

This study was supported by an Anti-Communicable Diseases Control Program of the National Institute of Health (NIH 348-6111-215), Ministry of Health and Welfare, the Republic of Korea. We thank Miss A-Ra Cho and Hae-In Ryu, Department of Parasitology, Gyeongsang National University School of Medicine, Jinju, Korea, for their help in fish examinations.

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