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▣ ORIGINAL ARTICLE

Pygidiopsis summa (Digenea: Heterophyidae): Status of Metacercarial Infection in Mullets from Coastal Areas in the Republic of Korea

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Abstract: To know the infection status of zoonotic trematode metacercariae in brackish water fish, we surveyed mullets collected from 18 coastal areas in the Republic of Korea. The metacercariae of *Pygidiopsis summa* were detected in 236 (68.2%) out of 346 mullets examined. They were found in mullets from 15 areas except for those from Boseong-gun (Jeollanam-do), Pohang-si, and Uljin-gun (Gyeongsangbuk-do). Especially in mullets from Taean-gun (Chungcheongnam-do) and Geoje-si (Gyeongsangnam-do), their prevalences were 100% and 95.5%, and the average metacercarial density was more than 1,000 per fish. They were also detected in mullets from 3 coastal lakes, Gyeongpoho, Songjiho, and Hwajinpo-ho, in Gangwon-do, and their average densities were 419, 147, and 672 per infected fish, respectively. The metacercariae of 5 other heterophyid species, including *Heterophyes nocens*, *Heterophyopsis continua*, *Metagonimus* sp., *Stictodora fuscata*, and *Stictodora lari*, were found in the mullets examined. The metacercariae of *H. nocens* were detected in 66.7, 100, 28.6, 81.6, 3.9, 61.5, and 27.3% of mullets from Muan-gun, Shinan-gun, Haenam-gun, Gangjin-gun, and Boseong-gun (Jeollanam-do), Hadong-gun, and Geoje-si (Gyeongsangnam-do), and their metacercarial intensities were 64, 84, 119, 99, 1, 24, and 24 per fish infected, respectively. From the above results, it has been confirmed that *P. summa* metacercariae are heavily infected in mullets from coastal areas of Korea. It is suggested that residents who frequently consume raw mullet dish can be easily infected with heterophyid flukes.

Key words: *Pygidiopsis summa*, heterophyid fluke, infection status, metacercaria, mullet, coastal area

INTRODUCTION

Pygidiopsis summa (Digenea: Heterophyidae) is a very small intestinal trematode. This fluke was first found from dogs experimentally fed the metacercariae from mullets, *Mugil cephalus*, in Japan [1]. Human infections with this fluke were confirmed by detection of eggs in the feces and also adult flukes in Japan [2,3]. In the Republic of Korea (Korea), human cases have been sporadically reported from western and southern coastal areas, i.e., Okgu-gun and Buan-gun (Jeollabuk-do), Shinan-gun, Muan-gun, Gangjin-gun, Haenam-gun, and Yeoungam-gun (Jeollanam-do) [4-11]. Some species of brackish water fish, namely, *M. cephalus, Liza haematocheila*, and *Acanthogobius flavimanus*, are known to be the source of human infections and second intermediate hosts of this fluke [12-17].

A total of 11 species in 8 genera have been reported as zoonotic heterophyid flukes (ZHF) in Korea. Among these, 7 including *P. summa* are contracted by consumption of raw flesh of brackish water fish, including *M. cephalus, L. haematocheila, A. flavimanus, Lateolabrax japonicus, Konosirus punctatus, Boleophthalmus pectinirostris*, and *Scartelaos* sp., in Korea [12]. Studies on the source of human infections with ZHF were performed mainly in western and southern coastal areas of Korea. Most of the surveys were carried out in limited areas and examined a small number of fish hosts [13-18]. Especially, Seo et al. [14] surveyed total 59 mullets (1-14; av. 4.2 per locality) from 14 localities, and Guk et al. [17] examined a total of 139 mullets (5-20; av. 12.6) from 11 areas. However, the number of examined fish was not enough for evaluation of the metacercarial endemicity in each surveyed area. Therefore, we examined

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mullets broadly collected from 18 coastal areas in 3 Korean seas, Yellow Sea, South Sea, and East Sea, to know the infection status of zoonotic trematode metacercariae.

MATERIALS AND METHODS

Collection sites of mullets

The collection sites of mullets were as follows: 1. Taean-gun, Gyeongsangnam-do: Byeongsulman in Ahnmyeon-eup (latitude: 36.478867; longitude: 126.342750); 2. Gochang-gun, Jeollabuk-do: Gusipo in Sangha-myeon (35.429330; 126.448270); 3. Muan-gun, Jeollanam-do: Haeje-myeon (35.092247; 126.304052); 4. Shinan-gun, Jeollanam-do: Aphae-eup (34. 841932; 126.363447); 5. Haenam-gun, Jeollanam-do: Hwangsan-myeon (34.596476; 126.460790); 6. Gangjin-gun, Jeollanam-do: Daegu-myeon (34.500880; 126.792037); 7. Boseong-gun, Jeollanam-do: Beolgyo-eup (34.830308; 127.370214); 8. Hadong-gun, Gyeongsangnam-do: Jingyo-myeon (35.009910; 127.918501); 9. Sacheon-si, Gyeongsangnam-do: Gonyangcheon in Gonyang-myeon (35.049422; 127.975090); 10. Goseong-gun, Gyeongsangnam-do: Baedun-myeon (35.048684; 128.372615); 11. Geoje-si, Gyeongsangnam-do: Dundeokcheon in Dundeok-myeon (34.838177; 128.508674); 12. Ulsan Metropolitan City: Taehwagang in Jung-gu (35.547438; 129.297372); 13. Pohang-si, Gyeongsangbuk-do: Hyeongsangang in Yeonil-eup (36.003818; 129.332359); 14. Yeongdeokgun, Gyeongsangbuk-do: Osipcheon in Ganggu-myeon (36.379421; 129.376851); 15. Uljin-gun, Gyeongs angbuk-do: Wangpicheon in Uljin-eup (36.965826; 129.394991); 16. Gangneung-si, Gangwon-do: Gyeongpoho (ho means lake) in

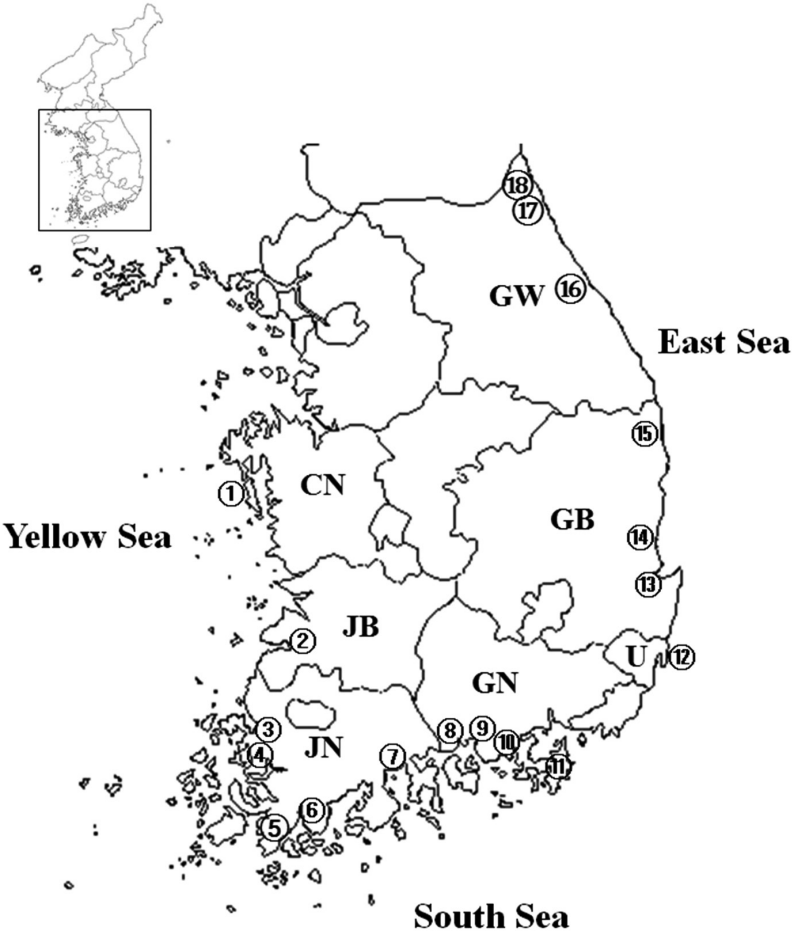

Fig. 1. Surveyed areas in South Korea. 1. Taean-gun, Chungcheongnam-do (CN); 2. Gochang-gun, Jeollabuk-do (JB); 3. Muan-gun; 4. Shinan-gun; 5. Haenam-gun; 6. Gangjin-gun; 7. Boseong-gun, Jeollanam-do (JN); 8. Hadong-gun; 9. Sacheon-si; 10. Goseong-gun; 11. Geoje-si, Gyeongsangnam-do (GN); 12. Ulsan Metropolitan City (U); 13. Pohang-si; 14. Yeongdeok-gun; 15. Uljin-gun, Gyeongsangbuk-do (GB); 16. Gangneung-si; 17. Goseong-gun (Songjiho); 18. Goseong-gun (Hwajinpoho), Gangwon-do (GW).

Woonjeong-dong (37.797481; 128.911507); 17. Goseong-gun, Gangwon-do: Songjiho in Jukwang-myeon (38.335426; 128.51339); 18. Goseong-gun, Gangwon-do: Hwajinpoho in Geojin-eup (38.466 792; 128.442357) (Fig. 1).

Examination of mullets

Total 346 mullets were collected in the above 18 coastal areas for 3 years (2013: 1-4; 2014: 5-11, 16, and 18; 2015: 12-15 and 17). The number of fish collected in each site was designated in Table 1. All collected mullets were transferred to our laboratory (Department of Parasitology and Tropical Medicine, Gyeongsang National University School of Medicine, Jinju, Korea) with ice, and examined by artificial digestion method. Each fish was finely ground with a mortar with a pestle or a grinder, the ground fish flesh was mixed well with artificial gastric juice, and the mixture was incubated at 36°C for 2 hr. The digested material was filtered through a 1×1 mm of mesh, and washed with 0.85% saline until the supernatant became clear. Trematode metacercariae were collected from sediments under a stereomicroscope, and categorized according to the size and morphological characteristics. The prevalence and intensities of infection were then calculated.

RESULTS

Metacercarial infection in mullets

The metacercariae of *P. summa* were detected in mullets from 15 areas except for 7. Boseong-gun (Jeollanam-do), 13. Pohang-si, and 15. Uljin-gun (Gyeongsangbuk-do). The overall infection rate was 68.2% (236 out of 346 mullets examined). They were found in 76 (93.8%) out of 81 mullets from 4 coastal areas (1-4) of Yellow Sea, 122 (69.3%) of 176 ones from 7 sites (5-11) of South Sea, and in 38 (42.7%) out of 89 mullets from 7 sites (12-18) of East Sea. Their average intensities were 496, 369, and 291 per fish infected, respectively. Especially in mullets from 1. Taean-gun (Chungcheongnam-do) and 11. Geoje-si (Gyeongsangnam-do), their prevalences were 100% and 95.5%, and intensities were 1,116 and 1,444 per fish infected. They were also detected in mullets from 3 coastal lakes, i.e., Gyeongpoho, Songjiho, and Hwajinpoho ('-ho' means lake), in Gangwon-do, and their average intensities were 419, 147, and 672 per fish infected, respectively. The infection status of *P. summa* metacercariae in mullets by collection sites is shown in Table 1.

Other heterophyid metacercariae

The metacercariae of 5 other species of heterophyids, includ-

Table 1. Infection status of *Pygidiopsis summa* metacercariae by the collection sites of mullet

Locality[a]	No. of mullets examined	No. (%) mullets infected	No. of metacercariae detected Average (Range)	Other heterophyid metacarcariae: Prevalence (average no. of metacercariae per fish infected)
1. Taean-gun, Chungcheongnam-do	30	30 (100)	1,116 (170-3,936)	-
2. Gochang-gun, Jeollabuk-do	15	12 (80.0)	62 (1-425)	Hc: 6.7% (2); Sl: 13.3% (2)
3. Muan-gun, Jeollanam-do	15	15 (100)	20 (4-82)	Hn: 66.7% (64)
4. Shinan-gun, Jeollanam-do	21	19 (90.5)	168 (5-712)	Hn: 100% (84); Hc: 4.8% (2)
5. Haenam-gun, Jeollanam-do	35	33 (94.3)	42 (1-367)	Hn: 28.6% (119); Hc: 5.7% (1)
6. Gangjin-gun, Jeollanam-do	38	17 (44.7)	12 (2-74)	Hn: 81.6% (99); Hc: 15.8% (3); Sl: 18.4% (4); Sf: 2.6% (1); Meta: 2.6% (1)
7. Boseong-gun, Jeollanam-do	26	0	-	Hn: 3.9% (1); Hc: 15.4% (2)
8. Hadong-gun, Gyeongsangnam-do	13	13 (100)	140 (2-815)	Hn: 61.5% (24); Hc: 7.7% (5)
9. Sacheon-si, Gyeongsangnam-do	17	16 (94.1)	25 (3-74)	-
10. Goseong-gun, Gyeongsangnam-do	25	22 (88.0)	495 (1-2,670)	-
11. Geoje-si, Gyeongsangnam-do	22	21 (95.5)	1,444 (21-4,520)	Hn: 27.3% (24); Hc: 4.6% (1)
12. Ulsan Metropolitan City	20	7 (35.0)	2 (1-4)	Meta: 5.0% (1)
13. Pohang-si, Gyeongsangbuk-do	20	0	-	-
14. Yeongdeok-gun, Gyeongsangbuk-do	8	3 (37.5)	8 (1-22)	Meta: 12.5% (1)
15. Uljin-gun, Gyeongsangbuk-do	9	0	-	-
16. Gangneung-si, Gangwon-do	8	8 (100)	419 (82-1,286)	Meta: 12.5% (1)
17. Goseong-gun (Songjiho), Gangwon-do	11	11 (100)	147 (32-678)	-
18. Goseong-gun (Hwajinpo), Gangwon-do	13	9 (69.2)	672 (2-3,050)	-

Hc, *Heterophyopsis continua*; Hn, *Heterophyes nocens*; Meta, *Metagonimus* sp.; Sf, *Stictodora fuscata*; Sl, *Stictodora lari*.
[a]Administative regions of mullet collection sites, which are revealed in detail in Materials and Methods.

ing *Heterophyes nocens, Heterophyopsis continua, Metagonimus* sp., *Stictodora fuscata*, and *Stictodora lari*, were found in mullets examined. The metacercariae of *H. nocens* were detected in 66.7, 100, 28.6, 81.6, 3.9, 61.5, and 27.3% mullets from Muan-gun, Shinan-gun, Haenam-gun, Gangjin-gun, and Boseong-gun (Jeollanam-do), Hadong-gun, and Geoje-si (Gyeongsangnam-do), respectively. Their metacercarial densities were 64, 84, 119, 99, 1, 24, and 24 per fish infected, respectively. *H. continua* metacercariae were found in mullets from Gochang-gun (Jeollabuk-do), Shinan-gun, Haenam-gun, Gangjin-gun, and Boseong-gun (Jeollanam-do), Hadong-gun, and Geoje-si (Gyeongsangnam-do). Their infection rates and intensities were relatively low. The infection status of heterophyid metacercariae by collection sites of fish is shown in Table 1.

DISCUSSION

By the present study, it has been confirmed that *P. summa* metacercariae are prevalent in mullets from lakes in eastern coastal areas as well as in mullets from western and southern coastal areas in Korea. Among 346 mullets collected from 18 surveyed areas, 236 (68.2%) from 15 areas, except for Boseong-gun (Jeollanam-do), Pohang-si, and Uljin-gun (Gyeongsangbuk-do), were infected with *P. summa* metacercariae. Especially mullets from Taean-gun (Chungcheongnam -do) and Geoje-si (Gyeongsangnam-do) were infected with more than 1,000 metacercariae per infected fish. On the other hand, Seo et al. [14] detected *P. summa* metacercariae in 17 (28.8%) out of 59 mullets collected from 14 surveyed areas, and they found them in mullets from only 4 localities, i.e., Okgu-gun (Jellabuk-do), Mokpo-si, Yeocheon-gun (Jellanam-do), and Sacheon-si (Gyeongsangnam-do). Metacercarial burdens were relatively low except for mullets from Okgu-gun [14]. Guk et al. [17] reported that *P. summa* metacercariae were infected in 88 (63.3%) out of 139 mullets collected from 11 surveyed areas, and their intensity per infected fish was not so high, i.e., 146 metacercariae in average. Moreover, the mullets from 3 localities, i.e., Masan-si (Gyeongsangnam-do), Yeongdeok-gun (Gyeongsangbuk-do), and Sokcho-si (Gangwon-do), were negative for *P. summa* metacercariae [17].

The prevalence and intensity of *P. summa* metacercariae in mullets have been shown to vary by geographical localities of 3 different seas in the Korean peninsula. In the present study, the metacercariae of *P. summa* were detected in 76 (93.8%) out of 81 mullets from 4 coastal areas of Yellow Sea, 122 (69.3%) of 176 mullets from 7 areas of South Sea, and in 38 (42.7%) out of 89 mullets from 7 sites of East Sea. The average intensities were 496, 369, and 291 metacercariae per fish infected, respectively. Whereas, in Guk et al. [17], the prevalences were 87.0, 54.1, and 4.0% in mullets from the coastal areas of Yellow Sea, South Sea, and East Sea, and their average intensities were 177, 47, and 8 per fish infected, respectively. The trends of metacercarial infection in this study, the higher prevalence and higher intensity in mullets from the coastal areas in Yellow Sea than in other 2 Seas, were similar to those of Guk et al. [17], although each data of this study was much higher. This endemic trend of *P. summa* metacercariae in mullets coincided well with the trend that human cases with this fluke have been mainly reported in the western and southern coastal areas, i.e., Okgu-gun and Buan-gun (Jeollabuk -do), Shinan-gun, Muan-gun, Gangjin-gun, Haenam-gun, and Youngam-gun (Jeollanam-do), in Korea [4-11]. Among 18 surveyed areas, 5 areas, including Muan-gun, Shinan-gun, Gangjin-gun, Sacheon-si, and Yeongdeok-gun, were the same regions as in Guk et al. [17]. The prevalence and metacercarial density in mullets from these 5 regions were somewhat different between the 2 studies. In the present study, the prevalences were 100, 90.5, 44.7, 94.1, and 37.5% in Muan-gun, Shinan-gun, Gangjin-gun, Sacheon-si, and Yeongdeok-gun, respectively, and intensities were 20, 168, 12, 25, and 8 metacercariae per fish infected, respectively. Whereas, in Guk et al. [17], the prevalences were 100, 100, 70, 77, and 0%, and the metacercarial densities were 59, 483, 2, 71, and 0, respectively. These differences were probably due to the collection time and site of mullets.

In previous studies [14,17], *P. summa* metacercariae were rarely detected in mullets from eastern coastal areas. Seo et al. [14] could not find them at all in mullets from Ulsan Metropolitan City, Pohang-si, Yeongdeok-gun (Gyeongsangbuk -do), and Gangneung-si (Gangwon-do). Guk et al. [17] detected total 8 *P. summa* metacercariae in only 1 mullet from Donghae-si (Gangwon-do). They could not detect them in mullets from Yeongdeok-gun (Gyeongsangbuk-do) and Sokcho-si (Gangwon-do) [17]. In the present study, we detected total 11,058 *P. summa* metacercariae (291 in average) in 38 (63.3%) out of 60 mullets from 5 eastern coastal sites, i.e., Ulsan Metropolitan City, Yeongdeok-gun (Gyeongsangbuk -do), and Gangneung-si, Goseong-gun (Songjiho and Hwajinpoho, Gangwon-do). Especially in mullets from 3 coastal lakes, Gyeongpoho, Songjiho, and Hwajinpoho, in

Gangwon-do, the prevalences were 100, 100, and 69.2%, and the intensities were 419, 147, and 672 metacercariae per fish infected, respectively. These interesting findings suggest that the life cycle of *P. summa* is actively maintained in the coastal lakes and adjacent areas of East Sea. Accordingly, residents who habitually eat raw flesh of mullets in these areas should pay attention to infection with this fluke.

In the present study, the metacercariae of *H. nocens* were detected in mullets from 7 localities, which included Muan-gun, Shinan-gun, Haenam-gun, Gangjin-gun, and Boseong-gun (Jeollanam-do), Hadong-gun, and Geoje-si (Gyeongsangnam -do), in western and southern coasts of Korea. Their prevalences were 66.7, 100, 28.6, 81.6, 3.9, 61.5, and 27.3%, and the intensities were 64, 84, 119, 99, 1, 24, and 24 metacercariae per fish infected, respectively. In Guk et al. [17], *H. nocens* metacercariae were found in mullets from 7 surveyed areas, i.e., Ganghwa-gun (Gyeonggi-do), Seocheon-gun (Chungcheongnam -do), Buan-gun (Jeollabuk-do), Muan-gun, Shinan-gun, Gangjin-gun (Jeollanam-do), and Sacheon-si (Gyeongsangnam -do), in western and southern coasts of Korea. Their prevalences were 42-100%, and the intensities were 4-271 metacercariae per fish infected [17]. On the other hand, it has been known that *H. nocens* is the dominant intestinal fluke species among the residents of western and southern coastal areas, i.e., Buan-gun, Muan-gun, Shinan-gun, Gangjin-gun, and Sacheon-si, in Korea [4-11]. Accordingly, by the present study, it became clear that the endemic areas of *H. nocens* are closely related to the distribution of mullets highly infected with the metacercariae of this species.

More than 18 fish species have been reported as the second intermediate hosts of *H. continua* in Asian countries, such as Japan, China, Korea, and Vietnam [15,16,19-27]. Among them, mullets are most widely distributed and highly important as the source of human infections. Human infection cases have been sporadically reported in western and southern coastal areas, i.e., Buan-gun (Jeollabuk-do), Muan-gun, Shinan-gun, Gangjin-gun, Haenam-gun, Youngam-gun (Jeollanam-do), and Sacheon-si, (Gyeongsangnam-do), in Korea [5,6,8-10,22,28]. Although infection rates and densities of *H. continua* metacercariae were relatively low, they were found in mullets from western and southern coastal areas, i.e., Gochang-gun (Jeollabuk-do), Shinan-gun, Haenam-gun, Gangjin-gun, Boseong-gun (Jeollanam-do), Hadong-gun, and Geoje-si (Gyeongsangnam-do) in the present study.

Collectively, heterophyid flukes, including *P. summa*, are prevalent in western and southern coastal areas, which developed the tideland in brackish zones. However, it has been confirmed for the first time that *P. summa* metacercariae are heavily infected in mullets from some lakes in eastern coastal areas of Korea.

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CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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