




Survey of Zoonotic Trematode Metacercariae in Fish from Irrigation Canal of Togyo-jeosuji (Reservoir) in Cheorwon-gun, Gangwon-do, Republic of Korea

Woon-Mok Sohn^{1,*} , Byoung-Kuk Na¹ , Shin-Hyeong Cho², Hee Il Lee², Jung-Won Ju² , Myoung-Ro Lee², Eun-Joo Lim³, Sung Yong Son³, Eunmi Ko³, Jaeseok Choi⁴

¹Department of Parasitology and Tropical Medicine, and Institute of Health Sciences, Gyeongsang National University College of Medicine, Jinju 52727, Korea; ²Division of Vectors and Parasitic Diseases, Korea Disease Control and Prevention Agency, Osong 28159, Korea; ³Infectious Disease Intelligence Division, Gangwon Institute of Health and Environment, Chuncheon 24203, Korea; ⁴Research Center for Fish Environmental Institute/Wildlife and Fish Research & Conservation Center, Kangwon National University, Chuncheon 24341, Korea

Abstract: The infection status of zoonotic trematode metacercariae (ZTM) was investigated in total 568 freshwater fishes (19 species) from the irrigation canal of Togyo-jeosuji (Reservoir) in Cheorwon-gun, Gangwon-do, the Republic of Korea for 3 years (2018-2020). All fishes were examined using the artificial digestion method. The metacercariae of *Clonorchis sinensis* (CsMc) were detected in 180 (43.8%) out of 411 fish of positive species, and their infection intensity was 38 per fish infected (PFI). Especially, in 2 fish species, i.e., *Pseudorasbora parva* and *Puntungia herzi*, the prevalence was 82.1% and 31.3%, and the infection intensity with CsMc was 88 and 290 PFI, respectively. *Metagonimus* spp. metacercariae (MsMc) were found in 403 (74.1%) out of 544 fish of positive species, and their infection intensity was 62 PFI. In the pale chub, *Zacco platypus*, the prevalence of MsMc was 98.6%, and their infection intensity was 144 PFI. *Centrocestus armatus* metacercariae were detected in 171 (38.9%) out of 440 fish of positive species, and their infection intensity was 1,844 PFI. *Echinostoma* spp. metacercariae were found in 94 (19.6%) out of 479 fish of positive species, and their infection intensity was 3 PFI. *Metorchis orientalis* metacercariae were detected in 43 (29.3%) out of 147 fish of positive species, and their infection intensity was 4 PFI. By the present study, it has been confirmed that some species of ZTM, including CsMc and MsMc, are prevalent in fishes from the irrigation canal of Togyo-jeosuji in Cheorwon-gun, Gangwon-do, Korea.

Key words: *Clonorchis sinensis*, *Metagonimus* spp., *Centrocestus armatus*, *Echinostoma* spp., *Metorchis orientalis*, zoonotic trematode metacercaria, Togyo-jeosuji, Cheorwon-gun, Gangwon-do

Nowadays, infection of fishborne zoonotic trematodes (FZT), including *Clonorchis sinensis*, is one of the important public health problems in the Republic of Korea (=Korea) [1]. The Division of Vectors and Parasitic Diseases of Korea DCPA (Korea Disease Control and Prevention Agency) has continuously paid attention to the decrease of the endemicity of FZT infections in endemic riverside areas of major rivers in southern regions of Korea [2-5]. The team also concentrated on the infection status of zoonotic trematode metacercariae (ZTM) in freshwater fishes, the infection sources of FZT, to surveil the epidemiological situations in water systems of major rivers in Korea [6-16]. In the water systems of Gangwon-do (do=province),

Cho et al. [6] investigated the infection status of *C. sinensis* metacercariae (CsMc) in fishes from Gangwon-do, where they surveyed fishes from 3 wide regions by the latitudinal levels of the Korean peninsula. Cho et al. [7] also surveyed the infection status of ZTM in fishes from Gangwon-do, Korea. The infections of digenetic trematode metacercariae (DTM), including *C. sinensis*, were investigated in freshwater fish from Hantan-gang (gang means river) and Imjin-gang in northern regions of Korea [8]. Recently, Sohn et al. [15] reported the infection status with DTM in fishes from coastal lakes in Gangwon-do, Korea.

Togyo-jeosuji (Reservoir) is a lake for agricultural water supply located in Dongsong-eup (eup=township), Cheorwon-gun (gun=county), Gangwon-do, Korea. This lake is famous for the visiting site of migratory birds, especially avian species at extinct crisis, crane and white-naped crane, *Grus* spp. [17]. An epidemiological survey on the infections of intestinal parasites among inhabitants and ZTM in fishes was performed in

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*Corresponding author (wmsohn@gnu.ac.kr)

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riverside areas of Hantan-gang in Cheorwon-gun, Gangwon-do [18]. A lot of fishes from Hantan-gang, adjacent river of Togyo-jeosuji, was also investigated to reveal the infection status of ZTM in previous studies [7,8]. However, the infection status of ZTM has not been examined in fishes from Togyo-jeosuji. Therefore, we intentionally investigated the infection status of ZTM in fishes from the irrigation canal of Togyo-jeosuji in Cheorwon-gun, Gangwon-do, Korea.

We collected lots of freshwater fishes in the irrigation canal of Togyo-jeosuji (Latitude: 35.980225; Longitude: 127.393880), in Dongsong-eup, Cheorwon-gun, Gangwon-do for 3 years (2018-2020). All collected fishes were transferred to the laboratory of the Department of Parasitology and Tropical Medicine, Gyeongsang National University College of Medicine, Jinju, Korea. The length and weight of fish were individually measured, and the fish species was identified [19]. Individual fish was finely ground in a mortar with pestle, the ground fish meat was mixed with artificial gastric juice, and the mixture was incubated at 36°C for about 2 hr. The digested material was filtered with 1 × 1 mm of mesh, and washed with 0.85% saline until the supernatant became clear. The sediment was carefully examined under a stereomicroscope. The metacercariae of each species (only ZTM) were separately collected viewing from the general morphological features and were counted to get hold of the prevalence (%) and intensity of infection (No. of ZTM per fish infected) by fish species [20].

A total of 200 freshwater fish in 15 species were examined in 2018. The fish species (no. of fish) examined were *Zacco platypus* (54), *Carassius auratus* (25), *Acheilognathus rhombeus*

(25), *Pseudorasbora parva* (24), *Hemibarbus labeo* (15), *Abbotina rivularis* (14), *Zacco koreanus* (12), *Rhodeus ocellatus* (10), *Microphysogobio longidorsalis* (8), *Pungtungia herzi* (4), *Coreoleuciscus splendidus* (2), *Iksookimia koreensis* (2), *Micropterus salmoides* (2), *Pseudogobio esocinus* (2), and *Odontobutis interrupta* (1). In 2019, a total of 188 freshwater fish in 12 species were examined. The fish species examined included *Z. platypus* (40), *H. labeo* (34), *C. auratus* (30), *P. esocinus* (28), *M. salmoides* (15), *Lepomis macrochirus* (11), *Hemibarbus longirostris* (8), *P. parva* (6), *O. interrupta* (6), *Acanthorhodeus gracilis* (5), *P. herzi* (3), and *R. ocellatus* (2). A total of 180 freshwater fish in 15 species were examined in 2020. The fish species examined were *Z. platypus* (45), *P. parva* (26), *H. labeo* (19), *C. auratus* (16), *R. ocellatus* (15), *Acheilognathus lanceolatus* (10), *M. salmoides* (10), *P. herzi* (9), *P. esocinus* (8), *Z. koreanus* (7), *O. interrupta* (5), *A. rivularis* (4), *L. macrochirus* (3), *M. longidorsalis* (2), and *H. longirostris* (1).

The metacercariae of *C. sinensis* were detected in 180 (43.8%) out of 411 fish of the positive species, and their intensity of infection was 38 PFI. Especially in 3 fish species, i.e., *P. parva*, *P. herzi*, and *A. gracilis*, the prevalence was 82.1%, 31.3%, and 100%, and the infection intensity was 88, 290, and 125 PFI, respectively. The overall infection status by the fish species was presented in Table 1. The prevalence of CsMc was gradually decreased by the year surveyed, 65.5% in 2018, 44.4% in 2019, and 26.7% in 2020. The intensity of infection with CsMc was highest in 2019 (49 PFI), followed by in 2020 (40 PFI) and 2018 (30 PFI) (Supplementary Table S1). *Metagonimus* spp. metacercariae were found in 403 (74.1%) out of 544 fishes of the positive species, and their intensity of infection was 62 PFI.

Table 1. Infection status of *Clonorchis sinensis* metacercariae (CsMc) in fishes from the irrigation canal of Togyo-jeosuji in Cheorwon-gun, Gangwon-do, Korea

| Fish species | No. of fish examined | No. (%) of fish infected | No. of CsMc detected | |
|--------------------------------------|----------------------|--------------------------|----------------------|---------|
| | | | Range | Average |
| <i>Zacco platypus</i> | 139 | 32 (23.0) | 1-22 | 3.7 |
| <i>Hemibarbus labeo</i> | 68 | 21 (30.9) | 1-10 | 2.4 |
| <i>Pseudorasbora parva</i> | 56 | 46 (82.1) | 1-511 | 87.9 |
| <i>Pseudogobio esocinus</i> | 38 | 10 (26.3) | 1-10 | 2.6 |
| <i>Rhodeus ocellatus</i> | 27 | 15 (55.6) | 1-11 | 3.1 |
| <i>Acheilognathus rhombeus</i> | 25 | 25 (100) | 1-45 | 9.9 |
| <i>Abbotina rivularis</i> | 18 | 13 (72.2) | 2-60 | 10.3 |
| <i>Pungtungia herzi</i> | 16 | 5 (31.3) | 18-654 | 290.0 |
| <i>Microphysogobio longidorsalis</i> | 10 | 2 (20.0) | 8-41 | 24.5 |
| <i>Hemibarbus longirostris</i> | 9 | 6 (66.7) | 1-8 | 3.0 |
| <i>Acanthorhodeus gracilis</i> | 5 | 5 (100) | 16-233 | 124.6 |
| Total | 411 | 180 (43.8) | 1-654 | 37.8 |

The infection status of MsMc by the fish species was designated in Table 2. Especially in case of the pale chub, *Z. platypus*, the prevalence was 98.6%, and infection intensity was 144 PFI.

The metacercariae of *Centrocestus armatus* (CaMc) were found in 171 (38.9%) out of 440 fishes of the positive species, and their intensity of infection was 1,844 PFI. Especially in 2 species of chubs, i.e., *Z. platypus* and *Z. koreanus*, the prevalence was 99.3% and 100%, and the infection intensity was 2,130 and 1,118 PFI, respectively. *Echinostoma* spp., Metacercariae (EsMc) were detected in 94 (19.6%) out of 479 fishes of the positive species, and their intensity of infection was 3 PFI. The metacercariae of *Metorchis orientalis* (MoMc) were detected in 43 (29.3%) out of 147 fishes of the positive species, and their intensity of infection was 4 PFI. The infection status of CaMc, EsMc, and MoMc by the fish species was as shown in Table 2.

In this study, more than 5 species of ZTM, i.e., *C. sinensis*, *Metagonimus* spp. *C. armatus*, *Echinostoma* spp., and *M. orientalis*, were detected in fishes from the irrigation canal of Togyo-jeosuji in Cheorwon-gun, Gangwon-do, Korea. Their individual prevalence was 43.8%, 74.1%, 38.9%, 19.6%, and 29.3%, and the infection intensity was 38, 62, 1,844, 3, and 4 PFI, respectively. The prevalence of MsMc was generally higher than the other 4 species of ZTM. Meanwhile, the intensity of infection was relatively high-level in CaMc. However, the susceptibility indices (SI: prevalence/100 × mean no. of ZTM PFI) in each ZTM were relatively low (16.6, 45.9, 717.3, 0.6, and 1.2).

A total of 568 fish in 19 species were examined in this study. Among them, *Z. platypus* (24.5%) was most dominantly examined, and followed by *C. auratus* (12.5%), *H. labeo* (12.0%), *P. parva* (9.9%), *P. esocinus* (6.7%), *R. ocellatus* (4.8%), *M. salmoides* (4.8%), *A. rhombeus* (4.4%), *Z. koreanus* (3.4%), *A. rivularis* (3.2%), and *P. herzi* (2.8%). The remaining 8 fish species, i.e., *L. macrochirus*, *O. interrupta*, *M. longidorsalis*, *A. lanceolatus*, *H. longirostris*, *A. gracilis*, *C. splendidus*, and *I. koreensis*, were examined less than 14 fish individuals (8 fish in average). Moreover, 2 fish species, *M. salmoides* (large mouthed bass; n = 27) and *L. macrochirus* (blue gill; n = 14), were included as the exotic fish species. From the aforementioned situation of fish examined, such as the small number of fish species, the biased number of fish by the fish species and the prevalence of exotic fish species, we could know that the fish ecology is not so good in this survey region.

The metacercariae of *C. sinensis* were detected in 180 (43.8%) out of 411 fishes in 11 (57.9%) positive species, and their in-

fection intensity was 38 PFI in this study. However, in previous studies on metacercariae in fish from Gangwon-do, CsMc were detected in only fish from Hantan-gang in Cheorwon-gun [6-8]. Cho et al. [6] detected CsMc in 2 fish species, *P. herzi* (60.0%) and *S. japonicus coreanus* (100%), and the intensity of infection was 2.8 and 2.0 PFI, respectively. Later, Cho et al. [7] also found CsMc in 4 fish species, i.e., *P. herzi*, *S. japonicus coreanus*, *A. rhombeus*, and *Ladislabia taczanowskii*, from Hantan-gang; the prevalence was 29.8% (28/94) in 4 positive fish species, and the intensity of infection was 2.0 PFI. Sohn et al. [8] detected total 13 CsMc in 7 (7.4%) out of 95 fishes in 3 species, i.e., *P. herzi*, *Microphysogobio longidorsalis*, and *Hemibarbus mylodon*, from Hantan-gang. When we compared the endemicity of CsMc in this study with the findings of previous studies performed in Hantan-gang, we could know that the endemicity of CsMc in fish from Togyo-jeosuji is much higher than that in fish from Hantan-gang. On the other hand, Sohn et al. [8] reported the similar prevalence (40.0%) and infection intensity of CsMc (36 PFI) in fishes from Munsan-cheon (a stream of Imjin-gang), in Paju-si, Gyeonggi-do.

In this study, MsMc were detected in 403 (74.1%) out of 544 fish in 15 (78.9%) positive species, and their infection intensity was about 62 PFI. Cho et al. [7] detected MsMc from 74.3% (324/436) of fish from Hantan-gang for 3 years (2010, 2012, and 2013), and the overall intensity of infection was 32 PFI. Sohn et al. [8] also detected MsMc in 61.1% (312/511) of fishes (in 19 spp.) from the water systems of Hantan-gang, and their intensity of infection was 48 PFI. Sohn et al. [8] also reported 59.0% (113/191) MsMc prevalence and about 10 in intensity of infection in fishes from the water systems of Imjin-gang. Recently, Sohn et al. [21] reported 55.4% and 57.7% prevalence, and 96 and 138 MsMc intensity in fishes from Seomjin-gang and Tamjin-gang, respectively. The prevalence of MsMc in this study is relatively higher than those of previous studies. However, infection intensities with MsMc are much higher in fish from Seomjin-gang and Tamjin-gang. One of the reasons is the presence of susceptible fish, *Plecoglossus altivelis*, in fishes from Seomjin-gang and Tamjin-gang.

On the other hand, the rasborinid fish, *Z. platypus*, *Z. temminckii*, *Z. koreanus*, and *Opsarichthys uncirostris amurensis*, are regarded as the index fish of MsMc survey in inland localities where the sweetfish, *P. altivelis*, are unavailable. In this study, the prevalence of MsMc in *Z. platypus* and *Z. koreanus* was 98.6% and 94.7%, and the intensity of infection was 144 and 57 PFI, respectively. The prevalence of MsMc in rasborinid fish

Table 2. Infection status of freshwater fish with zoonotic trematode metacercariae (ZTM) by fish species from the irrigation canal of Togyo-jeosuji, in Cheorwon-gun, Gangwon-do, Korea

| Fish species examined ^a | No. of fish examined | <i>Metagonimus</i> spp. | | <i>Centrocestus armatus</i> | | <i>Echinotoma</i> spp. | | <i>Metorchis orientalis</i> | |
|--------------------------------------|----------------------|-------------------------|-------------------------|-----------------------------|-------------------------|------------------------|-------------------------|-----------------------------|-------------------------|
| | | Fish infected (%) | Av (range) ^b | Fish infected (%) | Av (range) ^b | Fish infected (%) | Av (range) ^b | Fish infected (%) | Av (range) ^b |
| <i>Zacco platypus</i> | 139 | 98.6 | 144 (1-1,020) | 99.3 | 2,130 (2-11,750) | 1.4 | 3 (1-5) | 0 | - |
| <i>Carassius auratus</i> | 71 | 63.4 | 19 (1-100) | 1.4 | 1 (-) | 7.0 | 3 (1-5) | 0 | - |
| <i>Hemibarbus labeo</i> | 68 | 79.4 | 10 (1-57) | 5.9 | 2 (1-3) | 30.9 | 4 (1-15) | 0 | - |
| <i>Pseudorasbora parva</i> | 56 | 87.5 | 10 (1-78) | 3.6 | 1 (-) | 26.8 | 2 (1-4) | 50.0 | 3 (1-12) |
| <i>Pseudogobio esocinus</i> | 38 | 81.6 | 59 (1-240) | 0 | - | 39.5 | 3 (1-18) | 2.6 | 1 (-) |
| <i>Rhodeus ocellatus</i> | 27 | 74.1 | 7 (1-30) | 3.7 | 2 (-) | 0 | - | 0 | - |
| <i>Micropterus salmoides</i> | 27 | 18.5 | 4 (1-14) | 3.7 | 1 (-) | 48.2 | 2 (1-3) | 0 | - |
| <i>Acheilognathus rhombeus</i> | 25 | 100 | 13 (3-31) | 0 | - | 0 | - | 0 | - |
| <i>Zacco koreanus</i> | 19 | 94.7 | 57 (1-211) | 100 | 1,118 (342-2,190) | 0 | - | 0 | - |
| <i>Abbotina rivularis</i> | 18 | 33.3 | 3 (1-9) | 16.7 | 20 (3-39) | 5.6 | 1 (-) | 50.0 | 3 (1-8) |
| <i>Pungtungia herzi</i> | 16 | 12.5 | 3 (1-4) | 0 | - | 37.5 | 3 (1-7) | 18.8 | 9 (4-19) |
| <i>Lepomis macrochirus</i> | 14 | 7.1 | 2 (-) | 0 | - | 14.3 | 2 (1-2) | 0 | - |
| <i>Odontobutis interrupta</i> | 12 | 66.7 | 4 (1-5) | 0 | - | 42.9 | 3 (1-6) | 0 | - |
| <i>Microphysogobio longidorsalis</i> | 10 | 0 | - | 0 | - | 10.0 | 2 (-) | 10.0 | 36 (-) |
| <i>Acheilognathus lanceolatus</i> | 10 | 0 | - | 10.0 | 19 (-) | 0 | - | 0 | - |
| <i>Hemibarbus longirostris</i> | 9 | 88.9 | 7 (2-18) | 0 | - | 66.7 | 4 (1-10) | 11.1 | 1 (-) |
| <i>Acanthorhodeus gracilis</i> | 5 | 100 | 8 (1-24) | 20.0 | 1 (-) | 60.0 | 4 (1-10) | 0 | - |
| <i>Coreoleuciscus splendidus</i> | 2 | 0 | - | 0 | - | 0 | - | 0 | - |
| <i>Iksookimia koreensis</i> | 2 | 0 | - | 0 | - | 0 | - | 0 | - |
| Total | 568 | 74.1 ^c | 62 (1-1,020) | 38.9 ^c | 1,844 (1-11,750) | 19.5 ^c | 3 (1-18) | 29.3 ^c | 4 (1-36) |

^aA total of 568 freshwater fishes in 19 species were examined. ^bNo. of metacercariae detected (Av: average). ^cNo. of ZTM positive fish/No. of fish examined in ZTM positive species × 100.

from water systems of Hantan-gang and Imjin-gang was 73.9% and 72.1%, and their infection intensity was 43 and 19 PFI, respectively [8]. The MsMc prevalence in rasborinid fish from water systems of Seomjin-gang and Tamjin-gang was 89.2% and 89.0%, and their infection intensity was 74 and 42 PFI, respectively [21]. From the findings of previous studies, we can

suppose that the endemicity of MsMc in this study is much higher than those in previous studies performed in Seomjin-gang, Tamjin-gang, Hantan-gang, and Imjin-gang [7,8,21].

In this study, CaMc were detected in 171 (38.9%) out of 440 fish of 10 positive species, from the irrigation canal of Togyo-jeosuji in Cheorwon-gun, Gangwon-do, Korea. The mean in-

tensity of infection was 1,844 PFI. However, the prevalence was 99.3% and 100% in 2 species of chubs, *Z. platypus* and *Z. koreanus*, susceptible fish hosts of CaMc, and the mean intensity of infection was 2,130 and 1,118 PFI, respectively. The endemicity of CaMc in chubs from the irrigation canal of Togyo-jeosuji is higher than that from water systems of Seomjin-gang and Mangyeong-gang, but it is similar and/or lower than that from water systems of other major rivers in Korea [22].

The endemicity of EsMc was low in fish from the irrigation canal of Togyo-jeosuji in this study. They were detected in 94 (19.6%) out of 479 fish of 13 positive species, and their mean intensity of infection was 3 PFI. Moreover, in the dark sleeper, *O. interrupta*, the susceptible fish host for *Isthmiophora hortensis* [23], the EsMc prevalence was 42.9%, and the mean intensity of infection was 2.5 PFI in this study. Recently, Sohn et al. [14,24] reported that the prevalence of EsMc were 100% and 65.1% in Korean dark sleepers, *O. platycephala*, from Yugu-cheon (a branch of Geum-gang) in Gongju-si, Chungcheongnam-do, and Soyang-cheon (a branch of Mangyeong-gang) in Wanju-gun, Jeollabuk-do. The intensity of infection was about 48 and 12 PFI in Yugu-cheon and Soyang-cheon, respectively. On the other hand, the infection status of *M. orientalis* metacercariae was very low in fishes from the irrigation canal of Togyo-jeosuji in this study. They were detected in 43 (29.3%) out of 147 fish of 6 positive species, and their mean intensity of infection was 4 PFI.

Although more than 5 species of ZTM, i.e., *C. sinensis*, *Metagonimus* spp., *C. armatus*, *Echinostoma* spp., and *M. orientalis*, were detected in this study, their individual endemicity was relatively not so high. However, the endemicity of CsMc in 3 fish species, i.e., *P. parva*, *P. herzi* and *A. gracilis*, was relatively high (SI: 72.2, 90.8, and 124.6, respectively). The endemicity of MsMc was high in the pale chub, *Z. platypus*, (SI: 141.9), and that of CaMc was relatively high in 2 species of chubs, *Z. platypus* and *Z. koreanus* (SI: 2,115 and 1,118) from the irrigation canal of Togyo-jeosuji, in Cheorwon-gun, Gangwon-do, Korea.

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CONFLICT OF INTEREST

The authors have no conflicts of interest concerning the work reported in this paper.

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