

# Infection Status with *Clonorchis sinensis* Metacercariae in Fish from Tamjin-gang (River) in Jeollanam-do, Republic of Korea

Ki-Bok Yoon<sup>1</sup>, Hyun-Cheol Lim<sup>1</sup>, Doo Young Jeon<sup>1</sup>, Sook Park<sup>1</sup>, Shin-Hyeong Cho<sup>2</sup>, Jung-Won Ju<sup>2</sup>,  
Sung-Shik Shin<sup>3</sup>, Byoung-Kuk Na<sup>4</sup>, Woon-Mok Sohn<sup>4,\*</sup>

<sup>1</sup>Division of Microbiology, Jeollanam-do Institute of Health and Environment, Muan 58568, Korea; <sup>2</sup>Division of Vectors and Parasitic Diseases, Centers for Disease Control and Prevention, Osong 28159, Korea; <sup>3</sup>Department of Parasitology, College of Veterinary Medicine, Chonnam National University, Gwangju 61186, Korea; <sup>4</sup>Department of Parasitology and Tropical Medicine, and Institute of Health Sciences, Gyeongsang National University College of Medicine, Jinju 52727, Korea

**Abstract:** The present study was performed to investigate the infection status with *Clonorchis sinensis* metacercariae (CsMc) in fishes from 2 sites, the middle and lower reaches, of Tamjin-gang (River) in Jeollanam-do, the Republic of Korea. Total 1,132 fishes in 22 species were collected from the middle reaches in Jangheung-gun for 4 years (2014-2017) and 517 fishes in 17 species were also collected from the lower reaches in Gangjin-gun in 2014 and 2017. They were all individually examined with the artificial digestion method in our laboratory. CsMc were detected in 322 (28.5%) out of 1,132 fishes from Jangheung-gun, and in 161 (31.1%) out of 517 fishes from Gangjin-gun, and their densities were 51 and 57 per fish infected each. In the fish species with CsMc, positive rates were 61.5% in Jangheung-gun and 62.7% in Gangjin-gun. A total of 222 *Pungtungia herzi* were examined and they were all infected with CsMc. The average intensity was 103 CsMc in the index fish, *P. herzi* (95 in Jangheung-gun and 121 in Gangjin-gun). In *P. herzi* bimonthly examined in 2017, the intensity was commonly most higher in March in 2 surveyed sites, however the significant seasonal endemicity was not showed. Conclusively, it was confirmed that CsMc is more or less prevalent in fishes from Tamjin-gang and their endemicity is higher in fish from the lower reaches in Gangjin-gun than the middle reaches in Jangheung-gun in Jeollanam-do, Korea.

**Key words:** *Clonorchis sinensis*, metacercaria, freshwater fish, index fish, *Pungtungia herzi*, Tamjin-gang

## INTRODUCTION

In the nationwide survey on the helminthic infection in the Republic of Korea (Korea), the prevalence of clonorchiasis, *Clonorchis sinensis* infection, was 1.86%, 932,540 residents with this endemic disease estimated. Nowadays, it is the highest value among the prevalences of parasitic diseases in Korea [1]. The prevalence of this endemic disease has maintained at relatively high levels in residents of riverside areas in Korea [2-6]. Especially, Cho et al. [5] reported the egg positive rates of *C. sinensis* in residents living in the 4 river basins, Nakdong-gang (gang means River), Seomjin-gang, Yeongsan-gang and Geum-

gang, located in southern part of Korea. Recently, Jeong et al. [6] reported the prevalences of clonorchiasis in the adjacent residents of 5 major rivers, i.e., Nakdong-gang, Seomjin-gang, Geum-gang, Yeongsan-gang and Han-gang. However, the endemicity of this trematode infections is rarely reported in the riverside residents of Tamjin-gang except for the Seo et al. [2] performed in 1981.

Many Korean parasitologists examined freshwater fishes, the infection source of clonorchiasis, from various endemic areas to estimate the endemicities of liver fluke infections [7-16]. Especially, in 2008, Kim et al. [10] widely surveyed freshwater fishes from 34 localities to know the infection status with *Clonorchis sinensis* metacercariae (CsMc) in Korea. Cho et al. [12] investigated the infection status of CsMc in freshwater fish from 3 wide regions, which tentatively divided by the latitudinal levels of Korean peninsula in 2011. Cho et al. [13] also surveyed on the prevalence of zoonotic trematode metacercariae in freshwater fish from Gangwon-do (do = Province), Korea.

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

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Sohn et al. [14] investigated the infection status of digenetic trematode metacercariae including *C. sinensis* in freshwater fish from the water systems of Hantan-gang and Imjin-gang located in relatively northern regions of Korea. Recently, Sohn et al. [15] surveyed the prevalence of CsMc in freshwater fishes from the water systems of Seomjin-gang. Sohn et al. [16] also reported the infection tendency of CsMc by the subfamily groups in Cyprinidae fish hosts from a highly endemic site, Wicheon (a branch of Nakdong-gang), in Gunwi-gun, Gyeongsangbuk-do, Korea.

Tamjin-gang is one of the 3 major rivers in Jeollanam-do together with Seomjin-gang and Yeongsan-gang. It rises from a mountainous area between Yuchi-myeon (myeon = township) in Jangheung-gun (gun = county) and Geumjeong-myeon in Yeongam-gun, flows via Jangheung-gun and Gangjin-gun and flows into the South Sea in the Gangjin Bay, Jeollanam-do, Korea [17]. Its riverside areas have been known as the endemic area of intestinal fluke, *Metagonimus yokogawai* and heterophyid flukes [2,18-22]. However, the infection status with CsMc in fish from this river has not been widely and systematically examined yet. Therefore, we performed the present study to investigate the infection status with CsMc in fish from 2 local sites of Tamjin-gang in Korea.

## MATERIALS AND METHODS

### Fish collection

Total 1,132 fishes in 22 species were collected in the middle reaches of Tamjin-gang (River) in Jangheung-gun (Latitude: 34.4257; Longitude: 126.5432), Jeollanam-do, Korea for 4 years (2014-2017). Total 517 fishes in 17 species were also collected in the lower reaches of Tamjin-gang in Gangjin-gun (Latitude: 34.3805; Longitude: 126.4851), Jeollanam-do in 2104 and 2017.

### Fishes collected from the middle reaches of Tamjin-gang in Jangheung-gun

Total 167 fishes in 13 species were examined in 2014. Fish species (no. of fish) examined were *Zacco temminckii* (30), *Carassius auratus* (25), *Zacco platypus* (20), *Acheilognathus yamatsutae* (11), *Odontobutis platycephala* (6), *Micropterus salmoides* (5), *Acanthorhodeus gracilis* (1) including 6 ones with CsMc in Table 1. Total 243 fishes in 15 species were examined in 2015. Fish species (no. of fish) examined were *Z. temminckii* (32), *C. auratus* (30), *Z. platypus* (27), *Coreoperca kawamebari* (27),

**Table 1.** Infection status of *Clonorchis sinensis* metacercariae in fishes from the middle reaches of Tamjingang (River) in Jangheung-gun, Jeollanam-do

Year and fish sp.	No. of fish examined	No. (%) of fish infected	No. of CsMc detected	
			Range	Average
<b>2014</b>				
<i>Pseudogobio esocinus</i>	25	2 (8.0)	1-2	1.5
<i>Hemibarbus longirostris</i>	20	19 (95.0)	1-20	6.8
<i>Pungtungia herzi</i>	14	14 (100)	15-500	185.6
<i>Hemiculter eigenmanni</i>	6	4 (66.7)	1-27	8.0
<i>Coreoperca herzi</i>	3	1 (33.3)	-	1.0
<i>Sarcocheilichthys nigripinnis</i>	1	1 (100)	-	54.0
Subtotal	69	41 (59.4)	1-500	68.7
<b>2015</b>				
<i>Pungtungia herzi</i>	35	35 (100)	2-325	65.7
<i>Pseudogobio esocinus</i>	20	2 (10.0)	-	1.0
<i>Sarcocheilichthys variegatus</i>	14	14 (100)	1-66	17.2
<i>Odontobutis platycephala</i>	12	1 (8.3)	-	1.0
<i>Acheilognathus yamatsutae</i>	9	2 (22.2)	1-4	2.5
<i>Acheilognathus rhombeus</i>	5	4 (80.0)	2-29	9.0
<i>Hemibarbus longirostris</i>	4	2 (50.0)	1-5	3.0
<i>Squalidus gracilis majimae</i>	3	2 (66.7)	3-85	44.0
<i>Sarcocheilichthys nigripinnis</i>	3	2 (66.7)	1-16	8.5
Subtotal	105	64 (61.0)	1-325	42.1
<b>2016</b>				
<i>Pungtungia herzi</i>	36	36 (100)	2-950	181.3
<i>Zacco platypus</i>	30	2 (6.7)	-	1.0
<i>Pseudogobio esocinus</i>	29	1 (3.5)	-	1.0
<i>Acheilognathus lanceolatus</i>	27	2 (7.4)	-	1.0
<i>Hemibarbus longirostris</i>	21	8 (38.1)	1-29	7.6
<i>Sarcocheilichthys variegatus</i>	17	15 (88.2)	1-46	12.1
<i>Sarcocheilichthys nigripinnis</i>	15	15 (100)	1-116	32.2
<i>Acanthorhodeus gracilis</i>	9	9 (100)	1-28	8.2
Subtotal	184	88 (47.8)	1-950	82.3
<b>2017</b>				
<i>Pungtungia herzi</i>	67	67 (100)	1-240	45.0
<i>Hemibarbus longirostris</i>	32	17 (53.1)	1-12	3.6
<i>Acheilognathus lanceolatus</i>	25	9 (36.0)	1-4	1.3
<i>Sarcocheilichthys variegatus</i>	23	22 (95.7)	1-133	23.7
<i>Sarcocheilichthys nigripinnis</i>	10	8 (80.0)	1-5	3.0
<i>Acheilognathus rhombeus</i>	5	2 (40.0)	-	2.0
<i>Acheilognathus koreensis</i>	3	3 (100)	1-2	1.3
<i>Acanthorhodeus gracilis</i>	1	1 (100)	-	1.0
Subtotal	166	129 (77.7)	1-240	28.3
<b>Total</b>	<b>524</b>	<b>322 (61.5)</b>	<b>1-950</b>	<b>50.9</b>

*Plecoglossus altivelis* (18), *Acheilognathus koreensis* (4) including 9 ones with CsMc in Table 1.

Total 302 fishes in 16 species were examined in 2016. Fish species (no. of fish) examined were *Z. temminckii* (28), *C. kawamebari* (24), *P. altivelis* (22), *C. auratus* (20), *O. platycephala* (12), *Siniperca scherzeri* (6), *C. herzi* (3) and *M. salmoides* (3) including 8 ones with CsMc in Table 1. Total 420 fishes in 18 species were examined in 2017. Fish species (no. of fish) examined were *Z. temminckii* (62), *Z. platypus* (61), *C. auratus* (41),

*C. kawamebari* (33), *O. platycephala* (18), *P. altivelis* (12), *P. esocinus* (12), *C. herzi* (10), *A. yamatsutae* (3) and *Cyprinus carpio* (2), including 8 ones with CsMc in Table 1.

**Fishes collected from the lower reaches of Tamjin-gang in Gangjin-gun**

Total 110 fish in 12 species were examined in 2014. Fish species (no. of fish) examined were *Z. platypus* (20), *C. auratus* (19), *Z. temminckii* (5), *Lateolabrax japonicus* (2), *O. platycephala* (1) and *Mugil cephalus* (1) including 6 ones with CsMc in Table 3. Total 407 fishes in 14 species were examined in 2017. Fish species (no. of fish) examined were *Z. platypus* (55), *C. auratus* (43), *P. altivelis* (40), *C. kawamebari* (25), *O. platycephala* (25), *Z. temminckii* (21) and *C. herzi* (3), including 7 ones with CsMc in Table 3.

**Examination methods**

All collected fishes with ice were transferred to the laboratory of the Department of Parasitology and Tropical Medicine, Gyeongsang National University College of Medicine, Jinju, Korea. After the identification of fish species, they were individually ground with a mortar or grinder. Each ground fish meat was mixed with artificial gastric juice and the mixture was incubated at 36°C for 2 hr. The digested material was filtered with 1 × 1 mm of mesh, and washed with 0.85% saline until the supernatant is clear. The sediment was carefully examined under a stereomicroscope. CsMc were separately collected by the general feature [11], and they were counted to get hold of infection rates (%) and densities (No. of CsMc per fish infected) by fish species.

**Table 2.** Infection status of *Clonorchis sinensis* metacercariae in *Pungtungia herzi* from the middle reaches of Tamjingang (River) in Jangheung-gun, Jeollanam-do

Year	No. of fish examined	No. (%) of fish infected	No. of CsMc detected	
			Range	Average
2014	14	14 (100)	15-500	185.6
2015	35	35 (100)	2-325	65.7
2016	36	36 (100)	2-950	181.3
2017	67	67 (100)	1-240	45.0
March	15	15 (100)	9-240	113.4
May	20	20 (100)	4-75	20.7
July	20	20 (100)	1-90	30.2
October	12	12 (100)	4-100	24.9
Total	152	152 (100)	1-950	95.0

**RESULTS**

**Infection status of fish with CsMc in middle reaches of Tamjin-gang**

The metacercariae of *C. sinensis* (CsMc) were detected in 322 (61.5%) out of 524 fishes in 16 species from the middle reaches of Tamjin-gang in Jangheung-gun, Jeollanam-do, and their average density was 51 per fish infected. In the positive fish species with CsMc, prevalences were 59.4%, 61.0%, 47.8%, and 77.7% from 2014 to 2017 and their densities were 69, 42, 82 and 28 per fish infected respectively. The infection status with CsMc by the fish species and surveyed years was detailedly shown in Table 1. All 152 striped shinner, *P. herzi*, examined were infected with CsMc, of which intensities were 186, 66, 181, and 45 in average from 2014 to 2017. In case of this fish species examined in 2017, intensities with CsMc were 113 in March, 21 in May, 30 in July and 25 in October respectively (Table 2).

**Infection status of fish with CsMc in lower reaches of Tamjin-gang**

The CsMc were detected in 161 (62.7%) out of 257 fishes in 8 species from the lower reaches of Tamjin-gang in Gangjin-gun, Jeollanam-do, and their average density was 57 per fish infected. In the positive fish species, prevalences were 33.9%

**Table 3.** Infection status of *Clonorchis sinensis* metacercariae in fishes from the lower reaches of Tamjingang (River) in Gangjin-gun, Jeollanam-do

Year and fish sp.	No. of fish examined	No. (%) of fish infected	No. of CsMc detected	
			Range	Average
2014				
<i>Acanthorhodeus macropterus</i>	28	8 (28.6)	1-4	1.6
<i>Pseudogobio esocinus</i>	20	4 (20.0)	1-2	1.3
<i>Acheilognathus lanceolatus</i>	8	3 (37.5)	1-2	1.7
<i>Pungtungia herzi</i>	3	3 (100)	146-427	242.0
<i>Sarcocheilichthys variegatus</i>	2	2 (100)	28-34	31.0
<i>Acheilognathus rhombeus</i>	1	1 (100)	-	12.0
Subtotal	62	21 (33.9)	1-427	39.2
2017				
<i>Pungtungia herzi</i>	67	67 (100)	7-745	115.4
<i>Sarcocheilichthys nigripinis</i>	33	33 (100)	1-57	12.4
<i>Pseudogobio esocinus</i>	31	1 (3.2)	-	1.0
<i>Hemibarbus longirostris</i>	24	12 (50.0)	1-6	2.6
<i>Sarcocheilichthys variegatus</i>	18	18 (100)	1-33	11.1
<i>Acheilognathus lanceolatus</i>	14	3 (21.4)	-	1.0
<i>Acheilognathus rhombeus</i>	8	6 (75.0)	1-6	2.7
Subtotal	195	140 (71.8)	1-745	59.9
Total	257	161 (62.7)	1-745	57.2

in 2014 and 71.8% in 2017 and their densities were 39 and 60 CsMc per fish infected. The infection status with CsMc by the fish species and surveyed years was detailedly revealed in Table 3. All 70 striped shinner, *P. herzi*, examined were infected with CsMc, of which intensities were 242 and 115 in average in 2014 and 2017. In case of this fish species examined in 2017, intensities with CsMc were 195 in March, 64 in May, 59 in July and 136 in October respectively (Table 4).

#### Comparison of the infection status of fish with CsMc in 2 surveyed sites of Tamjin-gang

Total 1,649 fishes were examined in this study. Among them, 1,132 (68.7%) were caught from the middle reaches of Tamjin-gang in Jangheung-gun and remain 517 (31.4%) were from the lower reaches in Gangjin-gun, Jeollanam-do. The overall positive rate with CsMc was 29.3% in 1,649 fishes from Tamjin-gang. That was 28.5% in 1,132 fishes from Jangheung-gun and was 31.1% in 517 fishes from Gangjin-gun. A total of 222 *P. herzi* were examined and they were all infected with CsMc. The average density was 103 CsMc in the index fish, *P. herzi* (95 in Jangheung-gun and 121 in Gangjin-gun) (Table 5).

**Table 4.** Infection status of *Clonorchis sinensis* metacercariae in an index fish, *Pungtungia herzi*, from the lower reaches of Tamjin-gang (River) in Gangjin-gun, Jeollanam-do

Year	No. of fish examined	No. (%) of fish infected	No. of CsMc detected	
			Range	Average
2014	3	3 (100)	146-427	242.0
2017	67	67 (100)	7-745	115.4
March	20	20 (100)	7-745	194.8
May	15	15 (100)	7-267	63.6
July	19	19 (100)	12-217	58.6
October	13	13 (100)	28-265	136.2
Total	70	70 (100)	7-745	120.9

**Table 5.** Comparison of the infection status<sup>a</sup> with *C. sinensis* metacercariae in fish from 2 regions of Tamjin-gang (River)

Items	Infection status of <i>C. sinensis</i> metacercariae in fish from		
	Jangheung-gun	Gangjin-gun	Total
No. of fish examined	1,332 (68.7)	517 (31.4)	1,649 (100)
Overall positive rate (%)	322/1,132 (28.5)	161/517 (31.1)	483/1,649 (29.3)
Total metacercarial density	50.9	57.2	53.0
No. (%) <sup>b</sup> of <i>P. herzi</i> examined	152 (13.4)	70 (13.5)	222 (13.5)
Metacercarial density in <i>P. herzi</i>	95	120.9	103.2

<sup>a</sup>Positive rate: No. of fish infected/No. of fish examined ×100; metacercarial density: mean no. of CsMc per fish infected.

<sup>b</sup>No. of *P. herzi*/Total No. of fish examined ×100.

## DISCUSSION

By the present study, it was confirmed that CsMc are more or less prevalent in fish from Tamjin-gang. Total positive rate, 29.3%, and average metacercarial density, 53 per fish infected, were not so high (Table 5). However, the prevalence (100%) and metacercarial density (103) in the index fish, *P. herzi*, were shown the relatively higher level. In the positive fish species group, the endemicity of CsMc was slightly higher in fish from the lower reaches in Gangjin-gun (prevalence: 62.7%; density: 57) than in fish from the middle reaches in Jangheung-gun (61.5%; 51). In *P. herzi*, the metacercarial density was higher in the lower reaches (121) than in the middle reaches (95). These findings suggested that the endemicity with CsMc is more or less higher in fish from the lower reaches in Gangjin-gun than in fish from the middle reaches in Jangheung-gun, Jeollanam-do, Korea.

Cho et al. [12] examined fishes from 2 sites, Gangjin-gun (58 fish in 11 spp.) and Jangheung-gun (52 fish in 9 spp.), of Tamjin-gang in 2005. They detected 206 CsMc per infected fish in 39 (76.5%) out of 51 fishes (9 spp.) from Gangjin-gun, but they found no CsMc in 52 fishes from Jangheung-gun. From these findings, we can speculate that the endemicity of CsMc has been highly maintained in fish from Gangjin-gun, however, no CsMc in fish from Jangheung-gun unlike in this study is questionable. Two fish species, *P. herzi* and *S. variegatus wakiye*, were showed to be the highly susceptible fish hosts of *C. sinensis* in Cho et al. [12]. Their susceptibility index of CsMc, 420 in *P. herzi* and 356 in *S. variegatus wakiye*, were very high, when we compared with those of present study, 103 in *P. herzi*. On the other hand, Sohn et al. [16] reported that the susceptibility index of CsMc is 1,310 in 323 gobioninid fishes, i.e., 169 *P. herzi*, 117 *Squalidus* spp., 25 *S. variegatus wakiye*, and 12 *Pseudorasbora parva*, from Wicheon in Gunwi-gun, Gyeong-

sangbuk-do. Kim et al. [10] reported 81 CsMc in average density in 10 *P. herzi*, from Yangcheon in Sancheong-gun, Gyeongsangnam-do.

In the metacercarial surveys for the epidemiology of zoonotic trematode infections, the sampling of fish is one of the important factors to obtain the more precise information. Total 1,649 fishes were collected from 2 sites (1,132 from the middle reaches in Jangheung-gun and 517 from the lower reaches in Gangjin-gun, Jeollanam-do) of Tamjin-gang in this study. In the middle reaches, total 1,132 fishes in 22 species were examined for 4 years (2014-2017) and a total of 517 fishes in 17 species from the lower reaches were examined in 2014 and 2017. The difference of fish number examined in 2 surveyed areas is depends upon the number of examined years. The striped shinner, *P. herzi* (222: 13.5%), was the most dominant fish species, and followed by pale chub, *Z. platypus* (213: 12.9%), dark chub, *Z. temminckii* (178: 10.8%), crusian carp, *C. auratus* (178: 10.8%), and goby minnow, *P. esocinus* (137: 8.3%) among the fish species examined in this study. The numbers of striped shinner examined in each 2 reaches were 152 (middle: 13.4%) and 70 (lower: 13.5%). Those of pale chub were 138 (12.2%) and 75 (14.5%), and of crusian carp were 116 (10.3%) and 62 (12.0%). According to the above findings, major 4 fish species were collected in similar proportions in 2 surveyed areas. However, the dark chub, *Z. temminckii*, was collected more in the middle (152: 13.4%) than in the lower reaches (26: 5.0%).

Total 51 fish species (in 36 genera 9 families) have been reported as the second intermediate hosts of *C. sinensis* in Korea [7-16]. In the present study, CsMc were detected in 16 fish species, i.e., *Acanthorhodeus gracilis*, *A. macropterus*, *Acheilognathus lanceolatus*, *A. koreensis*, *A. rhombeus*, *A. yamatsutae*, *Coreoperca herzi*, *Hemibarbus longirostris*, *Hemiculter eigenmanni*, *Odontobutis platycephala*, *Pseudogobio esocinus*, *Pungtungia herzi*, *Sarcocheilichthys nigripinnis morii*, *S. variegatus wakiye*, *Squalidus gracilis majimae*, and *Zacco platypus*. They all had been listed as the second intermediate hosts of *C. sinensis* in Korea [9-16].

According to the recent studies, CsMc were found in fishes from the water system of Imjingang and Hantangang located in the northern part of Korea, although their prevalences and densities were very low [14]. Whereas CsMc were not detected at all in fishes from the water systems of Hangang in Gangwon-do [13]. In fishes from Geumgang, the prevalence and density of CsMc were also revealed in a low level [12]. They were showed in the moderate level in fishes from Yeongsan-

gang and Seomjingang in Jeollanam-do [12,15] like those in this study. However, fishes from 3 localities, i.e., Wicheon (in Gunwi-gun, Gyeongsangbuk-do), Banbyeoncheon (in Yeongyang-gun, Gyeongsangbuk-do) and Yangcheon (in Sancheong-gun, Gyeongsangnam-do), of Nakdonggang were highly and heavily infected with CsMc [12,16].

Some species of freshwater fish edible in raw practically act as the infection source of clonorchiasis in Korea. Among 22 fish species examined in this study, 5 ones, i.e., crusian carp (*C. auratus*), Korean and Japanese aucha perch (*C. herzi* and *C. kawamebari*), sweet smelt (*Plecoglossus altivelis*), and Korean dark sleeper (*O. platycephala*), are frequently eaten in raw in Korea. A total of 472 (28.6%) fish edible in raw, i.e., 178 crusian carp, 19 Korean and 109 Japanese aucha perch, 92 sweet smelt and 74 Korean dark sleeper, were examined in this study, but only 2 (0.4%) fish, 1 *C. herzi* and 1 *O. platycephala*, were infected with only 1 CsMc of each. In even such a highly endemic area, Wicheon, total 3 (8.8%) out of 34 *C. auratus* were infected with total 3 CsMc, 2 (11.1%) *C. herzi* were retained with 4 CsMc and only 1 (33.3%) *S. scherzeri* were infected with total 6 CsMc, respectively [16]. No CsMc were detected in 4 fish species, i.e., *C. herzi* (57), *C. auratus* (42), *S. scherzeri* (11), and *C. carpio* (2), from Seomjin-gang [15]. Fortunately, these fish species edible in raw are less prevalent with CsMc in Korea. Then, what kinds of factors are acted in maintenance of the endemicity of clonorchiasis in Korea? The chopping board contaminated with fish meat may be act as the other transmission route of clonorchiasis in the riverside endemic areas of Korea.

Conclusively, it is confirmed that the endemicity of CsMc is moderately high in fishes from Tamjin-gang, which is known to be the endemic area of heterophyid flukes, including *Metagonimus yokogawai* and *Heterophyes nocens* [2,18-22]. The surveillances on the zoonotic fish-borne trematode infections should be constantly done in this river basin.

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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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