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▣ ORIGINAL ARTICLE

Prevalence and Intensity of *Clonorchis sinensis* Metacercariae in Freshwater Fish from Wicheon Stream in Gunwi-gun, Gyeongsangbuk-do, Korea

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Abstract: The infection status of *Clonorchis sinensis* metacercariae (CsMc) was examined in freshwater fish from a highly prevalent site, Wicheon (a branch of Nakdong-gang), which is located in Gunwi-gun, Gyeongsangbuk-do, the Republic of Korea. Total 1,162 fish in 32 species were examined by the artificial digestion method through 6 years. CsMc were detected in 720 (67.5%) out of 1,067 fish (26 spp.) and their density was 610 per fish infected. In the susceptible gobioninid fish group, i.e., *Pungtungia herzi, Squalidus gracilis majimae, Squalidus japonicus coreanus, Sarcocheilichthys variegatus wakiyae* and *Pseudorasbora parva*, all of 323 fish were infected with an average of 1,310 CsMc. Total 23 (95.8%) gobioninid fish, i.e., *Pseudogobio esocinus, Abbottina springeri, Hemibarbus longirostris, Microphysogobio koreensis*, and *Microphysogobio jeoni*, were infected with 127 CsMc in average. In the acheilognathinid fish (bitterlings) group, the prevalence was 77.0%, and the density was 50 CsMc per fish infected. In the rasborinid fish (chubs) group, i.e., *Zacco platypus, Zacco temminckii, Zacco koreanus*, and *Opsariichthys uncirostris amurensis*, 147 (36.5%) out of 403 fish examined were infected with 15 CsMc in average. The susceptibility indices of CsMc were 412 in the overall positive fish group, 1,310 in the gobioninid group-1, 122 in the gobioninid group-2, 38.5 in the acheilognathinid group, and 5.5 in the rasborinid fish group. Conclusively, it was confirmed that CsMc are highly prevalent in fish from Wicheon, and their infection tendency varied according to the subfamily groups in Cyprinidae fish hosts.

Key words: *Clonorchis sinensis*, metacercaria, susceptibility index, gobioninae, acheilognathinae, rasborinae, cyprinidae fish host, Wicheon

INTRODUCTION

Nowadays *Clonorchis sinensis* infection is the most endemic parasitic disease in the Republic of Korea (=Korea). The prevalence of this endemic disease was 1.9%, and about 932,540 residents are estimated to be infected, which is the highest value among the prevalences in the nationwide survey on helminthic infections in Korea [1]. The endemicity of clonorchiasis has maintained at relatively high levels in riverside areas of Korea [2-8]. Especially, in 1981, high prevalences were reported from the riverside residents in 7 major rivers, i.e., Nakdong-gang (gang=River) (40.2%), Yeongsan-gang (30.8%), Seomjin-gang (17.3%), Tamjin-gang (15.9%), Han-gang (15.7%), Geum-gang (12.0%), and Mangyeong-gang (8.0%) [2]. Also in 2006 and 2007, the egg positive rates of *C. sinensis* were surveyed from riverside residents in major 5 rivers of Korea [5,7]. Recently, Jeong et al. [8] reported the prevalence of clonorchiasis from the residents of 5 major rivers as Nakdong-gang (11.7%), Seomjin-gang (9.9%), Geum-gang (6.5%), Yeongsan-gang (3.1%), and Han-gang (1.0%).

On the other hand, many Korean workers surveyed on freshwater fish, the infection sources of clonorchiasis, collected from various endemic riverside areas to estimate the endemicities of clonorchiasis. The results revealed that more than 49 fish species in 7 families have been reported as the second intermediate hosts of *C. sinensis* [9-18]. Especially, Kim et al. [12] examined 677 freshwater fish (21 spp.) collected from 34 localities to know the infection status of *C. sinensis* metacercariae (CsMc). In addition, Cho et al. [14] surveyed the infection status of CsMc in freshwater fish from 3 wide regions, which are tentatively divided by the latitudinal levels of the Korean peninsula. They examined total 136 freshwater fish (16 spp.) from

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Wicheon, the same area in this study, in 2008. Later, in 2014, Cho et al. [16] investigated the prevalence of zoonotic trematode metacercariae in freshwater fish from Gangwon-do (Province), Korea. Recently, Sohn et al. [17] surveyed the infection status of digenetic trematode metacercariae including *C. sinensis* in freshwater fish from the water systems of Hantan-gang and Imjin-gang in northern regions of Korea. Sohn et al. [18] also investigated the infection status with *C. sinensis* metacercariae in fish from water systems of Seomjin-gang.

Wicheon is one of the branch streams of Nakdong-gang, which arises from a mountainous area of Goro-myeon (myeon = township) in Gunwi-gun (gun = county), flows via Gunwi-gun and Euiseong-gun, and unites with the main stream at Sangju-si (si = city), Gyeongsangbuk-do. The water ecosystem of this stream is more or less healthy but the ecological conditions for fish is not so good [19]. However, it has been known that freshwater fish such as *Pungtungia herzi, Squalidus gracilis majimae, S. japonicus coreanus*, and *Pseudogobio esocinus* from this stream were heavily infected with CsMc [14]. Therefore, for a period of 7 years, we focused on the infection status of CsMc in fish from a highly prevalent site of Wicheon, Gyeongsangbuk-do, Korea, and analyzed the infection status of CsMc according to the subfamily groups of Cyprinidae fish hosts of *C. sinensis*.

MATERIALS AND METHODS

Fish collection site and freshwater fish examined

We collected total 1,162 freshwater fish in 32 species in Wicheon (a stream of Nakdong-gang), which is located in Woobo-myeon, Gunwi-gun (latitude: 38.43268; longitude: 127.4375), Gyeongsangbuk-do, Korea. The numbers and species of fish examined in each year were as follows:

In 2011, total 105 freshwater fish in 13 species (no. of fish examined), i.e., *Zacco platypus* (49), *Squalidus japonicus coreanus* (10), *Zacco temminckii* (10), *Squalidus gracilis majimae* (8), *Pungtungia herzi* (5), *Acheilognathus majusculus* (5), *Opsariichthys uncirostris amurensis* (5), *Carassius auratus* (3), *Pseudogobio esocinus* (3), *Acanthorhodeus macropterus* (3), *Sarcocheilichthys variegatus wakiyae* (2), and *Acheilognathus koreensis* (1), *Cobitis sinensis* (1), were examined . In 2013, total 107 freshwater fish in 12 species, i.e., *P. herzi* (20), *Z. platypus* (20), *Acheilognathus yamatsutae* (16), *Z. temminckii* (15), *A. koreensis* (10), *S. gracilis majimae* (9), *S. variegatus wakiyae* (6), *Coreoperca herzi* (5), *Abbottina springeri* (2), *C. sinensis* (2), *C. auratus* (1), and *Lepomis macrochirus* (1), were examined.

In 2014, total 338 freshwater fish in 24 species, i.e., *Z. platypus* (60), *Z. temminckii* (50), *P. herzi* (47), *A. koreensis* (29), *A. yamatsutae* (25), *S. japonicus coreanus* (14), *C. auratus* (13), *S. gracilis majimae* (13), *Acheilognathus rhombeus* (11), *S. variegatus wakiyae* (11), *A. majusculus* (10), *Micropterus salmoides* (8), *C. sinensis* (8), *C. herzi* (7), *P. esocinus* (7), *Misgurnus anguillicaudatus* (6), *Odontobutis platycephala* (4), *Pseudorasbora parva* (4), *L. macrochirus* (4), *Cobitis lutheri* (3), *Acheilognathus lanceolatus* (2), *Microphysogobio jeoni* (1), and *Microphysogobio koeensis* (1), were examined. In 2015, total 245 freshwater fish in 19 species, i.e., *Z. platypus* (50), *Z. temminckii* (49), *P. herzi* (41), *A. koreensis* (29), *A. yamatsutae* (16), *S. japonicus coreanus* (15), *S. gracilis majimae* (11), *C. auratus* (10), *A. rhombeus* (7), *A. springeri* (4), *S. variegatus wakiyae* (3), *O. uncirostris amurensis* (3), *P. parva* (1), *Rhynchocypris oxycephalus* (1), *A. macropterus* (1), *P. esocinus* (1), *C. herzi* (1), *M. koeensis* (1), and *A. lanceolatus* (1), were examined .

In 2016, total 168 freshwater fish in 17 species, i.e., *A. koreensis* (35), *Z. platypus* (30), *P. herzi* (30), *A. rhombeus* (15), *A. yamatsutae* (15), *Zacco koreanus* (14), *O. platycephala* (6), *C. auratus* (5), *S. japonicus coreanus* (4), *A. lanceolatus* (3), *C. herzi* (3), *P. esocinus* (2), *S. gracilis majimae* (2), *Hemibarbus longirostris* (1), *M. anguillicaudatus* (1), *Siniperca scherzeri* (1), and *M. koreensis* (1), were examined. In 2017, total 199 freshwater fish in 17 species, i.e., *Z. platypus* (50), *S. japonicus coreanus* (31), *A. yamatsutae* (27), *P. herzi* (26), *A. koreensis* (26), *Z. temminckii* (10), *P. parva* (7), *Acanthorhodeus gracilis* (5), *S. variegatus wakiyae* (3), *C. herzi* (2), *C. auratus* (2), *Z. koreanus* (2), *A. rhombeus* (2), *S. scherzeri* (2), *M. salmoides* (2), *C. lutheri* (1), and *Pseudobagrus fulvidraco* (1), were examined.

Examination methods

All collected fish with ice were transferred to the laboratory of the Department of Parasitology and Tropical Medicine, Gyeongsang National University College of Medicine, Jinju, Korea. After 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 became clear. The sediment was carefully examined under a stereomicroscope. The metacercariae of *C. sinensis* were separately collected by the general morphological feature [13,15], and they were counted to get hold of the infection rate and density by fish species. The susceptibility indices of

Table 1. Infection status of *Clonorchis sinensis* metacercariae in fish from Wicheon (a stream of Nakdong-gang) in Gunwi-gun, Gyeongsangbuk-do

Year and fish species	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
2011					
Pungtungia herzi	5	5 (100)	5,216	340-2,420	1,043
Squalidus japonicus coreanus	10	10 (100)	818	13-295	82
Squalidus gracilis majimae	8	8 (100)	4,960	9-1,035	620
Sarcocheilichthys variegatus	2	2 (100)	1,901	2-12	951
Pseudogobio esocinus	3	3 (100)	16	-	5
Acheilognathus majusculus	5	1 (20.0)	1	2	1
Acanthorhodeus macropterus	3	3 (100)	91	87	30
Acheilognathus koreensis	1	1 (100)	9	-	9
Zacco platypus	49	18 (59.3)	129	1-32	7
Subtotal	86	51 (59.3)	13,141	1-2,420	258
2013					
Pungtungia herzi	20	20 (100)	22,790	27-11,290	1,140
Squalidus gracilis majimae	9	9 (100)	8,687	33-2,720	965
Sarcocheilichthys variegatus	6	6 (100)	2,545	26-1,245	424
Abbottina springeri	2	2 (100)	95	44-51	48
Acheilognathus koreensis	10	10 (100)	58	1-14	6
Acheilognathus yamatsutae	16	6 (37.5)	23	1-10	4
Zacco platypus	20	8 (40.0)	129	1-70	16
Zacco temminckii	15	1 (6.7)	2	-	2
Subtotal	98	62 (63.3)	34,329	1-11,290	554
2014					
Pungtungia herzi	47	47 (100)	116,805	6-31,250	2,485
Squalidus japonicus coreanus	14	14 (100)	25,291	140-8,460	1,807
Squalidus gracilis majimae	13	13 (100)	20,285	132-6,130	1,560
Sarcocheilichthys variegatus	11	11 (100)	8,936	13-2,730	812
Pseudorasbora parva	4	4 (100)	2,911	483-1,180	728
Microphysogobio jeoni	1	1 (100)	365	-	365
Acheilognathus koreensis	29	29 (100)	1,099	1-184	38
Microphysogobio koeensis	1	1 (100)	12	-	12
Acheilognathus yamatsutae	25	22 (88.0)	560	1-136	26
Acheilognathus rhombeus	11	9 (81.8)	782	1-290	87
Acheilognathus majusculus	10	2 (20.0)	3	1-2	2
Acheilognathus lanceolatus	2	1 (50.0)	283	-	283
Zacco platypus	60	40 (66.7)	447	1-66	11
Zacco temminckii	50	1 (2.0)	1	-	1
Carassius auratus	13	1 (7.7)	1	-	1
Micropterus salmoides	8	1 (12.5)	1	-	1
Coreoperca herzi	7	1 (14.3)	3	-	3
Misgurnus anguillicaudatus	6	2 (33.3)	2	-	1
Subtotal	319	206 (64.6)	178,007	1-31,250	864
2015					
Pungtungia herzi	41	41 (100)	53,753	24-7,750	1,311
Squalidus japonicus coreanus	15	15 (100)	21,164	359-2,670	1,411
Squalidus gracilis majimae	11	11 (100)	34,255	146-7,680	3,114
Sarcocheilichthys variegatus	3	3 (100)	1,632	157-1,318	544
Pseudorasbora parva	1	1 (100)	1,538	-	1,538
Pseudogobio esocinus	1	1 (100)	1,325	-	1,325
Abbottina springeri	4	4 (100)	155	10-80	39
Microphysogobio koeensis	1	1 (100)	139	-	139
Acheilognathus koreensis	29	29 (100)	2,454	4-678	85
Acheilognathus yamatsutae	16	4 (25.0)	38	1-28	10
Acheilognathus rhombeus	7	7 (100)	1,081	70-295	154
Acheilognathus lanceolatus	1	1 (100)	245	-	245
Acanthorhodeus macropterus	1	1 (100)	79	-	79
Zacco platypus	50	33 (66.0)	965	1-420	29
Zacco temminckii	49	4 (8.2)	7	1-4	2
Opsariichthys uncirostris	3	2 (66.7)	2	-	1
Carassius auratus	10	2 (20.0)	2	-	1
Rhynchocypris oxycephalus	1	1 (100)	28	-	28
Subtotal	244	161 (66.0)	118,862	1-7,750	738

(Continued to the next page)

Table 1. Continued

Year and fish species	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
2016					
Pungtungia herzi	30	30 (100)	36,686	28-9,870	1,223
Squalidus japonicus coreanus	4	4 (100)	3,603	210-1,480	901
Squalidus gracilis majimae	2	2 (100)	3,323	653-2,670	1,662
Pseudogobio esocinus	2	2 (100)	540	36-504	270
Microphysogobio koreensis	1	1 (100)	40	-	40
Hemibarbus longirostris	1	1 (100)	13	-	13
Acheilognathus koreensis	35	34 (97.1)	871	2-287	26
Acheilognathus rhombeus	15	15 (100)	3,006	64-329	200
Acheilognathus yamatsutae	15	8 (53.3)	25	1-9	3
Acheilognathus lanceolatus	3	3 (100)	327	4-255	109
Zacco platypus	30	19 (63.3)	192	1-30	10
Siniperca scherzeri	1	1 (100)	6	-	6
Subtotal	139	120 (86.3)	48,632	1-9,870	405
2017					
Pungtungia herzi	26	26 (100)	26,736	22-8,950	1,028
Squalidus japonicus coreanus	31	31 (100)	13,161	4-2,750	425
Sarcocheilichthys variegatus	3	3 (100)	759	156-352	253
Pseudorasbora parva	7	7 (100)	5,260	365-2,225	751
Acheilognathus yamatsutae	27	15 (55.6)	45	1-2,030	7
Acheilognathus koreensis	26	19 (73.1)	129	1-30	5
Acanthorhodeus gracilis	5	5 (100)	26	2-12	1
Acheilognathus rhombeus	2	1 (50.0)	1	-	16
Zacco platypus	50	21 (42.0)	345	1-205	1
Coreoperca herzi	2	1 (50.0)	1	-	5
Micropterus salmoides	2	1 (50.0)	5	-	387
Subtotal	181	120 (66.3)	46,468	1-8,950	
Total	1,067	720 (67.5)	439,439	1-31,250	610

CsMc were calculated by the formula, prevalence/100× mean metacercarial density per fish infected.

Subfamily groups in Cyprinidae fish hosts of *C. sinensis*

The gobioninid (Gobioninae) group-1 (323 fish) included *P. herzi* (169), *S. gracilis majimae* (43), *S. japonicus coreanus* (74), *S. variegatus wakiyae* (25), and *P. parva* (12). The gobioninid group-2 (24 fish) included *P. esocinus* (13), *A. springeri* (6), *M. koreensis* (3), *M. jeoni* (1), and *H. longirostris* (1). The acheilognathinid (Acheilognathinae) group (296 fish) included *A. koreensis* (130), *A. yamatsutae* (99), *A. rhombeus* (35), *A. majusculus* (15), *A. macropterus* (4), *A. lanceolatus* (6), and *A. gracilis* (7). The rasborinid (Rasborinae) group (403 fish) included *Z. platypus* (259), *Z. temminckii* (136), and *O. uncirostris amurensis* (8).

RESULTS

Infection status of CsMc in overall examined fish

CsMc were detected in 720 (62.0%) out of 1,162 fish in 32 species examined, and their average density was 610 per fish infected. The infection status by fish species and surveyed years is shown in Table 1.

Infection status of CsMc in gobioninid fish group-1

CsMc were detected in all (100%) of 323 fish examined, and their average density was 1,310 per fish infected. The densities were highest in 2014 (1,958), followed by 2015 (1,582), 2016 (1,211), 2013 (972), 2017 (685), and 2011 (516) (Table 2). The infection status of CsMc by fish species, i.e., *P. herzi, S. gracilis majimae, S. japonicus coreanus, S. variegatus wakiyae* and *P. parva*, is revealed in Table 3.

Infection status of CsMc in gobioninid fish group-2

CsMc were detected in 23 (95.8%) out of 24 fish examined, and their average density was 127 per fish infected. The infection status by fish species, i.e., *P. esocinus, A. springeri, M. koreensis, M. jeoni* and *H. longirostris*, is shown in Table 4.

Infection status of CsMc in acheilognathinid fish group

CsMc were detected in 228 (77.0%) out of 296 fish examined, and their average density was 50 per fish infected. The densities were highest in 2015 (93), followed by 2016 (70),

Table 2. Infection status of *Clonorchis sinensis* metacercariae in susceptible gobioninid fish from Wicheon in Gunwi-gun, Gyeongsangbuk-do

Year examined	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
2011	25	25 (100)	12,895	9-2,420	516
2013	35	35 (100)	34,022	26-11,290	972
2014	89	89 (100)	174,228	6-31,250	1,958
2015	71	71 (100)	112,342	24-7,750	1,582
2016	36	36 (100)	43,612	28-9,870	1,211
2017	67	67 (100)	45,916	4-8,950	685
Total	323	323 (100)	423,015	4-31,250	1,310

Table 3. Infection status of *Clonorchis sinensis* metacercariae by fish species in the gobioninid group-1

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Pungtungia herzi	169	169 (100)	261,986	6-31,250	1,550
Squalidus japonicus coreanus	74	74 (100)	64,037	4-8,460	865
Squalidus gracilis majimae	43	43 (100)	71,510	9-7,680	1,663
Sarcocheilichthys variegatus	25	25 (100)	15,773	13-2,730	631
Pseudorasbora parva	12	12 (100)	9,709	365-2,225	809
Total	323	323 (100)	423,015	4-31,250	1,310

Table 4. Infection status of *Clonorchis sinensis* metacercariae by fish species in the gobioninid group-2

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Pseudogobio esocinus	13	12 (92.3)	2,101	1-1,325	175
Abbottina springeri	6	6 (100)	250	10-80	42
Hemibarbus longirostris	1	1 (100)	13	-	13
Microphysogobio koreensis	3	3 (100)	191	12-139	64
Microphysogobio jeoni	1	1 (100)	365	-	365
Total	24	23 (95.8)	2,920	1-1,325	127

Table 5. Infection status of *Clonorchis sinensis* metacercariae in acheilognathinid fish from Wicheon in Gunwi-gun, Gyeongsangbuk-do

Year examined	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
2011	9	5 (55.6)	101	1-87	20
2013	26	16 (61.5)	81	1-14	5
2014	77	63 (81.8)	2,727	1-290	43
2015	54	42 (77.8)	3,897	1-678	93
2016	68	60 (88.2)	4,229	1-329	70
2017	60	40 (66.7)	201	1-30	5
Total	296	228 (77.0)	11,372	1-678	50

2014 (43), 2011 (20), 2013 (5), and 2017 (5) (Table 5). The infection status of CsMc by fish species, i.e., *A. koreensis, A. yamatsutae, A. rhombeus, A. majusculus, A. macropteru, A. lanceolatus* and *A. gracilis*, is revealed in Table 6.

Infection status of CsMc in rasborinid fish group

CsMc were detected in 147 (36.5%) out of 403 fish examined, and their average density was 15 per fish infected. The densities were highest in 2015 (25), followed by 2017 (16), 2013 (15), 2014 (11), 2016 (10), and 2011 (7) (Table 7). The

Table 6. Infection status of *Clonorchis sinensis* metacercariae by fish species in the acheilognathinid group

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Acheilognathus koreensis	130	122 (93.9)	4,620	1-678	38
Acheilognathus yamatsutae	99	55 (55.6)	691	1-136	13
Acheilognathus rhombeus	35	32 (91.4)	4,870	1-295	152
Acheilognathus majusculus	15	3 (20.0)	4	1-2	1.3
Acanthorhodeus macropterus	4	4 (100)	170	2-87	43
Acheilognathus lanceolatus	6	5 (83.3)	855	4-283	171
Acanthorhodeus gracilis	7	7 (100)	162	2-82	23
Total	296	228 (77.0)	11,372	1-678	50

Table 7. Infection status of *Clonorchis sinensis* metacercariae in rasborinid fish from Wicheon in Gunwi-gun, Gyeongsangbuk-do

Year examined	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
2011	64	18 (28.1)	129	1-32	7
2013	35	9 (25.7)	131	1-70	15
2014	110	41 (37.3)	448	1-66	11
2015	102	39 (38.2)	974	1-420	25
2016	44	19 (43.2)	192	1-30	10
2017	62	21 (33.9)	345	1-205	16
Total	403	147 (36.5)	2,219	1-420	15

Table 8. Infection status of *Clonorchis sinensis* metacercariae by fish species in the rasborinid group

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Zacco platypus	259	139 (53.7)	2,207	1-420	16
Zacco temminckii	136	6 (4.4)	10	1-4	2
Opsariichthys uncirostris	8	2 (25.0)	2	-	1
Total	403	147 (36.5)	2,219	1-420	15

Table 9. Susceptibility index of *Clonorchis sinensis* metacercariae by surveyed year and fish groups

Group	Susceptibility index[a]						
	2011	2013	2014	2015	2016	2017	Total
Overall positive fish group	153	351	558	487	350	257	412
Gobioninid group-1	516	972	1,958	1,582	1,211	685	1,310
Acheilognathinid group	11.2	3.1	35.2	72.4	61.7	3.3	38.5
Rasborinid group	2	3.9	4.1	9.6	4.3	5.4	5.5

[a]Prevalence/100 × mean metacercarial density per fish infected.

infection status of CsMc by fish species, i.e., *Z. platypus*, *Z. temminckii* and *O. uncirostris amurensis*, is shown in Table 8.

Susceptibility index of CsMc by fish groups

The susceptibility indices of CsMc were 412 in the overall positive fish group; 1,310 in the gobioninid group-1, 122 in the gobioninid group-2, 38.5 in the acheilognathinid, and 5.5 in the rasborinid fish group (Table 9).

DISCUSSION

By the present study, it was reconfirmed that CsMc are highly prevalent in fish from Wicheon, Gyeongsangbuk-do, Korea. Moreover, the infection status showed a certain tendency ac-

cording to the subfamily groups of fish hosts, i.e., Gobioninae, Acheilognathinae, and Rasborinae, among the Cyprinidae. The prevalences were 100%, 79.7%, and 35.5%, and metacercarial densities were 1,310, 50, and 15 per fish infected in 3 fish groups, respectively. These findings also suggested that the endemicity of CsMc is closely related to the fish group, and this host-parasite relationship may originate from a long-lasting evolutional process.

The fish sampling is one of the important factors in metacercarial surveys for the epidemiology of fishborne zoonotic trematode infections. Total 1,162 fish in 32 species were collected through 6 years (2011 and 2013-2017) in the same site of Woobo-myeon in Gunwi-gun. Among them, the pale chub (259 *Z. platypus*: 22.2%) was the most dominant fish species like Sohn et al. [18] in Seomjin-gang, followed by the striped shinner (169 *P. herzi*: 14.5%), dark chub and/or Korean chub (136 *Z. temminckii* and/or *Z. koreanus*: 11.7%), oily bitterling (130 *A. koreensis*: 11.2%), Korean striped bitterling (99 *A. yamatsutae*: 8.5%), and short barbell gudgeon (74 *S. japonicus coreanus*: 6.4%). The number of fish examined was 867 (74.4%) in major 6 species and 298 (25.6%) in the remaining 26 species. The disproportion of the fish number examined was dependent upon the ecological conditions of each fish species, and moreover, we collected all fish only by the casting net in the daytime. If we used together with other methods for fish catching like a netting, we could have collected more various species of fish, including nocturnal ones.

Total 49 fish species (in 34 genera 7 families) have been reported as the second intermediate hosts of *C. sinensis* in Korea [9-18]. In the present study, CsMc were found in 26 fish species, i.e., *Abbottina springeri, Acanthorhodeus macropterus, A. gracilis, Acheilognathus koreensis, A. lanceolatus, A. majuscules, A. rhombeus, A. yamatsutae, Carassius auratus, Coreoperca herzi, Hemibarbus longirostris, Micropterus salmoides, Microphysogobio koeensis, M. jeoni, Misgurnus anguillicaudatus, Opsariichthys uncirostris, Pseudogobio esocinus, Pungtungia herzi, Pseudorasbora parva, Rhynchocypris oxycephalus, Sarcocheilichthys variegates wakiyae, Siniperca scherzeri, Squalidus gracilis majimae, S. japonicus coreanus, Zacco platypus*, and *Z. temminckii*. Among them, 2 fish species, i.e., *M. salmoides* (Centrachidae) and *M. anguillicaudatus* (Cobitidae), are newly added in the list of the second intermediate hosts of *C. sinensis* in Korea [9-18]. Accordingly, total 51 fish species in 9 families are listed as the second intermediate hosts of *C. sinensis* in Korea.

The large mouth bass, *Micropterus salmoides*, was imported from USA as an edible fish species in 1973. However, this fish is widely spread in the water systems of the whole country, and then became notorious as an agitating fish species of the ecosystem in Korea [20]. In this study, we examined total 10 *M. salmoides* and detected 1 and 5 CsMc from 2 fish. Although the number of fish examined and metacercariae detected were not so many, the fact, infection with CsMc in foreign fish species, is highly important. Thus, we should pay attention to the fish species from foreign countries in the metacercarial survey for the fishborne zoonotic trematodes.

Fish species, i.e., Mandarin fish (*S. scherzeri*), Korean aucha perch (*C. herzi*), common carp (*Cyprinus carpio*), and crusian carp (*C. auratus*), edible in raw conditions practically act as the infection source of clonorchiasis in Korea. Fortunately, these fish species are less prevalent with CsMc. Total 3 (8.8%) out of 34 *C. auratus* were infected with total 3 CsMc, 2 (11.1%) *C. herzi* was infected with a total of 4 CsMc, and only 1 (33.3%) *S. scherzeri* was infected with 6 CsMc, respectively, even in such a highly endemic area, Wicheon. In a survey of the fish from Seomjin-gang, no CsMc was detected in 4 fish species, i.e., *C. herzi* (57), *C. auratus* (42), *S. scherzeri* (11), and *C. carpio* (2), edible in raw conditions [18].

The striped shinner, *P. herzi*, is appropriate to be an index fish species to check the endemicity of *C. sinensis* infection. This fish species broadly lives in the water systems of rivers in Korea and is highly susceptible to CsMc. In the present study, all of 169 *P. herzi* examined were infected with an average of 1,550 CsMc per fish. The prevalences of CsMc in this fish species were 80.4%, 72.5%, and 30.6%, and metacercarial densities were 15.2, 46.1, and 175.9 per fish infected in the upper, middle, and lower reaches of Seomjin-gang, respectively [18]. The positive rates of CsMc were 6.2%, 31.8%, and 92.9%, and densities were 2.8, 214, and 409 per fish infected in *P. herzi* from the northern, middle, and southern regions of the Korean peninsula, respectively [14]. Especially, in this fish species from the streams of Nakdong-gang, i.e., Wicheon in Gunwi-gun, Banbyuncheon in Yeongyang-gun, Gyeongsangbuk-do, and Yangcheon in Sancheong-gun, Gyeongsangnam-do, the endemicities of CsMc were much higher like in this study [14].

Conclusively, it has been confirmed that the endemicity of CsMc is much higher in fish from Wicheon, and a strong tendency was shown in the prevalence and intensity of CsMc according to the subfamily groups of fish, i.e., Gobioninae, Acheilognathinae, and Rasborinae, in the family Cyprinidae fish hosts. Among the highly susceptible gobioninid fish, *P.*

herzi is recommended as a useful index fish to check the endemicity of clonorchiasis in a certain area of 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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