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▣ ORIGINAL ARTICLE

Prevalence of Zoonotic Trematode Metacercariae in Freshwater Fish from Gangwon-do, Korea

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Abstract: The infection status of zoonotic trematode metacercariae was investigated in a total of 2,293 freshwater fish collected from 11 rivers or streams in 9 administrative regions of Gangwon-do, Korea for 5 years (2009-2013). All fish were collected by netting methods and examined using the artificial digestion methods. *Clonorchis sinensis* metacercariae were detected in 4 fish species, i.e., *Pungtungia herzi*, *Squalidus japonicus coreanus*, *Acheilognathus rhombeus*, and *Ladislabia taczanowskii*, from only Hantangang in Cheorwon-gun. *Metagonimus* spp. metacercariae were found in 1,154 (50.3%) fish and their average number per infected fish was 55.8. Among the positive fish species, especially *Tribolodon hakonensis* from Namdaecheon in Yangyang-gun and *Plecoglossus altivelis* from Osipcheon in Samcheok-si were most heavily infected. *Centrocestus armatus* metacercariae were detected in 611 (26.7%) fish and the average metacercarial burden per infected fish was 1,032. Two chub species, *Zacco platypus* and *Zacco temminckii* were highly and heavily infected with *C. armatus* metacercariae in almost all regions surveyed. *Echinostoma* spp. metacercariae were also found in 24 fish from a few localities, but their numbers per fish infected were very low. From the above results, it is confirmed that the metacercariae of intestinal flukes, especially *Metagonimus* spp. and *C. armatus*, were heavily infected, while *C. sinensis* metacercariae were rarely found in fish from Gangwon-do, Korea.

Key words: *Clonorchis sinensis*, *Metagonimus* spp. *Centrocestus armatus*, metacercaria, zoonotic trematode, freshwater fish, Gangwon-do

INTRODUCTION

Soil-transmitted nematodiases were one of the national health problems in the Republic of Korea (=Korea) in old days, before 1980. However, they are no longer a public health problem these days. On the other hand, the prevalence of zoonotic trematode (mainly fishborne trematodes: FBTs) infections, including clonorchiasis, is maintained high, and they became the most important parasitic diseases in some endemic areas, especially in riverside areas of 7 major rivers, i.e., Hangang (River), Gumgang, Mangyeonggang, Yeongsangang, Tamjingang, Seomjingang, and Nakdonggang, in Korea [1-4]. Human infections with FBTs are usually caused by habitual consumption of raw fish containing infective larvae, metacercariae. The endemic areas of FBT infections are highly localized depending on the food habits of residents and on the presence of susceptible intermediate hosts [5]. Moreover, FBTs show low host-specificity, and then many kinds of reservoir hosts can contribute to the maintenance of their life cycles. Thus, the infection status of FBT metacercariae in intermediate hosts is one of the important epidemiological indices together with the status of adult worm infections in the definitive and reservoir hosts [6-10].

Gangwon-do (Province) is located at the northeast (between 37°02′ and 38°37′ N; 127°05′ and 129°22′ E) of Korea, and comprised of 7 si (City) and 11 gun (County). The landscape of this province is dominated by Taebaek Mountains, of which mountainous areas occupy the most areas of the province and retained the head streams of Hangang and Nakdonggang [11].

Epidemiological studies have been performed to investigate

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the infection status of FBTs, including *Metagonimus* spp. in Gangwon-do. Most of these studies were performed before an early 1990's and focused on the infection status with *M. yokogawai* metacercariae in sweetfish, *Plecoglossus altivelis*, from rivers and streams in the east coast of Gangwon-do [12-18]. In addition, metacercarial infections of *Metagonimus* spp. and *Echinostoma hortense* were investigated in some species of freshwater fish from several regions in Gangwon-do [19-23]. However, large-scale surveys on the infection status of FBT metacercariae in a variety of fish species have not been conducted in Gangwon-do. Therefore, in the present study, we investigated the infection status of zoonotic trematode metacercariae in freshwater fish from various regions of Gangwon-do for a period of 5 years.

MATERIALS AND METHODS

Surveyed areas

The survey was conducted in 13 localities of 9 administrative regions in Gangwon-do, from 2009 to 2013. The surveyed areas (year examined) were as follows: ① Sooipcheon in Yanggu-gun (2009); ② Namdaecheon in Yangyang-gun (2009); ③ Donggang in Yeongwol-gun (2009); ④ Osipcheon in Samcheok-si (2009); ⑤ Gagokcheon in Samcheok-si (2009); ⑥ Hwagang in Cheorwon-gun (2010); ⑦ Hantangang in Cheorwon-gun (2010); ⑧ Hongcheongang in Hongcheon-gun (2010); ⑨ Seomgang in Hoengseong-gun (2011); ⑩ Hantangang in Cheorwon-gun (2012); ⑪ Joyanggang in Jeongseon-gun: (2012); ⑫ Hantangang in Cheorwon-gun (2013); ⑬ Pyeongchanggang in Pyeongchang-gun (2013) (Fig. 1).

Fig. 1. The surveyed areas in Gangwon-do, Korea: ① Sooipcheon in Yanggu-gun (2009); ② Namdaecheon in Yangyang-gun (2009); ③ Donggang in Yeongwol-gun (2009); ④ Osipcheon in Samcheok-si (2009); ⑤ Gagokcheon in Samcheok-si (2009); ⑥ Hwagang in Cheorwon-gun (2010); ⑦ Hantangang in Cheorwon-gun (2010); ⑧ Hongcheongang in Hongcheon-gun (2010); ⑨ Seomgang in Hoengseong-gun (2011); ⑩ Hantangang in Cheorwon-gun (2012); ⑪ Joyanggang in Jeongseon-gun: (2012); ⑫ Hantangang in Cheorwon-gun (2013); ⑬ Pyeongchanggang in Pyeongchang-gun (2013).

Freshwater fish examined

We collected a total of 865 freshwater fish (32 species) in 5 localities, i.e., Sooipcheon (in Yanggu-gun), Namdaecheon (in Yangyang-gun), Donggang (in Yeongwol-gun), Osipcheon (in Samcheok-si), and Gagokcheon (in Samcheok-si), 2 times in a year, June and October 2009. We also collected 547 freshwater fish (29 species) in Hwagang (in Cheorwon-gun), Hantangang (in Cheorwon-gun), and Hongcheongang (in Hongcheon-gun) 2 times in a year, June and October 2010, and in Seomgang (in Hoengseong-gun) in September 2011. A total of 881 freshwater fish (30 species) were collected in Hantangang (in Cheorwon-gun) and Joyanggang (in Jeongseon-gun) 2 times a year in July and October 2012, and in Hantangang (in Cheorwon-gun) and Pyeongchanggang (in Pyeongchang-gun) 2 times a year in June and September 2013. The numbers and species of fish examined were shown in Tables 1, 2, and 3. As for fish collection methods, nettings with a gill net, casting net, and stake net were used in all surveyed areas.

Examination methods

All collected fish were transferred on ice to the laboratory of the Department of Parasitology and Tropical Medicine, Gyeongsang National University School of Medicine, Jinju, Korea. After identification of fish species, fish were individually ground with a mortar with pestle or in a grinder. Each 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 1×1 mm of mesh, and washed with 0.85% saline until the supernatant became clear. The sediment was carefully examined under a stereomicroscope. Each species of FBT metacercariae was separately collected by the general feature [5], and they were counted to obtain the infection rates and densities by fish species.

RESULTS

Infection status of *Clonorchis sinensis* metacercariae

The metacercariae of *C. sinensis* were detected only in 4 fish species, i.e., *Pungtungia herzi*, *Squalidus japonicus coreanus*, *Acheilognathus rhombeus*, and *Ladislabia taczanowskii*, collected from Hantangang in Cheorwon-gun. The infection rate of fish was 29.8% (28 out of 94 fish), and a total of 56 metacercariae were harvested (2.0 metacercariae per fish). The infection status of each fish species and each year is revealed in Table 4.

Table 1. Freshwater fish[a] collected from streams and rivers in Gangwon-do, Korea (2009)

Species of fish	No. of fish collected from 5 localities[b]					
	①	②	③	④	⑤	Total
Cypriniforms						
Zacco platypus	26	30	30	23	19	128
Zacco temminckii	23	30	30	-	20	103
Coreoleuciscus splendidus	27	-	30	30	8	95
Pungtungia herzi	11	15	16	-	17	59
Tribolodon hakonensis	-	30	-	21	-	51
Microphysogobio longidorsalis	25	-	25	-	-	50
Rhynchocypris oxycephalus	10	3	-	1	25	39
Hemibarbus longirostris	14	-	16	-	-	30
Pseudogobio esocinus	11	-	10	-	-	21
Gobiobotia brevibarba	-	-	18	-	-	18
Carassius auratus	14	2	-	-	-	16
Hemibarbus labeo	-	-	13	-	-	13
Koreocobitis rotundicaudata	-	-	13	-	-	13
Hemibarbus mylodon	2	-	10	-	-	12
Acanthorhodeus macropterus	-	-	10	-	-	10
Orthrias toni	-	-	-	8	-	8
Opsariichthys uncirostris	5	-	-	-	-	5
Misgurnus mizolepis	-	-	5	-	-	5
Ladislabia taczanowskii	-	-	-	3	-	3
Gobiobotia macrocephala	-	-	3	-	-	3
Iksookimia koreensis	-	-	2	-	-	2
Cyprinus capio	1	-	-	-	-	1
Pseudobagrus fulvidraco	-	-	1	-	-	1
Osmeriformes						
Plecoglossus altivelis	-	25	-	29	-	54
Salmoniformes						
Onchorhynchus masou masou	-	5	-	2	3	10
Siluriformes						
Liobagrus andersoni	10	-	3	-	-	13
Silurus asotus	-	-	1	-	-	1
Perciformes						
Coreoperca herzi	17	10	15	-	-	42
Chaenogobius urotaenia	-	-	-	19	8	27
Tridentiger brevispinis	-	-	-	15	9	24
Rhinogobius giurinus	-	-	-	4	-	4
Acanthogobius pflaumi	-	4	-	-	-	4
Total	196	154	251	155	109	865

[a]Total 865 freshwater fish of 32 species were examined.
[b]① Sooipcheon in Yanggu-gun, ② Namdaecheon in Yangyang-gun, ③ Donggang in Yeongwol-gun, ④ Osipcheon in Samcheok-si, ⑤ Gagokcheon in Samcheok-si.

Infection status of *Metagonimus* spp. metacercariae

The metacercariae of *Metagonimus* spp. were found in 1,154 (50.3%) fish, and their average number per infected fish was 55.8. Among the positive fish species, *Tribolodon hakonensis* from Namdaecheon in Yangyang-gun and *P. altivelis* from Osipcheon in Samcheok-si were most heavily infected. The in-

Table 2. Freshwater fish[a] collected from rivers in Gangwon-do, Korea (2010-2011)

Species of fish	No. of fish collected from 4 localities[b]				
	⑥	⑦	⑧	⑨	Total
Cypriniforms					
Zacco platypus	3	30	20	23	76
Zacco temminckii	10	30	16	5	61
Pungtungia herzi	13	7	25	14	59
Hemibarbus longirostris	12	3	20	20	55
Pseudogobio esocinus	3	8	23	13	47
Microphysogobio longidorsalis	8	-	20	2	30
Coreoleuciscus splendidus	2	-	18	6	26
Carassius auratus	5	3	2	15	25
Rhynchocypris oxycephalus	-	-	-	15	15
Acheilognathus majusculus	-	6	6	-	12
Gobiobotia brevibarba	-	-	-	10	10
Rhynchocypris steidachneri	-	3	-	6	9
Opsariichthys uncirostris	-	-	2	5	7
Acheilognathus lanceolatus	2	1	-	3	6
Pseudobagrus koreanus	-	-	-	6	6
Koreocobitis rotundicaudata	-	-	-	6	6
Acheilognathus rhombeus	-	5	-	-	5
Acheilognathus signifer	4	-	-	-	4
Hemibarbus mylodon	-	3	-	-	3
Squalidus japonicus coreanus	-	2	-	-	2
Hemibarbus labeo	-	-	1	-	1
Iksookimia koreensis	-	-	-	1	1
Sarcocheilichthys nigripinnis	-	1	-	-	1
Microphysogobio jeoi	-	1	-	-	1
Siluriformes					
Liobagrus andersoni	-	-	-	3	3
Perciformes					
Coreoperca herzi	7	5	20	15	47
Siniperca scherzeri	-	-	11	-	11
Odontobutis platycephala	-	-	2	1	3
Scorpaeniformes					
Cottus koreanus	-	-	-	15	15
Total	69	108	186	184	547

[a]Total 547 freshwater fishes in 29 species examined.
[b]⑥ Hwagang in Cheorwon-gun (2010), ⑦ Hantangang in Cheorwon-gun (2010), ⑧ Hongcheongang in Hongcheon-gun (2010), ⑨ Seomgang in Hoengseong-gun (2011).

Table 3. Freshwater fish[a] collected from rivers in Gangwon-do, Korea (2012-2013)

Species of fish	No. of fish collected from 4 localities[b]				
	⑩	⑪	⑫	⑬	Total
Cypriniforms					
Zacco temminckii	30	39	22	34	125
Pungtungia herzi	44	24	20	35	123
Microphysogobio longidorsalis	30	24	22	22	98
Zacco platypus	29	20	16	20	85
Pseudogobio esocinus	17	1	40	21	79
Coreoleuciscus splendidus	11	25	14	17	67
Hemibarbus longirostris	2	-	12	17	31
Pseudobagrus koreanus	6	15	7	-	28
Koreocobitis rotundicaudata	2	7	-	15	24
Hemibarbus mylodon	12	1	4	5	22
Acheilognathus rhombeus	20	-	-	-	20
Gobiobotia brevibarba	-	1	4	8	13
Acheilognathus signifer	11	-	1	-	12
Rhynchocypris oxycephalus	-	-	-	10	10
Hemibarbus labeo	8	1	1	-	10
Acanthorhodeus macropterus	5	-	2	-	7
Pseudopungtungia tenuicorpa	-	2	-	5	7
Carassius auratus	-	-	6	-	6
Ladislabia taczanowskii	1	-	-	4	5
Iksookimia koreensis	-	1	4	-	5
Acheilognathus majusculus	-	-	4	-	4
Misgurunus anguillicaudatus	-	-	4	-	4
Pseudobagrus fulvidraco	-	-	2	-	2
Rhodeus ocellatus	-	-	2	-	2
Sarcocheilichthys variegatus	-	-	1	-	1
Siluriformes					
Liobagrus andersoni	-	18	4	15	37
Liobagrus mediadoposalis	9	-	-	-	9
Liobagrus obesus	3	-	-	-	3
Perciformes					
Coreoperca herzi	15	17	5	2	39
Siniperca scherzeri	-	-	3	-	3
Total	255	196	200	230	881

[a]Total 881 freshwater fishes in 30 species examined. [b]⑩ Hantangang in Cheorwon-gun (2012), ⑪ Joyanggang in Jeongseon-gun (2012), ⑫ Hantangang in Cheorwon-gun (2013), ⑬ Pyeongchanggang in Pyeongchang-gun (2013).

fection status of each fish species, collection site, and examination year is designated in Tables 5, 6, and 7.

Infection status of *Centrocestus armatus* metacercariae

The metacercariae of *C. armatus* were detected in 611 (26.7%) fish, and the average metacercarial burden per infected fish was 1,032. Two species of chubs, *Zacco platypus* and *Zacco temminckii* were most highly and most heavily infected in almost all regions surveyed. The infection status by each fish species, collection site, and examination year is shown in Tables 8, 9, and 10.

Infection status of *Echinostoma* spp. metacercariae

A total of 102 metacercariae of *Echinostoma* spp. were detected in 24 (15.5%) out of 155 freshwater fish (9 species) collected from 5 localities of Gangwon-do. The infection status by fish species, collection site, and examination year is designated in Table 11.

Table 4. Infection status of *Clonorchis sinensis* metacercariae in fish from rivers in Gangwon-do, Korea (2010-2013)

Locality (year) and fish sp. examined	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
⑦ Hantangang in Cheorwon-gun (2010)[a]					
Pungtungia herzi	7	6 (85.7)	19	1-6	3.2
Squalidus japonicus coreanus	2	2 (100)	4	-	2.0
Subtotal	9	8 (88.9)	23	1-6	2.9
⑩ Hantangang in Cheorwon-gun (2012)					
Pungtungia herzi	44	16 (36.4)	27	1-3	1.7
Acheilognathus rhombeus	20	2 (10.0)	2	-	1.0
Ladislabia taczanowskii	1	1 (100.0)	2	-	2.0
Subtotal	65	19 (29.2)	31	1-3	1.6
⑫ Hantangang in Cheorwon-gun (2013)					
Pungtungia herzi	20	1 (5.0)	2	-	2.0
Total	94	28 (29.8)	56	1-6	2.0

[a]Previously reported by Cho et al., 2011.

DISCUSSION

In the present study, *C. sinensis* metacercariae were detected in 28 fish (4 species, i.e., *P. herzi*, *S. japonicus coreanus*, *A. rhombeus*, and *L. taczanowskii*) from Hantangang in Cheorwon-gun. In our previous study [10], *C. sinensis* metacercariae were also found in 2 fish species, *P. herzi* and *S. japonicus coreanus*, from Hantangang in Cheorwon-gun, Gangwon-do [10]. According to Kim et al. [9], all fish collected from upper regions of Cheongju-si, Chungcheongbuk-do were negative for *C. sinensis* metacercariae [9]. It is interesting to note that *C. sinensis* metacercariae were detected only in fish from limited areas of Hantangang among various regions surveyed in Gangwon-do. First of all, the presence of snail intermediate host, *Parafossarulus manchouricus*, in this river should be investigated.

Several studies have been performed to investigate on metacercariae of intestinal flukes in fish from Gangwon-do before the early 1990's [12-17,19-23]. Especially, the prevalence of *M. yokogawai* metacercariae was investigated in sweetfish from rivers and streams in the east coast of Gangwon-do [12-18]. Metacercarial infections of *Metagonimus* spp. (*M. miyatai* and *M. takahashii*) were also examined in freshwater fish from Seomgang, Jucheongang, Pyeongchanggang, Hongcheongang, Donggang, and Osipcheon, in Gangwon-do, and also from the upper reaches of Namhangang [21-23]. In addition, epidemiological studies on *E. hortense* infection were performed in some areas of Gangwon-do [19, 20].

Ahn and Ryang [21] detected 3-87 *Metagonimus* spp. metacercariae (37.6 in average) in 30 (68.2%) out of 44 *Z. platypus* from Hongcheongang [21]. In the present study, *Metagonimus* spp. metacercariae were found in 71 (39.9%) out of 178 fish (12 species), including *Z. platypus* from Hongcheongang, and their burden per infected fish was 14.3. In case of *Z. platypus*, all of 20 fish examined were infected with them, and the mean burden was 33.4. Therefore, when we compared the infection status of *Metagonimus* spp. metacercariae in *Z. platypus* with that in Ahn and Ryang [21], the prevalence is higher in the present study, although the metacercarial burden is almost similar.

Ahn [22] also detected *Metagonimus* spp. metacercariae in *Z. platypus* from Seomgang, Jucheongang, Pyeongchanggang, Hongcheongang, and Donggang in Gangwon-do [22]. The prevalence by the surveyed area was 75.7% (112/148 fish), 77.1% (37/48), 87.5% (28/32), 63.2% (12/19), and 81.5% (22/27), respectively. The metacercarial burden in total 69 *Z. platypus* ranged 3-1,218 (93.8 in average). In the present study, they were found in all *Z. platypus* from Seomgang (23 fish), Pyeongchanggang (20), Hongcheongang (20), and Donggang (30), and their burdens were 28.8, 13.1, 33.4, and 31.6 metacercariae per fish, respectively. From the above findings, it is indicated that the prevalence is higher in the present study, and the metacercarial burden is higher in Ahn's study [22]. Like in Ahn's study [22], *Metagonimus* spp. (*M. yokogawai*) metacercariae were also found in 2 fish species, *P. altivelis* (sweetfish) and *T. hakonensis* (sea rundace), from Osipcheon in Samcheok-si in the present study. All fish (2 species) examined were infected with them in both studies. In the present study, the metacercarial burden was 615 in 29 sweetfish and 82 in 21 sea rundace, whereas the burden was 729 in 5 sweetfish and 68 in 10 sea rundace in Ahn [22]. In Namdaecheon,

Table 5. Infection status of *Metagonimus* spp. metacercariae in fish from streams and rivers in Gangwon-do, Korea (2009)

Locality (year) and fish sp. examined	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
① Sooipcheon in Yanggu-gun					
Zacco platypus	26	17 (65.4)	53	1-16	3.1
Zacco temminckii	23	18 (78.3)	85	1-27	4.7
Pseudogobio esocinus	11	2 (18.2)	24	11-13	12.0
Liobagrus mediadiposalis	10	2 (20)	2	-	1.0
Hemibarbus longirostris	8	1 (12.5)	1	-	1.0
Subtotal	78	40 (51.3)	165	1-27	4.1
② Namdaecheon in Yangyang-gun					
Tribolodon hakonensis	30	27 (90.0)	12,134	2-2,250	449.4
Zacco platypus	30	25 (83.3)	223	1-58	8.9
Zacco temminckii	30	9 (30.0)	66	1-47	7.3
Plecoglossus altivelis	25	20 (80.0)	979	1-397	49.0
Pungtungia herzi	15	10 (66.7)	33	1-9	3.3
Coreoperca herzi	10	1 (10)	1	-	1.0
Onchorhynchus masou masou	5	4 (80)	95	11-37	23.8
Subtotal	145	96 (66.2)	13,531	1-2,250	140.9
③ Donggang in Yeongwol-gun					
Zacco platypus	30	30 (100)	949	2-148	31.6
Zacco temminckii	30	30 (100)	477	2-51	15.9
Coreoleuciscus splendidus	30	27 (90.0)	238	1-28	8.8
Microphysogobio longidorsalis	25	11 (44.0)	93	1-27	8.5
Hemibarbus longirostris	16	15 (93.8)	727	1-260	48.5
Pungtungia herzi	16	9 (56.3)	19	1-10	2.1
Hemibarbus labeo	13	12 (92.3)	201	1-70	16.8
Gobiobotia brevibarba	11	11 (100)	682	6-112	62.0
Pseudogobio esocinus	10	10 (100)	1,224	10-387	122.4
Hemibarbus mylodon	10	5 (50)	15	1-7	3.0
Coreoperca herzi	5	1 (20)	1	-	1.0
Liobagrus andersoni	3	2 (66.7)	5	1-4	2.5
Subtotal	199	163 (81.9)	4,631	1-387	28.4
④ Osipcheon in Samcheok-si					
Plecoglossus altivelis	29	29 (100)	17,820	6-3,380	614.5
Zacco platypus	23	23 (100)	303	1-75	13.2
Tribolodon hakonensis	21	21 (100)	1,730	1-275	82.4
Chaenogobius urotaenia	10	1 (10.0)	1	-	1.0
Ladislabia taczanowskii	3	3 (100)	16	1-12	5.3
Onchorhynchus masou masou	2	2 (100)	28	3-25	14.0
Rhynchocypris oxycephalus	1	1 (100)	17	-	17.0
Subtotal	89	80 (89.9)	19,915	1-3,380	248.9
⑤ Gagokcheon in Samcheok-si					
Zacco platypus	19	6 (31.6)	22	1-7	3.7
Pungtungia herzi	15	7 (46.7)	10	1-2	1.4
Zacco temminckii	15	2 (13.3)	5	1-4	2.5
Rhynchocypris oxycephalus	15	2 (13.3)	4	1-3	2.0
Tridentiger brevispinis	9	1 (11.1)	8	-	8.0
Onchorhynchus masou masou	2	2 (100)	68	20-48	34.0
Subtotal	75	20 (26.7)	117	1-48	5.9
Total	586	399 (68.1)	38,359	1-3,380	96.1

Table 6. Infection status of *Metagonimus* spp. metacercariae in fish from rivers in Gangwon-do, Korea (2010-2011)

Locality (year) and fish sp. examined	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
⑥ Hwagang in Cheorwon-gun (2010)					
Zacco temminckii	10	7 (70.0)	103	1-47	14.7
Pungtungia herzi	13	3 (23.1)	5	1-3	1.7
Hemibarbus longirostris	12	12 (100)	1,267	3-596	105.6
Microphysogobio longidorsalis	8	1 (12.5)	1	-	1.0
Acheilognathus signifier	4	4 (100)	106	18-50	26.5
Zacco platypus	3	3 (100)	82	8-46	27.3
Pseudogobio esocinus	3	3 (100)	2,078	78-1,680	692.7
Acheilognathus lanceolatus	2	2 (100)	2	-	1.0
Coreoleuciscus splendidus	2	2 (100)	11	1-10	5.5
Subtotal	57	37 (64.9)	3,655	1-1,680	98.8
⑦ Hantangang in Cheorwon-gun (2010)					
Zacco temminckii	30	29 (96.7)	342	1-56	11.8
Zacco platypus	30	30 (100)	1,013	2-184	33.8
Pseudogobio esocinus	8	8 (100)	383	4-125	47.9
Acheilognathus majusculus	6	6 (100)	46	3-10	7.7
Acheilognathus rhombeus	5	5 (100)	33	1-9	6.6
Hemibarbus mylodon	3	2 (66.7)	13	5-8	6.5
Hemibarbus longirostris	3	3 (100)	160	5-85	53.3
Rhynchocypris steidachneri	3	2 (66.7)	68	32-36	34.0
Squalidus japonicus coreanus	2	1 (50.0)	3	-	3.0
Subtotal	90	86 (95.6)	2,061	1-184	24.0
⑧ Hongcheongang in Hongcheon-gun (2010)					
Pungtungia herzi	25	1 (4.0)	1	-	1.0
Hemibarbus longirostris	20	7 (35.0)	16	1-4	2.3
Pseudogobio esocinus	23	16 (69.6)	92	1-19	5.8
Coreoperca herzi	20	1 (5.0)	1	-	1.0
Coreoleuciscus splendidus	18	4 (22.2)	6	1-2	1.5
Zacco platypus	20	20 (100)	667	7-123	33.4
Zacco temminckii	16	13 (81.3)	95	1-22	7.3
Microphysogobio longidorsalis	20	1 (5.0)	4	-	1.0
Siniperca scherzeri	11	4 (36.4)	5	-	1.0
Odontobutis platycephala	2	1 (50.0)	4	-	1.0
Opsariichthys uncirostris	2	2 (100)	22	8-14	11.0
Hemibarbus labeo	1	1 (100)	107	-	107.0
Subtotal	178	71 (39.9)	1,013	-1-123	14.3
⑨ Seomgang in Hoengseong-gun (2011)					
Zacco platypus	23	23 (100)	662	3-122	28.8
Hemibarbus longirostris	20	19 (95.0)	1,316	1-214	69.3
Pungtungia herzi	14	2 (14.3)	2	-	1.0
Pseudogobio esocinus	13	12 (92.3)	102	1-19	8.5
Gobiobotia brevibarba	10	6 (60.0)	23	1-12	3.8
Zacco temminckii	5	1 (20.0)	4	-	4.0
Opsariichthys uncirostris	5	5 (100)	116	5-75	23.2
Acheilognathus lanceolatus	3	1 (33.3)	3	-	3.0
Subtotal	93	69 (74.2)	2,228	1-214	32.3
Total	418	263 (62.9)	8,957	1-1,680	34.1

Yangyang-gun, *Metagonimus* spp. (*M. yokogawai*) metacercariae were detected in 27 (90.0%) *T. hakonensis* in the present study, with their burden being 449 metacercariae per infected fish. By the aforementioned findings, it is confirmed again that 2 fish species, *P. altivelis* and *T. hakonensis*, are highly suitable second intermediate hosts of *Metagonimus* spp. (*M. yokogawai*) in

Table 7. Infection status of *Metagonimus* spp. metacercariae in fish from rivers in Gangwon-do, Korea (2012-2013)

Locality (year) and fish sp. examined	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
⑩ Hantangang in Cheorwon-gun (2012)					
Pungtungia herzi	44	6 (13.6)	7	1-2	1.2
Zacco temminckii	30	28 (93.3)	834	1-135	29.8
Zacco platypus	29	29 (100.0)	907	3-115	31.3
Acheilognathus rhombeus	20	17 (85.0)	474	1-95	27.9
Pseudogobio esocinus	17	17 (100.0)	2,389	10-462	140.5
Hemibarbus mylodon	12	12 (100.0)	601	4-242	50.1
Acheilognathus signifer	11	8 (72.7)	30	1-7	3.8
Coreoperca herzi	11	1 (9.1)	2	-	2.0
Microphysogobio longidorsalis	10	1 (10.0)	1	-	1.0
Hemibarbus labeo	8	6 (75.0)	53	1-22	8.8
Pseudobagrus koreanus	6	2 (33.3)	12	2-10	6.0
Acanthorhodeus macropterus	5	3 (60.0)	8	1-4	2.7
Hemibarbus longirostris	2	2 (100.0)	151	36-115	75.5
Ladislabia taczanowskii	1	1 (100.0)	1	-	1.0
Subtotal	206	133 (64.6)	5,470	1-462	41.1
⑪ Joyanggang in Jeongseon-gun (2012)					
Zacco temminckii	39	38 (97.4)	4,939	10-403	130.0
Coreoleuciscus splendidus	25	25 (100.0)	424	1-71	17.0
Microphysogobio longidorsalis	24	20 (83.3)	185	1-42	9.3
Pungtungia herzi	24	10 (41.7)	23	1-9	2.3
Zacco platypus	20	20 (100.0)	1,333	5-169	66.7
Liobagrus andersoni	8	3 (37.5)	5	1-3	1.7
Pseudopungtungia tenuicorpa	2	2 (100.0)	2	-	1.0
Coreoperca herzi	2	1 (50.0)	1	-	1.0
Hemibarbus mylodon	1	1 (100.0)	1	-	2.0
Hemibarbus labeo	1	1 (100.0)	320	-	320.0
Gobiobotia brevibarba	1	1 (100.0)	95	-	95.5
Subtotal	147	122 (83.0)	7,329	1-403	60.1
⑫ Hantangang in Cheorwon-gun (2013)					
Pseudogobio esocinus	40	38 (95.0)	1,473	1-486	38.8
Zacco temminckii	22	18 (81.8)	199	2-32	11.1
Microphysogobio longidorsalis	22	1 (4.5)	1	-	1.0
Zacco platypus	16	16 (100)	559	1-67	34.9
Hemibarbus longirostris					10.1
Carassius auratus					10.8
Gobiobotia brevibarba	4	4 (100)	97	15-29	24.3
Hemibarbus mylodon	4	3 (75.0)	224	43-134	74.7
Liobagrus andersoni	4	2 (50.0)	3	1-2	1.5
Acheilognathus majuscules	4	2 (50.0)	2	-	1.0
Rhodeus ocellatus	2	2 (100)	17	7-10	8.5
Acanthorhodeus macropterus	2	2 (100)	102	38-64	51.0
Sarcocheilichthys variegatus	1	1 (100)	1	-	1.0
Hemibarbus labeo	1	1 (100)	153	-	153.0
Subtotal	140	105 (75.0)	2,985	1-486	28.4

(Continued to the next page)

Gangwon-do.

The infection status of *M. yokogawai* metacercariae was frequently investigated in sweetfish from Namdaecheon in Yangyang-gun. Song et al. [15] found 116 metacercariae per fish from 9 out of 10 sweetfish examined. Recently, Cho et al. [18] detected 170 metacercariae per fish from all of 20 (100%) fish.

Table 7. Continued

Locality (year) and fish sp. examined	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
⑬ Pyeongchanggang in Pyeongchang-gun (2013)					
Pungtungia herzi	35	2 (5.7)	5	1-4	2.5
Zacco temminckii	34	32 (94.1)	325	1-29	10.2
Pseudogobio esocinus	21	19 (90.5)	148	1-30	7.8
Zacco platypus	20	20 (100)	261	1-53	13.1
Coreoleuciscus splendidus	17	15 (88.2)	213	1-63	14.2
Hemibarbus longirostris	17	16 (94.1)	269	1-145	16.8
Rhynchocypris oxycephalus	10	6 (60.0)	9	1-3	1.5
Gobiobotia brevibarba	8	7 (87.5)	32	1-9	4.6
Pseudopungtungia tenuicorpa	5	1 (20.0)	1	-	1.0
Hemibarbus mylodon	5	1 (20.0)	1	-	1.0
Ladislabia taczanowskii	4	2 (50.0)	5	2-3	2.5
Subtotal	198	132 (66.7)	1,325	1-145	10.0
Total	691	492 (71.2)	17,109	1-486	34.8

Table 8. Infection status of *Centrocestus armatus* metacercariae in fish from streams and rivers in Gangwon-do, Korea (2009)

Locality (year) and fish sp. examined	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
① Sooipcheon in Yanggu-gun					
Zacco temminckii	23	22 (95.7)	7,231	77-1,035	329
Zacco platypus	26	25 (96.2)	3,937	1-887	158
Opsariichthys uncirostris	5	1 (20.0)	3	-	3.0
Subtotal	54	48 (88.9)	11,171	1-1,035	233
② Namdaecheon in Yangyang-gun					
Zacco temminckii	30	21 (70.0)	4,772	1-3,687	227
Zacco platypus	30	25 (83.3)	3,533	2-809	141
Tribolodon hakonensis	30	2 (6.7)	2	-	1.0
Subtotal	90	48 (53.3)	8,307	1-3,687	173
③ Donggang in Yeongwol-gun					
Zacco temminckii	30	30 (100)	6,178	31-663	206
Zacco platypus	30	30 (100)	10,699	54-1,006	357
Subtotal	60	60 (100)	16,877	31-1,006	281
④ Osipcheon in Samcheok-si					
Zacco platypus	23	22 (95.7)	2,973	10-576	135
Tribolodon hakonensis	21	5 (23.8)	16	1-10	3.2
Rhynchocypris oxycephalus	1	1 (100)	13	-	13.0
Orthrias toni	8	1 (12.5)	1	-	1.0
Subtotal	53	29 (54.7)	3,003	1-576	104
⑤ Gagokcheon in Samcheok-si					
Zacco temminckii	15	12 (80.0)	2,421	1-1,518	202
Zacco platypus	19	18 (94.7)	8,449	1-1,880	469
Subtotal	34	30 (88.2)	10,870	1-1,880	362
Total	291	215 (73.9)	50,228	1-3,687	234

In the present study, 1-397 (49 per infected fish) metacercariae were found from 20 (80.0%) out of 25 fish examined. However, no metacercariae were detected in 25 and 22 sweetfish examined by Seo et al. [13] and Ahn et al. [16], respectively. In sweetfish from Osipcheon in Samcheok-si, the infection status of *M. yokogawai* metacercariae was also surveyed by several workers. Seo et al. [13] reported 100% prevalence and 1,643 metacercariae per fish in 15 sweetfish examined. Ahn [14] and

Table 9. Infection status of *Centrocestus armatus* metacercariae in fish from rivers in Gangwon-do, Korea (2010-2011)

Locality (year) and fish sp. examined	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
⑥ Hwagang in Cheorwon-gun (2010)					
Zacco temminckii	10	10 (100)	2,208	108-406	221
Zacco platypus	3	3 (100)	1,149	154-774	383
Acheilognathus signifer	4	1 (25)	1	-	1.0
Subtotal	17	14 (82.4)	3,358	1-774	240
⑦ Hantangang in Cheorwon-gun (2010)					
Zacco temminckii	30	30 (100)	15,637	24-2,630	521
Zacco platypus	30	30 (100)	14,650	8-4,355	488
Carassius auratus	3	1 (33.3)	1	-	1.0
Subtotal	63	61 (96.8)	30,288	1-2,630	497
⑧ Hongcheongang in Hongcheon-gun (2010)					
Zacco temminckii	16	16 (100)	53,486	42-8,041	3,343
Zacco platypus	20	20 (100)	123,150	780-13,650	6,158
Opsariichthys uncirostris	2	2 (100)	3,750	1,460-2,290	1,875
Pungtungia herzi	25	10 (40)	56	1-24	5.6
Hemibarbus longirostris	20	3 (15)	4	1-2	1.3
Coreoperca herzi	20	2 (10)	7	1-6	3.5
Coreoleuciscus splendidus	18	3 (16.7)	3	-	1.0
Subtotal	121	56 (46.3)	180,456	1-13,650	3,222
⑨ Seomgang in Hoengseong-gun (2011)					
Zacco temminckii	5	3 (60.0)	1,755	2-1,385	585
Zacco platypus	23	23 (100)	41,599	21-8,720	1,809
Opsariichthys uncirostris	5	2 (40.0)	158	60-98	79
Subtotal	33	28 (84.8)	43,512	2-8,720	1,554
Total	234	159 (68.0)	257,614	1-13,650	1,620

Song et al. [15] detected 382 and 185 metacercariae per fish in 9 and 10 fish examined, respectively. In the present study, 29 sweetfish from Osipcheon in Samcheok-si were infected with 615 metacercariae per fish. Accordingly, the endemicity of *M. yokogawai* metacercariae was much higher in sweetfish from Osipcheon in Samcheok-si than those from Namdaecheon in Yangyang-gun.

As the second intermediate hosts of *Metagonimus* spp. (*M. miyatai* and *M. takahashii*), approximately 48 species of freshwater fish (37 genera) have been listed in Korea [5]. In the present study, *Metagonimus* spp. metacercariae were detected in a variety of fish species in Gangwon-do, from 2009 to 2013. Among the positive fish, 16 species, i.e., *Acanthorhodeus macropterus* (from ⑩, ⑫), *Acheilognathus majusculus* (⑦, ⑫), *Acheilognathus signifier* (⑥, ⑩), *Chaenogobius urotaenia* (④), *Hemibarbus mylodon* (③, ⑦, ⑩, ⑪, ⑫, ⑬), *Ladislabia taczanowskii* (④, ⑩, ⑬), *Liobagrus andersoni* (③, ⑪, ⑫), *Microphysogobio longidorsalis* (③, ⑥, ⑧, ⑩, ⑪, ⑫, ⑬), *Rhynchocypris steidachneri* (⑦), *Odontobutis platycephala* (⑧), *Onchorhynchus masou masou* (②, ④, ⑤), *Opsariichthys uncirostris* (⑧, ⑨), *Pseudobagrus koreanus* (⑩), *Pseudopungtungia tenuicorpa* (⑪, ⑫), *Squalidus japonicus coreanus* (⑦), and *Tridentiger brevispinis* (④), have never been listed as the second intermediate hosts of *Metagonimus* spp. in Korea [5]. Therefore, 64 fish species (42 genera) in total are included among the second intermediate hosts of *Metagonimus* spp. in Korea.

To date, 3 *Metagonimus* species, i.e., *M. yokogawai*, *M. takahashii*, and *M. miyatai*, are known to distribute in Korea [24]. As the second intermediate hosts for *M. yokogawai*, 3 fish species, i.e., *P. altivelis*, *Tribolodon taczanowskii* (=*T. hakonensis*), and *Lateolabrax japonicus*, were reported [25-27]. As for *M. takahashii*, 4 fish species, i.e., *Carassius auratus*, *P. altivelis*, *T. taczanowskii* (=*T. hakonensis*) and *L. japonicas*, have been known to be the second intermediate hosts [28-31]. Two species of chubs, *Z. platypus* and *Z. temminckii*, were recorded as the second intermediate hosts for *M. miyatai* [24]. Possible presence of another species of *Metagonimus* in Korea should be investigated in the near future through recovery of adult worms via experimental infection of animals with these metacercariae.

In the present study, the metacercariae of *C. armatus* were

Table 10. Infection status of *Centrocestus armatus* metacercariae in fish from rivers in Gangwon-do, Korea (2010-2012)

Locality (year) and fish sp. examined	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
⑩ Hantangang in Cheorwon-gun (2012)					
Zacco temminckii	30	30 (100)	17,982	28-2,340	599
Zacco platypus	29	29 (100)	28,640	23-6,620	988
Pseudogobio esocinus	17	2 (11.8)	3	1-2	1.5
Microphysogobio longidorsalis	20	1 (5.0)	1	-	1.0
Acheilognathus rhombeus	10	6 (60.0)	45	3-12	7.5
Hemibarbus longirostris	2	1 (50.0)	4	-	4.0
Subtotal	108	69 (63.9)	46,675	1-6,620	676
⑪ Joyanggang in Jeongseon-gun (2012)					
Zacco temminckii	39	39 (100)	40,537	163-3,205	1,039
Zacco platypus	20	20 (100)	6,361	53-978	318
Liobagrus andersoni	8	2 (25.0)	2	-	1.0
Coreoperca herzi	2	1 (50.0)	2	-	2.0
Pseudogobio esocinus	1	1 (100)	10	-	10.0
Koreocobitis rotundicaudata	1	1 (100)	5	-	5.0
Subtotal	71	64 (90.1)	46,917	2-3,205	733
⑫ Hantangang in Cheorwon-gun (2013)					
Zacco temminckii	22	22 (100)	14,315	112-2,760	651
Zacco platypus	16	16 (100)	29,603	370-3,735	1,850
Microphysogobio longidorsalis	22	1 (4.5)	2	-	2.0
Hemibarbus longirostris	12	1 (8.3)	2	-	2.0
Gobiobotia brevibarba	4	2 (50.0)	5	1-4	2.5
Rhodeus ocellatus	2	2 (100)	15	3-12	7.5
Subtotal	78	44 (56.4)	43,942	1-3,735	999
⑬ Pyeongchanggang in Pyeongchang-gun (2013)					
Zacco temminckii	34	34 (100)	148,770	96-11,470	4,376
Zacco platypus	20	20 (100)	36,420	240-8,080	1,821
Pseudogobio esocinus	21	1 (4.8)	2	-	2.0
Microphysogobio longidorsalis	22	3 (13.6)	3	-	1.0
Coreoleuciscus splendidus	17	1 (5.9)	2	-	2.0
Hemibarbus mylodon	5	1 (20.0)	1	-	1.0
Subtotal	119	60 (50.4)	185,198	1-11,470	3,087
Total	376	237 (63.0)	322,732	1-11,470	1,362

detected in various fish species from Gangwon-do. Especially in 2 species of chubs, *Z. platypus* and *Z. temminckii*, they were highly and heavily infected in almost all regions surveyed. Hong et al. [32] investigated the infection status of *C. armatus* metacercariae in *Z. platypus* and *Z. temminckii* collected from 19 sites in 5 major rivers, Hangang, Geumgang, Yeongsangang, Seomjingang, and Nakdonggang [32]. They could catch *Z. temminckii* only in 3 regions, Hongcheongang (in Hongcheon-gun), Soyanggang (Inje-gun), and Seocheon (Yanggu-gun), of Gangwon-do. According to them [32], *C. armatus* metacercariae were detected in 100% (20/20 fish), 35.0% (7/20), and 68.4% (13/19) of *Z. temminckii*, and their burdens were 65, 2, and 3 metacercariae, respectively. Therefore, it appears that the endemicity of *C. armatus* is currently much higher than in the past.

Ten species of freshwater fish (8 genera), i.e., *Aphyocypris chinensis*, *C. auratus*, *C. splendidus*, *Microphysogobio yaluensis*, *P. parva*, *Pseudobagrus fulvidraco*, *Rhodeus ocellatus ocellatus*, *R. uyekii*, *Z. platypus*, *Z. temminckii*, have been listed as the second intermediate hosts for *C. armatus* in Korea [5]. In the present study, 15 fish species, i.e., *Acheilognathus rhombeus* (from ⑩), *Acheilognathus signifier* (⑥), *Coreoperca herzi* (⑧, ⑪), *Gobiobotia brevibarba* (⑫), *Hemibarbus longirostris* (⑧, ⑩, ⑫), *Hemibarbus mylodon* (⑬), *Koreocobitis rotundicaudata* (⑪), *Liobagrus andersoni* (⑪), *Microphysogobio longidorsalis* (⑩, ⑫, ⑬) , *Opsariichthys uncirostris* (①, ⑧, ⑨), *Orthrias toni* (④), *Pseudogobio esocinus* (⑩, ⑪, ⑬), *Pungtungia herzi* (⑧), *Rhynchocypris oxycephalus* (④), and *Tribolodon hakonensis* (②, ④), are newly recorded as the second

Table 11. Infection status of *Echinostoma* spp. metacercariae in fish from streams and rivers in Gangwon-do, Korea

Locality (year) and fish sp. examined	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
⑥ Hwagang in Cheorwon-gun (2010)					
Pungtungia herzi	13	2 (15.4)	5	1-4	2.5
Acheilognathus signifer	4	1 (25.0)	1	-	1.0
Subtotal	17	3 (17.7)	6	1-4	2.0
⑧ Hongcheongang in Hongcheon-gun (2010)					
Pungtungia herzi	25	4 (16.0)	4	-	1.0
Pseudogobio esocinus	23	1 (4.3)	1	-	1.0
Siniperca scherzeri	11	1 (9.1)	1	-	1.0
Odontobutis platycephala	2	1 (50.0)	4	-	4.0
Subtotal	61	7 (11.5)	10	1-4	1.4
⑨ Seomgang in Hoengseong-gun (2011)					
Zacco platypus	23	8 (34.8)	75	3-18	9.4
Pungtungia herzi	14	2 (14.3)	5	2-3	2.5
Subtotal	37	10 (27.0)	80	2-18	8.0
⑩ Hantangang in Cheorwon-gun (2012)					
Pungtungia herzi	14	1 (7.1)	1	-	1.0
Coreoperca herzi	11	1 (9.1)	1	-	1.0
Subtotal	25	2 (8.0)	2	-	1.0
⑬ Pyeongchanggang in Pyeongchang-gun (2013)					
Rhynchocypris oxycephalus	10	1 (10.0)	3	-	3.0
Pseudopungtungia tenuicorpa	5	1 (20.0)	1	-	1.0
Subtotal	15	2 (13.3)	4	1-3	2.0
Total	155	24 (15.5)	102	1-18	4.3

intermediate hosts. Accordingly, 24 fish species (20 genera) in total are listed as the second intermediate hosts of *C. armatus* in Korea.

In the present study, *Echinostoma* spp. metacercariae (species undetermined) were detected in 9 fish species, i.e., *A. signifer* (from ⑥), *C. herzi* (⑩), *O. platycephala* (⑧), *P. esocinus* (⑧), *P. tenuicorpa* (⑬), *P. herzi* (⑥, ⑧, ⑨, ⑩), *R. oxycephalus* (⑬), *Siniperca scherzeri* (⑧), and *Z. platypus* (⑨). To date, 3 zoonotic *Echinostoma* species, i.e., *E. cinetorchis*, *E. hortense*, and *E. revolutum*, are distributed in Korea [33]. Among them, *E. hortense* is the dominant species and has 8 fish intermediate hosts, i.e., *Misgurnus anguillicaudatus*, *Misgurnus mizolepis*, *R. oxycephalus*, *Odontobutis interrupta*, *S. japonicus coreanus*, *Rhinogobius brunneus*, *A. macropterus*, and *Acanthogobius flavimanus*, whereas the remaining 2 species take snails as the second intermediate hosts [5]. Moreover, some inland areas, i.e., Eumseong-gun (Chungcheongbuk-do), Yeongwol-gun (Gangwon-do), Cheongsong-gun (Gyeongsangbuk-do), and Geochang-gun (Gyeongsangnam-do), have been reported as the endemic foci of *E. hortense* infection [20,34-36]. Therefore, the metacercariae of *Echinostoma* spp. detected in the present study are presumed to be *E. hortense*.

Conclusively, it is reconfirmed that Gangwon-do is a highly endemic area of intestinal flukes, i.e., *Metagonimus* spp., *C. armatus*, and *Echinostoma* spp., infections rather than clonorchiasis. The inhabitants residing in endemic areas should pay attention to infections with these intestinal flukes, and consumption of raw freshwater fish naturally produced should be avoided. In addition, species differentiation in 2 genera, *Metagonimus* and *Echinostoma*, should be done in the near future through experimental infection of these metacercariae to animal hosts.

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CONFLICT OF INTEREST

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

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