Korean J Parasitol. Vol. 46, No. 4: 247-251, December 2008 DOI: 10.3347/kjp.2008.46.4.247

Infection Status of Freshwater Fish with Metacercariae of *Clonorchis sinensis* in Korea

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Abstract: This study investigated freshwater fish for their current infection status with metacercariae of *Clonorchis sinensis* in Korea. Twenty-one species of freshwater fish (n = 677) were collected from 34 regions nationwidely from February 2007 to June 2008. They were individually examined by digestion technique. Eight species of freshwater fish from 17 different regions were recognized positive for the metacercariae of *C. sinensis*. The positive rates (range of metacercariae number per fish) of fish by the species were as follows: 48% (1-1,142) in *Pseudorasbora parva*, 60% (1-412) in *Pungtungia herzi*, 15.7% (1-23) in *Pseudogobio esocinus*, 29% (1-7) in *Acheilognathus intermedia*, 21% (1-4) in *Odontobutis interrupta*, 33% (1-6) in *Zacco temmincki*, 3.6% (1-4) in *Zacco platypus*, and 26.3% (1) in *Hemibarbus labeo*. The two species, *P. parva* and *P. herzi*, are able to be the index fish for estimation of *C. sinensis* transmission in a certain locality. Still several species of freshwater fish are briskly transmitting *C. sinensis* infection in many riverside areas of southern Korea.

Key words: *Clonorchis sinensis*, metacercariae, freshwater fish

INTRODUCTION

Clonorchiasis has been known to be endemic in Korea for a long time. *Clonorchis sinensis* has been known to be actively transmitted along major rivers, especially among the fish-eating residents. In Korea, various species of freshwater fish were reported as the second intermediate hosts of *C. sinensis*. Up to present, a total of 80 species of freshwater fish have been confirmed to be the second intermediate hosts of *C. sinensis* over the country [1]. Of these, *Pseudorasbora parva*, *Pungtungia herzi*, *Pseudogobio esocinus*, *Sarcocheilichthys sinensis*, and *Hemibarbus labeo* were confirmed as major intermediate hosts of *C. sinensis* in Korea. Recently, the pond smelt, *Hypomesus olidus*, became a newly recognized intermediate host of *C. sinensis* [2-4]. The pond smelt is a favorite freshwater fish which many people eat alive during winter.

Most of the major river basins in Korea were observed as endemic of clonorchiasis and metagonimiasis. Of these river basin areas, the Nakdong-gang and its tributaries flowing through the Gyeongbuk and Gyeongnam are well-known by heavy endemicity [5].

Even though praziquantel is supplied for effective cure of clonorchiasis in Korea, the population of clonorchiasis cases is estimated more than a million at present [6,7]. The present situation suggests that *C. sinensis* is still transmitted actively in Korea. Infection status of freshwater fish with metacercariae of *C. sinensis* is one of the important epidemiological indices, which reveals the status of sources of human infection. In order to evaluate transmission status of *C. sinensis* in Korea, this study investigated infection status of freshwater fish with the metacercariae.

MATERIALS AND METHODS

Subjected sites for fish collection

A total of 677 freshwater fish of 21 species were caught from 34 localities during the period from February 2007 to June 2008. Those sites were selected according to geographical locations and water flows (Fig. 1). We visited the fields and caught fish with fish traps by ourselves.

Fish examination

The collected fish were labeled and transferred to the laboratory in an ice-packed box. The fish were measured of length and weight, and digested with artificial gastric juice for 1 hr at 37°C individually. The digested fish materials were washed with 0.9% saline and examined by stereomicroscopy to identify *C. sinensis* metacercariae.

• Received 4 November 2008, accepted after revision 24 November 2008.

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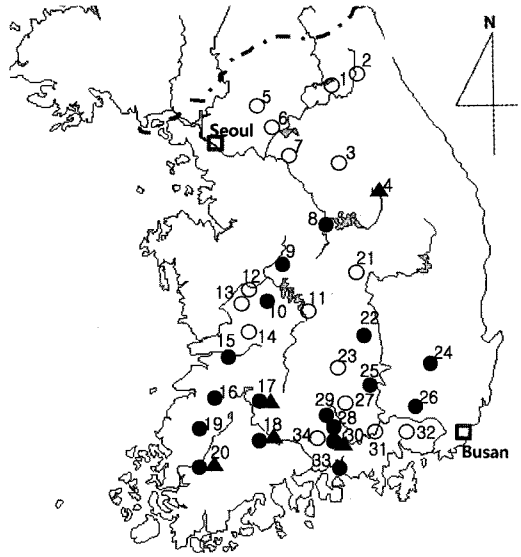

Fig. 1. The surveyed areas of the Republic of Korea for collection of freshwater fish. Numbers are area codes on Tables. Closed circles (●) indicate areas of *Clonorchis sinensis* metacercaria positive fish; closed triangles (▲) for *Metagonimus* sp.; open circles (○) for metacercaria negative areas.

RESULTS

A total of 8 species of freshwater fish were found to carry metacercariae of *C. sinensis* in the present study (Table 1). The positive fish were *P. parva, P. herzi, P. esocinus, Acheilognathus intermedia, Odontobutis interrupta, Z. temmincki,* and *Z. platypus*. Eleven species of fish and freshwater shrimps examined were negative for the metacercariae (Table 2). Of the *C. sinensis* positive fish, *P. parva* and *P. herzi* were collected in several localities and their positive rates were higher than those of other fish species. Also their infection burden was higher than that of the others (Table 1). The positive fish were collected from total 17 different sites over the country (Fig. 1).

Five species of the fish, *Z. platypus, P. parva, Carassius carassius, A. intermedia,* and *P. esocinus,* were observed to carry metacercariae of *Metagonimus* sp. (Table 3). The localities of *Metagonimus* positive fish were overlapped with those of *C. sinensis* except Yeongwol, Gangwon-do, where only *Metagonimus* metacercarie were recovered (Fig. 1).

Table 1. Infection status by *Clonorchis sinensis* metacercariae (MC) in freshwater fish by species and localities

Species of fish	Localities of fish collection (numbers in Fig. 1)	No. of examined	No. (%) of positive	Mean No. (range) of MC/fish
Pseudorasbora parva	Ochang in Chungbuk (9)	10	10 (100)	73.4 (3-383)
	Chungju in Chungbuk (8)	20	15 (75)	1.55 (1-3)
	Imsil in Jeonbuk (17)	3	3 (100)	344.6 (53-1142)
	Iksan in Jeonbuk (15)	10	5 (50)	1.8 (1-3)
	Jeongeup in Jeonbuk (16)	11	4 (36.4)	1 (1)
	Gokseong in Jeonnam (18)	4	3 (75)	5.5 (3-15)
	Jangseong in Jeonnam (19)	2	1 (50)	1 (1)
	Naju in Jeonnam (20)	3	2 (66.7)	3 (35-64)
	Seonsan in Gyeongbuk (22)	10	1 (10)	1 (1)
	Yeongcheon in Gyeongbuk (24)	10	8 (80)	2 (1-8)
	Jinju in Gyeongnam (33)	20	29 (100)	1.3 (1-2)
Pungtungia herzi	Jangseong in Jeonnam (19)	2	2 (100)	118 (44-192)
	Goryeong in Gyeongbuk (25)	1	14 (100)	56 (6-321)
	Gyeongho-gang in Gyeongnam (29)	13	13 (100)	31.5 (15-62)
	Yangcheon-gang in Gyeongnam (28)	10	10 (100)	80.9 (1-412)
Pseudogobio esocinus	Cheongwon in Chungbuk (10)	9	2 (22)	3.2 (1-6)
	Yangcheon-gang in Gyeongnam (28)	10	6 (60)	4 (1-23)
	Miryang in Gyeongnam (26)	6	2 (33.3)	9.2 (3-21)
Acheilognathus intermedia	Ochang in Chungbuk (9)	10	8 (80)	3 (1-7)
	Jeongeup in Jeonbuk (16)	10	1 (10)	2.4 (1-4)
	Imsil in Jeonbuk (17)	10	8 (80)	1.7 (1-6)
	Jangseong in Jeonnam (19)	9	7 (77.8)	1.89 (1-6)
	Yeongcheon in Gyeongbuk (24)	20	3 (15)	2.4 (1-7)
Odontobutis interrupta	Imsil in Jeonbuk (17)	10	3 (30)	3 (2-4)
	Gokseong in Jeonnam (18)	3	1 (33.3)	1 (1)
Zacco temmincki	Gokseong in Jeonnam (18)	1	1 (100)	6 (6)
Zacco platypus	Naju in Jeonnam (20)	5	1 (20)	4 (1-4)
Hemibarbus labeo	Iksan in Jeonbuk (15)	4	2 (50)	1 (1)

Table 2. Numbers of negative fish for *Clonorchis sinensis* metacercariae by species and localities

Species of fish	Localities of fish collection (numbers in Fig. 1)	No. examined
Hypomesus olidus	Chuncheon in Gangwon-do (2)	20
	Hapcheon-gun in Gyeongnam (27)	40
Oncorhynchus masou	Hwacheon (1) / Yeongwol (4) in Gangwon-do	10/1
Hemibarbus longirostris	Yeongwol in Gangwon-do (4)	1
	Shinan in Gyeongnam (34)	6
Silurus asotus	Yeongwol in Gangwon-do (4)	2
	Gokseong in Jeonnam (18)	1
Coreoperca herzi	Yeongwol in Gangwon-do (4)	1
Barbatula toni	Yeongwol in Gangwon-do (4)	1
Micropterus almoides	Imsil in Jeonbuk (17)	6
Carassius carassius	Gokseong (18) / Naju (20) in Jeonnam	5/2
	Gyeongho-gang in Gyeongnam (29)	7
Opsariichthys bidens	Gokseong in Jeonnam (18)	5
Siniperca scherzeri	Gokseong in Jeonnam (18)	1
Coilia ectense	Haman-gun in Gyeongnam (31)	20
Pseudorasbora parva	Pocheon (5) / Gapyeong (6) in Gyeonggi-do	34/18
	Okcheon in Chungbuk (11) / Gongju in Chungnam (13)	10/14
	Gimcheon in Gyeongbuk (23) / Miryang in Gyeongnam (26)	17/15
Pungtungia herzi	Yeongwol in Gangwon-do (4)	1
	Anheung-myeon in Gangwon-do (3)	11
	Chungju in Chungbuk (8) / Miryang in Gyeongnam (26)	10/10
Pseudogobio esocinus	Yeongwol in Gangwon-do (4)	1
	Okcheon (11) / Chungju (8) in Chungbuk	10/14
	Nonsan in Chungnam (14) / Yecheon in Gyeongbuk (21)	14/15
	Gyeongho-gang in Gyeongnam (29)	13
Acheilognathus intermedia	Yeongi (12) / Nonsan (14) in Chungnam	2/15
	Gokseong (18) / Naju (20) in Jeonnam	2/5
	Shian in Gyeongnam (34)	16
Odontobutis interrupta	Yeongwol in Gangwon-do (4) / Okcheon in Chungbuk (11)	1/3
	Yeongcheon in Gyeongbuk (24)	1
	Gyeongho-gang in Gyeongnam (29)	1
Zacco platypus	Yeongwol (4) / Anheung-myeon (3) in Gangwon-do (4)	1/10
	Gokseong in Jeonnam (18) / Gimhae in Gyeongnam (32)	10/2
	Gyeongho-gang in Gyeongnam (29)	2
Zacco temmincki	Yeongwol in Gangwon-do (4)	2
Hemibarbus labeo	Chungju in Chungbuk (8) / Gokseong in Jeonnam (18)	10/13
	Goryeong in Gyeongbuk (25)	1
	Gyeongho-gang in Gyeongnam (29)	1
Misgurnus mizolepis	Pocheon in Gyeonggi-do (5)	2
Freshwater shrimp	Yangpyeong in Gyeonggi-do (7) / Shinan in Gyeongnam (34)	7/13

DISCUSSION

The present findings confirm that *C. sinensis* metacerariae are still briskly transmitted by freshwater fish in southern Korea in 2007. The Korea Association of Health Promotion (KAHP) has been implementing detection and chemotherapy of infected people by clonorchiasis in Korea after introduction of praziquantel in the 1980s [6]. Furthermore, the Korea Center for Disease Control and Prevention (KCDCP) is running a control program of clonorchiasis in endemic areas after the 7th national survey of intestinal parasite infections [8]. Although the control activities have been implemented for more than 20 yr, clonorchiasis is still prevalent throughout the country in Korea because of continuous reinfection [9].

All of the 8 metacercaria-positive freshwater fish are known species as the intermediate hosts of *C. sinensis* in Korea [1]. Of the 8 positive species of fish, *P. herzi* was recognized 100% positive at every locality of fish collection. Also numbers of metacercariae in a fish were more in *P. herzi* than those in other species (Table 1). Until now the freshwater fish, *P. parva*, has been used as an index species for distribution of *C. sinensis* at a certain unknown areas, because the species is susceptible for the meta-

Table 3. Infection status with *Metagonimus* sp. metacercariae (MC) in freshwater fish by species and localities

Species of fish	Localities of fish collection (numbers in Fig. 1)	No. examined	No. (%) positive	Mean No. (range) of MC/fish
Zacco platypus	Yeongwol in Gangwon-do (4)	1	1 (100)	1 (1)
	Gokseong in Jeonnam (18)	5	4 (80)	11 (1-15)
	Naju in Jeonnam (20)	5	2 (40)	2 (2-4)
Pseudorasbora parva	Imsil in Jeonbuk (17)	10	2 (20)	3 (2-8)
	Gokseong in Jeonnam (18)	4	4 (100)	3 (1-6)
Carassius carassius	Gokseong in Jeonnam (18)	5	5 (100)	7 (2-12)
	Naju in Jeonnam (20)	2	2 (100)	12 (2-15)
Acheilognathus intermedia	Gokseong in Jeonnam (18)	2	2 (100)	3 (2-4)
Pseudogobio esocinus	Sancheong in Gyeongnam (30)	10	2 (20)	5 (1-9)

cercariae [1,10-12]. Although *P. parva* is the most susceptible species, this can be a rare source of human infection because this is not a favored fish for eating raw in Korea. In contrast, *P. herzi* is one of the favorite fish which people prefer eating raw because it lives in rather unpolluted water at upper streams of rivers and tastes good. Since *P. herzi* is susceptible for the metacercariae, it can be the real index of *C. sinensis* transmission in the field. In other words, detection of metacercariae from *P. parva* or *P. herzi* may reveal the transmission of clonorchiasis in unknown areas.

Another freshwater fish, the pond smelt (*Hypomesus olidus*) is the most favorite species for raw eating in Korea. About hundred tons of the fish are produced from several big lakes in Korea during winter annually. Most of lay Koreans believe that these fish are free from any parasite or pathogen because they are produced in clean icy water. Since several hundred-thousand people enjoy fishing and eating the fish every year at ice-fishing festivals or local markets, susceptibility of this species to the metacercariae of *C. sinensis* is a practically serious factor for risk of the infection.

Up to present, 3 studies have investigated infection status of pond smelts by metacercariae of *C. sinensis* [2-4]. Two studies examined hundreds of the fish by one batch of artificial digestion, and one reported positive results of the fish from Taehoman in 1998 and Soyangho in 1999. However, the risk of infection was regarded very low because 3 metacercariae were recovered from 62 pond smelts at Taehoman and 2 metacercariae were from 156 fish at Soyangho [2]. The other one reported that 161 *C. sinensis* metacercariae were recovered from 459 digested pond smelts from the Soyangho and 369 metacercariae from 100 fish from the Daecheongho in 2003 [3]. The positive result in 2003 provoked a serious social reverberation because ice-fishing and eating live pond smelts became a popular winter festival at the lake of Soyangho. The result raised the risk of clonorchiasis transmission by the winter festival. Consequently, the risk of clonorchiasis and the economical advantage of local residents were in a conflict by the report and the consistency of the positive finding of the pond smelts should have been confirmed. The KCDCP extensively examined a total of 3,920 pond smelts from the Soyangho in January of 2003, 2004, and 2005 continuously, but found no metacercariae of *C. sinensis* [4]. The study also examined the pond smelts from other lakes in Korea, such as Uiamho, Unamho, Jangseongho, Andongho, and Uirimji, but all of examined fish were negative and only a few unknown diplostomula were found from Jangseongho [4]. The results of the extensive survey could have soothed the social conflict of eating pond smelts in Korea. The KCDCP is monitoring the status of metacercariae in the pond smelts from the Soyangho annually up to present. The present study examined 20 pond smelts from the Soyangho and 20 from the Hapcheonho, and also 10 *Oncorhynchus masou* from the Paroho. Both fish are produced as a commercial strategy by local governments and openly encouraged to eat raw. Fortunately, all of the examined fish were negative for the metacercariae by this study. The natural environment required for growth of pond smelts and *O. masou* is cold, clean, and deep water in upper streams of rivers like the Soyangho while that of the first intermediate host of *C. sinensis*, *Parafossarulus manchouricus* is warm and shallow water, which is an environment of down streams of rivers. The locality around the Soyangho is also non-endemic of clonorchiasis and thus egg contamination of the water is least in Korea [1,6]. The examination data of the fish and the ecological characteristics of the locality suggest that pond smelts from the Soyangho have little risk of clonorchiasis transmission.

No metacercarial infections were found in 11 kinds of freshwater fish (Table 2). Most of them were insusceptible to those

of *C. sinensis*. Also numbers of examined fish were limited. Therefore, it is better to understand the present findings as minimum confirmed status. Those negative results do not necessarily mean no transmission in the surveyed areas.

The freshwater fish also mediate *Metagonimus* sp. as well as *C. sinensis*. The *Metagonimus* metacercariae were recovered from 5 species at 5 localities. Both metacercariae of *C. sinensis* and *Metagonimus* sp. were detected from 4 same localities although the first intermediate hosts are different. The two flukes are forming their transmission cycles together at several overlapped local areas (Fig. 1). The present study confirmed again that these two flukes are major fish-borne parasites in Korea.

Recently, water pollution in rivers is improved and forests in mountains are well preserved in Korea. Due to the improved environment and supply of praziquantel, many people tend to neglect the infection risk of clonorchiasis by eating raw freshwater fish. However, the present findings warn definitely that the risk should be brisk because metacercariae of *C. sinensis* are still transmitted by the fish in most of southern Korea.

ACKNOWLEDGEMENTS

This study was supported by a research grant from the Korea Association of Health Promotion (2007).

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