Korean Journal of Parasitology
Vol. 45, No. 1: 33-38, March 2007

A new endemic focus of *Heterophyes nocens* and other heterophyid infections in a coastal area of Gangjin-gun, Jeollanam-do

Jae-Hwan PARK[1], Jae-Lip KIM[1], Eun-Hee SHIN[1], Sang-Mee GUK[1], Yun-Kyu PARK[2] and Jong-Yil CHAI[1]*

[1]*Department of Parasitology and Tropical Medicine, Seoul National University College of Medicine, and Institute of Endemic Diseases, Seoul National University Medical Research Center, Seoul 110-799,*
[2]*Department of Parasitology and Institute for Medical Sciences, College of Medicine, Inha University, Incheon 440-712, Korea*

Abstract: To know the prevalence of heterophyid trematodes among inhabitants of a southern coastal village, i.e., Sacho-ri, Gangjin-gun, Jeollanam-do (Province), 82 stool samples were examined on helminth eggs and protozoan cysts using Kato-Katz and formalin-ether sedimentation techniques. Total 33 people (40.2%) were positive for trematodes (*Heterophyes nocens*; 15 people, *Pygidiopsis summa*; 3, *Metagonimus* sp.; 4, *Clonorchis sinensis*; 7, *Gymnophalloides seoi*; 6) and/or protozoa (*Entamoeba coli*; 3). Among intestinal trematode egg positive cases, 17 were treated with praziquantel and their whole diarrheic stools were collected after purgation. Adult flukes of *H. nocens* (number of specimens = 1,294), *P. summa* (386), *Stellantchasmus falcatus* (5), *Stictodora lari* (4), and *Heterophyopsis continua* (1) were collected using a stereomicroscope. To know the source of human *H. nocens* infections in this village, metacercarial infections in mullets (10) were examined and most *H. nocens* metacercariae (101/105, 96.2%) were found in the trunk portion. From above results, the surveyed coastal village has been newly known as an endemic focus of human *H. nocens* infection and consuming raw mullets was the presumable source of human heterophyid infections.

Key words: *Heterophyes nocens*, heterophyid, endemic focus, stool examination, mullet, metacercaria

INTRODUCTION

In the Republic of Korea, the prevalence and intensity of human soil-transmitted nematode infections were remarkably decreased during last decades through active parasite controls (Ministry of Health and Social Affairs and Korea Association of Health, 1997). However, high prevalences of intestestinal trematode infections have been reported consecutively in many coastal areas and islands (Chai et al., 2000, 2004; Chai and Lee, 2002). Human infections with heterophyids, particularly *Heterophyes nocens* and *Pygidiopsis summa*, were indigenous to western and southern coastal islands (Chai et al., 2004). Brackish water fishes including mullets, gobies, and perches are sources of human heterophyid infections (Chai and Lee, 2002) and among them mullets are most important for human infections with *H. nocens* and *P. summa* (Sohn et al., 1994; Kim et al., 2006). People residing in coastal areas can easily catch brackish water fishes and frequently consume their raw freshes

• Received 30 December 2006, accepted after revision 2 February 2007.
*Corresponding author (e-mail: cjy@snu.ac.kr)

in sashimi style. In other words, people in those areas are frequently exposed to re-infections with heterophyids. We expected some correlations between prevalences of human heterophyid infections and metacercarial intensities of fish hosts in a localized coastal area but little have been known about those. If they had some correlations each other, we could presume the endemicity of human heterophyid infections in coastal areas through metacercarial intensity in their fish hosts and the geographical distribution.

In this study, we selected a small coastal village, where the whole population was composed of 146 people and never studied about intestinal trematode infections. Many of them enjoyed consuming raw mullets in a sashimi style and therefore, strongly suspected heterophyid infections. In this area, we tried to know infection status of human intestinal trematodes among villagers. In addition, metacercarial infections in mullets caught at seashore near the studied village were also studied.

MATERIALS AND METHODS

Area surveyed

The surveyed area, Sacho-ri, Sinjeon-myon, Gangjin-gun, Jeollanam-do, is located on a southwestern coast of the Republic of Korea (Fig. 1). Most villagers were attending to the farming and favored eating raw mullets in sashimi style, a potential source of human infection with various species of heterophyids.

Fecal examinations

Total 82 fecal specimens, one specimen from each person, were collected from the studied village in June 2003. Specimens were transported to the Department of Parasitology and Tropical Medicine, Seoul National University College of Medicine and stored at 4°C until examined. Formalin-ether sedimentation and the Kato-Katz thick smear techniques were used to detect protozoan cysts and helminth eggs. Egg counting was done in Kato-Katz thick smears, which contained approximately 41.7 mg of a fecal sample per smear. Eggs per gram of feces (EPG) were calculated from egg counts in Kato-Katz thick smear × 24.

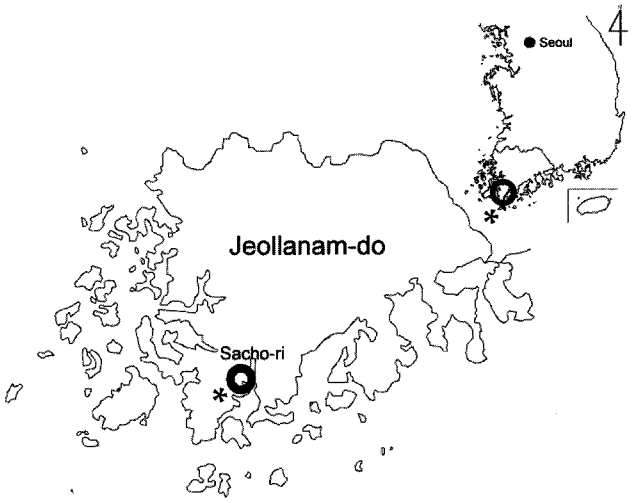

Fig. 1. Map showing the surveyed area, Sacho-ri (*), Sinjeon-myon (Gangjin-gun, Jeollanam-do Province).

Worm collection

For adult fluke collection, we visited the village again one month later (July 2003) and selected 17 cases which showed relatively high EPG in the previous fecal examination. They were treated with a single dose (10 mg/kg) of praziquantel (Shinpoong Pharmaceutical Co., Seoul, Korea) and purged with magnesium salt after obtaining informed consent from each person. Whole diarrheic stools passed successively 4-5 times were collected and then individual stools were washed several times with 20 volumes of water and sieved with a 2-mm mesh to discard large debris. After 10 min, the upper clean layer was discarded and the lower dark layers was fixed with 2% neutral-buffered formalin. Each sample was filled in an individual bottle and transported to the Department of Parasitology and Tropical Medicine, Seoul National University College of Medicine and examined for adult worms using a stereomicroscope. Collected worms were counted and morphologically identified under a light microscope.

Metacercarial infection in mullets

To know the metacercarial density according to body portions in each fish and the infection rate, 10 mullets *Mugil cephalus* were caught in the estuary nearby the surveyed village. The fish body was divid-

ed into head, gill, trunk, fin and tail, and visceral organs. Individual portions were digested in 0.6% pepsin-HCl solution and filtered through 1-mm mesh to discard large tissue debris. After 10 min, lower precipitates were examined for trematode metacercariae using a stereomicroscope. Collected metacercariae were identified morphologically by the size and shape of their cysts, and by the characteristic features of internal organs. Metacercariae of *H. nocens* were round or slightly ellipsoid measuring 0.16-0.20 by 0.13-0.19 mm. The excretory bladder was round and bluish brown or dark pinkish. The metacercarial cyst of *P. summa* was globular, round to elliptical measuring 0.19-0.29 by 0.16-0.29 mm. The excretory bladder was X- or I-shaped and dark brown in color. Metacercariae of *H. nocens* and *P. summa* as well as other unidentified metacercariae were infected to 8-week-old SD rats and tried to confirm or identify their species by the adult worms obtained at a week post-infection.

RESULTS

Prevalence of parasite infections among villagers

Of 81 fecal specimens examined, total cases of helminth egg and/or protozoan cyst positives were 33 (40.7%) and 6 of them were infected with more than 2 species of parasites (Table 1). The overwhelming majority of parasite infections among villagers was heterophid flukes (22 persons; 27.2%) including *H. nocens* and *P. summa*, followed by *Clonorchis sinensis* (8.6%) and *Gymnophalloides seoi* (7.4%). Cysts of *Entamoeba coli* were found in 3 specimens. Average egg count per gram of feces was the highest in *P. summa* followed by *C. sinensis*, *H. nocens*, *Metagonimus* sp., and *G. seoi* (Table 1). Men (40.5%) were more infected with various helminths than women (38.6%) ($P > 0.05$) and most (87.5%) infected people were over 40 year-old. However, about *H. nocens*, women (20.5%) were more infected than men (16.2%).

Table 1. Prevalence of parasites by fecal examination in residents of Sacho-ri, Sinjeon-myon (Gangjin-gun, Jeollanam-do)

Parasite	No. of positive (%)	EPG (average ± SD)[a]
No. examined	81	
No. positive (egg/cyst)[b]	33 (40.7)	
No. helminth egg positive	32 (39.5)	
Heterophyes nocens	15 (18.5)	48.0 ± 37.9
Pygidiopsis summa	3 (3.7)	72.0 ± 55.4
Metagonimus sp.	4 (4.9)	32.0 ± 13.9
Clonorchis sinensis	7 (8.6)	67.2 ± 59.8
Gymnophalloides seoi	6 (7.4)	24.0
Entamoeba coli	3 (3.7)	-

[a]EPG: eggs per gram of feces.
[b]Mixed infections with more than 2 species of parasites were found in 6 cases.

Worms collected from 17 villagers

Total 1,708 adult flukes were collected from 17 persons (average 101 flukes per person; 1-540 in range) (Table 2). Among the flukes, *H. nocens* was the most prevalent and heavily infected species. Total 1,294 adult flukes of *H. nocens* were recovered from 17 persons (average 76.1, 1-267 in range). Total 386 adult *P. summa* were collected from 1 person. Other trematodes including *S. falcatus*, *S. fuscata*, and *H. continua* were also collected (Table 2).

Metacercariae in mullets

A total of 499 trematode metacercariae were collected from examined mullets, of which 5 were infected with 105 *H. nocens* metacercariae and 7 were infected with 18 *P. summa* metacercariae (Table 3). According to body portions, *H. nocens* metacercariae were found in trunk and head, and *P. summa* metacercariae were found in gills, internal organs, head, and trunk (Table 3). Various metacercariae which were difficult to identify morphologically were collected from internal organs, head, body, gills, and tail and pins (Table 3). After infection to experimental animals, adult flukes of *H. nocens* and *P. summa* were recovered. However, no other flukes were found in the small intestine of experimental animals.

Table 2. Results of worm collection from villagers in Sacho-ri (Gangjin-gun)

Patient code	Age/sex	Worms collected					
		H.n*	P.s	S.fa	S.fu	H.c	Total
1	62/F	267	0	0	0	0	267
2	55/M	239	0	0	0	0	239
3	55/F	192	0	2	0	0	208
4	53/M	155	0	3	0	0	158
5	73/M	153	386	0	1	0	540
6	58/F	94	0	0	0	0	97
7	60/M	55	0	0	2	0	57
8	56/M	37	0	0	0	1	38
9	45/F	24	0	0	0	0	24
10	45/M	19	0	0	0	0	19
11	22/M	17	0	0	0	0	17
12	46/F	13	0	0	0	0	13
13	62/M	10	0	0	0	0	10
14	66/M	8	0	0	0	0	8
15	38/M	8	0	0	0	0	8
16	50/F	2	0	0	1	0	4
17	55/M	1	0	0	0	0	1
Total		1,294	386	5	4	1	1,708

*H.n: *Heterophyes nocens*, P.s: *Pygidiopsis summa*, S.fa: *Stellantchasmus falcatus*, S.fu: *Stictodora fuscata*, H.c: *Heterophyopsis continua*

Table 3. The distribution and intensity of trematode metacercariae in the body part of 10 mullets collected from the seashore of Sacho-ri (Gangjin-gun)

Species of metacercaria	Part of mullet					
	Head	Gill	Body	Intestine	Tail & pin	Total
Heterophyes nocens	4	0	101	0	0	105
Pygidiopsis summa	2	12	1	3	0	18
Unidentified	123	2	41	208	2	376

DISCUSSION

From this study, a coastal village of Gangjin-gun, Jeollanam-do was proven to be an endemic focus of various heterophyid flukes through the stool examination and worm collection. Out of 32 (39.5%) helminth egg positive cases, 20 (24.7%) were infections with heterophyids including *H. nocens* (18.5%) and *P. summa* (3.7%). This prevalence of human heterophyid infection was lower than those from southwestern coastal areas including Muan-gun (75.0%) and Sinan-gun (42.9%) (Lee et al., 1994; Chai et al., 1997), but higher than those from southern coastal areas, Sacheon-gun (18.8%) and Haenam-gun (10.3%) (Chai et al., 1998; Guk et al., 2006). Among adult flukes collected from 17 villagers, the majority (1,294/1,708; 75.8%) was *H. nocens*, even though a few number of 3 heterophyid species including *P. summa*, *S. falcatus*, and *H. continua* were also found. Therefore, we knew that *H. nocens* was the most prevalent intestinal parasite among villagers.

Since the first case report of a human infection with *H. nocens* (Seo et al., 1981a), human infections have been found sporadically in different coastal areas (Chai et al., 1984, 1985; Sohn et al, 1989; Hong et al., 1996; Guk et al., 2006). In a recent survey on western

and southern coastal islands, human *H. nocens* infections were widely distributed even though infection rates varied on each island (Chai et al., 2004). In that survey, low prevalences of *H. nocens* infection were observed among people residing on Pogildo (Island) (6.8%), Nopdo (2.0%), and Nohwado (1.8%) in Wando-gun (Chai et al., 2004), where locate nearby the area of the present study. Prevalences of heterophyid infections in such islands were lower than that of the present study (18.5%).

Even in a small district, Gangjin-gun, the pattern of parasite infections was different between northern areas, where Tamjingang (river) passes through, and southern coastal areas. In previous epidemiological studies, northern villages of Gangjin-gun were known to be endemic foci of human metagonimiasis and no human infections with *H. nocens* and *P. summa* were reported (Chai et al., 1977; Seo et al., 1981b). However, in this study, the major human intestinal parasite was *H. nocens*. Different facilities to catching and consuming fresh water fishes or brackish water fishes should influence on this different pattern of parasite infection. People in northern areas can easily catch and consume sweetfishes, an important source of human metagonimiasis, and people residing in southern coastal areas can easily catch and consume mullets, an important source of human *H. nocens* infection. Sweetfishes caught from Tamjingang were highly infected with *Metagonimus* metacercariae (Seo et al., 1981c, 1982). In the present study, mullets caught nearby the surveyed area were infected with many (105) *H. nocens* metacercariae. Particularly, most (101, 96.2%) *H. nocens* metacercariae were found in the trunk of mullets, which were commonly consumed in sashimi style.

In the Republic of Korea, human infections with intestinal and liver flukes were more prevalent among aged men over 40 year-old who had more chances to consuming raw freshwater fishes or brackish water fishes (Rim, 1990; Chai and Lee, 2002). In this study, men were more infected with various helminths than women. However, *H. nocens* infections were more prevalent among men than women. It is not clear whether women had more chances to consume raw mullets than men in this village or not. In this village, most human infections with intestinal flukes (87.5%) including *H. nocens* (93.3%) were over 40 year-old. These age prevalences can be explained that aged people had more chances to consuming raw brackish water fishes than young people and most people (91.5%) residing in the surveyed village were old ages.

According to above results, we knew that many inhabitants in the surveyed coastal village were infected with heterophyid flukes and among them *H. nocens* infections were most prevalent. Mullets caught from the seashore nearby the surveyed village were highly infected with *H. nocens* metacercariae, particularly in the trunk portion, and regarded as the source of *H. nocens* infection.

REFERENCES

Chai JY, Cho SY, Seo BS (1977) Study on *Metagonimus yokogawai* (Katsurada, 1912) in Korea. IV. An epidemiological investigation along Tamjin River basin, South Cholla Do, Korea. *Korean J Parasitol* **15:** 115-120.

Chai JY, Hong SJ, Sohn WM, Lee SH, Seo BS (1985) Further cases of human *Heterophyes nocens* infection in Korea. *Seoul J Med* **26:** 197-200.

Chai JY, Kim IM, Seo M, Guk SM, Kim JL, Sohn WM, Lee SH (1997) A new endemic focus of *Heterophyes nocens*, *Pygidiopsis summa*, and other intestinal flukes in a coastal area of Muan-gun, Chollanam-do. *Korean J Parasitol* **35:** 233-238.

Chai JY, Lee GC, Park YK, Han ET, Seo M, Kim J, Guk SM, Shin EH, Choi MH, Lee SH (2000) Persistent endemicity of *Gymnophalloides seoi* infection in a southwestern coastal village of Korea with special reference to its egg laying capacity in the human host. *Korean J Parasitol* **38:** 51-57.

Chai JY, Lee SH (2002) Food-borne intestinal trematode infections in the Republic of Korea. *Parasitol Int* **51:** 129-154.

Chai JY, Park JH, Han ET, Shin EH, Kim JL, Guk SM, Hong KS, Lee SH, Rim HJ (2004) Prevalence of *Heterophyes nocens* and *Pygydiopsis summa* infections among residents of the western and southern coastal islands of the Republic of Korea. *Am J Trop Med Hyg* **71:** 617-622.

Chai JY, Seo BS, Lee SH (1984) Studies on intestinal trematodes in Korea. XI. Two cases of human infection by *Heterophyes heterophyes nocens*. *Korean J Parasitol* **22:** 37-

42.

Chai JY, Song TE, Han ET, Guk SM, Park YK, Choi MH, Lee SH (1998) Two endemic foci of heterophyids and other intestinal fluke infections in southern and western coastal areas in Korea. *Korean J Parasitol* **36:** 155-161.

Guk SM, Park JH, Shin EH, Kim JL, Lin A, Chai JY (2006) Prevalence of *Gymnophalloides seoi* infection in coastal villages of Haenam-gun and Yeongam-gun, Republic of Korea. *Korean J Parasitol* **44:** 1-5.

Hong SJ, Chung CK, Lee DH, Woo HC (1996) One human case of natural infection by *Heterophyopsis continua* and three other species of intestinal trematodes. *Korean J Parasitol* **34:** 87-89.

Kim DG, Kim TS, Cho SH, Song HJ, Sohn WM (2006) Heterophyid metacercarial infections in brackish water fishes from Jinju-man (Bay), Kyongsangnam-do, Korea. *Korean J Parasitol* **44:** 7-13.

Lee SH, Chai JY, Lee HJ, Hong ST, Yu JR, Sohn WM, Kho WG, Choi MH, Lim YJ (1994) High prevalence of *Gymnophalloides seoi* infection in a village on a south-western island of the Republic of Korea. *Am J Trop Med Hyg* **51:** 281-285.

Ministry of Health and Social Affairs and Korea Association of Health (1997) Prevalence of intestinal parasitic infections in Korea. The sixth reports in monographic series (in Korean).

Rim HJ (1990) Clonorchiasis in Korea. *Korean J Parasitol* **28** (suppl.): 63-78.

Seo BS, Hong ST, Chai JY (1981a) Studies on intestinal trematodes in Korea. 3. Natural human infections of *Pygidiopsis summa* and *Heterophyes heterophyes nocens*. *Seoul J Med* **22:** 228-235.

Seo BS, Hong ST, Chai JY, Cho SY (1981b) Studies on intestinal trematodes in Korea. IV. Geographical distribution of *Pygidiopsis* and *Heterophyes* metacercariae. *Seoul J Med* **22:** 236-242.

Seo BS, Lee SH, Cho SY, Chai JY, Hong ST, Han IS, Sohn JS, Cho BH, Ahn SR, Lee SK, Chung SC, Kang KS, Shim HS, Hwang IS (1981c) An epidemiologic study on clonorchiasis and metagonimiasis in riverside areas in Korea. *Korean J Parasitol* **19:** 137-150.

Seo BS, Hong ST, Chai JY, Lee SH (1982) Study on *Metagonimus yokogawai* (Katsurada, 1912) in Korea. VI. The geographical distribution of metacercarial infection in sweetfish along the east and south coast. *Korean J Parasitol* **20:** 28-32.

Sohn WM, Chai JY, Lee SH (1989) Two cases of natural human infection by *Heterophyes nocens* and the infection status of heterophyid metacercariae in mullets from Samcheonpo, Kyongnam Province. *Inje Med J* **10:** 443-452.

Sohn WM, Han GG, Kho WG, Chai JY, Lee SH (1994) Infection status with the metacercariae of heterophyid flukes in the brackish water fish from Haenam-gun, Chollanam-do, Korea. *Korean J Parasitol* **32:** 163-169.