

A new endemic focus of *Gymnophalloides seoi* infection on Aphae Island, Shinan-gun, Jeollanam-do

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Abstract: A new endemic focus of *Gymnophalloides seoi* infection has been discovered on Aphae Island (Shinan-gun, Jeollanam-do), Republic of Korea. This area, which is referred to as Bokyeong-ri, is a small seashore village located in the northern portion of the island. Fecal samples were collected from a total of 57 residents and examined by the Kato-Katz and formalin-ether sedimentation techniques. Helminth eggs were detected in 37 samples (64.9%); 33 samples were positive for *G. seoi* eggs (57.9%), 4 for *Pygidiopsis summa* (7.0%), 13 for other heterophyids (22.8%), 1 for *Clonorchis sinensis* (1.7%), and 1 for *Trichuris trichiura* (1.7%). Women (70.4%) revealed higher rates of *G. seoi* infection than did men (46.7%), and individuals older than 50 years of age also evidenced higher rates of *G. seoi* infection than in other age groups ($P < 0.05$). In worm collection from 13 *G. seoi* egg positive cases, *G. seoi* (total 86,558 specimens), *Heterophyes nocens* (278), *Stictodora* sp. (10), *Heterophyopsis continua* (3), *P. summa* (3), and unidentified flukes (42) were collected. Oysters, the source of *G. seoi* infection, were collected from an area proximal to the village and 50 were examined for metacercariae; 47 (94%) were found infected and the observed metacercarial density was 9.5 ± 8.9 per oyster. The results of this study indicate that Bokyeong-ri is a new endemic area of *G. seoi* infection, with high rates of infection in humans and oysters.

Key words: *Gymnophalloides seoi*, *Pygidiopsis summa*, heterophyid fluke, fecal examination, Aphae Island (Jeollanam-do), oyster

INTRODUCTION

Gymnophalloides seoi Lee, Chai and Hong, 1993 (Trematoda; Gymnophallidae) is a minute intestinal fluke that infects humans and migratory birds, including the Palearctic oystercatcher *Haematopus ostralegus* (Lee et al., 1993; Lee and Chai, 2001; Ryang et al., 2000; Chai et al., 2003). The oyster, *Crassostrea gigas*, has

been identified as the source of human infections (Lee et al., 1995a). Until the present, *G. seoi* has been detected only in the Republic of Korea (Lee et al., 1993, 1995, 1996; Sohn et al., 1998; Lee et al., 1999; Chai and Lee, 2002).

The first human *G. seoi* infection was detected in 1988 from a 66-year-old woman suffering from acute pancreatitis (Lee et al., 1993). A small village on Aphae Island, Sinjang-3-ri, the residence of the first patient, was determined to be highly endemic for *G. seoi* infection. Half of the villagers residing in this area

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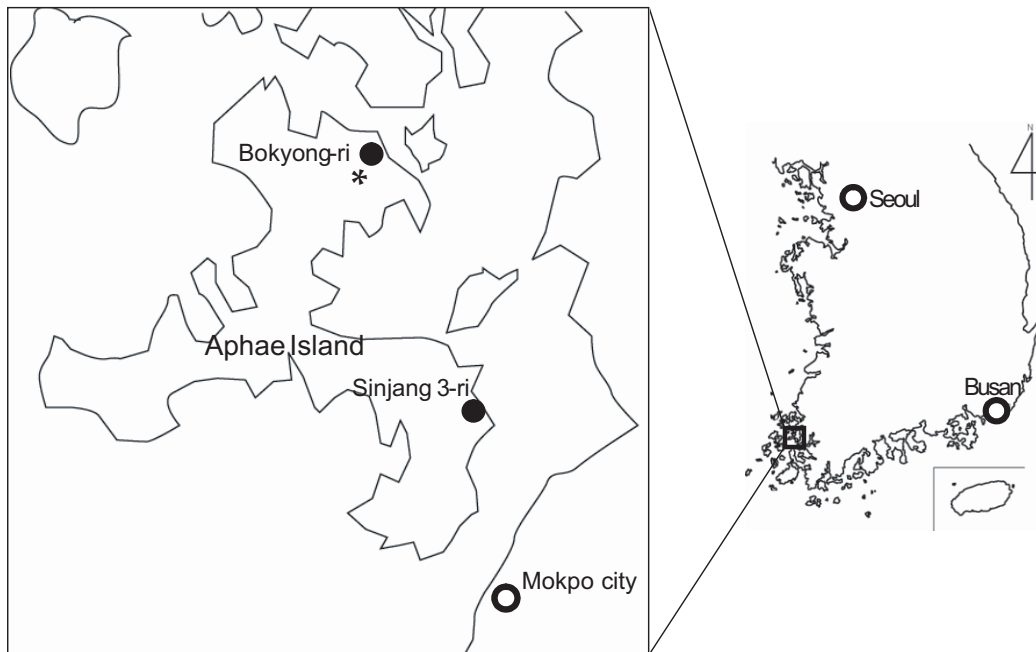


Fig. 1. The area surveyed in this study (Bokyong-ri, astrix) in comparison with Sinjang 3-ri, a known endemic area of *Gymnophalloides seoi* infection.

were found to be infected (Lee et al., 1994). Thereafter, oysters infected with *G. seoi* metacercariae were detected in 14 western coastal areas and more human infections were discovered in 24 southeastern coastal areas (Lee and Chai, 2001). It is now generally thought that human *G. seoi* infections may be present in a broader distribution than previously expected, as natural oysters are available all along the southwestern coast, and people can readily procure and consume raw oysters in these regions.

The prevalence and intensity of *G. seoi* metacercarial infection in oysters appears to vary substantially according to geographical area (Lee et al., 1996; Chai et al., 2001b). Therefore, even on Aphae Island, in which the endemic focus of *G. seoi* was initially discovered, the status of metacercarial infection in oysters might differ according to locality on the island. In addition, the status of human *G. seoi* infection may also differ in accordance with locality on the island. Up to the present, on Aphae Island, epidemiological studies have concentrated on Sinjang 3-ri, and no other information has been available regarding human *G. seoi* infections on different regions of Aphae Island. The objective of this study was, then, to deter-

mine the infection status of *G. seoi* among residents of a small coastal village on Aphae Island, and among oysters caught in a region near this village.

MATERIALS AND METHODS

Fecal examination and worm collection

A total of 57 residents in Bokyong-ri, Aphae Island, were subjected to fecal examinations. Bokyong-ri is located in the northern portion of Aphae Island and is quite far (approximately 10 km) from Sinjang 3-ri, in which *G. seoi* infection is endemic (Fig. 1). The fecal samples were collected and examined for helminth eggs using formalin-ether sedimentation and the Kato-Katz thick smear techniques in June, 2004. Adult flukes were collected from 13 persons, all of whom evidenced relatively high numbers of eggs per gram of feces. They were treated with 10 mg/kg of praziquantel and purged with magnesium sulfate (Sigma, St. Louis, Missouri, USA). The whole diarrheic stools were collected, washed 2 times after filtration, and fixed in 2% formalin solution. Worms were collected and morphologically identified with a stereomicroscope.

Questionnaire regarding the eating habits of residents and clinical complaints

In order to characterize the relationships between parasite infections, eating habits, and clinical complaints, a paper questionnaire was distributed to inhabitants who had submitted stool samples. The contents of the questionnaires included questions concerning; 1) consumption of raw oysters, mullets, and gobies, 2) clinical complaints, including anorexia, abdominal pain, constipation, diarrhea, fatigue, indigestion, polyuria, or thirst, 3) suffering (or not) from diabetes mellitus, as adjudged via blood glucose examinations and history taking. In blood examinations, a glucose level in excess of 126 mg/dl on an empty stomach was regarded as a positive result, a level of 110 to 125 mg/dl was considered 'suspected', and a level of less than 110 was regarded as a negative result.

Oyster examination

Oysters were harvested from a tidal flat near Bokyong-ri in July 2004, and 50 oysters were selected for further evaluation, all of which were within a range of 4-6 cm in length and 20-40 g in weight. The animal part of oysters was digested for 30 min in 0.6% pepsin-HCl solution. After filtration through 1 mm diameter mesh, *G. seoi* metacercariae were collected and counted with a stereomicroscope.

Statistical analysis

The relationships between trematode infections, eating habits, physical complaints, and diabetes mellitus were analyzed using Spearman's correlation coefficient test. Sex-dependent prevalence was analyzed using the chi-square test. A value of $P < 0.05$ was considered to be significant.

RESULTS

Helminth infection status in residents

Out of 57 fecal samples, 37 (64.9%) revealed helminth eggs and the majority of them (33 samples, 57.9%) were positive for *G. seoi* eggs (Table 1). A total of 86,558 *G. seoi* adults were collected from 13 individ-

Table 1. Results of fecal examination of residents in Bokyong-ri, Aphae Island (Shinan-gun)

Items/Parasite species	No. of residents (%)
No. of residents examined	57
No. of helminth egg positive ^{a)}	37 (64.9)
<i>Gymnophalloides seoi</i>	33 (57.9)
<i>Pygidiopsis summa</i>	4 (7.0)
Other heterophyid flukes	13 (22.8)
<i>Clonorchis sinensis</i>	1 (1.7)
<i>Trichuris trichiura</i>	1 (1.7)

^{a)}A total of 11 persons had 2-3 different parasite eggs.

uals who showed high EPGs in the stool examination (Table 2). Three residents were infected with more than 15,000 worms, i.e., 25,015, 20,155, and 15,023, respectively. Other parasites were also recovered, i.e., *Heterophyes nocens*, *Heterophyopsis continua*, *Pygidiopsis summa*, and *Stictodora* sp. The rate of *G. seoi* infection in women (70.4%) was higher than that in men (46.7%). However, these sex-dependent differences were statistically not significant ($P > 0.05$). All of the helminth positive cases were more than 30 years old and the infection rate in the 50-69 year age group (overall helminth: 95.8%; *G. seoi*: 83.3%) was significantly higher than those in the other age groups ($P < 0.05$).

Results of questionnaire

Of the 20 inhabitants that completed the questionnaire, only one was not infected with any intestinal trematode. The others (19) were infected with either *G. seoi* (19), *P. summa* (1), other heterophyids (7), or *Clonorchis sinensis* (1). The number of persons who reported consuming raw oysters, mullets, and gobies were 17, 10, and 10, respectively (Table 3). The inhabitants complained of several symptoms on the questionnaire, including anorexia (6), abdominal pain (8), constipation (6), diarrhea (7), fatigue (2), indigestion (7), polyuria (5), and thirst (9). Out of 20 subjects examined, 4 suffered from diabetes mellitus, and 2 were assigned to the 'suspected' category. Via statistical analysis, *G. seoi* infection was closely associated with the consumption of raw oysters ($P = 0.017$). However, heterophyid infections and consumption

Table 2. Results of fecal examination and worm collection in 13 residents of Bokyeong-ri, Aphae Island (Shinan-gun)

Resident code	Age/Sex	Fecal examination		No. of worms collected				
		Kato-Katz ^{a)}	F-E ^{b)}	Gs ^{c)}	Hn ^{c)}	Hc ^{c)}	Ps ^{c)}	Ssp ^{c)}
1	48/F	Gs123	Gs	25,015	39	0	0	0
2	69/M	Gs43	Gs	20,155	24	0	3	0
3	68/M	Gs29	Gs	15,023	1	0	0	0
4	66/M	Gs39	Gs	6,524	0	0	0	0
5	65/F	Gs12	Gs	6,386	0	0	0	0
6	57/M	Gs2	Hd)	3,772	0	0	0	0
7	44/M	Gs10	Gs, H	3,036	160	3	0	10
8	61/F	Gs14	Gs	2,313	8	0	0	0
9	50/F	Gs32, H2	Gs, H	1,706	0	0	0	0
10	64/M	Gs29	Gs, H	1,309	43	0	0	0
11	62/F	Gs21	Gs	921	3	0	0	0
12	56/M	Gs16, H2, Ps2	Gs, H	377	0	0	0	0
13	51/F	Gs2, H2, Cs ^{d)} 1	Gs, Cs	21	0	0	0	0
Total				86,558	278	3	3	10

^{a)}Number represents the number of eggs detected on one Kato-Katz smear.

^{b)}F-E: formalin-ether sedimentation

^{c)}Gs: *Gymnophalloides seoi*, Hn: *Heterophyes nocens*, Hc: *Heterophyopsis continua*, Ps: *Pygidiopsis summa*, Ssp: *Stictodora* sp.

^{d)}H: eggs of heterophyids, Cs: eggs of *Clonorchis sinensis*

habits, physical complaints, and diabetes mellitus were not found to be significantly related.

Metacercarial infection in oysters

Among the 50 oysters collected from a nearby estuary in Bokyeong-ri, 47 (94%) were determined to be infected with *G. seoi* metacercariae. However, the metacercarial density per oyster was not so high (9.5 ± 8.9).

DISCUSSION

In the present study, Bokyeong-ri was identified as a new endemic focus of *G. seoi* infection. Among 57 residents examined, 33 (57.9%) appeared to be positive for *G. seoi* eggs. This rate is higher than that reported in Sinjang 3-ri, in which the *G. seoi* egg positive rate was 49% (Lee et al., 1994). The mean number of worms per infected individual was also higher than that recorded for Sinjang 3-ri (Lee et al., 1994).

The infection rate and intensity of *G. seoi* metacercariae in oysters differed significantly between the oysters collected in Bokyeong-ri (present study) and

previously in Sinjang 3-ri (Sohn et al., 1998). However, the mean number of metacercariae detected in the oysters from Bokyeong-ri (9.5) was much lower than that observed in the oysters from Sinjang 3-ri (1,339) (Sohn et al., 1998). The number of metacercariae infected in oysters was small but residents were heavily infected. This result may be attributed to the repeated and frequent consumption of raw oysters infected with the metacercariae (Chai et al., 2000).

Among 13 areas, in which metacercarial infections were detected in oysters, the highest metacercarial density was discovered in Chungdo (203.0). In other areas, metacercarial densities tended to be lower than 67.6 (Lee et al., 1996). In the present study, the metacercarial density in the oysters was lower than that observed in the oysters from Chungdo, but the infection rate and the number of worms recovered (or EPG) was far higher than was seen in Chungdo (Chai et al., 2001b). Therefore, metacercarial infections in oysters and human infections do not appear to evidence a consistent positive relationship. More detailed studies regarding the life span of *G. seoi* in humans might provide more detailed information regarding

Table 4. Summary of questionnaire from residents of Bokyong-ri, Aphae Island (Shinan-gun)

Resident code	Age/sex	Results of stool exam. ^{a)}	Consuming raw			Complaints ^{b)}	Hx of diabetes mellitus ^{c)}
			Oysters	Mullets	Gobies		
A	43/M	Gs, H	+	+	+	AP, D, F, P, T	+
B	65/M	Gs	+	-	-	AP, P, T	+
C	61/F	Gs	-	-	-	AP	+
D	64/M	Gs, H	+	+	+	D, T	+
E	68/M	Gs	+	+	+	A, P, T	±
F	70/M	Gs	+	-	-	A, C, I, P, T	±
G	56/M	Gs, H, Ps	+	+	+	-	-
H	51/F	Gs, H, Cs	+	+	+	D, I	-
I	68/M	Gs	+	+	+	T	-
J	66/F	Gs	+	+	-	A, I	-
K	58/M	Gs, H	+	+	+	AP, D, I	-
L	49/F	Gs, H	+	+	+	AP, C, F, P	-
M	47/F	Gs	+	+	+	A, AP, C, D, T	-
N	62/F	Gs	+	-	+	AP, C	-
O	65/F	Gs	+	-	-	A, AP, I	-
P	63/F	Gs, H	+	-	-	C, D	-
Q	75/M	Gs	+	-	-	D, I, T	-
R	85/F	Gs	+	-	-	-	-
S	72/F	Gs	-	-	-	A	-
T	76/M	Gs	-	-	-	C, I, T	-

^{a)}Gs: *G. seoi*, H: heterophyids, Ps: *P. summa*, Cs: *C. sinensis*

^{b)}A: anorexia, AP: abdominal pain, C: constipation, D: diarrhea, F: fatigue, I: indigestion, P: polyuria, T: thirst

^{c)}History of diabetes mellitus (+: positive, ±: suspected, -: negative)

the negative relationships between human infection rates and metacercarial infection in oysters.

The small intestine of *G. seoi*-infected mice showed villous atrophy and crypt hyperplasia in the duodenum and jejunum, with inflammatory reactions in the villous stroma and crypt, probably due to mechanical and chemical irritations by the worms (Chai et al., 2001a). Therefore, this fluke is considered to be enteropathogenic to its definitive hosts, most notably humans. From the questionnaire study, the major complaints of the individuals infected with *G. seoi* included thirst and abdominal pain (42.1%), followed by diarrhea (36.8%), anorexia (31.6%), indigestion (31.6%), constipation (26.3%), polyuria (26.3%), and fatigue (10.5%). *G. seoi* infections were significantly associated with the consumption of raw oysters, as the oyster is the only known source of human *G. seoi* infection.

One of the interesting things determined in this

study was is that 6 (21.1%) out of 19 individuals infected with *G. seoi* were suffering from or suspected of diabetes mellitus. The majority of them complained of thirst (5) or polyuria (4), both of which have been implicated as clinical signs of diabetes mellitus. Diabetes mellitus and *G. seoi* infection are suspected to be related in some way, as 2 cases of diabetes mellitus were accompanied by *G. seoi* infection (Lee et al., 1995b; Chai and Lee, 2002). The first such case in a human subject was diagnosed as acute pancreatitis (Lee et al., 1993). Therefore, caution should be exercised in such cases, as *G. seoi* infection might eventually damage the pancreatic duct and nearby tissues. Further studies are required in order to elucidate this issue.

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