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A nationwide survey of naturally produced oysters for infection with *Gymnophalloides seoi* metacercariae

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Abstract: A nationwide survey was performed to know the geographical distribution of *Gymnophalloides seoi* (Digenea: Gymnophallidae) metacercariae in Korea, by examining the infection status of locally produced oysters, *Crassostrea gigas*. A total of 24 coastal areas (myons) of 14 guns (= counties) in Kyonggi-do, Chollabuk-do, Chollanam-do, Kyongsangnam-do, Kyongsangbuk-do, or Kangwon-do, where natural oysters are produced but *G. seoi* has never been reported, and 13 areas (myons) of Shinan-gun, Chollanam-do, nearby the known endemic area, were surveyed. Oysters from non-endemic areas were free from *G. seoi* infection, except Byonsan-myon of Buan-gun, Chollabuk-do, where one of 50 oysters examined was infected with 15 metacercariae of *G. seoi*. In Shinan-gun, oysters from 10 areas including Aphae-myon (= township) and Anjwa-myon were infected with the metacercariae, with the infection rate ranging from 1.7% to 100% by areas. The intensity of infection was the highest in Aphae-myon, 785.9 metacercariae per oyster. The results indicate that high prevalence of *G. seoi* is confined to Shinan-gun, but low grade prevalence is also present in adjacent areas such as Buan-gun, Chollabuk-do.

Key words: *Gymnophalloides seoi*, oyster (*Crassostrea gigas*), metacercaria, epidemiological survey, Shinan-gun, Buan-gun

INTRODUCTION

Gymnophalloides seoi Lee, Chai and Hong, 1993 (Digenea: Gymnophallidae) is a peculiar trematode infecting the gastrointestinal tract of humans in Korea. Human infection with this fluke is contracted by eating raw oysters, *Crassostrea gigas* (Lee *et al.*, 1995a). A highly endemic area of human *G. seoi* infection was discovered on a southwestern coastal island of Shinan-gun, Chollanam-do (Lee *et al.*, 1994). However, distribution of *G. seoi* in other areas has not yet been studied.

Recently, three human cases of *G. seoi* infection were found among the inhabitants of a coastal area of Muan-gun, Chollanam-do, 25 km northwards from Shinan-gun (unpublished data). Therefore, *G. seoi* infection might be present in other localities. So as to understand geographical distribution of this fluke we performed a nationwide survey of naturally

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produced oysters for infection with *G. seoi* metacercariae.

MATERIALS AND METHODS

1. Areas surveyed

A total of 37 areas (myons) along the western, southern, and eastern coasts of Korea (Figs. 1 & 2) were surveyed.

1) Areas where *G. seoi* has never been reported

A total of 24 myons (area code A-X in Fig. 2) in various localities were selected for this study.

Kyonggi-do: Kangwha-gun, Kanghwa-up (A)

Chollabuk-do: Buan-gun Dongjin-myon (B), Buan-gun Byonsan-myon (C)

Chollanam-do: Yonggwang-gun Yomsan-myon (D), Muan-gun Haejae-myon (E) Hampyong-gun Hampyong-up (F), Muan-gun Chongye-myon (G) Hampyong-gun Sonbul-myon (H), Haenam-gun Hwangsan-myon (I) Chindo-gun Chindo-up (J), Chindo-gun Uishin-myon (K), Chindo-gun Inchun-myon (L), Wando-gun Nowha-up (M), Wando-gun Wando-up (N)

Kyongsangnam-do: Kosong-gun Hai-myon (O), Kosong-gun Koong-up (P) Koje-gun Ilun-myon (Q), Koje-gun Jangsungpo (R) Koje-gun Dongbu-myon (S), Yangsan-gun Kijang-up (T)

Kyongsangbuk-do: Kyongju-gun Kampo-up (U).

Kangwon-do: Yangyang-gun Ssonyang-myon (V), Sokcho-shi Yongumchong (W), Myongju-gun Kandong-myon (X)

2) Shinan-gun, Chollanam-do (nearby the known endemic area)

Oysters were collected from 13 myons (area code in Fig. 2) nearby the reported endemic area (Lee *et al.*, 1995a); Aphae-myon (1), Anjwa-myon (2), Amtae-myon (3), Chungdo-myon (4), Jido-up (5), Palgum-myon (6), Jangsan-myon (7), Haeui-myon (8), Docho-myon (9), Shineui-myon (10), Bigum-myon (11), Jaeun-myon (12), and Amja-myon (13).

2. Examination of oysters and identification of the metacercariae

The oysters collected were transported to the labaratory, and weighed after removal of their shell. The soft body of oysters were digested with artificial gastric juice for 3-4 min. After washing three times with physiological saline, the presence of *G. seoi* metacercariae was investigated under stereomicroscopy. If metacercariae were present, they were identified under light microscopy. The number of metacercariae per oyster was counted. Some of the metacercariae were experimentally infected to C3H mice, and adult worms were recovered 5 days later.

RESULTS

1. Infection status of oysters from non-endemic areas

None of the oysters collected from 23 out of 24 myons (Fig. 1) was infected with the metacercariae of *G. seoi* (Table 1). Only one area, Buan-gun Byonsan-myon, Chollanam-do (Fig. 1), revealed the infected oyster, although the infection rate was very low, only one oyster among 50 examined (Table 1). A total of 15 metacercariae were isolated from the infected oyster.

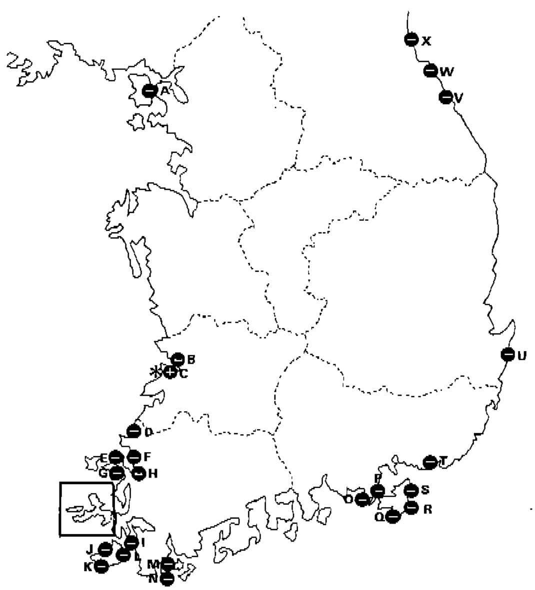

Fig. 1. Map showing the areas where naturally produced oysters were collected and examined for *G. seoi* metacercariae. Boxed area shows Shinan-gun, the known endemic area, which is shown in detail in Fig. 2 (⊕: positive for *G. seoi* infection, ⊖: negative for *G. seoi* infection).

Table 1. Results of oyster examination for *G. seoi* metacercariae in non-endemic areas

Province	Area	Area code[a]	No. of oysters examined	Weight of oysters in gram (av.)	No. of oysters infected
Kyonggi-do	Kangwha-gun Kanghwa-up	A	15	16.1-90.3 (45.0)	0
Chollabuk-do	Buan-gun Dongjin-myon	B	50	11.0-37.0 (20.0)	0
	Buan-gun Byonsan-myon	C	50	10.0-42.0 (21.0)	1[b]
Chollanam-do	Yonggwang-gun Yomsan-myon	D	40	15.0-26.0 (23.0)	0
	Muan-gun Haejae-myon	E	30	17.0-31.0 (27.0)	0
	Hampyong-gun Hampyong-up	F	40	23.0-36.0 (32.0)	0
	Muan-gun Chongye-myon	G	30	20.0-35.0 (31.0)	0
	Hampyong-gun Sonbul-myon	H	30	20.0-35.0 (31.0)	0
	Haenam-gun Hwangsan-myon	I	31	—	0
	Chindo-gun Chindo-up	J	24	6.0-17.5 (9.5)	0
	Chindo-gun Uishin-myon	K	22	10.0-25.5 (16.6)	0
	Chindo-gun Inchun-myon	L	26	8.0-34.0(16.5)	0
	Wando-gun Nowha-up	M	38	—	0
	Wando-gun Wando-up	N	50	—	0
Kyongsangnam-do	Kosong-gun Hai-myon	O	70	—	0
	Kosong-gun Kosong-up	P	50	—	0
	Koje-gun Ilun-myon	Q	37	7.0-36.0 (14.0)	0
	Koje-gun Jangsungpo-shi	R	25	86.0-92.0 (120.0)	0
	Koje-gun Dongbu-myon	S	26	33.0-92.0 (55.0)	0
	Yangsan-gun Kijang-up	T	12	117.0-238.0 (191.0)	0
Kyongsangbuk-do	Kyongju-gun Kampo-up	U	17	135.0-376.0 (246.0)	0
Kangwon-do	Yangyang-gun Ssonyang-myon	V	2	—	0
	Sokcho-shi Yongumchong	W	35	20.0-81.9 (48.4)	0
	Myongju-gun Kandong-myon	X	23	130.0-410.0 (236.1)	0

[a]area code in Fig. 1.
[b]infected with 15 metacercariae

2. Infection status of oysters from Shinan-gun, the known endemic area

Ten of 13 surveyed areas (myons) of Shinan-gun were found to have oysters infected with *G. seoi* metacercariae (Fig. 2, Table 2). The positive rate was 100% in Aphae-myon and Anjwa-myon, followed by Amtae-myon (67.5%), Chungdo-myon (58.3%), Jido-up (54.5%), Palgum-myon (27.0%), Jangsan-myon (14.1%), Haeui-myon (8.1%), Shineui-myon(1.7%), and Docho-myon (1.3%).

The intensity of infection was the highest in Aphae-myon, 785.9 metacercariae per oyster (Table 2). The next high intensity was found in oysters collected from Chungdo-myon, 203.0 per oyster. In Anjwa-myon and Amtae-myon the average number of metacercariae was 67.6 and 62.9, respectively. In other 6 myons the numbers were lower than 20 per oyster (Table 2). Three remaining myons were negative for the metacercariae.

There is a strong tendency that the infection is distributed mainly in the central areas of Shinan-gun (= coastal islands). Remote islands tend to show low grade infection or negative results (Fig. 2, Table 2).

3. Morphology of the metacercariae and adults

All of the metacercariae collected from the oysters had a large oral sucker, prominent ventral pit, and other characterisitc features consistent with the descriptions of *G. seoi* metacercariae (Lee *et al.*, 1995a). The adult flukes harvested from C3H mice 5 days after infection were also characterized by the presence of a large oral sucker, muscular ventral pit and genital pore located close to the ventral sucker, vitellaria, and uterine tubule with characteristic eggs. These features were consistent with the descriptions of *G. seoi* adult flukes (Lee *et al.*, 1993).

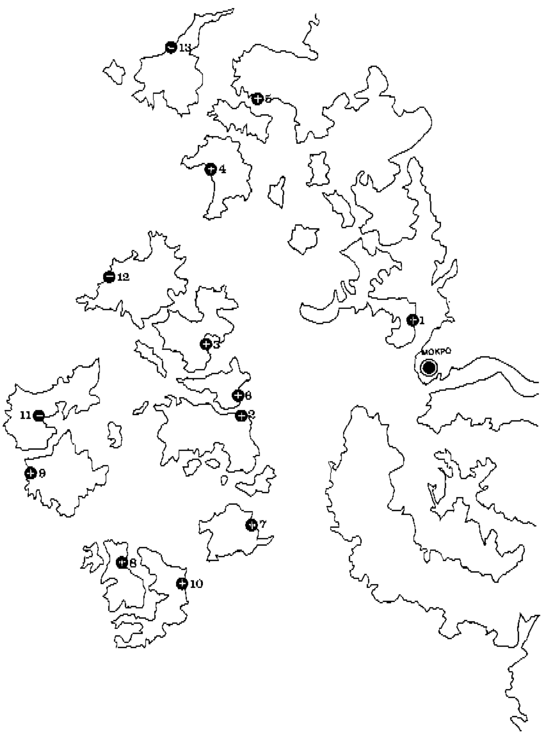

Fig. 2. Geographical distribution of *G. seoi* infection in oysters from Shinan-gun (⊕: positive for *G. seoi* infection, ⊖: negative for *G. seoi* infection).

DISCUSSION

Until present only Shinan-gun, Chollanam-do has been known to be the endemic area of *G. seoi* infection (Lee *et al.*, 1994). However, one patient living in Inchon City (Lee *et al.*, 1995b) and three residing in Muan-gun (unpublished observation) were verified to be infected with *G. seoi*, which strongly suggested that this fluke infection might be quite widely distributed along the coastal areas of Korea. Interestingly, however, the present study revealed that *G. seoi* metacercariae are not so widely distributed beyond the boundary of Shinan-gun. Only one area of Buan-gun, north to Shinan-gun, revealed low density infection with *G. seoi* metacercariae in one of 50 oysters examined.

Based on the present results of oyster examination and occurrence of human infections so far, it could be concluded that *G. seoi* infection is highly prevalent on the central areas (= mainly coastal islands) of Shinan-gun, and low grade endemicity occurs in adjacent localities such as Muan-gun and Buan-gun.

Table 2. The infection status of oysters from Shinan-gun, Chollanam-do, with *G. seoi* metacercariae

Area	Area code[a]	No. of oysters examined	No. of oysters infected (%)	Total No. of metacercariae collected	Mean No. of metacercariae per oyster
Aphae-myon	1	20	20 (100.0)	1,572	785.9
Anjwa-myon	2	16	16 (100.0)	1,082	67.6
Amtae-myon	3	40	27 (67.5)	1,698	62.9
Chungdo-myon	4	36	21 (58.3)	4,262	203.0
Jido-up	5	44	24 (54.5)	342	14.3
Palgum-myon	6	37	10 (27.0)	44	4.4
Jangsan-myon	7	78	11 (14.1	35	0.5
Haeui-myon	8	62	5 (8.1)	83	16.6
Docho-myon	9	238	3 (1.3)	3	1.0
Shineui-myon	10	59	1 (1.7)	20	20.0
Bigum-myo	11	28	0 (0.0)	0	0.0
Jaeun-myon	12	15	0 (0.0)	0	0.0
Amja-myon	13	11	0 (0.0)	0	0.0

[a]area code in Fig. 2.

One area of Yonggwang-gun, located between Muan-gun and Buan-gun, was negative for the metacercariae in oysters in this study, although, further study is needed. Kangwha-myon is also suspected as a low grade endemic area, since one patient living in Inchon consumed raw oysters collected from Kangwha-myon (Lee *et al.*, 1995b). In the present study, however, the oysters from Kangwha-myon were free from *G. seoi* metacercariae. Repeated surveys on oysters produced from Kangwha-myon are also needed.

It is very difficult to explain properly the specificity in geographical distribution of this fluke infection. If migrating birds were playing the major role for natural final hosts, this phenomenon is more enigmatic. The distribution of this infection should have been greatly wider than it is seen now. Two kinds of speculations seem possible. First, there is a possibility that the major final host of *G. seoi* might be humans, not migratory birds, which could explain the limited geographical distribution of this fluke infection. Second, the snail intermediate host shedding the cercariae, the species of which is not known yet, could be a peculiar one which might be distributed around the endemic area only.

Various kinds of marine bivalves were reported to carry the metacercarial stage of gymnophallid trematodes in Korea and other countries, although *G. seoi* is the only species infecting humans. *Macoma balthica*, a clam from the North Sea, for example, was reported to harbour larval stages of three gymnophallid species, *Gymnophallus gibberosus*, *Lacunovermis macomae*, and *Parvatrema affinis* (Loss-Frank, 1971). In Korea, metacercariae of *Parvatrema timondavidi* were found from *Tapes philippinarum*, one of the most common marine clams (Yu *et al.*, 1993). In addition, the razor clams collected from Yŏnggwang-gun, Chollanam-do, were found infected with the metacercariae of *Meiogymnophallus* sp. (unpublished observation). The metacercariae of *G. seoi* were found only from oysters. However, any possibility for other kinds of marine bivalves to play the role for a source of human *G. seoi* infection should be verified.

A positive correlation was reported between the infection rate of *Parvatrema affinis* and the size of *Macoma balthica* in the Netherlands (Hulscher, 1973). Such correlation was also observed in *M. balthica* infected with *L. macomae* (Pekkarinen, 1984). In this study, the relationship between the shell size of oysters and intensity of metacercarial infection was not studied extensively. However, when it was observed on the oysters collected from Aphae-myon, no positive correlation was recognized between the density of infection and the length of oysters (data not shown).

Seasonal variation of metacercarial density in marine bivalves was reported in a few species of gymnophallid trematode. Pekkarinen (1983) found a slight increase in the number of *L. macomae* metacercariae in the extrapallial space of *M. balthica* in summer season. Erasmus (1972) stated that seasonal variation is not so evident with metacercarial stages of trematodes in general, and it is correlated with longevity of metacercariae in the host. But Erasmus (1972) acknowledged that the incidence will rise in summer after exposure to a second cercarial population. In our study, the oysters from Aphae-myon did not show any significant seasonal variation in the metacercarial density of *G. seoi* (data not shown).

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=초록=

전국 여러 지역산 굴의 참굴큰입흡충 피낭유충 감염 상황

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참굴큰입흡충(*Gymnophalloides seoi*)의 국내 분포상황을 알기 위하여 동해안, 남해안 및 서해안 등 여러 해안 지역에서 참굴을 채집한 후 참굴큰입흡충 피낭유충 감염상황을 조사하였다. 전라남도 신안군의 13개 면과 신안군 외 14개 군(경기도, 전라북도, 전라남도, 경상남도, 경상북도, 강원도)의 24개 면을 대상지역으로 하였고, 굴의 피낭유충 감염 여부 및 감염량을 조사하였다. 신안군 13개 면 중 압해면 및 안좌면을 포함한 10개 면의 굴에서 피낭유충 감염이 확인되었고 지역별 감염률은 1.7-100%이었다. 그 중 압해면산 굴이 개체당 평균 785.9개의 피낭유충을 보유하고 있어 감염량이 가장 높았다. 신안군 이외의 24개 면 중에서는 전라북도 부안군 변산면만 제외하고 모두 음성의 결과를 나타내었으며, 부안군 변산면에서는 굴 50개 중 1개에서 피낭유충 15마리가 검출되었다. 이번 연구 결과로 보아 참굴큰입흡충의 농후한 유행은 전라남도 신안군에 국한된 것으로 보이며, 전라북도 부안군 등 인근 지역에 약간의 유행이 있는 것으로 판단되었다.

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