

## Surveys on *Gymnophalloides seoi* Infection in the Gogunsan Gundo (Islands) of Korea

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### ABSTRACT

The present study aimed to elucidate whether an intestinal fluke, *Gymnophalloides* sp. exist in Gogunsan-gundo located at western part of Korean peninsula; about 100 km apart from Gunsan city, Jeollabuk-do, Korea. The survey was carried out two times each in 1997 and 1998, examining stool samples by means of formalin-ether concentration method. Results are summarized as follows : of 411 fecal samples examined, *Gymnophalloides* sp. positives were 19 (8.9%); 9.5% in Munyeodo, 1% in Seonyudo, but none in Jangjado. The positive cases did not show any specific clinical complaints. Prevalence rate of *Gymnophalloides seoi* metacercariae from oysters, *Crassostrea gigas* were 80.8%

**Key words:** *Gymnophalloides seoi*, infection rate, *Crassostrea gigas*, Gogunsan islands

### INTRODUCTION

Human infection of *Gymnophalloides seoi* (Digenea, Gymnophallidae) was first reported from Shinan-gun, Chollanam-do, Korea, as a new

human intestinal trematode (Lee *et al.*, 1993). Subsequently, additional reports followed from other islands along the south-western coast of Korea (Lee *et al.*, 1994; Lee *et al.*, 1996). Oysters, *Crassostrea gigas*, one of the popularly collected oyster species along the west and south coast, were proven as the second intermediate host (Lee *et al.*, 1996). This oyster was strongly suspected as the source of infection since it has been a favored food by most of the villagers in the endemic area (Lee *et al.*, 1994). As an animal model for *G. seoi* metacercariae, hamsters, mice, gerbils and cats were recognized as experimentally-infectable hosts (Sohn *et al.*, 1995, Ryang *et al.*, 1996).

In this study, surveys were conducted in two ways; human infection and metacercarial incidences of *Gymnophalloides* sp. in the islands among Gogunsan-gundo which is located in yellow sea about 40km apart from the west coast of Korean peninsula; E. 126 30', W. 35 50' 1km from Seonyudo (Fig. 1). At first, stool examination of the inhabitants was conducted in voluntary base. The examinees in each island were roughly 30% of the inhabitants, and oyster examination was followed. Adult worms and metacercariae were examined for the species identification.

### MATERIALS AND METHODS

#### 1. Human stool

The stool samples were collected from

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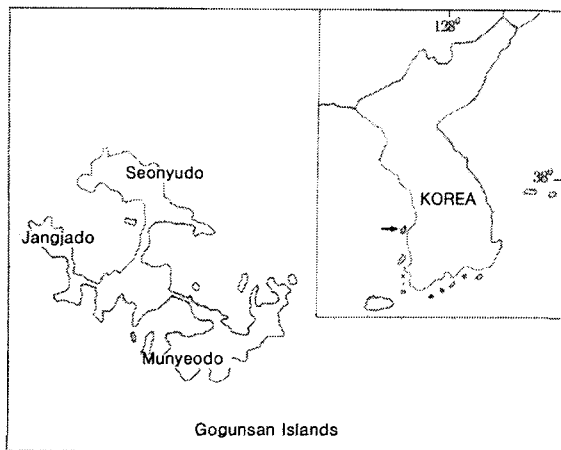


Fig. 1. A map showing the areas where naturally produced oysters were collected and examined for *G. seoi* metacercariae.

Gogunsan Islands, comprising the smaller islands of Seonyudo, Munyeodo and Jangjado (Fig. 1). Stools of the inhabitants were collected by a local health center and examined at Wonkwang University by formalin-ether concentration method.

Egg-positive cases of intestinal flukes were treated with praziquantel, 15 mg/kg, single dose, and purged with magnesium sulfate. Minute flukes, if any, were collected from the stool under a stereomicroscope.

For extensive morphological examination, the adult worms were washed in tap water, fixed in 10% formalin for two days, stained with Semicon's aceto-carmine and mounted with Canada balsam.

Some specimens were paraffined, sectioned and stained with hematoxylin and eosin for more detailed internal structures.

## 2. Intermediate host

The oysters were easily collected from tideland at ebb tide. Oysters, *Crassostrea gigas*, were collected from Munyeodo. After opening the shells with a dull knife, the mantle surfaces of the oysters were carefully examined under a stereomicroscope to determine whether or not they contained gymnophallid metacercariae, which are usually unencysted. In order to isolate the metacercariae, the oysters were treated with an artificial digestive fluid mainly composed of pepsin and hydrochloric acid, for 12 hr at 37°C. Metacercariae were collected under a stereomicroscope, washed in saline, then fixed in 10% formalin. For species identification, morphological characteristics of metacercariae were examined. The remaining metacercariae were fed to seven BALB/c mice, and were sacrificed at seven days post-infection. The adult worms from the small intestine were collected, fixed with 70% alcohol under cover slip pressure, and observed after stained with carmine for 10 min. Some flukes were fixed in 2.5% glutaraldehyde at 4°C and observed with a scanning electron microscope.

## RESULTS

Intestinal parasites in 411 stool samples

Table 1. Infection status\* of *Gymnophalloides seoi* among the inhabitants of Gogunsan-gundo (islands), Jeollabuk-do, Korea in April 1997 and August 1998.

Collection sites (islands)	In 1997			In 1998		
	Male	Female	Total	Male	Female	Total
Munyeodo	63(5)**	63(7)	126(12)	27(1)	21(1)	51(3)
Seonyudo	51(0)	49(1)	100(1)	57(3)	36(0)	93(3)
Jangjado	13(0)	16(0)	29(0)	4(0)	8(0)	12(0)
Total	127(5)	128(8)	255(13)	91(4)	65(2)	156(6)

\*Other intestinal parasites were: *Heterophyes* sp. 6, *Strongyloides stercoralis* 1, *Trichocephalus trichiurus* 1, *Entamoeba coli* 1.

\*\*Parenthesis means positive cases of *Gymnophalloides seoi* infection

collected in April 1997 and in August 1998 included 19 intestinal flukes containing three other parasites, *Strongyloides stercoralis*, *Trichocephalus trichiurus*, and *Entamoeba coli*. Intestinal flukes from the stained specimens were identified as *Gymnophalloides seoi* 13 individuals, 5.1% and *Heterophyes heterophyes* 6 individuals, 2.7%. The incidences in 1997 varied: 9.5% 12 out of 126 at Munyeodo, 1.0% 1 out of 100 at Seonyudo and none at Jangjado (Table 1). The prevalence showed a similar trend for the first and second survey in 1997 and in 1998.

The positive cases were in above 30 up to 80 of age. They were treated with praziquantel in 1997. Among the negative samples in 1998, eight positive cases from 1997 were included. They were positive but treated with praziquantel. Other positive cases in 1997 could not be followed because of poor cooperation in the stool collection (Table 2).

Oysters, *Crassostrea gigas*, were collected mainly from Munyeodo in 1998, and 80.8% were found *G. seoi* metacercariae positive out of 52 examined (Table 3). The size of the oysters varied from 4.0-9.9 cm in length and the mean number of *G. seoi* metacercariae was 3.8 per oyster. The size of metacercariae was 275 x 165 µm, and the

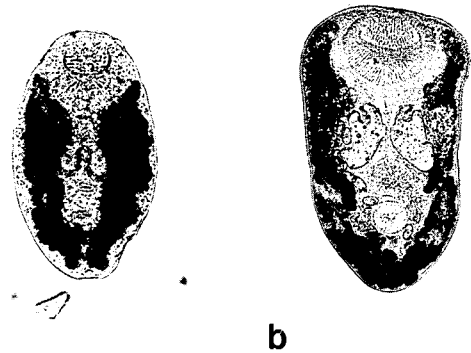


Fig. 2. a. A living metacercaria of *Gymnophalloides seoi* unstained. b. A living adult worm of *G. seoi* collected from a mouse. Scale bar indicates 50 µm.

location of testes was on the lower part of both sides of the body. The ventral pit and vitellaria showed the characteristic criteria as *G. seoi*.

All of the metacercariae collected from the oysters had a large oral sucker, ventral sucker and prominent ventral pit (Fig. 2a). The anterior end of a living adult worm was round and the posterior-end was slightly narrowed (Fig. 2b). The metacercariae of *G. seoi* under scanning electron microscope showed an oral sucker with fine spines and ventral pit located posterior to the midline, and the ventral sucker was located at about 1/4 body length from the posterior end (Fig. 3). Testis, ovary and vitellaria in the posterior part were easily observed by carmine stain (Fig. 4).

Table 2. Infection status of *Gymnophalloides seoi* by age group of the inhabitants living in the 3 islands in 1997 and 1998.

Age group	In 1997 / in 1998	
	No. examined	No. infected (%)
-10	12 / 5	- / -
11-20	29 / 6	- / -
21-30	13 / 9	- / -
31-40	32 / 32	3(9.4) / 4(12.0)
41-50	56 / 42	5(8.9) / 1(2.4)
51-60	48 / 33	3(6.3) / -
61-70	44 / 22	1(2.3) / 1(4.5)
71-80	13 / 7	1(7.7) / -
81-	8 / -	- / -
Total	255 / 156	13(5.1) / 6(3.8)

Table 3. The infection rate of *Gymnophalloides seoi* metacercariae by shell length of oysters from Munyeodo, Jeollabuk-do, in 1998.

Size in length(cm)	No. examined	No. infected(%)	No. <i>G. seoi</i> metacercariae
4 - 4.9	4	3(75.0)	14
5 - 5.9	23	19(82.6)	94
6 - 6.9	6	5(83.3)	24
7 - 7.9	13	10(76.9)	50
8 - 8.9	5	4(80.0)	12
9 - 9.9	1	1(100)	3
Total	52	42(80.8)	197

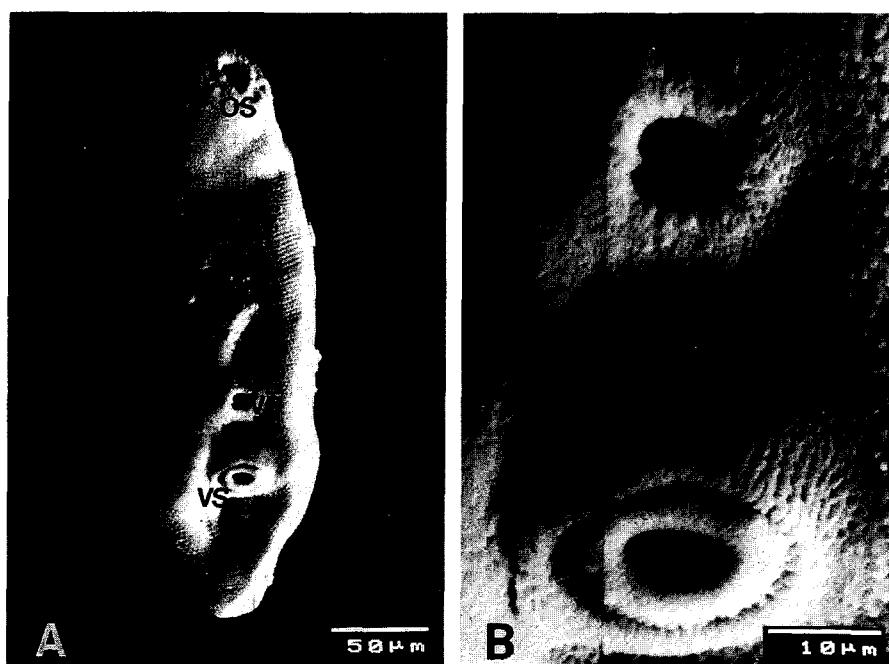


Fig. 3. Scanning electron microscopic features of *Gymnophalloides seoi* metacercariae collected from an oyster. A. Ventral view. B. Enlarged viewing of a ventral pore and a ventral sucker. OS, oral sucker; VP, ventral pit; VS, ventral sucker.

The fecal samples were collected by a routine method, without clinical prejudices. In interviews, the positive cases of intestinal trematode did not complain of any specific clinical sign that might hamper their daily activities.

#### DISCUSSION

In this study, the gymnophallid metacercariae in oysters, *C. gigas*, collected near Gunsan city were identified as those of *G. seoi*. Oysters collected from the eastern and northwestern coasts of the Korean peninsula are known to be infection-free from *G. seoi* (Lee *et al.*, 1993). Only 50 oysters from Byeonsan-myeon, on the mid-section of the west coast were positive with metacercariae of *G. seoi* (Lee *et al.*, 1996). The islands in the present survey are located about 30 km north of Byeonsan-myeon. The purpose of this survey was simply investigate whether *G. seoi* exist in the area. Through this survey at Gogunsan Islands, in 1997 and in 1998, it is known that relatively high infection of an intestinal fluke *G. seoi* was recognized. Further studies will be required to

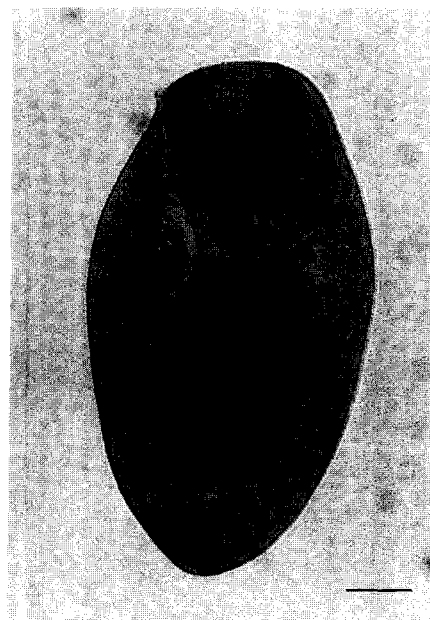


Fig. 4. Adult worm of *Gymnophalloides seoi* with carmine stain. EP, excretory pore; OS, oral sucker; T, testis; V, vitellaria; VP, ventral pit; VS, ventral sucker. Scale bar indicates 50 μm.

determine the clinical and epidemiological significance of *G. seoi* infection. In addition, there are several aspects to be clarified. Even though the three islands are close together and connected by a bridge, no *Gymnophalloides* positive cases were found at Jangjado, during the two surveys. It will be required to perform a detailed survey in the future. Seasonal fluctuation of the metacercarial density in oysters needs to be studied in connection with the breeding season of oysters and other mollusks (Lee *et al.*, 1996). A study on the migratory birds could be important to confirm whether this intestinal fluke is indigenous or exogenous, as well as to determine intermediate hosts other than *C. gigas*. From an epidemiological viewpoint, it was noted that the age group under 30 were negative among those examined; 54 of 256 in 1997 and 20 of 156 in 1998 (Table 2).

One morphological point to be rechecked is the ventral sucker and vitellaria. In some specimens vitellaria overlapped the ventral sucker (Fig. 3), which needs to be clarified whether this phenomenon is caused by a simple technical error or a step in the maturing process in the host.

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