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Three Confirmed and Five Suspected Human Cases of *Gnathostoma doloresi* Infection Found in Miyazaki Prefecture, Kyushu

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Abstract

Eight cases of gnathostomiasis patients were found in the central part of Miyazaki Prefecture since 1985. None of them have past history of eating snakeheads nor loaches. In one case, a whole parasite was, as two pieces, dissected out directly from formalin-fixed, biopsied skin. In two cases, an existence of the parasite was at first noted in the slide preparate for histopathology, and subsequently a part of the parasite was dissected out from each paraffin-embedded block of the biopsied skin. Fortunately the head bulb of each parasite remained intact and they were morphologically identified as the third stage larvae of *Gnathostoma doloresi*. These results indicate that *G. doloresi* is clinically important as the causative agent of zoonosis.

Key words: *Gnathostoma doloresi*, human cases, creeping eruption, larva migrans, Miyazaki Prefecture

Introduction

Human gnathostomiasis is not a rare parasitic disease in the Southeast Asia (Daengsvang, 1981), and more than 1,000 cases were reported in Japan. The majority of these previously reported cases were believed to be caused by *Gnathostoma spinigerum*, of which the third stage larvae were ingested by eating raw slices ("Sashimi") of snakehead, *Channa argus*. In addition, as an imported parasitic disease, recently gnathostomiasis cases caused by *G. hispidum* have been reported in patients having common past history of eating raw ("Odori-gui") loaches, *Misgurnus anguillicaudatus*, imported from Taiwan, the mainland China, or Korea (Tsushima *et al.*, 1980; Nishimura *et al.*, 1981; Akahane *et al.*, 1982; Morita *et al.*, 1984; Araki, 1986). In Japan, two other *Gnathostoma* species are, even nowadays, known to be distributed naturally in wild animals at a high frequency; *G. doloresi* in wild boars, *Sus scrofa leucomystax* (Ashizawa *et al.*, 1979; Sakaguchi *et al.*, 1985) and *G. nipponicum* in Japanese weasels, *Mustela sibirica itatsi* (Gyouten and Nishida, 1978; Ashizawa *et al.*, 1978; Koga and Ishii, 1981a). Therefore, the possibility of their infection in human has been suggested. Recently we have encountered the first confirmed human case of infection with *G. doloresi* (Nawa *et al.*, 1988). Subsequently, we have made a retrospective survay for gnathostomiasis cases in Miyazaki Prefecture and found additional two confirmed and five suspected cases of *G. doloresi* infection. In this paper we describe these cases including the first confirmed case.

Case Report

Eight cases of clinically diagnosed gnathostomiasis were found in Miyazaki Prefecture since 1985. Geographical distribution of the patients is shown in Fig. 1. All patients are living in the central part of Miyazaki Prefecture.

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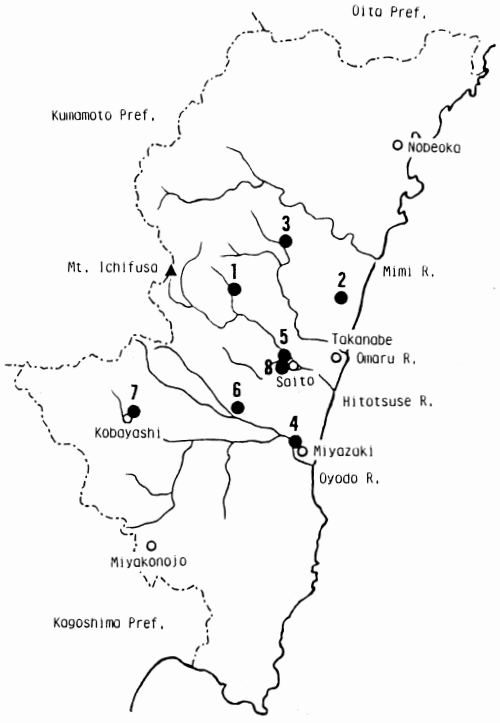


Fig. 1. Geographical distribution of the patients found in Miyazaki Prefecture.

Clinical data related to parasitic diseases are summarized in Table 1. The patients were six males and two females and the mean age was 48.4 y.o. They presented to the Department of Dermatology, Miyazaki Medical College with the chief complaint of creeping eruption (5/8 cases) or mobile, localized swelling with redness (so-called "Quincke's edema" type: 4/8 cases) appeared mainly on the trunk skin. The patient No. 2 showed both type of symptoms. Time of the onset of the disease of these patients was mostly spring to early summer. All patients, except No. 3, showed moderate eosinophilia with normal or slightly elevated peripheral blood leukocyte count. The patient No. 3 showed extremely high eosinophil count and serum IgE level. He has a past history of gnathostomiasis more than 40 years ago when he was in the mainland China during World War II. Total IgE level in serum was measured in 7

Table 1 Clinical data of the patients

No.	Age	Sex	Symptoms*	Onset (date)	Total WBC (/mm³)	Eosino (%)	IgE (IU/ml)	Immunodiagnosis D.D.†	Immunodiagnosis S.T.‡	Parasite in biopsy	Raw materials ate
1	51	M	C	17/5/1985	11,600	14.0	4,800	−	ND	−	freshwater fish§, beef
2	40	M	C+Q	20/4/1985	9,800	12.0	<500	+	ND	−	beef, snake
3	70	M	C	15/7/1985	12,200	67.0	16,000	+	ND	+	freshwater fish, pork liver
4	38	F	C	13/4/1986	4,700	12.0	ND	−	ND	ND	freshwater fish§
5	35	M	Q	20/4/1986	6,000	21.2	681.9	−	ND	+	freshwater fish§, wild boar
6	58	M	Q	28/3/1987	7,800	14.2	83.4	ND	ND	−	freshwater fish§
7	34	F	Q	1/1988	5,000	12.0	485.5	−	−	−	freshwater fish§, deer
8	61	M	C	20/5/1988	7,200	6.0	>4,000	−	+	+	freshwater fish§

*: creeping eruption (C); so-called Quincke's edema (Q).
†: double diffusion by Ouchterlony's method (D.D.).
No. 1, 2, 3, and 5 were done by Dr. M. Tsuji, Hiroshima University, No. 4 by Dr. H. Akahane, Fukuoka University, and No. 7 and 8 were done in our laboratory using antigen prepared by Dr. Y. Horii, Nagasaki University.
‡: skin test (S.T.).
Test antigen was supplied by Dr. T. Mimori, Kumamoto University.
§: brook trout, *Oncorhynchus masou*
ND: not done

cases. Although serum IgE level was variable among these cases, 5 cases (No. 1, 3, 5, 7, and 8) showed elevated serum IgE level. In addition to general examinations, immunodiagnosis for gnathostomiasis was performed in some cases. As shown in Table 1, only 2 out of 7 cases were positive by an Ouchterlony's double diffusion test and 1 out of 2 cases was positive by skin test.

As to the source of infection, all patients stated that none of them ever had eaten snakeheads nor loaches, which were known as the causative agent of infection of *G. spinigerum* and *G. hispidum*, respectively. Instead, 6 out of 8 patients (No. 1, 4, 5, 6, 7, and 8) have common past history of eating raw slices (locally called "Segoshi") of brook trout (common Japanese name "Yamame", *Oncorhynchus masou*) several months before the onset of the disease. The patient No. 3 had other kinds of freshwater fishes in the same manner. The patient No. 2 has previous history of eating various kinds of raw materials such as snake and beef. In addition to freshwater fish, some of the patients have previous history of eating raw meat of different kind of animals (No. 1, 5, 7) or pork liver (No. 3).

Description of the parasites

Among these cases, a whole or a head part of parasite was directly demonstrated in the biopsied skin of the patients No. 3, 5, and 8. In the case of patient No. 8, which is the first record of definite human case of *G. doloresi* infection (Nawa *et al.*, 1988), a whole length of larva was, as two pieces, dissected out from formalin-fixed biopsy specimen before processed for pathology. In other two cases (No. 3 and 5), a cross section of the parasite was at first noted by the pathologist in the slide preparates without identification of parasite species. Therefore, remaining paraffin-embedded tissue blocks containing parasite were dewaxed with three changes of xylene, rehydrated with the descending series of ethanol, and then finally immersed in 10% buffered formalin. Fortunately in both cases the head part of parasite was dissected out from each tissue block.

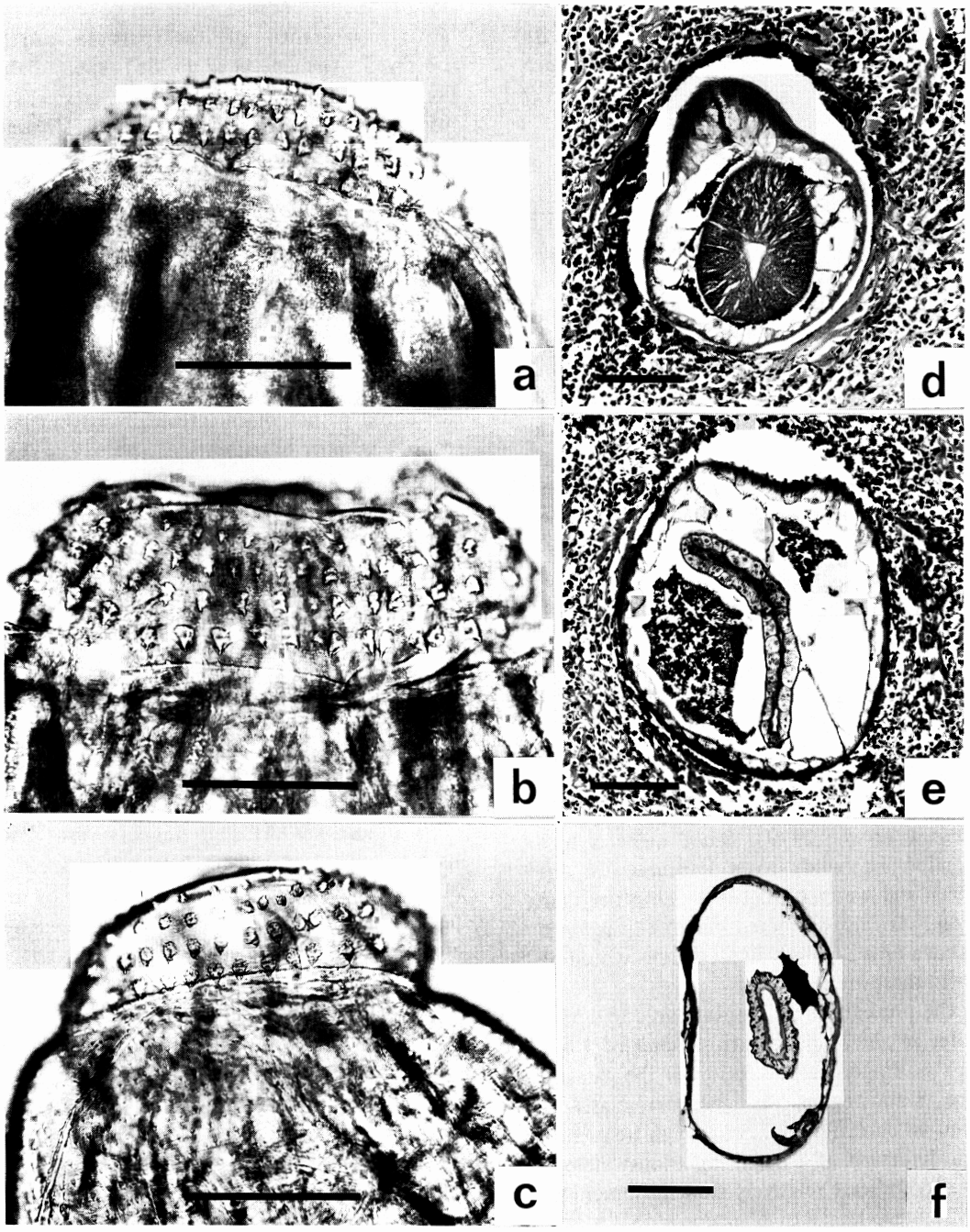
These parasites were identified as the third stage larvae of *G. doloresi* based on their morphological characteristics. The identification of the parasite was confirmed further by Dr. H. Akahane, Associate Professor, Department of Parasitology, School of Medicine, Fukuoka University.

Figs. 2a–2c (from patients No. 3, 5, and 8, respectively) shows the head bulb of each parasite. All of them had four lines of hooklets. The number of hooklets in each row was less than 40, and the number in fourth row was, in common, less than that in other three rows (Table 2). Furthermore, the size of the hooklets in the first row was considerably smaller than that of other three rows. Each hooklet had an irregular four sided base. These features were essentially identical to the morphological characteristics of the head bulb of *G. doloresi* described previously (Miyazaki and Ishii, 1952; Miyazaki, 1960).

Figs. 2d (from patient No. 3) and 2e (from patient No. 5) are the tissue sections of biopsied skin containing cross section of the parasite. In Fig. 2d, the parasite was cross-sectioned at the height of oesophagus, whereas in Fig. 2e, an intestinal region of the parasite was cross-sectioned and the number of nuclei in the intestinal epithelial cells was 1 or 2 with the dominance of binucleated cells. Different from Figs. 2d and 2e, Fig. 2f (from patient No. 8) shows the cross section of the parasite after having been dissected out from biopsied skin.

Table 2 Number of hooklets on the head bulb of the third stage larvae of *G. doloresi* dissected out from the patients

Patient No.	No. of hooklets in			
	1st row	2nd row	3rd row	4th row
3	36	36	34	31
5	35	35	35	33
8	34	36	34	31
mean	35.0	35.7	34.3	31.7



Figs. 2a–2c Head bulb of each parasite obtained from patient No. 3, 5, and 8, respectively. Scale bar: 0.1 mm
Figs. 2d–2f Cross section of each parasite.
Figs. 2d and 2e are those found in tissue sections of the biopsied skin of patients No. 3 and 5, respectively, for histopathology. Note massive accumulation of eosinophils around the parasite. Fig. 2f is the cross section of the parasite after dissecting out from the biopsied skin of patient No. 8. Scale bar: 0.1 mm

As same as Fig. 2e from patient No. 5, the intestinal epithelial cells have 1 or 2 nuclei with the dominance of binucleated cells. Such characteristics are of *G. doloresi* (Akahane *et al.*, 1986).

Discussion

G. doloresi is a parasite of wild boars and pigs in nature, and the adult worms parasitize in the gastric wall of these animals (Miyazaki, 1960). Soon after the discovery of this species in Japan, Miyazaki (1954) pointed out the possibility of infection with this parasite in human, because immature worms are occasionally found in liver or muscle of wild boar. Recently Koga and Ishii (1981b) strengthened this possibility further by demonstrating that monkey was susceptible to infection with *G. doloresi*. Our results directly demonstrate that human also is susceptible to this species and that Miyazaki Prefecture is an endemic area in terms of human gnathostomiasis doloresi. The area where the patients are distributed is well known from earlier times as the endemic area of *G. doloresi* in wild boars (Ishii, 1956), and even recently a quite high incidence of this parasite in wild boars has been reported (Ashizawa *et al.*, 1979). Since quite a high proportion of wild boars captured in the southern and western part of Japan other than Miyazaki is also infected with *G. doloresi* (Ashizawa *et al.*, 1979), human cases would be found in these areas.

In the present study, we were able to detect parasites in the biopsied skin samples of 3 out of 7 cases. In general, detection of the parasite in the skin regions of gnathostomiasis is believed to be difficult. Thus, a high frequency of the detection of parasite by biopsy in our study may indicate relatively slow movement of the third stage larvae of *G. doloresi* in the skin of patients.

The results reported in this paper show that it is easy to dissect out parasites from paraffin-embedded tissue blocks in two cases by dewaxing and rehydration. This method seems to be useful because both cross section and gross appearance of the parasite can be observed. Akahane *et al.* (1986) reported that, in addition to the morphological characteristics of the hooklets on the head bulb (Miyazaki, 1960), the number of nuclei in the intestinal epithelial cell is helpful for the morphological identification of *Gnathostoma* species.

Although immunodiagnosis for gnathostomiasis was performed, only 2 out of 7 cases were positive by an Ouchterlony's double diffusion test and 1 out of 2 cases was positive by skin test. Evaluation of these immunodiagnostic methods should be postponed until we could gather more information from large enough number of patients.

As for the sources of infection, 7 out of 8 patients have previous history of eating raw freshwater fishes. Among them, 6 patients stated that they had eaten raw slice of brook trout, *Oncorhynchus masou*, within few months before the onset of the disease, suggesting that this fish species is likely to be a source of infection. Similar to our cases, gnathostomiasis patient having past history of eating locally obtained brook trout but never eating snakeheads nor loaches was found in Kumamoto Prefecture (Mimori, T., personal communication). As a preliminary survay, we have examined about 100 brook trouts, about 30 of which were caught and brought by the patients, 20 from people living in the endemic area, and about 50 were purchased from a local fish-nursery for brook trout. However, none of them were infected with the third stage larvae of *G. doloresi*. Thus, care should be taken to draw any definite conclusion until direct evidence is obtained, because involvement of freshwater fishes in the life cycle of *G. doloresi* as the second intermediate host or paratenic host has never been proven. Since Miyazaki and Ishii (1952) first reported salamanders, *Hynobius* species, as the second intermediate host for *G. doloresi*, various reptiles and/or amphibians were reported as the second intermediate host or the paratenic host for this parasite (Miyazaki and Kawashima, 1962; Tada *et al.*, 1969; Hasegawa *et al.*, 1981; Hasegawa *et*

al., 1982; Mako and Akahane, 1985). In the present study, some of the patients stated that, in addition to, or instead of, brook trout, they have a past history of eating raw meat of various kind of animals or even raw pork liver. Therefore, the exact route of infection and the natural life cycle of the parasite in Miyazaki Prefecture should be urgently clarified.

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