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Gnathostoma doloresi Larvae Found in Snakes, *Agkistrodon halys*, Captured in the Central Part of Miyazaki Prefecture

JUN-ICHI IMAI[1], YUJIRO ASADA[2], YOICHIRO HORII[3] AND YUKIFUMI NAWA[1]

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Abstract

The advanced third stage larvae of *Gnathostoma doloresi* were found in poisonous snakes, *Agkistrodon halys*, captured in the central part of Miyazaki Prefecture during July-August 1988. A total of 6 snakes were examined and all of them harboured many encysted larvae of *G. doloresi* mainly in the muscles. This is the first record of *G. doloresi* larvae from *A. halys*. The importance of snakes in the life cycle of *G. doloresi* and also as the possible source of human infection with this parasite was discussed.

Key words: *Gnathostoma doloresi*, advanced third stage larvae, snake, *Agkistrodon halys*, Miyazaki Prefecture, Kyushu

Introduction

Recently we have reported three confirmed and five suspected human cases of infection with *Gnathostoma doloresi* in the central part of Miyazaki Pref. and emphasized a clinical importance of *G. doloresi* as the zoonotic parasite (Nawa *et al.*, 1988; Ogata *et al.*, 1988). Since the exact route of infection to human has not been determined yet, our efforts are currently directed to elucidate the life cycle including paratenic host of *G. doloresi* in the endemic area. *G. doloresi* is parasitic to wild boars and pigs in adult stage (Miyazaki, 1960), and, even nowadays, practically almost all wild boars, *Sus scrofa leucomystax*, captured in the mountainous area of Kyushu District were shown to harbour this parasite (Ashizawa *et al.*, 1979; Sakaguchi *et al.*, 1985). The first intermediate hosts for *G. doloresi* were experimentally determined to be some copepods (Ishii, 1956). As to the second intermediate host, Miyazaki and Ishii (1952) first found the advanced third stage larvae in salamanders. Subsequently, based on the survey in Nansei (Amami and Okinawa) Islands, various reptiles and amphibians were added as the second intermediate host or as the paratenic host (Miyazaki and Kawashima, 1962; Tada *et al.*, 1969; Toshioka, 1970; Hasegawa *et al.*, 1981; Hasegawa *et al.*, 1982; Mako and Akahane, 1985). Thus, a wide range of animals, such as fishes, amphibians and reptiles, are the objects of our survey in the endemic area. In this paper, we report the first finding of *G. doloresi* larvae in the poisonous snakes, *Agkistrodon halys*, captured in the central part of Miyazaki Pref.

Materials and Methods

During July–August 1988, a total of 6 poisonous snakes, *A. halys* (common Japanese name: Mamushi) were captured in Shiromi-Village, Saito-City, Miyazaki Pref., where is located in the center of the endemic area of human gnathostomiasis doloresi. They were anesthetized by hypothermia in ice. After measuring their body length and body weight,

Departments of [1]*Parasitology and* [2]*Pathology, Miyazaki Medical College, Kiyotake, Miyazaki 889-16, Japan*

[3]*Department of Medical Zoology, Nagasaki University, School of Medicine, Sakamoto-machi, Nagasaki 852, Japan*

今井淳一　名和行文（宮崎医科大学寄生虫学講座）
浅田祐士郎（同　病理学第一講座）
堀井洋一郎（長崎大学医学部医動物学講座）

their head was cut off and the skin was peeled. The viscera were removed and the carcase was cut into equal length of 10 pieces. The thin abdominal wall of each piece was pressed between two thick glass plates to search for the encysted larvae under a dissecting microscope. Then, the muscles and viscera were separately homogenized by a blender, and digested in an artificial gastric juice (pepsine, Difco, 1:10,000 1 g, conc. HCl 7 ml in 1,000 ml distilled water) at 37°C for 3 hr. The residues were examined for remaining larvae. The larvae were removed from the cysts and fixed in hot 10% buffered formalin.

For counting and morphological observation of the hooklets on the head-bulb, the heads of some larvae were cut off from body and mounted in lactophenol solution. Some larvae were embedded in murine liver and cross sections of the larvae were prepared for microscopic observation of intestinal epithelial cells (Akahane *et al.*, 1986).

Some larvae were examined by scanning electron microscope. For this purpose, formalin-fixed larvae were washed in phosphate buffer and postfixed with 2% osmium tetroxide for 1 hr. Then they were dehydrated in an ascending series of ethanol, critical-point-dried using liquid CO_2, and sputter-coated with platinum-palladium at 10 mA for 5 min. Observations were done with a scanning electron microscope (Hitachi S-800) operated at 15 kV.

Results

Six snakes, *A. halys* (Fig. 1), were examined and the results were summarized in Table 1. Their body length ranged from 49 to 54 cm and their body weight, from 72 to 120 g (the heaviest one had four embryos in the uterus). All six snakes harboured encysted larvae of *Gnathostoma*. The number of larvae per snake was rather constant with the minimum 11 and maximum 48.

Almost all encysted larvae were found in the muscles (Fig. 2) and only two in the viscera of the snake No. 6. The average size of the cysts was 1.0 x 0.9 mm. In the snake body, the majority of larvae were located in the middle part corresponding to the location of liver, stomach, and duodenum.

Morphometric observations were done on randomly chosen 10 excysted larvae (Fig. 3). The body of the larvae was almost colorless except for the brownish intestine. The average body length was 2.5 mm, ranging from 2.2–3.0 mm, and the average width 0.33 mm, ranging from 0.29–0.36 mm. All larvae had four lines of hooklets on the head-bulb, and each hooklet had an irregular square base (Figs. 4, 5, 7, and 9). The size of the hooklets in the first row was considerably smaller than that of other three rows (Figs. 4, 5, 7, and 9). The average number of hooklets in each row was 37.0, 36.9, 34.0, and 34.4 from the first to the fourth row, respectively. The number in the fourth row was, in common, less than that in the first row (Table 2). The surface of the body was covered with small cuticular spines forming 158–192 transverse rows, which become minute in the posterior part of the body (Figs. 7–11). Cervical papilla (Figs. 7 and 10) was noted at around 14–17th row from the neck. These morphological characteristics were essentially identical to those of the advanced third stage larvae of *G. doloresi* described previously by Miyazaki (1960). In addition, the cross section of the larvae (Fig. 6) revealed that each intestinal epithelial cell had one or two nuclei of round-oval shape. This agreed with the results of Akahane *et al.* (1986), in that three species of *Gnathostoma, G. spinigerum, G. hispidum*, and *G. doloresi* could be distinguished by the number of nuclei in the intestinal epithelial cells in cross sections.

Discussion

The results reported here show that six poisonous snakes, *A. halys*, captured in the central part of Miyazaki Pref. harboured 11–48 encysted larvae mainly in the muscles with a 100% prevalence. Based on their morphological and morphometric characteristics, all these larvae were identified as the advanced third stage larvae of *G. doloresi*.

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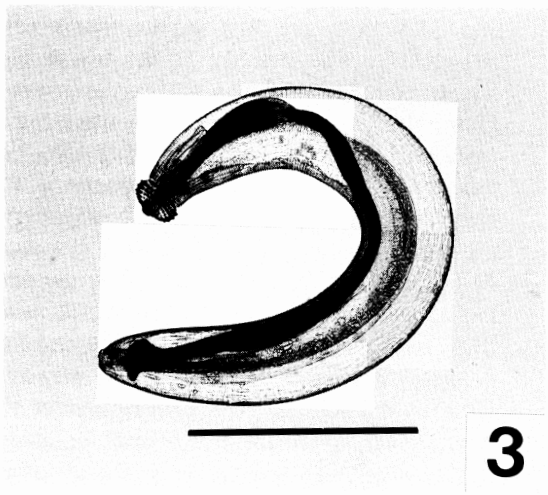

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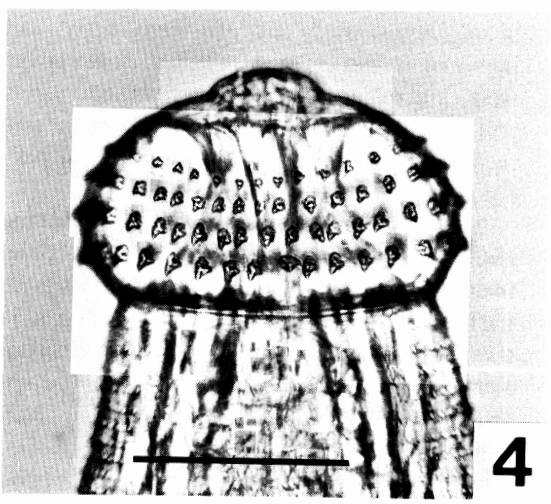

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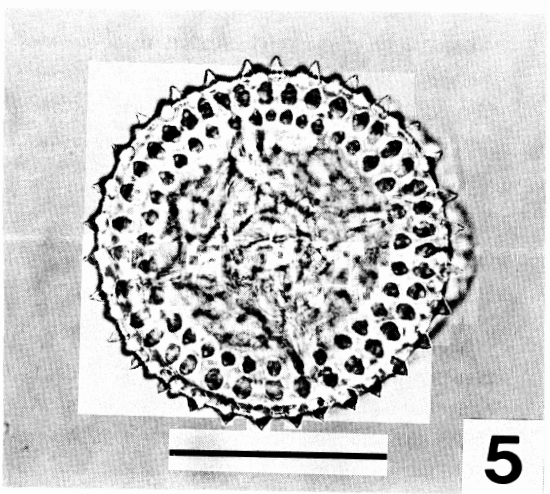

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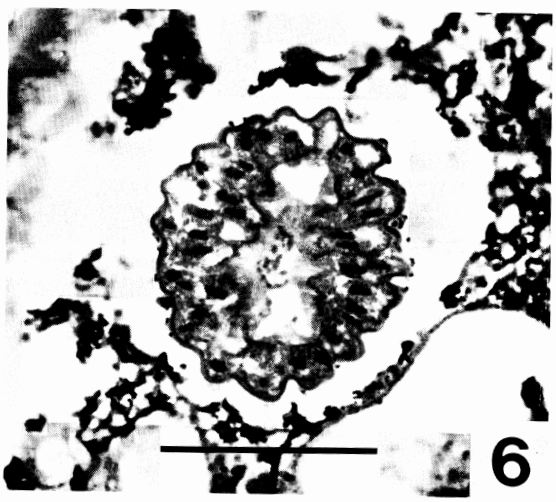

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Table 1 Number of *Gnathostoma doloresi* larvae recovered from *Agkistrodon halys*

Snake No.	Sex	Body length (cm)	Body weight (g)	No. of encysted larvae	
				muscles	viscera
1	M	54	72	22	0
2	F	54	120*	11	0
3	M	52	76	48	0
4	F	52	86	20	0
5	M	51	80	12	0
6	M	49	N D	37	2

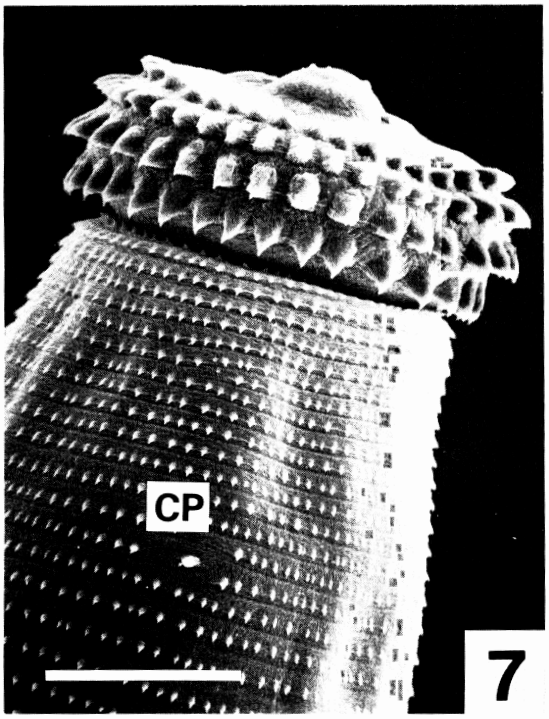
The snakes were captured in Shiromi-Village, Saito-City, Miyazaki Pref. during July-August 1988.
*Four embryos in the uterus.
ND: not determined

Table 2 Number of hooklets on the head-bulb of 10 larval *Gnathostoma doloresi* obtained from *Agkistrodon halys*

Larva No.	1st row	2nd row	3rd row	4th row	4th-1st
1	40	39	37	35	−5
2	38	37	35	36	−2
3	37	39	36	36	−1
4	39	38	36	36	−3
5	36	35	35	33	−3
6	35	38	32	34	−1
7	36	34	31	33	−3
8	37	34	34	32	−5
9	38	37	33	36	−2
10	34	38	31	33	−1
Mean±SD	37.0±1.7	36.9±1.8	34.0±2.0	34.4±1.5	−2.6

More than 30 years ago, Miyazaki and Ishii (1952) found the third stage larvae of *G. doloresi* in two species of salamanders captured in the mountaineous area of the border of Miyazaki and Kumamoto Pref. and proposed that these salamanders were the second intermediate host of *G. doloresi*. However, in their results, the occurrence of *G. doloresi* larvae in the salamanders was less than 10%. Furthermore, according to Mr. Isozaki, the Chief of Art and Science Division of Miyazaki Prefectural Museum, the habitat of the salamanders in the mountaineous area of Miyazaki Pref. is limited. Nevertheless, practically almost all wild boars

Fig. 1. *Agkistrodon halys* captured in Shiromi-Village, Saito-City, Miyazaki Prefecture.
Fig. 2. An encysted larva of *Gnathostoma doloresi* in the muscle of *A. halys*. Scale bar = 0.5 mm
Fig. 3. Total view of excysted larva. Scale bar = 1.0 mm
Fig. 4. Lateral view of the head-bulb of the larva. Scale bar = 0.1 mm
Fig. 5. Apical view of the head-bulb of the larva. Scale bar = 0.1 mm
Fig. 6. Cross section of the larva showing intestinal epithelial cells. Scale bar = 0.05 mm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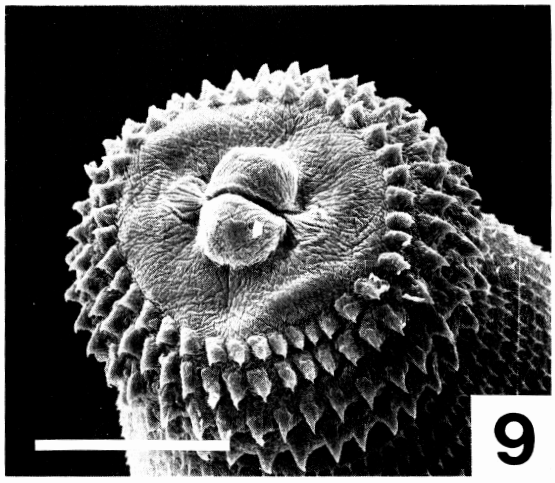
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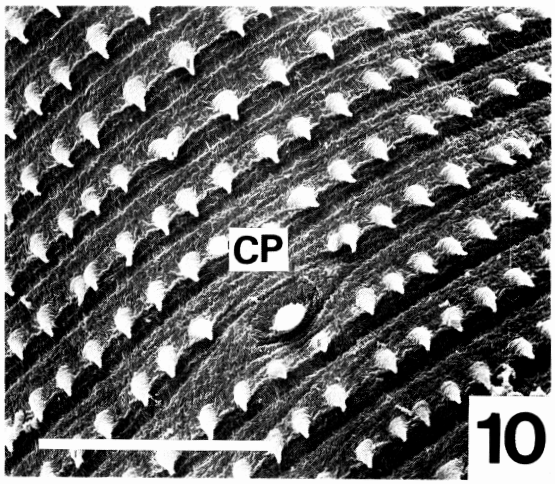
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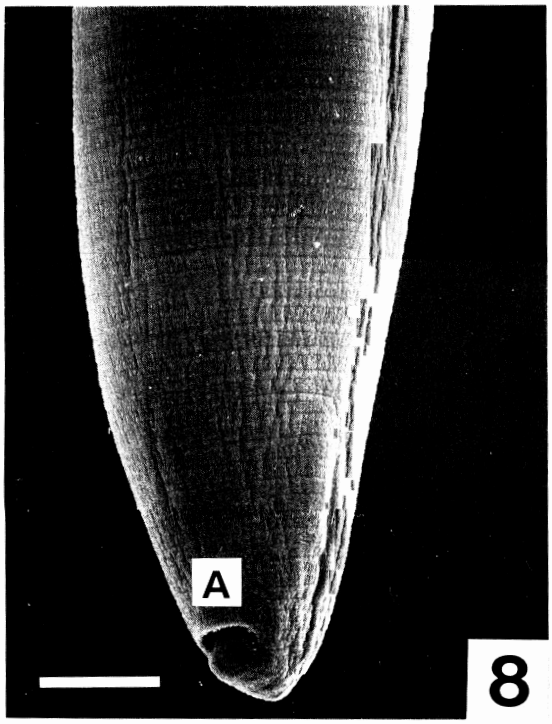
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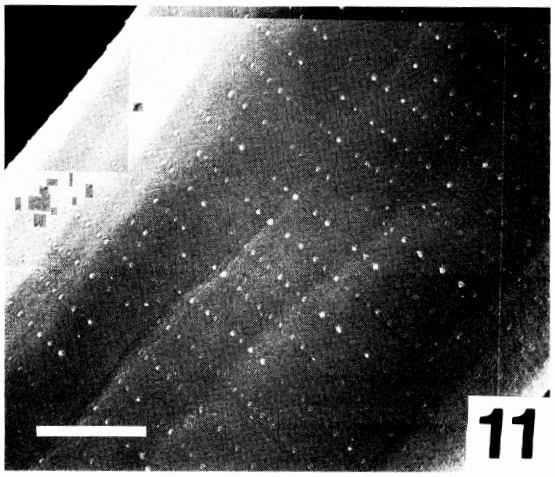
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recently captured in the mainland Kyushu were infected with *G. doloresi* (Ashizawa *et al.*, 1979; Sakaguchi *et al.*, 1985). Therefore, the presence of more appropriate intermediate hosts and/or paratenic hosts as the source of infection to wild boars has been expected.

In Nansei (Amami-Okinawa) Islands, *G. doloresi* larvae were found in various reptiles and amphibians, especially in snakes (Miyazaki and Kawashima, 1962; Tada *et al.*, 1969; Toshioka, 1970; Hasegawa *et al.*, 1981, Mako and Akahane, 1985). Mako and Akahane (1985) reported that, in Amami Islands, wild boars, *Sus riukiuensis,* often ate snakes. Similarly, Mr. Ishikawa, a hunter, and Mr. Hamasuna, a forestrial worker, who are living in Shiromi-Village and provided us with snakes, stated that they often observed wild boars eating poisonous snakes, *A. halys,* and that they sometimes found bodies of snakes in the gastric contents of wild boars. Such findings are also noted in various places in the mountaineous area of Miyazaki Pref. (Isozaki, personal communication). Thus, it is likely that snakes are, in common, the most important sources of infection with *G. doloresi* in wild boars.

Recently we have found three confirmed and five suspected human cases of *G. doloresi* infection in Miyazaki Pref. (Nawa *et al.*, 1988; Ogata *et al.*, 1988). Among these cases, one of the patients had a past history of eating raw meat of *A. halys.* This poisonous snake is widely distributed in the farm-field and mountaineous area of Japan. Some people eat its meat in raw, because its blood, meat, or alcholic extract is believed to have mysterious effects on health. Thus, the present results indicate that *A. halys* could be one of the possible sources of human infection with *G. doloresi.*

At the present, how *A. halys* acquire *G. doloresi* larvae is not clear. Ishii (1956) determined experimentally that the first intermediate host of *G. doloresi* is copepods. However, it is unlikely that *A. halys* ingests copepods directly. Uchida and Imaizumi (1939) reported that the major foods of *A. halys* were small mammals (about 60%) and frogs (about 20%). The presence of *G. doloresi* larvae in frogs was already noted in Okinawa (Hasegawa *et al.*, 1981; 1982). To elucidate the life cycle of *G. doloresi* in conjunction with the identification of the source of human infection in the endemic area, a survey for *G. doloresi* infection in frogs and also in various other possible intermediate and/or paratenic hosts is currently undergoing by our hands.

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Figs. 7–11. Scanning electron micrographs of the larvae. Each scale bar is 50 μm except for Fig. 10 (20 μm)
Fig. 7. Lateral view of the head-bulb. CP: cervical papilla
Fig. 8. Tail part of the larva. A: anus
Fig. 9. Apical view of the head-bulb.
Fig. 10. Cuticular spines of the anterior part. CP: cervical papilla
Fig. 11. Cuticular spines of the middle part.

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