[Jpn. J. Parasitol., Vol. 44, No. 2, 128–132, April, 1995]

The First Record of *Gnathostoma nipponicum* in Aomori Prefecture

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(Accepted for publication; April 7, 1995)

Abstract

In January 1993, two Japanese weasels, *Mustela sibirica itatsi*, were captured in Tenmabayashi-mura, in eastern Aomori Prefecture, and were examined for *Gnathostoma*. Three adults and 8 larvae of *G. nipponicum* were recovered from esophageal tumors and the muscles of the 2 animals. In addition, fertilized eggs of the parasite were demonstrated in fecal samples of weasels collected in the same locality. The present report is the first record of *G. nipponicum* in Aomori Prefecture. It also is the first time *G. nipponicum* larvae have been detected in the muscles of naturally-infected weasels.

Key words: *Gnathostoma nipponicum*, weasel, *Mustela sibirica itatsi*, geographical distribution

Introduction

Nematodes of the genus *Gnathostoma* are important zoonotic parasites (Miyazaki, 1960). Four species of *Gnathostoma* have been implicated in human gnathostomiasis in Japan (Akahane, *et al.*, 1982; Ando *et al.*, 1991; Nawa *et al.*, 1989; Sato *et al.*, 1992; Taniguti *et al.*, 1991). Three species native to Japan, *G. spinigerum* Owen, 1836, *G. doloresi* Tubangui, 1925, and *G. nipponicum* Yamaguti, 1941, and larval *G. hispidum* Fedtchenko, 1872 in imported loaches, have been reported. The native species are mainly distributed in the central, western, and southern parts of Japan (Ando *et al.*, 1988; Gyouten and Nishida, 1978; Katagiri and Oturu, 1957).

G. nipponicum is a common parasite of *Mustela sibirica itatsi*, the definitive host, and is distributed widely as far as Iwate Prefecture, in northern Japan (Katagiri and Oturu, 1957). Until now, members of the genus *Gnathostoma* have not been found in weasels or other hosts in Aomori Prefecture. Recently, however, Sato *et al.* (1992) reported 5 human cases of *G. nipponicum* infection in Akita (2 cases) and Aomori (3 cases) Prefectures. This suggested the possibility of infections in weasels in northern Honshu. Accordingly, we made a survey of Japanese weasels for *G. nipponicum* in Aomori Prefecture.

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Materials and Methods

Two male weasels, 426 g (No. 1) and 405 g (No. 2) in body weight, were captured in Tenmabayashi-mura, Kamikita-gun, Aomori Prefecture, and autopsied in January, 1993. The viscera and muscles were examined for larvae after artificial digestion of tissues. The recovered worms were washed in physiological saline, fixed in 10% hot formalin, and cleared and mounted in lactophenol for taxonomical identification. For histological examination, tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (HE). A total of 10 fecal samples from weasels collected in the same locality were examined for *G. nipponicum* eggs. For the detection of eggs in feces, the formalin-ether method (MGL) was used.

Morphological studies were performed using a light microscope and a micrometer.

Results

From the 2 captured weasels, 3 adult and 8 larval nematodes of *Gnathostoma* were recovered from esophageal tumors and the muscles (Table 1). All worms were identified as *G. nipponicum*. The weasels harbored two and one adult males of *Gnathostoma* in esophageal tumors (Fig. 1). The dimensions

of the adults were as follows (mm): length, 19.0–26.0; width, 0.75–0.90; head-bulb width, 0.65–0.67; head-bulb height, 0.25–0.34; esophagus length, 2.8–5.1; cervical sac length, 0.8–1.2; tail length, 0.26; caudal alae length, 0.47–0.50. As shown in Table 2, they had eight rows of hooklets on the head-bulb. The worms possessed a pair of lateral lips on the head-bulb, and each of the lips had two labial papillae. The spicules were unequal in length, the left one was 1.8–2.2 mm and the right one 0.4–0.5 mm. There were four pairs of caudal papillae and three pairs of small papillae on the ventral surface of the posterior extremity. The body surface was covered with cuticular spines extending from immediately behind the head-bulb to almost the middle of the body. The largest spines had three teeth, with the middle tooth being much longer than the other two teeth.

Seven larval *Gnathostoma* were recovered from the muscles of one weasel, and one larva from the other weasel. Measurements of six of the 8 larvae are summarized in Table 3. The stage of development of the larvae was different and the worms exhibited either three or four rows of hooklets on the head-bulb. The number of hooklets on the 1st to 3rd rows were 31–36, 35–40, and 38–43, and those on the 4th rows were 19 and 27. The most developed larva had two layers of cuticle and 8 rows of hooklets on the inner cuticle of the head-bulb (sheathed larva) (Figs. 4 and 5). The whole worm was encircled by transverse striations consisting of single-toothed minute cuticular spines. Extending posteriorly, the spines gradually decreased in size and density, and finally disappear near the tip of the tail.

Table 1 Distribution of *Gnathostoma nipponicum* in two Japanese weasels

Weasel No.	No. of worms recovered from			
	esophagus	muscles of		
		thoracic wall	abdominal wall	legs
1	2	3	2	2
2	1	1	0	0

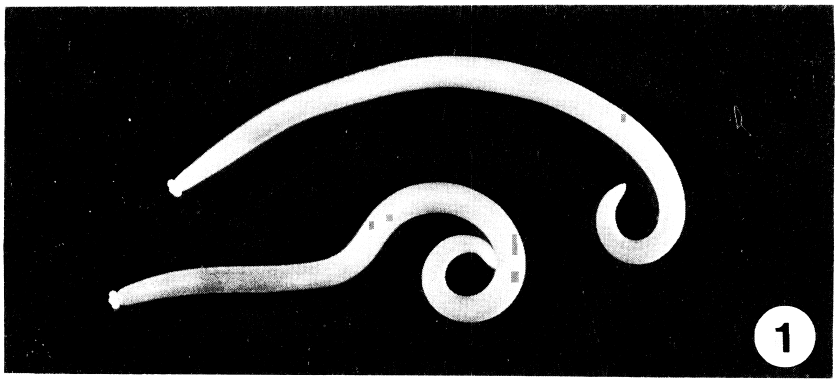
Table 2 Measurements (mm) and number of hooklets on the head-bulb of *Gnathostoma nipponicum* adult males

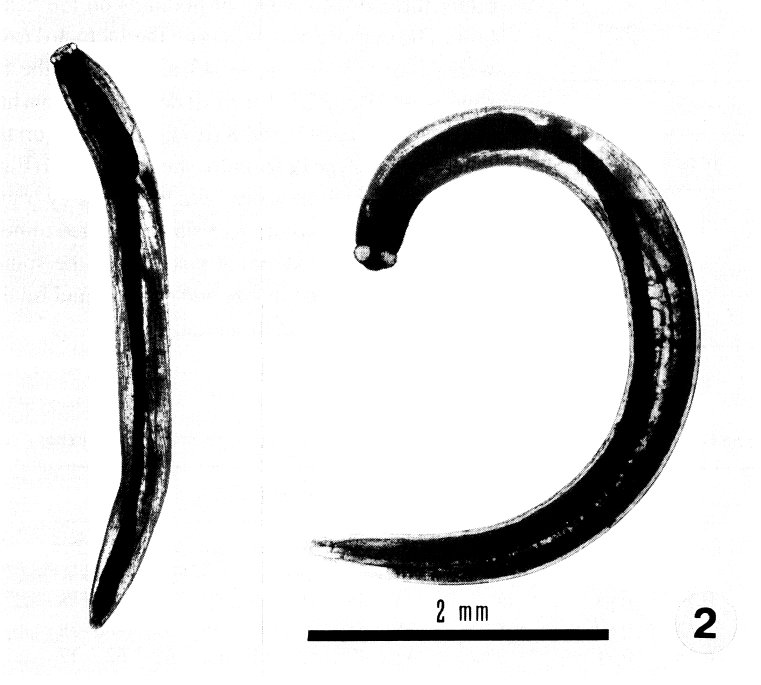
Worm No.	Body		Head-bulb		No. of rows of hooklets	No. of hooklets on each row							
	length	width	height	width		1st	2nd	3rd	4th	5th	6th	7th	8th
1	19.0	0.75	0.25	0.64	8	34	45	46	60	59	63	56	8
2	24.0	0.80	0.32	0.60	8	35	45	52	50	56	56	48	32
3	26.0	0.90	0.34	0.71	8	43	47	51	61	62	61	62	17

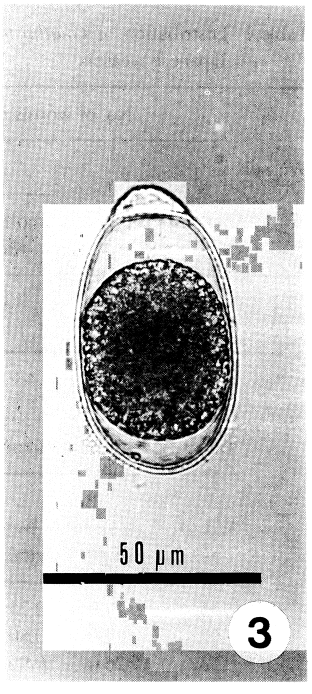
Table 3 Measurements (mm) of *Gnathostoma nipponicum* larval worms recovered from muscle tissue of Japanese weasels

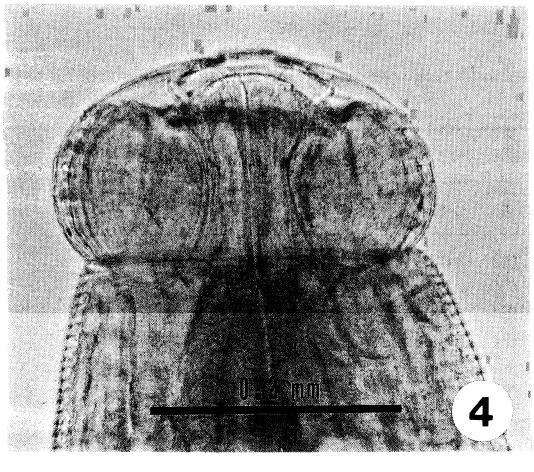
Larva No.	Body length	Head-bulb		Length of			No. of rows of hooklets	No. of hooklets			
		height	width	esophagus	c. sac*	tail		1st	2nd	3rd	4th
1	1.5	0.05	0.10	0.5	0.2	0.04	3	35	36	39	
2	2.6	0.08	0.15	0.9	0.5	0.04	4	35	35	38	19
3	3.0	0.06	0.18	0.9	0.4	0.07	3	35	36	40	
4	3.1	0.09	0.21	0.9	0.4	0.04	3	36	38	40	
5	3.9	0.09	0.71	1.1	0.5	0.06	3	36	40	42	
6	6.2	0.11	0.30	1.4	0.5	0.16	4	31	37	43	27

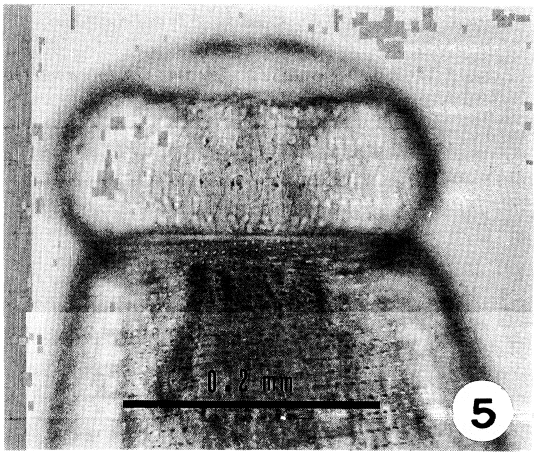
*: cervical sac


1


2 mm
2


50 µm
3


0,2 mm
4


0,2 mm
5

Pathologically, the esophageal tumors caused by the adult worms were present on the outer side of the esophageal wall in the thoracic cavity. The distance from the cardium was 2.0–3.5 cm, and the size of the tumors were about 3.5×1.5 cm and 1.5×0.5 cm. Histologically, the tumors were seen to be formed by a remarkable proliferation of connective tissue accompanied by infiltration of lymphocytes, plasma cells, neutrophils, and eosinophils. Necrosis and homogenous substances in the inner wall of the parasitic canal induced by tissue perforation of the worms were seen. In addition, scattered in the connective tissue, granuloma formation around cuticular substances seemingly derived from the molting of the nematodes were also noticed. Moreover, hyperplastic or degenerative changes in the esophageal epithelium and glands, and haemosiderin deposition, were found in some parts of the granulation tissue.

In the fecal examinations, many fertilized eggs of *G. nipponicum* were detected in the 8 samples. The eggs were colorless or slightly yellowish-brown, and usually contained an unsegmented ovum. The surface of the shells were finely granulated and had a operculum (cap-like thickening) at one pole (Fig. 3). The sizes (μm) were 64–80 (mean: 71.1) in length and 38–40 (mean: 42.1) in width.

Discussion

In this study, a total of three adults and eight larvae of *Gnathostoma* were found in two weasels. The adult and larval worms were identified as *G. nipponicum*. Thus, this report is the first record of *G. nipponicum*-infected weasels in Aomori Prefecture. From these results, it seems this parasite is widely distributed in Japan.

The pathological findings in the esophageal tumors were essentially the same as those previously reported by Ashizawa *et al.* (1978a, b). Furthermore, the morphological features of the adult worms coincided with the description of *G. nipponicum* adult males by Yamaguti (1941) and Miyazaki (1960), except for the lesser number of hooklets on the head-bulb. The discrepancy in the number of hooklets seemed to be related to worm maturity. In addition, 8 larval worms of different sizes, including one still ensheathed, were recovered from the muscles. They were compared with advanced third-stage larvae (AdL_3) of *G. nipponicum* obtained from loaches (Ando *et al.*, 1988; Oyamada *et al.*, 1994), and their morphological similarities were confirmed. In natural infection, only one *G. spinigerum* larva has been reported from muscle tissue of the Japanese weasel (Arita, 1953; Umetani, 1950). Our report is the first instance that *G. nipponicum* larvae have been found in the muscles of the weasel. Based on our findings, we suggest that migration into the muscles might be very important for larval development. Recently, Ando *et al.* (1994) outlined details concerning the body migration and development of the advanced third-stage larva to the adult in the weasel. The results of the fecal examinations clearly show that *G. nipponicum* is endemic in the Temmabayashi-mura district in Aomori Prefecture.

Much has been learned about the life cycle of *G. nipponicum*. Three species of copepods can serve as the first intermediate host, and some of fishes, amphibians, and mammals can act as the second intermediate and/or paratenic host (Ando *et al.*, 1992; Koga and Ishii, 1981; Miyazaki, 1954). Larvae were recently recovered from naturally infected loaches, catfish, and snakes (Ando *et al.*, 1988; Koga and Ishii, 1981; Oyamada *et al.*, 1994). It is important to know the life cycle of *G. nipponicum* to prevent human infection, and to elucidate the source of infection with gnathostomes.

Acknowledgement

We thank Prof. Haruo Kamiya, Department of Parasitology, Hirosaki University School of Medicine, for his important suggestions to the present study.

Fig. 1 Lateral view of adult male worms of *Gnathostoma nipponicum* (24mm and 26mm length) recovered from esophageal tumors.

Fig. 2 Larval *Gnathostoma nipponicum* obtained from the muscles.Left, 3.9mm, right, 6.2mm in length.

Fig. 3 Fertilized egg of *Gnathostoma nipponicum* in feces of a Japanese weasel.

Figs. 4, 5 Head-bulb of a larval worm recovered from abdominal wall of a weasel.

Fig. 4 Larva has two layers of cuticule.

Fig. 5 Eight rows of hooklets on the head-bulb can be seen under the sheath.

References

1) Akahane, H., Iwata, K. and Miyazaki, I. (1982): Studies on *Gnathostoma hispidum* Fedchenko, 1872 parasitic in loaches imported from China. Jpn. J. Parasitol., 31, 507–516 (in Japanese with English Abstract).
2) Ando, K., Tanaka, H., Taniguti, Y., Shimizu, M. and Kondo, K. (1988): Two human cases of gnathostomiasis and discovery of a second intermediate host of *Gnathostoma nipponicum* in Japan. J. Parasitol., 74, 623–627.
3) Ando, K., Tanaka, H. and Ohkawa, C. (1988): A survey of geographical distribution of *Gnathostoma nipponicum* in Mie, Nara, Kyoto and Shiga Prefectures, Japan. Jpn. J. Parasitol., 37, 263–267 (in Japanese with English Abstract).
4) Ando, K., Hatsushika, R., Akahane, H., Matsuoka, H., Taylor, D., Miura, K. and Chinzei, Y. (1991): *Gnathostoma nipponicum* infection in the past human case in Japan. Jpn. J. Parasitol., 40, 184–186.
5) Ando, K., Tokura, H., Matsuoka, H., Taylor, D. and Chinzei, Y. (1992): Life cycle of *Gnathostoma nipponicum* Yamaguti, 1941. J. Helminthol., 66, 53–61.
6) Ando, K., Sato, Y., Miura, K., Matsuoka, H. and Chinzei, Y. (1994): Migration and development of the larvae of *Gnathostoma nipponicum* in the rat, second intermediate host or paratenic host, and the weasel, definitive host. J. Helminthol., 68, 13–17.
7) Arita, M. (1953): Studies on two species of *Gnathostoma* parasitic in the weasels. Igakukenkyu, 23, 1729–1749 (in Japanese with English Abstract).
8) Ashizawa, H., Kugi, G., Nosaka, D., Tateyama, S. and Yanai, T. (1978a): Natural infection of weasels with *Gnathostoma nipponicum* in Oita Prefecture, Japan. Bull. Fac. Agr. Miyazaki Univ., 25, 85–92 (in Japanese with English Abstract).
9) Ashizawa, H., Kugi, G., Nosaka, D., Tateyama, S. and Yanai, T. (1978b): Pathological findings on esophageal tumor caused by parasitization of *Gnathostoma nipponicum* in weasels. Bull. Fac. Agr. Miyazaki Univ., 25, 297–306 (in Japanese with English Abstract).
10) Gyouten, J. and Nishida, H. (1978): On the *Gnathostoma nipponicum* in Kagawa Prefecture. Jpn. J. Parasitol., 27, 411–416 (in Japanese with English Abstract).
11) Katagiri, S. and Oturu, M. (1957): Distribution of *Gnathostoma nipponicum* in Hokuriku and Ou districts, Japan (Nematoda: Gnathostomatidae). Medicine and Biology, 43, 199–202 (in Japanese).
12) Koga, M. and Ishii, Y. (1981): Larval gnathostomes found in reptiles in Japan and experimental life cycle of *Gnathostoma nipponicum*. J. Parasitol., 67, 565–570.
13) Miyazaki, I. (1954): Studies on *Gnathostoma* occurring in Japan (Nematoda: Gnathostomidae). II. Life history of *Gnathostoma* and morphological comparison of its larval forms. Kyushu Mem. Med. Sci., 5, 123–139.
14) Miyazaki, I. (1960): On the genus *Gnathostoma* and human gnathostomiasis, with special reference to Japan. Exp. Parasitol., 9, 338–370.
15) Nawa, Y., Imai, J. and Ogata, K. (1989): The first record of a confirmed human case of *Gnathostoma doloresi* infection. J. Parasitol., 75, 166–169.
16) Oyamada, T., Kudo, N., Sano, T. and Narai, H. (1994): An epidemiological survey of the third-stage larvae of *Gnathostoma nipponicum* in loach, *Misgurnus anguillicaudatus*, in eastern district, Aomori Prefecture. Jpn. J. Parasitol., 43, (Suppl.) 73 (in Japanese).
17) Sato, H., Kamiya, H. and Hanada, K. (1992): Five confirmed human cases of gnathostomiasis nipponica recently found in northern Japan. J. Parasitol., 78, 1006–1010.
18) Taniguti, Y., Hashimoto, K., Ichikawa, S., Shimizu, M., Ando, K. and Kotani, Y. (1991): Human gnathostomiasis. J. Cutan. Pathol., 18, 112–115.
19) Umetani, T. and Arita, M. (1950): Adult and larvae of *Gnathostoma* parasitic in the weasel in Kyushu. Medicine and Biology, 18, 154–157 (in Japanese).
20) Yamaguti, S. (1941): Studies on helminth fauna of Japan. Part 35. Mammalian nematodes, II. Jpn. J. Zool., 9, 409–438.