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Prevalence of the Advanced Third-stage Larvae of *Gnathostoma nipponicum* in Loaches (*Misgurnus anguillicaudatus*), in Aomori Prefecture, Northern Part of Honshu, Japan

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Abstract

From June 1993 to December 1994, a total of 9,017 loaches, *Misgurnus anguillicaudatus*, captured in Kamikita-gun in Aomori Prefecture, were examined for the third-stage larva of *Gnathostoma nipponicum*. The prevalence was different among 14 localities, with infection rates ranging from 0 to 2.1%. All infected loaches were larger than 7.0 cm in body length. As the body length of the loaches increased, the prevalence also increased. A total of 102 larvae were recovered and those larvae were identified as the advanced third-stage larvae of *G. nipponicum*.

The present report is the first record that *G. nipponicum* larvae were found in naturally-infected loaches in Aomori Prefecture, and confirmed that the Kamikita-gun in Aomori Prefecture is an endemic area for *G. nipponicum*.

Key words: *Gnathostoma nipponicum*; advanced third-stage larva; loach; *Misgurnus anguillicaudatus*; survey.

Introduction

Helminthes of the genus *Gnathostoma* are important zoonotic parasites (Miyazaki, 1960; Nawa *et al.*, 1989; Ando *et al.*, 1988a; Ando *et al.*, 1991; Sato *et al.*, 1992). *G. nipponicum* is found in esophageal tumors in the Japanese weasel, *Mustela sibirica itatsi*, which is distributed widely in Kyushu, Shikoku, and throughout Honshu, Japan (Yamaguti, 1941; Katagiri and Otsuru, 1957; Miyazaki, 1960; Ashizawa *et al.*, 1978; Gyoten and Nishida, 1978; Ando *et al.*, 1988b). Until recently, Aomori Prefecture had been regarded as non-endemic of *G. nipponicum*, although human cases of this nematode infection were reported by Sato *et al.* (1992). Since 1992, we have performed several surveys the definitive hosts for *G. nipponicum* in Aomori Prefecture, and have found the Japanese weasels infected with adults and larvae of this nematode (Oyamada *et al.*, 1995).

There have been few reports on the life cycle of *G. nipponicum* in nature. On the other hand, it was experimentally proved that some species of copepods could serve as the first intermediate hosts (Arita, 1953; Mabuchi, 1957; Koga and Ishii, 1981), and various species of fishes, amphibians, and mammals could serve as the second intermediate and/or paratenic hosts (Koga and Ishii, 1981; Ando *et al.*, 1992). In addition, larvae were observed in loaches, catfish and snakes, and these animals are regarded as the second intermediate and/or paratenic host in nature (Koga and Ishii, 1981; Ando *et al.*, 1992). Loaches especially seemed to be the most suitable second intermediate host of *G. nipponicum*.

We examined native loaches for the prevalence of larval *G. nipponicum* in eastern Aomori Prefecture.

Materials and Methods

From June 1993 to December 1994, a total of 9,017 loaches, *Misgurnus anguillicaudatus*, were

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captured in rice fields in 14 localities in Kamikita-gun (Fig. 1), eastern Aomori Prefecture, in which *G. nipponicum* had previously been found in the Japanese weasels (Oyamada *et al.*, 1995). Before dissection, each loach was weighed and its body length was measured. Each of 8,607 loach was cut into small pieces, and digested one by one in an artificial gastric juice solution (pepsin, 1:10,000, 8 g; HCl, 8 ml in 1,000 ml distilled water) at 37°C for 45–60 minutes. To determine the location of larvae, 410 loaches were divided into 7 parts, i.e., head, skin, fins, viscera, and 3 portions of musculature (anterior, abdominal, and posterior), and each part digested as described above. After sedimentation of the digested homogenate for about 15 minutes, the residues were repeatedly washed in physiological saline (about 4 times), and were examined under a dissecting microscope. The larvae were readily found, and collected larvae were fixed in hot 10% formalin, cleared, and mounted in lactophenol for taxonomical identification.

Results

The prevalence of gnathostome larvae in the loaches is summarized in Table 1. As shown in Table 1, examinations revealed that 10 of 14 localities were positive. The larvae were found in 84 of

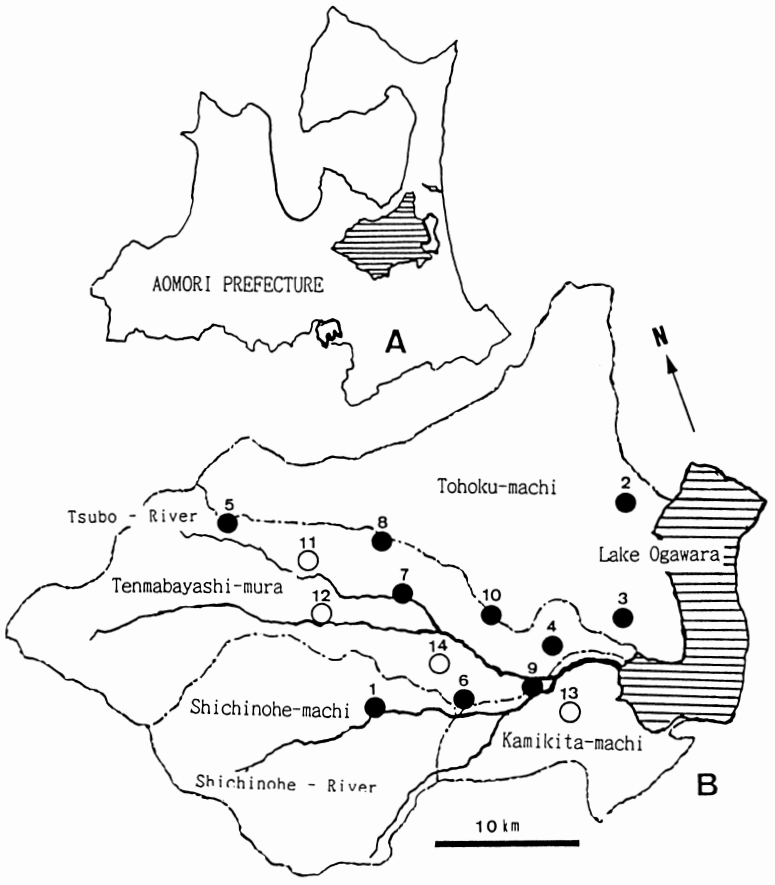

Fig. 1 A: Map showing the location where the present survey was carried out.
B: Map showing 14 location in the Kamikita-gun district, Aomori Prefecture where the loaches were captured for the present survey.
● , positive for larval *Gnathostoma nipponicum* ; ○ , negative.

8,607 loaches (1.0%), and a total of 92 larvae were recovered. The infection rates differed among the localities, and ranged from 0 to 2.1%. The mean intensity in each locality ranged from 0 to 1.2. The relationship between the body length of 7,051 loaches from 10 positive localities and the prevalence of larvae is summarized in Table 2. The larvae were found in loaches larger than 7.0 cm in body length, and as body length increased, so did the prevalence. However, the intensity of larvae was about 1.0 in each body length range of loaches.

In another examination, only 10 larvae were obtained from the muscles of 410 loaches, i.e., 4, 5, and 1 larvae from the anterior, abdominal, and posterior muscles, respectively. No larva was recovered from another parts such as head, skin, fins, and viscera.

Morphological features of the larvae are summarized in Table 3. The body of the larva was almost colorless except for the brownish intestine (Fig. 2). The body dimensions were 745–1,684 μm in length, and 98–186 μm in width. Two pairs of cervical sacs

Table 1 Prevalence and intensity of larval *Gnathostoma nipponicum* in loaches from eastern Aomori Prefecture

No.*	Locality	No. loaches		Prevalence (%)	Intensity (mean)
		examined	positive		
1	Ezobana	1,834	38	2.1	1–4 (1.2)
2	Tanosawa	443	7	1.6	1 (1.0)
3	Toyota	647	10	1.5	1–2 (1.1)
4	Toriguti	230	3	1.3	1 (1.0)
5	Komata	1,652	17	1.0	1 (1.0)
6	Nagasawa	339	2	0.6	1 (1.0)
7	Tsukuta	515	3	0.6	1 (1.0)
8	Futatsumori	485	2	0.4	1 (1.0)
9	Hanamatsu	326	1	0.3	1 (1.0)
10	Syowa	580	1	0.2	1 (1.0)
11	Morinokami	346	0	0	
12	Yojyokai	342	0	0	
13	Saichida	404	0	0	
14	Nakaguki	464	0	0	
	Total	8,607	84	1.0	1–4 (1.1)

* The Nos. are indicated in Fig. 1.

Table 2 Relationship between the body length of loaches and the prevalence of larval *Gnathostoma nipponicum*

Body length range (cm) of loaches	No. loaches		Prevalence (%)	No. larvae recovered	Mean of intensity
	examined	positive			
≦ 6.0	464	0	0	0	0
6.1– 9.0	2,868	20	0.7	21	1.1
9.1–12.0	2,932	44	1.5	50	1.1
12.1–15.0	736	18	2.5	19	1.1
15.1≦	51	2	3.9	2	1.0
Total	7,051	84	1.2	92	1.1

Table 3 Measurements (μm) of *Gnathostoma nipponicum* larvae recovered from loaches

Body length	1,158	(745–1,684)
width	141	(98–186)
Head-bulb height	42	(28–60)
width	90	(54–108)
Esophagus length	461	(321–594)
Cervical sac length	243	(156–363)
Tail length	36	(15–62)
Number of transverse striation	229	(188–267)
Number of hooklets on head-bulb		
1st row	34	(28–39)
2nd row	36	(30–42)
3rd row	39	(25–46)

Larval size determined from 38 specimens, data on hooklets from 34 specimens.
( ): Range

were clearly observed in the region of the club-shaped esophagus. The larvae had three hooklet rows on the head-bulb (Fig. 3). The numbers of hooklets on the 1st to 3rd rows were 28–39, 30–42, and 25–46 respectively, and each one appearing rectangular at its base. The whole larvae were encircled by 188–267 transverse striations consisting of single-pointed minute cuticular spines. Extending posteriorly, the spines gradually decreased in size and density, and finally disappeared near the tip of the tail.

No relationship was detected between the morphological features of larvae and the body length of infected loaches.

Discussion

The advanced third-stage larvae of *G. nipponicum* were first found in naturally-infected in snakes (*Rhabdophis tigrinus*) by Koga and Ishii (1981), afterward, were observed in loaches (*M. anguillicaudatus*), catfish (*Silurus asotus*) and snakes (*Elaphe quadrivirgata*) by Ando *et al.* (1988a, 1992). Therefore, these cold-blooded animal species were considered as the second intermediate and/or paratenic hosts in the natural life cycle of *G. nipponicum*.

In the present study, we found a total of 102 gnathostome larvae in 9,017 loaches. The larvae were 745–1,684 μm in length, and had three hooklet rows on the head-bulb. These and other morphological characteristics of the larvae described above are similar to the descriptions of the advanced third-stage larvae (AdL3) of *G. nipponicum* reported by previous investigators (Miyazaki, 1954; Koga and Ishii, 1981; Ando *et al.*, 1992). Miyazaki (1954) emphasized that the number and shape of the hooklets of the third-stage larvae were very useful in making a specific identification of the genus *Gnathostoma*.

On the other hand, it seemed that the prevalence of larval *G. nipponicum* in loaches were higher in Aomori Prefecture than those of Mie Prefecture (Ando *et al.*, 1992). As to local differences, we considered the distribution and density of infected weasels and the existence of the intermediate hosts of *G. nipponicum* to be essential factors. Even in this survey, the infection rates differed among the localities. And, 4 of 14 localities were negative, although each locality was located adjacent to positive localities as shown in Fig. 1. The results may be related to the tributaries and small streams emanating from contaminated places with the ova of this parasite.

As the body length of loaches increased, the prevalence showed a rise. A consideration of the feeding habits of the loach may help explain the observed relationship between the length of the loach and the higher prevalence. In general, the juvenile loach is usually carnivorous, and then becomes herbivorous during adult period. Although the adult loach is essentially herbivorous, however, it temporarily becomes carnivorous just after the spawning season, and actively feed on *Cyclops*,

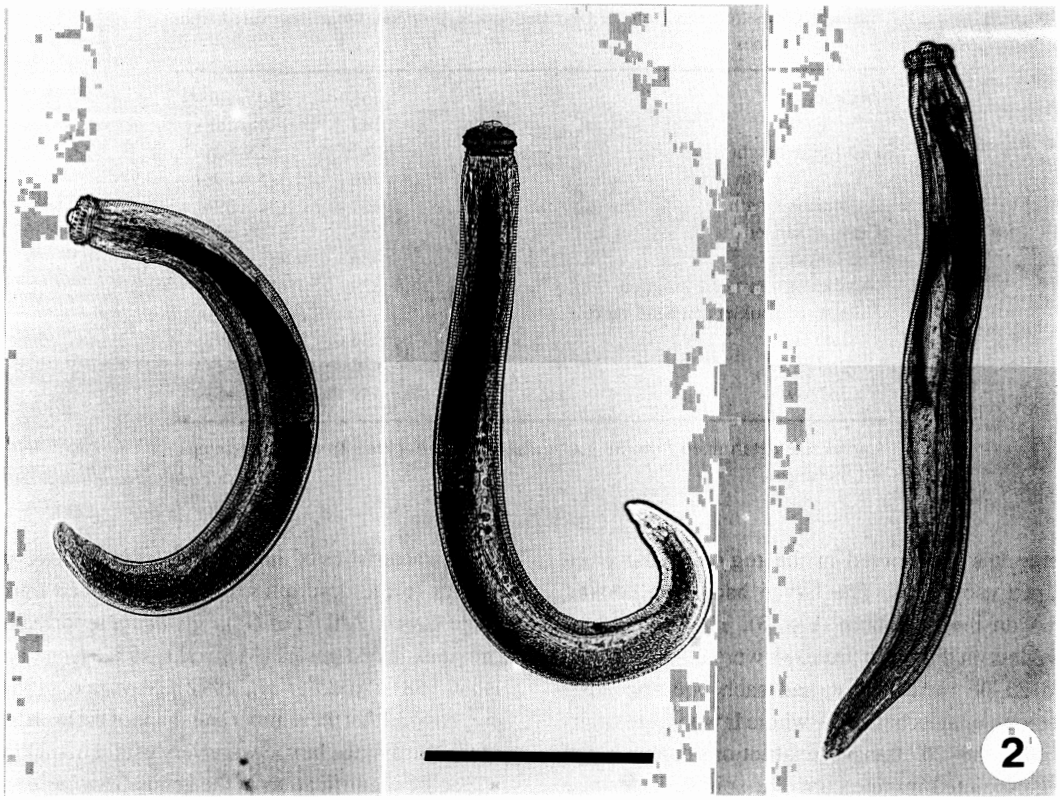

Fig. 2 Lateral view of three *Gnathostoma nipponicum* larvae recovered from loaches (bar=500 μm).
Fig. 3 Lateral view of head-bulb, showing three rows of hooklets on the surface (bar=100 μm).

Bosmina, insect larvae, and small crustaceans (Kubota, 1961). Some species of copepods, including genus *Cyclops*, are known to serve as the first intermediate hosts of *G. nipponicum* (Koga and Ishii, 1981). Therefore, because of feeding habits of loaches as a juvenile and adult, it seems likely that both loaches can become infected with this larvae. Nevertheless, loaches less than 6.0 cm in body length (juvenile) were not found to be infected. This relationship may be related to the quantity of foods,

including the first intermediate hosts such as copepods. A possible explanation might be that adult loaches are infected with larvae by eating many of copepods than juvenile loaches in its after spawning season.

In the examinations to determine the location of larvae within the loach, 10 larvae were only obtained from the muscles. Ando *et al.* (1988a) found encapsulated AdL3 of *G. nipponicum* in the muscles of experimentally-infected loaches, but because we made artificial digestion to recover larvae, it was uncertain whether the larvae were encapsulated or not.

Based on these results, it is confirmed that the Kamikita-gun district in Aomori Prefecture is an endemic area of *G. nipponicum*. Although much has been learned about the life cycle of this nematode, it remains unclear in nature. Further additional investigations are necessary to elucidate the source of infection with this parasite, and to prevent the further occurrence of human gnathostomiasis nipponica.

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