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Prevalence of *Gnathostoma nipponicum* Larvae in *Oncorhynchus masou* (Salmonidae) and *Tribolodon hakonensis* (Cyprinidae) Collected from Eastern Aomori Prefecture, Japan

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Abstract

From July 1992 to September 1995, to clarify the exact source of human gnathostomiasis nipponica occurred in northern part of Honshu, a total of 1,427 freshwater fishes consisting two species was caught in an endemic area of *Gnathostoma nipponicum* in Aomori Prefecture, and was examined for this larvae. Eight of 291 (2.75%) *Oncorhynchus masou* (Salmonidae) and 2 of 1,136 (0.18%) *Tribolodon hakonensis* (Cyprinidae) were infected with gnathostome larvae, and 12 larvae were recovered from 10 fishes. Taxonomically, all the larvae were identified as the advanced third-stage larvae of *G. nipponicum*. From these results, it would seem that Salmonidae and Cyprinidae freshwater fishes, including *O. masou* and *T. hakonensis*, may be important as the source of human gnathostomiasis nipponica in conjunction with custom of eating raw freshwater fishes among the inhabitants at northern Honshu. In addition, it also confirmed newly that two freshwater fish species, which could serve as the second intermediate and/or paratenic hosts of this nematode in nature. This is the first record of naturally-infected *O. masou* and *T. hakonensis* with *G. nipponicum* larvae in Japan.

Key words: *Gnathostoma nipponicum*; human gnathostomiasis; advanced third-stage larva; *Oncorhynchus masou*; *Tribolodon hakonensis*; epidemiology.

Introduction

Gnathostoma nipponicum is a common nematode found in esophageal tumors of weasels in Japan (Yamaguti, 1941; Miyazaki, 1954, 1960; Ashizawa *et al.*, 1978; Ando *et al.*, 1992). Recently, *G. nipponicum* is known as a cause of human gnathostomiasis, as in the case of *G. spinigerum* (Miyazaki, 1960), *G. doloresi* (Ogata *et al.*, 1988), and *G. hispidum* (Kondo *et al.*, 1986). Until now, 3 probable cases (Ando *et al.*, 1988, 1991) and 10 confirmed cases (Sato *et al.*, 1992, five cases unpublished) of human infection with *G. nipponicum* have been recorded in Japan. As the possible agents of human gnathostomiasis nipponica, native loaches (two cases of Mie Prefecture and one case of Aomori Prefecture) and catfish (one case of Okayama Prefecture) were suspected. In the cases of Akita and Aomori prefectures in northern Honshu, however, 9 patients who had eaten neither raw loach nor catfish, although had a common past history of eating raw flesh of several kinds of freshwater fish, including kakone (*Salmo nerca nerca*), a kind of salmonoid fish, have been reported (Sato *et al.*, 1992; unpublished data). Consequently, from the latter cases, it suggests the possibility of larval infection in another commonly eaten freshwater fishes. On the other hand, in human cases of *G. doloresi* infection in Miyazaki Prefecture, over 20 patients who often ate various wild mammals and freshwater fishes including brook trout (Salmonidae) had been reported

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(Ogata *et al.*, 1988, 1992; Miyamoto *et al.*, 1994). Subsequently, Nawa *et al.* (1993) found a freshwater fish, *Lepomis macrochirus* (common name: bluegill), infected with larvae of *G. doloresi* in Miyazaki Prefecture.

The natural life cycle of *G. nipponicum* is incompletely known. It is important to clarify the exact source of human infection and to prevent the further occurrence of this parasitic zoonoses. We examined two species of freshwater fish which have been often eaten customarily as a raw or under-cooked flesh among the inhabitants at Akita and Aomori prefectures.

Materials and Methods

From July 1992 to September 1995, a total of 1,427 freshwater fishes consisting two species, *Oncorhynchus masou* and *Tribolodon hakonensis* (Table 1), was collected from the rivers located in eastern Aomori Prefecture, an endemic area of *G. nipponicum* confirmed by previous surveys (Oyamada *et al.*, 1995a, b, c). Prior to dissection, each fish was weighted and its body length was measured. And then, each fish was cut into small pieces or minced, and digested individually in an artificial gastric juice (Oyamada *et al.*, 1995b) at 37°C for 50–70 minutes. After digestion, the homogenate was passed through a stainless mesh, sedimented for about 15 minutes, and the sediments were examined under a dissecting microscope. In 40 *O. masou* larger than 18 cm in length of fish collected from June to September 1995, the stomachs were examined to know their ingested food, and each fish was divided into three parts (head and viscera, abdominal wall, and other sites of musculature) to determine the location of larvae before artificial digestion. The recovered larvae were readily fixed with hot 10% buffered formalin, cleared and mounted with lactophenol for taxonomical observation.

Results

As shown in Table 1, we found infected fishes with gnathostome larvae in 8 of 291 *O. masou* (common Japanese name: "Yamame" and/or "Sakuramasu") (Fig. 1) and 2 of 1,136 *T. hakonensis* (common Japanese name: "Ugui"). The infection rate in each fish species was 2.75% in the former and was 0.18% in the latter. Each infected fish harbored one or two larvae, from which a total of 12 larvae was recovered. The relationship between fish body length of the two species and seasonal prevalence of infection is summarized in Table 2. Eight infected *O. masou* were larger than 22 cm in body length, and these were largely limited to the fishes collected in September–November (Table 2). The positive cases were 6 males, 1 female, and not determined 1 fish. While, in two infected *T. hakonensis*, each body length was 19 cm and 11 cm, and each fish was collected in August and November, respectively.

To determine the location of larvae in 5 *O. masou* collected from June to September 1995, 5 of 7 larvae were found in the abdominal wall, and 2 were in the head and visceral portions. By examinations of the stomach in 40 *O. masou*, various food such as insect, insect larvae, small fishes, and frogs were found in 26. Additionally, one to three loaches were seen in 5 *O. masou* collected in August and September 1995 (Fig. 1). Moreover, two gnathostome larvae were recovered from one of them which ate three loaches.

Table 1 Prevalence of *Gnathostoma nipponicum* larvae in *Oncorhynchus masou* and *Tribolodon hakonensis* collected from eastern Aomori Prefecture

Examined fish species	Body length range of fish (cm)	No. of fish		No. of larvae recovered	Incidence (mean)
		examined	infected (%)		
Oncorhynchus masou	8–37	291	8 (2.75)	10	1, 2 (1.25)
Tribolodon hakonensis	7–31	1,136	2 (0.18)	2	1 (1.00)
Total		1,427	10	12	

Table 2 Relationship between seasonal prevalence of infection with *Gnathostoma nipponicum* larvae and body length of two fish species, *Oncorhynchus masou* and *Tribolodon hakonensis*, collected from July 1992 to September 1995

Fish species	Collected season	Body length range (cm)					Total
		≦10	11–14	15–18	19–22	23≦	
O. masou	Mar. – May	–	–	0/7	0/11	0/2	0/20
	June – Aug.	0/2	0/21	0/15	0/22	0/29	0/89
	Sept. – Nov.	0/1	0/58	0/64	1/21	7/38	8/182
	Total	0/3	0/79	0/86	1/55	7/69	8/291
T. hakonensis	Mar. – May	0/47	0/8	0/9	0/3	0/4	0/71
	June – Aug.	0/54	0/98	0/140	1/71	0/19	1/382
	Sept. – Nov.	0/149	1/196	0/231	0/81	0/26	1/683
	Total	0/250	1/302	0/380	1/155	0/49	2/1,136

No. of positive specimen/No. of specimen examined.

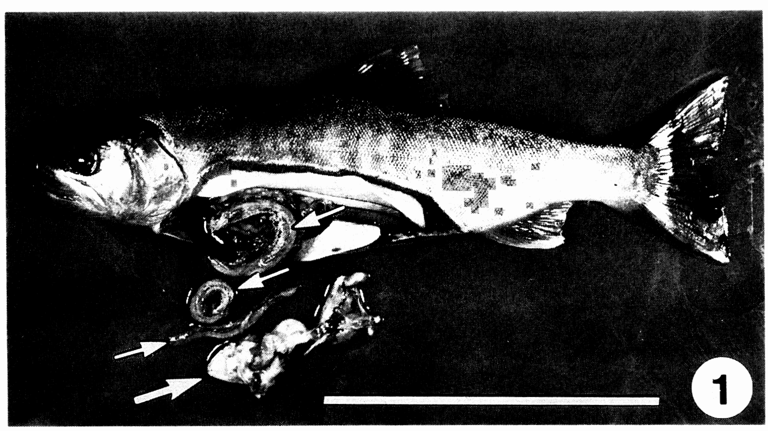

Fig. 1 View of the stomach in a *Oncorhynchus masou* (Bar = 10 cm). Ingested food showing three loaches (small arrows) and one frog (large arrow).

The morphological features and the dimensions of the larvae are summarized in Table 3, and compared with those of AdL3 of *G. nipponicum* from loaches reported previously by present authors (Oyamada *et al.*, 1995b). As shown in Table 3, the taxonomical features of the present larvae from both *O. masou* and *T. hakonensis* were similar to each other. The larvae were almost colorless except for the brownish intestine (Fig. 2a). Their mean size (μm) were: body 1,434 × 130, head-bulb 96 × 47, esophagus 499, cervical sac 257, and tail 31 in length, respectively. They had three rows of hooklets on head-bulb, and the number of hooklets of each row from 1st to 3rd was 30, 34, and 39 (Fig. 2b). The whole body was encircled by 230 transverse striation of single-pointed minute spines. As they extend posteriorly, the spines gradually decreased in size and density, and finally disappeared near the tip of

Table 3 Comparison of measurements (μm) of *Gnathostoma nipponicum* larvae obtained from three freshwater fish species collected from eastern Aomori Prefecture

	Present paper		Oyamada *et al.* (1995b)
Host (No. of specimens)	*O. masou* (10)	*T. hakonensis* (2)	*M. anguillicaudatus* (79)
Body length	1,068–1,872	960, 1,468	745–1,684
width	110–151	108, 128	98–186
Head-bulb height	38–53	39, 56	28–60
width	85–107	87, 113	54–108
Esophagus length	356–617	342, 514	321–594
Cervical sac length	186–309	212, 279	156–363
Tail length	18–68	33, 51	15–62
No. of transverse striation	214–253	226, 197	188–267
No. of hooklets on head-bulb			
1st row	29–36	26, 29	28–39
2nd row	31–38	30, 31	30–42
3rd row	33–44	36, 38	25–46

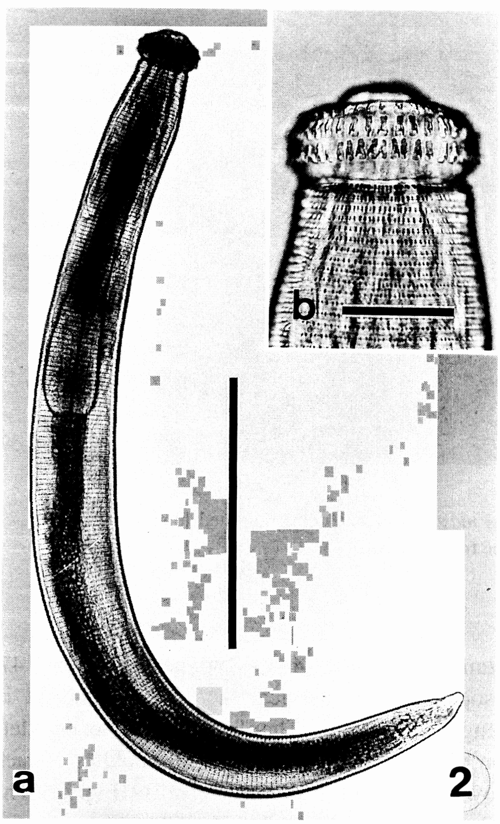

Fig. 2 a: Lateral view of a larva recovered from the *Oncorhynchus masou* (Bar = 500 μm). b: Head-bulb showing three rows of hooklets on the surface (Bar = 50 μm).

the tail. From these findings, all of 12 recovered larvae were identified as the advanced third-stage larvae (AdL3) of *G. nipponicum*.

Discussion

In Japan, human gnathostomiasis is one of the important food-borne parasitic zoonoses caused by Japanese custom of eating raw or under-cooked flesh of freshwater fish, prepared as "sashimi", "arai", or "nuta". Although the diseases caused by *G. doloresi* and *G. nipponicum* are suspected to be infected by eating raw native freshwater fish, the exact route of infection in many instances are not completely cleared. Since 1992, to determine the direct source of infection to human beings and to elucidate the natural life cycle of *G. nipponicum*, we have performed conducting surveys for this nematode in an endemic area of eastern Aomori Prefecture.

In the present study, eventually, we found naturally-infected *O. masou* (Salmonidae) and *T. hakonensis* (Caprinidae), and obtained a total of 12 AdL3 of *G. nipponicum*. The morphological features and dimensions of the larvae coincide well with the descriptions of the AdL3 obtained from loaches (*Misgurnus anguillicaudatus*) reported by previous investigators (Ando *et al.*, 1988; Oyamada *et al.*, 1995b). The infection rate was 2.75% in *O.*

masou and was 0.18% in *T. hakonensis*. The prevalence of AdL3 was higher in *O. masou* than that in loaches reported previously from the same endemic area (Oyamada *et al*., 1995b). Although the detection of AdL3 from *T. hakonensis* was very low level, it clearly confirmed that this fish species was also susceptible to AdL3 of *G. nipponicum*. In the past several cases of human gnathostomiasis, a kind of traut, *O. m. masou* for *G. doloresi* (Ogata *et al*., 1988; Nawa *et al*., 1989; Miyamoto *et al*., 1994) and *S. n. nerka* for *G. nipponicum* (Sato *et al*., 1992) have been suspected as the source of infection. Nevertheless, these gnathostome larvae had not been found in the salmonoid freshwater fishes before our present discovery of *G. nipponicum* larvae from *O. masou*. We could also detect the AdL3 of *G. nipponicum* from *T. hakonensis*. Thus, this is the first record of *O. masou* and *T. hakonensis* infected with AdL3 of *G. nipponicum* in Japan. And, it also confirmed newly that two freshwater fish species, which could serve as the second intermediate and/or paratenic host of this nematode in nature. From these results, it was supposed that *G. nipponicum* larvae were more widely spreaded in freshwater fishes in this endemic area.

According the location of the larvae, 5 of 7 larvae were found in the abdominal walls of *O. masou*. These findings suggested that the AdL3 migrated into the muscles of the abdominal wall with high rate. Ando *et al*. (1988) reported encapsulated AdL3 of *G. nipponicum* in the muscles of loaches. However, it was uncertain whether the larvae obtained at this time were encapsulated or not, because we used artificial digestion to recover larvae from the tissues.

We found the high prevalence in *O. masou* larger than 22 cm in body length. In general, adults and large-sized fish (larger than about 20 cm) of Salmonidae prey chiefly on small fishes by their feeding habits (Miyadi *et al*., 1988). In fact, ingested loaches were observed in 5 *O. masou*, and the prevalence of AdL3 was only found in large-sized fishes. Based on these findings, we speculated that the direct sources to *O. masou* might be loaches. In addition, the prevalence was limited to the season between September and November. It seems likely that this fact attributes to the feeding habits of *O. masou* and the ecological characteristics of loach in this season. In Aomori Prefecture, paddy fields and ditches around irrigation creeks constitute temporary waters flooded during May to August. Saitoh *et al*. (1988) reported that several small fishes, including loaches, frequently entered the temporary waters, and some of them utilized these waters as spawing sites. However, the waters decreased gradually in Fall season in this survey areas. For this reason, it was considered that many loaches infected with AdL3 of *G. nipponicum* in this area, moved from paddy fields and irrigation creeks into the permanent waters, such as rivers, ponds, and lakes, where salmonoid fishes are living. Therefore, it would seem that *O. masou* prey upon various small fishes, including infected loaches with AdL3, in this season. As to *T. hakonensis*, the AdL3 infection was found in a small-sized (10 cm) and a middle-sized (19 cm) fish. Generally, *T. hakonensis* is known as an omnivorous fish (Miyadi *et al*., 1988). Because of its habitats and feeding habits, it seems that this fish will have the larvae by eating both copepods and loaches. Recently, we confirmed experimentally that *T. hakonensis* was susceptible to the early third-stage larvae (EaL3) from copepods, and it developed into the AdL3 in this fish (Ohta *et al*., 1995).

In human gnathostomiasis nipponica occurred in Akita and Aomori prefectures, the Salmonidae and Cyprinidae fishes seems to be a good possibility as the sources of infection, because these freshwater fishes are more commonly eaten as a raw flesh. Loach and catfish, which were infected with *G. nipponicum* larvae in eastern Aomori Prefecture (Oyamada *et al*., 1995a, b), were not eaten as a raw flesh among the inhabitants in these areas. Furthermore, it speculated that not only *O. masou* but also another salmonoid fishes, such as *O. mykiss* ("Nijimasu") and *S. leucomaenis* ("Iwana"), might be infected with *G. nipponicum* larvae, because of their feeding habits, habitats, and another ecological similarities. In addition, it is also suspected that the another species of Cyprinidae fishes are possibly susceptible to the larvae and may be able to preserve it in nature.

The freshwater fish of these Salmonidae and Cypridae are native and popular fish in freshwater reservoirs, rivers, and lakes, and they are distributed widely in Japan. They had often been eaten as a raw flesh among the inhabitants because of Japanese custom of eating. Therefore, to prevent the further occurrence of human gnathostomiasis, the public should be informed of the danger of eating raw freshwater fishes. As the involvement of these fishes in the natural life cycle of genus *Gnathostoma* is rather a recent event, we consider that this report is useful to prevent the further occurrence of human gnathostomiasis in Japan.

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