[Jpn. J. Parasitol., Vol. 43, No. 2, 105–109, April, 1994]

Prevalence of Larval *Gnathostoma* in Snake-Head Fish from Northeast India with Reference to Their Morphological Findings

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(Accepted for publication; March 16, 1994)

Abstract

A total of 24 snake-head fish (21 *Channa striata* and 3 *C. marulius*) purchased in the towns of Jalpaiguri and Muzaffarpur, in Northeast India, in February 1989, were examined for the prevalence of the advanced third-stage larvae of the genus *Gnathostoma*. Twenty-one larval *Gnathostoma* were discovered in 16 *C. striata* from Jalpaiguri, whereas, the larvae were not found in *C. striata* nor *C. marulius* from Muzaffarpur. The shape and number of hooklets on the head-bulb closely resembled those of *G. spinigerum*. The number of nuclei in each intestinal cell of these larvae was also similar to that of *G. spinigerum*. According to these results, the advanced third-stage larvae from Jalpaiguri were considered to be *G. spinigerum*.

Key words: *Gnathostoma spinigerum*, Northeast India, advanced third-stage larvae, hooklet number, intestinal cell

Introduction

We have previously carried out morphological and ecological observations of the genus *Gnathostoma* from various areas in the world (Setasban *et al.*, 1991; Akahane *et al.*, 1994). It is well known that the genus *Gnathostoma* is distributed in Bangladesh (Bashirullah, 1972; Rahaman and Khan, 1978), while in Pakistan, there have been few reports on the positive prevalence of gnathostomes. We have examined for the prevalence of the larval *Gnathostoma* in Hyderabad, Southern part of Pakistan, however, no larva was found in any fresh-water fish (Sano *et al.*, 1988). A few years ago, we had an opportunity to examine fresh-water fish for the gnathostomes in Northeast India. It is thus considered to be of interest to examine the fresh-water fish for larval *Gnathostoma* in India, which is located between Bangladesh and Pakistan. We herein report the results of those observations on the Indian *Gnathostoma*.

Materials and Methods

In February 1989, snake-head fish were purchased at several markets in the towns of Jalpaiguri, West Bengal Province, and Muzaffarpur, Bihar Province, in Northeast India. The muscles of these fish were sliced, pressed between two glass plates and examined either with naked eyes and/or under a stereomicroscope for the presence of *Gnathostoma* larvae. Hooklet features in some larvae were observed without fixation under a light microscope.

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This study was supported in part by a Grant-in Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (No. 63041161).

The morphology of the hooklets and the presence of body spines at the terminal end of the worm were examined by using a scanning electron microscope. The methods for preparation of the specimens for scanning electron microscopy were the same as those described previously (Koga *et al.*, 1991).

Some larvae fixed in 10% formalin solution were inserted into the unfixed intestinal canals of rats and re-fixed in 10% formalin solution. The tissues of intestinal canals containing larvae were dehydrated, cleared and embedded in paraffin by a routine procedures. The cross sections 5 μm in thickness were made and stained with hematoxylin and eosin. In order to count the number of hooklets, the head-bulb was cut off from the body and embedded in Faure's solution.

Results

Twenty-four snake-head fish (16 *Channa striata* from Jalpaiguri, and 5 *C. striata*, 3 *C. marulius* from Muzaffarpur) were examined. The prevalence of larval *Gnathostoma* in these fish is summarized in Table 1. A total of 21 advanced third-stage larvae were found in *C. striata* from Jalpaiguri whereas no larva was detected in either *C. striata* or *C. marulius* from Muzaffarpur. The prevalence of *C. striata* with larval *Gnathostoma* was 50% in Jalpaiguri, and the intensity of infection varied from 1 to 7 (Table 1).

The advanced third-stage larva of the Indian *Gnathostoma* were 2.5–3.5 mm in body length, 280 μm in body width, and 1.1 mm in the distance between the anterior end of body and the posterior end of esophagus (Fig. 1). The base of the hooklets was oblong in shape (Fig. 2). The hooklet features under scanning electron microscopy showed also oblong base and almost equal in size (Fig. 3). The number of hooklets in the 2 larvae were 42 and 40 (mean: 41.0) in the first row, 44 and 43 (43.5) in the second, 45 and 43 (44.0) in the third, 46 and 44 (45.0) in the fourth, respectively. The whole larval body was covered with transverse rows of single pointed spines, but their size and density gradually decreased toward the posterior end (Figs. 3, 4). A pair of cervical papillae were situated between the 13th and the 15th transverse rows of the body spines (Fig. 3).

The intestinal wall in cross section consisted of a single layer with many intestinal cells (Fig. 5). Out of a total of 107 cells in cross sections, 21 cells were not observed any nucleus, while the other 86 cells contained from 1–6 (mean: 2.6) nuclei.

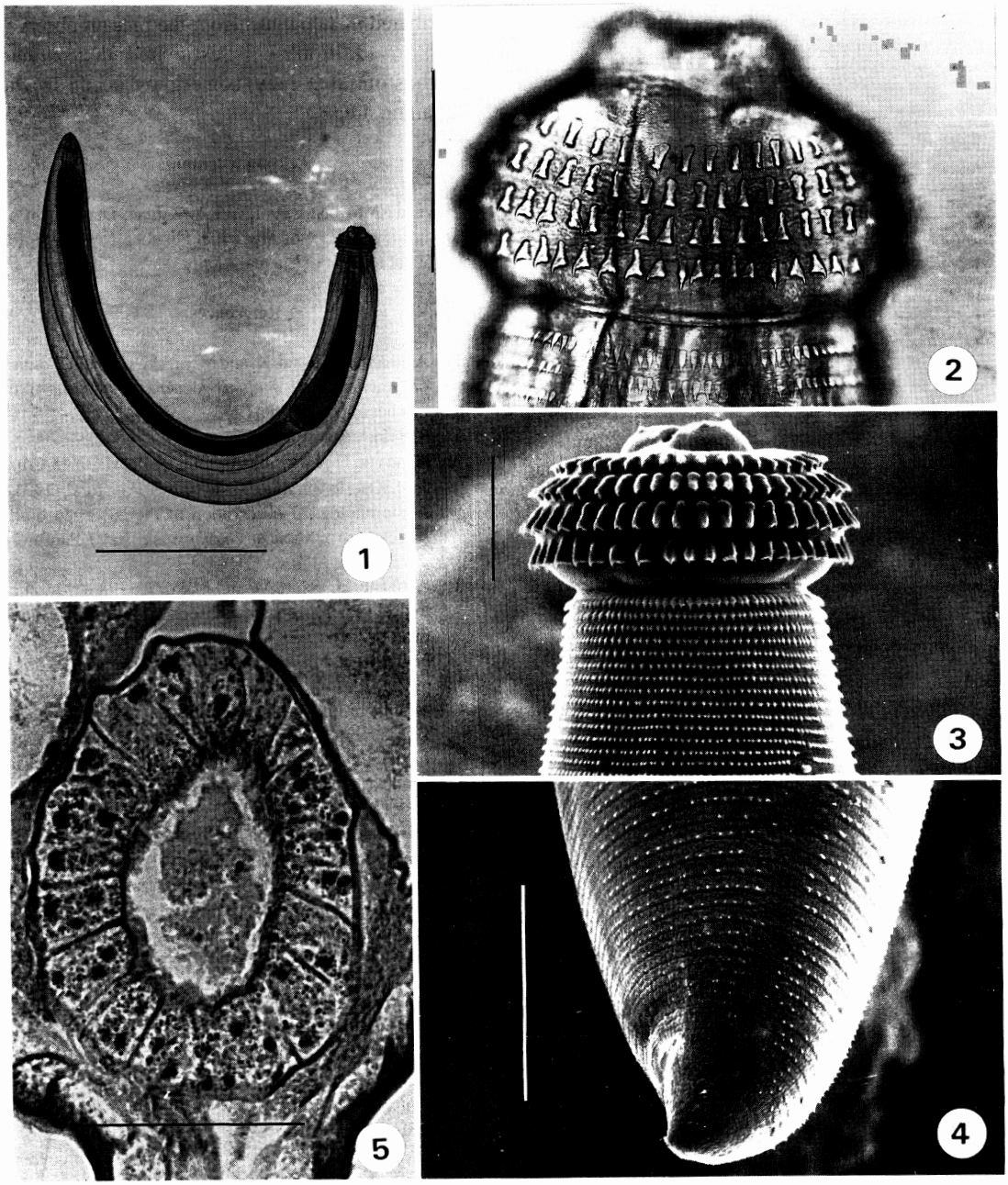
Discussion

The hooklet features of the Indian larvae were very similar to those of *G. spinigerum* described by Miyazaki (1960) and Anantaphruti *et al.* (1982). However, the Indian larval *Gnathostoma* had fewer hooklets on the head-bulb than *G. spinigerum* because the average number of hooklets in *G. spinigerum* distributed in Thailand and Japan are 43–44, 46–47, 48–50, 52–53 in the first, second, third and the fourth rows, respectively (Table 2). The average number of nuclei in each intestinal cell of the Indian *Gnathostoma* larvae was also fewer than that of *G. spinigerum*, which has 1–7 (mean: 3.4) nuclei per cell (Akahane *et al.* 1994).

Miyazaki (1960) reported that the number and the shape of the hooklets on head-bulb in the ad-

Table 1 Prevalence of larval *Gnathostoma* in snake-head fish purchased at two towns in Northeast India

Town	Fish			*Gnathostoma* larva	
	Species	No. examined	Total Length range (mean) (cm)	prevalence (%)	intensity range (mean)
Jalpaiguri	*Channa striata*	16	36–56 (44.3)	50	1–7 (2.6)
Muzaffarpur	*Channa striata*	5	47–53 (49.2)	0	0
Muzaffarpur	*Channa marulius*	3	28–48 (39.7)	0	0



Figs. 1–5 Advanced third-stage larvae of the Indian *Gnathostoma*.
Fig. 1 Lateral view of a whole larva (scale: 1 mm).
Fig. 2 A head-bulb under light microscopy (scale: 100 μm).
Fig. 3 A head-bulb under scanning electron microscopy (scale: 100 μm).
Fig. 4 The terminal end of the body under scanning electron microscopy (scale: 50 μm).
Fig. 5 A cross section of the intestine (scale: 50 μm).

Table 2 The average number of hooklets in the Indian larval *Gnathostoma*

Row	1st	2nd	3rd	4th
Indian larvae (present record)				
No. 1	42	44	45	46
No. 2	40	43	43	44
G. spinigerum				
Thailand*	43.3	46.0	48.5	52.7
Japan†	44.3	47.3	49.6	52.0
G. hispidum‡	38.3	40.5	41.8	46.0
G. doloresi§	38.3	37.9	35.6	35.7
G. vietnamicum‖	46–48	46–48	46–48	46–48

*From Akahane *et al.* (1994) †From Miyazaki (1960)
‡From Akahane *et al.* (1982) §From Mako & Akahane (1985)
‖From Daengsvang (1980)

vanced third-stage larvae were very useful in making a specific identification of the genus *Gnathostoma*. Akahane *et al.* (1986) noticed that the number of nuclei in the intestinal cells in advanced third-stage larvae differs among the various species generally. As the morphology of the hooklet and intestinal cell of the Indian larvae closely resembled those of *G. spinigerum*, the advanced third-stage larvae from Jalpaiguri were therefore identified to be *G. spinigerum*. It is thus considered that the difference in the number of hooklets and nuclei in the intestinal cells between the Indian larval *Gnathostoma* and larval *G. spinigerum* is merely an intraspecific variation due to geographic distribution.

Mitter (1910) and Maplestone (1930) first found the adult worms of *G. spinigerum* and *G. doloresi* from the respective final hosts in India. However, no systematic study has ever been done on the prevalence of larval *Gnathostoma* in the intermediate hosts in India. Our study is the first to detect larval *Gnathostoma spinigerum* in snake-head fish from Jalpaiguri. The first human gnathostomiasis in India was reported by Maplestone (1929) in Jalpaiguri. According to his study, the larval *Gnathostoma* was removed from a patient but no taxonomical identification was made. The agent of the first human case of India is estimated to have most likely been the advanced third-stage larva of *G. spinigerum*, because it have been clarified that *G. spinigerum* is distributed in Jalpaiguri from the present observation. In addition, it is estimated that human gnathostomiases may sometimes occur in the Jalpaiguri area.

Acknowledgments

We wish to thank Professor T. Kifune, Department of Parasitology, School of Medicine, Fukuoka University, for his critical reading of the manuscript.

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