[Jpn. J. Parasitol., Vol. 36, No. 6, 424–426, December, 1987]

Research Note

Morphological Features of "Large-Type" Larval *Gnathostoma* in Loaches from Mainland China

HIROSHIGE AKAHANE[1] AND TOSHIHIRO MAKO[2]

(Received for publication; July 3, 1987)

Key words: larval *Gnathostoma*, "large-type" larva, imported loach, cross section, number of hooklets, intestinal epithelial cell

In recent years, many living loaches (*Misgurnus anguillicaudatus*) have come to be imported to Japan from foreign countries such as Mainland China, Korea and Taiwan. Since 1980, many cases of human gnathostomiases have occurred in Japan due to eating these raw loaches (Morita *et al.*, 1984).

In loaches from Mainland China, there are at least two types of *Gnathostoma* larvae, one remarkably small in size measuring about 610 μm in body length, and the other, larger measuring 2–4 mm in length. The former is the "small-type" to which most larvae found in imported loaches belong. The latter, the "large-type", is rather rare. The "small-type" larva was identified by the authors as the early third stage larva of *G. hispidum*, which is not distributed in Japan (Akahane *et al.*, 1982). The "large-type" larva is considered the advanced third-stage larva belonging to the genus *Gnathostoma* but its specific identification remains to be made.

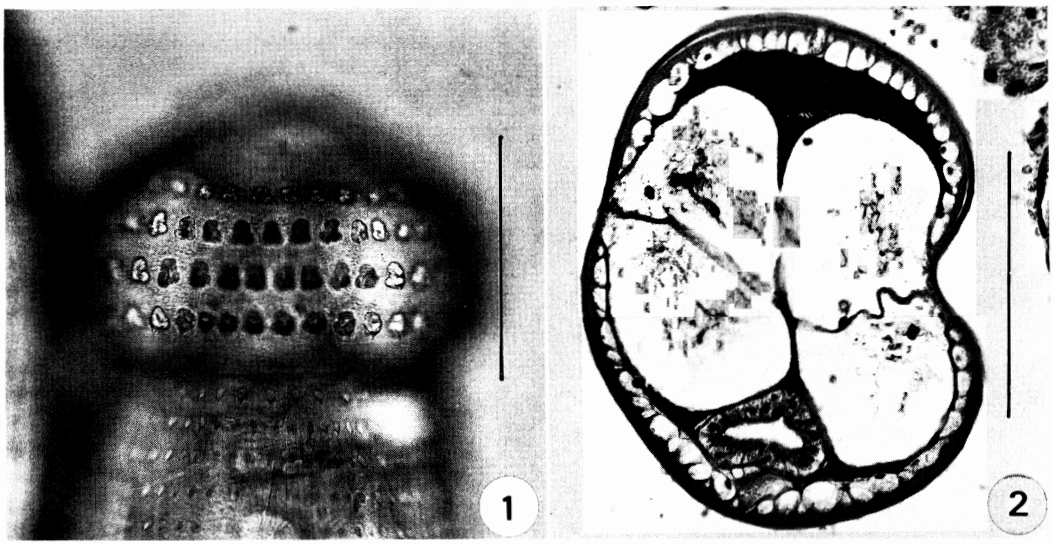
According to Miyazaki (1960), advanced third-stage larvae of the genus *Gnathostoma* can be distinguished on the basis of differences in the number and features of hooklets on the head bulb. Recently, the authors reported morphological differences in the cross-sectioned intestinal walls of advanced third-stage larvae among 3 species of *G. spinigerum*. *G. hispidum* and *G. doloresi* (Akahane *et al.*, 1986). *G. spinigerum* had 3–7 nuclei in each intestinal epithelial cell whereas only one large nucleus was observed in that of *G. hispidum*.

In the present study, taxonomical analysis of "large-type" larval *Gnathostoma* was carried out.

Some examinations were made of the number and features of hooklets on the head bulb and of the number of nuclei of intestinal epithelial cells in the "large-type" larval *Gnathostoma*. Some larvae collected from loaches imported from Mainland China were observed microscopically for hooklet features without fixation, and other larvae were fixed in 10% buffered formalin solution. Two larvae were cut at the junction of the head-bulb and body. The separated head bulbs were enface viewed using Faure's embedding solution to count the hooklets. Several fixed larvae were embedded in pig liver and were fixed again in formalin solution. The pieces of liver were dehydrated, cleared and embedded in paraffin by a routine procedure. Serial sections 4 μm in thickness were stained with hematoxylin and eosin.

The hooklets on the head bulb are shown in Fig. 1. Each hooklet had an irregular four-sided base resembling those of *G. hispidum* and *G. doloresi* as described by Miyazaki (1960) and the present authors (Akahane *et al.*, 1982; Mako and Akahane, 1985). However,

[1] *Department of Parasitology, School of Medicine, Fukuoka University, Fukuoka 814-01, Japan.*
[2] *Fukuoka City Institute of Public Health, Fukuoka 810, Japan.*
赤羽啓栄（福岡大学医学部寄生虫学教室）
真子俊博（福岡市衛生試験所）



Figs. 1–2. Micrographs of the "large-type" larva (scale = 100 μm).
Fig. 1 Head bulb.
Fig. 2 Cross section through intestinal region.

the size of the hooklets in the first row was conspicuously smaller than that of other rows. This feature was noted more in *G. doloresi* than in *G. hispidum*. The number of hooklets in 2 "large-type" larvae was 36 or 37 in the first row, 37 in the second row, 41 in the third row, and 44 or 46 in the fourth row, as shown in Table 1. These findings indicated the number of hooklets of "large-type" larvae to be essentially the same as that of larval *G. hispidum*.

The intestinal wall of "large-type" larvae consisted of a single layer of many elongate epithelial cells. Although a few cells contained more than two nuclei, most columnar cells each possessed only a single nucleus, as shown in Fig. 1. This feature was also observed in advanced third-stage larvae of *G. hispidum*. It is thus clear that the "large-type" larval *Gnathostoma* does not belong to *G. spinigerum* which has 2–7 nuclei in each intestinal epithelial cell. "Large-type" larvae in loaches imported from Mainland China appear to be the advanced third-stage larvae of *G. hispidum*.

The present results are consistent with those of scanning electron microscopic observations by Koga *et al.* (1987), who could find no micromorphological difference between "large-type" gnathostome larvae and larval *G. hispidum*. Furthermore, the authors noted that "large-type" larvae from imported loaches

Table 1 Number of hooklets of the "large-type" larvae

Row	Large-type		*G. spinigerum*	*G. hispidum*
	No. 1	No. 2		
1	36	37	44.3	38.3
2	37	37	47.3	40.5
3	41	41	49.6	41.8
4	44	46	52.0	46.0

1) From Miyazaki (1960) based on measurements of 23 samples.
2) From Akahane *et al.* (1982) based on measurements of 4 samples.

failed to infect cat, a common final host of *G. spinigerum*.

Assuming the above observations to be valid, it may be said that there are two different stages of the *G. hispidum* larva, early and advanced third-stages, in loaches imported from Mainland China. But according to Wang *et al.* (1976) and Akahane *et al.*, (1983), the early third stage-larvae of *G. hispidum*, which were experimentally fed to freshwater fish such as gold fish, remained in the same stage without any subsequent development. Thus, an attempt at this time to make a specific identification of "large-type" larvae appears premature.

In general, taxonomy of a parasite must be conducted on the basis of the morphology of the adult worm. It would be necessary to infect pig experimentally in order to identify "large-type" larvae using adult specimens.

The authors wish to thank Professor T. Kifune, Department of Parasitology, School of Medicine, Fukuoka University, for his critical reading of the manuscript. Thanks are also due to Miss E. Yamamoto for her technical assistance.

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)
2) Akahane, H., Iwata, K., Miyazaki, I. and Mako, T. (1983): Studies on the life cycle of *Gnathostoma hispidum* Fedchenko, 1872. (1) Experimental studies on susceptibility of various vertebrates to the early third-stage larvae from loaches. Jpn. J. Parasitol., 32, 459–464. (in Japanese)
3) Akahane, H., Sano, M. and Mako, T. (1986): Morphological difference in cross sections of the advanced third-stage larvae of *Gnathostoma spinigerum*. *G. hispidum* and *G. doloresi*. Jpn. J. Parasitol., 35, 465–467.
4) Koga, M., Ishibashi, Y., Ishii, Y. and Nishimura, T. (1987): *Gnathostoma* and human gnathostomiasis caused by ingesting imported fresh water fish. 5. Scanning electron microscopic observations on the large type of gnathostome larvae obtained from loaches. Jpn. J. Parasitol., 36 (2, suppl.), 76. (in Japanese)
5) Mako, T. and Akahane, H. (1985) On the larval *Gnathostoma doloresi* found in a snake, *Dinodon semicarinatus* from Amami-Oshima Is., Japan. Jpn. J. Parasitol., 34, 493–499. (in Japanese)
6) Miyazaki, I. (1960): On the genus *Gnathostoma* and human gnathostomiasis with special reference to Japan. Exp. Parasit., 9, 338–370.
7) Morita, H., Segawa, T., Nishiyama, T., Yamada, S., Yagi, J., Chin, I., Shimazu, K., Uno, T., and Araki, T. (1984): Gnathostomiasis cases caused by imported loaches. J. Nara Med. Assoc., 35, 607–619. (in Japanese)
8) Wang, P., Sun, Y. and Zhao, Y. (1976): On the development of *Gnathostoma hispidum* in the intermediate host with special reference to its transmission route in pigs. Acta Zool. Sinica, 22, 45–52. (in Chinese with English summary)