[Jpn. J. Parasitol., Vol. 35, No. 5, 465–467, October, 1986]

Research Note

Morphological Difference in Cross Sections of the Advanced Third-Stage Larvae of *Gnathostoma spinigerum*, *G. hispidum* and *G. doloresi*

HIROSHIGE AKAHANE[1], MOTOHITO SANO[2] AND TOSHIHIRO MAKO[3]

(Received for publication; April 14, 1986)

Key words: *Gnathostoma spinigerum, G. hispidum, G. doloresi,* advanced third-stage larva, cross section

It is well known that three species of the genus *Gnathostoma, G. spinigerum, G. doloresi* and *G. nipponicum* are distributed in Japan. Recently several workers reported that *G. hispidum* was imported with living loaches, *Misgurnus anguillicaudatus,* from foreign countries (Akahane *et al.,* 1982; Akahane and Mako, 1984; Koga *et al.,* 1984, 1985; Takakura *et al.,* 1985).

According to Miyazaki (1960), the advanced third-stage larvae of the genus *Gnathostoma* are distinguishable by the difference of the number of rows and features of hooklets. But those taxonomical characteristics were rarely observed in biopsy for the identification of larvae removed from human skin.

In the present study, cross sections of the advanced third-stage larvae are compared among 3 species, *G. spinigerum, G. hispidum* and *G. doloresi.* The results proved that the cross sections of intestinal regions of larvae presented characteristics of distinguishing 3 species.

This study was supported in part by the research grant No. 59372001 from the Ministry of Education, Science and Culture of Japan.

[1]*Department of Parasitology, School of Medicine, Fukuoka University, Fukuoka 814-01, Japan.*

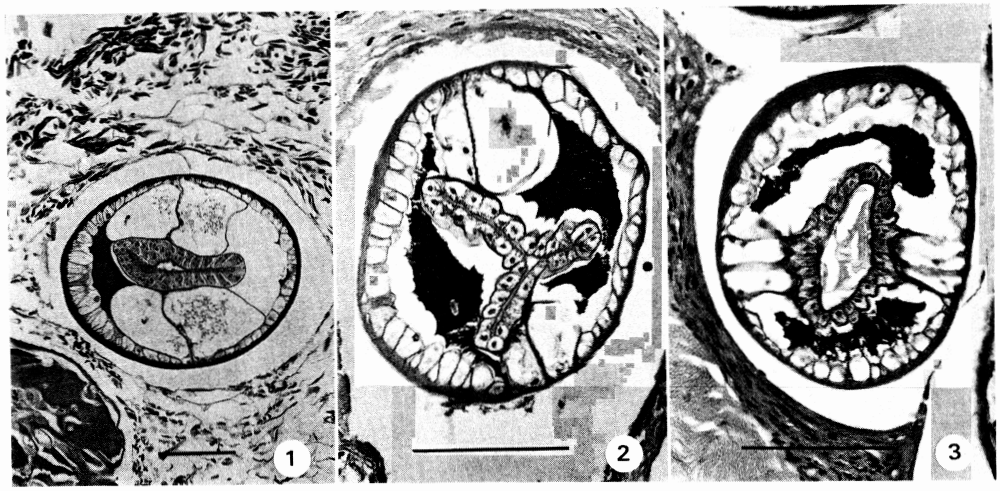
[2]*Department of Parasitology, Hamamatsu University School of Medicine, Handa-cho, Hamamatsu 431-31, Japan.*

[3]*Fukuoka City Institute of Public Health, Fukuoka 810, Japan.*

The advanced third-stage larvae of *G. spinigerum* were obtained from the muscle of mice experimentally infected with the larvae collected from Thai freshwater fishes. Those of *G. hispidum* were collected form the muscle of rats experimentally infected with the early third-stage larvae from viscera of loaches imported from the mainland China. Larvae of *G. doloresi* were brought from the muscle of a snake, *Dinodon semicarinatus,* which is an intermediate host of this parasite in Amamioshima Island (Mako and Akahane, 1985).

The larvae collected from the muscle of the experimental rodents and the snake were fixed in 10% buffered formalin solution and were embedded in a pig liver. Pieces of the liver containing the larvae and/or those encapsulated with host tissues were fixed in the formalin solution then, in routine procedures, were processed in dehydration, clearing and embedding in paraffin. Serial sections at 4 micron thickness were stained with hematoxylin and eosin.

In the microscopical observations, the majority of cross sections of advanced third-stage larvae are similar to each other and are indistinguishable except the transverse sections through intestinal regions. The intestinal wall of *G. spinigerum* is composed of many elongate cells of columnar simple epithelium, and most of those cells have 3–7 nuclei. There are a few cells containing less than three nuclei or more than seven. Then, in the intestinal wall



Figs. 1–3 Cross sections of the advanced third-stage larvae of the genus *Gnathostoma*. Scale = 100 μm.
Fig. 1 *G. spinigerum* larva collected from the muscle of experimentally infected mouse.
Fig. 2 *G. hispidum* larva collected from the muscle of an albino rat experimentally infected with the early third-stage larvae.
Fig. 3 *G. doloresi* larva collected from the muscle of a naturally infected snake. *Dinodon semicarinatus*.

of larval *G. hispidum*, a large nucleus is found at the central region of each epithelium cell as shown in Fig. 2. There are a few cells containing more than two nuclei. In larval *G. doloresi*, most of cells had 2 nuclei as shown in Fig. 3, though a few variations are noticed. As mentioned above, the cross sectional findings through intestinal regions are obviously different among the three species of larval *Gnathostoma*.

It has been believed that human gnathostomiasis is caused by infection of only one species, *Gnathostoma spinigerum*. Previously in Japan, most patients of gnathostomiasis took raw flesh of a freshwater fish, *Opicephalus argus* (Miyazaki, 1960). In the last ten years, however, larval *G. spinigerum* has not been detected from the fish in Japan, and there is a few reports of the human gnathostomiasis. Recently, human gnathostomiasis has been reported prevalently in Japan and the majority of those patients ate raw loaches imported from foreign countries. From the imported loaches, the early third-stage larvae of *G. hispidum* were observed by several workers (Akahane *et al.*, 1982; Akahane and Mako, 1984; Koga *et al.*, 1984, 1985; Takakura *et al.*, 1985). But no larvae identified as *G. spinigerum* was found in the imported loaches. Although it is estimated that most of the recent human cases of gnathostomiasis were caused by larval *G. hispidum*, the species has not yet been surely identified because of difficulty to identifying the larva in skin biopsy.

Chitwood and Chitwood (1974) published that the genus *Gnathostoma* is polynucleate. Also, Morita (1955) already reported that the intestine of larval *G. spinigerum* contained 2–8 nuclei in an epithelial cell, and according to Takeichi (1956), the adults of *G. spinigerum* had 2–8 nuclei in an epithelial cell of the intestine. Those figures completely agree with our observation on the *G. spinigerum* from Thailand.

On the other hand, there have been no literatures on the number of nuclei in larval *G. hispidum* and *G. doloresi*. By the present observation, it is proved that the figures of Chitwood and Chitwood (1974) are not appli-

cable, at least, to the larvae of *G. hispidum* and *G. doloresi*. Especially in advanced third-stage larvae, the number of nuclei in intestinal epithelial cells is significantly different among three species of *Gnathostoma* mentioned in the present paper.

It is expected that the first case of human gnathostomiasis caused by larval *G. hispidum* will be proven in Japan by the cross sectional findings of the intestinal region of the larva.

The authors wish to thank Prof. I. Miyazaki, Prof. T. Kifune, and Associate Prof. T. Soji, School of Medicine, Fukuoka University for the continuing guidance and the helpful advice. Thanks are also due to Miss N. Kaneda and Miss E. Yamamoto for their kind 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. and Mako, T. (1984): Infection patterns of *Gnathostoma hispidum* in loaches imported from mainland China. Jpn. J. Parasitol., 33, 509–513 (in Japanese).
3) Chitwood, B. G. and Chitwood, M. B. (1974): Introduction to nematology. University Park Press, Baltimore, London, Tokyo, 102–115.
4) Koga, M., Ishibashi, J., Ishii, Y., Hasegawa, H., Dong-Wik Choi and Tsan-Ying Lo (1985): Morphology and experimental infections of Gnathostome larvae from imported loaches, *Misgurnus anguillicaudatus*. Jpn. J. Parasitol., 34, 361–370 (in Japanese).
5) Koga, M., Ishii, Y., Akahane, H. and Shou-Pai Mao (1984): Scanning electron microscopic comarison of adult *Gnathostoma hispidum* Fedtschenko, 1972 from China with a male *Gnathostoma* sp. obtained experimentally from a pig in Japan. Jpn. J. Parasitol., 33, 407–414 (in Japanese).
6) 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).
7) Miyazaki, I. (1960): On the genus *Gnathostoma* and human gnathostomiasis with special reference to Japan. Exp. Parasit., 9, 338–370.
8) Morita, T. (1955): A study on the third stage larva of *Gnathostoma spinigerum*. I. Morphological features. Acta Med., 25, 411–431 (in Japanese).
9) Takakura, Y., Ohnishi, T., Akao, N., Kondo, K. and Yoshimura, H. (1985): Studies on experimental infection to the piglets with *Gnathostoma hispidum* larvae and morphology of the worms. Jpn. J. Parasitol., 34, 211–218 (in Japanese).
10) Takeichi, T. (1956): A morphological study on the adult form of *Gnathostoma spinigerum*. Acta Med., 26, 2600–2634 (in Japanese).

短　報

Gnathostoma 属幼虫の断端構造 ——
有棘顎口虫，剛棘顎口虫，ドロレス顎口虫における第3後期幼虫の断端構造の差異

赤羽啓栄[1)]　佐野基人[2)]　真子俊博[3)]
([1)]福岡大学医学部寄生虫学数室，[2)]浜松医科大学寄生虫学教室，[3)]福岡市衛生試験所)

有棘顎口虫 *G.spinigerum*, 剛棘顎口虫 *G.hispidum* ドロレス顎口虫 *G.doloresi* の3種顎口虫の第3後期幼虫 advanced third-stage larva の断端構造の差異について検討した．その結果，腸管の円柱上皮細胞の核数に差異が認められた．すなわち，1つの円柱上皮細胞内にみとめられる核数は若干の変異はあるが，有棘顎口虫では3～7個，ドロレス顎口虫では2個，剛棘顎口虫では1個と明らかな種間差をみとめた．従って本形質による3種顎口虫幼虫の鑑別が可能であった．