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A Morphological Observation of the Advanced Third-stage Larvae of Mexican *Gnathostoma*

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Abstract

In spite of the recent prevalence of human gnathostomiasis in northern and southern parts of Mexico, no taxonomically satisfactory identification of the agent has yet been made. We observed the morphology of the advanced third-stage larvae discovered in pelicans collected at the "Presidente Miguel Alemán" Dam, in Temascal, Oaxaca, Mexico. Both the hooklet shape and the number of nuclei in each intestinal cell of the Mexican *Gnathostoma* larvae were similar to those of *Gnathostoma spinigerum*. However, the Mexican *Gnathostoma* has about 4 fewer hooklets on each row than *G. spinigerum*. The authors were unable to conclude whether or not the difference in the hooklet number between the larvae of Mexican *Gnathostoma* and of *G. spinigerum* was due to an intra- or interspecific variation.

Key words: *Gnathostoma*, Mexico, advanced third-stage larva, hooklet number, cross section, intestinal cell

Introduction

Many human gnathostomiasis have occurred in Mexico since Peláez and Pérez (1970) reported the first case. Recently, the endemic areas of gnathostomiasis throughout the world include not only the countries of southeast Asia and Japan but Mexico as well. In Mexico, advanced third-stage larvae have been found in fresh water fish, aquatic birds and human patients (Peláez & Pérez, 1970; Lamothe *et al.*, 1989; Almeyda, 1991). However, up to now no taxonomical satisfactory identification of the larvae has been made. From January to March 1993, one of the authors, Akahane stayed in Mexico, and collected specimens of advanced third-stage larvae from pelicans with Mexican coworkers. Based on our findings, we herein report the morphological observations of Mexican larval *Gnathostoma*.

Materials and Methods

Two pelicans were caught at the "Presidente Miguel Alemán" Dam, in Temascal, Oaxaca, Mexico on 19 March 1993. Thereafter, approximately 60 advanced third-stage larvae of the genus *Gnathostoma* were collected from the muscles of the pelicans by artificial digestive methods. The features and number of hooklet rows on the headbulbs of the larvae were then observed by light microscope. On the other hand, the fixed larvae were embedded in rat liver tissue and then were again fixed in a 10% formalin solution. The tissue specimens containing larvae were dehydrated, cleared and embedded in paraffin by routine procedures. Cross sections were made at a thickness of 5 μm and then were stained with hematoxylin and eosin.

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Results

The average body length of the Mexican *Gnathostoma* larvae was 4.83 mm for the 15 examples which naturally died in cold Ringer's solution. The hooklets of the advanced third-stage larva are shown in Fig. 1. The hooklets have an oblong base, which are almost equal in size for the four rows. The hooklet shape of the Mexican larval *Gnathostoma* was found to be similar to that of *G. spinigerum*. In the Mexican *Gnathostoma*, the average number of hooklets in the 8 examined larvae were 38.3 in the first row, 41.3 in the second, 44.7 in the third and 47.4 in the fourth, respectively, as shown in Table 1.

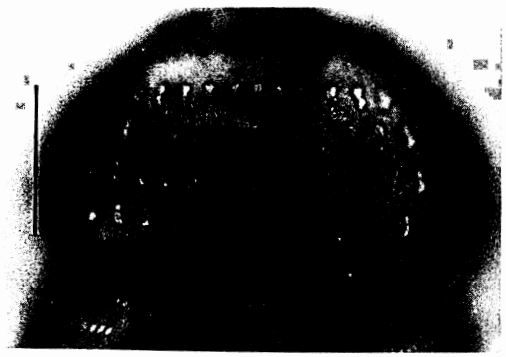

Fig. 1 Hooklets of the advanced third-stage larva of the Mexican *Gnathostoma* (scale: 100 μm).

Table 1 The mean number of hooklets in the larval *Gnathostoma*

Row	1st	2nd	3rd	4th
Mexican *Gnathostoma*				
Present record	38.3	41.3	44.7	47.4
Lamothe (1989)	40.1	42.8	46.0	49.3
Almeyda (1991)	38.7	42.4	44.7	48.2
G. spinigerum				
Thailand[1)]	43.3	46.0	48.5	52.7
Japan[2)]	44.3	47.3	49.6	52.0
Equador[3)]	42	46	50	54
G. procyonis[4)]	32.7	36.6	41.0	45.0
G. hispidum[5)]	38.3	40.5	41.8	46.0
G. doloresi[6)]	38.3	37.9	35.6	35.7

[1)]From Akahane (unpublished) [2)]From Miyazaki (1960) [3)]From Ollague (1987)
[4)]From Ash (1962) [5)]From Akahane *et al.* (1982) [6)]From Mako & Akahane (1985)

Fig. 2 A cross section through the intestine of the Mexican larval *Gnathostoma* (scale: 100 μm).

Fig. 3 A cross section of the intestine of the Mexican larval *Gnathostoma* (scale: 50 μm).

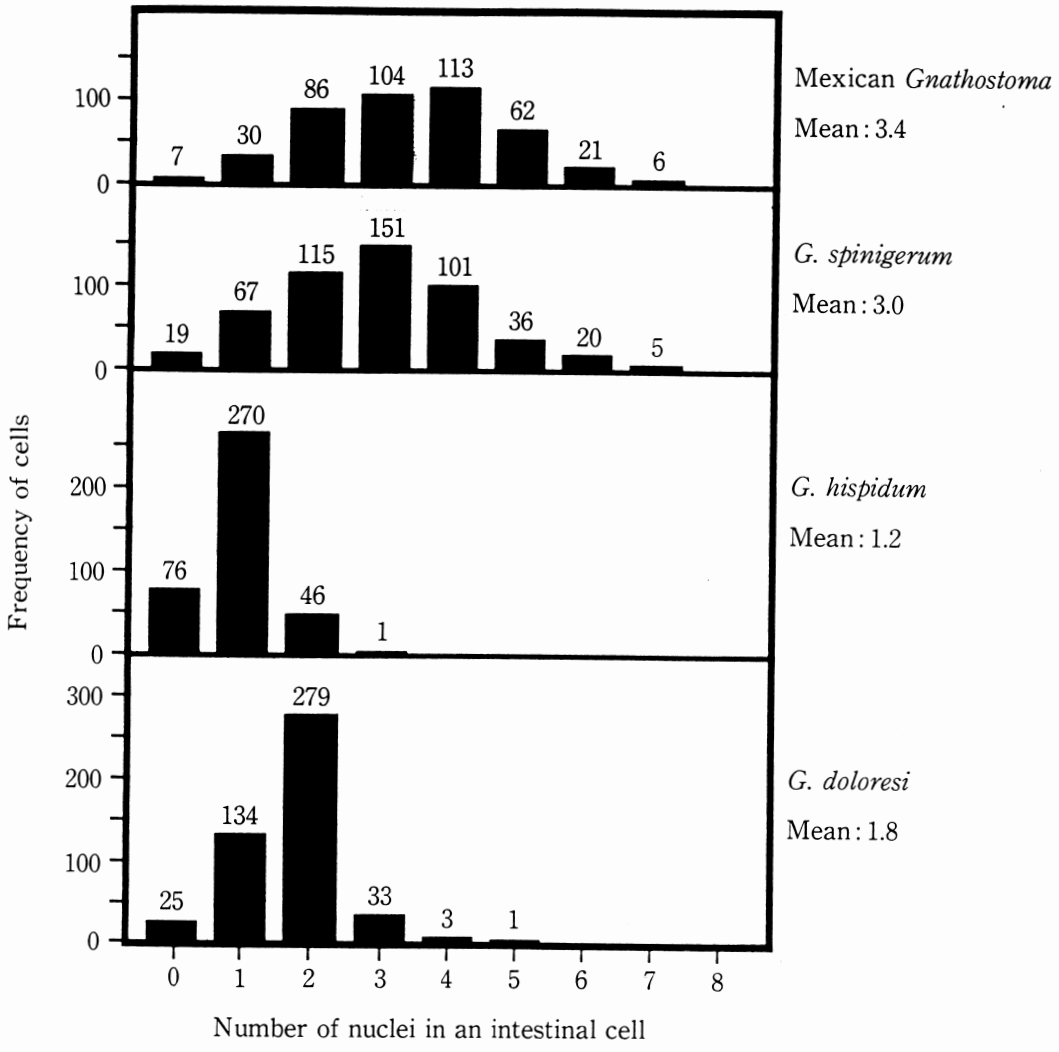

Fig. 4 A comparison of the nuclei number in an intestinal cell of the advanced third-stage larvae among the Mexican *Gnathostoma*, *G. spinigerum*, *G. hispidum*, and *G. doloresi*.

A cross section of the Mexican larva is shown in Figs. 2 and 3. The intestinal wall of the larva consisted of a single layer with many intestinal cells. Some nuclei were observed in the intestinal cells. Out of a total of 429 cells, most columnar cells (113 cells) had 4 nuclei, while 104, 86, 62, 21, 6 cells had 3, 2, 5, 6, 7 nuclei, respectively, as shown in Fig. 4. Thirty cells contained a nucleus while no nucleus was observed in seven cells. In the Mexican larvae, the average number of nuclei in each intestinal cell was 3.4 except for the cells without any nucleus.

Discussion

Miyazaki (1960) stated that the number and the shape of the hooklets on the head-bulb were very useful in making a specific identification of the genus *Gnathostoma*. The hooklet shape of the Mexican larval *Gnathostoma* was found to be similar to that of *G. spinigerum* as mentioned above. One of the present authors, Lamothe *et al.* (1989), and Almeyda (1991) previously reported on the number of hooklets in the Mexican larval *Gnathostoma*. The

present data were similar to the number of hooklets observed by them. The hooklet number in the Mexican larval *Gnathostoma* was similar to that of *G. hispidum* and was clearly larger in number than those of *G. procyonis* and *G. doloresi* as shown in Table 1. In addition, the Mexican larvae had an obviously smaller number of hooklets than that of *G. spinigerum*. It is taxonomically very important that the Mexican larval *Gnathostoma* has about 4 fewer hooklets on each row than *G. spinigerum*.

On the other hand, the present data of nuclei number of Mexican *Gnathostoma* was similar to that of *G. spinigerum* as mentioned above. However, this value was significantly higher than that for either *G. hispidum* or *G. doloresi*. Akahane *et al.* (1986) reported that the intestinal cells of the advanced third-stage larvae have a different number of nuclei among *G. spinigerum, G. hispidum* and *G. doloresi*, therefore, it was possible to identify the agent of human gnathostomiasis by examining the cell number in the intestinal cells of the larvae. In addition, Ando *et al.* (1991) stated that the number of the nuclei and the morphology of the intestinal cells of larval *G. nipponicum* could be easily distinguished from *G. spinigerum, G. hispidum* and *G. doloresi*. Moreover, Almeyda (1991) reported that many advanced third stage-larvae of the Mexican *Gnathostoma* had two nuclei in the intestinal cells. According to those characteristics, he proposed that the Mexican *Gnathostoma* was a new species, which he named *G. binucleatum*. However, we were unable to directly agree with his proposal, because most of the Mexican larvae examined had four nuclei in the intestinal cells as described above. The features of the advanced third stage larvae of the Mexican *Gnathostoma* were very similar to those of *G. spinigerum*, except for the number of hooklets on each row. The authors were not able to explain that the difference in the hooklet number between the larvae of Mexico and *G. spinigerum* was caused merely by either an individual variation in same species or some other interspecific phenomena. In this study, we observed the morphology of the advanced third-stage larva by light microscopy. We intend to investigate the external shape of the larvae by using a scanning electron microscope in the future.

In a review of the literature, Daengsvang (1980) reported that there were 12 species of the genus *Gnathostoma* in the world. Miyazaki (1991), however, considered that the genus *Gnathostoma* contains only 10 distinct species; i.e. *G. spinigerum, G. hispidum, G. turgidum, G. americanum, G. doloresi, G. nipponicum, G. procyonis, G. miyazakii, G. vietnamicum* and *G. malaysiae*. Our attempt to make a specific identification of the Mexican *Gnathostoma* still appears to be somewhat premature. The authors hereafter intend to obtain an adult of the Mexican *Gnathostoma* by conducting an experimental infection of the final hosts. If we can successfully obtain adult specimens of the Mexican *Gnathostoma*, then we would be able to identify it precisely.

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