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Morphological Differences between the Third-Stage Larvae of *Angiostrongylus cantonensis* and Those of *Filaroides martis*

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Key words: *Angiostrongylus cantonensis, Filaroides martis,* third-stage larva, *Biomphalaria glabrata*

Introduction

Human eosinophilic meningoencephalitis, caused by larvae of *Angiostrongylus cantonensis,* is one of the important parasitic zoonoses in the world. In Japan the rat lungworm has been found in *Rattus rattus* and/or *R. norvegicus,* and additionally in several molluscs from Okinawa (Nishimura *et al.,* 1964; Nishimura, 1966), Kagoshima (Yamashita *et al.,* 1978; Sato *et al.,* 1980; Noda *et al.,* 1982), Hiroshima (Tanaka *et al.,* 1982), Aichi (Makiya and Onitake, 1983), Shizuoka (Sano *et al.,* 1977), Kanagawa (Hori *et al.,* 1969), Tokyo (Hori and Kusui, 1972) and Hokkaido (Ohbayashi and Orihara, 1968). On the other hand, another weasel lungworm, *Filaroides martis,* has been detected from *Mustela sibirica* and *M. nivalis* in Hikkaido (Yamashita and Azuma, 1964; Kamiya and Ishigaki, 1972), Miyazaki (Ashizawa *et al.,* 1980) and Kagoshima (Uchikawa *et al.,* 1983).

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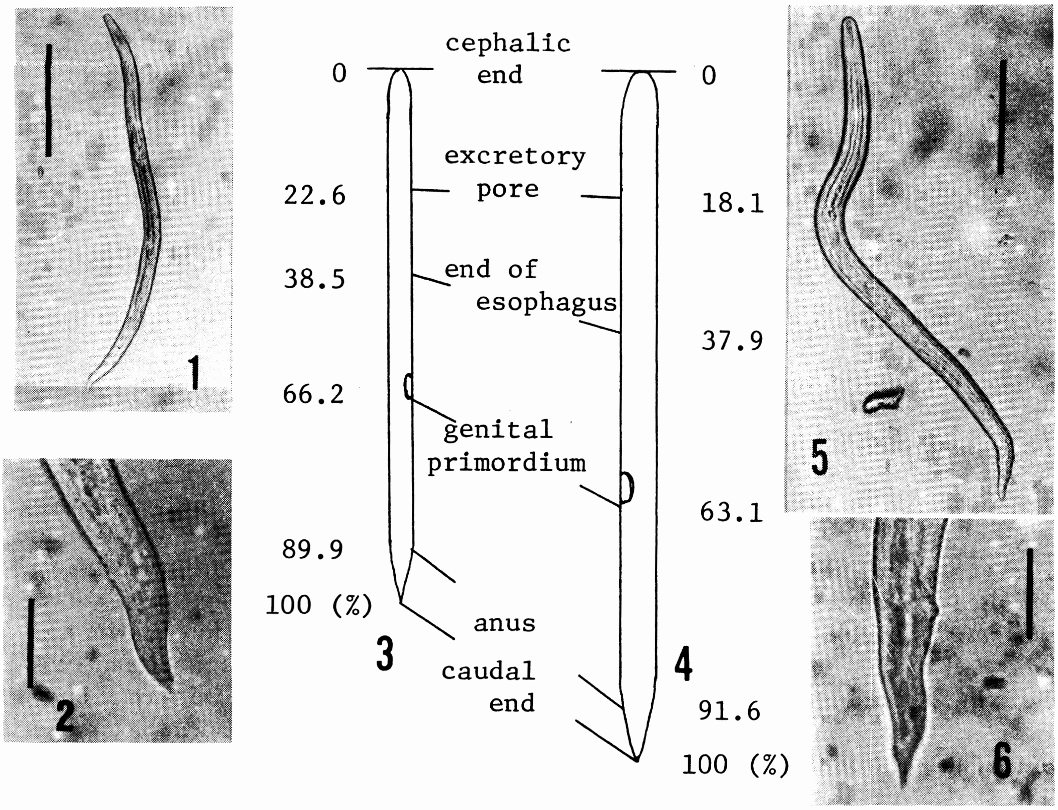
The two species of metastrongyles are known to permit many kinds of molluscs playing a role of their intermediate host, so that it is possible that the prasites easily meet their common intermidate hosts in Japan. Though it is important to distinguish between the two species larvae in molluscs during the epidemiological survey on *A. cantonensis,* there is no report comparing their morphological features. The present study has been undertaken to make criteria on the specific differences between the third-stage larvae of *A. cantonensis* and those of *F. martis.*

Materials and Methods

A. cantonensis derived from naturally infected *R. rattus* trapped in Yoronjima, Kagoshima, was maintained in *Biomphalaria glabrata*—rat in the laboratory. The first-stage larvae were collected from lungs of infected rats. Those of *F. martis* were obtained from the nodules in lungs of *M. sibirica* captured in Tanegashima, Kagoshima.

Two groups of laboratory-reared *B. glabrata* were exposed to either larvae of lungworm species in water for 12 hours. The exposed snails were kept in aquarium at 25C for 60 days and then minced snail tissues were digested in 1% HCl-1% pepsin



Figs. 1–3 Third-stage larva of *Filaroides martis*: 1; whole larva, 2; caudal end, 3; schematic summary of whole larva.
Figs. 4–6 Third-stage larva of *Angiostrongylus cantonensis*: 4; schematic summary of whole larva, 5; whole larva, 6; caudal end.
Scale bar shows 0.02 mm in Fig. 2 & 6 and 0.1 mm in Fig. 1 and 5, respectively.

solution for 2 hours. The third-stage larvae of each species were heat-killed on the slide glass and observed under microscope.

Results

The first-stage larvae of both species developed to the third-stage in *B. glabrata*. Through dissecting microscopic observation, the infective larvae of *A. cantonensis* moved more actively than those of *F. martis* at room temperature (15–20C).

General morphological features shown by the larvae of both species were almost similar to one another except the tails. In *F. martis* the tail bended dorsally, but this feature was not observed in *A. cantonensis* (Figs. 1, 2, 5 and 6). The prominent serrations, such as seen in larvae of *Anafilaroides rostratus* (Ash, 1970), were not clear in *F. martis* while they were not at all in *A. cantonensis*.

Measurements of 20 third-stage larvae of both species were summarized in Table 1. The size of body in *A. cantonensis* (0.436–0.482 mm in length and 0.023–0.029 mm in width) was larger than that in *F. martis* (0.345–0.403 mm in length and 0.015–0.021 mm in width). The level of excretory pore as % of body length from cephalic end was 18.1% (16.4–20.8%) in *A. cantonensis* and 22.6% (22.1–24.5%) in *F. martis*, respectively (Figs. 3 and 4).

Though the third-stage larvae of *F.*

Table 1 Comparative measurements between 20 third-stage larvae of *Angiostrongylus cantonensit* and those of *Filaroides martis* (in mm)

Dimention	*A. cantonensis*		*F. martis*	
	mean	(range)	mean	(range)
Length of body	0.464	(0.436–0.482)	0.358	(0.345–0.403)
Maximum width of body	0.027	(0.023–0.029)	0.017	(0.015–0.021)
Length of esophagus	0.176	(0.170–0.190)	0.138	(0.133–0.150)
Cephalic end to excretory pore	0.084	(0.079–0.091)	0.081	(0.079–0.091)
Caudal end to genital primordium	0.171	(0.158–0.192)	0.121	(0.116–0.133)
Length of tail	0.039	(0.033–0.042)	0.036	(0.033–0.042)

martis were experimentally fed to puppies, which were recognized as one of most possible final host in the laboratory, no adult worms were recovered in the lungs of the animals 50 days after feeding.

Discussion

The larvae of several mammaliam lumgworm species are found in molluscan intermediate hosts and their geographical distributions occasionally overlap that of *A. cantonensis* (Malek, 1980). In Japan both of *A. cantonensis* and *F. martis* were reported from Hokkaido and Kyushu. Ashizawa *et al.* (1980) suggested that *F. martis* probably distributed also in Honshu. Once epidemiological study on *A. cantonensis* is carried out, it becomes important to distinguish the larvae from those of other nematode species in molluscs.

The third-stage larvae of *A. cantonensis* were previously compared morphologically with *A. rostratus, Aelurostrongylus abstrusus* and *A. vasorum* (Alicata, 1963; Ash, 1970). According to these reports, *A. cantonensis* and *A. rostratus* have finely pointed tails, while the tip of tail is digitiform in *A. vasorum* and rounded knob in *A. abstrusus,* respectively. *A. cantonensis* has no prominent serrations of tail which are observed in *A. rostrusus.* Furthermore, *A. cantonensis* is smallest in body size among the 4 species.

Ash (1970) indicated, however, that "Size alone of larvae is not a sufficient criterion for precise identification." and that "The size attainde by lungworm larvae may possibly be influenced by at least two factors: numbers of larvae developing in the intermediate host and age of larvae in the mollusk."

In the present study the third-stage larvae of *A. cantonensis* were obviously larger than those of *F. martis,* when they were compared at same age and in same host species. These measurements obtained from both species coincided with those in previous reports on either lungworm species. (Anderson, 1962; Katakura *et al.,* 1981). Therefore the difference in size was a useful criterion between at least the two species. On the other hand, as the larvae of other three lungworm species mentioned above are larger than those of *A. cantonensis,* species identification by the lungworm larvae alone should be made after careful morphological observations on them. The present study suggested that the larvae of *A. cantonensis* and *F. martis* were also distinguishable by observing the shape of tail and the position of excretory pore.

It is possible that potential molluscan intermediate hosts of *A. cantonensis* harbor larvae of other nematode species in Japan. Recently, the authors met an incident (unpublished) just like that reported by Kurihara *et al.* (1979): In authors' case, some larval nematodes which looked like *A. cantonensis* morphologically were obtained from *Achatina fulica* in Tokunoshima, Kagoshima. Though these larvae were fed to laboratory rats, no adult worms were recovered from lungs of the rats. Thus, there is the possibility that larvae of other species than *A. cantonensis* and *F. martis* may parasitize in molluscs of Amami Islands.

More investigations on larval nematodes parasitizing in molluscs and paratenic hosts are needed to distinguish the larvae of *A. cantonensis* from those of other species. The distribution of *A. cantonensis* from those of other species. The distribution of *A. cantonensis* should be established by the detection of adult worms from wild rats and from laboratory rats which are experimentally fed on the larvae obtained from molluscs and/or paratenic hosts. When the larvae alone are available for species identification, careful and exact observations are needed.

Summary

Third-stage larvae of *Angiostrongylus cantonensis* and *Filaroides martis* experimentally developing in *Biomphalaria glabrata* were morphologically compared on day 60 post infection. The larvae of *A. cantonensis* were obviously larger than those of *F. martis* in body size. Larvae of both species were also distinguished from one another by the shape of tail and the position of excretory pore.

In cases where larval nematodes of other species may occur in molluscs and/or paratenic hosts collected in the field, larval *A. cantonensis* should be identified by detailed observations. The most reliable method for the identification is to detect adult worms from laboratory rats experimentally given the larvae.

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Angiostrongylus cantonensis および *Filaroides martis* の第3期幼虫における形態的比較

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広東住血線虫 *Angiostrongylus cantonensis* およびイタチの肺より得られた *Filaroides martis* の第1期幼虫を *Biomphalaria glabrata* に感染させ，60日後にそれぞれの第3期幼虫の形態を比較した．

両種の一般的形態は互いに類似しているが，広東住血線虫は体長，体幅ともに *F. martis* より大きかった．また両者は排泄孔の位置，尾部の形態でも判別されたが，生きたままの状態では鑑別が困難である．

さらに，広東住血線虫の中間宿主となりうる軟体動物が他の幼線虫を保有していることも想定されるので，広東住血線虫幼虫の同定には詳細な形態的観察が必要であり，最も確実な方法は幼虫をラットに投与し成虫を得ることである．