MORPHOLOGICAL VARIATION AND ABNORMALITY OF CEPHALIC HOOKLETS OF *GNATHOSTOMA SPINIGERUM* HEPATIC STAGE LARVAE FROM LABORATORY INFECTED MICE

Wichit Rojekittikhun and Somchit Pubampen

Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand

Abstract. One thousand advanced third-stage larvae of *Gnathostoma spinigerum* from laboratory infected mice, two to five weeks after being fed with infected cyclops, were examined specifically for the morphology of their cephalic hooklets. Among these, only the 15-day old (early hepatic-stage) larvae and the 30-day old (late hepatic-stage) larvae were measured for the size of their body and hooklets. The average body size of the 15-day old and 30-day old larvae were $3.4 \pm 0.4 \times 0.4 \pm 0.04$ mm and $4.9 \pm 0.4 \times 0.5 \pm 0.04$ mm, respectively. The average size of the hooklets from rows one to four of the 15-day old larvae was $14.6 \pm 1.7 \times 6.8 \pm 0.6$ μm, $15.6 \pm 2.0 \times 7.2 \pm 0.5$ μm, $16.0 \pm 1.8 \times 7.4 \pm 0.6$ μm and $15.9 \pm 1.9 \times 7.3 \pm 0.6$ μm, respectively. Those of the 30-day old larvae were $15.1 \pm 1.7 \times 7.1 \pm 0.6$ μm, $16.3 \pm 1.6 \times 7.5 \pm 0.7$ μm, $16.5 \pm 1.7 \times 7.8 \pm 0.6$ μm and $16.3 \pm 1.7 \times 7.6 \pm 0.8$ μm, respectively. The average number of cephalic hooklets from rows one to four of the two- to five-week old larvae were 42.8 ± 2.6, 45.3 ± 2.8, 46.9 ± 2.8 and 50.2 ± 2.9, respectively. Several types of morphological variation and abnormality of the cephalic hooklets were observed. The most common ones were extra rudimentary hooklets below row four or within the four rows of hooklets (10.8%), the present of a fifth row of hooklets (1.9%), abnormal hooklets in only row four (1.2%), lobed or branched hooklets (0.5%), spiral arrangement of the four rows of hooklets (0.4%), and fragmented hooklets (0.4%).

INTRODUCTION

Gnathostoma spinigerum advanced third-stage larvae (GsAL3), the causative agent of human gnathostomosis in Thailand, is morphologically divided into 3 parts: lips, head-bulb and body. The head-bulb normallly bears four transverse rows of well-developed single-point hooklets. The hooklets on each row are practically the same in size and morphology except that those of row one are somewhat smaller. There are 40 or more cephalic hooklets in each row and each hoooklet has an oblong base (Daengsvang, 1980; Miyazaki, 1991). *G. spinigerum* can be maintained in the laboratory via 3 essential hosts: fresh-water cyclops (first intermediate host), mice (second intermediate host) and cats (final host). In mice, the overall recovery rate of the advanced third-stage larvae (AL3) one week after being fed with infected cyclops containing early third-stage larvae (EL3) is only 9.3%. The AL3 accumulate mostly in the liver from week two (63.3%) to week four (24.0%) after infection (Anantaphruti *et al*, 1986) or on days 15-30 post-infection (Daengsvang, 1980). Most of the AL3 are then recovered in the muscle on week five (37.3%). Encystation of the AL3 also starts on week five (1.3% in the muscle) and is completed on week 10 post-infection (Anantaphruti *et al*, 1986). The infectivity of the 3-week old hepatic-stage larvae in mice is 60.0%. This is nearly similar to the infectivity of the 8-week, 6-month and 12-month old encysted muscle-stage larvae which are 61.3%, 66.0% and 69.3%, respectively (Rojekittikhun *et al*, 1989).

As far as it is known, there is no report on the morphological variation and abnormality of the cephalic hooklets of GsAL3 from laboratory infected mice. The aim of this work was to study all possible features of the hooklets of the hepatic-stage larvae obtained from infected mice. The size of the body an cephalic hooklets of the 15-day (early hepatic-stage) larvae and the 30-day (late hepatic-stage) larvae is also discussed.

MATERIALS AND METHODS

A number of mice were each infected with quite a number of infected cyclops containing EL3. Some of these animals began to die in about one week post-infection, or they were sacrificed two to five weeks after the infection. The AL3 were collected

Tel:+94-1-867404; Fax:+94-1-866854; Email: w.van-der-hoek2cgnet.com

from mice livers by the press preparation technique, washed several times in normal saline and distilled water (DW), and then fixed and preserved in 10% formalin. These hepatic-stage larvae were later softened in DW and examined specifically for the morphology of their cephalic hooklets. Only the 15-day old and the 30-day old larvae were measured for the size of their body and cephalic hooklets using a camera lucida.

RESULTS

Of the 1,000 (hepatic-stage, two- to five-week old) AL3 examined, 155 (15.5%) were found to be morphological variants or abnormal (Table 1, Fig 1). The most common unusual features were extra rudimentary hooklets below row four or within the four rows of hooklets (10.8%) (Fig 1e), the presence of a fifth row of hooklets (1.9%) (Fig 1c, 1d), abnormal hooklets in only row four (1.2%) (Fig 1f), lobed or branched hooklets (0.5%) (Fig 1h), spiral arrangement of the four rows of hooklets (0.4%) (Fig 1g), and fragmented hooklets (0.4%). The average number of cephalic hooklets from rows one to four of the 250 AL3 examined were 42.8 ± 2.6 (range 36-52), 45.3 ± 2.8 (38-53), 46.9 ± 2.8 (39-55) and 50.2 ± 2.9 (43-59), respectively (Table 2). Among these, 19 AL3 (1.9%) were found with five rows of hooklets, the fifth row comprising 8-54 (averaging 25.5) hooklets. Table 3 and Fig 2 show the frequency and relative frequency (%) distribution of the number of cephalic hooklets in the four rows. The highest frequency of hooklet numbers in rows one to four fell between 40-44 (64.8%), 45-49 (54.0%), 45-49 (59.6%) and 50-54 (53.6%) hooklets, respectively.

The average size of the 15-day old and 30-day old larvae (50 AL3 in each group) were 3.4 ± 0.4 × 0.4 ± 0.04 mm (2.7 - 4.4 × 0.3 - 0.4 mm) and 4.9 ± 0.4 × 0.5 ± 0.04 mm (4.1 - 5.8 × 0.4 - 0.6 mm), respectively, (Table 4). The average size of the hooklets from rows one to four of the 15-day old larvae (n = 160, Table 5) were 14.6 ± 1.7 × 6.8 ± 0.6 μm (11.4 - 18.5 × 5.5 - 8.9 μm), 15.6 ± 2.0 × 7.2 ± 0.5 μm (11.6 - 20.2 × 6.2 - 8.6 μm), 16.0 ± 1.8 × 7.4 ± 0.6 μm (12.4 - 19.8 × 6.4 - 8.6 μm) and 15.9 ± 1.9 × 7.3 ± 0.6 μm (12.7 - 20.2 × 6.2 - 8.8 μm), respectively. And those of the 30-day old larvae (n = 160, Table 5) were 15.1 ± 1.7 × 67.1 ± 0.6 μm (11.1 - 18.5 × 6.1 - 8.6 μm), 16.3 ± 1.6 × 7.5 ± 0.7

Table 1

Morphological variation and abnormality of cephalic hooklets of *G. spinigerum* hepatic-stage larvae from laboratory infected mice.

Type of variation or abnormality	No. of larvae	%
Fragmented	4	0.4
Rounded	1	0.1
Lobed or branched	5	0.5
Four rows variable		
-row 1, not completely encircling the head-bulb	1	0.1
-row 4, not completely encircling the head-bulb	7	0.7
-row 4, outgrowing/jagged hooklets	5	0.5
Extra rudimentary hooklets		
-few pieces below row 4	40	4
-several to numerous pieces below row 4	65	6.5
-few to numerous pieces in between the 4 rows	3	0.3
Three rows	1	0.1
Five rows		
-all rows completely encircling the head-bulb	8	0.8
-row 5 not completely encircling the head-bulb	11	1.1
Four rows spirally arranged	4	0.4
Total (n = 1,000)	155	15.5

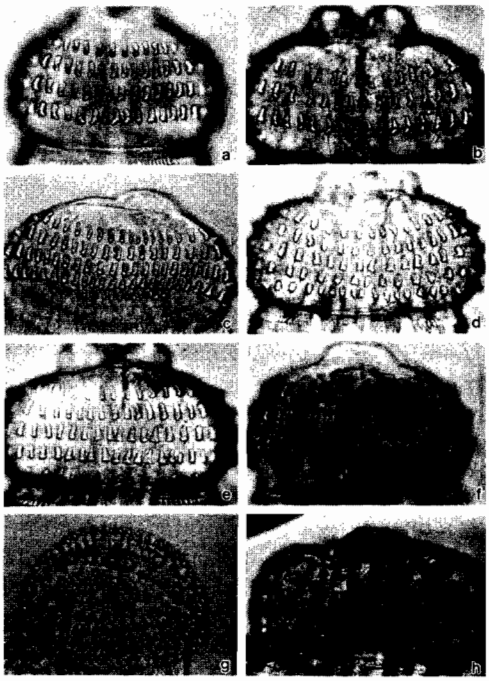

Fig 1–Morphological variation and abnormality of cephalic hooklets of *G. spinigerum* hepatic-stage larvae from laboratory infected mice: a) normal feature of the four rows of hooklets, b) three rows of hooklets, c) five rows of hooklets, hooklets in row five not completely encircling the head-bulb, d) five complete rows of hooklets, e) four rows of hooklets with numerous rudimentary hooklets below row four, f) abnormal hooklets in row four, g) spirally arranged row of hooklets, h) lobed hooklets.

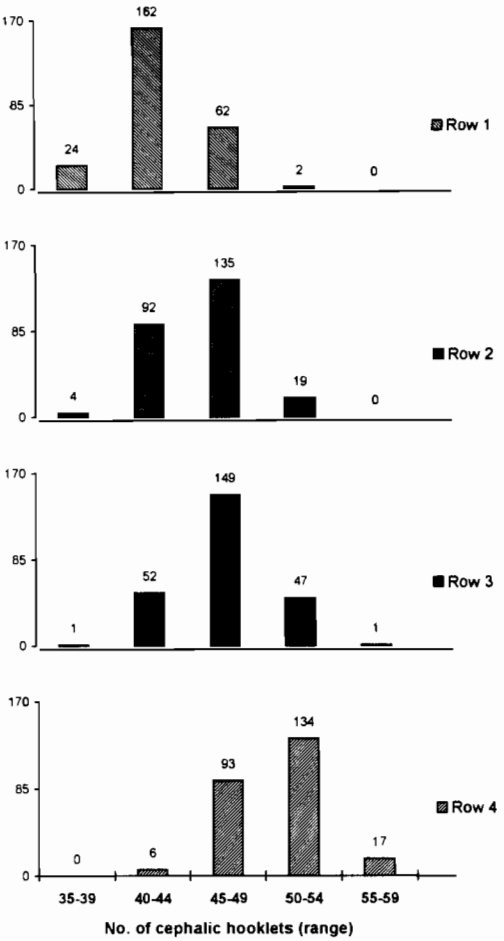

Fig 2–Frequency distribution of cephalic hooklet number of *G. spinigerum* hepatic-stage (2 to 5-week-old) larvae (n = 250).

μm (13.6 - 20.3 × 6.5 - 9.5 μm), 16.5 ± 1.7 × 7.8 ± 0.6 μm (13.0 - 19.7 × 6.5 - 10.1 μm) and 16.3 ± 1.7 × 7.6 ± 0.8 μm (13.0 - 21.5 × 5.9 - 10.2 μm), respectively.

DISCUSSION

Daengsvang (1980) reported that the average measurement of 29 encysted AL3 after dissection from the cyst wall obtained from the muscles of

Table 2

Number of cephalic hooklets of *G. spinigerum* hepatic-stage (2 to 5-week-old) larvae (n = 250).

Row No.	No. of hooklets (range)	Average ± SD
Row 1	36-52	42.8 ± 2.6
Row 2	38-53	45.3 ± 2.8
Row 3	39-55	46.9 ±2.8
Row 4	43-59	50.2 ± 2.9

Table 3

Frequency and relative frequency (%) distributions of cephalic hooklet number of *G. spinigerum* hepatic-stage (2 to 5-week-old) larvae (n = 250).

No. of hooklets (range)	Frequency (%)			
	Row 1	Row 2	Row 3	Row 4
35 -39	24 (9.6)	4 (1.6)	1 (0.4)	0 (0.0)
40-44	162 (64.8)	92 (36.8)	52 (20.8)	6 (2.4)
45-49	62 (24.8)	135 (54.0)	149 (59.6)	93 (37.2)
50-54	2 (0.8)	19 (7.6)	47 (18.8)	134 (53.6)
55-59	0 (0.0)	0 (0.0)	1 (0.4)	17 (6.8)

Table 4

Body size of 15-day old and 30-day old *G. spinigerum* hepatic-stage larvae (n = 50).

Larva age	Size range (mm)	Average size ± SD (mm)
15 days	2.7 - 4.4 × 0.3 - 0.4	3.4 ± 0.4 × 0.4 ± 0.04
30 days	4.1 - 5.8 × 0.4 - 0.6	4.9 ± 0.4 × 0.5 ± 0.04

Table 5

Size of cephalic hooklets of 15-day old and 30-day old *G. spinigerum* hepatic-sage larvae (n = 160).

Row No.	Size of cephalic hooklets (μm)			
	15-day old larvae		30-day old larvae	
	Range	Average ± SD	Range	Average ± SD
Row 1	11.4 - 18.5 × 5.5 - 8.9	14.6 ± 1.7 × 6.8 ± 0.6	11.8 - 18.1 × 6.1-8.6	15.1 ± 1.7 × 7.1 ± 0.6
Row 2	11.6 - 20.2 × 6.2 - 8.6	15.6 ± 2.0 × 7.2 ± 0.5	13.6 - 20.3 × 6.5-9.5	16.3 ± 1.6 × 7.5 ± 0.7
Row 3	12.4 - 19.8 × 6.4 - 8.6	16.0 ± 1.8 × 7.4 ± 0.6	13.0 - 19.7 × 6.5-10.1	16.5 ± 1.7 × 7.8 ± 0.6
Row 4	12.7 - 20.2 × 6.2 - 8.8	15.9 ± 1.9 × 7.3 ± 0.6	13.0 - 21.5 × 5.9-10.2	16.3 ± 1.7 × 7.6 ± 0.8

infected white mice sacrificed 78-742 days after experimental feeding (primary infection) was 3.95 × 0.42 mm (ranging from 2.8 - 5.2 × 0.3 - 0.8 mm). The hooklets on each row found in 69 AL3 taken from the experimentally fed rodents measured about 16.3 × 8.0 μm except those of row one which were smaller, about 13.0 × 6.6 μm. The number of hooklets normally increases posteriorly from rows one to four, averaging 41.5, 43.3, 46.6 and 49.2 respectively. From the present study, an average size of 4.1 - 5.8 × 0.4 - 0.6 mm of the late hepatic-stage (unencysted, 30-day old) larvae reveals that this stage has almost reached the maximum size. The average number of hooklets in the four rows – 42.8, 45.3, 46.9 and 50.2 – were also almost identical as compared to those of the previous report. Though the average size of the 15-day old larvae was much smaller than that of the 30-day old larvae, their hooklet size was not much different. We found, however, the hooklet size in the first row to be slightly bigger than that reported by Daengsvang (1980).

Daengsvang (1980) also reported that each larva had 40 or more cephalic hooklets in each row, noting that 24.6% of row one, 4.3% of row two,

1.5% of row three and 2.9% of row four had less than 40 hooklets, and there was only one larva bearing 36-38 cephalic hooklets in each of the four rows. In the present study, 9.6% of row one, 1.6% of row two, 0.4% of row three and 0% of row four had less than 40 hooklets. None of the 250 larvae examined bore less than 40 hooklets in each of the four rows.

So far as is known, there is no report on the variation or abnormality of the cephalic hooklets of the AL3 obtained from laboratory infected mice. However, Daengsvang (1980) did mention the fifth row of hooklets that was observed in one larva consisting of a few hooklets from an experimental white mouse, and in one unencysted AL3 found in the liver of an experimental tree-shrew sacrificed 30 days after being fed with the EL3 in cyclops. This AL3 bore five complete rows of cephalic hooklets. In the present study, we found 15.5% of the 1,000 AL3 examined to be morphological variants or abnormal. Among these, 19 larvae (1.9%) were found to bear the fifth row of hooklets which consists of 8-54, averaging 25.5, hooklets. Setasuban *et al* (1991) and Akahane *et al* (1995) have also reported a new type of the AL3 of the genus *Gnathostoma* in fresh-water eels. The authors distinguished these larvae from other species by the shape of their hooklets, which branched in a complex manner at the base, and suggested that the larvae belong to either *G. vietnamicum, G. malaysiae* or a new species. In the present study, 0.5% (5/1,000) of *G. spinigerum* advanced third-stage larvae were found to have a lobed or branched type of cephalic hooklets of which is very similar to that described, as belonging to a new type of the AL3, by Setasuban *et al* (1991) and Akahane *et al* (1995). This needs further investigation and clarification.

REFERENCES

Akahane H, Setasuban P, Nuamtanong S, Horiuchi S, Koga M, Kojima S. A new type of advanced third-stage larvae of the genus *Gnathostoma* in freshwater eels, *Fluta alba*, from Nakhon Nayok, Central Thailand. *Southeast Asian J Trop Med Public Health* 1995; 26 : 743-7.

Anantaphruti M, Waikagul J, Nithi-Uthai S, Pubampen S, Rojekittikhun W. Detection of humoral immune response to *Gnathostoma spinigerum* in mice. *Southeast Asian J Trop Med Public Health* 1986; 17 : 172-6.

Daengsvang S. A monograph on the genus *Gnathostoma* and gnathostomiasis in Thailand. Tokyo: Southeast Asian Medical Information Center SEAMIC, International Medical Foundation of Japan, 1980: 85.

Miyazaki Y. An illustrated book of helminthic zoonosis. Tokyo: International Medical Foundation of Japan, 1991: 483.

Rojekittikhun W, Pubampen S, Pojanayon A, Setasuban P. Infectivity of advanced third-stage larvae of *Gnathostoma spinigerum* in mice. *J Trop Med Parasitol* 1989; 12 : 29-30.

Setasuban P, Nuamtanong S, Rojanakittikoon V, *et al*. Gnathostomiasis in Thailand: A survey on intermediate hosts of *Gnathostoma* spp with special reference to a new type of larvae found in *Fluta alba*. In: Cross JH, ed. Proceedings of the 33rd SEAMEO-TROPMED regional seminar. Emerging problems in food-borne parasitic zoonosis: Impact on agriculture and public health. Bangkok: SEAMEO Regional Tropical Medicine and Public Health Project, 1991: 220-4.