SEASONAL VARIATION IN THE INTENSITY OF *GNATHOSTOMA* LARVAE IN SWAMP EELS (*FLUTA ALBA*) SOLD IN A LOCAL MARKET IN BANGKOK

Wichit Rojekittikhun, Somchit Pubampen and Jitra Waikagul

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

Abstract. The viscera of swamp eels were obtained from a local market in Bangkok twice a month from June 1996 to May 1997. The livers were separated, weighed and counted. Gnathostome larvae were recovered from the livers by the digestion technic, examined, identified, and counted. A total of 12,278 *Gnathostoma* larvae were obtained from 18,561.1 g (15,264 pieces) of eel livers. The overall average number of larvae/g liver and the overall average number of larvae/liver are 0.91 and 0.94, respectively. The greatest number of larvae/g liver (on average) was in December (high levels of infection during the months of October to December) whereas the lowest was in April (lowest levels of infection during the months of March to April). Thus there was a marked decrease in the average number of larvae/g liver during January to April, which then started to rise in May. This finding suggests that the level of infection abruptly decreases soon after the completion of the rainy season, starts to rise when the rain has come, and reaches its peak when the amount of rainfall is highest. More than 99% of the total gnathostome larvae recovered were identified to be *G. spinigerum*, and 25.4% of the entire larvae recovered bore variant or abnormal cephalic hooklets. The most common unusual feature was that there were extra rudimentary hooklets above row one, below row four and in between the four rows of hooklets which comprised 21.4%. In addition, the body size and the number of cephalic hooklets of *G. spinigerum* are also discussed.

INTRODUCTION

Gnathostoma spinigerum is one of the five species of the genus *Gnathostoma* causing human gnathostomiasis (Daengsvang, 1980; Ogata *et al*, 1988; Ando *et al*, 1988; Nawa *et al*, 1989; Almeyda-Artigas, 1991). The disease is still prevalent in a number of countries, especially Thailand. In Thailand, *G. spinigerum* is the only species that has been proven to cause gnathostomiasis in man (Daengsvang, 1986; Radomyos and Daengsvang, 1987). People usually get the infection by eating raw or improperly cooked meat, particularly fish harboring advanced third-stage larvae (AL3) – the infective stage of the parasite (Daengsvang, 1980; Miyazaki, 1991). In gnathostomiasis research at the Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, the AL3 are usually obtained from two main sources – laboratory infected mice and the naturally infected eels (Rojekittikhun *et al*, 1996, 1997a). Mice, as the second intermediate host, are one of the three essential hosts in the life cycle of *G. spinigerum* maintained in the laboratory. Although a large quantity of AL3 can be obtained from infected mice, they are not available at all times because the complete life cycle of *G. spinigerum* in the laboratory is rather long, taking approximately 9-12 months (Rojekittikhun *et al*, 1996). For research purposes, therefore, eels and eel livers are often purchased from the local markets or from endemic areas. In Thailand, there are five species of *Gnathostoma* (Daengsvang, 1980; Kamiya *et al*, 1987), and swamp eels (*Fluta alba*) have been reported to naturally harbor at least four species of them, namely: *G. spinigerum, G. hispidum, G. doloresi* and *G. vietnamicum* (Rojekittikhun *et al*, 1989, 1997a; Nuamtanong *et al*, 1997). Moreover, swamp eels have also been reported to be one of the most heavily infected natural intermediate hosts of *G. spinigerum* (Daengsvang, 1980; Rojekittikhun *et al*, 1989; Setasuban *et al*, 1991; Akahane *et al*, 1995; Nuamtanong *et al*, 1997). The infection rates are as high as 40-100% with the maximum number of larvae recovered to be 28-105 per eel (Daengsvang, 1980; Rojekittikhun *et al*, 1989) and 2,283-2,582 per eel (Setasuban *et al*, 1991; Nuamtanong *et al*, 1997). The larvae in the eels were found to be mostly concentrated in the liver: 34.7-46.7% in heavy infections, and 33.3-100% in light infections (Setasuban *et al*, 1991; Rojekittikhun *et al*, 1997a). However, the number of AL3 in the eels purchased during various seasons is unpredictable. This observation suggests that there is only a certain period of time within a year that the number of the AL3 are most abundant in eels, while at other times there may not be many. The purpose of this study was to describe the intensity of *Gnathostoma* AL3 during each month of the year in swamp eels captured and sold for local consumption.

MATERIALS AND METHODS

The most convenient and economical way to obtain *Gnathostoma* AL3 from eels is to obtain only the viscera of eels since it was found that the AL3 are concentrated in encysted form, mostly in eel livers (Setasuban *et al*, 1991; Rojekittikhun *et al*, 1997a). The viscera of eels were purchased from a single market in Bangkok twice a month on week one and week three throughout the investigating year. The organs were washed, and the livers were separated from the rest. After the livers were weighed and counted, they were then minced, digested with artificial gastric juice (1% HCl in 1% pepsin), and incubated in a water bath at 37°C for 2-3 hours with frequent stirring. The digested livers were washed several times in physiological saline by a simple sedimentation technic, and the sediments were then examined for AL3 under a stereomicroscope. All the recovered larvae were thoroughly washed in saline, counted, examined and identified under a light microscope. The number of cephalic hooklets of some *G. spinigerum* larvae were counted, and the size of their body was also measured using a camera lucida.

RESULTS

A total of 12,278 gnathostome larvae were recovered from 18,561.1 g (15,264 pieces) of eel liver over a period of one year. The overall average number of larvae/g liver and the overall average number of larvae/liver were 0.91 and 0.94, respectively (Table 1). The highest and lowest number of larvae/g liver (on average) occurred during December and April, respectively, while the highest and lowest number of larvae/liver (on average) occurred during October and March. Regardless of the parameters referred to, both exhibit a similar trend, with peaks between October to December and the lowest between March and April. It is evident that a marked decrease in the average number of larvae/g liver occurred from January to April, after which it started to increase again in May (Fig 1).

The number of cephalic hooklets of *G. spinigerum* AL3 is tabulated in Table 2, and the frequency and relative frequency distributions of the

Table 1

The intensity of *Gnathostoma* advanced third-stage larvae in swamp eels sold in a local market in Bangkok (June 1996 - May 1997).

Month and year	Total weight of eel livers (g)	Total no. of eel livers	Total no. of larvae recovered	Average no. of larvae/g liver	Average no. of larvae/liver
June 1996	1,938.1	2,270	1,288	0.49	0.44
July	949.5	1,201	658	0.73	0.58
August	1,193.1	1,147	875	0.80	0.76
September	2,182.9	1,949	1,638	0.96	0.92
October	1,198.8	1,034	1,990	1.70	2.89
November	978.4	878	1,534	1.57	1.75
December	677.8	986	2,807	3.80	2.62
January 1997	1,632.6	983	434	0.25	0.45
February	2,905.5	1,571	474	0.18	0.32
March	2,760.7	1,330	87	0.04	0.07
April	404.6	71	8	0.02	0.11
May	1,739.1	1,844	485	0.40	0.34
Total	18,561.1	15,264	12,278	0.91	0.94

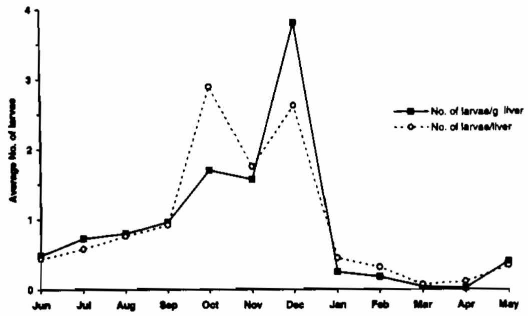

Fig 1–The intensity of *Gnathostoma* advanced third-stage larvae in swamp eels (June 1996-May 1997).

Table 2

Number of cephalic hooklets of *G. spinigerum* advanced third-stage larvae recovered from swamp eels (n = 250).

Row No.	No. of hooklets (range)	$\bar{X} \pm SD$
Row 1	36 - 53	42.2 ± 3.0
Row 2	38 - 57	44.6 ± 3.1
Row 3	39 - 55	46.8 ± 3.1
Row 4	42 - 59	50.0 ± 3.0

hooklet numbers are shown in Table 3 and Fig 2. The average number of the hooklets from rows one to four were 42.2 ± 3.0 (range 36-53), 44.6 ± 3.1 (38-57), 46.8 ± 3.1 (39-55) and 50.0 ± 3.0 (42-59), respectively. The highest frequency of hooklet numbers in rows one to four fell between 40-44 (60.8%), 45-49 (45.6%), 45-49 (57.2%) and 50-54 (50.0%), respectively. No larvae having more than 54 hooklets in row one, and having less than 40

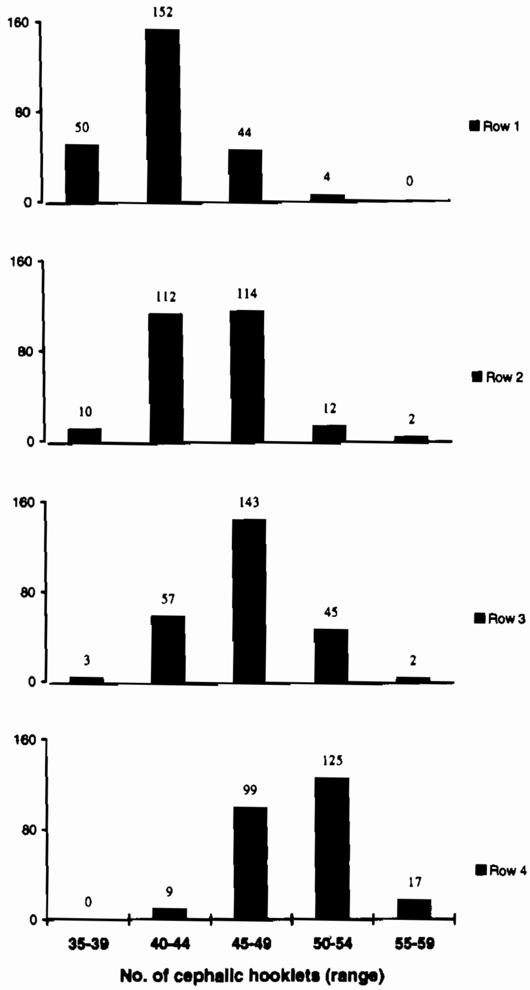

Fig 2–Frequency distribution of cephalic hooklet number of *G. spinigerum* advanced third-stage larvae from swamp eels (n = 250).

Table 3

Frequency and relative frequency (%) distributions of cephalic hooklet number of *G. spinigerum* advanced third-stage larvae from swamp eels (n = 250).

No. of hooklets	Frequency (%)			
(range)	Row 1	Row 2	Row 3	Row 4
35 - 39	50 (20.0)	10 (4.0)	3 (1.2)	0 (0.0)
40 - 44	152 (60.8)	112 (44.8)	57 (22.8)	9 (3.6)
45 - 49	44 (17.6)	114 (45.6)	143 (57.2)	99 (39.6)
50 - 54	4 (1.6)	12 (4.8)	45 (18.0)	125 (50.0)
55 - 59	0 (0.0)	2 (0.8)	2 (0.8)	17 (6.8)

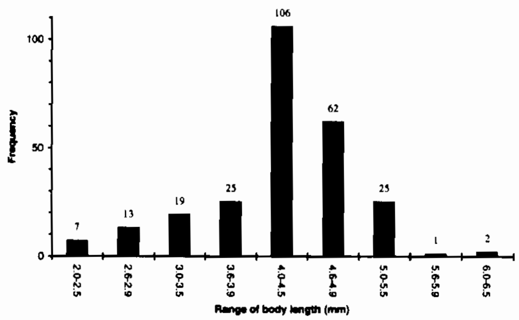

Fig 3–Frequency distribution of the body length of *G. spinigerum* advanced third-stage larvae from swamp eels (body size = 2.1-6.2 × 0.3-0.6 mm, average = 4.2 ± 0.7 × 0.4 ± 0.04 mm, n = 260).

hooklets in row four were found. Surprisingly, 1.3% of all the AL3 recovered had a fifth row of cephalic hooklets consisting of 3-48 (average 18.3 ± 13.1) hooklets. The average size of *G. spinigerum* AL3 was 4.2 ± 0.7 × 0.4 ± 0.04 mm (range 2.1-6.2 × 0.3-0.6 mm), and the most common body length (40.8%) fell between 4.0-4.5 mm (Fig 3).

Of the 12,278 gnathostome larvae examined, 3,117 (25.4%) were found to possess morphologically variant or abnormal cephalic hooklets (Table 4, Fig 4). The most common unusual features are that there were few to numerous extra rudimentary hooklets in between the four rows of hooklets (21.4%, Fig 4e, 4f), the presence of a fifth row of hooklets (1.3%, Fig 4d), abnormal hooklets in any of the four rows of hooklets (1.0%, Fig 4g), lobed or branched hooklets (0.7%, Fig 4j), spiral arrangement of the four rows of hooklets (0.4%, Fig 4h), rounded hooklets (0.2%), larvae having only three rows of hooklets (0.07%, Fig 4c), and some other features including five rows of incomplete/imperfect hooklets, spiral arrangement of the five rows of hooklets, overlapping rows of hooklets, hooklets without the base but with only the pointed spine (Fig 4i), and an unidentified species. Among these variants only one larva was identified as *G. doloresi* (0.01%, Fig 4b).

Table 4

Morphological variation and abnormality of cephalic hooklets of gnathostome larvae recovered from swamp eels (n = 12,278).

Type of variation or abnormality	No. of larvae	%
Rounded	31	0.2
Lobed or branched (several to all hooklets)	89	0.7
Four rows variable		
- row 4, not completely encircling the head-bulb	62	0.5
- row 4, outgrowing/jagged hooklets	33	0.3
- outgrowing/jagged hooklets in all rows	16	0.1
- fewer hooklets in all rows	4	0.03
- defect at the tip of the hooklets	3	0.02
Extra rudimentary hooklets (few to numerous)		
- above row 1	70	0.6
- below row 4	2,246	18.3
- in between the four rows	312	2.5
Three rows	9	0.07
Five rows		
- all rows completely encircling the head-bulb	39	0.3
- row 5 not completely encircling the head-bulb	128	1.0
Four rows spirally arranged	48	0.4
Six incomplete/imperfect rows	9	0.07
Miscellaneous	18	0.1
Total	3,117	25.4

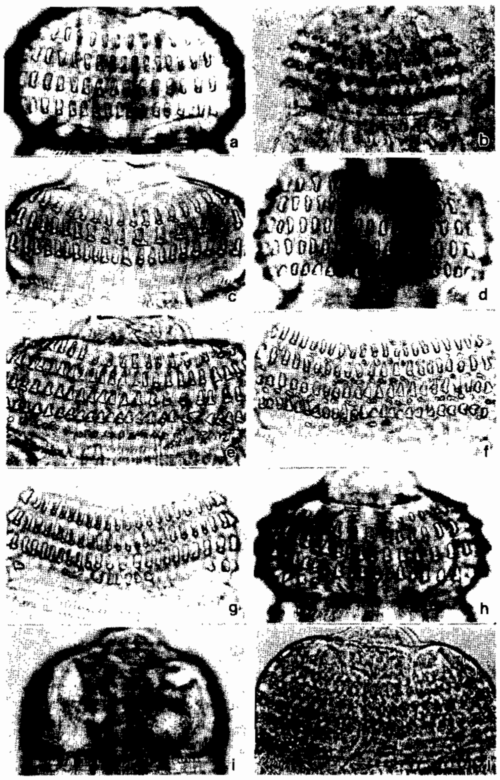

Fig 4–Morphological variation and abnormality of cephalic hooklets of gnathostome larvae from swamp eels sold in a local market in Bangkok: a) normal feature of the four rows of hooklets of *G. spinigerum* larva, b) normal cephalic hooklets of *G. doloresi* larva, c) three rows of hooklets, d) five complete rows of hooklets, e) four rows of hooklets with rudimentary hooklets below row four, f) numerous rudimentary hooklets in between the four rows of hooklets, g) abnormal hooklets in row four, h) spirally arranged row of hooklets, i) hooklets with only the pointed spine and no base, j) five rows of branched hooklets.

DISCUSSION

In general, the rainy season, the cool season, and hot season in Thailand are between May and October, October and February, and February and May, respectively. According to data from the Local Climatology Subdivision, Meteorological Department the highest amount of rainfall was between May to November 1996, and there was no rain at all between December 1996 and January 1997. In the present study, it is apparent that the highest intensity of gnathostome larvae (*G. spinigerum* larvae since more than 99% of the total larvae recovered were identified as this species) in swamp eels was found between October and December with the average number of larvae/g liver between 1.57-3.80, while the period with low intensity commenced from January. It is suggested that the level of the infection abruptly decreased soon after the completion of the rainy season, started to rise again when the rain had come, and reached the peak in the season with the highest amount of rainfall. The reason for this might be that the development period of *G. spinigerum* from ova in cat or dog feces to the encysted form of AL3 in eel livers requires about 1-2 months. If there is no rain then it is impossible for the ova to reach canals or any natural water reservoirs where cyclops, the first intermediate host, are present. It takes about 1-2 weeks for the ova to become embryonated and to hatch in water, and needs at least seven days to become fully developed early third-stage larvae in cyclops. The AL3 can then be found in eel livers two weeks after ingestion of the infected cyclops, and as the encysted form in about 4-5 weeks post-ingestion (Daengsvang, 1980).

In preparing swamp eels for consumption, Thai people actually discard eel viscera where most of the AL3 are concentrated. These infected viscera, if improperly disposed, can be ingested by snakes, rats or chickens (the paratenic hosts) and also by dogs and cats (the natural definitive hosts) (Daengsvang, 1980). If the dish containing eel flesh is well cooked, the chance of getting the infection is nil. Therefore, the principal role played by swamp eels in this zoonotic disease is that of a second intermediate host or paratenic host in the animal cycles. However, it is, with no doubt, a good source of infection for people who prefer raw dishes.

In the present study, the size of *G. spinigerum* AL3 recovered from eels (2.1-6.2 × 0.3-0.6 mm) was similar to that of the hepatic-stage AL3 obtained from laboratory infected mice 15 and 30 days post-infection, that is, 2.7-5.8 × 0.3-0.6 mm (Rojekittikhun *et al*, 1997b). The number of cephalic hooklets in rows one to four of the AL3 from eels in the present study (36-53, 38-57, 39-55 and 42-59, respectively) were almost identical to those of the AL3 from mice (36-52, 38-53, 39-55, and 43-59, respectively) reported by Rojekittikhun *et al* (1997b).

In laboratory infected mice, 15.5% of the hepatic-stage AL3 were found to possess variant or abnormal cephalic hooklets. The most common unusual feature (10.8%) was that there are extra rudimentary hooklets below row four and in between the four rows of hooklets (Rojekittikhun *et al*, 1997b). In the present study, the extra rudimentary hooklets appearing above row one, below row four and in between the four rows of hooklets are as high as 21.4%, and the overall morphological variation and abnormality of the hooklet is much higher (25.4%). This may be because more species of gnathostomes are involved as well as due to the longer infection period in natural life cycles.

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.

Almeyda-Artigas RJ. Finding of *Gnathostoma binucleatum* n sp (Nematoda: Spirurida) in wild felids and the role of fresh-water and oligohaline fishes as human gnathostomiasis vectors in the lower basin of Papaloapan river, Oaxaca-Veracruz, Mexico. *An Inst Cienc Mar Limnol Univ Nal Auton Mexico* 1991; 18 : 137-55. (Spanish, English abstract.)

Ando K, Tanaka H, Taniguchi Y, Shimuzu M, Kondo K. Two human cases of gnathostomiasis and discovery of a second intermediate host of *Gnathostoma nipponicum* in Japan. *J Parasitol* 1988; 74 : 623-7.

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

Daengsvang S. *Gnathostoma spinigerum* and human gnathostomiasis. A review. In: Sucharit S, *et al*, eds. The 25th Anniversary of the Faculty of Tropical Medicine, Mahidol University. Bangkok: Krung Siam Press, 1986: 124-47.

Kamiya H, Kamiya M, Ohbayashi M, Klongkamnuankarn K, Vajrasthira S. *Gnathostoma malaysiae* Miyazaki and Dunn, 1965 from *Rattus surifer* in Thailand. *Southeast Asian J Trop Med Public Health* 1987; 18 : 121-6.

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

Nawa Y, Imai J, Ogata K, Otsuka K. The first record of confirmed human case of *Gnathostoma doloresi* infection. *J Parasitol* 1989; 75 : 166-9.

Nuamtanong S, Waikagul J, Anantaphurti MT. Gnathostome infection in swamp eels, *Fluta alba*, in central Thailand. *Southeast Asian J Trop Med Public Health* 1997 (in press).

Ogata K, Imai J, Nawa Y. Three confirmed and five suspected human cases of *Gnathostoma doloresi* infection found in Miyazaki Prefecture, Kyushu. *Jpn J Parasitol* 1988; 37 : 358-64.

Radomyos P, Daengsvang S. A brief report on *Gnathostoma spinigerum* specimens obtained from human cases. *Southeast Asian J Trop Med Public Health* 1987; 18 : 215-7.

Rojekittikhun W, Pubampen S, Hiranyachattada P, Chiamratana B, Setasuban P. A survey on the infective larvae of *Gnathostoma spinigerum* in fresh water fish sold in the local markets in Bangkok. *J Trop Med Parasitol* 1989; 12 : 7-12. (Thai, English abstract.)

Rojekittikhun W, Pubampen S, Waikagul J. Twelve-years laboratory infection of cats with *Gnathostoma spinigerum*. *J Trop Med Parasitol* 1996; 19 : 63-4.

Rojekittikhun W, Pubampen S, Waikagul J. Swamp eels (*Fluta alba*), the genuine second intermediate host of *Gnathostoma* in Thailand. *J Trop Med Parasitol* 1997a (in press).

Rojekittikhun W, Pubampen S. Morphological variation and abnormality of cephalic hooklets of *Gnathostoma spinigerum* hepatic-stage larvae from laboratory infected mice. *Southeast Asian J Trop Med Public Health* 1997b (in press).

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.