A SURVEY OF INFECTIVE LARVAE OF *GNATHOSTOMA* IN EELS SOLD IN HO CHI MINH CITY

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Abstract. To investigate the distribution of *Gnathostoma* spp in Ho Chi Minh City (HCM city), 1,081 eels were purchased from a local market twice a month from March 1998 to February 1999. Infective larvae of *Gnathostoma* spp detected from the flesh and liver of eels by the press preparation technique were examined and identified. Three hundred and fifty advanced third-stage larvae were recovered from liver, none from the flesh. The average rate of infection was 0.11; a high rate of infection was found from August to November and a low rate of infection from February to May. The average number of larvae/eel was 2.9; the greatest number of larvae/eel was in January whereas the lowest was in March and April. There was a marked decrease in both prevalence and intensity of infection from February to May, followed by a rise from June. The finding suggests that in HCM city, the infection rate abruptly decreases soon after the end of the rainy season and starts to rise when the rain comes and reaches its peak at the end of the rainy season. All recovered larvae were identified as *G. spinigerum*.

INTRODUCTION

Human gnathostomiasis is endemic in Southeast Asia and most frequently caused by *Gnathostoma spinigerum*. The normal route of infection is ingestion of infected second intermediate or paratenic hosts such as fishes, amphibians, eels. In Vietnam, four species of *Gnathostoma* were detected in the final hosts, namely: *G. spinigerum*, *G. doloresi*, *G. hispidum* and *G. vietnamicum* (Le *et al*, 1963). The first case of human gnathostomiasis was reported in 1963 (Le *et al*, 1963) and since then new cases were sometimes observed (Tran 1992), all of the pathogens were identified as *G. spinigerum*. The understanding of the distribution of *Gnathostoma* infective larvae in second intermediate hosts is probably helpful to clarify the source of infection in Vietnam. There are several species of fresh water fishes serving as second intermediate hosts of *Gnathostoma* (Daengsvang, 1981; Rojekittikhun *et al*, 1989). It has been found that eels (*Fluta alba*) might be important in transmitting the infection either among human and reservoir animals (Daengsvang, 1981; Rojekittikhun *et al*, 1989; Setasuban *et al*, 1991; Akahane *et al*, 1995; Nuamtanong *et al*, 1997). The infection rates are high (40-100%) and most of the larvae concentrate in the liver (Setasuban *et al*,1991; Rojekittikhun *et al*, 1997). The purpose of this study was to investigate the prevalence of larval *Gnathostoma* and describe their intensity during each month of a year in eels sold for local consumption in HCM city.

MATERIALS AND METHODS

One thousand eighty-one eels were purchased from a local market in District 5 in HCM city twice a month from March 1998 to February 1999. The eels were sacrificed, their livers were separated and washed several times in normal saline, their flesh was sliced into small pieces. Both liver and flesh were pressed between two glass plates and examined under a dissecting microscope. The detected larvae were collected, counted, examined for the morphology under a light microscope.The number of cephalic hooklets of some *G. spinigerum* larvae were counted.

RESULTS

A total of 350 gnathostome larvae were recovered from 1,081 eels in one year. All of them were found in liver, none in flesh. The overall infection rate (prevalence) and the overall number of larvae/eel (intensity) were 0.11 and 2.90, respectively (Table 1). The highest infection rate occurred in October and the lowest in March-April. The highest numbers of larvae/eel was found in January and the lowest in March - April. Both prevalence and intensity of infection exhibited a seasonal fluctuation, the parasites were more abundant in the rainy season, with peaks between August to November, and August to January, respectively; the lowest levels of infection were between March and April. There was a marked decrease in infec-

Table 1
Seasonal variation of prevalence and intensity of *Gnathostoma* advanced third-stage larvae in eels sold in a local market in HCM city (March 1998-February 1999).

Month and year	No. positive/ No. examined	% Positive	No. of larvae recovered	Mean no. of larvae/eel	No. of larvae/eel (Range)	Amount of rain in HCM city (mm)
March 1998	0 /30	0	0	0	0	0
April	0 /35	0	0	0	0	8.3
May	3 /150	2	3	1	1-1	219.5
June	13 /150	8.66	26	2	1-6	466.5
July	6 /90	6.60	16	2.6	1-7	240.7
August	14 /70	18.66	40	2.9	1-14	400.9
September	11 /135	8.10	30	2.7	1-10	349.4
October	31 /110	28.18	109	3.5	1-47	208.3
November	24 /135	17.7	73	3.0	1-15	422.4
December	7 /118	5.93	16	2.3	1-6	177.4
January 1999	9 /90	10.00	35	3.9	1-12	72.2
February	2 /105	1.90	2	1	1-1	55.0
Total	120 /1,081	0.11	350	2.9		
		R* : 80.5		R* : 141		

R* = Test rank of Spearman
N=12, $R_{spearman}$ = 80.5 < 116 ⩾ there is correlation between infection rate and amount of rain.
N=12, $R_{spearman}$ = 141 > 116 ⩾ there is no correlation between intensity of infection and amount of rain.

Table 2
Infection rate and the number of *G. spinigerum* larvae/eel in the eel, *Fluta alba*.

Weight (g)	No. positive/ No. examined	% positive	No. of larvae recovered	Mean No. of larvae/eel	No. of larvae/eel (Range)
100 - 200	24/129	18.6	64	2.7	1 - 10
201 - 300	91/262	11.8	108	3.5	1 - 47
301 - 400	32/301	10.6	92	2.9	1 - 12
401 - 500	19/236	8.0	55	2.9	1 - 7
> 500	14/153	9.2	31	2.2	1 - 5
Total	120/1,081		350		
		R*: 38		R*: 27	

R* = Test rank of Spearman
$R_{spearman}$ = 38 > ⩾ 0, there is no correlation between prevalence of infection and weight of eels.
$R_{spearman}$ = 27 > ⩾ 0, there is no correlation between intensity of infection and weight of eels.

Table 3
Number of cephalic hooklets of *G. spinigerum* advanced third-stage larvae recovered from eels (n=50).

Row No.	No. of hooklets (range)	$\bar{X}$ ± SD
Row 1	36-46	40.8 ± 2.1
Row 2	39-49	43.8 ± 2.2
Row 3	42-51	45.9 ± 2.0
Row 4	44-55	49.9 ± 2.2

tion rate and intensity from February to May, followed by an increase from June (Fig 1).

The advanced third-stage larvae were 2.5-4.5 mm in length and 0.3-0.4 mm in width. The average size of these larvae was 3.4 ± 0.6 x 0.3 ± 0.05 mm. The number of cephalic hooklets of advanced third-stage larvae is presented in Table 2, and the frequency and relative frequency distribution of hooklets number are shown in Table 3. The average number of hooklets from rows one to four were 40.8 ± 2.1 (range 36-46), 43.8 ± 2.2 (range

Table 4
The average number of cephalic hooklets of *G. spinigerum* third-stage larvae.

Row Larvae from	1st	2nd	3rd	4th
Vietnam (Le, 1999)	40.8	43.8	45.9	49.9
Thailand (Rojekittikhun, 1997)	42.2	44.6	46.8	50.0
Japan (Miyazaki, 1960)	44.3	47.3	49.6	52.0

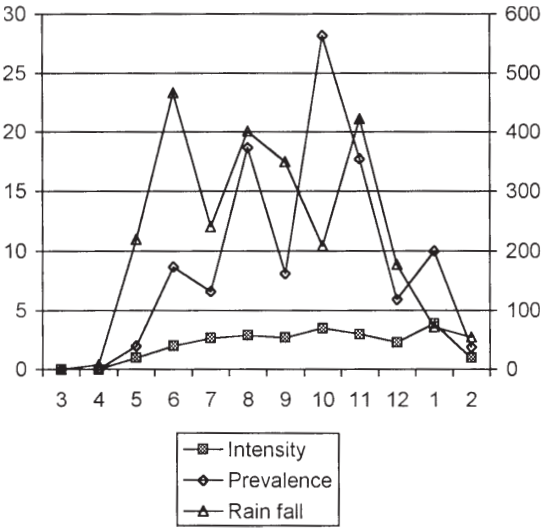

Fig 1–The prevalence and intensity of *Gnathostoma* advanced third-stage larvae in eels (March 1998-February 1999).

39-49), 45.9 ± 2.0 (range 42-51) and 49.9 ± 2.2 (range 44-55), respectively. The highest frequency of hooklets numbers in rows one to four was between 40-44 (76.0%), 40-44 (60.0%), 45-49 (72.0%) and 50-54 (56.0%), respectively. No larvae having more than 47 hooklets in row one, and having less than 44 hooklets in row four were found.

Of the 350 gnathostome larvae examined, all were found to possess morphologically normal cephalic hooklets, the base of hooklets was oblong in shape and almost equal in size. Three hundred forty-eight larvae had four rows of hooklets; and two others had a fifth rudimentary row of cephalic hooklets (range 9-12). The whole larval body was covered with transverse rows of single pointed spines, but their size and density gradually decreased toward the posterior end. All these larvae were identified as *G. spinigerum*.

DISCUSSION

In general, in south provinces of Vietnam, the rainy season begins in May and ends in November and the hot (dry) season is between December and April. According to data from the Meteorological Department in HCM city, the highest amount of rainfall was between June to November 1998 and there was no rain at all in March 1998.

This study shows that the prevalence of gnathostome larvae starts to increase in May and attains its peak around October. The intensity of gnathostome larvae is also increased in this period of time, however, the time trend is not remarkable (Table 1, Fig 1).

The result can be explained as follows: first, several domestic animals such as cats and dogs, are definitive hosts of *Gnathostoma spinigerum* (all of the larvae recovered were identified as this species), in which the prevalence and worm burden of *G. spinigerum* exhibited a seasonal fluctuation. The parasites were more abundant in the rainy season and the early winter (August-December) than in summer (April-March), most parasites were sexually mature between August and December while immature worms were observed during March and April (Maleewong *et al*, 1992). During this period, more eggs were excreted, and the soil, polluted with animal fecal matter, was more contaminated with the gnathostome eggs. Secondly, in the dry season, the feces and gnathostome eggs remain on the surface soil and can not continue the life cycle. When the rainy season comes, the water flow flushes the *Gnathostoma* eggs to the surface reservoirs and the eggs then hatch into first-stage larvae. The larvae are then ingested by cyclops spp and develop into the second-stage larvae. Eels, fishes, frogs which have eaten these infective cyclops spp will be infected and larvae can be found in flesh and liver. The development period of *G. spinigerum* from ova in cats or dogs

feces to the encysted form of third-stage larvae in eel livers requires about 1-2 months.

This explains why the prevalence of gnathostome larvae among eels is increased in the rainy season. It is remarkable that the peak of prevalence of gnathostome larvae is around the end of rainy season. This is because the cumulative effects of contamination of gnathostome eggs in surface water reservoirs. The concentration of infective cyclops increase by time in the rainy season. In addition, in the rainy season, the population of cyclops may be more abundant thanks to the availability of fresh water flora and fauna. Therefore, people should be warned to be careful in preparation of food, especially around the end of the rainy season. In additon, the infection rate increased according to amount of rain whereas the number of larvae did not increase (Table 1).

These findings were almost similar to those reported by Rojekittikhun *et al* (1998) in Thailand, with a peak between October and December and the lowest between March and April, the level of infection started to rise when the rain had come, and reached the peak in the rainy season with the highest amount of rainfall, then abruptly decreased soon after the completion of the rainy season.

Because the prevalence of gnathostome larvae is a function of time, we strongly recommend scientists report the findings on prevalence of larvae along with information of the time of the conducting a study.

The variation of infection rate and number of larvae/eel to weight of eels was also considered, however, both did not correlate with weight (Table 2), suggesting that the infection of eels might occur at any period of life but not uniformly.

Theoretically, larvae are located in flesh and liver. However, we could not find any larvae in eel flesh. This is consistent with the finding of Setasuban (1991) and Rojekittikhun (1997) who found that in low infection areas, most larvae are in liver rather than in flesh. Because in Vietnam most housewives discard eel viscera during food preparation, this implies the risk of infection with *Gnathostoma* is rather low. However, since some people prefer roasted eels, the chance of undercook is high and then the risk of infection is high. Therefore these dishes should be discouraged. Since *Gnathostoma* larvae were found to be concentrated in the liver of fresh water eels, the discarded viscera should be carefully controlled in order to avoid transmitting a new infection to dogs and cats.

In the present study, most larvae recovered had four rows of hooklets in the cephalic bulb, two had some hooklets (range 8-12) in the fifth row, which are rudimentary. This may be due to the longer infection in natural life cycles. The number of cephalic hooklets of advanced third-stage larvae from rows one to four (36-46, 39-49, 42-51 and 44-55) (Table 3) were almost identical to those of the third-stage larvae from eels (36-53, 38-57, 39-55 and 42-59) reported by Rojekittikhun *et al* (1998). and slightly fewer than those of the third-stage larvae reported by Miyazaki (1991) (Table 4).

ACKNOWLEDGEMENTS

This study was financially supported by SEAMEO TROPMED-FRANCE Programme of Co-operation.

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