GNATHOSTOMA INFECTION IN FISH CAUGHT FOR LOCAL CONSUMPTION IN NAKHON NAYOK PROVINCE, THAILAND. II. SEASONAL VARIATION IN SWAMP EELS

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Abstract. From August 2000 to August 2001, 1,844 swamp eels (*Monopterus albus*) were purchased from several local markets in Nakhon Nayok Province, Thailand, and examined for the presence of *Gnathostoma* advanced third-stage larvae. The overall prevalence was 30.1% and the mean number of larvae/eel (infection intensity) was 10.0. The highest infection rate (44.1%) was found in August 2000 and the lowest (10.7%) in March 2001. The greatest mean number of larvae/eel (75.1) was found in August 2000, whereas the fewest (2.3) was in July 2001. It is suggested that the prevalence and intensity of infection decreased within two months after the end of the rainy season and started to rise again about two months after the next rainy season began. A total of 5,532 *Gnathostoma* larvae were recovered from 555 infected eels, with a maximum number of 698 larvae/eel. The highest rates of *Gnathostoma* infection according to eel body length and weight were 87.5% in the group 91-100 cm, and 100% in groups of 901-1,100 g, respectively. There were significant correlations between eel body lengths and infection rates, body lengths and infection intensities; eel body weights were also significantly correlated with infection rates and infection intensities. It was noted that the longer/heavier the eels were, the higher would be the infection rates and the greater the infection intensities. Tissue distributions of *Gnathostoma* larvae in the livers and muscles of swamp eels were as follows: 43.0% of the total number of larvae were found in the muscles and 57.0% were in the liver; 29.7, 51.7, and 18.6% were in the anterior, middle, and posterior parts, respectively; 35.1% were in the dorsal part, while 64.9% were in the ventral part; 9.0, 18.7, 7.4, 20.6, 33.1, and 11.2% were in the anterodorsal, mediodorsal, posterodorsal, anteroventral, medioventral and posteroventral parts, respectively. Of the 5,532 *Gnathostoma* larvae examined, 1,101 (19.9%) were found to possess morphological variants or abnormal cephalic hooklets. The most common unusual feature was that there were few to numerous extra rudimentary hooklets below row 4 and between the 4 rows of hooklets (7.6%), the presence of a fifth row of hooklets (3.5%), abnormal hooklets in any of the 4 rows of hooklets (5.2%), spiral arrangement of the 4 rows of hooklets (1.8%), and larvae having only 3 rows of hooklets (0.3%).

INTRODUCTION

Swamp eels (*Monopterus albus*, previously *Fluta alba*) are a very important intermediate/paratenic host of *Gnathostoma* in Thailand (Rojekittikhun *et al*, 1998a). The prevalence rate was normally the highest among other fresh-water fish species, ranging from 10-100% (Daengsvang *et al*, 1964; Daengsvang, 1980; Setasuban *et al*, 1991; Nuamtanong *et al*, 1998; Rojekittikhun *et al*, 1989, 2002b, 2004; Saksirisampant *et al*, 2002a,b; Sugaroon and Wiwanitkit, 2003). The infection intensity in swamp eels was also the greatest among all infected animal species, up to 2,582 larvae/eel (Setasuban *et al*, 1991; Rojekittikhun *et al*, 2001, 2002a; Rojekittikhun, 2002). Moreover, they were found to harbor at least four species of *Gnathostoma*: *G. spinigerum* (Daengsvang, 1964; 1980; Rojekittikhun *et al*, 1998b), *G. hispidum* (Nuamtanong *et al*, 1998), *G. doloresi* (Rojekittikhun *et al*, 1998a), and *G. vietnamicum* (Nuamtanong *et al*, 1998).

In Thailand, high numbers of gnathostome larvae in infected swamp eels were found between October and December, with low numbers during the period March to April (Rojekittikhun *et al*, 1998b). This result was based especially upon infection intensity. The prevalence rate in each month could not be obtained since the number of recovered larvae had been counted from pools of

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livers. Furthermore, the recovered larvae were collected only from eels' livers, not from the whole bodies of the eels. An attempt to investigate both the prevalence and intensity of *Gnathostoma* infection in swamp eels for each month of the year for at least one year was, therefore, our first objective. The other purpose was to study the infection in eels caught for local consumption in Nakhon Nayok Province, where gnathostomiasis is endemic.

MATERIALS AND METHODS

Swamp eels were purchased 2-3 times a month from several local markets in Nakhon Nayok Province, between August 2000 and August 2001. They were immediately transported to the laboratory of the Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok. Live eels were transferred into an aquarium, while dead ones were kept in a freezer. Before being subjected to the next preparation stage, each eel was measured and weighed.

The visceral organs were taken out; the liver was cut into small pieces and firmly pressed between two thick glass plates, then examined under a dissecting microscope or a large hand lens for the presence of gnathostome larvae. Eel muscle was roughly divided into six parts according to body region: the dorsal and ventral parts, which were then separated into anterior, middle and posterior portions. All the muscles were cut and scraped out of bone and skin, and examined as for the liver.

The recovered *Gnathostoma* larvae were collected, cleaned, counted, and fixed in 70% ethanol. Their lengths and widths were measured using a camera lucida.

RESULTS

The prevalences and infection intensities of *Gnathostoma* advanced third-stage larvae in swamp eels, and the amounts of rainfall in Nakhon Nayok Province between August 2000 and August 2001 are shown in Table 1 and Figs 1 and 2. The overall infection rate (prevalence) was 30.1% and the average number of larvae/eel (infection intensity) was 10.0 (Table 1). The highest infection rate (44.1%) was found in August 2000 and the lowest rate (10.7%) in March 2001. The rates seemed to drop during the months February to April 2001. The greatest mean number of larvae/eel (75.1) was found in August 2000, and the fewest (2.3) in July 2001. The infection intensities became lower from December 2000 and seemed to rise again in August 2001 (Table 1 and Fig 2).

A total of 5,532 gnathostome larvae were recovered from 555 infected eels over one year, with a maximum of 698 larvae/eel (Table 1). The

Table 1
Prevalences and infection intensities of *Gnathostoma* advanced third-stage larvae in swamp eels, and rainfall in Nakhon Nayok Province, August 2000 to August 2001.

Year and month	Eels			No. of larvae recovered	No. of larvae/eel		Rainfall in Nakhon Nayok Province (mm)
	No. examined	No. Positive	% Positive		Mean	Range	
2000 August	59	26	44.1	1,953	75.1	1-698	157.3
2000 September	189	83	43.9	1,081	13.0	1-56	262.5
2000 October	121	41	33.9	435	10.6	1-43	171.1
2000 November	132	56	42.4	687	12.3	1-65	10.8
2000 December	136	57	41.9	396	6.9	1-41	1.7
2001 January	117	39	33.3	160	4.1	1-67	0.6
2001 February	150	30	20.0	105	3.5	1-16	10.8
2001 March	150	16	10.7	40	2.5	1-15	135.2
2001 April	140	20	14.3	61	3.1	1-16	104.0
2001 May	137	43	31.4	136	3.1	1-12	227.6
2001 June	136	32	23.5	130	4.1	1-21	206.2
2001 July	262	78	29.8	178	2.3	1-11	103.0
2001 August	115	34	29.6	170	5.0	1-12	135.3
Total	1,844	555	30.1	5,532	10.0	1-698	1,526.1

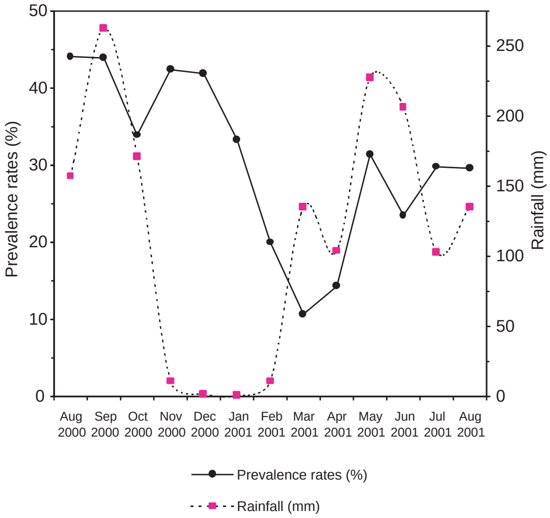

Fig 1–Prevalences of *Gnathostoma* infection in swamp ells and rainfall in Nakhon Nayok Province, August 2000 to August 2001.

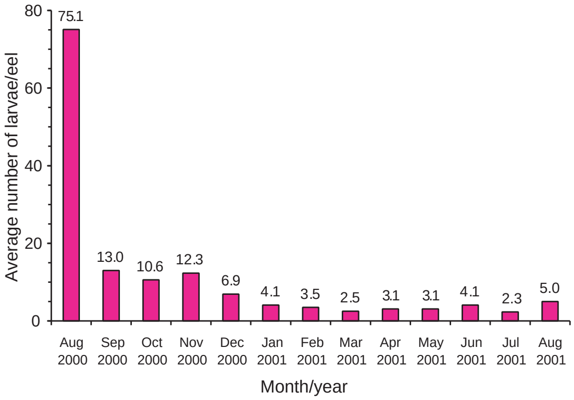

Fig 2–Average number of *Gnathostoma* larvae per ell (infection intensity), August 2000 to August 2001.

highest rates of *Gnathostoma* infection according to eel body length and weight were 87.5% (7/8) in the group 91-100 cm (Table 2), and 100% (2/2) in groups 901-1,100 g (Table 3), respectively. The maximum number of 698 larvae/eel was recovered from an eel 86 cm long and 850 g in body weight. There were statistically significant correlations between eel body lengths and infection rates (r = 0.786, p-value = 0.021), and also between eel body lengths and infection intensities (r = 0.910, p-value = 0.002) (Table 2). Table 3 shows that eel body weight was highly significantly correlated with infection rate (r = 0.897, p-value = 0.000), and eel body weight was also significantly correlated with infection intensity (r = 0.770, p-value = 0.006).

The distributions of *Gnathostoma* larvae in the livers and muscles, and in the muscles according to the body regions of swamp eels are shown in Tables 4 and 5: 43.0% (2,381/5,532) of the total number of larvae were found in the muscles and 57.0% (3,151/5,532) in the liver (Table 4); 29.7% (706/2,381), 51.7% (1,232/2,381) and 18.6% (443/2,381) were in the anterior, middle and posterior parts, respectively (Table 5); 35.1% (836/2,381) were in the dorsal part, while 64.9% (1,545/2,381) were in the ventral part; 9.0% (215/2,381), 18.7% (445/2,381), 7.4% (176/2,381), 20.6% (491/2,381), 33.1% (787/2,381), and 11.2% (267/2,381) were in the anterodorsal, mediodorsal, posterodorsal, anteroventral, medioventral, and posteroventral parts, respectively (Table 5). Tables 4 and 5 also show the comparison of tissue distribution between the larvae recovered from one infected eel, which harbored the highest number of 698 larvae, and those from the total infected eels (555),

Table 2
Infection rates and numbers of *Gnathostoma* larvae per eel, by eel body length.

Eel body length (cm)	No. examined	No. positive	% positive	No. of larvae recovered	No. of larvae/eel	
					Mean	Range
21-30	14	4	28.6	17	4.3	1-10
31-40	89	24	27.0	137	5.7	1-26
41-50	237	48	20.3	265	5.5	1-41
51-60	648	159	24.5	1,067	6.7	1-56
61-70	827	300	36.3	2,567	8.6	1-87
71-80	14	7	50.0	47	6.7	1-15
81-90	7	6	85.7	1,287	214.5	5-698
91-100	8	7	87.5	145	20.7	1-65
Total	1,844	555	30.1	5,532	10.0	1-698

Table 3
Infection rates and numbers of *Gnathostoma* larvae per eel, by eel body weight.

Eel body weight (g)	No. examined	No. positive	% positive	No. of larvae recovered	No. of larvae/eel	
					Mean	Range
101 - 200	19	5	26.3	16	3.2	1 - 5
201 - 300	260	51	19.6	302	5.9	1 - 26
301 - 400	692	168	24.3	1,075	6.4	1 - 56
401 - 500	842	311	36.9	2,672	8.6	1 - 87
501 - 600	3	1	33.3	3	3.0	3
601 - 700	11	6	54.5	32	5.3	1 - 15
701 - 800	4	2	50.0	21	10.5	5 - 16
801 - 900	6	5	83.3	1,317	263.4	41 - 698
901 - 1,000	1	1	100	11	11.0	11
1,001 - 1,100	1	1	100	11	11.0	11
1,101 - 1,200	5	4	80.0	72	18.0	1 - 65
Total	1,844	555	30.1	5,532	10.0	1 - 698

Table 4
Distribution of *Gnathostoma* advanced third-stage larvae in the livers and muscles of one infected swamp eel, which harbored the highest number of 698 larvae, and the total infected eels (555), harboring altogether 5,532 larvae.

Organ	No. of larvae recovered (%)	
	One infected eel (%)	Total infected eels (%)
Liver	339 (48.6)	3,151 (57.0)
Muscle	359 (51.4)	2,381 (43.0)
Total	698	5,532

Table 5
Distribution, by body region, of *Gnathostoma* advanced third-stage larvae in the muscles of one infected swamp eel, which harbored the highest number of larvae, and total infected eels.

Eel body regions	No. of larvae recovered (%)	
	One infected eel (%)	Total infected eels (%)
Dorsal	166 (46.2)	836 (35.1)
Anterodorsal	54 (15.0)	215 (9.0)
Mediodorsal	71 (19.8)	445 (18.7)
Posterodorsal	41 (11.4)	176 (7.4)
Ventral	193 (53.8)	1,545 (64.9)
Anteroventral	65 (18.1)	491 (20.6)
Medioventral	82 (22.8)	787 (33.1)
Posteroventral	46 (12.8)	267 (11.2)
Total	359	2,381
Anterior	119 (33.1)	706 (29.7)
Middle	153 (42.6)	1,232 (51.7)
Posterior	87 (24.2)	443 (18.6)
Total	359	2,381

harboring altogether 5,532 larvae.

Of the 5,532 *Gnathostoma* larvae examined, 1,101 (19.9%) were found to possess morphological variants or abnormal cephalic hooklets (Table 6). The most common unusual feature was that there were few to numerous extra rudimentary hooklets below row 4 and between the 4 rows of hooklets (7.6%), the presence of a fifth row of hooklets (3.5%), abnormal hooklets in any of the 4 rows of hooklets (5.2%), spiral arrangement of the 4 rows of hooklets (1.8%), and larvae having only 3 rows of hooklets (0.3%).

DISCUSSION

The overall prevalence (30.1%) of *Gnathostoma* infection in swamp eels in Nakhon Nayok in the present study, is a little lower than that (38.1%) of our previous report (Rojekittikhun *et al*, 2002b). The average number of larvae/eel (10.0) is also a little lower than that (11.0) of the report in 2002. However, these figures (30.1% and 10.0 larvae/eel) are about one-half and one-third of those (68.7% and 29.7) reported by Nuamtanong *et al*, in 1998. It was arbitrarily estimated that the rates of the infection in this prov-

Table 6
Morphological variation and abnormality of cephalic hooklets of 5,532 *Gnathostoma* larvae recovered from 555 infected swamp eels.

Type of variation or abnormality	No. of larvae	%
Normal features of the 4 hooklet rows	4,431	80.1
Four rows variable		
- row 4, not completely encircling the head-bulb	131	2.4
- defect at the tip of the hooklets	56	1.0
- four rows spirally arranged	101	1.8
Extra rudimentary hooklets (few to numerous)		
- above row 1	1	0.02
- below row 4	214	3.9
- in between the 4 rows	206	3.7
Three rows	19	0.3
Five rows		
- all rows completely encircling the head-bulb	60	1.1
- row 5 not completely encircling the head-bulb	82	1.5
- five rows spirally arranged	51	0.9
Overall abnormality	1,101	19.9
Total	5,532	100

ince decreased about 20% at about each 5-8 year interval (Rojekittikhun *et al*, 2001).

Rojekittikhun *et al* (1998b) reported that high and low intensities of *Gnathostoma* infection in eel livers, bought from a local market in Bangkok, occurred between October and December 1996, and March and April 1997, respectively (the highest was in October, the lowest in March). Similarly, as reported by Saksirisampant *et al* (2002b), the highest and lowest prevalences were observed in September 1999 and April 2000, respectively, and low prevalences and intensities were both between March and May 2000. It was suggested that the level of infection abruptly decreased soon after the end of the rainy season, started to rise again when the rain came, and reached a peak in the season with high rainfall (Rojekittikhun *et al*, 1998b).

In general, the rainy season, the cool season, and the hot season in Thailand are between May and October, November and February, and February and May, respectively. However, according to data from the Local Climatology Subdivision, Meteorogical Department, Thailand, heavy rainfall in Nakhon Nayok Province in 2000-2001 was between March and October, and light rainfall was between November and February. In the present study, it is apparent that the high prevalence and intensity of *Gnathostoma* infection in swamp eels was between August and December 2000, while the period with low intensity commenced from January 2001. It is suggested that the prevalence and intensity of infection decreased within two months after the completion of the rainy season and started to rise again about two months after the next rainy season began. The reason for this may be that the development period of *G. spinigerum* from eggs in cats' or dogs' feces to encysted advanced third-stage larvae in swamp eels requires about 1-2 months (Rojekittikhun *et al*, 2001). However, we found that there were no correlations between infection rate and amount of rainfall ($r = 0.110$, p-value = 0.720), or between infection intensity and amount of rainfall ($r = 0.188$, p-value = 0.539). This lack of correlation coincides with a similar study in Ho Chi Minh City, Vietnam (Xuan and Rojekittikhun, 2000). The reason for this may be that it is impossible to differentiate between new and old infections in infected eels.

The present study examined correlations between the body lengths/weights of swamp eels, and *Gnathostoma* infection rates and intensities. It is evident that there were statistically significant correlations between all of the following pairs: body length and infection rate, body length and infection intensity, body weight and infection rate, and body weight and infection intensity. Therefore, it is interesting to note that the longer/heavier the eels were, the higher the infection rates and the greater the infection intensities. Bigger eels may feed more and live longer, so that the chance of becoming infected increases accordingly. In contrast to the present study, our previous studies and data from reports by other investigators suggested that the prevalence and intensity of *Gnathostoma* infections in swamp eels were not associated with the size of the infected eels (Xuan and Rojekittikhun, 2000). The prevalence and intensity of infection appeared to vary directly with endemicity (Rojekittikhun *et al*, 2002b). This was illustrated by cases of rather small eels (64-67 cm long) that were naturally infected with 2,283-2,582 gnathostome larvae (Setasuban *et al*, 1991; Nuamtanong *et al*, 1998; Rojekittikhun *et al*, 2001).

The distribution of *Gnathostoma* larvae in the livers and muscles of swamp eels in the present study follows the distribution patterns that have been described before (Setasuban *et al*, 1991; Rojekittikhun *et al*, 2001; 2002b). About half of the total number of larvae was concentrated in the liver, whereas the remaining half was distributed throughout the muscles. Negligible numbers of larvae were found in other visceral organs. The most preferred region, among the six divided parts of the eels, was the medioventral.

Rojekittikhun *et al* (1998b) have reported morphological variation and abnormality of cephalic hooklets of *Gnathostoma* larvae recovered from swamp eels. The most common unusual feature (21.4%) was extra rudimentary hooklets above row 1, below row 4, and between the 4 rows of hooklets. The overall morphological variation and abnormality of the hooklets was 25.4%. In the present study, the most common unusual feature was extra rudimentary hooklets below row 4 and between the 4 rows of hooklets, but at a lower rate (7.6%). The overall morphological variation and abnormality of the hooklets (19.9%) was also lower. This may be because all of the infected eels were captured from only one small province (Nakhon Nayok) compared with several provinces in the previous report.

As swamp eels are voracious predators and are remarkably tolerant to harsh environments, they truly play an important role in maintaining and spreading *Gnathostoma* infection in Thailand. Nakhon Nayok Province has been, and remains, the area with the highest and heaviest records of infection, although both prevalence and intensity of infection have gradually decreased over time.

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