GNATHOSTOMA INFECTION IN NAKHON NAYOK AND PRACHIN BURI, CENTRAL THAILAND

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Abstract. *Gnathostoma* infection in Nakhon Nayok and Prachin Buri Provinces, Central Thailand, was investigated. The prevalence and intensity of infection of swamp eels were determined; dog fecal samples and fresh-water copepods were examined for evidence of infection. The overall prevalence of eel infection was 38.1% (117/307) in Nakhon Nayok and 24.0% (74/308) in Prachin Buri – the former rate being significantly higher than the latter. Most of the positive Nakhon Nayok eels (53.8%) harbored only 1-9 larvae; only one eel bore more than 50 larvae. In Prachin Buri, 67.6% of the positive eels harbored 1-9 larvae; again, only one eel bore more than 50 larvae. The mean number of 11.0 ± 10.4 larvae/eel in Nakhon Nayok was not significantly different from that of Prachin Buri (9.3 ± 11.4).

A total of 1,292 gnathostome larvae were recovered from 307 eels in Nakhon Nayok. Of these, 52.3% had accumulated in the liver and 47.7% had spread throughout the muscles. In eels from Prachin Buri, 50.6% and 49.4% of the total of 688 larvae (from 308 eels) were found in the liver and muscles, respectively. The larvae preferred encysting in ventral of muscles rather than dorsal part; they preferred the middle portion to the anterior and posterior portions. The average length of gnathostome larvae recovered from Nakhon Nayok eels was 4.0 ± 0.5 mm (range 2.5-5.1 mm) and the average body width was 0.40 ± 0.05 mm (range 0.29-0.51 mm). Those from eels in Prachin Buri were 3.9 ± 0.5 mm (range 2.2-5.1 mm) and 0.34 ± 0.05 mm (range 0.20-0.48 mm), respectively. The mean body length and width of the larvae from eels in Nakhon Nayok were significantly greater than those of the larvae from eels in Prachin Buri.

In Ban Phrao, Nakhon Nayok, none of the first 44 fecal specimens examined was positive. Of the second (68) and the third (70) specimens, one (1.5%) and two (2.9%) samples were positive. However, six months after the third fecal collection, no eggs were found. In Tha Ngam, Prachin Buri, no eggs were found in all three batches (109, 115, and 100 fecal samples). A cyclops survey of 4,000-5,000 crustacea from each of two areas (Ban Phrao and Tha Ngam) found no evidence of natural cyclops infection.

INTRODUCTION

Gnathostomiasis is a zoonotic disease caused by roundworms of the genus *Gnathostoma*, 23 species of which have been described. However, to date, only 11-13 species are considered to be valid (Daengsvang, 1980; 1982; Miyazaki, 1991; Almeyda-Artigas *et al*, 2000), and five of these – *G. spinigerum* Owen 1836, *G. hispidum* Fedtschenko 1872, *G. doloresi* Tabangui 1925, *G. nipponicum* Yamaguti 1941 and *G. binucleatum* Almeyda-Artigas 1991 – have been shown to be responsible for human disease (Daengsvang, 1983; Ando *et al*, 1988; Ogata *et al*, 1988; Nawa *et al*, 1989; Almeyda-Artigas, 1991). Human infections, cutaneous and/or visceral larva migrans, are usually acquired by consumption of raw or improperly cooked fresh-water fish or meat that harbor infective (advanced third-stage) larvae. Several thousand cases of human gnathostomiasis caused by the five *Gnathostoma* species mentioned above have been reported from various parts of the world. Most cases were documented in Thailand

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(Daengsvang, 1980, 1982, 1986; Radomyos and Daengsvang, 1987), Japan (Miyazaki, 1960, 1966, 1991; Kagei, 1991; Nawa, 1991; Ando *et al*, 1991; Sato *et al*, 1992; Taniguchi *et al*, 1992; Nawa *et al*, 1997; Akahane, 1998; Akahane *et al*, 1998; Kurokawa *et al*, 1998) and Mexico (Camacho *et al*, 1998; Koga *et al*, 1998; Ogata *et al*, 1998; Rojas-Molina *et al*, 1999; Leon-Regagnon *et al*, 2000), although some cases were reported from India, Bangladesh, Burma, Lao PDR, Cambodia, China, Vietnam, Malaysia, Indonesia, the Philippines, Palestine, Israel, Australia, Ecuador, and Argentina (Daengsvang, 1982; Miyazaki, 1991; Almeyda-Artigas *et al*, 2000).

In Thailand, from 1961 to 1963, about 900 patients were clinically diagnosed each year as having gnathostomiasis, and about 10 deaths were reported between 1967-1981 (Daengsvang, 1980; 1986). In each year from 1985 to 1988, about 300-600 suspected gnathostomiasis patients came to the gnathostomiasis clinic at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok. From 1989 until now, about 100-400 new cases of suspected gnathostomiasis have seen each year at the Hospital for Tropical Diseases (Rojekittikhun, unpublished data). Thailand has now, as it had in the past, the highest burden of recorded gnathostomiasis.

There are at least five species of *Gnathostoma* in Thailand: *G. spinigerum* Owen 1836, *G. hispidum* Fedtschenko 1872, *G. doloresi* Tabangui 1925, *G. vietnamicum* Le-Van-Hao 1965, and *G. malaysiae* Miyazaki and Dunn 1965 (Dissamarn *et al*, 1966; Daengsvang, 1973, 1980; Kamiya *et al*, 1987). However, only *G. spinigerum* is known to cause human gnathostomiasis in Thailand (Daengsvang, 1980, 1986; Radomyos and Daengsvang, 1987; Sirikulchayanonta and Viriyavejakul, 2001). The life cycle of *G. spinigerum* involves three essential hosts: first intermediate hosts (mainly freshwater cyclops); second intermediate and paratenic hosts (sources of the infection for the definitive host and man); and definitive hosts (normally cats and dogs). The second intermediate/paratenic hosts are numerous. Forty-eight species of vertebrate: fish (20), frogs (2), reptiles (11), avians (11) and mammals (4), are reported to be naturally infected with *G. spinigerum* advanced third-stage larvae (Daengsvang, 1980; Rojekittikhun *et al*, 1989a, b). Among these, swamp eels (*Monopterus albus*) are found to be the best second intermediate/paratenic hosts of the parasite: these eels are highly prevalent and give rise to the greatest intensity of infection. These eels are invariably present in Nakhon Nayok and Prachin Buri Provinces, Central Thailand, where gnathostomiasis is endemic (Daengsvang, 1980; Rojekittikhun *et al*, 1989a, 1998a, b; Setasuban *et al*, 1991; Nuamtanong *et al*, 1998).

The purpose of this study is to investigate the current status of *Gnathostoma* infection in Nakhon Nayok and Prachin Buri by means of: 1) determining the prevalence and intensity of the infection in swamp eels; 2) examining the positivity of the fecal samples of stray and domesticated dogs, and of fresh-water cyclops in specified areas of the two provinces.

MATERIALS AND METHODS

Swamp eels

Swamp eels that had grown naturally, rather than those that had been reared commercially, were purchased from several local markets in Nakhon Nayok and Prachin Buri between September and November 2000. Most of the areas where the eels were caught (for sale) could be identified – at least at subdistrict level; some of the fishing sites were not identified; in a few cases, the exact location of catching or trap setting could be determined. Eels were transported to the laboratory in Bangkok, about one hundred kilometers away. They were then killed, measured and weighed. The visceral organs were taken out; only the liver was cut into small pieces and examined by a press preparation (compression) technique under a dissecting microscope or a big hand lens. Eel muscle was roughly divided into six parts according to body regions: the dorsal and ventral parts, which were then separated into anterior, middle and posterior portions. All the muscles were cut and scraped clear of bone and skin, and examined by the method used for the liver. Gnathostome larvae were collected, counted, cleaned, and fixed with 70% ethanol; their length and width were measured using a camera lucida.

Dog fecal samples

Two areas, Ban Phrao in Nakhon Nayok and Tha Ngam in Prachin Buri, were selected for the collection of dog fecal samples. These two sites were chosen because the eels that had been caught there contained gnathostome larvae. The sites also featured natural or man-made irrigation canals and roadside paddy fields; many stray/domesticated dogs were available. As many fresh-looking fecal samples as possible were collected; very dry samples were ignored. The specimens were examined for *Gnathostoma* eggs by a formalin-ether centrifugal sedimentation method. Four collections of fecal specimens were undertaken between September 2000 and May 2001. The first three collections were at monthly interval; the last collection was six months after the third.

Cyclops

Cyclops specimens were collected in the same two areas. The copepods were taken from canals using an insect catching stick fitted with a finer net. Cyclops and some other same-sized crustacea were filtered and then identified and examined under a light microscope for the presence of early third-stage gnathostome larvae.

RESULTS

The prevalence and intensity of *Gnathostoma* infections in the swamp eels are shown in Table 1. The average prevalence of infection in Nakhon Nayok was 38.1% (117/307 eels) but only 24.0% (74/308 eels) in Prachin Buri. In Nakhon Nayok, the highest prevalence rate (50.0%) was found in Phrom Mani, but the greatest intensity of infection (65 larvae/eel)

Table 1
Prevalence and intensity of *Gnathostoma* infection in swamp eels *(Monopterus albus)*.

Province and subdistrict	No. examined	No. positive (%)	Mean no. of larvae/eel (range)
Nakhon Nayok			
Ban Phrao	18	5 (27.8)	9.8 (3-16)
Bang-O	56	26 (46.4)	10.1 (1-34)
Don Yo	8	1 (12.5)	8.0
Dong Lakhon	47	16 (34.0)	7.3 (1-31)
Phrom Mani	48	24 (50.0)	12.4 (1-41)
Sinawa	42	14 (33.3)	15.9 (1-41)
Tha Chang	6	0	0
Wang Krachom	8	3 (37.5)	2.0 (1-3)
Unspecified areas	74	28 (37.8)	11.8 (1-65)
Total	307	117 (38.1)	11.0 (1-65)
Prachin Buri			
Ban Phra	53	11 (20.8)	5.4 (1-11)
Khok Makok	42	16 (38.1)	12.4 (1-59)
Nong Nam Khao	55	24 (43.6)	11.4 (1-45)
Taphan Hin	62	10 (16.1)	8.8 (1-29)
Tha Ngam	59	9 (15.2)	4.1 (1-9)
Unspecified areas	37	4 (10.8)	7.5 (1-20)
Total	308	74 (24.0)	9.3 (1-59)

was in the unknown areas. Only one batch of eels, from Tha Chang, gave a negative result (0/6 eels). In Prachin Buri, all five subdistricts and unspecified areas were positive for the infection. The highest prevalence (43.6%) was observed in Nong Nam Khao, while the greatest intensity of infection (59 larvae/eel) was in Khok Makok.

Table 2 shows the frequency and relative frequency distributions of the body lengths and infection rates of eels infected with gnathostome larvae in the two provinces. The range of length of the eels and the range of length of the positive eels in Nakhon Nayok were 30.0-97.0 cm and 40.0-97.0 cm, respectively; the corresponding figures in Prachin Buri were 30.0-71.0 cm and 34-70 cm. In Nakhon Nayok, it was found that most of the eels examined, 87.3% (47.6% + 39.7%), were in the range of 50-69 cm in length, which was very similar to that of 89.0% of the eels (44.8% + 44.2%) in Prachin Buri. The average body length (58.8 ± 7.6 cm; range 30-97 cm) and weight (399.8 ± 76.2 g; range 210-1,200 g) of the examined eels in Nakhon Nayok were not significantly different from those of the Prachin Buri eels (average length 57.9 ± 6.7 cm; range 30-71 cm; average weight 396.9 ± 55.1 g; range 200-500 g) (Table 5). However, the average body length (62.7 ± 7.2 cm; range 40-97 cm) and weight (433.8 ± 95.0 g; range 260-1,200 g) of the eels positive for gnathostome larvae in Nakhon Nayok were significantly greater (p = 0.000 and 0.014, respectively) than those of the Prachin Buri positive eels (average length 58.6 ± 6.5 cm; range 34-70 cm; average weight 403.8 ± 51.9 g; range 250-500 g) (Table 5). The infection rates of eels in Nakhon Nayok seemed to correlate well with their size: the bigger the eel the higher its infection rate. The three largest eels (range 80-99 cm) had a 100% infection rate; the second highest rate (54.8%) was in the 60-69 cm group. However, this ostensible correlation was not seen in Prachin Buri, where almost all groups of eels showed about the same rate of infection. The mean infection rate of the Nakhon Nayok eels (38.1%) was significantly higher (p = 0.000) than that of the Prachin Buri eels (24.0%) (Tables 2 and 5).

The intensity of the eels' *Gnathostoma* infection was arbitrarily categorized into five groups (Table 3). Most of the positive eels in Nakhon Nayok (53.8%) harbored 1-9 larvae; 30.8% of these infected eels were 60-69 cm long. Only one eel bore more than 50 larvae. In Prachin Buri, 67.6% of the positive eels harbored 1-9 larvae; 59.4% (32.4% + 27.0%) of them were 50-69 cm long. Only one eel bore more than 50 larvae. In both provinces, the eels' intensity of infection was not associated with

Table 2
Frequency and relative frequency (%) distributions of the body lengths, and infection rates of swamp eels infected with gnathostome larvae.

Eel body length (cm)	Nakhon Nayok			Prachin Buri		
	No. examined (%)	No. positive (%)	Infection rate	No. examined (%)	No. positive (%)	Infection rate
30 - 39	4 (1.3)	0	0	3 (1.0)	1 (1.4)	33.3
40 - 49	28 (9.1)	5 (4.3)	17.8	28 (9.1)	4 (5.4)	14.3
50 - 59	122 (39.7)	27 (23.1)	22.1	136 (44.2)	34 (45.9)	25.0
60 - 69	146 (47.6)	80 (68.4)	54.8	138 (44.8)	34 (45.9)	24.6
70 - 79	4 (1.3)	2 (1.7)	50.0	3 (1.0)	1 (1.4)	33.3
80 - 89	2 (0.6)	2 (1.7)	100	0	0	0
90 - 99	1 (0.3)	1 (0.8)	100	0	0	0
Total	307 (100)	117 (100)	38.1	308 (100)	74 (100)	24.0

Table 3
The intensity of infection and frequency and relative frequency (%) distributions of the number of *Gnathostoma* larvae per eel according to the size of eel.

Eel body length (cm)	No. of positive eels (%)							Total no. of larvae recovered	Mean no. of larvae/eel (range)
	Larval group (no. of larvae/eel)						Total		
	1-9	10-19	20-29	30-39	40-49	≥ 50			
Nakhon Nayok									
30 - 39	0	0	0	0	0	0	0	0	0
40 - 49	4 (3.4)	1 (0.8)	0	0	0	0	5 (4.3)	31	6.2 (1-11)
50 - 59	20 (17.1)	3 (2.6)	2 (1.7)	2 (1.7)	0	0	27 (23.1)	223	8.2 (1-34)
60 - 69	36 (30.8)	33 (28.2)	5 (4.3)	3 (2.6)	3 (2.6)	0	80 (68.4)	949	11.9 (1-43)
70 - 79	2 (1.7)	0	0	0	0	0	2 (1.7)	7	3.5 (3-4)
80 - 89	1 (0.8)	1 (0.8)	0	0	0	0	2 (1.7)	17	8.5 (1-16)
90 - 99	0	0	0	0	0	1 (0.8)	1 (0.8)	65	65.0
Total	63 (53.8)	38 (32.5)	7 (5.9)	5 (4.3)	3 (2.6)	1 (0.8)	117 (100)	1,292	11.0 (1-65)
Prachin Buri									
30 - 39	1 (1.4)	0	0	0	0	0	1 (1.4)	1	1.0
40 - 49	4 (5.4)	0	0	0	0	0	4 (5.4)	10	2.5 (1-5)
50 - 59	20 (27.0)	8 (10.8)	3 (4.0)	0	2 (2.7)	1 (1.4)	34 (45.9)	416	12.2 (1-59)
60 - 69	24 (32.4)	7 (9.4)	1 (1.4)	1 (1.4)	1 (1.4)	0	34 (45.9)	260	7.6 (1-45)
70 - 79	1 (1.4)	0	0	0	0	0	1 (1.4)	1	1.0
80 - 89	0	0	0	0	0	0	0	0	0
90 - 99	0	0	0	0	0	0	0	0	0
Total	50 (67.6)	15 (20.3)	4 (5.4)	1 (1.4)	3 (4.1)	1 (1.4)	74 (100)	688	9.3 (1-59)

Table 4
Distribution of *Gnathostoma* infective larvae in muscles according to body regions of swamp eels (expressed as number and percentage).

	Eel body region	Anterior	Middle	Posterior	Total
Nakhon Nayok					
	Dorsal	53 (8.6)	98 (15.9)	50 (8.1)	201 (32.6)
	Ventral	122 (19.8)	222 (36.0)	71 (11.5)	415 (67.4)
	Total	175 (28.4)	320 (51.9)	121 (19.6)	616 (100)
Prachin Buri					
	Dorsal	38 (11.2)	44 (12.9)	28 (8.2)	110 (32.4)
	Ventral	91 (26.8)	116 (34.1)	23 (6.8)	230 (67.6)
	Total	129 (37.9)	160 (47.1)	51 (15.0)	340 (100)

Table 5
Summary and comparison of vital statistics of swamp eels and their gnathostome larvae in Nakhon Nayok and Prachin Buri.

	Nakhon Nayok	Prachin Buri	p-value
Examined eels			
Total no. examined	307	308	-
Length range (cm)	30 - 97	30 - 71	-
Average length (mean ± SD)	58.8 ± 7.6	57.9 ± 6.7	> 0.05
Weight range (g)	210 - 1,200	200 - 500	-
Average weight (mean ± SD)	399.8 ± 76.2	396.9 ± 55.1	> 0.05
Positive eels			
Total no. positive	117	74	-
Infection rate (%)	38.1	24.0	0.000
Length range (cm)	40 - 97	34 - 70	-
Average length (mean ± SD)	62.5 ± 7.2	58.6 ± 6.5	0.000
Weight range (g)	260 - 1,200	250 - 500	-
Average weight (mean ± SD)	433.8 ± 95.0	403.8 ± 51.9	0.014
Gnathostome larvae			
Total no. recovered	1,292	688	-
No. of larvae/eel (range)	1 - 65	1 - 59	-
No. of larvae/eel (mean ± SD)	11.0 ± 10.4	9.3 ± 11.4	> 0.05
Only 1 larva/eel (%)	16.2	28.4	> 0.05
Distribution in eel liver:muscle (%)	52.3 : 47.7	50.6 : 49.4	> 0.05
Found in the liver only (%)	29.1	43.2	> 0.05
Found in muscles only (%)	0	0	-
Max no. of larvae found only in the liver of one eel	6	10	-
Body length range (mm)	2.5 - 5.1	2.2 - 5.1	-
Average body length (mean ± SD)	4.0 ± 0.5	3.9 ± 0.5	0.023
Body width range (mm)	0.29 - 0.51	0.20 - 0.48	-
Average body width (mean ± SD)	0.40 ± 0.05	0.34 ± 0.05	0.000

Table 6
Number and percentage of fecal samples of dogs positive for *Gnathostoma spinigerum* eggs in Ban Phrao, Nakhon Nayok and in Tha Ngam, Prachin Buri.

Date of specimen collection	Ban Phrao, Nakhon Nayok			Tha Ngam, Prachin Buri		
	No. examined	No. positive	% positive	No. examined	No. positive	% positive
2000, September	44	0	0	109	0	0
2000, October	68	1	1.5	115	0	0
2000, November	70	2	2.9	not done	-	-
2001, May	50	0	0	100	0	0

their size. Although the biggest eel in Nakhon Nayok (97.0 cm, 1,200 g) was found to contain the greatest number of larvae (65), a much smaller eel in Prachin Buri (53 cm, 440 g) was found to contain 59 larvae. The mean number of larvae in Nakhon Nayok eels (11.0 ± 10.4) was not significantly different from that of Prachin Buri eels (9.3 ± 11.4) (Tables 3 and 5).

The distribution of gnathostome larvae in eel tissue is shown in Fig 1 and Table 4. A total of 1,292 larvae were recovered from 307 eels in Nakhon Nayok. Of these, 52.3% were accumulated in the liver and 47.7% were spread throughout the muscle. In the Prachin Buri eels, 50.6% and 49.4% of 688 larvae (from 308 eels) were detected in the liver and muscle, respectively (Fig 1). There was no significant difference in the tissue distribution of larvae between the two provinces (Table 5). The larvae preferred encysting in ventral rather than dorsal muscle; they also preferred middle rather than anterior or posterior muscle (Table 4). Most of the Nakhon Nayok larvae (36.0%) were found in the medioventral part of their eels; while only a few (8.1%) were in the posterodorsal part. These figures were similar to those of the Prachin Buri eels, most of whose larvae were medioventral (34.1%); again, only a few (8.2%) were posterodorsal (Table 4).

Table 5 shows the vital statistics of the swamp eels and their gnathostome larvae. It was interesting to find that 16.2% of Nakhon Nayok eels and 28.4% of Prachin Buri eels

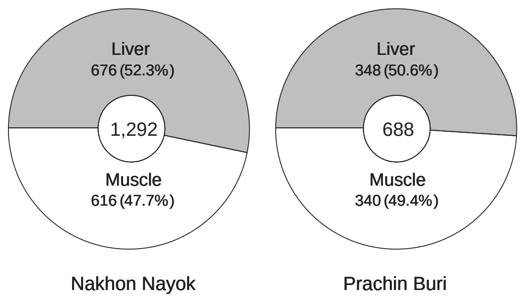

Fig 1–Tissue distribution of *Gnathostoma* infective larvae in swamp eels (expressed as number and percentage).

harbored only a single larva; there was no significant difference between provinces in the number of eels harboring only one larva. It was also noted, in both provinces, that if eels were negative for hepatic gnathostome larvae, then no larvae would be found in their muscles: 29.1% of Nakhon Nayok eels harbored larvae in their livers only (six larvae were found in the liver of one infected eel); in Prachin Buri, a rather higher rate (43.2%) was found (one eel had 10 hepatic larvae). However, there was no significant difference between provinces in the number of eels harboring hepatic larvae only.

The average body length and width of the larvae recovered from the Nakhon Nayok eels were 4.0 ± 0.5 mm (range 2.5-5.1 mm) and 0.40 ± 0.05 mm (range 0.29-0.51 mm), respectively. Prachin Buri larvae were 3.9 ± 0.5 mm (range 2.2-5.1 mm) and 0.34 ± 0.05 mm (range 0.20-0.48 mm), respectively. The mean body length and width of the Nakhon Nayok larvae were significantly greater (p = 0.023 and 0.000, respectively) than those of the Prachin Buri larvae (Table 5).

Table 6 presents the number and percentage of positive and negative dog fecal samples. In Ban Phrao, none of the first 44 fecal specimens examined was positive. Of the second (68) and the third (70) specimens, one (1.5%) and two (2.9%) samples, respectively, were positive. However, at the fourth collection, six months after the third, no gnathostome eggs were found. In Tha Ngam, three batches (109, 115, and 100 samples) were examined: no eggs were recovered.

The cyclops survey called for the examination of 4,000-5,000 crustacea from each of the two selected areas, Ban Phrao and Tha Ngam. The tiny fresh-water crustacea were species of water-fleas (*eg*, *Daphnia*) and copepods (*eg*, *Cyclops* and *Diaptomus*). Careful examination enabled the separation of the cyclops species, in which no infection was found.

DISCUSSION

The prevalences of *Gnathostoma* infections in swamp eels in Nakhon Nayok (38.1%) and Prachin Buri (24.0%) in the present study are much lower than those reported by other investigators. In 1987-1989, the infection rates were 80-100% (24/30, 27/33, 4/4 eels) in Nakhon Nayok and 100% (13/13 eels) in Prachin Buri (Setasuban *et al*, 1991). A few years later, in 1992, the rates in Nakhon Nayok and Prachin Buri decreased to 68.7% (300/437 eels) and 50.0% (28/48 eels), respectively (Nuamtanong *et al*, 1998). The average numbers of gnathostome larvae/eel in Nakhon Nayok (11.0) and Prachin Buri (9.3) in the present study are about one half of those reported by Nuamtanong *et al* (1998), who found 29.7 in the former and 21.7 in the latter province. However, both the prevalence and intensity of infections in Nakhon Nayok have remained higher than in Prachin Buri. The striking difference between these studies is that the maximum numbers of larvae per eel were 2,283-2,582 (Nakhon Nayok) and 133 (Prachin Buri) (Setasuban *et al*, 1991; Nuamtanong *et al*, 1998), whereas in the current study, maxima of 65 and 59 were found. The dramatic decrease in both the prevalence and intensity of infections, especially in Nakhon Nayok, may possibly be due to the more frequent and heavier rainfall that affected the province in the few years before, and during the study.

Our current data, our unpublished data, and data from earlier reports by other investigators, suggest that both the prevalence and intensity of gnathostome infections in swamp eels are probably not associated with the size of the infected eels. The prevalence and intensity of infection appears to vary directly with endemicity: endemic areas have greater numbers of infected definitive hosts that their feces contaminate canals and ponds in which the first intermediate host, cyclops, is found. This is illustrated by the case of a rather small eel (only 64 cm long) that was naturally infected with 2,283 gnathostome larvae (Rojekittikhun *et al*, 2001).

About 51-52% of the gnathostome larvae recovered from eels in Nakhon Nayok and Prachin Buri were found to have concentrated in the liver, 48-49% were distributed throughout the musculature. This finding agrees with the 53.3%:46.7% distribution reported by Setasuban *et al* (1991) and the 52.3%:47.7% distribution cited by Nuamtanong *et al* (1998). These three reports are consistent in another respect: about 61-71% of the larvae in eel muscles were found in the ventral parts and about 47-54% of larvae were located in the middle parts. However, Setasuban *et al* (1991) found that the ratio of liver to muscle larval distribution was 65.3%:34.7%; our findings were much different. There is no good explanation for this difference. In addition, it is interesting to note that less than 2% of the positive eels had gnathostome larvae in their head muscles; less than 4% of positive eels had larvae in the muscles of the tail end (from the cloacal opening to the tail tip). Generally, only one larva is found in both the head and the tail of infected eels.

Almost all of the gnathostome larvae recovered from eels in Nakhon Nayok and Prachin Buri Provinces were in their encysted form; very few of them were found to be unencysted. This implies the chronic infection of eels or the fast encystation of the larvae in eel second intermediate/paratenic hosts. A similar phenomenon was reported by Anantaphruti *et al* (1986) and Rojekittikhun *et al* (1989a), who found that in mice infected with *G. spinigerum* early third-stage larvae (EL3), encystation started to occur within 4-5 weeks and was completed within 8-10 weeks of infection; in mice infected with *G. spinigerum* advanced third-stage larvae (AL3), encystation occurred in 2-4 weeks and was completed within five weeks of infection; in rats infected with *G. spinigerum* AL3, encystation started in the fourth week and was completed within the following week. A quick glance at the morphology of all individual collected larvae under a dissecting microscope revealed no difference in parasite species but *G. spinigerum*.

Daengsvang (1980) has summarized the prevalence of *G. spinigerum* infection in stray dogs in Bangkok, as follows: in 1933, the infection rate was 100% (from 5 stomachs); in 1962, 10% (100 stomachs) and 18% (1,000 stomachs); and in 1965-1970, 1.1% (17,855 stomachs and 277 fecal samples). Similar reports by other investigators have shown prevalence rates of: 1.6% from 1,000 fecal samples (Sirisumpan, 1962); 10.0% from 100 dogs (Ito *et al*, 1962); 2% of 2,599 dogs (Manning *et al*, 1969); and 2.8% of 107 dogs (Hinz, 1980). However, Rojekittikhun *et al* (1998c) recovered no *G. spinigerum* from 200 dog stomach samples. In northeast Thailand, 4.1% of 2,940 dogs were found to be infected with *G. spinigerum* (Maleewong *et al*, 1992). Although there has been no such report from Prachin Buri, there has been one article about dog gnathostomiasis in Nakhon Nayok. The overall positive rate for gnathostome eggs in dog fecal samples was 1.2%, with the highest rate of 4.2% in Don Yo, Muang district (Rojekittikhun *et al*, 2000). In the present study, a negative result was obtained in Tha Ngam in Prachin Buri, and the positive rate in Nakhon Nayok was much lower than expected. These findings are surprising considering the prevalence rates of 24.0% and 38.1% in the swamp eels of these provinces. One reason that may account for this is that *G. spinigerum* displays a distinct annual fluctuation in its prevalence in dogs – no explanation has given for this, but it should be taken into consideration when one-time surveys are made of any parasite population (Manning *et al*, 1969). Only one and two fecal samples of the second and the third collections, respectively were positive for *G. spinigerum* eggs. It is likely that the two positive samples in the third batch were from the same infected dog, and they may possibly belong to the animal that produced the one positive specimen in the second batch.

In Thailand, there are at least four species of cyclops that can, at least in experiments, serve as the first intermediate host of *G. spinigerum* (Sooksri, 1967). To date, no evidence of naturally infected cyclops has been found in Bangkok, Nakhon Nayok, and Prachin Buri (Muennoo *et al*, 1991; Maipanich, personal communication; Rojekittikhun, unpublished data). In the current study, we failed to find natural cyclops infection in Tha Ngam and especially in Ban Phrao, where swamp eels were positive for gnathostome larvae and dog fecal samples were positive for the parasite eggs.

In conclusion, *Gnathostoma* infection is still prevalent in swamp eels in Nakhon Nayok and Prachin Buri, although the rate of positive dog feces is low in Nakhon Nayok and zero in Prachin Buri. The search for naturally infected first intermediate hosts of the *Gnathostoma* remains challenging.

REFERENCES

Akahane H. Human gnathostomiasis caused by *Gnathostoma doloresi*, with particular reference to the parasitological investigation of the causative agent. *Ann Trop Med Parasitol* 1998; 92: 721-6.

Akahane H, Sano M, Kobayashi M. Three cases of human gnathostomiasis caused by *Gnathostoma hispidum*, with particular reference to the identification of parasitic larvae. *Southeast Asian J Trop Med Public Health* 1998; 29: 611-4.

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 with English abstract).

Almeyda-Artigas RJ, Bargues MD, Mas-Coma S. ITS-2 rDNA sequencing of *Gnathostoma* species (Nematoda) and elucidation of the species causing human gnathostomiasis in the Americas. *J Parasitol* 2000; 86: 537-44.

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

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

Ando K, Hatsushika R, Akahane H, *et al*. *Gnathostoma nipponicum* infection in the past human cases in Japan. *Jpn J Parasitol* 1991; 40: 184-6.

Camacho SPD, Ramos MZ, Torrecillas EP, *et al*. Clinical manifestations and immunodiagnosis of gnathostomiasis in Culiacan, Mexico. *Am J Trop Med Hyg* 1998; 59: 908-15.

Daengsvang S. First report on *Gnathostoma vietnamicum* Le-Van-Hoa, 1965 from urinary system of otters (*Aonyx cinerea*, Illiger) in Thailand. *Southeast Asian J Trop Med Public Health* 1973; 4: 63-70.

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.

Daengsvang S. Gnathostomiasis. In: Schultz MG, ed. Section C: Parasitic zoonoses, vol II. Florida: CRC Press, 1982: 147-80.

Daengsvang S. The world' s first-story of *Gnathostoma spinigerum* infection in the young Thai woman. *J Parasitol Trop Med Assoc Thai* 1983; 6: 1-10 (Thai with English abstract).

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.

Dissamarn R, Thirapat K, Aranyakanada P, *et al*. Studies on morphology and life history of *G. doloresi* and *G. hispidum* in Thailand. *J Thai Vet Med Assoc* 1966; 17: 1 (Thai with English abstract).

Hinz E. Intestinal helminths in Bangkok stray dogs and their role in public health. *Zentralbl Bakteriol Mikrobiol Hyg* 1980; 17: 79-85.

Ito J, Papasarathorn T, Tongkoom B. An investigation of parasitic helminths of stray dogs in Bangkok. *Jpn J Med Sci Biol* 1962; 15: 53-60.

Kagei N. Morphological identification of parasites in biopsied specimens from creeping disease sections. *Jpn J Parasitol* 1991; 40: 437-45.

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

Koga M, Akahane H, Ogata K, *et al*. *Gnathostoma* sp as an etiological agent in Mexican gnathostomiasis: a morphological study by SEM. *Parasitol Int* 1998; 47(suppl): 291.

Kurokawa M, Ogata K, Sagawa S, *et al*. Cutaneous and visceral larva migrans due to *Gnathostoma doloresi* infection via an unusual route. *Arch Dermatol* 1998; 134: 638-9.

Leon-Regagnon V, Garcia-Prieto L, Osorio-Sarabia D, *et al*. Gnathostomiasis in fish from Tres Palos Lagoon, Guerrero, Mexico. *Emerg Infect Dis* 2000; 6: 429-30.

Maleewong W, Pariyanonda S, Sitthithaworn P, *et al*. Seasonal variation in the prevalence and intensity of canine *Gnathostoma spinigerum* infection in northeastern Thailand. *J Helminthol* 1992; 66: 72-4.

Manning GS, Sukhawat K, Viyanant V, *et al*. Zoonotic potential of the parasites of dogs in Bangkok, Thailand. *J Med Assoc Thai* 1969; 52: 425-31.

Miyazaki I. On the genus *Gnathostoma* and human gnathostomiasis, with special reference to Japan. *Exp Parasitol* 1960; 9: 338-70.

Miyazaki I. *Gnathostoma* and gnathostomiasis in Japan. In: Morishita K, Komiya Y, Matsubayashi H, eds. Progress of medical parasitology in Japan. Tokyo: Meguro Parasitological Museum, 1966: 531-86.

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

Muennoo C, Hiranyachattada P, Setasuban P. *Gnathostoma spinigerum*: a search for naturally infected first intermediate host. Bangkok: Mahidol University Annual Research Abstracts 1991: 490.

Nawa Y. Historical review and current status of gnathostomiasis in Asia. *Southeast Asian J Trop Med Public Health* 1991; 22 (suppl): 217-9.

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

Nawa Y, Maruyama H, Ogata K. Current status of gnathostomiasis doloresi in Miyazaki Prefecture, Japan. *Southeast Asian J Trop Med Public Health* 1997; 28 (suppl 1): 11-3.

Nuamtanong S, Waikagul J, Anantaphruti MT. Gnathostome infection in swamp eels, *Fluta alba,* in central Thailand. *Southeast Asian J Trop Med Public Health* 1998; 29: 144-7.

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.

Ogata K, Nawa Y, Akakane H, *et al*. Short report: gnathostomiasis in Mexico. *Am J Trop Med Hyg* 1998; 58: 316-8.

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.

Rojas-Molina N, Pedraza-Sanchez S, Torres-Bibiano B, *et al*. Gnathostomiasis, an emerging foodborne zoonotic disease in Acapulco, Mexico. *Emerg Infect Dis* 1999; 5: 264-6.

Rojekittikhun W, Nuamtanong S, Pubampen S, *et al*. Circumoval and larval microprecipitation reactions in experimental and human gnathostomiasis. *Southeast Asian J Trop Med Public Health* 1989a; 20: 81-6.

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

Rojekittikhun W, Daengswang S, Pubampen S, *et al*. A survey on the infective larvae of *Gnathostoma spinigerum* in rats at Nakhon Nayok and Prachin Buri. *J Trop Med Parasitol* 1989c; 12: 13-6 (Thai with English abstract).

Rojekittikhun W, Pubampen S, Waikagul J. Swamp eels (*Fluta alba*), the genuine second intermediate host of Gnathostoma in Thailand. *J Trop Med Parasitol* 1998a; 21: 44-5.

Rojekittikhun W, Pubampen S, Waikagul J. Seasonal variation in the intensity of *Gnathostoma* larvae in swamp eels (*Fluta alba*) sold in a local market in Bangkok. *Southeast Asian J Trop Med Public Health* 1998b; 29: 148-53.

Rojekittikhun W, Nuamtanong S, Anantaphruti MT, *et al*. *Toxocara* and *Gnathostoma* among stray canines in Bangkok. *Southeast Asian J Trop Med Public Health* 1998c; 29: 744-7.

Rojekittikhun W, Chaiyasith T, Yaemput S. Dog gnathostomosis in Nakhon Nayok province. *J Trop Med Parasitol* 2000; 23: 43-52.

Rojekittikhun W, Chaiyasith T, Nuamtanong S, *et al*. Record numbers of *Gnathostoma* larvae in swamp eels. *J Trop Med Parasitol* 2001; 24: 79-82.

Sato H, Kamiya H, Hanada K. Five confirmed human cases of gnathostomiasis nipponica recently found in northern Japan. *J Parasitol* 1992; 78: 1006-10.

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. Southeast Asian J Trop Med Public Health* 1991; 22 (suppl): 220-4.

Sirikulchayanonta V, Viriyavejakul P. Various morphologic features of *Gnathostoma spinigerum* in histologic sections: report of 3 cases with reference to topographic study of the reference worm. *Southeast Asian J Trop Med Public Health* 2001; 32: 302-7

Sirisumpan P. A survey of intestinal helminths in stray dogs in Bangkok and Dhonburi. *J Med Assoc Thai* 1962; 45: 567-79 (Thai with English abstract).

Sooksri V. Studies on different species of cyclops in Bangkok and neighbouring areas with reference to those acting as first intermediate host of *Gnathostoma spinigerum*. Bangkok: University of Medical Sciences, 1967 (MSc Thesis).

Taniguchi Y, Ando K, Isoda K, *et al*. Human gnathostomiasis: successful removal of *Gnathostoma hispidum. Int J Dermatol* 1992; 31: 175-7.