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Positivity and Intensity of *Gnathostoma spinigerum* Infective Larvae in Farmed and Wild-Caught Swamp Eels in Thailand

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Abstract: From July 2008 to June 2009, livers of the swamp eels (*Monopterus alba*) were investigated for advanced third-stage larvae (AL3) of *Gnathostoma spinigerum*. Results revealed that 10.2% (106/1,037) and 20.4% (78/383) of farmed eels from Aranyaprathet District, Sa Kaeo Province and those of wild-caught eels obtained from a market in Min Buri District of Bangkok, Thailand were infected, respectively. The prevalence was high during the rainy and winter seasons. The infection rate abruptly decreased in the beginning of summer. The highest infection rate (13.7%) was observed in September and absence of infection (0%) in March-April in the farmed eels. Whereas, in the wild-caught eels, the highest rate (30.7%) was observed in November, and the rate decreased to the lowest at 6.3% in March. The average no. (mean ± SE) of AL3 per investigated liver in farmed eels (1.1 ± 0.2) was significantly lower ($P = 0.040$) than those in the caught eels (0.2 ± 0.03). In addition, the intensity of AL3 recovered from each infected liver varied from 1 to 18 (2.3 ± 0.3) in the farmed eels and from 1 to 47 (6.3 ± 1.2) in the caught eels, respectively. The AL3 intensity showed significant difference ($P = 0.011$) between these 2 different sources of eels. This is the first observation that farmed eels showed positive findings of *G. spinigerum* infective larvae. This may affect the standard farming of the culture farm and also present a risk of consuming undercooked eels from the wild-caught and farmed eels.

Key words: *Gnathostoma spinigerum*, third-stage larva, positivity, intensity, farmed eel, wild-caught eel, Thailand

INTRODUCTION

Gnathostomiasis is endemic mainly in Thailand, Japan, and Mexico [1]. The disease has also been reported in China, Korea, Loas, Myanmar, Vietnam, India, Bangladesh, Malaysia, Indonesia, the Philippines, and Israel [1]. There were a few case reports from Australia, Ecuador, Spain, and Africa [2-10]. Due to the expansion of international traveling, there are increasing imported cases described in many countries, including Asia, Europe, and America [11-13].

Seven *Gnathostoma* species have been described in human infections. *G. spinigerum* is the main causative agent in Thailand [1,3]. Domestic and wild felines and canines are the definitive hosts. The adult worms inhabit in a tumor where they produce in the gastric wall and lay eggs. The eggs are passed in the feces and then into water and later hatch into first-stage larvae. These larvae are ingested by the first intermediate host (copepod) and develop into second-stage larvae. Following ingestion of the infected copepods by the second intermediate hosts (fish, frogs, snakes, and fowls), the second-stage larvae develop into third-stage and later into advanced third stage larvae (AL3). An AL3 finally develops into an adult worm if the second intermediate host is ingested by a definitive host. Man is its accidental host by ingestion of undercooked fish or poultry containing AL3 [14]. The infection causes cutaneous larva migrans, i.e., intermittent migratory pain and pruritic swellings. In addition, an AL3 can invade the eye, brain, and other visceral organs which can cause serious illnesses [12,14-16].

In Thailand, there are 48 species of vertebrates in natural habitats reported to serve as the second intermediate and/or paratenic hosts for *G. spinigerum*. These include fish (20 species), amphibians (2 species), reptiles (11 species), avians (11 species), and mammals (4 species). Among these hosts, fresh water fish; particularly, swamp eels (*Monopterus albus*, previ-

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ously *Fluta alba*) had the highest prevalence of *G. spinigerum* AL3 infection, whereas *Channa striata* (*pla-chon* in Thai) and *Clarias batruchus* (*pla-duk-dan* in Thai) showed the second- and the third high prevalence rates of the parasite [14]. In addition, the swamp eels show the greatest infection intensity of *G. spinigerum* AL3 [17]. From 1987 to 1989, larvae of *G. spinigerum* were found in 80-100% of the wild-caught eels obtained from a local market in Nakhon Nayok province, with a maximum recovery of 2,582 larvae per eel [18]. Many other studies on AL3 prevalence and intensity in eels sold in local markets were reported [19,20].

It has been reported that the annually AL3 infection rate in eels from a local market in Bangkok, Thailand is high in the rainy season and drops abruptly during the winter, and becomes negative in the summer [19]. However, our survey of the prevalence rate of AL3 in eels purchased from another market in Bangkok, Thailand from June 1999 to May 2000 demonstrated a high prevalence of 21.5%, 38.3%, and 31.2% during the rainy season (August-October) and was still high with 28.7%, 23.0%, 12.3%, and 28.1% of the positive rate during the cool season (November-February). Thereafter, it decreased and showed the lowest in April (7.0%) [20]. There was another report that showed 30.1% overall prevalence of AL3 in wild-caught eels from Nakhon Nayok Province with the highest infection rate in August 2000 (44.1%) and the lowest in March 2001 (10.7%) [21]. The infection rate of 12.2% was reported in wild-caught eels obtained from markets in Ho Chi Minh City, Vietnam [22].

Interestingly, eel farming has started and grew extensively in many countries throughout Southeast Asia in the last 2 decades. In Vietnam, there was a survey of AL3 infection in 1,020 eels from a farm and no infection was reported [22]. Also in Thailand, there was no report of *G. spinigerum* infection in farm eels to date. However, continuous surveys are required to monitor *G. spinigerum* infection in farm eels. It is known that about half (43-52%) of the total AL3 number was accumulated in the livers of fresh water fish, including eels, cat fish, snake fish, and others, while the remaining half distributed in the whole body muscles [23]. Therefore, in the present study, we studied the burden of *Gnathostoma* AL3 in swamp eels purchased from a farm in the district of Aranyaprathet Sa Kaeo Province and compared with the results obtained from wild-caught eels sold in a market in Min Buri District, Bangkok, Thailand by using the liver as the target organ to quantify the prevalence and intensity of AL3 population.

MATERIALS AND METHODS

Collection of swamp eel livers

A total of 1,420 swamp eel livers from 2 different sources were collected at different time intervals during July 2008 and June 2009.

A: One thousand and thirty-seven (1,037) eels purchased from a farm in Aranyaprathet District, Sa Kaeo Province (APSK) was studied for the AL3 infection. This farm was located 300 km sub-northeast away from Bangkok.

B: Three hundred eighty-three (383) wild-caught eels were purchased from a local market in Min Buri (MBBK) District which was located 75 km north from the center of Bangkok.

Harvesting *Gnathostoma spinigerum* AL3

The livers of the swamp eels were separated from other visceral organs and washed with tap water. Each liver was then digested with 20 ml of artificial gastric juice (0.05% HCl in 1.5% pepsin) in a screw-cap tube and incubated in a water bath at 37˚C for 4 hr. The digestion was performed with frequent agitation. The digested liver tissue was washed with normal saline and then allowed to settle. Lighter materials could then be decanted off, leaving the heavier particles including any AL3 in the sediment. The washing and sedimentation were repeated 3 times. The final sediment was then examined under a stereomicroscope for gnathostome AL3. The AL3 from each liver were identified into species and counted. The species identification was done based on the criteria of morphology and number of cephalic hooklet rows as described [24].

Statistical analysis

The number of AL3 harvested was calculated for prevalence and intensity with mean and standard error (SE). The significant difference of the prevalence and the intensity between the 2 sources was analyzed by student's *t*-test with 95% confidence interval.

RESULTS

Positivity of AL3 *G. spinigerum*

The infection rates and the average rainfall (mm) from the inter-annual report of Thai Meteorological Department, Ministry of Information and Communication Technology [25] during our study period were presented (Fig. 1).

Among 1,420 investigated eels' livers from 2 different sourc-

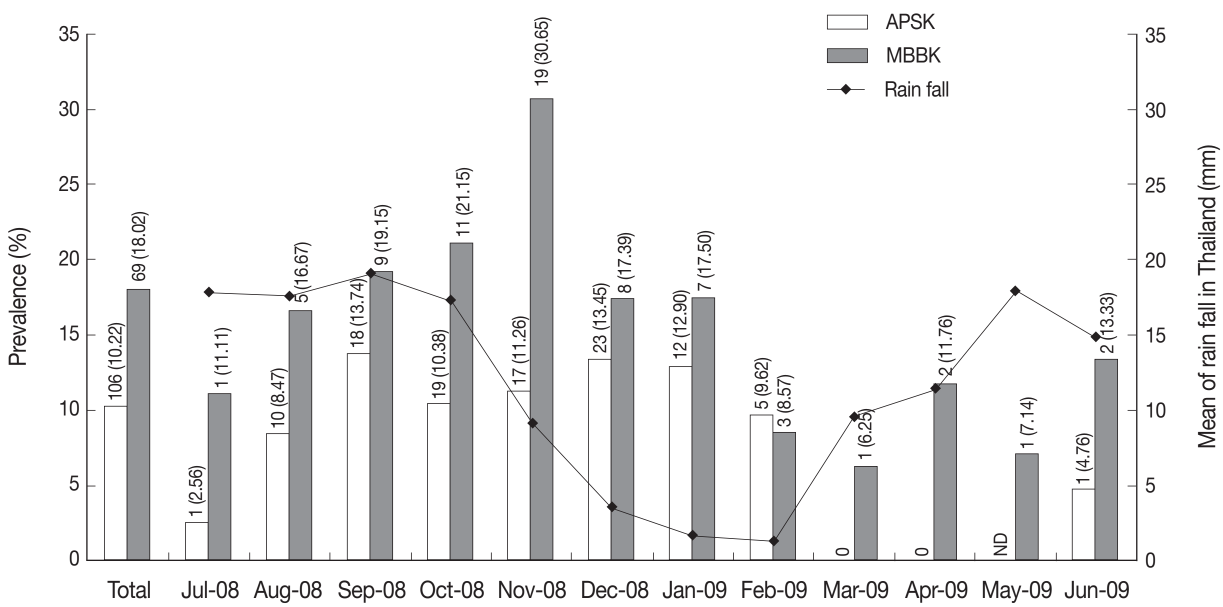

Fig. 1. The prevalence of AL3 in farmed eels from Aranyaprathet District, Sa Kaeo (APSK) and wild-caught swamp eels from Min Buri District, Bangkok (MBBK), Thailand during July and December 2008 and during January and June 2009. Lines represent mean rainfall in Thailand (mm); Bars represent the prevalence in percentage (%). ND, not done.

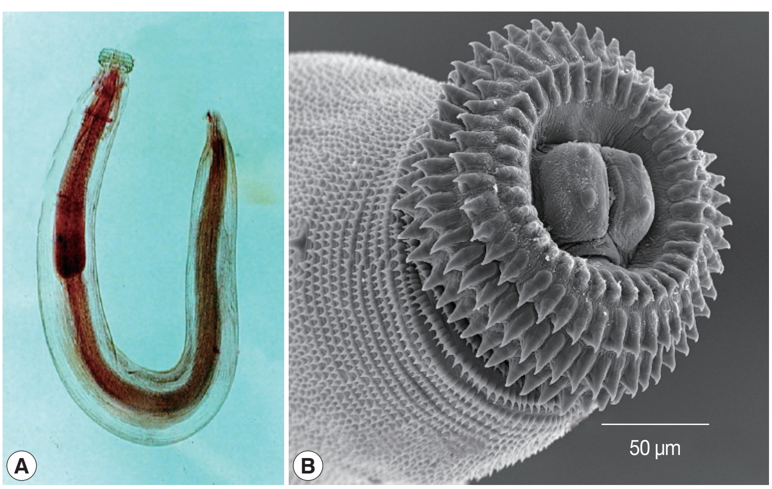

Fig. 2. Image of the advanced third stage larva of *Gnathostoma spinigerum* harvested from the investigated eel's liver. (A) Whole larva. (B) Close up of the head bulb.

es, a total of 674 larvae were harvested. According to the source of eels, 10.2% (106/1,037) and 20.4% (78/383) of the eels from a farm in the district of Aranyaprathet, Sa Kaeo Province (APSK) and those from wild-caught eels of Min Buri District, Bangkok (MBBK) were infected, respectively (Fig. 1). On the criteria of morphology and number of cephalic hooklet rows on the head bulb, all *Gnathostoma* AL3 harvested were *G. spinigerum* (Fig. 2).

The prevalence of AL3 infection in the eels from both sources were high between August and January with a peak of 13.7% (September) in the APSK farmed eels and 30.7% (November) in the MBBK caught eels (Fig. 1). The positivity of *G. spinigerum* AL3 in the wild-caught eels from August to January (16.7%, 19.2%, 21.2%, 30.7%, 17.4, and 17.5%) was higher than those of the farmed eels (8.5%, 13.7%, 10.4%, 11.3%, 13.5%, and 12.9%). The infection rate dropped abruptly from 13.7% in September to 9.6% in February in APSK farm. Similarly, it decreased from 16.7% in August to 8.6% in February in MBBK which was the end of the winter. In March, the infection bcame negative (0%) in APSK farm and showed the lowest (6.3%) in MBBK wild-caught eels.

Table 1. The intensity of L3 in eels from a farm in Aranyaprathet Sa Kaeo (APSK) and from wild-caught swamp eels in Min Buri Bangkok (MBBK) provinces Thailand from July to December 2008 and from January to June 2009

		Sources	Purchased date in 2008						Purchased date in 2009						Total
			Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	
No. of L3 recovered	In each positive liver range	SK	1	1-3	1-12	1-14	1-18	1-4	1-8	1-3	0	0	ND	1	1-18
		MB	1	1-13	1-21	1-28	1-47	1-29	1-13	1-3	2	1-3	4	2-3	1-47
	Per investigated liver Mean (SE)	SK	0.03 (0.03)	0.14 (0.05)	0.31 (0.11)	0.28 (0.10)	0.36 (0.15)	0.23 (0.05)	0.34 (0.13)	0.13 (0.07)	0.00 (0.00)	0.00 (0.00)	ND	0.05 (0.05)	0.23 (0.03)[a]
		MB	0.11 (0.11)	0.63 (0.44)	0.77 (0.46)	1.50 (0.70)	3.21 (1.16)	1.09 (0.66)	0.70 (0.37)	0.14 (0.09)	0.13 (0.13)	0.24 (0.18)	0.29 (0.29)	0.33 (0.23)	1.13 (0.24)[a]
	Per positive liver Mean (SE)	SK	1 (0.00)	1.60 (0.27)	2.28 (0.63)	2.68 (0.72)	3.18 (1.11)	1.74 (0.21)	2.67 (0.69)	1.40 (0.40)	0.00 (0.00)	0.00 (0.00)	ND	1 (0.00)	2.29 (0.26)[b]
		MB	1 (0.00)	3.80 (2.33)	4.00 (2.17)	7.09 (2.80)	10.47 (3.28)	6.25 (3.36)	4.00 (1.66)	1.67 (0.67)	2.00 (0.00)	2.00 (1.00)	4.00 (0.00)	2.50 (0.50)	6.25 (1.17)[b]
	Total	SK	1	16	41	51	54	40	32	7	0	0	ND	1	243 674
		MB	1	19	36	78	199	50	28	5	2	4	4	5	431

[a]*P*-value, 0.040; [b]*P*-value, 0.011; ND, Not done.

Intensity of AL3 *G. spinigerum*

The number of AL3 per investigated liver varied from month to month. The minimum number of AL3/liver was 0.03 in July and the maximum 0.36 in November from the APSK farmed eels while the minimum-maximum data of the MBBK wild-caught eels were 0.11-3.21 in the same period of time (July and November) (Table 1). The average (mean ± SE) of AL3 per investigated liver in APSK farmed eels (1.1 ± 0.2) was significantly lower ($P = 0.040$) than that of MBBK wild-caught eels (0.2 ± 0.03). In addition, the density of AL3 recovered from each infected liver varied from 1 to 18 with 2.3 ± 0.3 in APSK farmed eels and from 1 to 47 with 6.3 ± 1.2 in the MBBK caught eels, respectively. These overall infection intensity (larvae recovered per positive liver showed significant difference ($P = 0.011$) between the eels from the 2 different sources.

DISCUSSION

Among fresh water fish in natural habitats studied in Thailand, swamp eels had the highest prevalence and infection intensity of *G. spinigerum* [14]. Wild-caught and farmed eels are the 2 major sources of the eel consumption in Thailand. The APSK farm in this survey is closed to Cambodia and is now the only formal and biggest farm. The eels from this farm are sold throughout the country. MBBK market is the big source of wild-caught eels. The catching areas around MBBK are the small canals which join to big rivers, Chao Phraya and Bang Pakong Rivers. Therefore, we investigated the eels from both APSK farm and MBBK market as the representatives of the consumed eels in Thailand. Firstly, we expected no infection in farmed eels which signify the standard farming.

In our study, the AL3 were harvested from the individual eel's livers. This is different from other studies which did from pools of livers. The highest numbers of AL3-infected livers observed were 47 and 18 from MBBK caught and APSK farmed eels, respectively. These numbers were lower than the density found in a caught eel reported in Nakhon Nayok province (2,258 AL3 per wild eel in 1987-1989) and from Klong Toey market, a local market in Bangkok (55 AL3 per liver in 2000) [18,20]. Our study demonstrated a higher AL3 infection rate (18.0%) in the MBBK caught eels than those of APSK farmed eels (10.2%). Overall, the mean intensity or number of AL3 recovered per investigated liver, and also AL3 per infected liver, from MBBK wild-caught eels were significantly higher than those from APSK farmed eels (Table 1).

There are 3 seasons in Thailand: a hot season or summer (February-May), a rainy season (May-October), and a cool season or winter (October-February). MBBK and APSK are 230 km far from each other in the same latitude [26]. There was a report showing that the infections were commonly found in rainy season between June and October and suddenly drop in November after the end of rainy season [19]. However, the prevalence of AL3 in eels from both places of our results interestingly showed positive finding of AL3 throughout the rainy and winter seasons both in farmed and wild-caught eels. A drop of infection was observed at the end of winter or at the beginning of summer (February). After the rainy season ends parasites in the intermediate hosts seem to be still growing and therefore could be detected during 2-3 months later. We hypothesize that in the beginning of winter, the aquatic environment or water level was still high enough for supporting the survival of all the intermediate hosts. When water decreases in the late winter, some copepods which serve as the first intermediate hosts might not be able to survive. Thus, the parasite life cycle may be interrupted and result in low infection rates or negativity for AL3 in summer. In contrast, eels can dig into mud burrows; breathe atmospheric air and live in anoxic environments. They are nocturnal predator devouring crustaceans, copepod, fishes, and other small aquatic animals [27]. They often survive in habitats which are droughty or other extreme environments [28]. Thus, the infective larvae could be still detected in eels during the winter season.

This study is the first observation in Thailand that farmed eels showed positive findings of *G. spinigerum* infective larvae. This may reflect a wrong standard of culture farms. Therefore, consuming well cooked foods is recommended.

Humans are accidental host of this tissue nematode. The disease is now considered an emerging imported disease in Europe and Western countries. Our result demonstrates the potential infection of zoonotic *G. spinigerum* larvae which could be found both in wild-caught eels of Min Buri District, Bangkok and in an eel raised farm of Sa Kaeo Provinces, Thailand. This study presents a risk to consume undercooked eels during the annual rainy and winter seasons and could support the necessity of improvements in public health surveillance programs.

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