On the Biology of *Gnathostoma spinigerum*

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Abstract

Gnathostoma spinigerum, the main causative agent of human gnathostomosis, is actually a nematode parasite of carnivores. The life cycle of the worm essentially involves 3 hosts: a definitive host, the first intermediate host and the second intermediate host. In Thailand, the natural definitive hosts of *G. spinigerum* are normally cats and dogs. Four species of cyclops serve experimentally as the first intermediate host. Forty-eight species of vertebrates serve naturally as the second intermediate (and/or paratenic) hosts. Among these animals, fish, especially swamp eels, have been found to be the best second intermediate/paratenic hosts of *G. spinigerum* on the basis of having the highest prevalence rate and the greatest infection intensity. Swamp eels have also been found to harbor at least 4 species of *Gnathostoma*.

This paper reviews *G. spinigerum* natural definitive hosts; natural second intermediate hosts and paratenic hosts; the record numbers of *G. spinigerum* larvae in second intermediate/paratenic hosts; tissue distribution of the larvae in naturally infected swamp eels; experimental first intermediate hosts; experimental primary infection in vertebrate hosts; experimental secondary infection in vertebrate hosts; experimental infection in cats and dogs; infectivity of the larvae in mice; and the effects of temperature, chemicals, radiation and some native Thai foods upon the viability of the larvae.

Keywords: *Gnathostoma spinigerum*, gnathostomosis, biology, early third-stage larvae, advanced third-stage larvae, hosts

Gnathostoma spinigerum, the main causative agent of human gnathostomosis, is actually a nematode parasite of carnivorous animals, especially felines and canines. The life cycle of the worm essentially involves 3 hosts: 1) a definitive host (*eg*, cat, dog, tiger), 2) the first intermediate host (mainly fresh-water cyclops), and 3) the second intermediate host (*eg*, fresh-water fish and other vertebrates) (Fig 1). The second intermediate hosts and paratenic hosts are the primary sources of infection for the definitive host and man [1-2].

Natural definitive hosts

In Thailand, the common natural definitive hosts of *G. spinigerum* are cats and dogs. However, the following vertebrates are also considered to be natural hosts: tigers, leopards, golden cats, leopard cats and jungle cats [1].

Natural infections of *G. spinigerum* in stray dogs and cats normally range from about 1.1%-6.9% and 1.9%-8.3%, respectively, with the maximum number of adult worms found being 10 worms/cat (in 1 tumor) and 72 worms/dog (30 in 1 tumor plus 42 in another tumor) [1, 3-5]. In an investigation of 1,000 stray dogs in Bangkok and Thonburi, 17.2%, 0.6% and 0.2% of the gastric, esophageal and duodenal tumors, respectively, were caused by adult *G. spinigerum*, with a variation of 1-8 tumors in each dog [6]. This is the first time on record of tumors of the esophagus and duodenum parasitized by adult *G. spinigerum*.

Natural second intermediate hosts and paratenic hosts

Forty-eight species of vertebrates – fish (20), amphibians (2), reptiles (11), avians (11) and mammals (4) – serve naturally as the second intermediate (and/or paratenic) hosts of the worm (Table 1) [1, 7-9]. Among these animals, fish, especially swamp eels (*Monopterus albus*, previously *Fluta alba*) have been found to be the best second intermediate/paratenic hosts of *G. spinigerum* on the basis of having the highest prevalence rate and the greatest infection intensity. Moreover, they have been found to harbor at least 4 species of *Gnathostoma*: *G. spinigerum, G. hispidum, G. doloresi* and *G. vietnamicum* [10-13].

Record numbers of *Gnathostoma* larvae in second intermediate/paratenic hosts

The maximum number of 2,582 gnathostome larvae was collected from one eel (the heaviest load ever recorded) in Nakhon Nayok Province, Thailand, by Akahane *et al* in 1987 [10]. The record numbers of *G. spinigerum*

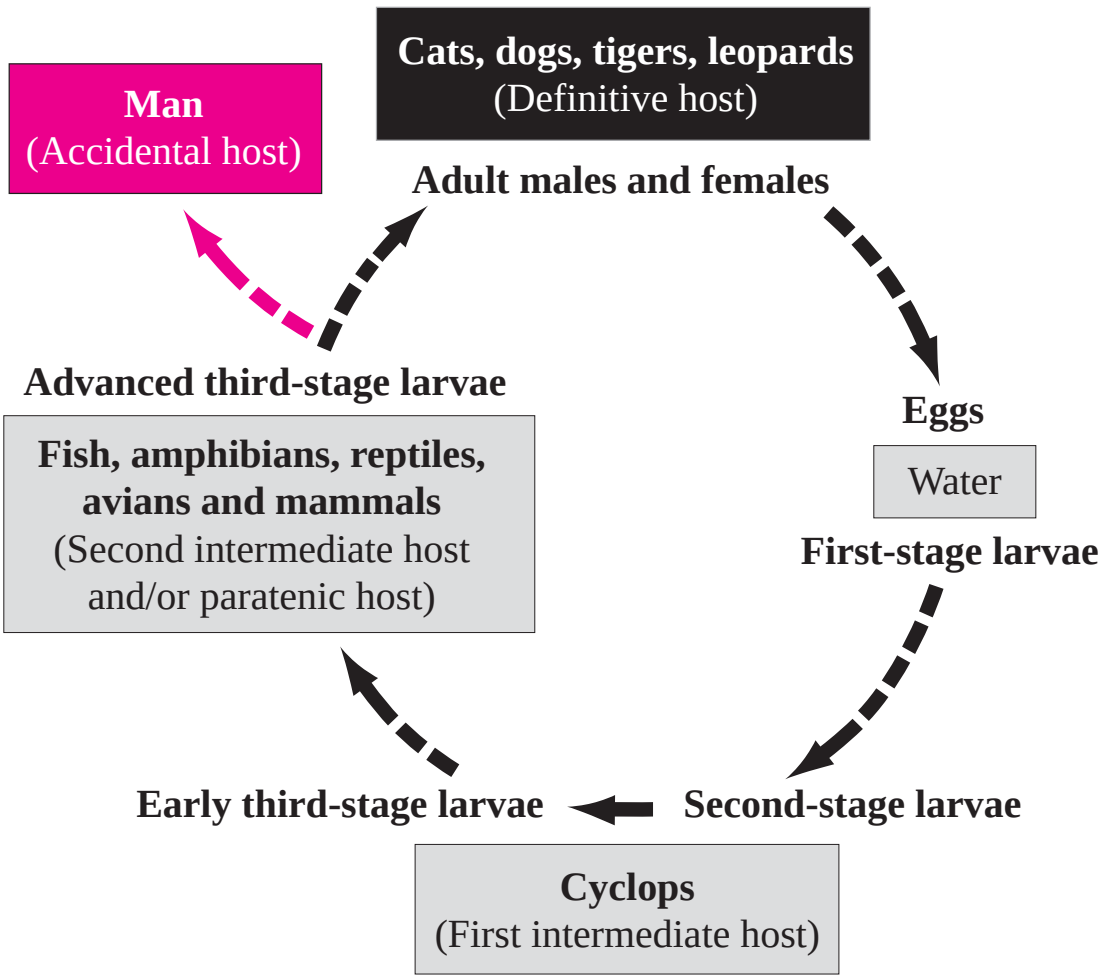

Fig 1 The life cycle of *Gnathostoma spinigerum* in Thailand (modified from Daengsvang, 1980).

larvae in other classes of vertebrates were summarized by Daengsvang in 1980, as follows: 1,808 larvae in a cobra (*Naja naja*); 83 larvae in a frog (*Rana rugulosa*); 17 larvae each in bittern (*Gorsachius* sp and *Ixobrychus* sp), jacana (*Hydrophasianus chirurgus*) and hawk (*Circus melanoleucos*); and 10 larvae in a great bandicoot (*Bandicota indica*) (Table 1).

Tissue distribution of *Gnathostoma* larvae in naturally infected swamp eels according to body regions

Almost all of the gnathostome larvae recovered from eels have been in their encysted form; very few of them were found to be unencysted. About half (43%-52%) of the total number of larvae were concentrated in the liver, whereas the remaining half (48%-57%) were in the whole muscles. Negligible numbers of larvae have been found in other visceral organs. In the muscles, it has been noted that the larvae preferred encysting in the ventral part (54%-71%) to the dorsal part (29%-46%), and in the middle portion (43%-53%) rather than the anterior (32%-33%) or posterior (15%-24%) portions of the eels. The most preferred region among the 6 divided parts of the eels was the medioventral region, where about 23%-30% of the larvae were present [13].

Experimental first intermediate hosts

There are at least 4 species of cyclops, *Mesocyclops leuckarti*, *Eucyclops agilis*, *Cyclops varicans* and *Thermocyclops* sp, which serve experimentally as the first intermediate host of *G. spinigerum* [14-15].

In the study by Daengsvang [1], it was found that immediately after ingestion, the first-stage larvae appeared in the stomachs of the cyclops as the second stage (without sheath). About 0.5-2 hours post-infection, they were in the body cavity and measured 293 x 13 μm; at 24 hours, 260 x 17 μm; on day 6, developed to early third-stage larvae, 460 x 45 μm; on day 7, at 29-31 °C, almost all became early third-stage larvae; also on day 7, only a few became early third-stage larvae if the temperature was about 25-29 °C. On days 8-9, at 29-31 °C, all the larvae became early third stage; on day 10, they measured 274-445 x 46-69 μm (average, 356 x 54 μm).

Experimental primary infection in vertebrate hosts

The following 20 species of vertebrates have been found to be susceptible to experimental infection with infected cyclops harboring *G. spinigerum* early third-stage larvae: 3 species of fresh-water fish (*Channa striata, Clarias batrachus* and *Gambusia holbrooki*), 3 species of amphibian (*Rana rugulosa, R. limnocharis limnocharis* and *Bufo melanostictus*), 3 species of reptile (tree lizard, cateye snake and monitor lizard), 1 species of avian (domestic fowl), 10 species of mammal (Norway rat, white rat, black rat, little house rat, white mouse, domestic pig, tree shrew, hamster, monkey and gibbon).

In fish, on the first few days of the feeding experiment, the larvae were found in the stomach wall and a few in the intestinal wall. Subsequently, they were mainly found in the muscles and some in the liver. Encystation occurred 6-61 days post-infection.

In mice, 1.5-24 hours after feeding, the larvae were found in the mucous membrane of the upper part of the small intestine and in the mucosa of the large intestine. None was found in the stomach wall. On the other hand, most of the free early third-stage larvae penetrated through the intestinal mucosa and a few may have penetrated the mucous membrane of the stomach. Then the larvae migrated to the liver where they were found mostly on days 15-30, the earliest being day 2 and the latest day 60. Later, they established infection in the muscles (first found on day 8). Normally, 1 larva was coiled in 1 cyst wall, at the earliest about 3 weeks post oral infection [1].

Experimental secondary infection in vertebrate hosts

Thirty species of vertebrates became infected after being fed with *G. spinigerum* advanced third-stage larvae that were obtained from the same or different species of vertebrates. These were: 2 species of fresh-water fish (*Channa*

Table 1 Summary of vertebrates (48 species) found naturally infected with *Gnathostoma spinigerum* advanced third-stage larvae in Thailand (modified from Daengsvang, 1980).

Host (Scientific name)	Common name	Thai name	Percent infected	Max no. of larvae/host
Fish				
1. *Anabas testudineus*	Common climbing perch	Pla mo Thai	0.9-64	14
2. *Arius caelatus*	Engraved catfish	Pla kot	3	5
3. *Boesemania microlepis*	River drumfish	Pla ma	5	1
4. *Channa striata*	Striped snake-head fish	Pla chon	7-72	153
5. *Channa micropeltes*	Giant snake-head fish	Pla chado	6-54	12
6. *Channa lucius*	Blotched snake-head fish	Pla krasong	4-50	12
7. *Clarias batrachus*	Batrachian walking catfish	Pla duk dan	2-30	6
8. *Clarias macrocephalus*	Gunther's walking catfish	Pla duk ui	3-50	16
9. *Chitala ornata*	Spotted featherback	Pla krai	7-11	1
10. *Kryptopterus cryptopterus*	?	Pla kha kai	0.9	5
11. *Macrognathus siamensis*	Spotted spiny eel	Pla lot chut	2	8
12. *Mastacembelus armatus*	Armed spiny eel	Pla krathing dam	6	1
13. *Micronema apogon*	Common sheatfish	Pla nam ngoen	2	?
14. *Monopterus albus*	Swamp eel	Pla lai na	7-100	2,582
15. *Ompok krattensis*	Sheatfish	Pla nua on	1-25	1
16. *Ophisternon bengalense*	Eel-like fish	Pla lat, pla lai	13-33	40
17. *Systomus orphoides*	Red-cheek barb	Pla kaem cham	0.4	1
18. *Trichogaster pectoralis*	Snake skin gourami	Pla salit	2-17	5
19. *Trichogaster microlepis*	Moonlight gourami	Pla kradi nang	3	5
20. *Trichogaster trichopterus*	Three-spot gourami	Pla kradi mo	3	?
Amphibians				
1. *Rana rugulosa*	Frog	Kop	10-92	83
2. *Rana limnocharis*	Small frog	Kop lek	0.3	1
Reptiles				
1. *Acrochordus javanicus*	Elephant-trunk snake	Ngu nguang chang	100	7
2. *Bungarus fasciatus*	Banded krait	Ngu sam liam	64-85	29
3. *Cylindrophis rufus rufus*	Red-tailed pipe snake	?	100	200
4. *Naja hannah*	King cobra	Ngu chong-ang	67-83	1,020
5. *Naja naja*	Cobra	Ngu hao	3-63	1,808
6. *Python reticulatus*	Python	Ngu lam	33-100	80
7. *Trimeresurus gramineus*	Green pit viper	Ngu khieo	20	1
8. Unidentified species ?	Fresh-water snake ?	Ngu kin pla	100	12
9. *Varanus salvator*	Water monitor	Takuat	100	52
10. *Varanus nebulosus*	Clouded monitor	Takuat	100	4
11. *Vipera russellii*	Russell's viper	Ngu maeo-sao	5	1
Avians				
1. *Anas platyrhynchos domestica*	Domestic duck	Pet	22	5
2. *Bubulcus ibis coromandus*	Cattle egret	Nok yang khwai	100	2
3. *Butorides striatus*	Little green heron	Nok yang daeng yai	100	1
4. *Circus melanoleucos*	Hawk (Pied harrier)	Yieo	100	17
5. *Corvus macrorhynchos*	Large-billed crow	Ka	80	9
6. *Egretta garzetta garzetta*	Little egret	Nok yang pia	50	1
7. *Gallus gallus domesticus*	Domestic chicken	Kai	56	10
8. *Gorsachius* sp	Bittern	Nok yang lai sua	100	17
9. *Hydrophasianus chirurgus*	Jacana	Nok i-chaeo	100	17
10. *Ixobrychus* sp	Little bittern	Nok yang	75	17
11. *Pelecanus philippensis*	Spot-billed pelican	Nok krathung	100	4
Mammals				
1. *Bandicota indica*	Great bandicoot	Nu phuk yai	3.4-8	10
2. *Herpestes javanicus*	Mongoose	Phangphon	50	4
3. *Rattus rattus*	Roof rat (black rat)	Nu thong khao	0.8	2
4. *Tupaia glis*	Tree shrew	Kratae	4	3

striata and *Clarias macrocephalus*), 5 species of amphibian (frogs and toads), 5 species of reptile (ground lizard, tree lizard, gecko, skink and turtle), 3 species of avian (domestic fowl, domestic duck and quail), 13 species of mammal (Norway rat, white rat, black rat, little house rat, white mouse, hamster, guinea pig, tree shrew, squirrel, domestic pig, Macaque monkeys and leaf monkey), 2 species of crustacean (fresh-water crab: *Paratelphusa sexpunctatum* and *Potamon smithanus*) [1].

Infections of *G. spinigerum* advanced third-stage larvae have also been successful in brackish-water fish, *Mugil* sp and *Chanos chanos*, by both forced and self-feeding. The percentages of infection were 56.0% and 80.0% in forced feeding and 53.3% and 30.0% in self-feeding, respectively [15]. In *Oreochromis mossambica* (*Tilapia mossambica*), the percentages of infection were about 63.6%-71.4%. The worms were found in the liver, tissues of the alimentary tract and abdominal muscle [16].

In mice, encystation of the advanced third-stage larvae occurred in 2-4 weeks, and was completed in 5 weeks post-infection; in rats, encystation started in the fourth week and was completed within the following week [17-18].

Experimental infection in cats and dogs

When eaten by the definitive hosts (cats, dogs), the advanced third-stage larvae penetrated the gastric wall or rarely the intestinal wall of the host and entered the liver. Then, they wandered through the muscle and connective tissue. Subsequently, the immature worms entered the gastric wall of the hosts from outside and became mature adults. There they caused the host reaction, the characteristic tumor, usually 1, and rarely 2 or more in number. The prepatent period in cats infected with *G. spinigerum* advanced third-stage larvae by skin penetration was about 2-7 months (59-227 days), with the maximum number of adult worms per cat being 52, and the infectivity 29%-94%. In a feeding experiment, the prepatent period was 2.7-12 months (81-363 days), the period of excretion of eggs 1.2-7.4 months (37-223 days), the infectivity 3%-100%, and 6% had no infection. In a dog feeding experiment, the prepatent period was about 3-8 months (84-247 days) and the infectivity 63% [1].

In our laboratory, from 1983 to 1995, 38 cats were individually infected by skin penetration with 25-85 advanced third-stage larvae of *G. spinigerum*. Twelve cats were dead before positivity and 2 (8.3%) showed no infection. The worm recovery rates ranged from 28.3% (17/60) to 87.9% (51/58), with the maximum number of worms recovered from 2 tumors of 1 cat being 51. The prepatent period was 160-273 days (5.3-9.1 months) except for 2 cases where it was only 55 and 77 days. The period of oviposition (excretion of eggs) was 103-232 days (3.4-7.7 months). In most cases, 1 tumor was formed inside the stomach of the cats (86.7%) or rarely 2 tumors (13.3%), and it was commonly found around the proximal part of the greater curvature. The average sex ratio between males and females obtained from 15 cats was about 1:1.4 [19].

Infectivity of *G. spinigerum* advanced third-stage larvae in mice

The infectivity of *G. spinigerum* advanced third-stage larvae at different ages in mice was once evaluated [20]. Mice were infected either with unencysted larvae or the encysted form, 5 larvae per mouse. Unencysted larvae at the age of 3 weeks and encysted larvae at the age of 8 weeks, 6 months and 12 months were obtained from laboratory mice infected with cyclops harboring *G. spinigerum* early third-stage larvae. The encysted larvae of unknown age were from naturally infected eels. The average number of larvae recovered from mice infected with unencysted/encysted larvae at the ages of 3, 8, 24, 48 weeks and at unknown age were 3.00, 3.07, 3.30, 3.47 and 3.10, respectively; while their infectivities were 60.0%, 61.6%, 66.0%, 69.3% and 62.0%, respectively. It was concluded that when mice were fed with unencysted larvae at the age of 3 weeks, encysted forms at the age of 8 weeks, 6 months and 12 months from laboratory infected mice; and the encysted larvae

from naturally infected eels (unknown age), there were no significant differences in infectivity (*ie*, the number of larvae recovered from the infected mice did not depend upon the age of the larvae infected) [20].

Effects of temperature, chemicals and some native Thai foods upon the viability of *G. spinigerum* larvae

Encysted larvae of *G. spinigerum* could survive 8 days in vinegar (4% acetic acid), 5 days in lime juice, 18 hours in fish sauce (23% NaCl), 20 hours in brine (30% NaCl), 6 days in syrup (20% sugar), and 8 and 9 days in 28% and 35% ethyl alcohol, respectively. The larvae survived 3 hours under desiccation at room temperature, 6 days in dechlorinated tap water, 12 days in physiological saline, 12 days at -4 °C, 9 days at -9 °C and 5 hours at 65 °C. Survival of these larvae in foodstuffs was over 8 days if they were mixed in minced meat salads; 25 days, 2 days and 1 day in fermented fish, dry fish with and without salt, respectively. Boiling and steaming of fish harboring encysted larvae (2 cm depth) for a few minutes were effective means of killing the larvae [21]. It was reported that, although *G. spinigerum* encysted larvae could survive several days in the freezer of a household refrigerator (about -4 °C), they lost their infectivity only after 1-2 days. However, the mimetic infected meat containing encysted larvae resisted this freezing temperature for at least 48 hours [22].

A living larva, 1 cm deep in the muscle of a fish, could be killed by treatment for at least 5 minutes in boiling water and could also be killed after being left in vinegar containing 4% acetic acid for 5.5 hours. However, larvae were found to be alive in lime juice after 5 days at room temperature. They were also still alive after being kept at 4 °C for 1 month [1, 23]. Encysted larvae, 2-4 mm deep in the flesh of a fish and a frog were killed within 7 days after being mixed with fresh lime juice containing 7.7% citric acid [24]. Excysted larvae were killed within 5 minutes in water at over 70 °C, 6 hours in vinegar, and 12 hours in soy sauce [25].

Effects of Cobalt-60 gamma radiation on *G. spinigerum* larvae

Advanced third-stage larvae of *G. spinigerum* (encysted and/or unencysted) were found to be very resistant to gamma rays. No changes in the morphology, viability or motility of the larvae were observed after exposure to 0-7 kGy of gamma rays. No larvicidal effect has been observed even at 10.0 kGy. The infectivity rates in mice were 72%, 78%, 64%, 54%, 28%, 42%, 26%, 14%, 5%, 0% and 0% when the larvae were pre-treated with radiation at doses of 0, 0.3, 0.6, 1.0, 1.5, 2.0, 3.0, 5.0, 7.0, 8.0 and 10.0 kGy, respectively. Irradiation of the larvae mixed in food prepared from fresh-water fish (Somfag) at 8.0 kGy inhibited their infectivity in mice. Therefore, 8.0 kGy was suggested as the minimum effective dose to inhibit the infectivity of the larvae [26].

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