RESEARCH NOTE

PILA AMPULLACEA AND *POMACEA CANALICULATA*, AS NEW PARATENIC HOSTS OF *GNATHOSTOMA SPINIGERUM*

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Abstract. Aquatic snails, *Pila ampullacea* and *Pomacea canaliculata* were experimentally found to be suitable paratenic hosts for advanced third-stage larvae (L3) of the nematode *Gnathostoma spinigerum*, the causative parasite of gnathostomiasis in humans. *G. spinigerum* (L3) were found to be encapsulated in the tissue of the snail's foot and its internal organs. The infection, intensity and survival of third-stage larvae of *G. spinigerum* in both species of aquatic snails are described. This is the first evidence to reveal that not only vertebrates but also invertebrates (snails) can serve as paratenic hosts to this parasite. Aquatic snails are one of several sources of human gnathostomiasis in Thailand.

INTRODUCTION

Gnathostoma spinigerum is the major causative agent of human gnathostomiasis, especially Thailand and Japan. It has also been reported in other countries including India, Vietnam, Myanmar, Lao PDR, Cambodia, China, Bangladesh, Malaysia, Indonesia, Philippines, Palestine, Israel, Australia, USA, Mexico and Ecuador (Rojekittikhun *et al*, 2005). This worm requires three types of hosts (a definitive host, a first intermediate host and a second intermediate host) for completion of its life cycle. 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 [fish (20 species), amphibians (2), reptiles (11), avians (11) and mammals (4)] serve naturally as second intermediate (and/or paratenic) hosts of the worm (Rojekittikhun, 2002). Second intermediate hosts and paratenic hosts, containing advanced third-stage larvae (L3) are the primary sources of infection for the definitive host. Humans are an accidental host, who may become infected by eating undercooked fish, poultry, amphibians or reptiles that harbor the infective larva of *G. spinigerum*.

Each year several hundred suspected cases of gnathostomiasis come to the Gnathostomiasis clinic, Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. Serum samples to diagnose gnathostomiasis were also sent to the Immunodiagnostic Unit for Helminthic Infection, Department of Helminthology. Many patients were diag-

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nosed clinically and confirmed serologically as having *G. spinigerum* infection. Patients with positive serological tests for gnathostomiasis were also questioned about their food intake, especially raw and rare animals, during the 30 days prior to the onset of first symptoms. Some patients revealed an intake history that included consuming raw or undercooked *Pila* and *Pomacea* snails. Questions regarding this history led to this study to determine whether the infective larva of *G. spinigerum* can survive in aquatic snails.

MATERIALS AND METHODS

Eggs of aquatic snails *Pila ampullacea* and *Pomacea canaliculata* (Fig 1A, 2A) were collected from rice fields and ponds in Nakhon Pathom Province, and maintained in a wet laboratory, at the Department of Helminthology Mahidol University, Bangkok, Thailand. After hatching, the snails were then reared in an aquarium until used for the experiment. The infective stage or advanced third stage larvae (L3) of *G. spinigerum* were separated from the livers of swamp eels (*Fluta alba*) which were purchased from a market in Bangkok, Thailand. All larvae obtained were morphologically identified as *G. spinigerum*. Twenty aquatic snails of each species of *Pomacea canaliculata* (50-65 mm) and *Pila ampullacea* (55-72 mm) were used in the feeding experiment. Each snail was fed orally with 10 L3 *G. spinigerum* in Petri dishes (150 x 25 mm) filled with dechlorinated water; all the snails were then maintained in an aquarium. Every fifth snail of each species were killed and their tissues were examined microscopically on Days 7, 14, 30 and 60 for possible infection by compression between two glass slides.

RESULTS

Both species of aquatic snails examined were found to be infected with L3 *G. spinigerum* (Figs 1, 2). The infection rates with L3 *P. ampullacea* were 80% (4/5) on Day 7, 80% (4/5) on Day 14, 60% (3/5) on Day 30, and 50% (2/4) on Day 60. The infection rates with L3 *P. canaliculata* were 80% (4/5) on Day 7, 80% (4/5) on Day 14, 60% (3/5) on Day 30, and 75% (3/4) on Day 60. The numbers of L3 per snail in *P. ampullacea* were 2-6, 1-5, 1-6 and 1-5 in the snails examined on Days 7, 14, 30 and 60, respectively, and in *P. canaliculata* were 4-8, 2-7, 2-6 and 2-5 in the snails examined on Days 7, 14, 30 and 60, respectively. Most of the larvae were encapsulated in snails' tissues *eg* foot, internal organs in both species. The morphology, size and shape of L3 obtained from snails were species identical with those L3 obtained from the eels.

In order to determine whether aquatic snails may act as paratenic hosts of this parasite, L3 obtained on day 14 from infected snails of both species were transferred to uninfected snails (2 snails per species, 2 larvae per snail) by the same technique. Seven days later, two L3 from *P. ampullacea* and three L3 from *P. canaliculata* were also found to be encapsulated in the snail's tissues; the L3 were still actively moving when removed. The morphology of the L3 obtained was the same as those of the conspecific L3 obtained from the eels.

DISCUSSION

The infection, intensity and survival of the infective larva of *G. spinigerum* in aquatic snails were demonstrated in this experiment. These may vary by one of various factors involved. During feeding some infected snails of both species discard the worm in a little mass of mucus that was discharged from their shell. This acts as a natural defense mechanism of the snail against parasite invasion. When this occurs, plenty of mucus is seen with parasite in snails of both species. One experiment demonstrated the

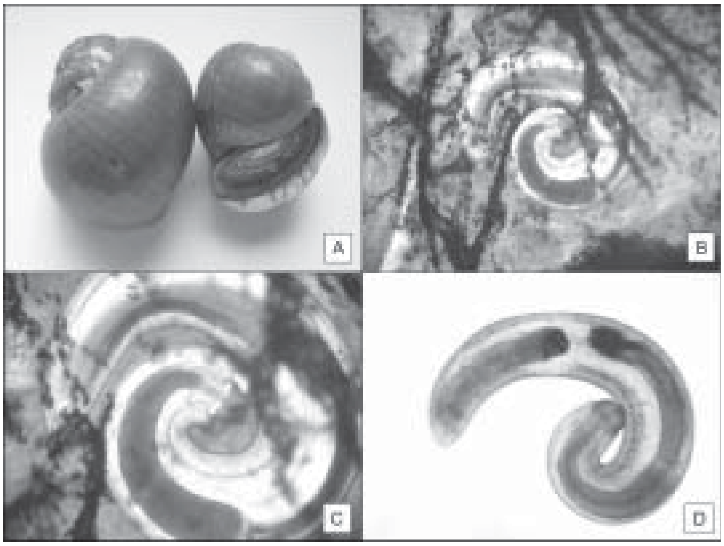

Fig 1–A. Aquatic snail, *Pila ampullacea*.
B., C. Infective larva of *Gnathostoma spinigerum* in snail tissue.
D. Infective larva removed from snail tissue.

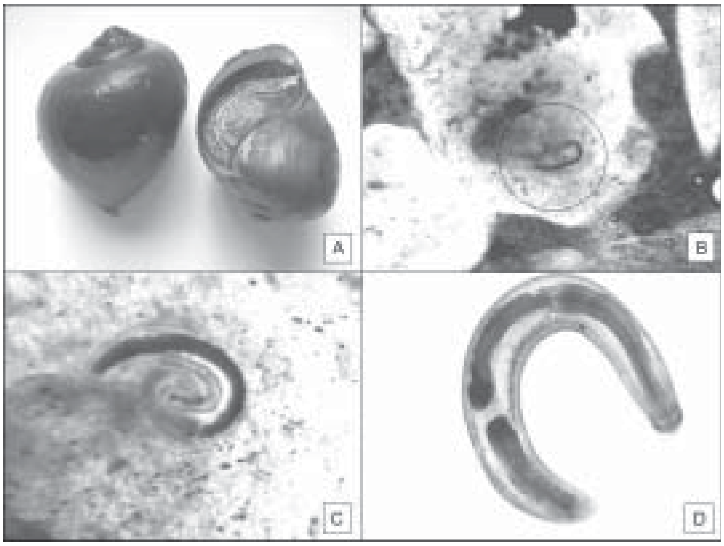

Fig 2–A. Aquatic snail *Pomacea canaliculata*.
B., C. Infective larva of *Gnathostoma spinigerum* in snail tissue.
D. Infective larva removed from snail tissue.

infectivity of L3 *G. spinigerum* in mice was different based on the age of the larvae (Rojekittikhun *et al*, 1989). The L3 recovered from naturally infected eels was of unknown age. The infectivity of the L3 in snails may vary by condition. We found the infective larvae (L3) of *G. spinigerum* can survive for 2 months (or longer) in aquatic snails.

The "apple snails" *P. ampullacea* and *P. canaliculata* are species of fresh water snails in the family ampullariidae. These snails are not selective and eat almost everything available in their environment. In general, they prefer soft or digestible vegetables. During prolonged starvation, apple snails are known to deploy cannibalistic behavior, and are able to consume all kinds of dead animals, such as dead fish, frogs, crustaceans and insects. Many animals are known as apple snail predators, such as insects, fish, amphibians, crocodilians, reptiles, snakes, crayfish, turtles, rodents and birds (Ghesquiere, 1998). Therefore, infection and transmission of L3 through snails may occurred in nature.

In Thailand the aquatic snails *P. ampullacea* and *P. canaliculata* are commonly found throughout the country. They are a good source of food for several kinds of animals, including humans. For people living in northern and northeastern Thailand, these snails are usually eaten raw or improperly cooked and often served with alcohol. Both species of aquatic snails have been found to be susceptible to the nematode, *Angiostrongylus* *cantonensis*, the causative agent of human eosinophilic meningites in Thailand (Tesana *et al*, 2008). Aquatic snails have been demonstrated as paratenic hosts for parasitic nematodes and cestodes (Moravec, 1996; Latham *et al*, 2003; Nikolov *et al*, 2008). Until now, aquatic snails (invertebrate) has not been reported to be in the life cycle of *G. spinigerum*. This experiment is the first evidence to reveal that not only vertebrates but also invertebrates (snails) may serve as paratenic hosts for this parasite. *P. ampullacea* and *P. canaliculata* are likely to be one of several sources of human gnathostomiasis in Thailand.

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