EXPERIMENTAL INFECTION OF *GNATHOSTOMA SPINIGERUM* LARVAE IN PRAWNS AND TADPOLES

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Abstract. Naturally captured Lanchester's freshwater prawns (*Macrobrachium lanchesteri*) and farm-bred *Rana regulosa* tadpoles were assessed for their capability of being the first or second intermediate hosts of *Gnathostoma spinigerum*. Seventy specimens from each animal group were randomized into a control group and investigated for larvae of *G. spinigerum* by pressing them between two pieces of glass and examination under stereomicroscope. No *Gnathostoma* larvae were found in the entire control group. Another 120 specimens of each animal were used in two treatment groups; 60 being exposed to the first-stage larvae, *G. spinigerum*, and 60 exposed to cyclops containing the third-stage larvae for 7 days. No larvae of *G. spinigerum* were found in the prawns of both treatment groups that resembled tadpoles exposed to the first-stage larvae. In contrast, 18.3% of tadpoles, which were exposed to cyclops containing third-stage larvae, were infected. Lanchester's freshwater prawns cannot serve as intermediate host of *G. spinigerum*, while *R. regulosa* can serve as the second intermediate host.

INTRODUCTION

Gnathostoma spinigerum is a human pathogenic nematode found mainly in Asia, especially Thailand and Japan (Miyazaki, 1960; Daengsvang, 1980). Migration of this parasite through superficial soft tissue can cause localized intermittent migratory cutaneous swelling, which may lasts for over a year in most patients; the most common clinical manifestation of gnathostomiasis (Daengsvang, 1980; Bunnag, 1984). Occasionally, this disease can be fatal due to cerebral invasion by the parasite (Punyagupta *et al*, 1968).

The complete life cyclè of *G. spinigerum* has been studied and described by Prommas and Daengsvang (1933, 1936). Several domestic and wild cats and dogs serve as definitive hosts. Among *Gnathostoma* spp, cats have been recorded as the definitive host for only *G. spinigerum* (Daengsvang, 1980; Miyazaki, 1991). Only four species of cyclops; *Mesocyclops leuckarti, Eucyclops agilis, Cyclops varicans* and *Thermocyclops* sp, have been experimentally shown to be the first intermediate host (Sooksri, 1967). Cockroach, dog flea (*Ctenocephalides canis*), cat flea (*C. cati*) and mice, have been proved experimentally unable to serve as a host for *G. spinigerum* (Heydon, 1929; Prommas and Daengsvang, 1933). Forty-four animals have been reported in Thailand (Daengsvang, 1980) to serve as second intermediate hosts, with freshwater eel (*Fluta alba*), cat fish (*Chana striata*) and frog (*Rana regulosa*) being the primary host containing a large number of advanced third-stage larvae (Setasuban *et al*, 1991). Since there are several animal species that can serve as intermediate host, the objective of this study was, therefore, to assess the possibility of other freshwater animals being involved in the life cycle of *G. spinigerum*. In view of having the same habitat as cyclops and the second intermediate host of this parasite, Lanchester's freshwater prawns (*Macrobrachium lanchesteri*) and *R. regulosa* tadpoles were investigated.

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MATERIALS AND METHODS

Ten domestic cats (≈ 3 kg body weight) were used to obtain *G. spinigerum* eggs. All were kept and nurtured in an animal room for at least 3 months before the experiments. Rabies and measles vaccinations were given to all cats to prevent disease transmission to researchers and reduce the mortality rate of animals. Four animal stool examinations, using the formalin-ether concentration method (Ritchie, 1984), were performed once a week for 4 consecutive weeks. Cats with parasitic infections were treated with appropriate medications and stool specimens were reexamined. Cats with 2 consecutive negative stool examinations were declared as parasite-free.

Advanced third-stage larvae of *Gnathostoma* spp were collected from the liver of freshwater eels (*F. alba*) by compressing the liver between two pieces of thick transparent glasses before examining it under a stereomicroscope. Eight collected larvae were fed to each cat using forced oral inoculation. The problem of

vomiting was observed for 30 minutes after inoculation. Stool examination was carried out once a week until the presence of one-knob *Gnathostoma* eggs was detected. Among cats with *Gnathostoma* spp, those with one-knob eggs were definitive hosts for *G. spinigerum* only (Daengsvang, 1980; Miyazaki, 1991). These eggs were then identified as *G. spinigerum* eggs.

After a stool was found positive for *G. spinigerum,* eggs were recovered by the brine floatation method (Beaver *et al*, 1984). The harvested eggs were washed three times in deminerized water, then left in a 250 ml beaker containing deminerized water at room temperature (17˚-30˚C). Development of the eggs was observed daily until the first-stage larvae hatched. The larvae were then separated into two groups; the first group for testing the capability of prawns and tadpoles to serve as intermediate hosts, while the other group was fed to cyclops (*M. leuckarti*).

Prawns were collected from paddy fields in Muang and San Pa Thong districts, Chiang Mai Province, northern Thailand. Tadpoles were kindly provided by the frog farm of the Royal Project at Huay Hong Krai, Doi Saket district, Chiang Mai Province. Both animal species were transferred in plastic bags with oxygen to the Department of Parasitology, Faculty of Medicine, Chiang Mai University. All animals were gently transferred to separated aquariums equiped with an oxygen pump. The animals were nurtured in the same aquarium for 3 days in order to adapt them to laboratory conditions. All animals were fed with commercial fish food.

For the control group, 70 specimens of prawns and tadpoles were randomly examined for *G. spinigerum* larvae by compressing them individually between two pieces of glass and examining under a stereomicroscope.

Sixty active prawns were used to determine whether they can be a first intermediate host. After 3 days of feeding in an aquarium, all were transferred into 3 trays at a density of 20 prawns per tray (50 cm in length, 20 cm in width and 10 cm in depth) and immersed in 10 cm of clean water. All trays were oxygenated using an oxygen pump and the trays were covered with a screen to prevent the prawns from jumping out. All the prawns fasted for 12 hours and 120 first-stage larvae were released into each tray, thus, indicating a ratio of prawns to larvae of 1:6. Fish food was given to the prawns two days after releasing the larvae. Prawns and the first-stage larvae were left together in the same trays for 7 days. All prawns were then individually sacrificed by crushing them between 2 pieces of glass and examined for *G. spinigerum* larvae. The capability of tadpoles to serve as the first intermediate host was determined in the same manner as previously described.

To determine the possible second intermediate host of *G. spinigerum*, another 60 prawns were used under the same environmental conditions as for determining the first intermediate host. All prawns were fasted for 12 hours and 60 cyclops infected with third-stage *G. spinigerum* larvae were released into each tray, indicating a ratio of prawns to cyclops of 1:3. After seven days, all prawns were individually sacrificed and examined for larvae, as in the previous experiment. The capability of tadpoles to serve as the second intermediate host was determined in the same manner.

RESULTS

Twenty-one prawns and 4 tadpoles died in the determinating of the possibility of serving as first intermediate host while 15 prawns and 10 tadpoles died in the determination for possible second intermediate host. All were negative for *G. spinigerum* larvae. However, metacercariae of unidentified trematodes were found in prawns.

No *G. spinigerum* larva was found in the animal examined in both control and treatment groups determined as the first intermediate host. Similar result for prawns was obtained for the second intermediate host, however, 11 of 60 tadpoles (18.3%) possessed a small advanced third-stage *G. spinigerum* larvae (one larva per tadpole).

DISCUSSION

Several species of vertebrates, *eg* fish, birds, reptiles, amphibians and mammals, have been reported as the natural second intermediate host of *G. spinigerum* in Southeast Asia, China and Japan (Komaya *et al*, 1945; Miyazaki, 1960; Daengsvang, 1980). Only the report of Miyazaki (1954) indicated that some crustaceans such as crayfish and crab naturally harbor advanced third-stage larvae of this parasite. In Thailand, 16 species of freshwater fishes, 2 species of frogs, 11 species of snakes, 11 species of avian and 4 species of rodents naturally contain advanced third-stage larvae of *G. spinigerum*, indicating a low host specificity. A high prevalence of infection was detected in frog (*R. regulosa*), eel (*F. alba*), snake-headed fish (*Opicephalus striatus*) and catfish (*Clarias batrachus*) (Daengsvang and Tansurat, 1938).

It is likely that prawns collected from the Chiang Mai region are free from immature infection with *G.*

spinigerum. It has been suggested that Lanchester's freshwater prawns cannot serve as intermediate hosts of *G. spinigerum*. The absence of larvae in both the 70 specimens of the control and 120 of the experimental groups, indicate that this particular crustacean species (*M. lanchesteri*) is not a good host. There is a delicious Thai dish of fresh uncooked prawns called "Kung Ten", which is made by mixing spices with small live prawns. This particular food must be eaten immediately after preparation, since it will not be so tasty after a period of time. Therefore, the route of human *G. spinigerum* infection is probably not caused from "Kung Ten"; however this dish can be contaminated with other pathogens such as enteric bacteria. Interestingly, a recent report indicated that a creeping eruption in Japanese men who had eaten raw freshwater shrimp in Myanmar was caused by *G. malaysiae* (Nomura *et al*, 2000). Little is known about animals serving as hosts in the food chain that account for completing the life cycle of this parasite. In Thailand, *G. malaysiae* has been recorded in rat definitive hosts, *Rattus surifer* (Kamiya *et al*, 1987). Setasuban *et al* (1991) proposed that the peculiar species of *Gnathostoma* larvae, which is probably the advanced third-stage of *G. malaysiae*, appeared in *F. alba* collected in Nakhon Nayok Province, central Thailand. Eating fresh uncooked prawns or shrimps is not recommended when trying to prevent infections such as *Gnathostoma* spp.

However, tadpoles of *R. regulosa* can serve as second intermediate host of *G. spinigerum*. In this study, 18.3% of tadpoles were experimentally infected with *G. spinigerum*, with the advanced third-stage larvae being observed in each infected tadpole. Natural infections of *R. regulosa* in Thailand ranged from 1-91.7% according to previous reports (Daengsvang, 1980; Setasuban *et al*, 1991). As many as 83 advanced third-stage *G. spinigerum* larvae were found in frog (Daengsvang, 1980). In Bangladesh, the infective rate of *G. spinigerum* larvae in frog (*Rana tigrina*) was relatively high (90%; 27/30) (Bashirullah, 1972). Miyazaki (1991) proposed that frogs can be infected with gnathostomes during both the tadpole period and as an adults.

Regarding the feeding behavior observed in this study, *R. regulosa* tadpoles are fierce and cannibalistic. Although *R. regulosa* tadpoles can be the second intermediate host, this animal is not properly used as an animal model for maintaining *G. spinigerum* larvae in a small-area laboratory.

In conclusion, Lanchester's freshwater prawns cannot serve as the first or second intermediate host of *G. spinigerum*, while *R. regulosa* can serve as the second intermediate host, beginning at the tadpole period.

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