Studies of New Experimental Hosts, Life Cycles and Modes of Transmission of Gnathostomes

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OBJECTIVE: During this reporting period continuation of the study to determine the seasonal variation in the prevalence of adult G. spinigerum infections in dogs in the Bangkok and Thonburi areas and of advanced third-stage larval infections in snake-headed fish (Ophicephalus striatus), bought at the Ayuthaya and Phetburi markets, was undertaken. Poisonous snakes provided by the Thai National Red Cross Snake Farm were also studied. Studies of adult G. spinigerum and other gnathostome species obtained from infected pig stomachs at the Bangkok Slaughter House were continued. Additional studies of the susceptility of some common fresh water animals and other vertebrates as intermediate and paratenic hosts of G. spinigerum, G. hispidum and G. doloresi were performed.

DESCRIPTION: Examination of the gastrointestinal tracts of stray dogs killed at The Bangkok-Thonburi Municipality Rabies Control Unit was performed in the month of April of this reporting year to complete a study on the seasonal prevalence of G. spinigerum in the definitive host (dogs) in the Bangkok and Thonburi areas. This study was initiated in 1965 for G. spinigerum, 1967 for G. hispidum and G. doloresi and 1968 for G. vietnamicum. Gnathostome worms were obtained from infected pig stomachs for species identification. This was made possible with the full cooperation and assistance of the Bangkok Slaughter House authorities.

Weekly stool examinations for gnathostome ova by the formalin-ether concentration method were done on cats and dogs brought to the SMRL animal house for experimental purposes; all cats and dogs that died were autopsied and examined for the presence of gnathostome worms in the gastrointestinal tracts and musculature. Stool samples collected from 40 young domestic pigs at a private Nakhornpathom pig farm by Dr. Markapol Tingpalapong of the SMRL Veterinary Medicine Department were examined by the formalin-ether concentration method for gnathostome ova.

In the month of April, 1969, about 2 kg. of fresh water snake-headed fish (22 fish) purchased at the markets in Ayuthaya and Phetburi (endemic areas) and posionous snakes from the Thai National Red Cross Snake Farm in Bangkok, which died spontaneously, were autopsied for G. spinigerum and other gnathostome larvae for the completion of a study on the seasonal prevalence rate of infection with G. spinigerum advanced third-stage larvae.

To avoid the experimental use of naturally infected animals with gnathostome larvae, autopsies were done on samples of snake-headed fish (Ophicepahalus striatus), catfish (Clarias batrachus), top minnow fish (Gambusia hollbrooki), small fighting fish (Trichopsis vittatus), toad tadpoles (Bufo melanostictus), frogs (Rana rugulosa) and fresh water crabs (Paratelphusa sexpunctatum), of which some were collected in the Bangkok area from public and private fresh water ponds and ditches, and others were bought at the markets at Bangkok and Thonburi.

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* Retroactive in the previous reports.
1 Worked for some month during the year before resignation for further education.
2 Replacement for 1.

Experimental infection was also continued on many vertebrates to determine additional potential second intermediate and paratenic hosts G. spinigerum and of G. hispidum. Additional numbers of white mice and one monitorlizard were fed with fully developed larvae of G. doloresi in cyclops to determine their ability to act as the second intermediate host of the worm.

PROGRESS: Table 1 summarizes the April, 1969, findings on examination of dog stomachs from the Bangkok Thonburi Municipality Rabies Control Unit, snake-headed fish purchased at the fish markets in Ayuthaya and Phetburi, and snakes from the Thai National Red Cross Snake Farm in Bangkok.

All 1,289 dog stomachs examined were found negative. During April of 1967 and 1968, 408 and 356 dog stomachs respectively, obtained from the Rabies Control Unit were also negative. Advanced third-stage larvae of G. spinigerum were found in 2 (9%) of 22 fresh water snake-headed fish bought at Ayuthaya aud Phetburi markets showing 1 and 2 encysted G. spinigerum larvae each (at Ayuthya 1 large fish negative and 1 of 6 small fish positive with 2 encysted larvae; at Phetburi 1 of 5 small fish positive with 1 encysted larva). These findings may be compared with 60.0% positive on examination of 8 small fish and 2 large fish (5 small fish and 1 large fish were positive) during April 1968. All 37 poisonous snakes obtained from the Thai National Red Cross snake farm (26 Naja naja, 1 Naja hannah, 8 Vipera russelli, and 2 Trimerus gramineus) were found negative as compared with 6% positive on examination of 17 poisonous snakes in April 1968 (1 of 17 snakes was positive with the larva). Also during this year 4 small and 1 large snake-headed fish obtained from Bangkok and Thonburi markets (2 small fish bought at Thonburi

Table 1. Result of study in April 1969 on the incidence of G. spinigerum infection in Bangkok domestic dogs and infection of second intermediate or paratenic hosts (snake-headed fish and poisonous snakes) with advanced third-stage larvae in endemic areas near Bangkok (Ayuthaya-Phetburi)

	Total
Dog stomach	
(No. pos./No. exam.)	0/1289
Infection with adult G. spinigerum	
% Positive	0
Ophicephalus striatus (snake-headed fish)	
(No. pos./No. exam.) Large*	0/1
Small**	2/21
Total	2/22
Infection with advanced third-stage larvae (in Ayuthaya – Phetburi)	
% Positive	9
Poisonous snakes (Naja naja & hannah, Vipera russelli, Trimeresus gramineus)	
(No. pos./No. exam.)	0/37
Infection with advanced third-stage larvae	
% Positive	0

* Large Ophicephalus striatus = 500 grams and over

** Small Ophicephalus striatus = Less than 500 grams

market and 1 large and 2 small bought at Bangkok market) were found negative for gnathostome larvae. Of an additional 15 young snake-headed fish weighing not more than 5 grams each and measuring about 1.5 cm – 6.0 cm × 0.2 cm – 0.8 cm collected from a roadside fresh water ditch about 15.0 kilometers north of SMRL, 1 was found infected with a non–encapsulated G. spinigerum advanced third–stage larva in its liver measuring 2.2 × 0.2 mm.

2 small catfish (Clarias batrachus) bought from a Thonburi market were found on autopsy to be negative for gnathostome infection.

Stool examinations of 28 domestic cats (#97 to #124) newly obtained from Rajburi Province and nearby areas for experimental infection with gnathostomes; 2 (#102, #103) were found positive with gnathostome ova (7.2%) but 11 of them died of unknown causes of which one only (#104) showed on autopsy one immature male measuring 13.3 × 0.3 mm in the stomach tumor of about 0.5 cm in diameter. This animal never showed gnathostome ova on frequent stool examinations.

Stool samples collected from 40 young domestic pigs at a private Nakhonpathom pig farm were found to be negative for gnathostome infection.

A total of 659 adult gnathostomes were recovered from infected pig stomachs from the Bangkok Slaughter House of wich none were G. spinigerum; however, 488 (206 males and 282 females) were identified as G. doloresi and 171 (58 males, 113 females) as G. hispidum, as compared with a total of 979 worms (359 G. hispidum = 80 males and 279 females; 620 G. doloresi = 86 males and 534 females) for 1967–68, and 1773 worms (425 G. hispidum = 182 males and 243 females); 1348 G. doloresi = 414 males and 934 females) for 1968 – 69. There were 3411 gnathostomes found in 3 years from infected pig stomachs; none were G. spinigerum, but 955 and 2456 were identified as G. hispidum and G. doloresi respectively. It is then reasonable to conclude that pigs are not the definitive host of, or naturally infected with, adult G. spinigerum.

Experimental infection with G. spinigerum larvae.

(a) Further determination of new second intermediate host and paratenic host on some animals commonly found in fresh water ditches and ponds was experimentally undertaken as follows:

After finding no natural infection with gnathostome larvae on examination of 3 small top–minnow fish (Gambusia holbrooki), 10 toad tadpoles (Bufo melanosticuts) and 6 fresh water crabs (Paratelphusa sexpundtatum) caught from a fresh water pond near the animal house of SEATO Medical Research Laboratory, 3 other top-minnow fish of the same size and from the same pond were experimentally in a 1000 ml beaker containing about 600 ml of fresh water with 18 cyclops infected with 37 fully developed larvae of G. spinigerum. These 3 fish on autopsies 5–6 days later were found to be infected with 2, 3 and 4 advanced third–stage larvae respectively in the skeletal muscle of which three larvae, one from each fish, were found dead. Measurements of all larvae revealed a variation in length of from 0.3–1.66 mm and in width of from 0.03–0.13 mm.

Four toad tadpoles of about the same size from the same pond were experimentally infected in a 500 ml glass beaker containing about 300 ml of fresh water with 10 cyclops infected with 30 fully developed larvae of G. spinigerum 10 days old. Of the tadpoles autopsied on different days, 2 examined on days 11 and 28 of the experiment were negative for the infection; however, the other 2 tadpoles examined on days 11 and 21 were found to be infected each with 2 and 4 live advanced third–stage larvae of the worm in the digestive tract measuring in the range of 0.4–1.0 mm × 0.03–0.12 mm.

The results of experimental investigation showed for the first time that top–minnow fish and toad tadpoles, commonly found in fresh water ditches and wells, also act as additional second intermediate hosts of G. spinigerum. Moreover it is reasonable to assume that snake–headed fish may be infected with advanced third–stage larvae of the worm by feeding on the infected top–minnow and toad tadpoles (secondary infection or paratenic host).

Fifteen adult fresh water crabs were experimentally fed with different numbers of advanced third–stage larvae (3 to 40) obtained from experimentally infected white mice on autopsies at various days (8–29) after infection; all were negative for the infection. This study will be continued until a total of 50 crabs have been examined or until some experimental crabs are found positive.

The life cycle of G. hispidum.

(a) Experimental study for determining second intermediate host by feeding vertebrates with G. hispidum fully developed larvae in cyclops.

Six adult snake–headed fish and 2 catfish were found negative after being fed with fully developed larvae in cyclops as reported in the SMRL Annual Report for 1969.

During the reporting year the following food animals of man were studied to determine their ability to act as second intermediate hosts of the worm with consequent possible transmission to man. The results are as follows:

Of 2 young snake–headed fish (Ophicephalus striatus) weighing about 3 grams (#5 and #6) and each fed with 15 fully developed 10 day old larvae in 4 and 8 cyclops respectively, one fish was found on autopsy 7 days after the experiment to harbor 1 living third–stage larva of G. hispidum measuring 345 microns × 66 microns located in the liver. The other fish autopsied 32 days after the experiment showed no infection. Two small snake–headed fish weighing 100 gram and 140 grams respectively were infected; one (#7) fed with with 50 fully developed 16 day old larvae in 29 cyclops and the other (#8) with 40 larvae of the same age in 21 cyclops. On autopsy, one of the fish (#8) 5 days after the experiment showed 12 advanced third–stage larvae located in the stomach wall of which 10 were in the range of 662–800 microns × 85–110 microns. To determine further the infectivity, 8 larvae were then fed to one white mouse (#36); autopsy 21 days later showed no infection. The other experimental fish (#7) was sacrificed 41 days after the experiment. On necropsy it was also negative

Four cat fish (Clarias batrachus) fed with 50, 46, 30 and 30, 10–12 day old fully developed larvae in cyclops showed on autopsies 24, 28, 46 and 47 days after the experiment 21, 7, 3, and 4 living larvae in the stomach wall each of which was surrounded by a thin cyst wall measuring 266–400 × 71–194 microns. The dimensions of 7 larvae after being freed from the cyst wall were 1.2–1.4×0.2–0.3 mm. To determine further the infectivity of these larvae in some warm–blooded vertebrates, 2 laboratory bred tree shrews (Tupaia glis) were fed with 9 and 10 larvae obtained from one of the infected fish. On autopsy 11 days later the tree shrews were negative for the infection.

Of 6 small fighting fish (Trichopsis vittatus) sacrificed 9 to 53 days after exposure to 68 cyclops infected with 89 G. hispidum fully developed larvae, 4 (66%) showed on autopsies 9 to 53 days after the experiment a total of 26 (30%) advanced third–stage larvae (25 in the stomach wall and 1 in the body flesh) of which 13 were dead and 13 living. The dimensions of 19 larvae were 0.4–0.5 × 0.05–0.08 mm. Eleven living larvae discovered in the stomach wall were then fed to a white mouse to determine their further infectivity; autopsy 14 days later revealed no infections.

Three frogs (Rana rugulosa) were previously reported negative with the infection after being fed with fully developed larvae in cyclops (Annual Progress Reports 1968 and 1969). During this reporting year each of 9 frogs was fed with 50 G. hispidum fully developed larvae 10–16 days old in cyclops ; an additional frog was given 30 larvae. On autopsies of these frogs, 4–50 days after the experiment, 4 (40%) sacrificed 48–50 days after being fed with the larvae were found positive with 1, 1, 2, and 11 advanced third–stage larvae of the worm encysted in the flesh of the abdominal wall, leg, back and anterior chest wall of the animals.

Two tree shrews were fed with 30 fully developed 15 day old larvae each in cyclops. On autopsy 83 days later, one was found negative for the infection and another had 1 encysted larvae measuring 1.0 mm × 0.8 mm in the flesh of its right hind leg.

A monitor lizard (Varanus nebulosus) was found negative on autopsy after being fed with a total of 62 fully developed larvae in cyclops.

Snake–headed fish (2), catfish (4), and small fighting fish (4) were found to be experimentally susceptible to G. hispidum; advanced third–stage larvae could be found developing several days in the stomach wall; in addition, one larva was found in the flesh of an infected fish. Four frogs and 1 tree shrew were also found to be experimental hosts in which G. hispidum advanced third–stage larvae could develop after being fed with larvae in cyclops; survival in the flesh of the leg, abdominal wall and back for some days was noted.

These findings show that snake–headed fish, catfish, small fighting fish, frog and tree shrew can act as the second intermediate host of the parasite.

Further study to determine second intermediate hosts of G. hispidum is to be undertaken.

(b) Experimental study for determining paratenic hosts (secondary infection) by feeding vertebrates with G. hispidum advanced third–stage larvae.

Three young snake–headed fish (Ophicephalus straitus), two weiging 3 grams and another 6 grams, were experimentally fed wtih 4, 5 and 6 G. hispidum advanced third–stage larvae obtained from an infected fish and two infected white mice; the results were as follows: one young fish showed on autopsy 3 days after being fed with the 6 larvae obtained from the mouse, 4 living larvae of which 1 was found in the body flesh and 3 in the liver. A few hours later these 4 larvae were fed to another young fish which was sacrificed 33 days later and showed on examination 3 living larvae in its intestinal wall. The third fish, sacrificed 41 days after being fed with 5 living advanced third–stage larvae 179 days old obtained from an infected white mouse, was found to be negative for the infection on autopsy.

Two adult laboratory bred tree–shrews (Tupaia glis) were fed with 9 and 10 G. hispidum 24 day old advanced third–stage larvae obtained from a catfish infected with fully developed larvae in cyclops. Autopsy 11 days after the experiment was negative for the infection.

It is clear that the advance third–stage larvae when obtained from infected mice could infect snake–headed fish and later removed from the fish could be transmitted to a second fish. On the other hand third–stage larvae from fish did not become well established when fed to a mouse or a tree shrew. The problem is to be further studied. The study on the life cycle of G. doloresi.

This is an experimental study for determining the second intermediate host by feeding vertebrates with G. doloresi fully developed larvae in cyclops of which the results are as follows:

31 laboratory bred adult white mice (Mus musculus) which had been fed a total of 1407 G. doloresi fully developed larvae 10–12 days old in cyclops were autopsied 12–225 days after feeding. 12 mice (39%) yielded 23 encysted G. doloresi third–stage larvae from the musculature. When considered with the 6 laboratory infections obtained in mice previously (1969 Annual Report) a total of 18 (40%) of 45 white mice have been experimentally infected with a total of 34 larvae.

One monitor lizard (Varanus nebulosus) was fed with 90 G. doloresi fully developed 10 day old larvae in cyclops. On autopsy 32 days later it was negative for the infection.

This study is continuing.

SUMMARY: 1289 stomachs from stray dogs killed at the Bangkok and Thonburi Municipality Rabies Control Unit were examined during April 1969. All were negative. Thirty seven poisonous snakes obtained from the Thai National Red Cross Snake Farm were also found to be negative for gnathostome larvae. Two (9.0%) of 22 fresh water snake–headed fish bought at Ayuthaya and Phetburi markets were found positive with 1 and 2 encysted G. spinigerum larvae. Many fresh water fish, frogs, and toad tadpoles obtained from Bangkok and nearby areas were examined for the presence of natural infection with gnathostome larvae; only 1 young snake–headed fish was found positive with 1 non–encapsulated G. spinigerum larva.

On frequent examinations of 28 domestic cat stools (#97 to #124 obtained for experimental purposes during this period from Rajburi Province and nearby areas, 2 (7.2%) were found positive with G. spinigerum ova. Of the 28 cats, 11 which died in captivity, showed autopsies only 1 immature adult male G. spinigerum in a small gastric tumor. Of 659 gnathostome adults recovered from pig stomachs at the Bangkok Slaughter House, 488 were identified as G. doloresi and 171 as G. hispidum. Forty pig stool samples collected at Rajburi Province were found negative for gnathostome ova.

Experimental infection with G. spinigerum larvae for determination of additional second and paratenic hosts during this period showed for the first time 3 top minnow fish and 4 toad tadpoles acting as second intermediate host of the worm. Fifteen fresh water crabs were found negative after being fed with the advanced third-stage larvae obtained from white mice.

The study on the life cycle of G. hispidum has proven for the first time the development of advanced third-stage larvae in snake-headed fish, catfish, small fighting fish, frogs and a tree shrew after being fed with many fully developed larvae in cyclops. However, the advanced third-stage larvae removed from infected fish could not be recovered after being fed to 2 white mice and 2 tree shrews for determining further infectivity of the larvae for mammals. Two snake-headed fish were found to be infected with advanced third-stage larvae first removed from white mice and from the fish after being first infected with the larvae from a white mouse.

Additional study on the life cycle of G. doloresi in laboratory bred white mice fed with many fully developed larvae of the worm in cyclops showed 12 (39.0%) of 31 experimental mice positive with 23 (1.6%) encysted advanced third-stage larvae of the worm.

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