

Title: Gnathostomiasis in Thailand.

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OBJECTIVES

The primary objectives of this study are to determine the prevalence of Gnathostoma spinigerum in man and animals, to carry out clinical and epidemiologic studies, and to investigate the pathology, diagnostic methodology, treatment and prophylaxis of gnathostomiasis in Thailand. Additional objectives are to further delineate the life cycle and mode of transmission of the parasite and to identify intermediate hosts, as well as to compare G. spinigerum with G. hispidum, G. doloresi and other gnathostomes of animals which are also present in Thailand that might be transmitted to man.

DESCRIPTION

The gastro-intestinal tracts of dogs killed at the Bangkok-Thonburi Municipality Rabies Control Unit were regularly examined for the seasonal prevalence of the gnathostome during the report period. Periodically, examinations were made of stomachs from pigs obtained from the Bangkok Slaughter House and once from the slaughter houses in the provinces of Chiangmai, Supanburi, and Rajburi for infection with pig gnathostomes and G. spinigerum. As opportunity permitted, stool examinations for gnathostome ova and examination of gastro-intestinal tracts were carried out on various species of animals, including domestic cats brought to the SMRL animal house for experimental purposes as well as on many wild-caught carnivores at the Bangkok Zoo.

Each month, about 2 kg. of fresh snake-headed fish were purchased in the markets of Ayuthaya and Pheiburi, endemic areas for human gnathostomiasis. These fish were examined for the presence of advanced third-stage larvae of G. spinigerum. The data was used for estimation of monthly or seasonal prevalence rates in these areas. Additionally, examinations of many young snake-headed fish collected in the Bangkok area from public fresh-water ponds and ditches during the breeding season were made for a determination of the earliest age at which natural infection by advanced third-stage larvae can occur. Poisonous snakes from the Thai National Red Cross Snake Farm which died spontaneously were regularly examined for G. spinigerum and other gnathostome larvae.

Occasionally some vertebrates obtained from the Bangkok area and a few provincial villages were examined for the larvae. Skin penetration in definitive hosts (cats and dogs) by G. spinigerum advanced third-stage larvae and their development in these hosts were investigated. Moreover, penetration of rodent skin by fully developed larvae of G. spinigerum from cyclops was studied. Studies of skin sensitivity, peripheral blood cells changes, and biochemical changes in the blood after oral infection with G. spinigerum advanced third-stage larvae are continuing on monkey #19.

An investigation on the chemotherapy of G. spinigerum infection in cats by parenteral Ancylool Disophenol (American Cyanamid) was initiated.

A comparative study on the size and morphological characters for the identification of G. spinigerum, G. hispidum, and G. doloresi began in the previous year was continued during this period on additional numbers of the adults and larvae.

Experimental infection was continued from the previous year to determine potential second intermediate or paratenic hosts of G. hispidum and second intermediate host of G. doloresi. Moreover, an experimental study was initiated by feeding known numbers of fully developed larvae of G. hispidum and G. doloresi in cyclops to definitive host animals (domestic pigs) to determine if direct development of the larvae into adult worms without the need for the second intermediate host could occur.

A study was continued from the previous year on G. vietnamicum obtained from infected river otters (Aonyx cinerea Illiger) obtained from Songkhla and Nakornsrihammarat areas for the determination of the incidence and localization of the worm in the animal as well as the gross pathological changes of the infected organs. Moreover, the significant morphological characters, including size of the adult, for its differentiation from other gnathostomes as well as the life cycle were investigated.

PROGRESS

Investigations of gnathostomiasis during this year have concentrated primarily on infections in animals.

NATURAL INFECTIONS

Table I summarizes the seasonal distributions of findings upon examination of dog stomachs from the Bangkok-Thonburi Municipality Rabies Control Unit, snake-headed fish purchased at fish markets in Ayuthaya and Phetburi, and snakes from the Thai National Red Cross snake farm.

12 (0.3%) of 4731 dog stomachs were positive for adult G. spingerum as compared with 24 (0.8%) of 5,372 stomachs positive last year (1967-1968). Advanced 3rd stage larvae of G. spinigerum were found in 13.4% of snake-headed fish from Ayuthaya and Phetburi as compared with a prevalence of 23.8% from the same places for the year 1967-1968. One snake-headed fish weighing about 600 grams contained 153 encysted advanced third-stage larvae in the liver and muscles. This is at present considered to be the heaviest infection ever recorded in one fish in this country. Of an additional 949 young snake-headed fish collected in October and November weighing not more than 5 grams each and measuring about 1.5 cm—6.0 cm × 0.2 cm—0.8 cm from a small fresh-water pond near the animal house of the Faculty of Tropical Medicine, Rajavithi Road and from a roadside fresh-water ditch about 15.0 kilometers north of SMRL, 11 (1.2%) were found to be infected as compared with 127 (25.7%) of 495 young snake-headed fish collected from roadside fresh-water ditches in Bangkok during 1967-1968. Each young infected fish contained from 1-3 encysted advanced third-stage larvae in the liver and muscles. Among poisonous snakes listed on Table I encysted advanced third-stage larvae of G. spinigerum were found in 11 (3.2%) of 345 as compared with 6 snakes (1.5%) found infected last year.

The findings, both as to seasonal distributions and species of the definitive and intermediate or paratenic hosts infected with the worm were consistent in 1967-1968 and in this reporting year but generally the percentage of infection for last year was found to be much higher than this year (Fig. 1,2,).

Additional vertebrates in which the third-stage larvae of G. spinigerum were found includes Rana rugulosa (frog) from the Bangkok area with 3 of 85 specimens positive. One of 5 frogs from Supanburi was also positive. A Viverricula indica (civet cat) from Nakhornayok was infected with 3 encysted advanced third-stage larvae and 1 monitor lizard (Varanus nebulosus) from Choburi province had 4 encysted larvae in its flesh. Three of 52 snake-headed fish examined in Supanburi and Rajburi were positive also.

Table 1. Result of study for 12 months on the incidence of infection of Bangkok Domestic dogs with adult G. spinigerum and infection of second intermediate or paratenic (snake-headed fish and poisonous snakes) with the advanced third-stage larvae in few endemic areas nearby Bangkok (Ayuthaya—Phetburi).

	Total	Apr68	Ma	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan69	Feb	Mar
Dog's stomach	12/4731	0/356	0/355	0/249	3/424	4/331	1/258	0/641	2/311	0/75	0/349	2/835	0/574
(Infection with adult <u>G. spinigerum</u>) % positive	0.3	0	0	0	0.7	1.2	0.4	0	0.6	0	0	0.2	0
<u>Ophicephalus striatus</u>													
(Snake-headed fish) large*	7/22	1/2	1/2	1/2	1/4	0	0/1	0	1/1	1/2	0/5	0	1/3
(No. pos./No. exam) small**	16/150	5/8	7/13	0/19	1/10	0/5	0/21	0	2/33	1/13	0/10	0	0/18
Total (Ayuthaya—Phetburi)	23/172	6/10	8/15	1/21	2/14	0/5	0/22	—	3/34	2/15	0/15	0	1/21
Infection with advanced third-stage larvae	13.4	60.0	53.3	4.8	14.3	0	0	0	8.8	13.3	0	0	4.8
<u>Poisonous snakes</u>													
(No. pos./No. exam.)	11/345	1/17	0/5	1/21	0/20	0/6	0/13	1/96	7/60	0/15	1/43	0/29	0/20
(Naja naja & hannah, Viperarusselli, Bungarus fasciatus) Infection with advanced third-stage larvae													
% positive	3.2	5.9	0	4.8	0	0	0	1.0	11.7	0	2.3	0	0

* Large Ophicephalus striatus = 500 grams and over

** Small Ophicephalus striatus = Less than 500 grams

Figure 1. Monthly distribution of infection with adult *G. spinigerum* in stomachs of Bangkok-Thonburi stray dogs killed at the Bangkok-Thonburi Municipality Rabies Control Unit

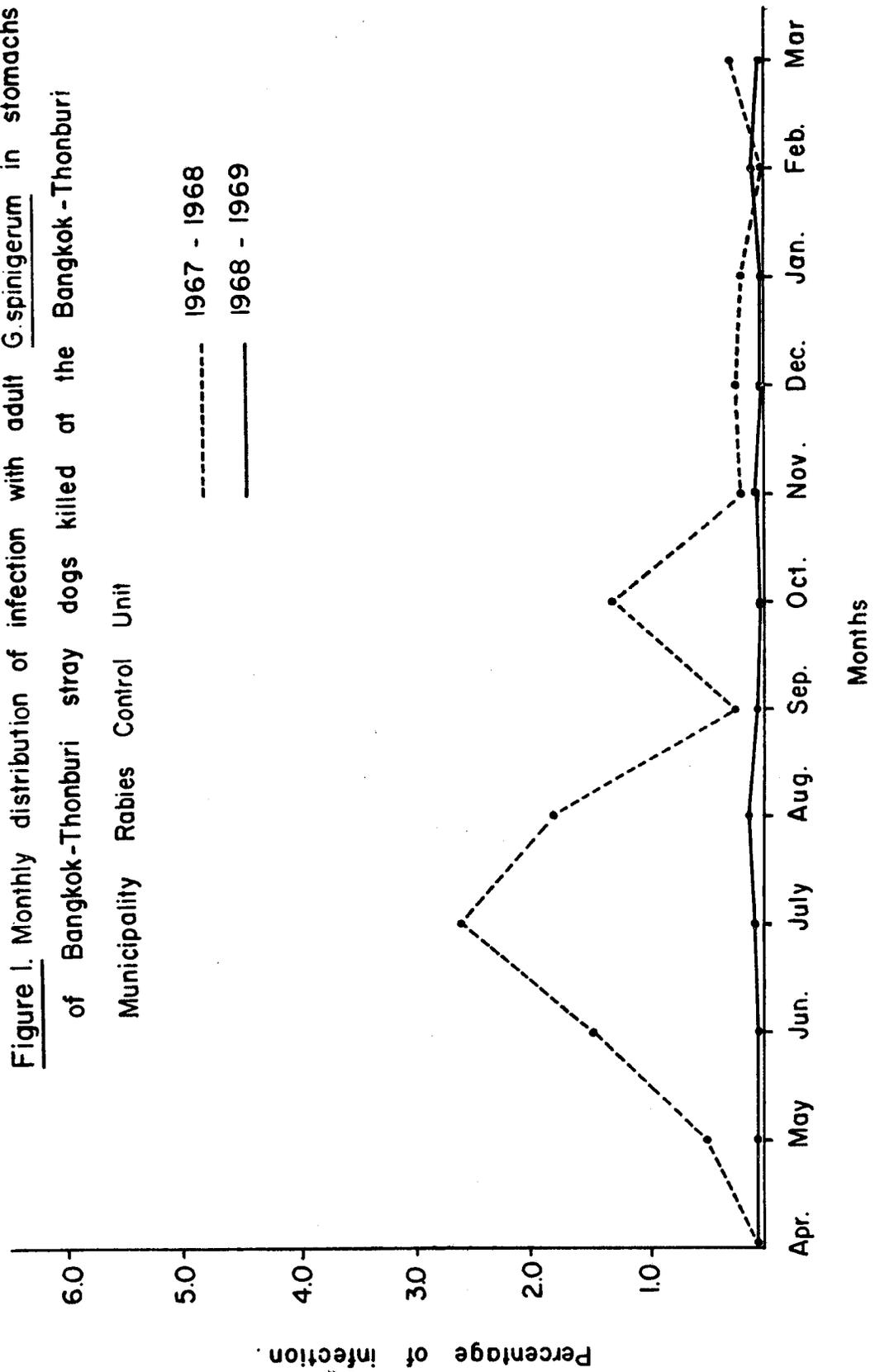


Figure 2. Monthly distribution of infection with G. spinigerum advanced third-stage larvae in snake-headed fish obtained from Phetburi and Ayuthaya areas

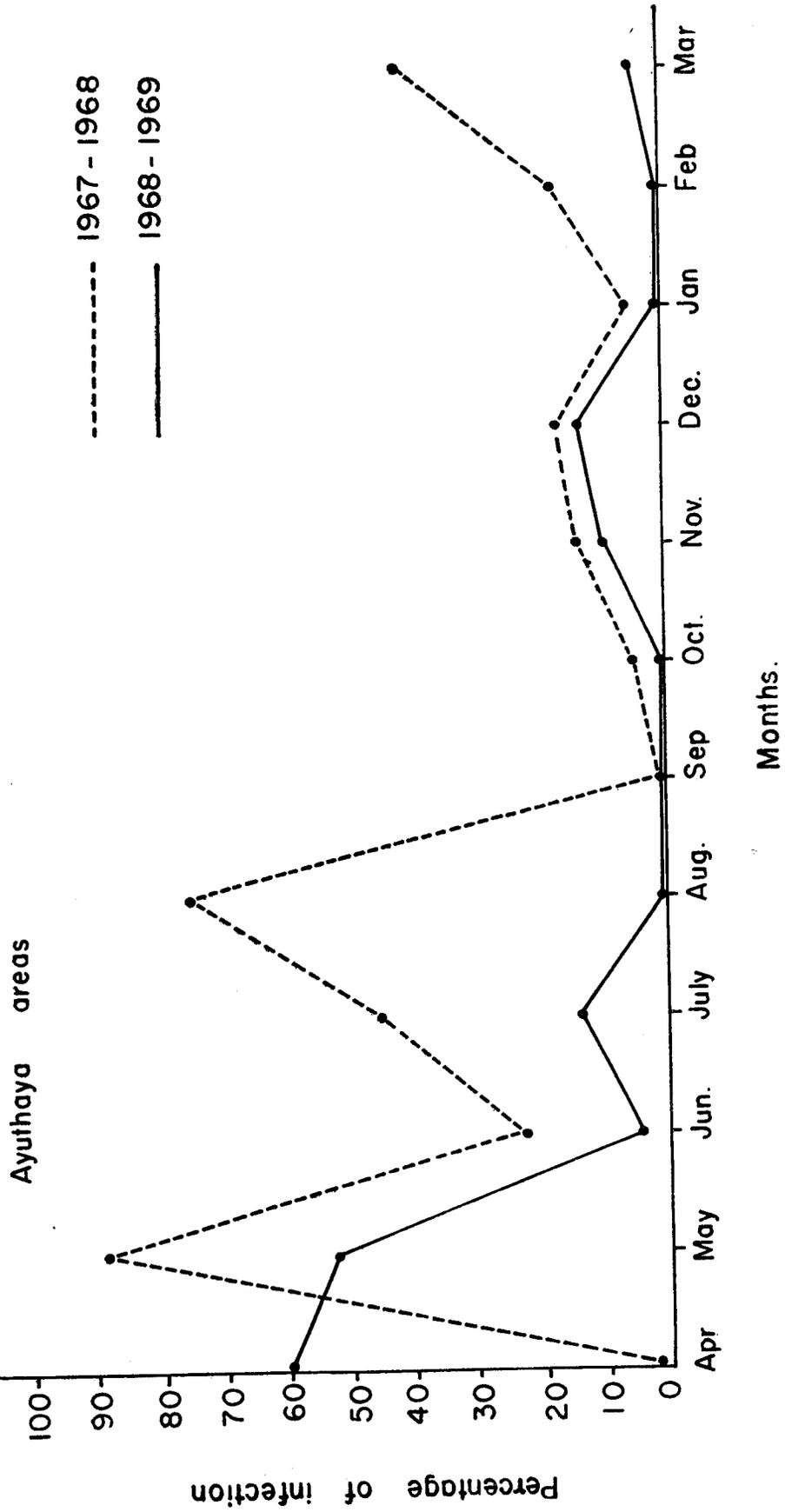


TABLE 2 summarizes species of vertebrates investigated which have not been found to harbour the 3rd stage larvae.

TABLE 2. Animals found to be negative for G. spinigerum third—stage larvae.

<u>Class</u>	<u>Species</u>	<u>Number Examined</u>
Pisces	<u>Clarias batrachus</u>	99
	<u>Monopterus albus</u>	2
	<u>Puntius gonionotus</u>	21
Amphibia	<u>Bufo melanostictus</u>	4
Reptilia	<u>Xenopeltis unicolor</u>	1
	<u>Cylindrophis rufus</u>	2
Aves	<u>Lonchura punctulata</u>	5
	<u>Rhipidura javanica</u>	1
	<u>Pycnonotus goiavier</u>	12
Mammalia	<u>Rattus norvegicus</u>	3
	<u>Rattus rattus</u>	16
	<u>Rattus exulans</u>	12
	<u>Tupaia glis</u>	2
	<u>Callosclurus erythraeus</u>	1

An experimental study made on one domestic cat (# 68) has shown 20 adult G. spinigerum in one gastric tumor of the animal, therefore clearly confirming the previous study reported in the Annual Progress Report for 1967—1968 that the wild—caught specimen of a golden cat (Profilis temmincki) was found for the first time in this country acting as an additional natural definitive host of G. spinigerum.

Stool examinations of 9 domestic cats from Bangkok were negative but one of them which died of intestinal obstruction showed on autopsy an adult male in the stomach cavity and 3 adult males (G. Spinigerum) in one small gastric tumor. This case indicated that a stool negative for the ova does not exclude the presence of infection.

On 24 species of wild—caught carnivores kept at the Bangkok Zoo studied by stool examinations, one Felis chaus (jungle cat) and one Felis bengalensis (leopard cat) were found positive for G. spinigerum ova. Subsequently one dead adult male G. spinigerum was discovered passing out with stool of a jungle cat. Therefore the jungle cat is reported for the first time to be naturally infected with the adult worm.

Examination of the stomachs of a civet otter (Cinogale dennetti) and a leopard cat (Felis bengalensis) obtained from Bangkok Zoo revealed no parasites.

A total of 1773 adult gnathostomes were recovered from infected stomachs of pigs from the Bangkok slaughter house of which none were G. spinigerum, 425 (182 males, 243 females) were identified as G. hispidum and 1348 (414 males, 934 females) as G. doloresi.

Of 422 stomachs examined in Chiangmai slaughter house 1 was found positive with 1 immature G. doloresi and of 415 stomachs examined in Rajaburi 8 were found to harbour 7 adult G. hispidum (5 males and 2 females) and 15 adult G. doloresi (5 males, 10 females) but none of 107 stomachs examined in Supanburi were positive for gnathostomes. Examinations of stools obtained from 544 domestic pigs in provinces of Supanburi and Rajburi showed no gnathostome ova.

To determine the incidence of infection with adult G. hispidum and G. doloresi among domestic pigs imported from many provinces of the country to be slaughtered at the Bangkok slaughter-house, examinations at random of 5841 stomachs were undertaken with the full co-operation and assistance of the slaughter-house authorities for which we are thankful. The result showed that 82 (1.4%) were found to be infected with a total of 71 adult G. hispidum (37 males, 34 females) in 31 stomachs (0.5%) and with a total of 246 adult G. doloresi (105 males and 141 females) in 51 stomachs (0.9%). The average numbers of adult worms per infected stomach were 2.3 for G. hispidum and 4.8 for G. doloresi. The variation in the number of worms per stomach was 1-12 and 1-34 for G. hispidum and G. doloresi respectively. Of all infected stomachs none showed double infection with both species.

An investigation on skin penetration of G. spinigerum advanced third-stage larvae in cats and dogs.

It was reported in the Annual Report for 1967-1968 that skin penetration of G. spinigerum advanced third-stage larvae was possible in white mice and white rats and the infections were proved to have become established. However, no experimental study has been as yet done on the definite hosts, (cats and dogs) to ascertain to what extent the development of the larvae into adult stages can occur.

To study this problem, the shaved intact skin of 10 negative cats and 8 negative dogs were exposed. The back of 2 cats (#46, #71) and the abdominal wall of the others were exposed to known numbers of G. spinigerum advanced third-stage larvae removed in most cases from infected white mice and in a few cases from naturally infected fish, snakes etc. The larvae were placed on the skin in drops of fresh water to maintain them in a moist state. The results obtained in each cat are as follows:

Cat # 38 was given 3 skin exposures at 3 and 5 day intervals with a total of 53 (62%) from 86 larvae in the experiment completing penetration (the third skin penetration accomplished by all 16 larvae, 100%). Weekly examinations of the stool were always negative up to the date of sacrifice which was 158 days after the first skin penetration or 149 days after the last (about 5 months). On necropsy we found a total of 27 G. spinigerum immature adults and larvae (infectivity rate of 51.0%) including 12 immature males and females (1 male and 1 female in a gastric tumor of about 2.0 cm. in diameter, 1 male and 8 females in the flesh of anterior abdominal wall and 1 immature worm, sex unidentified, in the diaphragm) and 15 advanced third-stage larvae (7 in the flesh of anterior abdominal wall, 3 in the omentum nearby the stomach, 2 and 3 in the flesh of the anterior chest wall and the back respectively). The range in size of the immature males and females was 6.2-10.0 mm. \times 0.7-0.9 mm. and the larvae 2.2-6.5 mm. \times 0.4-0.5 mm.

Cat # 46 was exposed 6 times at one day intervals with a total of 43 (83%) of 52 larvae in the experiment completing the penetration. Weekly examinations of the stools revealed the first preparation positive for G. spinigerum ova about 71 days after the first skin penetration or 59 days (about 2 months) after the last. Two months later the animal was sacrificed to test the one day result of chemotherapy with parenteral Ancylool Disophenol, and on necropsy a total of 22 G. spinigerum adults and larvae (infectivity rate of 51.0%) were found. Fourteen adult males and females (5 males were alive) were located in one gastric tumor of about 2.0 cm. \times 3.0 cm. and in the lumen of the small intestine. In addition, 8 living advanced third-stage larvae were found in the liver, diaphragm and abdominal flesh.

Cat # 71 was skin infected 2 times at 3 day interval with all 41 larvae (100%) in the experiment completing the penetration. Weekly stool examinations first became positive for ova of the worm about 63 days after the first skin penetration or 60 days after the last (about 2 months). This cat harbored a total of 12 G. spinigerum adults and larvae (infectivity rate of 29%), including the necropsy findings of 1 adult female in the greater omentum and 5 living larvae in the liver, muscle of the right hind leg and the diaphragm. A small gastric tumor of about 2.0 cm. \times 2.5 cm. containing no worms was noted at the greater curvature of the stomach. Six males and females had already been passed in the stools within 3 days of treatment with Ancylool Disophenol.

Cat # 73 was given 2 skin infections on 2 successive days with a total of 42 (93%) of 45 larvae on the experiment completing the penetration. Weekly stool examinations first became positive for G. spinigerum ova 176 days (about 6 months) after the first skin penetration.

Cat # 74 was given one skin infection with a total of 46 (90%) of 52 larvae in the experiment completing the penetration. Weekly stool examinations are negative up to 31 March (195 days after initiation of the experiment).

Cat # 77 was also given one skin infection with a total of 85 larvae (100%) completing the penetration within a period of only one hour. Weekly stool examinations up to 31 March (153 days after exposure) are still negative.

Cat # 83 was given one skin infection with a total of 61 larvae (100%) completing the penetration within 45 minutes. Weekly stool examinations up to 31 March 1969 (152 days) are still negative.

Cat # 84 was given 2 skin infections at 3 days intervals with a total of 44 (67.0%) of 66 larvae in the experiment completing the penetration (the second exposure, using 19 larvae was completed with 100% penetration within a period of 30 minutes). Up to 31 March, or 34 days after the first skin penetration, weekly stool examinations are still negative.

Cat # 87 and Cat # 89 were each given one skin penetration, with all 18 larvae (100%) and 45 (75%) of 60 larvae in the experiment completing the penetration respectively. Weekly stool examinations of both cats are negative up to 31 March (21 days after exposure).

With regard to skin penetration by the advance third-stage larvae in 8 experimental dogs, the following details are reported.

Dog # 2 was given 2 skin exposures at one week interval with a total of 76 larvae (100.0%) completing the experiment. Weekly stool examinations showed still negative up to 31 March (195 days after the first experiment).

Dog # 9 was given 2 skin infections at 10 days interval with a total of 192 (81.0%) from 236 larvae under the experiment completing the penetration. Weekly stool examinations showed still negative up to 31 March (158 days after the first experiment).

Dog # 10 was given 2 skin infections at 2 week intervals with a total of 119 (72%) of 166 experimental larvae completing the penetration. Weekly stool examinations up to 31 March are still negative (164 days after the first experiment and 138 days after the last).

Dog # 11 died about 15 hours after being infected by skin penetrations, with 33 (97%) of 34 larvae completing the penetration. Necropsy showed a total of 28 undeveloped larvae (infectivity rate of 85%) of which 1 larvae was still located in the skin and 27 were found in the muscle near the area penetrated.

Dog # 13 was given 3 skin infections a few days apart, with a total of 64 (39%) of 166 larvae in the experiment completing the penetration. Weekly stool examinations first became positive for G. spinigerum ova 112 days after the first exposure or 93 days after the last (about 3-4 months).

Dog # 14 was given 2 skin infections a few days apart, with a total of 68 (46%) of 147 larvae in the experiment completing the penetration. Weekly stool examinations first became positive for G. spinigerum ova 96 days after the first exposure and 84 days after the last (about 3 months).

Dog # 1 and # 12 were each given 1 skin infection with successful penetrations through the skin of 65 (79.3%) of 82 larvae and 88 (96.7%) of 91 larvae in the experiment respectively, on 15 and 16 October 1968. Weekly stool examinations are still negative up to 31 March (166 days for dog #1 and 165 days for dog #12 after exposure).

Skin reaction was always produced by penetration of the larva in both cats and dogs. Immediately around the penetrated area, a small wheal with an irregular margin of about 0.4 cm.—0.6 cm. in diameter developed and persisted for about one hour after finishing the 2-hour experiment. Hyperemia around the wheal occurred in some cases but not in others.

The total penetration time (TT.), which is the time required by the larva for completion of penetration after the moment of exposure, and the actual penetrating time (PT.) which is recorded from the first moment each larva begins penetrating until it completely passes through the skin were determined. Seventy-six larvae, mostly obtained from experimentally infected white mice and 4 larvae from a naturally infected snake and a snakeheaded fish were employed. Observations were made on the shaved intact skin of the back of 2 uninfected cats (#46 and #71) of which the detailed result was already reported in the Quarterly study Report covering the period 1 April 1968—30 June 1968. Briefly, the TT. for 50% of the larvae in the group was 16—50 minutes whereas the PT. for 90.8% of the group was 6—50 minutes.

This study makes it clear that infection of the definitive hosts (cats and dogs) by the worm can take place by skin penetration and that adult worms can develop (after 2 to 6 months in three cats and 3 to 4 months in 2 dogs).

However, it remains to be proved whether or not the fully developed larvae (early third-stage larvae) from cyclops can penetrate the skin and produce infection in vertebrates. In this connection, a study was initiated to investigate infection through the skin by such larvae. Seven and 5 fully developed larvae (10—12 days old) were placed on the ears of two white mice immediately after removal from experimentally infected cyclops. Observations were made under low magnification (10×10) for two hours during which the larvae were kept moist by repeated additions of water. Although the larvae remained active throughout the observation period, no penetration was observed.

The study on skin sensitivity, peripheral blood cell changes and biochemical blood changes in infected monkey #19 (*Macaca irus*).

During this year only infected monkey #19 was continually studied. The animal was orally infected with 17 advanced third-stage larvae on 1 March 1968. The following results have been obtained:

The skin test first became positive after 99 days and continued to be positive up to 120 days. Thereafter there was a negative period of about 50 days, a brief period of positivity (about one-week) and then continued negativity up to the present. Thus, from this animal, if the 50-day negative period is disregarded, it appears that the skin test becomes positive at about 3 months after the infection, remains positive for about 3 months and then becomes negative. This finding is consistent with the findings in infected monkeys reported in the 1966—1967 Annual Progress Report.

No significant peripheral blood cell changes were observed during the reporting period.

The preliminary study on biochemical changes of the blood kindly performed by CPT Peter K. Iber, Chief of Laboratory Services, SMRL showed a slight increase in the total blood protein beginning in the first week of the experiment (8.2 gm% as opposed to an average of 7.8 gm% blood protein observed before the animal was fed with the larvae). The average has now increased to about 8.4 gm%. Also total blood globulin tested during the same period revealed a variation of 3.4 to 5.7 gm% averaging about 4.3 gm% as compared with a range of 3.6—3.8 gm% before the experiment. It thus appears, that the total blood globulin is also increased by the infection.

The study is still in progress.

A preliminary study of the chemotherapy of *Gnathostoma spinigerum* infections in cats with Ancylos Disphenol (2,6-Diiodo-4-nitrophenol).

The chemical, Ancylos Disphenol parenteral 4.5% is prepared by the American Cyanamid Company for the treatment of hookworm infection in dogs and cats. Drug was supplied by the SMRL Veterinary Department and was given subcutaneously according to the directions given by the Company to 7 cats (#45, #46, #69, #71, #90, #57, and #80) infected with various numbers of adult *G. spinigerum*, the results are as follows:

Two infected cats (# 45 and # 46) showing in their stools about 183,600 eggs and 63,600 eggs of G. spinigerum per day respectively were sacrificed 1 day after treatment. One of them (# 45) was found to harbour a total of 11 dead adult G. spinigerum of which 10 worms (4 males and 6 females) were still located in a gastric tumor measuring 2.0 cm. × 3.0 cm. in size, and 1 dead female was in the lumen of the upper part of the small intestine. The second cat had in total 14 dead and living adult worms of which 9 (3 dead males, 3 living males and 3 dead females) were still in a gastric tumor measuring 2.0 cm. × 3.0 cm. and 5 worms (2 living males, 2 dead males and 1 dead female) were found in the lumen of the upper part of the small intestine. The latter cat had also 8 living advanced third—stage larvae in other organs (1 in liver, 2 in abdominal flesh and 5 in the diaphragm). It is obvious that the drug successfully acted on almost all the adult worms in 1 day after treatment.

The other 5 treated cats containing in their stools about 66,990 to 1,823,500 eggs of G. spinigerum per day were sacrificed 4 to 10 days after drug administration. Examination revealed a 1.0—2.0 cm. × 1.0—2.5 cm. gastric tumor produced by the worms in each cat, but none of the tumors contained gnathostome larva or adult worms. In total there were 19 dead adult G. spinigerum (6 males and 13 females) obtained from these animals of which 15 (4 males and 11 females) were passed out in the daily stools during the first 3 days. Seven of these worms (2 males and 5 females) were passed on day 1 after treatment, 5 worms (2 males and 3 females) on day 2 and 3 female worms on day 3. In addition to 1 dead female worm passed by cat #69, 2 more dead worms (1 male, 1 female) were eliminated by vomiting on day 4, the day of sacrifice. From another cat (#71), also sacrificed 4 days after treatment, 1 dead adult female worm was found in the greater omentum and 5 living advanced third—stage larvae were discovered in the liver, skeletal muscle and diaphragm. An additional cat (#90) that had already passed 2 dead females on day 1 was sacrificed 6 days after the treatment and showed on autopsy 1 dead adult male in its stomach cavity. Two cats (#57 and #80) that previously passed 6 dead adult worms on day 1—3 were sacrificed 10 days after the treatment and showed no gnathostome worms in their gastrointestinal tracts.

These results showed clearly that the drug successfully killed all the adult worms locating in the gastric tumors within about 3 days its parenteral administration to the infected animals. However, all the larvae found in liver, diaphragm and skeletal muscle seemed to resist the action of the drug during the same period. Close observation of the seven treated cats from the beginning of treatment up to 10 days showed no toxic or other ill effects resulting from the action of the drug.

Further investigation on the problem is in progress.

Comparative study on the size and morphology of G. spinigerum, G. hispidum and G. doloresi.

The study designed to delineate additional characters for the specific identification of G. spinigerum, G. hispidum and G. doloresi has already been mentioned in the last Annual Progress Report. Additional numbers of the adults and the larvae of these parasites have now been studied. The characteristics investigated include the variation in size of adult worms, the number of cephalic hooklets in each row, the number of rows formed by the hooklets, as well as the number of cephalic hooklets in each row on the head bulb of the advanced third—stage larva.

Size: All adult G. spinigerum, G. hispidum and G. doloresi collected from stomachs of naturally infected animals were measured after being fixed in warm 70% ethanol. The results of such measurements of both sexes during this period briefly showed as follows:

G. spinigerum:

Head and body of 34 males showed an average length and width of 24.3 mm x 1.9 mm.

Head of 35 males showed an average of 0.4 mm in length and 0.7 mm. in width and of 26 females 0.5 mm. in length and 0.9 mm. in width.

Table 3. Measurements of mature adult Gnathostoma spinigerum, G. hispidum, and G. doloresi obtained from stomach walls of naturally infected domestic dogs and pigs.

Measurements		<u>G. spinigerum</u> in mm.	<u>G. hispidum</u> in mm.	<u>G. doloresi</u> in mm.	
<u>Head</u> <u>and</u> <u>body</u>	Males:	Total	34	86	120
	width	range	1.0—2.0	1.0—3.0	1.0—3.0
		average	1.9	1.7	2.1
	length	range	16.0—35.0	9.0—36.0	9.0—37.0
		average	24.3	17.3	18.5
	Females:	Total	—	19	—
width	range	—	2.0—3.0	—	
	average	—	2.5	—	
length	range	—	15.0—36.0	—	
	average	—	26.2	—	
<u>Head</u>	Males:	Total	35	109	114
	width	range	0.28—0.93	0.55—0.93	0.20—0.99
		average	0.71	0.72	0.77
	length	range	0.16—0.57	0.23—0.68	0.10—0.79
		average	0.40	0.41	0.31
	Females:	Total	26	47	52
width	range	0.69—1.09	0.46—0.99	0.30—1.33	
	average	0.94	0.83	0.93	
length	range	0.40—0.73	0.28—0.63	0.24—0.83	
	average	0.52	0.48	0.46	

G. hispidum:

Head and body of 86 males showed an average length and width of 17.0 mm. × 1.7 mm. and of 19 females showed an average length and width of 26.2 mm. × 2.5 mm.

Head of 109 males showed an average of 0.4 mm. in length and 0.7 mm. in width and of 47 females 0.5 mm. in length and 0.8 mm. in width.

G. doloresi:

Head and body of 129 males showed an average length and width of 18.1 mm. × 2.1 mm.

Head of 114 males showed an average of 0.35 mm. in length and 0.8 mm. in width, and of 52 females an average of 0.46 in length and 0.93 mm. in width. (Table 3).

The results of study on the size of adult of these three species of *Gnathostoma* during last year and this reporting year can be reasonably concluded as follows:

G. spinigerum:

Head and body 73 males showed an average of 26.2 mm. × 1.8 mm. 87 females had an average of 34.7 mm. × 2.3 mm.

Head 55 males showed an average of 0.40 mm. in length and 0.65 mm. in width. 46 females an average of 0.56 mm. in length and 0.97 mm. in width.

The averages of head and body, and of the head alone of female *G. spinigerum* are longer and larger than those of the male.

G. hispidum:

Head and body: 104 males showed an average of 18.9 mm. × 1.7 mm. 104 females an average of 26.1 mm. × 2.2 mm.

Head 126 males showed an average of 0.44 mm. in length and 0.71 mm. in width. 126 females an average of 0.49 mm. in length and 0.82 mm. in width. The average of head and body and the head only of females are longer and larger than those of the male.

G. doloresi:

Head and body: 189 males showed an average of 19.8 mm. × 2.1 mm. 189 females average of 34.0 mm. × 2.7 mm.

Head 123 males showed an average of 0.37 mm. in length and 0.78 mm. in width. 100 females an average of 0.48 mm. in length and 0.96 mm. in width.

The average of head and body and head only of females are longer and larger than those of males.

Head: Adult cephalic hooklets. 45 adult male and female *G. spinigerum* have 7 to 9 cephalic hooklet rows (2 with 7 rows, 36 with 8 rows, 7 with 9 rows) and all rows are parallel. The number of cephalic hooklets generally increase from row 1 to row 8. Most of heads studied were provided with a range of 40–80 hooklets in rows 2, 3, 4, and 8 but a range of 60–100 hooklets was shown in rows 5, 6 and 7. The minimum number of hooklets was 3 and the maximum was 66 in row 1 each shown in one worm. The maximum number of hooklets was 131 in row 8 discovered also in one worm (Table 4).

Seventy-four adult male and female *G. hispidum* have 9 to 12 rows of hooklets (3 had 9 rows, 20 had 10 rows, 30 had 11 rows and 21 had 12 rows) of which most are parallel but a few diverging here and there. Also, it was found that the first and last rows of hooklets are smaller than the others and showed characters similar to those described in the last Annual Progress Report. The numbers of hooklets increase from 5 in row 1 to 192 in row 12. Most of 74 heads studied were provided with a range of 60–120 hooklets in rows 2, 3 and 4, a range of 80–120 hooklets in rows 5, 6, 7, 8, and 9, 80–140 in row 10, row 11 had 100–140 and row 12 was reduced to 40–60 hooklets (Table 5).

TABLE 4 Cephalic bulbs of 45 adult male and female *G. spinigerum* removed from stomachs of naturally infected dogs; numbers of cephalic hooklets by row.

Row No. Nos. cephalic hooklets in groups	Nos. of adult <i>G. spinigerum</i>								
	1	2	3	4	5	6	7	8	9
1-19	5	0	0	0	0	0	0	1	2
20-39	19	5	0	0	2	0	2	5	1
40-59	19	28	18	11	7	9	7	13	2
60-79	2	11	24	22	18	14	21	18	2
80-99	0	1	2	8	16	19	13	5	0
100-119	0	0	1	2	1	3	1	0	0
120-139	0	0	0	0	1	0	1	1	0

TABLE 5 Cephalic bulbs of 74 adult male and female G. hispidum removed from stomachs of naturally infected pigs; numbers of cephalic hooklets by row.

Row No. Nos. cephalic hooklet in groups.	Nos. of adult <u>G. hispidum</u>											
	1	2	3	4	5	6	7	8	9	10	11	12
1-19	10	0	0	3	0	2	0	0	1	0	0	1
20-39	18	2	1	1	0	0	0	0	1	0	0	1
40-59	29	9	5	4	2	2	1	0	1	2	3	6
60-79	8	30	14	17	6	3	0	1	2	6	6	1
80-99	8	25	34	28	33	26	19	17	20	16	5	3
100-119	1	5	17	16	27	30	44	40	31	21	14	1
120-139	0	2	3	4	4	9	8	14	13	18	13	5
140-199	0	1	0	1	2	2	2	2	5	8	10	3

The result of additional study to determine the size of the advanced third-stage larvae and the number of cephalic hooklets in each row on the head bulb of the larvae of three species of Gnathostoma during this period is as follows:

Size (larvae)

The average sizes of 2 G. spinigerum, 52 G. hispidum and 6 G. doloresi advanced third-stage larvae were 3.57 mm. × 0.34 mm., 2.03 mm. × 0.27 mm. and 2.70 mm. × 0.36 mm. respectively.

In summary for two-year study on the average size of the larvae showed as follows:—

69 G. spinigerum larvae have the average size of 3.8 mm. × 0.4 mm.

112 G. hispidum larvae have the average size of 2.1 mm. × 0.3 mm.

15 G. doloresi larvae have the average size of 2.7 mm. × 0.3 mm.

Cephalic hooklets (larvae):

Nineteen, 82 and 8 advanced third-stage larvae of G. spinigerum, G. hispidum and G. doloresi respectively were removed from experimentally infected mice for the present study. Each G. spinigerum larva had 40 or more cephalic hooklets in each row except that row I of 7 larvae, row II of 3 larvae, and row III and IV of 1 larva had less than 40 hooklets. There is one larva showing 36 to 38 cephalic hooklets in each of 4 cephalic hooklets rows. Each G. hispidum larva had less than 40 cephalic hooklet in every row except that row I of 1 larva, row II of 3 larvae, row III of 12 larvae and row IV of 24 larvae had 40 to 51 hooklets. Of 8 G. doloresi studied 5 had more hooklets formed on row I than row IV. Each larva had less than 40 cephalic hooklets in every row except that rows I, III and IV of 2 larvae each and row II of 3 larvae had 40–43 hooklets (Table 7,8,9,). At least 35 more G. doloresi advanced third-stage larvae will be measured and investigated before this study is concluded. However the present study has shown that G. spinigerum advanced third-stage larvae is the longest. The total of 69, 112 and 15 advanced third-stage larvae of G. spinigerum, G. hispidum and G. doloresi respectively studied in 2 years (1967–1968, and 1968–1969) for the variation of their cephalic-hooklets showed as follows on page 155.

TABLE 7 Distribution of cephalic hooklets of 19 advanced third-stage larvae of G. spinigerum.

Row No. Larva No.	I	II	III	IV	IV-I	Age (days)	Size (mm.)
1	44	46	48	55	11	294	3.49×0.33
2	35	39	43	46	11	294	3.66×0.36
3	42	45	44	47	5	294	—
4	35	37	40	44	9	294	—
5	39	42	43	46	7	294	—
6	40	41	42	43	3	294	—
7	39	40	40	44	5	294	—
8	36	38	37	37	1	294	—
9	31	41	41	42	11	294	—
10	42	46	47	47	5	107	—
11	47	53	56	61	14	107	—
12	39	44	52	50	11	107	—
13	47	48	47	50	3	107	—
14	40	41	45	50	10	107	—
15	47	48	49	53	6	107	—
16	43	49	53	60	13	107	—
17	47	46	53	51	4	107	—
18	41	45	53	52	11	107	—
19	40	43	49	53	13	107	—
Average	42.3	43.3	46.4	46.5	8.1	195.6	3.57×0.34

Note: All the larvae are obtained from experimentally infected white mice.

TABLE 8 Distribution of cephalic hooklets of 82 advanced third-stage larvae of G. hispidum.

Larva No. / Row No.	I	II	III	IV	IV-I	Age (days)	Size (mm.)
1	33	36	39	43	10	45	1.7×0.3
2	31	35	38	43	12	45	1.5×0.2
3	36	35	39	45	9	45	1.3×0.2
4	32	33	36	40	8	45	1.4×0.2
5	32	32	35	41	9	45	1.5×0.2
6	33	35	35	40	7	45	1.7×0.2
7	35	35	36	38	3	46	1.1×0.2
8	34	33	35	43	9	241	1.3×0.3
9	38	36	35	40	8	241	1.4×0.3
10	39	40	39	44	5	241	1.3×0.2
11	41	42	41	51	10	241	1.5×0.3
12	35	34	35	43	8	230	1.1×0.2
13	35	35	38	42	7	199	1.0×0.2
14	34	33	35	41	7	199	1.0×0.2
15	35	38	40	28	-7	199	1.1×0.2
16	33	34	47	43	10	199	1.0×0.2
17	32	35	35	38	6	81	1.8×0.3
18	31	35	33	35	4	28	1.8×0.3
19	34	36	38	43	9	28	2.5×0.3
20	35	36	41	37	2	28	2.0×0.2
21	34	31	36	35	1	42	3.1×0.3
22	33	32	30	33	0	42	2.4×0.2
23	32	30	30	32	0	42	2.7×0.2
24	31	28	33	31	0	42	2.6×0.2
25	32	30	34	36	4	42	2.2×0.2
26	28	29	32	35	7	42	1.7×0.2
27	28	30	31	32	4	42	2.8×0.3
28	33	35	36	33	0	42	2.3×0.2
29	31	33	37	37	6	56	3.1×0.3
30	32	30	32	38	6	56	1.9×0.2
31	34	34	37	37	3	56	—
32	37	35	40	44	7	56	—
33	33	35	35	40	7	27	2.1×0.3
34	28	31	30	35	7	29	—
35	30	32	30	19	-11	29	—
36	36	38	41	45	9	29	1.9×0.3
37	32	35	37	41	9	29	2.0×0.3
38	32	30	35	39	7	57	2.3×0.3
39	35	34	39	38	3	57	—
40	32	34	39	44	12	42	—
41	34	32	34	44	10	42	—
42	36	41	43	43	7	42	—
43	36	37	42	45	9	42	—
44	38	36	41	43	5	42	—
45	30	34	36	40	10	42	—
46	35	33	37	40	5	42	2.7×0.3
47	28	33	35	37	9	42	2.9×0.3
48	35	34	34	44	9	33	—
49	31	33	37	40	9	33	—
50	33	38	40	43	10	49	—
51	35	36	36	42	7	49	—
52	31	35	39	41	10	49	—

TABLE 8 (Cont'd)

Larva No.	Row No.	I	II	III	IV	IV-I	Age (days)	Size (mm.)
53		32	35	36	35	3	41	—
54		38	36	38	38	0	41	—
55		30	30	34	35	5	41	—
56		34	38	39	46	12	35	—
57		32	33	35	34	2	35	2×0.33
58		39	37	40	46	7	35	2.4×0.3
59		33	31	31	32	-1	35	2.5×0.3
60		38	36	37	38	0	135	2.4×0.3
61		34	34	36	36	2	135	—
62		39	34	36	42	3	135	—
63		33	35	37	33	0	135	—
64		33	31	35	36	3	135	—
65		34	35	38	35	1	135	—
66		29	33	32	31	2	135	—
67		30	30	34	38	8	135	—
68		33	36	41	37	4	135	—
69		27	36	33	40	13	135	—
70		30	30	32	34	4	135	—
71		31	33	37	42	11	15	—
72		34	36	36	38	4	10	2.4×0.4
73		33	34	36	40	7	29	4.3×1.03
74		36	36	37	44	8	35	2.7×0.34
75		33	34	35	39	6	35	1.8×0.32
76		33	34	36	42	9	35	2.23×0.29
77		31	33	31	36	5	35	2.51×0.33
78		37	37	37	45	8	232	2.28×0.32
79		37	39	38	46	9	232	1.91×0.22
80		31	35	36	37	6	232	1.82×0.27
81		35	35	37	40	5	232	2.2×0.27
82		31	38	35	38	7	232	2.3×0.25
								2.2×0.22
Average		32.2	34.3	36.3	39.0	5.7	84.1	2.0×0.3

Note: All the larvae are obtained from experimentally infected white mice.

B. Development of fully developed larvae of *G. doloresi* (early 3rd stage larvae) in cyclops in some vertebrates (second intermediate host).

Of 14 white mice (*Mus musculus musculus*) fed 8–61 fully developed larvae in cyclops each (a total of 374 larvae) and sacrificed 25 to 66 days after the experiment 6 (43.0%) were found on examination to be infected with 11 (only 3 encysted) advanced third-stage larvae in the muscles of the back, legs and costal area.

Of 2 roof rats (*Rattus rattus*) fed with 95 and 80 larvae respectively showed on examination 43 and 66 days after exposure 1 encysted third-stage larva in the rat fed with 95 larvae.

However, 2 *Rattus birdmorie* fed with 149 and 78 larvae and 1 domestic rat (*Rattus exulans*) fed with 10 larvae on autopsies 14, 18 and 35 days respectively after the experiment showed no infection with the larvae.

This experiment is still in progress.

C. The experimental investigation of the development of the early 3rd stage larvae of *G. hispidum* and *G. doloresi* in the domestic pig (definitive host).

(1) *G. hispidum*:

A newly weaned domestic pig (*Sus scrofa domestica*) obtained from the pig farm of Kasetsart University and initially negative for infection with gnathostomes, was subjected to 8 different feedings at about one week intervals with various numbers of fully developed *G. hispidum* larvae in cyclops (14–292 larvae) (a total of 625 larvae). On necropsy 210 days after the first feeding or 152 days after the last, in spite of weekly negative stool examinations for several weeks before being sacrificed showed 20 immature and mature adult *G. hispidum* in the stomach as follows:

1. In the stomach cavity there were 7 adults (2 mature males, 4 mature females and 1 immature female).

2. 13 adult worms (2 immature and 1 mature males, 9 immature and 1 mature females) each attached to a small ulcerated area of about 0.5 cm. in diameter found scattered throughout the mucosa of the greater curvature of the stomach. These worms could be easily removed with dissecting forceps. Every ulcer contained a small amount of thick mucous exudate and dark red blood clots. Some ulcers contained no worms.

Many fertilized ovoidal and transparent ova in the 1- or 2-cell stage with a knob at one end could be obtained from the mature females after being placed in a petri dish with a few drops of water for one day at room temperature (25–29°C). Subsequently some ova become embryonated; the first in 6 days and all by 8 days. Five days later some embryos were seen hatching through the knobs of the ova as the first-stage larvae. Each was covered with a thin transparent voluminous sheath. The larvae exhibited active and progressive movement in water and could undergo further development into early third-stage larvae in the body cavity of cyclops. Therefore the experiment has left little doubt that direct development of mature adult *G. hispidum* can occur in the stomach of its definitive host, the pig. One more pig is needed for a repeated experiment on this problem in the next year.

(2) *G. doloresi*:

Another young domestic pig also obtained from the same pig farm and proved to be negative for infection with gnathostomes was fed 12 different feedings at intervals of a few days with 7–290 fully developed *G. doloresi* larvae in cyclops (a total of 705 larvae). Thereafter, weekly stool examinations performed by the concentration method were always negative. Necropsy 210 days after the first feeding or 150 days after the last, proved to be negative for the infection with a normal gastric mucosa. Thus although the domestic pig is usually found to act as a definitive host of both *G. hispidum* and *G. doloresi*, only adult *G. hispidum* has been experimentally shown to develop directly from ingested fully developed larvae in cyclops. Further investigation of this problem is planned.

A STUDY ON *G. VIETNAMICUM* FROM RIVER OTTERS (*AONYX CINEREA* ILLIGER) IN THAILAND.

In the previous year (1967-1968) 4 (44%) of 9 river otters obtained from Songkhla and Nakorn-srithammarat areas were found to be infected in the renal pelvis with 17 adult gnathostomes tentatively identified as *G. vietnamicum*.

The study in this reporting year is concentrated on the following problems of this parasite:

(a) Incidence of *G. vietnamicum* in river otters (*Aonyx cinerea* Illiger).

During this year 18 more dead river otters (Figure 3) were obtained from the same areas mentioned last year with the full cooperation of Mr. Sompob Mitravicharn, veterinary officer of region 8. Included in this collection were 8 young otters weighing 0.3 kg-1.3 kg (average 0.47 kg), measuring 20.0 cm-35.0 cm. (average 24.0 cm.) for the body length and 10 adults weighing 1.5 kg-6.5 kg (average 2.5 kg.) measuring 40.0-65.0 cm. (average 46.6 cm.) for the body length.

The 8 young otters showed no gnathostomes. Of the 10 adults, 8 (80.0%) were infected in the urinary system with 17 adult males, 27 adult females and 7 larvae tentatively identified as *G. vietnamicum* of which the maximum number found in one otter was 16 (9 adults and 7 larvae).

(b) Localization of the parasite in the otters.

One adult otter (# 2) was infected with 2 adult worms (one male and one female) only in its kidney.

Two adult otters (# 16 and # 20) were infected with 9 adult worms (5 males and 4 females) only in their ureters.

Four adult otters (# 11, # 13, # 14, and # 23) had a total of 27 adults (8 males and 19 females) and 7 larvae of the gnathostome in their kidneys and ureters.

One otter (# 15) had a total of 6 adults (3 males and 3 females) of the gnathostome in its kidney, ureter and urinary bladder.

(c) Gross pathological changes of the infected organs.

The kidneys showed ulceration with some fibrous tissue formation. Small areas of calcified tissue were noted in some. In one infected pelvis a small calcified area containing a small stone was seen together with ulceration of the tissue (Figure 4).

The ureters showed a fibrous tissue-like tumor together with some ulceration and calcification with or without marked enlargement of the infected ureter (Figure 5).

The bladder showed also a small fibrous tissue-like tumor with some small superficial ulcers and calcified areas on sections (Figure 6).

(d) Study on adults and larvae.

General appearance: The adult gnathostome after being fixed in A.F.A. solution was slender at its anterior third after which the body was bigger with a tapering posterior part covered with swollen cuticular folds (Figure 6).

Size: The average measurement of the head and body of 17 adult males was about 29.7 mm. in length and 2.2 mm. in width (posterior part). However the width of the anterior part ranged from about 0.7 mm.-1.4 mm. (average 1.1 mm.).

The average measurement of the head and body of 24 adult females was about 34.6 mm. in length and 2.4 mm. in width (posterior part). The width of the anterior part ranged from about 0.8 mm.-1.6 mm. (average 1.1 mm.).

The average measurements of the heads of males was about 0.30 mm. × 0.54 mm. and of females 0.33 mm. × 0.59 mm. (Table 10).

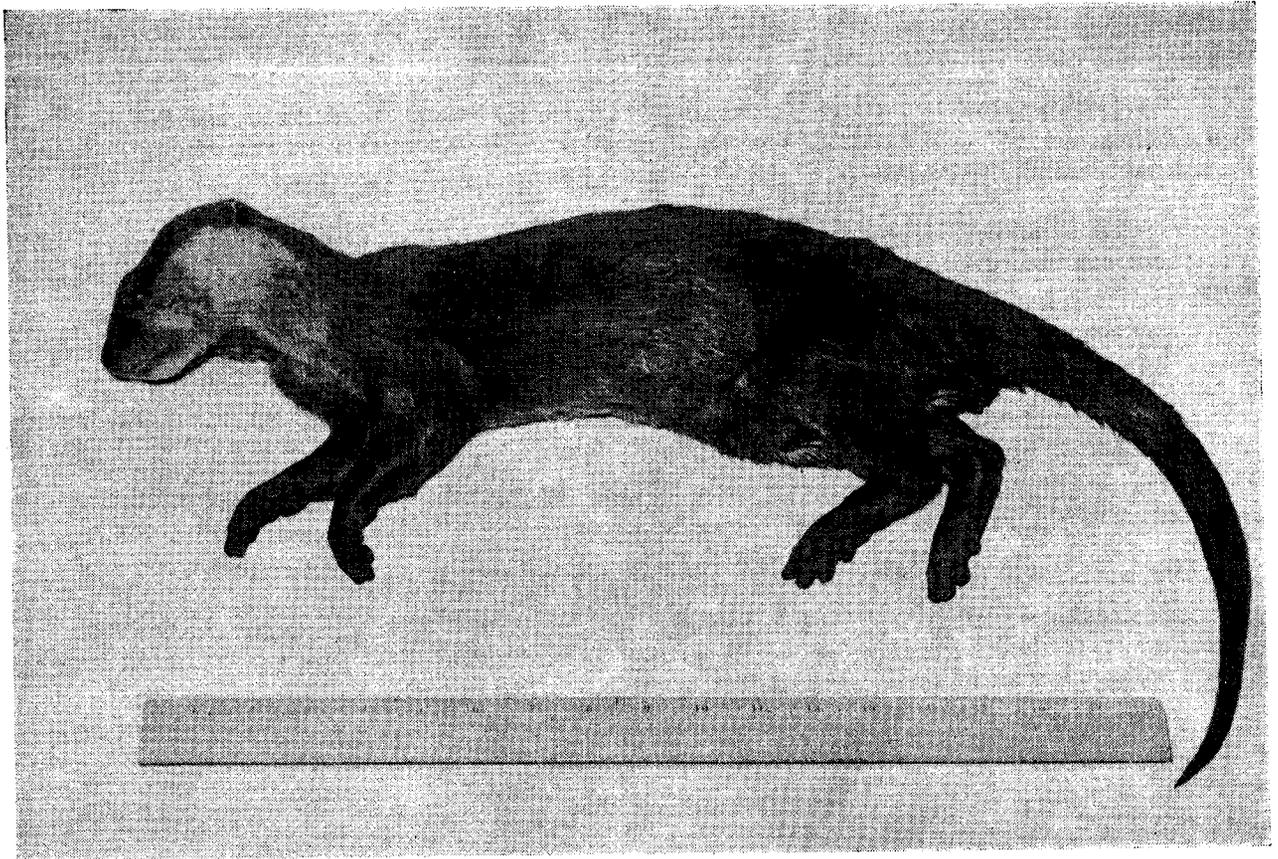


Figure 3. showing an adult female river otter (Aonyx cinerea Illiger),
from Nakornsrihamarat.

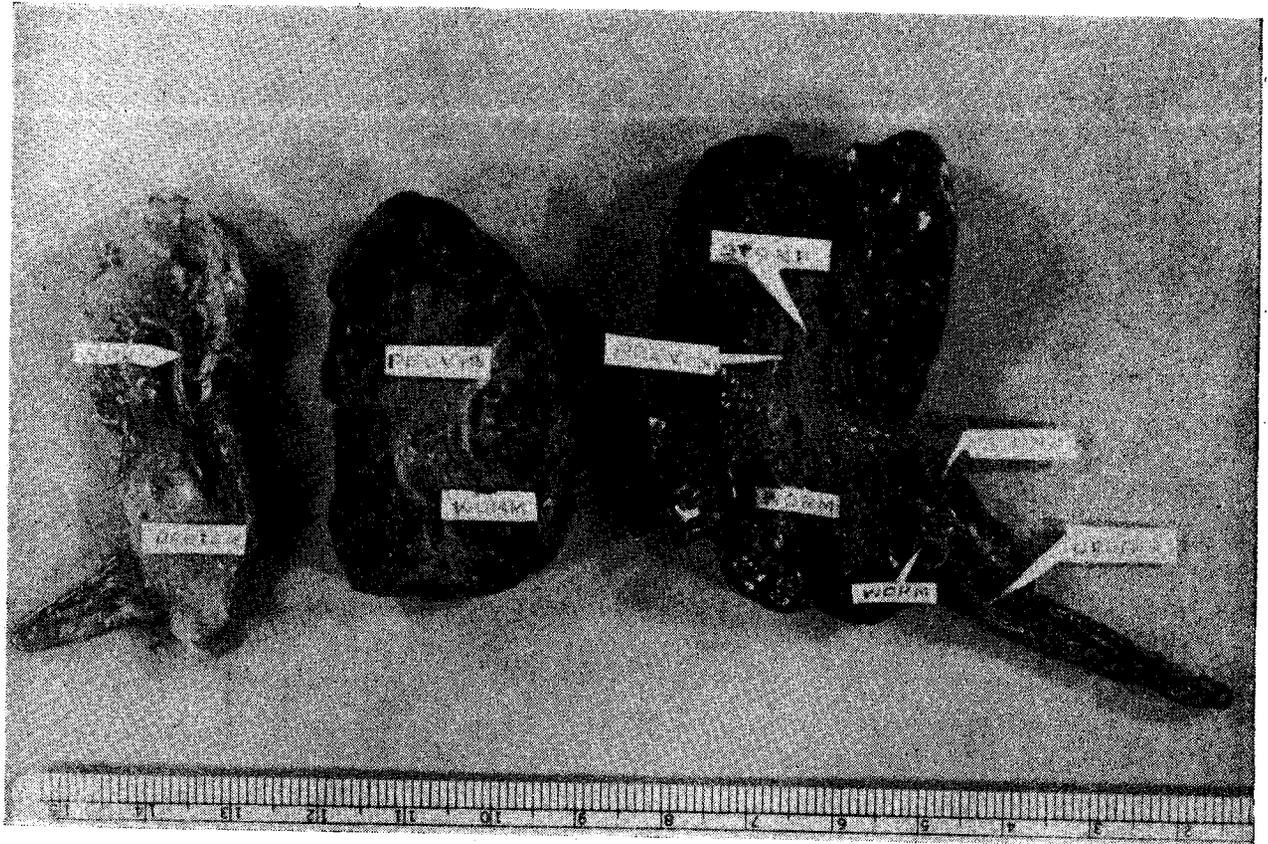


Figure 4. showing kidneys and the upper parts of ureters of an otter naturally infected with adult G. vietnamicum. Note the presence of a kidney stone and a calcified area in one kidney. The infected wall of the ureters are thickened with small areas of ulceration and slight enlargement.

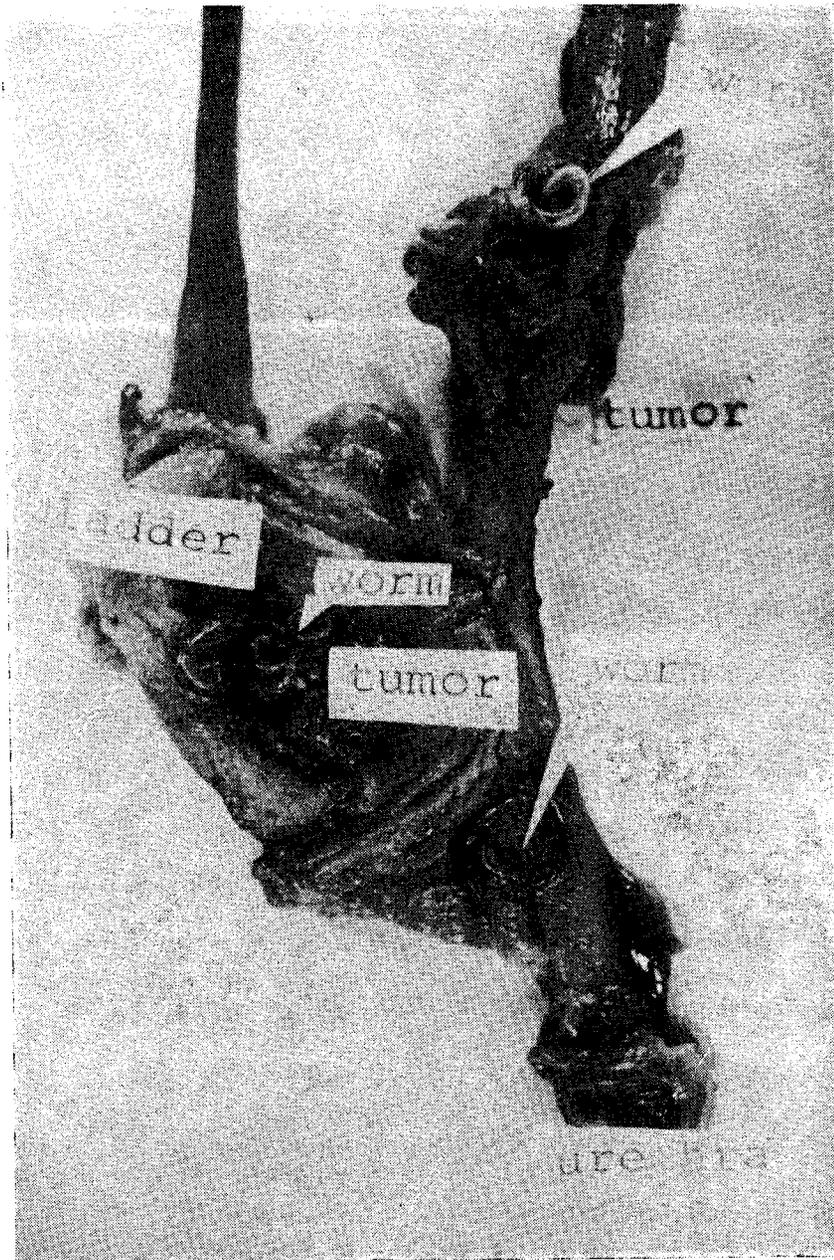
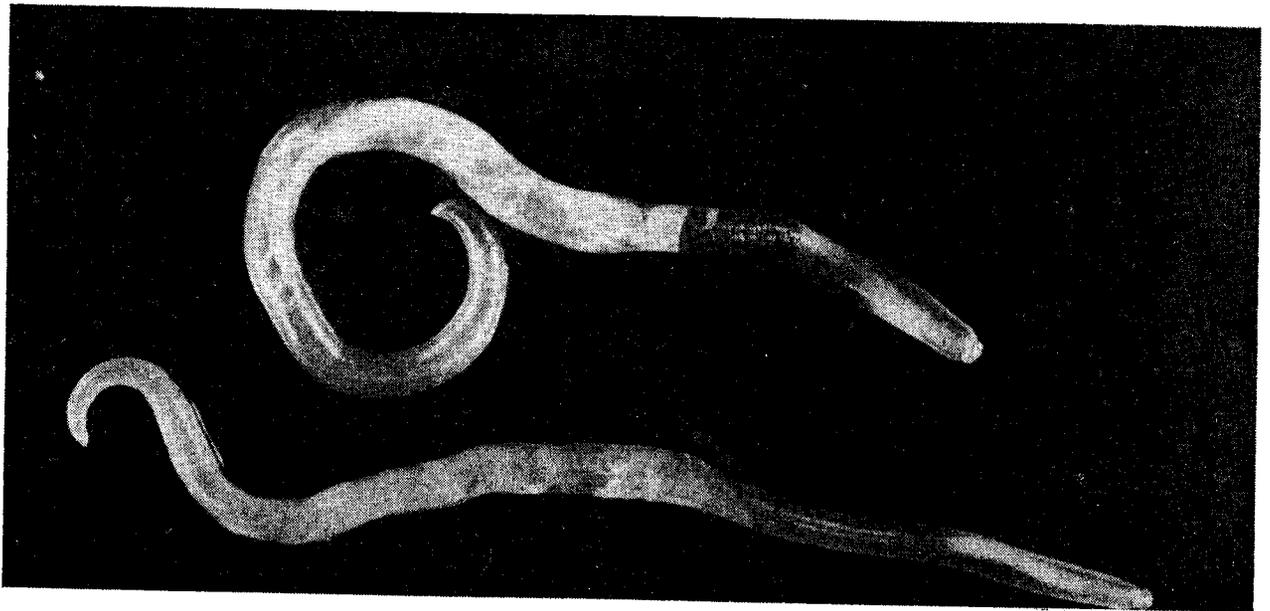


Figure 5. showing the lower parts of the left and right ureters and the urinary bladder of a naturally infected river otter. At each infected area of the bladder and in the left ureter a fibrous tissue—like tumor possibly caused by the worm is clearly seen. Note also the enlargement of the infected left ureter.

MALE



FEMALE

Figure 6. A male and female *G. vietamicum* obtained from a naturally infected river otter in Nakornsrithamarat. Note the slender anterior third and tapering posterior part covered with swollen cuticular folds.

TABLE 10 The dimensions of mature adult Gnathostoma vietnamicum obtained from urinary systems of river otters (Aonyx cinerea Illiger).

<u>G. vietnamicum</u>	Measurement (mm.)	
	Head & Body	Head
<u>Male</u>	<u>17 adults</u>	<u>14 heads</u>
Length	10.0–40.0	0.19–0.39
av.	29.7	0.30
Width		
(posterior part)	1.2–3.0	0.44–0.66
av.	2.2	0.54
<u>Female</u>	<u>24 adults</u>	<u>17 heads</u>
Length	17.0–55.0	0.20–0.48
av.	34.6	0.33
Width	1.4–3.2	0.43–0.72
(posterior part)		
av.	2.4	0.59

The measurement of 4 larvae ranged from 12.0 mm.–14.0 mm. × 0.6 mm.–0.7 mm. (average 13.0 mm. × 0.68 mm.).

Cephalic hooklets. On examination, 20 males and 26 females showed at each cephalic bulb a variation of 12–18 transverse rows of cephalic hooklets except that one adult female was provided with only 7 hooklet rows arranging in a somewhat parallel manner. About 67% of these heads were provided with 14–16 cephalic hooklet rows. The number of cephalic hooklets present in each of row 1–14 of the 2 cephalic bulbs studied ranged from 24–158. Each hooklet was about 16 microns × 8 microns in size and located about 18 microns apart from the adjacent hooklet. (Figure 7).

Cuticular body spines. 3–9 toothed cuticular spines were conspicuous, densely and transversely covering the area from immediately behind the head bulb to a little beyond the anterior third of the body. The cuticle thereafter was covered with vestigial single pointed spines which were less conspicuous but were also arranged in transverse rows almost to terminal part of the body. All cuticular spines were seen to be pointed posteriorly except at the ventral surface of the posterior end of the male worm, where the rudimentary spines become densely arranged in a transverse manner with anteriorly pointed tips. In the male, a somewhat Y-shaped spineless cuticular area is also seen on the ventral surface of the terminal end. (Figure 8).

The shape and size of the cuticular spines as studied in one female worm may be divided into five different zones from the anterior end to the posterior end of the body as shown in Figure 9.

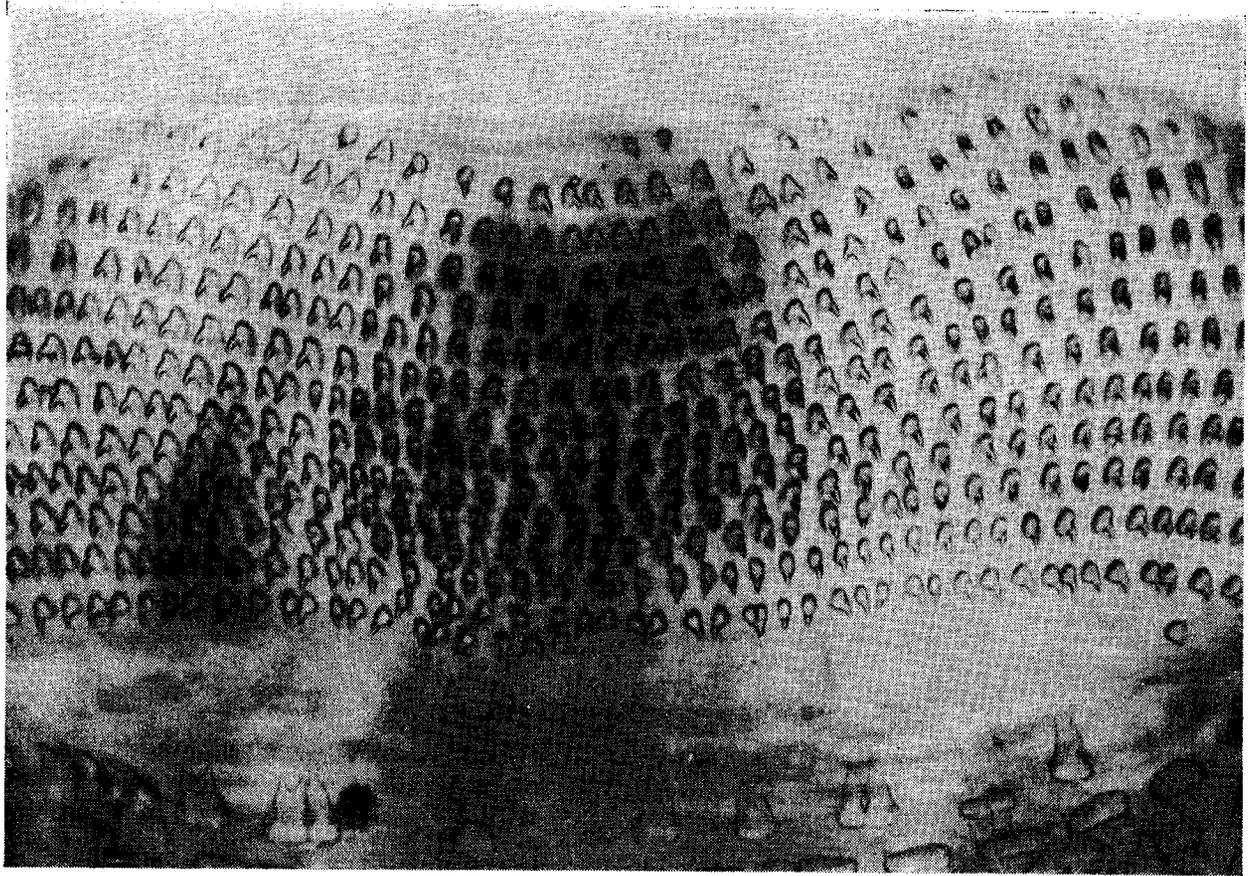


Figure 7. Photomicrograph of G. vietnamicum cephalic blub. Fourteen hooklet rows are arranging in an approximately parallel manner. The number of cephalic hooklets presented in each row ranges from 24 to 158 from rows 1 to 14. Each hooklet is provided with a short single spine pointed posteriorly.

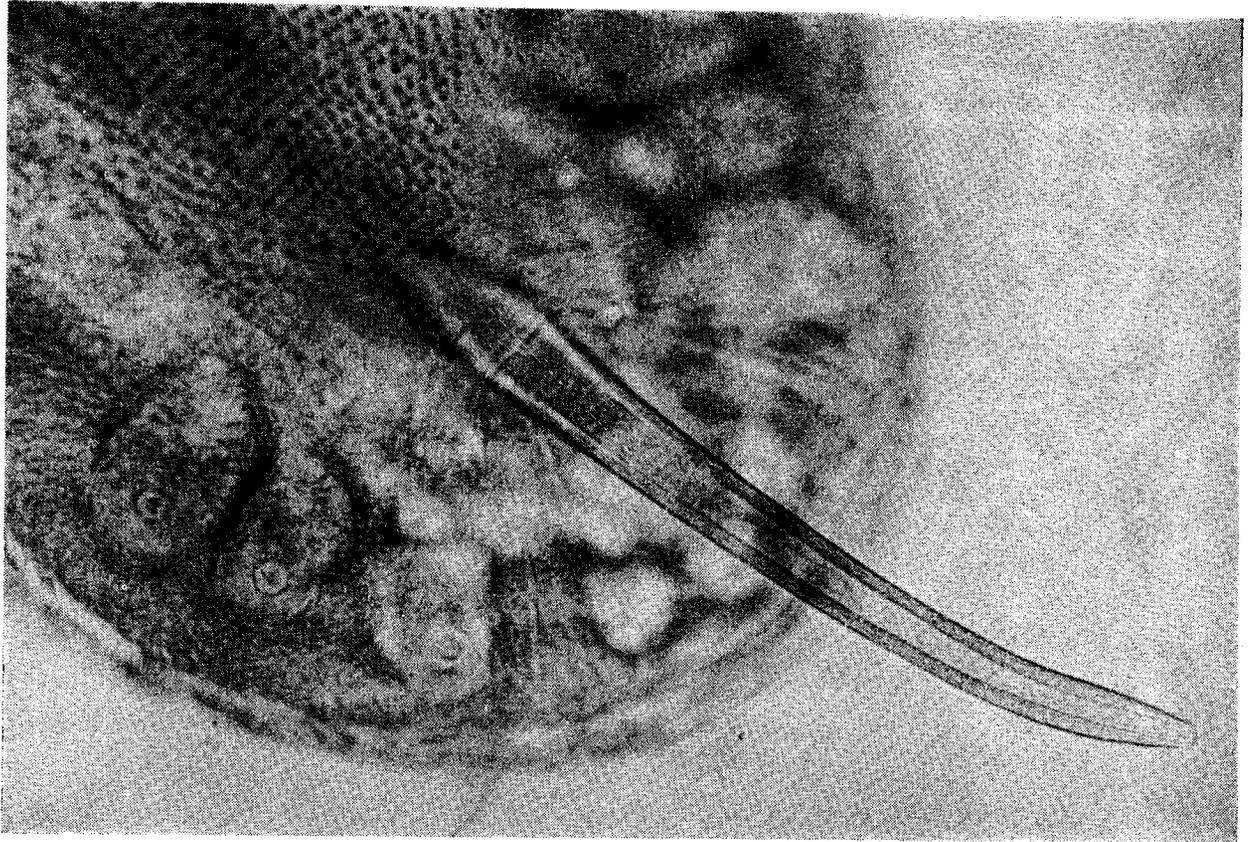


Figure 8. Photomicrograph of the ventral surface of the posterior end of a male *G. vietnamicum* showing rudimentary single pointed spines densely arranged in a transverse manner with anteriorly pointed tips and an approximately Y-shaped spineless area at the terminal end.

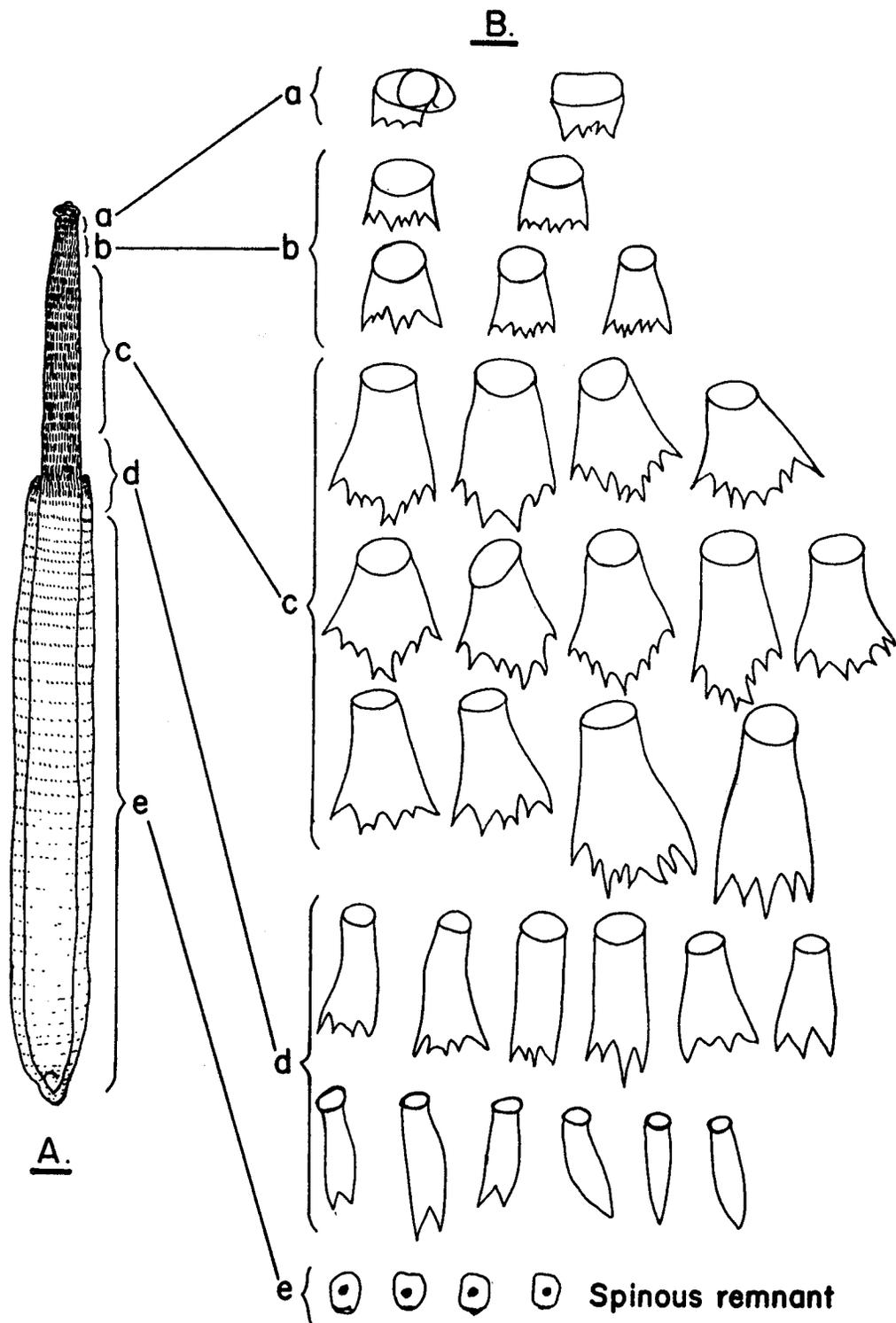


Figure 9 Extent and shape of cuticular spines of a female *G. vietnamicum*.
A. = Body diagram.
B. = Cuticular spines diagram.

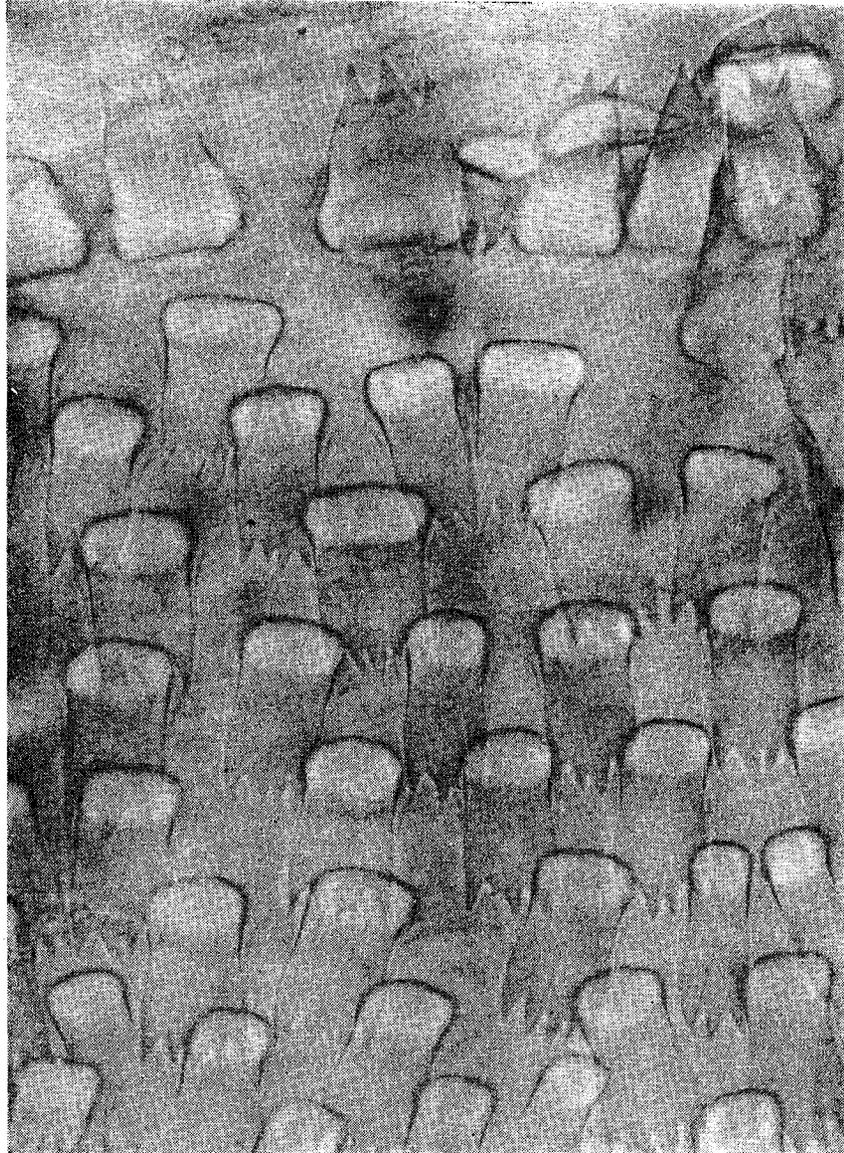


Figure 10. Photomicrograph of cuticular body spines of G. vietnamicum in Zone "a" provided with about 6 transverse row of posteriorly pointed spines each being provided with about 3-5 unequal teeth and measuring an average of about 21.3 microns \times 18.6 microns.

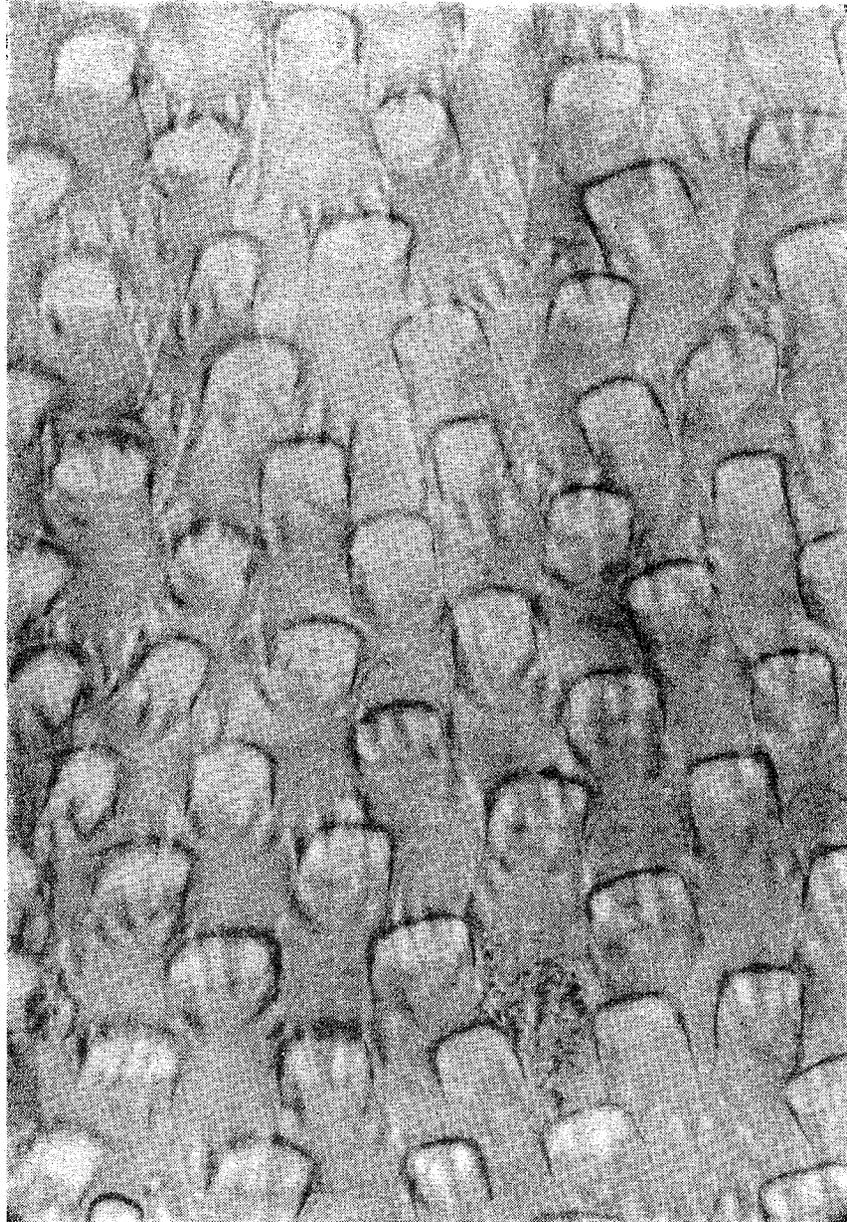


Figure 11. Photomicrograph of cuticular body spines of G. vietnamicum in Zone "b" provided with about 10 transverse rows of posteriorly pointed spines each being provided with about 6-8 unequal teeth and measuring an average of about 24.8 microns \times 22.0 microns.



Figure 12. Photomicrograph of cuticular spines of G. vietnamicum in Zone "c" provided with about 330 transverse rows of posteriorly pointed spines each being provided with 6-9 unequal teeth and measuring an average of 41.7 microns \times 29.4 microns.

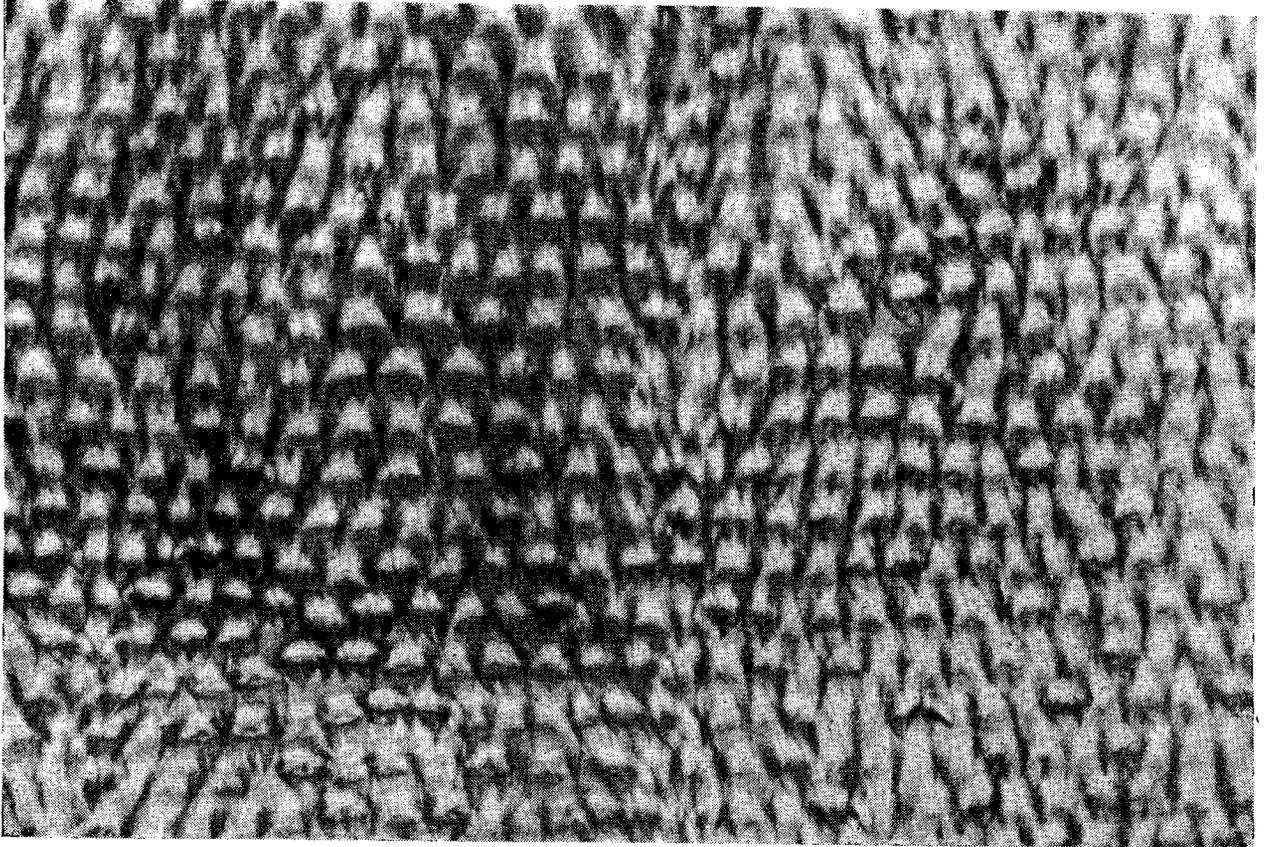


Figure 13. showing photomicrograph of cuticular spines of G. vietnamicum in Zone "d" covered with about 50 transverse rows of spines each being provided with 1-4 unequal teeth pointed posteriorly and measuring an average of about 46.8 microns \times 18.2 microns.

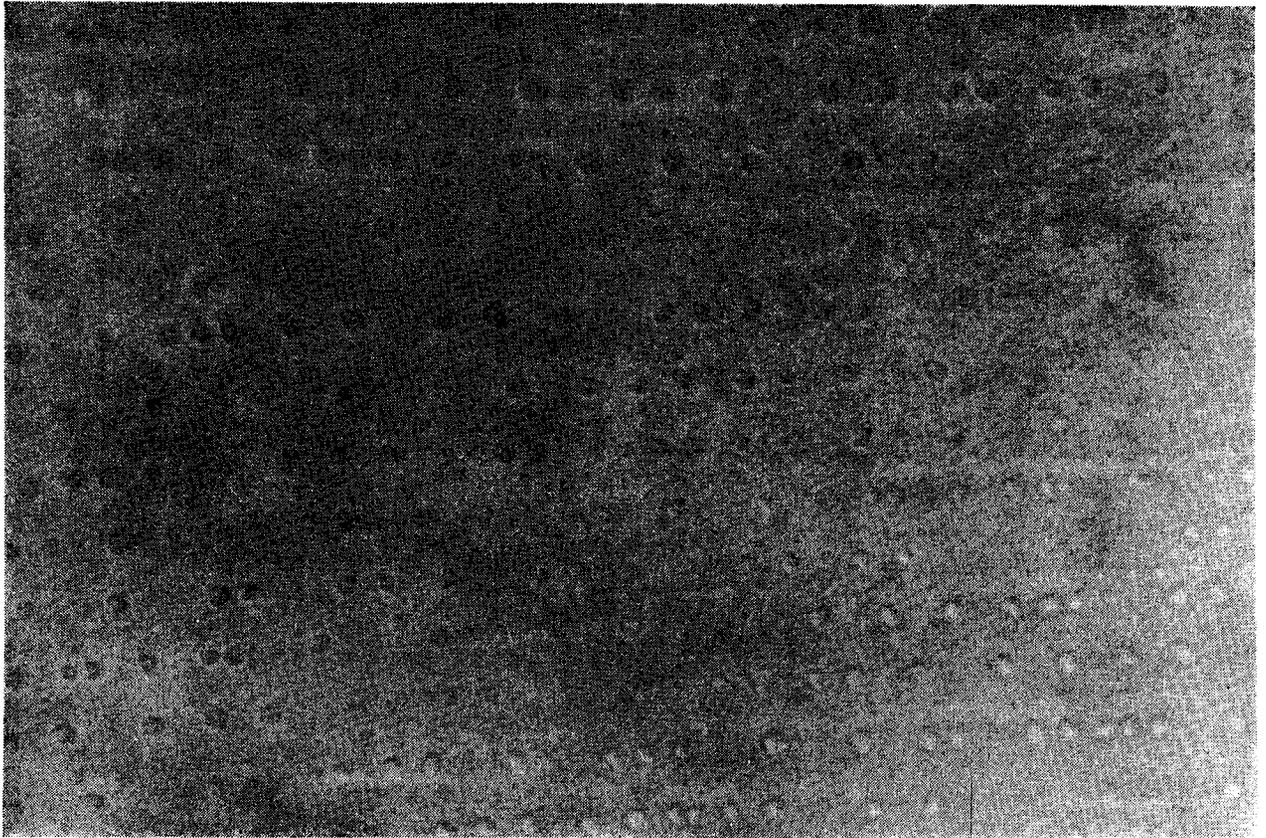


Figure 14. Photomicrograph of minute single-pointed cuticular body spines G. vietnamicum in Zone "e" covering the posterior part of the body, and measuring in an average of about 10.6 microns \times 5.3 microns.

Zone "a" measuring about 0.07 mm. in length from the neck, is covered with about 6 transverse rows of spines each being provided with 3–5 unequal teeth measuring on the average about 21.3 microns × 18.6 microns (Figure 10).

Zone "b", measuring about 0.7 mm. in length from zone "a", is covered with about 10 transverse rows of spines, each being provided with 6–8 unequal teeth measuring an average of about 24.8 microns × 22.0 microns (Figure 11).

Zone "c", measuring about 6.0 mm. in length from zone "b", is covered with about 330 transverse rows of spines each being provided with 6–9 unequal teeth measuring in the range 26.6 microns–53.2 microns × 21.3 microns–37.2 microns (average 41.7 microns × 29.4 microns) (Figure 12).

Zone "d", measuring about 2.3 mm. in length from zone "c", is covered with about 50 transverse rows of spines each being provided with 1–4 unequal teeth pointed posteriorly and measuring an average of about 46.8 microns × 18.2 microns (Figure 13).

Zone "e", continuing from Zone "d", to posterior end of the body is covered with minute single pointed spines measuring an average of about 10.6 microns × 5.3 microns being found and being reduced in number and size posteriorly (Figure 14).

(e) Study on the life cycle.

Ova. The average size of 200 newly laid ova mostly at the 1–4 cell–stage of development, was 77.8 microns × 42.7 microns (61–82 microns × 35–43 microns). They are ovoidal, colorless and superficially pitted each with a mucoid plug or knob at one end. At room temperature (29°C–31°C) in fresh water these ova developed to an embryonated stage in 7 days, to the actively moving embryos in 14 days and 2 days later some become free–living larvae with a characteristic active movement. Hatching then continued for about 10 more days.

Free–living larva. A newly hatched larva is covered with a voluminous transparent sheath extending beyond the anterior and posterior ends with a spinelike structure at the less tapered anterior end and rounded posterior tip. The esophageal tract is clearly seen separating from the intestine. Morphologically this first stage free–living larva of G. vietnamicum is similar to that of G. spinigerum, G. hispidum and G. doloresi and its average measurements based on 64 newly hatched larvae was 262.0 microns × 14.0 microns (range: 212–292 × 10–15 microns)

The larva in cyclops. The free–living larva after being ingested by cyclops (identified at present as Mesocyclops leuckarti Claus) lost its sheath and penetrated through the stomach wall into the body cavity. It then gradually developed at room temperature (29.0°C to 31.0°C) increasing in size and changing in morphology up to 11 days at which time it became a fully developed larva measuring on the average about 740 microns × 27 microns (4 larvae). Each of these larvae is armed with 4 cephalic–hooklet rows similar to those found in case of G. spinigerum. Further study on the life cycle of this worm is being continued.

SUMMARY

Of 4731 dog stomachs examined 12 (0.3%) were positive for adult G. spinigerum infection as compared with the 42 (0.8%) of 5372 stomachs reported last Year. Seasonally, the rate of infection in the definitive host dogs was higher during the middle and last parts of the rainy season (July–November, 10 stomachs) than in the other months of the year. Of 172 snake–headed fish examined 23 (13.4%) were positive as compared with 41 (23.8%) of 172 examined last year. Seasonally, the rate of infection with advanced third–stage larvae among snake–headed fish obtained from an area highly endemic for human gnathostomiasis (Ayuthaya and Phetburi) proved to be higher in the 5 months of the last part of the dry and first half of the rainy seasons (March–July). The above findings as to seasonal distributions are somewhat consistent in both years. Of 345 dead poisonous snakes from the National Red Cross Society 11

(3.2%) were positive as compare with 1.5% last year. Additional vertebrates examined and found positive with the larvae were 3 Rana rugulosa (Bangkok), 1 Viverricula indica (civet cat, Nakornnayok), and 1 Varanus nebulosus (monitor lizard) from Choburi.

An experimental study made on cat #68 has further confirmed the previous report (SMRL Annual Progress Report 1967-68) that Profilis temmincki (the golden cat) can act as the definitive host of the worm in this country.

One of 9 domestic cats caught in Bangkok during this year was found positive with the infection.

Of 24 species of wild-caught animals examined for G. spinigerum infection by stool examinations and stomachs of a civet otter (Cinogale dennetti) and a leopard cat (Felis bengalensis) from the Bangkok Zoo, 2 (1 Felis chaus, the jungle cat and 1 Felis bengalensis) were positive with ova presumably of G. spinigerum. Later an adult G. spinigerum was passed in the stool of the jungle cat. Therefore Felis chaus (jungle cat) was first found to be an additional definitive host this year.

Of a total of 1773 gnathostomes collected from the Bangkok slaughter house 425 (24.0%) were identified as G. hispidum and 1348 (76.0%) as G. doloresi. None were identified as G. spinigerum. The study on skin penetration by G. spinigerum advanced third-stage larvae was continued on 10 cats and 8 dogs. The results at present can be summarised as follows:

10 cats infected by skin penetration showed a variation in rates of successful penetration from 62.0% to 100.0%. In this connection the experiment for determining the total time (TT.) and the actual penetrating time (PT.) of each of 76 experimental larvae has shown that 16-20 minutes and 6-10 minutes are the minimum times respectively. On autopsies 3 cats (#38, #46 and 71) showed the infectivity rates ranging from 29.0% to 51.0%. Two of the animals (#46, #71) also showed G. spinigerum ova in the stool for the first time at about 2 months after exposure. A gastric tumor containing adult males and females was found on autopsy of one cat but the other cat had no worm in its stomach tumor. The other cat (#38) showed no ova in its stools, but had many immature worms and larvae in its muscles and a gastric tumor harbouring an immature male and female. One cat (#73) showed ova in its stools for about 6 months and is still kept for further observation. In total, 7 cats (#73, #74, #77, #83, #84, #87, and #89) are now still being kept for further study.

Eight dogs were skin infected with a variation in successful penetration of the larvae into each animal of from 39.0% to 100.0%.

One dog (#11) showed on autopsy 15 hours after the experiment an 85.0% infectivity rate.

2 dogs (#13 and #14) showed G. spinigerum ova 3-4 months after skin penetration. None of the other 5 dogs showed G. spinigerum ova up to now (about 5-6½ months after the exposure). They are kept for further study.

Skin reaction was observed immediately around the area penetrated by each larva. This consisted of a small wheal with a somewhat irregular margin measuring 0.4 cm-0.6 cm in diameter with or without a hyperemic appearance. This lasted for about 1 hour after finishing the 2-hour experiment.

Early third-stage, or fully developed larvae in cyclops were proved to make no skin penetration during a 2-hour experiment on the ears of two white mice.

This study showed that there is little doubt at present that skin penetration by advanced third-stage larvae of G. spinigerum is possible and that the adult worm can also develop in the stomach of the cat in about 2-6 months and in the stomach of dog in about 3-4 months. However the fully developed larvae in cyclops has no such capability.

The additional study of the size and significant morphological characters for the identification of adults and larvae of G. spinigerum, G. hispidum and G. doloresi showed that adult G. spinigerum is the longest and adult G. doloresi the largest. The larva of G. spinigerum is longer and larger than the others and G. hispidum is the shortest. Morphologically, adult G. spinigerum are provided with 7-9 (mostly 8) cephalic hooklet rows, G. hispidum with 9-12 (mostly 11-12) and G. doloresi with 7-12 (mostly 9-10). These hooklets were found arranged in a parallel manner in G. spinigerum and G. doloresi but a few rows were divergent in G. hispidum. The size of the hooklets in first and last rows were smaller than the other rows in G. hispidum while the size of all cephalic hooklets of the other two species were approximately equal. With regard to the numbers of cephalic hooklets in each row, it was found that G. spinigerum adults had in most specimens 40-99 as compared with 40-139 for G. hispidum, and 60-119 for G. doloresi. Regarding cephalic hooklets of the larvae, most specimens of G. spinigerum had 40 and more hooklets in each of the 4 rows and G. hispidum and G. doloresi had less than 40 hooklets in each row. However a few exceptions were recorded in the number of cephalic hooklets of the larvae in each species.

The study on the life cycle of G. hispidum and G. doloresi during this report period showed the following additional features:

With respect to G. hispidum, all experimental white mice were found positive with advanced third-stage larvae after being fed with various numbers of fully developed larvae in cyclops and advanced third-stage larvae removed from other vertebrates.

Five of 6 experimental roof rats (Rattus rattus) and both of 2 domestic rats (Rattus exulans) become positive with advanced third-stage larvae after being fed with fully developed larvae in cyclops. All of 3 roof rats showed worms in their organs after being fed with the advanced third-stage larvae; however the recovered larvae had developed no significant changes in size or morphology during the period in the roof rats. A few animals were found negative for advanced third-stage larvae when sacrificed after being fed fully developed larvae in cyclops. These consisted of 2 catfish, 6 snake-headed fish, 3 toads, 1 frog and 1 monitor lizard. However 3 catfish and 1 of 5 frogs were found infected with the advanced third-stage larvae after being fed another group of larvae 1 snake-headed fish fed from the same group of larvae was found to be negative.

An initially uninfected domestic pig (definitive host) was found to harbour 20 adult G. hispidum in its stomach when sacrificed 210 days after beginning a series of feedings consisting of a total of 625 fully developed larvae in cyclops.

In the study on G. doloresi, 6 (43.0%) of 14 white mice and 1 of 2 roof rats were found to be infected with the advanced third-stage larvae after being fed with fully developed larvae of the worm in cyclops. However none of 2 Rattus birdmorie and 1 domestic rat were found positive in the same experiment. Moreover an initially uninfected domestic pig was negative for the infection on necropsy 210 days after beginning a series of feedings consisting of 705 fully developed larvae in cyclops.

A preliminary study on the chemotherapy of G. spinigerum in 7 infected cats with Ancylosol Disophenol showed clearly that the drug successfully killed all the adult worms located in the gastric tumors within about 3 days after its parenteral administration to the animals. However all the larvae found in the liver, diaphragm and muscles seemed to resist the action of the drug during the same period. Observations from the beginning of treatment up to 10 days showed no toxic or other ill effects resulting from the action of the drug. Further investigation of the problem is to be continued.

Skin sensitivity tests on monkey #19, done during this reporting period about 3 months after the infection showed positive for a short period, a result consistent with the findings in other infected monkeys as reported in 1966-1967 Annual Progress Report. The examination of the peripheral blood cells showed no significant changes in this monkey. The preliminary biochemical study revealed that the total blood protein increased to an average level of 8.4 gm% as compared with an average of 7.8 gm% before feeding the worms. The total blood globulin after infection showed a variation of 3.4 to 5.7% (average 4.3 gm%) as compared with a range of 3.6-3.8 gm% with an average of 3.7 gm% before infection.

The monkey is to be further studied.

G. Vietnamicum

Of 8 young and 10 adult river otters examined, 8 (80.0%) of adults were found infected with 44 adult males and females and 17 larvae. The worms, which were identified as G. vietnamicum, were found in the urinary system and involved the kidney, ureters and bladder. This is the first time that the ureters and bladder of the animal have been reported to be infected with this species of gnathostome. Stone formation in the kidneys and calcified areas in the bladder and ureters was also observed.

The study of the size of the worm showed that on the average the adult male was 29.7 mm. x 2.2 mm. and the adult female was 34.6 mm x 2.4 mm. The cephalic bulb was provided with 12-18 cephalic hooklet rows but about 67.0% of the worm had 14-16 rows and generally each was provided with 24-158 hooklets from the first row to the last.

The cuticular body spines were divided into zones of different morphology designated as zones a, b, c, d, and e. The maximum numbers of teeth were found in Zone c (6-9 unequal teeth) located in most of the anterior third and provided with about 330 transverse rows of spines. Approximately the posterior $\frac{2}{3}$ of the body was found to be covered with minute single pointed spines. All body spines were directed posteriorly except for a few rows of single-pointed spines on the ventral surface near the posterior end of the male worm; these were pointed anteriorly.

The study of the life cycle of this worm has been initiated with the finding that ova develop in fresh water at room temperature (29°C-31°C) into moving embryos within 14 days and about 2 days later the embryos hatched into actively motile larvae each covered with a voluminous sheath; the tapering anterior end was provided with a spine-liked structure.

The esophagus and intestine were clearly seen in the larva. The free-living larvae after being ingested by Mesocyclops leuckarti developed within 11 days into fully developed larvae in the body cavity of the infected cyclop to a size of about 740 microns x 27 microns. These larvae are provided with 4 transverse rows of cephalic spines.

Further study on the life cycle of this species is being continued at present.