SEATO Medical Research Studies on Gnathostomiasis in Thailand.

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Period of Report:	1 April 1966 to 31 March 1967.

General Information

Human and animal gnathostomiasis caused by *Gnathostoma spinigerum* is highly endemic in Thailand and appears to be on the increase. Recently, an autopsy done on a female patient who died of symptoms referable to the central nervous system at Thonburi Mental Disease Hospital showed damage done to her CNS by an adult male *G. spinigerum.* After World War II, many hundreds of human cases of gnathostomiasis were found in the Prefecture of Kyushu, Hanshu and Shikoku in Japan and the disease seems to be extending gradually in that country. Human cases of infection with *G. spinigerum* have been reported from many countries in Asia. Twenty species of *Gnathostoma* have been recorded in the literature although only 8 seem to be recognized at present as distinct species. Among them, only *Gnathostoma spinigerum* was reported as a cause of human gnathostomiasis in Thailand and other countries except for one case each from Japan and Canton and two cases from India which were infected with *G. hispidum.* Most of the human infections have been with mature male *G. spinigerum* found in subcutaneous, visceral organs and other tissues.

The life cycle of *G. spinigerum* and some methods of its spread and prevention had been worked out in this country before the Second World War. In the Annual Progress Report for 1 April 1965 to 31 March 1966 it was noted that aspects of its epidemiology, individual and community preventive measures, diagnosis, pathological changes and treatment have not been fully studied. The present studies on gnathostomiasis therefore aim to achieve solutions for the above-mentioned problems.

Recently, domestic pigs slaughtered in Bangkok, Nakornpathom, Rajaburi, Nakornsrithammarat and Phuket provinces were found infected with *G. hispidum* in their stomachs. This problem also should be given further consideration concerning the possibility of human infection with this species of which its transmission reported by Golovin (1956) in Russia to the final host (pig) was claimed to be experimentally possible either by drinking water containing the infected cyclops or by eating flesh of the reservoir hosts e.g. fish, amphibians or reptiles. (Helminthological Abstracts, Vol. 25, 265-266, 1956).

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Study Reports

Title: Gnathostomiasis in Thailand

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Objective: The purpose of these studies is to determine the prevalence of Gnathostoma spinigerum among man and animals in Thailand and to carry out further clinical and epidemiological studies including individual and community preventive measures, methods of diagnosis, pathological changes of infected or ans and treatment of the disease. Several years before World War II, dogs and cats were shown to be natural or definitive hosts of the parasite in this country. Therefore tigers and leopards were found infected with adult worms in their stomachs. One leopard cat (Felis bengalensis) among 32 wild-caught carnivorous animals in Bangkok Dusit Zoo was found positive with presumably G. spinigerum ova in its stool as appeared in the last Annual Progress Report, 1 April 1965 to 31 March 1966. These domestic and wild animals therefore can act as reservoir hosts by passing eggs of the worm in their stools. The life cycle and method of transmission of this helminth were worked out in 1936, but many unsolved problems including epidemiological aspects, intermediate hosts and preventive measures remain to be further clarified.

Human cases of gnathostomiasis have been recognized in this country as due to only one species of Gnathostoma, that is G. spinigerum. The effect in man has been varied including lesions of respiratory and visceral organs, lesions of skin, eyes, mucous membranes, meninges and central nervous system damage in one instance. This parasitic helminth has been found in various parts of human body but pathological changes of the infected organs caused by the worm have not been fully clarified. There is, as yet, no effective chemotherapy; surgical removal of the worm from the infected organs was successful in only a few human cases in which non-vital organs were involved and the worm could be definitely located.

Description: The regional prevalence of human gnathostomiasis was investigated last year by collecting cases reported on prepared data-report forms from hospitals and personal communication with medical practitioners, health officers, hospital doctors and nurses as many as possible. This year parts of the Southern region of the country were visited by the team for obtaining more information about the prevalence of this disease in man from local workers and people. Further determination of the significance of animals that may ask as reservoir hosts of the disease was carried out by examination of stools or gastro-intestinal tracts of cats, dogs and other suspected domestic and wild-caught animals in Bangkok. Concurrently, studies to determine the natural animal sources of infection to definitive hosts and to man were continued from last year by examination of fresh-water fish and other animals. Much attention also was paid to examination of animal foods and domestic animals frequently eaten by definitive hosts. Experimental infection of many laboratory and other animals with the third-stage and second-stage larvae of the parasite was further undertaken to determine possible or potential second intermediate and paratenic hosts, to determine more

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susceptable animals than found in the previous year that could be properly used as laboratory hosts, to study detailed development and viability of the larvae in vivo and vitro, to determine the limitation of the number of the third-stage larvae after being fed repeatedly by paratenic host and the infectivity rate of the larvae in cats and dogs. Studies were also designed to determine pathological changes in detail of the infected organs and prenatal infection caused by the third-stage and second-stage larvae of the worm in non-pregnant and pregnant laboratory animals.

Progress: To further determine the prevalence of human gnathostomiasis in the Southern region, some selected areas of Provinces of Nakornsrithammarat, Phuket. Ranong and Prachuabkhirikhan were visited during the period. Many local medical practitioners, health officers, hospital doctors and nurses as well as some lay people were interviewed. Information proveded by the above-mentioned people indicated that some cases of the disease were diagnosed yearly but the symptoms were mild, mostly they suffered from off and on migratory swellings of the skin accompanied by a slight degree of pain and itching. Approximately 15-20 cases were found yearly in those four different provinces and usually some sought diagnosis and treatment from medical private practitioners and many were self-treated by coating the affected areas with indigenous medications. A few were removed from the skin by finger pressure. The incidence of Human gnathostomiasis appears to be higher in the Southern region and appears to be higher than given on prepared data-report forms subitted by the hospital authorities because cases of the disease were not sufficiently ill to seek for hospital assistance.

Due to the need for detailed information concerning the spread of G. spinigerum among animals and subsequently to man, the studies to determine the significance of animals acting as definite or reservoir hosts was continued from the previous year as appeared in Annual Progress Report for the year 1965-1966 with the co-operation and assistance of the Rabies Control Units of Bangok and Thonburi Municipalities, Provincial and Municipal Health Offices of the Southern Thailand including Nakornsrithammarat, Phuket, Ranong and Prachuabkhirikhan with following details:

In Bangkok area, of 4,509 dog stomachs 105 (2.3%) were found positive with adult male and female G. spinigerum during the year. It is interesting to note that dog stomachs at Bangkok and Thonburi Rabies Control Unit were found to be positive only during the months of June to January which is the rain and early part of dry seasons. Examinations of 1,276 dog stomachs during dry season of the year (February to May) were negative. Only 1 of 44 dog stomachs examined in February in the South at Nakornsrithammarat was positive with one immature G. spinigerum.

Of total 423 pig stomachs examined in 4 provinces of Southern region, 37 were found to be infected with G. doloresi and 7 with G. hispidum of which the details are as follows:

In Nakornsrithammarat, of 140 pig stomachs 6 were positive with G. hispidum and 18 with G. doloresi. (2 stomachs, one of a leopard cat and one of an otter were negative.)

In Phuket of 273 stomachs from pigs, 1 was positive with G. hispidum and 18 with G. doloresi.

In Ranong only 1 stomach of wild pig examined was positive with one adult female G. doloresi.

In Prachuabkhirikhan, all 9 pig stomachs were found negative.

The result of stool examinations of 127 cats (Bangkok 72, Ayuthaya 3, Nakornsrithammarat 16, Phuket 14, Ranong 9, Prachuabkhirikhan 13), 7 dogs (Nakornsrithammarat, Phuket, Ranong and Prachuabkhirikhan) and 18 wild-caught animals in Nakornsrithammarat were found to be negative.

In Summary 106 (2.3%) of 4,553 dogs examined were positive mostly in Bangkok area during rainy or wet season of the year but all 127 domestic cats and 20 wild-caught animals were negative.

Determination of natural infection of more fresh-water fish and other animals carrying the third-stage or infective larvae of G. spinigerum to human beings and definite hosts were also undertaken. Due to the regular supply in many months of the year of dead snakes, made possible by the Science Division, National Red Cross Society special attention was paid to the study on seasonal variation of the incidence of this infection among them. These snakes were all obtained from provinces in central Thailand except one king cobra and one cobra from the South. In future if possible it is also planned to determine seasonal distribution of other animals acting as the source of infective larvae to man and animal especially in central Thailand which is the highly endemic area of the parasite. Concurrently, experimental feedings with third-stage larvae removed from second intermediate hosts as well as second-stage larvae or fully developed larvae in cyclops were carried out on many species of animals to determine possible or potential second intermediate or paratenic hosts of the parasite, what animals could be appropriately used as laboratory hosts and lastly more detailed studies of the development and viability of the larvae in vivo and invitro. All these animals were examied for the third-stage or infective larvae in their flesh and other organs by using an electrical illumination apparatus and the identification of the larvae were then confirmed by microscopic examination. Examination for the natural infection with G. spinigerum third-stage larvae of many species of animals showed them encysted in the flesh and visceral organs of two more species of animals than previously recorded namely Ophicephalus lucius and Trimeresus gramineus (green pit viper). These animals were obtained from many provinces in central, southern and eastern regions of the country. The result of examination for natural infection of the infective larvae of the parasite during the period 1 April 1966 to 31 March 1967 showed that of 573 fresh-water fish examined 121 (21.5%) were found to be infected among which snakeheaded fish (Ophicephalus) showed still the highest rate of infection about 20%. Theincidence of infection of fresh-water fish found in last year was only 3.13% of which 68 (32.0%) of 212 snake-headed fish examined were positive. The maximum number of larvae in one infected Ophicephalus fish was 14 (49 last year). Of 255 snake-headed fish examined 111 (43.0%) were positive which is higher than that of the last year. At lower rates of infection were eel (Synbranchus bengalensis) 13.0% and cat-fish (Clarias Sup.) 2.7%. The species of various fish and other animals from which the infective larvae were recovered are listed in Table I. Fish and other animals from which larvae were not recovered by this year survey and of which more than ten specimens were examined are listed in Table II. In addition, the species of which only a few specimens were examined and negative are listed in Table III.

Among 44 amphibians examined, only 6 frogs of one species (Rana rugulosa) were positive. The result of examination of snakes obtained by the Bangkok Snake Farm of the National Red Cross Society from Samutprakarn, Angthong and Ayuthaya except one king cobra collected from Nakornsrithammarat and one cobra from Phuket was as follows: Of 3 king cobras (Naja hannah) 2 were positive with an average of 16 larvae and in 12 of 430 cobras found to be positive with an average of 248 larvae per animal. A single larvae was found in a green pit viper (Trimeresus gramineus) which is the first to be recorded in this snake. No larvae were recovered from 87 Vipera russelli, 39 Agkistrodon blomhoffi, 36 Bungarus fasciatus, 16 Boiga, one Python reticulatus and one Cylindromhis rufus. The monthly incidences of snakes found to be positive with the larvae seemed to be higher in the rainy season than other months of the year.

Table 1 Natural infection with G. spinigerum third-stage larvae No. positive/No. examined.

Species of animal	Bangkok	Thonburi	Samut prakarn	Cha-choeng-sao	Trat	Ayu-tha-ya	Kan-chana buri	Ang-thong	Sara-buri	Udon	Phetburi	Pra-chuap-khiri-khan	Ra-nong	Nakhon-sitham-marat	Phuket
Ophicephalus striatus (Snake-headed fish) Large	1/8		7/7	7/10		5/7		6/6			0/1		1/7	12/28	5/21
Small		1/14	34/80	29/51				3/6			0/9				
Ophicephalus micropeltes			1/1											2/8	
Ophicephalus lucius*			2/5											1/1	
Clarias macrocephalus	0/2		1/32	1/1							0/2	0/17	0/11	2/57	0/25
Synbranchus bengalensis (Eel)	0/4			1/2								0/1		1/9	
Rana rugulosa (Frog)	0/2	1/2	2/5						3/12	0/11					
Naja hannah (King cobra)						1/1		0/1						1/1	
Naja naja (cobra)	12/428				0/1										0/1
Trimeresus gramineus* (Green pit vipper)	1/5														
Bubulcus coromandus (Cattle egret)														1/3	
Corvus macrorhynchos (Crow)					2/2										
Anas plytyrhynchus domestica (Domestic duck)	0/2											0/1	0/1	1/1	
Bandicota indica (Bandicoot rat)	1/62						0/5							0/2	

* New species found infected.

Table II Animals Negative for G. spinigerum.
(10 and more specimens of each species examnied.)

Class	Species	Number
Crustacea	Paratelphus sexpunctatum	73*
	Palaemon potamiscus	13694
Pisces	Ompok bimaculatus	14*
	Trichopodus trichopterus	25*
	Anabus testudineus	18
	Notopterus notopterus	36
	Puntius gonionotus	11
	Tilapia mossombica	10
Reptilia	Vipera russelli	87
	Bungarus fasciatus	36
	Agkistrodon blomhoffi	39
	Boiga	16
	Leiolepis belliana belliana	17
	Calotes versicolor	22
Aves	Cynopterus brachyotis	215*
	Gallus gallus domesticus	11
Mammalia	Rattus exulans	51*
	Rattus rattus	169*
	Rattus norvegicus	164*
	Rattus rajah	16
	Mus famulus	10
	Tupaia glis	17*
	Sus scrofa domestica	900**

** Only 100 grams of fat and muscles from one hind leg and chest of each pig were examined through the kind assistance of Livestock Trading Co-operation (Bangkok Pig Slaughter House).

* Represents collection from more than two provinces.

Table III Animals Negative for G. spinigerum
(Less than 10 specimens of each species examined)

Class	Species	Number
Pisces	Catopra siamensis	7
	Puntius orphoides	8
	Trichopsis vittatus	1
	Osteogeneiosus militaris	2
	Hampala macrolepidota	2
	Macrognathus aculeata	2
	Labiobarbus siamensis	2
	Bagroides macrocanthus	1
	Trichogaster pectoralis	1

Amphibia	Rana limnocharis limnocharis	8
	Bufo melanostictus	4
Reptilia	Python reticulatus	1
	Cylindromhis rufus	1
	Riopa herberti	2
	Varanus nebulosus	1
Aves	Arachuothera longirostra	5
	Phycnonotus blanfordi	4
	Maeronous guraris moltingadult	1
	Lonchura striata	3
	Longhura punctulata	4
	Passer montaneus	3
	Passer flaveolus	1
	Ploceus philippinus	9
	Strunus trislis	1
	Pycnomlus blangferdi	1
	Ardeola grayii	1
	Haliastur indus	4*
	Pycnonotus goiavier	2
	Rhipidura javanica	1
	Hypothymis azurea	1
	Dicunm cruentatum	1
	Trichastoma abbotti	1
	Dicrurus paradiseus	1
	Unidentified fish-eating bird	2
Mammalia	Rattus norvegicus var albinus	1
	Rattus niviventor	1
	Mus cervicolor	1
	Menetus berdmoici	1
	Suncus murinus	4

* Represents collection from more than two provinces.

Among birds examined, 1 of 3 cattle egrets (Bubulcus coromandus), 2 crows (Corvus macrorhynchos) and 1 of 5 domestic ducks (Anas platyrhynchus domestica) were found positive (Table 1.) Specimens of 21 other species were found negative as shown in Table II and III.

Only one of 69 bandicoot rats, mostly trapped in Bangkok was found positive. One thousand three hundred and thirty five mammals of several species were found to be negative as listed in Table II and III.

The animals collected from the central area of the country had a higher prevalence of infection with the third-stage larvae than those from the southern other areas.

In general, it is obvious that among animals found to be naturally infected with the larvae, snake-headed fish, eels and frogs showed higher incidence of infection than other species. The detailed findings of naturally infected animals are shown in Table I.

Experimental infection of certain animals with known numbers of the third-stage larvae of the parasite for determination of possible or potential second intermediate hosts as well as paratenic hosts showed that 23 species of animals in five classes had the larvae in liver, lung, heart, heart blood, kidney, pancreas, stomach, intestinal and esophageal walls, mesentery, omentum, subcutaneous tissue and flesh (Table IV). Of these experimentally infected animals, 8 species including Ophicephalus striatus (snake-headed fish), Clarias macrocephalus (cat-fish), Rana rugulosa (frog), Rana limnocharis* (small frog), Anas platyrhynchus domestica (domestic duck), Gallus gallus domesticus* (domestic chicken), Tupaia glis* (tree shrew) and Rattus rattus* (rattus rat or roof rat) were also found naturally infected with the third-stage larvae in the previous year and this year (*negative larvae this year). The larvae were found renetrate the stomach walls of the experimented mammals within a few minutes to a few days after experimental feeding. However subsequently some of these larvae could be found in visceral and other organs including heart blood, heart muscles, lung, trachea, intercostal and other muscles, and subcutaneous tissue as early as about one hour after the feeding experiment. Thereafter they were commonly seen singly in the flesh with early formation of a thin fibrotic wall around each living larva about 30 days after the infection. This wall was seen gradually to increase in thickness. If the host was sacrificed at much longer period after the nfection especially in one infected chicken the fibrotic cyst well around the healthy living larva was found 0.2 mm. thick in its leg's muscles when the animal was sacrificed 548 days after the experiment (Figure 1). The preliminary findings of such an experiment on certain animals was reported in the last year Annual Progress Report. The third-stage larvae were found in the livers of experimentally infected animals as long as 30 days after the feeding. In the present experiment larvae were fed to white mice. Upon sacrafice larvae were found encysted in the livers within 5 to 154 days. It may be assumed from this result that encysted larvae may also persist in human livers for many months.

Further experimental feeding were carried on to determine if species of animal other than fresh-water fish act as second intermediate hosts when feed on cyclops harboring second-stage or fully developed larvae of the worm and to confirm the previous findings of the same experiment of which the results are as follows:

Table IV Experimental infection of certain animals with third-stage larvae of G. spinigerum.

Species of animal	No.	Source and Number of infective larvae			Days after feeding	No. larvae positive			Remarks
		Fish	Snake	others		in liver	in muscle	in others	
Pisces (Fresh-water fishes)									
Ophicephalus striatus (Snake-headed fish)	3	—	40	6-small frog	1-11	3	2	0	2 fish positive
Clarias macrocephalus (Catfish)	8	5	—	32-chickens, frog, toad, rat	3-15	2	15	0	6 fish positive
Total	11	5	40	38	1-15	5	17	0	
Amphibia (Amphibians)									
Bufo melanostictus (Toad)	2	—	—	14-turtle, tree shrew, white mouse	7-20	0	3	4-stomach wall	
Rana rugulosa (Frog)	5	6	—	35-duck, rats, white mouse	5-26	0	11	2-stomach wall	
Rana limnocharis limnocharis (Small frog)	4	—	10	6-lizard	7	0	12	0	
Total	11	6	10	55	5-26	0	26	6	
Reptilia (Reptiles)									
Leiolepis belliana belliana (Ground lizard)	1	—	—	3-small frog	7	0	1	0	
Calotes versicolor (Tree lizard)	1	—	—	3-small frog	7	0	1	0	
Physignathus cocincinus (Agamid lizard)	3	—	—	16-monkeys, white mouse	7-25	0	7	2-stomach wall	

Species of animal	No.	Source and Number of infective larvae			Days after feeding	No. larvae positive			Remarks
		Fish	Snake	others		in liver	in muscle	in others	
Domonia subtrijuga (Turtle)	1	—	—	22-white mouse, rat	30	0	4	0	
Total	6	0	0	44	7-30	0	13	2	
Aves (Birds)									
Passer montanus (Tree sparrow)	1	—	—	9-white mouse	3	0	0	0	to be repeated.
Ploceus philippinus (Weaver bird)	2	—	—	11-white mice	9-13	0	2	0	1 bird positive.
Coturnix coturnix (Quail)	4	—	—	35-white mice, rats	7-31	0	7	0	2 birds positive
Anas platyrhynchus domestica (Domestic duck)	6	41	—	16-chicken, frog, white mouse	8-17	1	7	0	
Gallus gallus domesticus (Domestic chicken)	3	—	41	11-frog, toad	14-67	0	9	0	2 chickens positive
Gallus gallus domesticus (Domestic chicken)	1	—	—	—	—	0	0	0	Control chicken.
Gallus gallus domesticus (Domestic chicken)	13 (egg-laying)	251	723	1406-white mice, rats, quail lizards, small frog.	119-548	0	60	35-subcutaneous tissue	They were experimented for eggs examination. So far 796 eggs collected were found to be negative.

Species of animal	No.	Source and Number of infective larvae			Days after feeding	No. larvae positive			Remarks
		Fish	Snake	others		in liver	in muscle	in others	
Aves (Bird) continued.									During the period 7 chickens died 119-548 days after the experiment and showed 95 encysted G. spinigerum larvae.
Total	30	292	764	1488	3-548	1	85	35	
Mammalia (Mammals)									
Tupaia glis (Tree shrew)	2	—	—	11-frog, hamster	7-14	4	4	0	
Rattus rattus (Rattus rat)	7	17	154	3-duck	10 hours to 42days	31	45	36-lung, stomach heart, heart blood.	
Rattus exulans (Domestic rat)	5	22	23	43-white mice, rat, bird	13-116	1	36	0	
Mesocricetus auratus (Hamster)	34	—	6	145-rats, hamsters	5-156	0	98	1-stomach	
Rattus norvegicus var albinus (White rat)	71	—	278	914-white mice, rats, pigs, civet cat, turtle, lizard, frog.	5 mins. to 195 days	54	228	237-stomach, lung, diaphragm, esophagus, omentum, fatty tissue, trachea, heart, mesentery, kidney, blood.	

Species of animal	No.	Source and Number of infective larvae			Days after feeding	No. larvae positive			Remarks
		Fish	Snake	others		in liver	in muscle	in others	
Mus musculus musculus (White mouse)	161	95	1545	1069-white mice, rats, monkeys, rabbits, chicken, quail, guinea pig, tree shrew, lizards frogs.	15 hours to 155 days	778	1086	132-intestine mesentery, stomach omentum, fatty tissue, subcutaneous tissue, lung, pancreas, diaphragm.	
Mus musculus musculus (Pregnant white mouse)	28	44	150	129-white mice, rats, tree shrew, rabbit	5—19	99	122	13-intestine, diaphragm, stomach skin, kidney uterus, babies	Pregnant white mice, 27 mothers were positive. Of 208 babies examined two were positive with two larvae in costal region (un-encysted)
Veverricula indica (Civet cat)	1	8	50	—	287	0	71	1 adult male G. spinigerum from stomach. 1 larvae 1 male and 2 females (immature) from mesentery.	
Paradoxurus hermaphroditus canus (Palm civet cat)	1	—	30	—	241	0	8	0	
Macace irus (Crab—eating monkey)	2	—	—	23-white mice, chicken	3	2	0	1-esophagus	1 monkey positive.
Total	312	186	2236	2337	5 mins. to 287 days	969	1698	424	
Grand total	370	489	3050	3962	5 mins. to 548 days.	975	1839	467	

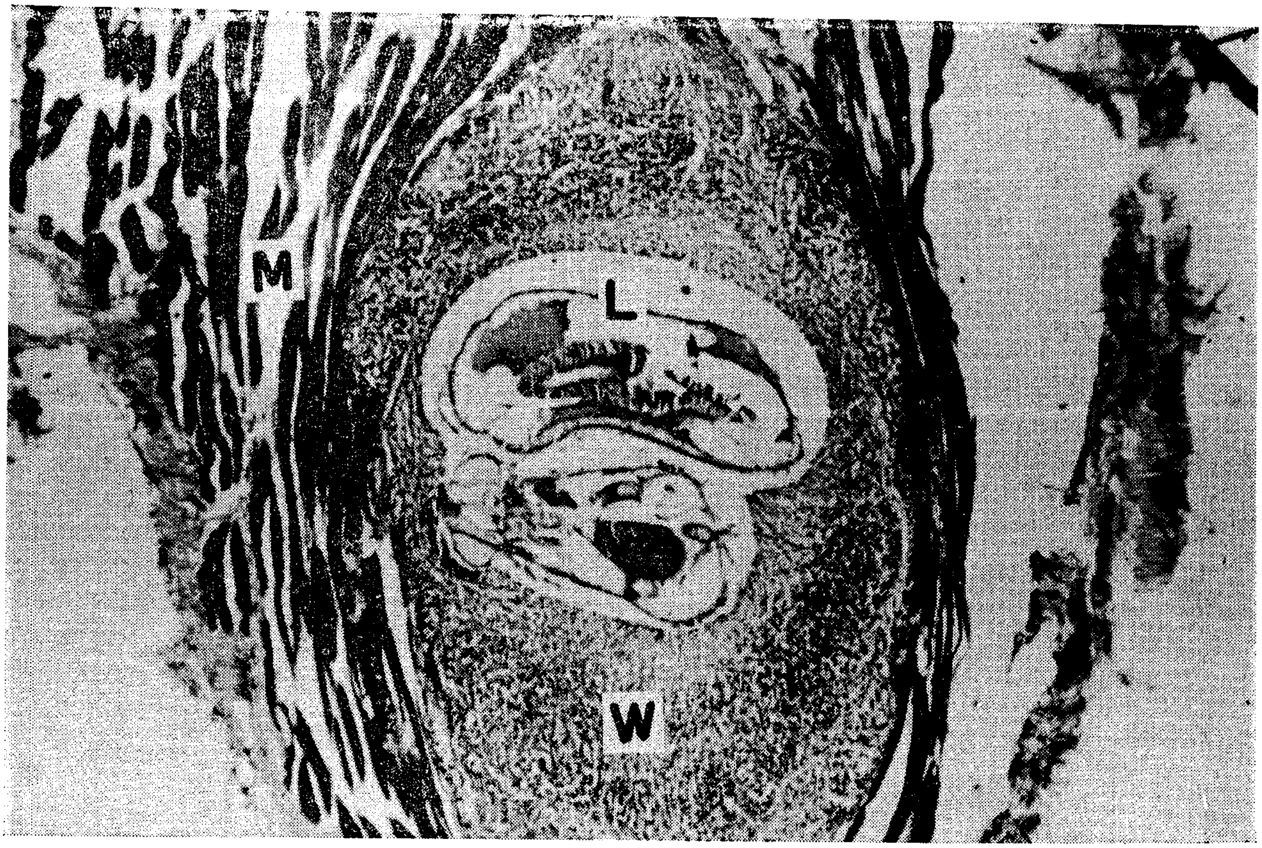

Figure 1. A microphotograph of the leg's muscles of a chicken sacrificed 548 days after being fed with G. spinigerum third-stage larvae shows an encysted living third-stage larva being surrounded with fibrotic cyst wall of about 0.2 mm. in thickness. (L=larva, M=muscle, W=cyst wall.)

Table V Experimental infection of certain animals with G. spinigerum second-stage larvae (fully developed larvae in cyclops).

Species of animal	No.	No. of larvae	No. of inf. cyclops	Days after feeding	No. larvae positive			Remarks
					in livers	in muscles	in others	
Amphibia (Amphibians)								
Bufo melanostictus (Toad)	4	280	136	8-19	0	2	26-stomach, intestine	2 toads positive.
Rana rugulosa (Frog)	17	950	426	1-22	4	9	0	4 frogs positive.
Total	21	1230	562	1-22	4	11	26	
Reptilia (Reptiles)								
Boiga (Cat-eye snake)	1	75	42	17	0	1	0	
Varanus nebulosus (Monitor lizard)	1	100	56	21	0	1	0	
Physignathus cocincinus (Agamid lizard)	3	800	325	18-28	0	0	0	
Calotes versicolor (Tree lizard)	9	525	267	3-17	0	0	20-stomach, intestine.	3 lizards positive.
Total	14	1500	690	3-28	0	2	20	
Aves (Birds)								
Gallus gallus domesticus (Domestic chicken)	31	4977	2275	4-59	2	0	0	Only one chicken was positive 32 days after feeding.
Lonchura punctulata (Spotted munia)	2	60	31	7-9	0	0	0	to be repeated
Passer montanus (Tree sparrow)	2	62	25	3	0	0	0	to be repeated
Ploceus philippinus (Weaver bird)	5	231	133	3-5	0	0	0	to be repeated
Coturnix coturnix (Quail)	6	325	191	6-30	0	0	0	to be repeated
Total	46	5655	2655	3-59	2	0	0	

Species of animal	No.	No. of larvae	No. of inf. cyclops	Days after feeding	No. larvae positive			Remarks
					in livers	in muscles	in others	
Mammalia (Mammals)								
Tupaia glis (Tree shrew)	3	142	75	15-60	4	5	1-fatty tissue under external abdominal wall.	
Rattus rattus (Rattus rat)	3	158	76	15-49	2	12	1-subcutaneous tissue of abdominal wall	
Rattus exulans (Domestic rat)	2	150	56	17	9	1	0	
Mesocricetus auratus (Hamster)	1	76	37	90	0	6	0	
Sus scrofa domestica (Domestic pig)	7	1923	940	2-108	9	7	0	Three pigs were positive.
Rattus norvegicus var albinus (White rat)	2	118	63	90-180	0	11	0	
Mus musculus musculus (White mouse)	86	5904	2624	4 hours to 169 days	1028	704	99-intestine, stomach, lung, kidney, fatty tissue, subcutaneous tissue.	
Mus musculus musculus (Pregnant white mouse)	10	656	281	6-33	110	27	3-intestine, stomach, baby	9 mothers were positive. 78 babies were examined of which one of five unborn babies was positive with one third-stage larva in abdominal wall.
Total	114	9077	4119	4 hours to 180 days	1162	764	104	
Grand total	195	17462	8026	4 hours to 180 days	1168	777	150	

Of 21 amphibians 6 (29.0%) were positive for the third-stage larvae in gastro-intestinal tracts, livers and flesh from 1 to 22 days.

Of 14 reptiles 5(36.0%) were positive with the third-stage larvea in the muscles from 17-21 days and in their gastro-intestinal walls from 3-17 days. Of 46 avians only 1 two month-old chicken (2.2%) showed 2 third-stage larvae in its liver when sacrificed 32 days after feeding. Of 114 experimented mammals sacrificed from 4 hours to 180 days after feeding 110 (96.5%) (3 tree shrews, 3 rattus rats or roof rats, 2 domestic or polynesian rats, 1 hamster, 3 domestic pigs, 2 white rats and 96 white mice) showed the third-stage larvae in one or more organs namely liver, flesh, gastro-intestinal tract, subcutaneous tissue, kidney and lung (Table V).

In this respect it is interesting to note that 3 of 7 experimented young domestic pigs were positive with 1 and 8 third-stage larvae in the livers of two pigs sacrified 33 and 45 days after feeding and the third pig showed 7 encysted third-stage larvae in the flesh 108 days after the experiment. The results of previous experimental feeding on 8 domestic pigs fed with the third-stage larvae showed positive larvae in livers and or flesh of 7 pigs sacrificed from 8 to 222 days after the experiment. Pigs therefore by these two experiments can be considered acting as paratenic and second intermediate host for the parasite. It is especially desirable to determine any natural infection of these animals in order to confirm the experiments because in many parts of Thailand, people eat fermented or pickled raw pork prepared in various forms of which the most popular one is called "Moo-nham" or in brief "Nham".

One of 5 fetuses of an infected pregnant white mouse was positive with one third-stage larvae in its abdominal wall 18 days after the pregnant mouse was fed on 100 second-stage larvae in 48 cyclops.

This present study has clearly proved for the first time that toad (Bufo melanostictus), frog (Rana rugulosa), cat-eye snake (Boiga), monitor lizard (Varanus nebulosus), tree lizard (Calotes versicolor) domestic chicken (Gallus gallus domesticus), rattus rat or roof rat (Rattus rattus), polynesian or domestic rat (Rattus exulans) and domestic pigs (Sus scrofa domestica) can serve as additional second intermediate hosts. It also confirmed the experimental findings reported last year that the hamster (Mesocricetus), white rat (Rattus norvegicus var albinus), and tree shrew (Tupaia glis) were proved to act as possible second intermediate host of the parasite as well as being suitable laboratory hosts for further studies on gnathostomiasis problems.

In summary more species of animals other than previously reported in the literatures have been added to the list of second intermediate and paratenic hosts by this year study as follows:

A. Additional species of animal proved to be second intermediate hosts after being fed on cyclops harboring second-stage larvae are toad (Bufo melanostictus), frog (Rana rugulosa), cat-eye snake (Boiga), monitor lizard (Varanus nebulosus), tree lizard (Calotes versicolor), domestic chicken (Gallus gallus domesticus), rattus rat or roof rat (Rattus rattus), domestic rat or polynesian rat (Rattus exulans) domestic pig (Sus scrofa domestica).

B. Additional species of animal proved to be paratenic hosts of the parasite in which the third-stage larvae of the worm could be transmitted by oral feeding and survived in livers and later in the flesh of animals without showing any morphological changes except a slight increase in size and reddish in color are the snake-headed fish (Ophicephalus striatus), cat-fish (Clarias macrocephalus), frog (Rana rugulosa), agamid lizard (Physignathus cocincinus), weaver bird (Ploceus philippinus), quail (Coturnix coturnix) roof rat rattus rat (Rattus rattus), polynesian rat or domestic rat (Rattus exulans) and palm civet cat (Paradoxurus hermaphroditus canus).

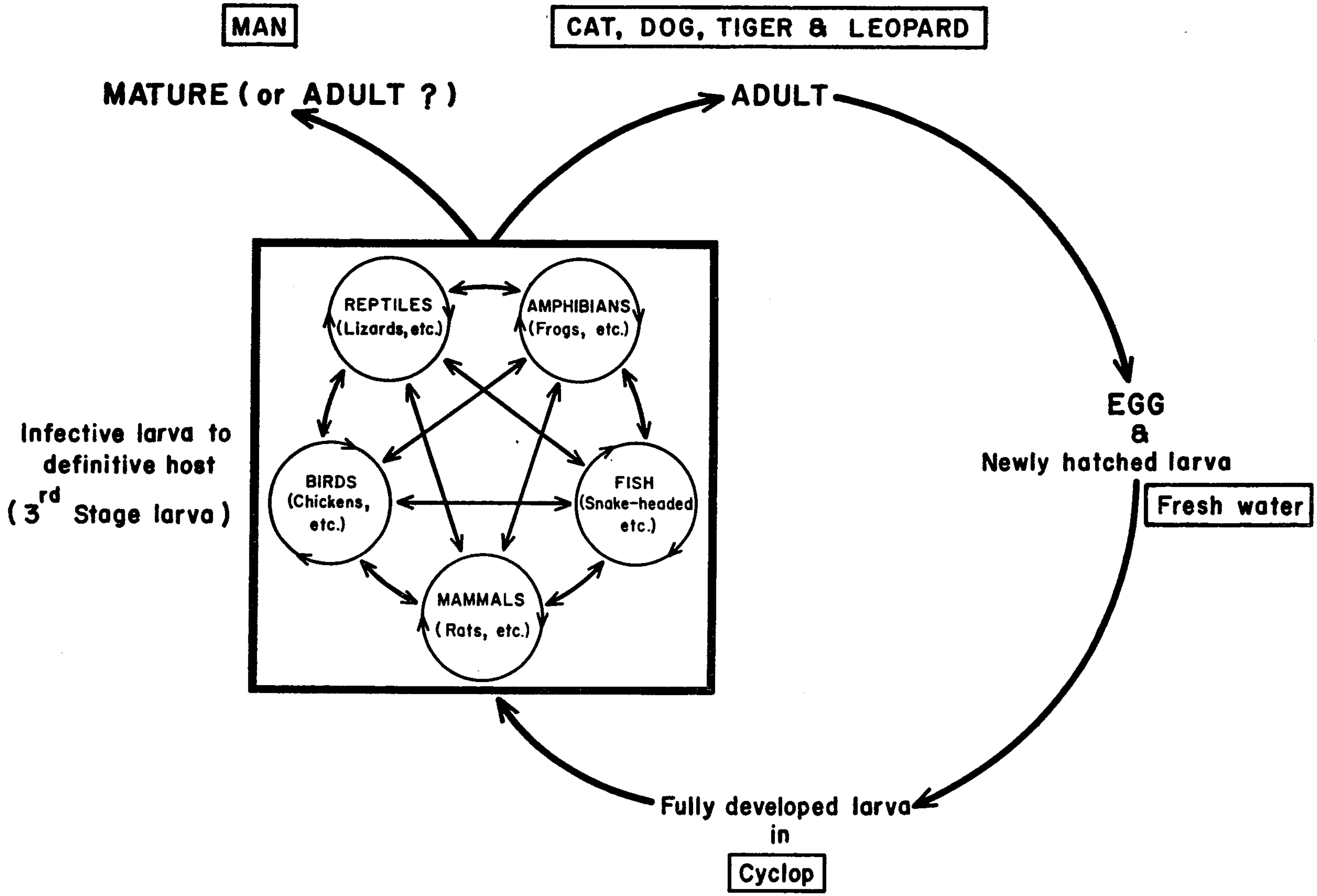

FIGURE 2. A DIAGRAM OF LIFE CYCLE OF GNATHOSTOMA SPINIGERUM IN THAILAND

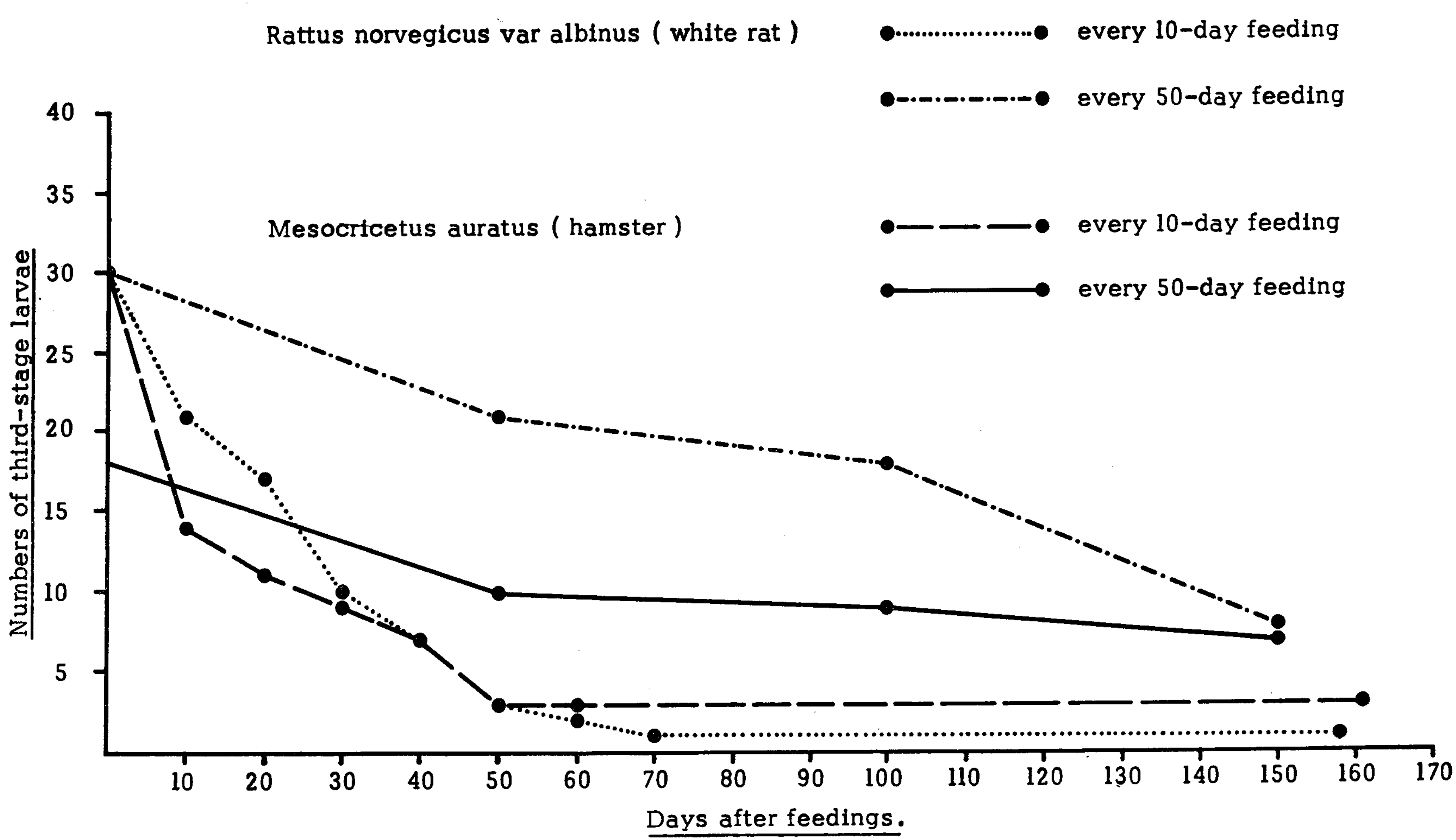

Figure- **3** Numbers of G. spinigerum third-stage larvae found in each group of 3 Rattus norvegicus var albinus (white rat) and Mesocricetus auratus (hamster) (the hamsters fed under 50-day plan were grouped in 2) sacrificed after being fed with the original 30 and 18 larvae and followed by successive feedings with the larvae obtained from preceding groups.

The life cycle of G. spinigerum therefore now needs to be revised because of the additional discoveries and can be briefly shown in figure 2.

A new study was initiated to determine possibility of prenatal transmission in the experimental host. Twenty-eight pregnant white mice obtained from the Department Veterinary Medicine were each fed with 2 to 6 third-stage larvae. Some of the animals were given infective feeds every 2 to 6 days until the birth of the young. Two days after the birth of offspring, mothers and youngs were sacrificed and examined for the presence of the larvae. Of 208 suckling mice born from 27 infected mothers 2 were positive of which each had one unencysted third-stage larvae in costal muscles. The third mother had a larvae in the uterine muscles. This experiment therefore has clearly proved for the first time that prenatal transmission of larvae from mothers to offspring can occur in mice. Similarly, human gnathostomiasis may possibly be prenatally transmitted through infected mothers. The study on determination of the possible migration of the third-stage larvae into chicken eggs was continued with full co-operation of Kasetsatra University in providing on request free of charge the egg-laying hens and the feed for which we rae thankful. If these experiments prove to be positive, the infected hen's egg may constitute another important source of human gnathostomiasis. Each hen was repeatedly fed with about 5 third-stage larvae at intervals of two to three weeks. One hen was served as control. During the period covered by this report there were 555 eggs and 241 eggs for the last year all were found negative but more eggs need to be studied.

Another experiment was designed to determine whether the paratenic hosts may limit the number of the larvae infected after successive feedings of the parasite. For this purpose, groups of three Rattus norvegicus var albinus and groups of two to three Mescricetus (hamster) were used. In one experiment the initial 30 third-stage larvae were fed to 3 white rats of each plan and only under the 10-day plan the initial 30 larvae were given to 3 hamsters while initial 18 larvae were fed to 2 hamsters under 50-day plan. Every 10 days and 50 days the animals were sacrificed and the larvae found from the first groups of experimented animals under the two plans (10-day and 50-day) were given orally to the second groups and subsequently all the larvae discovered from the second groups were fed to the third groups until both plans of feeding and sacrifice could be completed 150-160 days after the first infection. This experiment shows that there was a significant reduction of larvae after successive feedings and that this was more marked in the group given were fed to the third groups until both plans of feeding and sacrifice could be completed 150-160 days after the first infection. This experiment shows that there was a significant reduction of larvae after successive feedings and that this was more marked in the group given larvae under 10-day plan as compared to the group infected under 50-day program (Figure 3).

A further study on then number of eggs produced per day per female G. spinigerum was continued from the previous year on 8 more experimentally infected cats. The results showed each female worm in each cat produced daily the following numbers of eggs: 14,000, 18,000, 39,000, 45,000, 48,000, 64,000, 65,000. Tentatively the results of eggs-count made on 8 experimental cats for the present showed much variation from 14,000-65,000 eggs, however eggs production in 4 cats varied slightly from 39,000 to 48,000 eggs per day per female worm. During this period a naturally infected cat showed 95,000 eggs per day per female worm. In 3 experimentally infected dogs the eggs-count were about 42,000, 90,000, 75,000 per day per female worm.

The infectivity rate of the third-stage larvae fed to 8 cats and 3 dogs showed the following variations for cats 12%, 27%, 97%, 52%, 80%, 40%, and 13% and in 3 dogs showed 1.8%, 11.8%, 43.%. The patent period in 6 experimentally infected cats were varied from 65 to 223 days.

With regard to the spontaneous cure of the infection with the worm in infected dogs and cats, it was shown that 5 experimentally and 1 naturally infected cats killed on 18 to 64 days after stools negative for gnathostome ova showed healthy stomachs and on worm in 3 cats killed on 40, 57 and 64 days but in two cats there was still in each stomach a small thickend area at the cardiac part when sacrificed 29 and 49 days after negative stool examinations. No worms were found in these old lesions. Only one cat killed after 18 days of negative stools showed one immature female in a small thickened area at cardiac part of the stomach. It is obviously proved by this preliminary experiment in cats that spontaneous cure of infection with G. spinigerum is possible after patent periods of 65 to 223 days. Further study on this problem is still in progress.

The effect of infection with G. spinigerum third-stage larvae on changes of peripheral white blood cells was again studied in 8 experimentally infected monkeys (Macaca speciosa) and Macaca irus and one control Macaca speciosa (#5) with the following results.

Monkey #1 showed slight increase of white blood cells and also an increase in eosinophilic cells after being experimentally fed 25 third-stage larvae obtained from a mouse. A few days later the animal was sacrificed and found to be infected with 11 encysted larvae of the worm in its flesh.

Monkey #7 fed with 500 fully developed larvae in cyclops also showed a slight increase of WBC from 43 days up to 53 days and a moderate increase in number of eosinophilic cells from 36 days up to 53 days, three days later it was killed and found to be infected with 3 encysted and 1 un-encysted third-stage larvae in its flesh and liver respectively.

Monkey #8 fed with 85 third-stage larvae obtained from a snake showed also a moderate increase of white blood cells from 15 days to 49 days but a great increase in number of eosinophilic cells from 15 days to 63 days and it showed 11 encysted third-stage larvae in the flesh when sacrificed 2 days after the last examination.

Monkey #10 fed with 85 third-stage larvae removed from a snake showed only a slight increase of WBC from 15 days to 21 days after feeding but a great increase in number of eosinophilic cells from 15 days to 34 days. This animal is to be further studied.

Monkey #6 fed with 537 second-stage larvae in cyclops showed no increase in WBC or eosinophilic cells when examined from 48 days up to 65 days after the feeding. This monkey showed 2 encysted third-stage larvae of G. spinigerum in its flesh when it was sacrificed on 68 days after the infection.

Monkey #4 was given intraspinally 2 third-stage larvae obtained from a snake. It showed neither changes in total white blood cells nor eosinophilic cells when examined at intervals up to 528 days. It showed no infection on autopsy.

The other monkeys (#5, #9, and #13) are to be further studied.

The plan for preliminary determination of skin sensitivity of some laboratory animals experimentally infected with the third-stage larvae by intradermal testing with unfractionated lyophilized antigens prepared from adults and the third-stage larvae of G. spinigerum was further developed as described in the previous Annual Progress Report for 1 April 1965 to March 1966. During this period 8 monkeys (Macaca speciosa #1, #4, #6 and#7, Macaca irus #8, #9, #10 and #13) were fed with known numbers of G. spinigerum larvae and tested at interval with the antigens. The results of the experiment showed Monkey #1 (Macaca speciosa) save a positive skin test from 3 days up to 51 days of the feeding. This animal showed 11 encysted third-stage larvae in its flesh when killed.

Monkey #6, #7 fed with 537 and 500 fully developed larvae in cyclops respectively and monkey #6 was skin tested positive on 48-65 days with 2 encysted third-stage larvae in its flesh on autopsy and monkey #7 was skin tested positive 36-53 days with 3 encysted larvae in the flesh and 1 unencysted larva in its liver.

Monkey #4 was skin tested positive on 356 days up to 531 days after intraspinal an inoculation with 2 larvae obtained from a snake. However, the animal showed no lesions and no worm when sacrified on 567 days of the experiment.

Monkey #8 was fed with 85 third-stage larvae and an intraspinal inoculation of 5 larvae obtained from snakes showed negative skin test up to 365 days after intraspinal inoculation and 64 days after feeding.

Monkey #9 was intraspinally inoculated with 4 larvae obtained from a rat showed positive skin test on 153 days up to 293 days of the experiment. This animal is to be further studied.

Monkey #10 was fed with 85 larvae obtained from a snake showed positive for a short period when skin tested on 105 days of the experiment. It is also to be further studied. Monkey #13 was fed with 200 fully developed larvae in cyclops showed positive skin test on 21-96 days after feeding. The monkey is to be further investigated.

In summary on the result of skin test on monkey showed for 8 animals, 4 (#1, #6, #7, and #13) positive skin reaction appear about 3 week up to 96 days of the feeding with third-stage and second-stage larvae. 2 monkeys (#4 and #19) were skin test positive about 150 days up to 531 days after intraspinal inoculation wtih 2 and 4 third-stage larvae but one monkey (#4) showed no lesion and no worm when sacrificed few days after the last test. 2 smonkeys (#8 and #10) were both given intraspinal inoculation followed by oral feeding with the third-stage larvae obtained from snakes showed negative skin test in one monkey (#8). This negative monkey had infection with 11 encysted third-stage larvae in its flesh when sacrificed about 65 days after feeding or 456 days after intraspinal inoculation. The positive monkey (#10) is still kept for further study.

The results of skin test made on 6 white rats (Rattus norvegicus var albinus) and 4 rabbits (Oryctolagus cuniculus L.) are as follows:

Of 6 white rats 3 showed positive on 55 days up to 92 days after each being fed with 20 third-stage larvae and 3 others were negative when tested up to 146 days in 2 rats and 150 days in one rat after the experimental feeding each with 20 third-stage larvae. All rats were proved to be infected with 3 to 13 encysted third-stage larvae in their flesh on autopsy.

Of 4 rabbits, 3 were skin test positive on 71 to 104 days after being fed with 4, 20, 20 third-stage larvae respectively. The negative rabbit was fed with 20 larvae and died 43 days after the experiment. All rabbits were proved to be infected 2 to 14 encysted third-stage larvae on autopsy.

Pathological study

A detailed study to determine pathological changes of organs in white mouse (Mus musculus musculus) after being experimentally fed with 5-8 third-stage larvae and sacrificed on 1 day up to 6 months after the feeding was undertaken of which the preliminary results of 1 day up to 12 days are summarised as follows: Of 6 white mice sacrificed 1-3 days, one stomach showed 2 larvae in its submucous and muscular layers which had congestion of blood vessels, early pressure atrophy of muscles around the larvae with few lymphocytic neutrophic polymorphonuclear leucocyte infiltration. The small intestine showed at few places of similar reaction to that of the infected stomach with the presence of the larvae. Localized peritonitis was found around the infected areas of stomach and intestine and mainly infiltrated with lymphocytes, some polymorphonuclear leucocytes and few eosinophilic cells. All 6 livers showed a few congested and hemorrhagic areas together with a few yellowish white tracts of about 1.0 to 2.0 mm. X 0.5 to 1.0 mm. in size which were first seen at the lower surface and followed later at the upper surface of the left lobe. On the lower surface of left lobe of some livers there were little grayish white exudates similar to that found around the infected stomach and intestine. One to 4 third-stage larvae were found in only 3 of 6 livers examined. Microscopically, sections of the liver revealed cystic spaces of varying size. The cyst is composed of fibrinoid material as a wall and contain lymphocytes, neutrophilic polymorphonuclear leucocytes and a few eosinophiles. Some of these cysts contain purulent exudates consisting mainly of neutrophilic polymorphonuclear leucocytes and cell debris thus becoming abscess of varying size. The liver cells around the abscesses showed not much change except for some pyknotic nuclei. The blood vessels are severely congested and in few places there are small collections of red blood cells outside the vessels. In two mice there were a few small hemorrhagic areas scattered on the upper part of the costal surface of the left lungs and also of the right lung in one mouse.

Larvae were also found lying freely in costal muscles of 2 mice and in abdominal muscles and right hind leg muscles of one mouse with congestion of blood vessels around the larvae.

In the mesentery of 1 mouse a slight congestion of blood vessels was seen around the unencysted larvae.

Of 6 mice sacrificed 4-7 days after the feeding experiment each with 5-8 third-stage larvae, 3 showed 3 to 4 larvae in costal muscles and livers, 2 had 1 to 5 larvae in the livers and 1 had 1 larvae in its costal muscles. Pathologically the livers showed similar changes as seen in the group infected for 1-3 days except more damage was seen in the left lobes of livers and in one mouse (#123) the right lobe started to show few small areas of congestion and fibrinous changes at its left side adjacent to the left lobe otherwise normal.

Of 7 mice sacrificed 8-12 days after each being fed with 5 third-stage larvae, 4 showed 2 to 4 larvae in livers and muscles of neck, fore-leg, intercostal and abdominal wall and in mesentery, and 3 had 2-4 larvae in the muscles of fore-leg, abdominal wall and costal region.

On the upper and lower surfaces of left and right lobes of all livers there were many small irregular grayish white tracts of about 1.0 to 2.0 mm. X 0.5 to 1.5 mm. among which a few small hemorrhagic and congested spots were seen. Infected muscles and mesentery were slightly congested around the larvae other-wise normal. Microscopically the livers, muscles and mesentery showed similar pathological changes to those of 4-7 days after being infected except there were more areas of the liver tissue involved.

To help elucidate the epidemiological aspects of gnathostomiasis, an investigation for naturally infected cyclops with fully developed larvae (second-stage larvae) of G. spinigerum was undertaken by collecting and examining 5150 cyclops from few selected ponds of the South and from some ponds in Bangkok area. No infected cyclops were found.

Summary: Human gnathostomiasis in Thailand is highly endemic. The disease seems to be least endemic in the southern region, however it can be reasonably assumed as a result of personal visit and communication to four southern provinces that approximately 15 to 20 cases may be expected yearly. With regard to animal definitive hosts acting as reservoirs of the infection discovered during this year. 106 (2.3%) dogs in Bangkok and Thonburi except one in Nakornsrithammarat were positive only during the rainy and early part of dry seasons of the year. Other animals were negative.

To determine the natural infection of cyclops with gnathostome larvae 5,150 cyclops from Bangkok and the South were found to be negative.

Examination for natural infection of animals with G. spinigerum third-stage larvae showed two more species of animals than previously recorded namely Ophicephalus lucius (snake-headed fish) and Trimeresus gramineus (green pit viper), This year among animals found to be naturally infected with the larvae, snake-headed fish, eels and frogs still showed higher a prevalence of infection than other species.

Experimentally 23 species of animals in five classes were found to be acted a sparatenic hosts of which 9 were newly recorded. Of these 9 species, 4 including Physignatus cocincinus (weaver bird), Coturnix coturnix (quail) and Rattus exulans (polynesian rat) were not as yet found naturally infected with the larvae 9 species of animal also were newly proved to be infected with the third-stage larvae after being fed with. fully developed larvae in cyclops (second intermediate hosts).

Additionally some of these experimentally infected animals were common laboratory amimals and can be used effectively as experimental hosts.

An experimental study to determine prenatal transmission of the larvae in pregnant white mice has definitely shown that 2 suckling mice from two pregnant white mice were positive, each with one unencysted third-stage larvae in its costal muscles.

The present findings of new natural and experimental second intermediate and paratenic hosts are expected to further contribute to the existing knowledge on life cycle and some epidemiological aspects of G. spinigerum. For clarification of the above-mentioned knowledge gained by this study, a revised diagram of life cycle of the helminth is also presented.

The investigation on egg production and spontoneous disappearance of adult worms from infected cats being continued from last year and egg production of the worms in infected dogs also initiated this year has given further knowledge on the spread of infection. Infectivity rates of the third-stage larvae in cats and dogs showed much variations from 13.0% to 97% in cats and 1.8% to 43% in dogs.

The preliminary result of the study on detailed pathological changes of the infected organs of experimented mice were presented from 1 up to 12 days of infection. A plan of study on the development of fully developed larvae in cyclops after being fed to mice and pathological changes of the infected organs caused by their presence on different days was also initiated of which the result will be presented in the future. These experiments are still in progress.

Results of preliminary studies of skin sensitivity on experimentally infected laboratory animals including 6 white rats, 4 rabbits and 8 monkeys were presented. This study is to be continued.

The result of a study made on white blood cell changes in peripheral blood of 8 monkeys after fed or and inoculated with the third-stage larvae obtained from second intermediate hosts or after being fed with second-stage larvae in cyclops it has been shown that some infected monkeys had a short period of leucocytosis and eosinophilia. Further study on this problem is to be undertaken before any conclusion is made.

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