SEATO Medical Research Studies on Gnathostomiasis in Thailand

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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 at Thonburi Mental Disease Haspital showed damage ta the CNS by a gnathostome. After World War 11, many hundreds of human cases of gnathostomiasis were found in the Prefectures of Kyushu, Hanshu and Shikoku in Japan and the disease seems ta be extending gradually in that country. Few human cases of infection with gnathostomes have been reported from other countries in Asia. 18 species of Gnathostoma have been recorded in the literature of which 6 seem to be recognized as distinct species. Among them, only Gnathostoma spinigerum was reported frequently as the causative agent 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 the mature worms in subcutaneous 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 but many aspects of its epidemiology, individual and community preventive measures, diagnosis, pathological changes and treatment have not been fully studied. The present studies on gnathostomiasis aim to achieve solutions for the above - mentioned problems.

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 study including individual and community preventive measures, methods of diagnosis, pothological changes of infected organs and treatment of the disease. Several years before World War 11, dogs and cats were shown to be natural or definitive hosts of the parasite in this country. Later, tigers and leopards were found infected with adult worms in their stomachs. These animals can act as reservoir hosts by passing eggs of the worm in their stools. The life cycle and the method of transmission of this helminth were worked out in 1936, but many unsolved problems including epidemiological aspects and preventive measures remain to be clarified.

Human cases of gnathostomiasis have been recognized in this country as due to only only one species of Gnathostoma, that is G. spinigerum. The effect has been lesions of respiratory and visceral organs, lesions of skin and mucous membranes and central nervous syctem damage in one instance. This 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 not yet any effective chemotherapy; surgical removal of the worm from the infected organs was successful in only a few haman cases in which non - vital organs were involved.

Discription : The regional prevalence of haman gnathostomiasis was investigated by collecting cases reported on prepared data - report forms from hospitals and by personal communication with medical practitionsers, health officers, and nurses. To determine the significance of animals as definite or reservoir hosts of the disease, examinations of stools for the presence of ova of the worm and gastrointes - tinal tracts for adult worms among cats, dogs and other suspected wild and domestic animals in Bangkok and other areas were made. Concurrently, studies to determine the natural animal sources of infection to definite hosts and to man were done by examination of fresh - water fish and other animals. Much attention was paid to examination of animal foods. Experimental infection of many laboratory and other animals with the third - stage and second - stage larvae of the parasite was performed to determine possible or potential second intermediate hosts, to dedtermine susceptible animals that could be used as laboratory hosts and lastly to study detailed development and viabilty of the larvae in vivo and in vitro.

Skin testing with unfractionated antigen made from the worm and hematology changes were investigated in monkeys, rats and rabbits. Pathological changes of infected organs and prenatal infection caused by the third - stage and second stage larvae of the worm were studied in non - pregnant and pregnant laboratory animals. A plan for the study of the migration of the infective larvae to chicken eggs by feeding the hens at the egg - laying stage with the larvae was initiated.

Progress : To determine the yearly prevalence of human gnathostomiasis by regions of the country many local medical practitioners in highly endemic areas including the Provinces of Lopburi, Saraburi, Ayuthya,

Supanburi, Angthong, Samut Prakarn, Nondnburi, Nakorn Pathom, Bangkok and Thonburi in the central part and five North-east provinces namely Ubolratchathani, Nakorn-ratchasima, Roi-ed, Udorn-thani, and Nougkai and the northern provinces of Chiengmai, Lampang and Maesariang District of Mae-hongsorn province were interviewed. 85 provincial hospitals submitted the reports together with one rural health center and 7 hospitals in Bangkok. They reported a total of 2786 suspected cases of gnathostomiasis during three-year period (1961, 1962, and 1963) of which 2242 cases were from central Thailand while only 6 cases were from the southern part of the country. These data suggest a higher prevalence of the disease in central Thailad (Fig 1). Approximately 900 suspected cases are identified annually for the whole country. However as a result of personal communications with medical practitioners, health officers, hospital doctors and nurses in Lopburi, Ayuthya, Saraburi, Supanburi, Ubol and Roi-ed provinces, it was reasonably assumed that the actual cases were perhaps 10 times as many as reported since the symptoms are not continuous but may have long intervening periods in wihch the patient feels well. Also many cases may be treated at private clinics or at home.

The studies to detemine the significance of animals as definite or reservoir hosts were undertaken with the co-operation of the Rabies Control Units of Bangkok and Thonburi Municipalities, Provincial Health Offices of Ayuthya, Supanburi, Roe-ed, Maesariang, Chiengmai and Lampang as well as Bangkok and Chiengmai Zoological gardens. In Bangkok area, of 1422 dogs 32 (2.2%) showed positive, only one leopard cat (Felis bengalensis) among 32 wild carnivorous animals in Bangkok Zoo showed presumably ova of G. spinigerum in its stool., 143 cats examined were negative. In Ayuthya, 7 dogs and 7 pigs examined were negatiue but one cat was positive. In Supanburi, of 37 cats, 2 (5.0%) were positive but 115 pigs were negative. In Ubol Province, 5 (12.0%) of 40 domestic cats were positive but 37 dogs, 142 pigs and one civet cat (Paguma larvata) were negative. In Roi-ed, of 22 cats examined, 2 (9.0%) were positive but 18 dogs and 21 pigs were negative. In summary, 32 (2.0%) of 1623 dogs and 10 (3.0%) of 306 cats examined were positive and only one leopard cat (Felis bengalensis) among 57 wild carnivorous animals examined. (Table 1)

Determination of natural infection of fresh-water fish and other animals carrying the third-stage or infective larvae of G. spinigerum to human beings and definite hosts were undertaken. Concurrently, experimental fexdings with third-stage larvae were carried out on many species of ainimal determine possible or potential seccond intermediate hosts, what animals could be appropriately used as laboratory hosts and lastly detailed studies of development and viability of the larvae in vivo and in vitro. All animals were examined for the infective larvae in their flesh and visceral organs.

Examination for natural infection of animals with G. spinigerum third-stage larvae showed them encysting in the flesh and mesenteries of more species of animals than previously reported. These animals were collected from many provinces in the central, north-eastern and northern regions. Ot 2298 fresh water fish examined, 72 (3.13%) were found infected among which snake-headed fish (Ophicephalus) showed highest rate of infection. The average number of larvae per infected fish

Table III

Animals Negative for G. spinigerum

Class	Species	Number	Species	Number
Crustacea	Paratelphus sexpunctatum (Fresh water crab)	81	Palaemon potamiscus (Fresh water shrimp)	2410*
Pisces	Clarias batrachus	59*	Anabus testudineus	109*
	Puntius bramoides	25*	Notopterus notopterus	72
	Puntius gonionotus	38*	Cyclocheiliethys siaja	497
	Osteogeneiosus militaris	14	Tilapia mossombica	11
	Trichopodus trichopterus	31	Trichopsis vittatus	42
	Bagroides macrocanthus	65	Kryptopterus apogon	13
	Cultrop siamensis	150	Penaeopsis ovirostris	547
	Osteochilus hasseltii	31	Synbranchus bengalensis	12
	Catopra siamensis	10	Labiobarbus leptocheilus	78
	Dangila leptochilus	78*		
Amphibia	Bufo melanostictus	60	Callulutula pulchra	47
	Rana erythrea	40*		
Mammalia	Rattus exulans	137*	Callosciuris erythraeus	18
	Rattus norvegicus	709*		

* Represents collection from more than two provinces.

Table I

Examination of animals for natural infection with adult Gnathostoma spinigerum by stool or stomach examination

SPECIES	BANGKOK	AYUTHYA	UBOL	ROI-ED	CHIENGMAI	LAMPANG	MAESARIENG	LOPBURI	SUPANBURI	TOTAL
Canis domestica										
Examined	1422	7	37	18	75	59	5	-	-	1623
Positive	1422	7	37	18	75	59	5	-	-	1623
	32	0	0	0	0	0	0	-	-	32
%	2.2%									2.0%
Felis domestica										
Examined	143	1	40	22	38	18	3	4	37	306
Positive	0	1	5	2	0	0	0	0	2	10
%			12%	9%					5%	3.26%
Sus scrofa domestica										
Examined	-	7	142	21	330	150	-	-	115	765
Positive	-	0	0	0	0	0	-	-	0	0
%										

Table II

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

	BANGKOK	THON-BURI	AYTHYA	UBOL	ROI-ED	CHIENG-MAI	LAMPANG	MAE-SARIENG	LOPBURI	SUPAN-BURI	PETCH-BURI	SARA-BURI	RAJBURI	ANG-THONG	PRATUM-THANI	NAKORN-RACHA-SIMA	PITSA-NULOKE	UDORN	CHA CHOENG SAO	NAKORN-NAYOK
Ophicephalus striatus																				
Snake headed fish Large	6/11	6/10	19/23	3/5	0/1	1/14	0/2		2/2	3/3	1/1		4/12	17/26	2/3	1/5	0/6	1/4	1/5	
Small	0/4			0/3								4/75								
Clarias macrocephalus* Fish			1/3	0/5						1/5										
Ompok bimaculatus* Fish			2/138																	
Rana rugulosa Frog	2/3			0/289	0/20					1/4								0/19		
Rana limnocharis limnocharis* Frog		1/292																0/38		
Gallus gallus domestica* Domestic chicken			1/2	2/3	1/3	0/6	0/4	0/2	1/2	1/3		1/3		0/3				1/2		
Anas platyrhyncus domestica* Domestic duck	0/1		1/2	1/3	0/3	0/2	0/3	0/2	1/3	0/3		1/3		1/4				0/1		
Bandicota indica*	4/66		1/5	0/5		0/8				1/9		0/2				1/33		0/10		1/30

* Not previously recorded in literature

varied from 1 to 49. Of 212 snake-headed fish (Ophicephalus) examined, 68 (32.0%) were positive. At lower rates were cat-fish (Clarias) 2.8% and sheatfish (Ompok) 1.5% (Fig.2). The fish from which larvae were recovered are listed in Table II. Fish from which larvae were not recovered and of which more than ten specimens were examined are listed in Table III. In addition the following species of which only a few specimens were examined were negative; Plotosus lineatus, Bagroides macroterus, Zenachopterus, Notopterus chitala, Wallagonia attu, Trichogaster pectoralis, Trichogaster trichogaster, Oxyeleostris siamensis, Pangasius fowleri, Nematolosa nasus, Hampala dispar, Morulins chrysophekadion, Oxygaster oxygastroides. Among 812 amphibians examined, only 4 frogs of two species (Rana regulosa and Rana limnocharis) were positive. The results in snakes from Bangkok were as follows. In 4/5 cobras (Naja hannah) an average of 31 larvae were found, in 94/524 cobras (Naja naja) an average of 42 larvae per animal were present. A single larva was found in a wart snake Achrochordus javanicus which is the first time it has been recorded in this snake.

Also for the first time, two lizards Varanus salvator from Ayuthya were positive with an average of 37 larvae.

No larvae were recovered from seven Vipera russelli, one Cylindrophis rufus, one Python reticulatus one Simotes cyclurus, three fresh water snakes, two banded kraits (Bungarus fasciatus) and two turtles (Domonia subtrijuga).

Among birds, domestic chickens and ducks (Table II) have been shown for the first time to be natural hosts. Also for the first time, the crow (Corvus macrorhynchus) was found positive in six out of nine specimens. Four other birds were found positive for the first time; one specimen of the cattle egret (Bubulcus ibis coromandus), one of two little egrets (Egretta gazetta gazetta), one little green heron (Butorides striatus) and one pelican (Pelecanus philippensis). Specimens of faurteen other species were negative. A list of all species and the location where collected is available on request.

In addition to the bandicoot rat (Table II) three other mammals have been found positive for larvae for the first time: of 135 specimens of the tree shrew (Tupaia glis) representing five provinces, two were positive; one of three mongoose (Herpestes javanicus); of 1442 specimens of Rattus rattus from thirteen provinces one rat from Lopburi was positive. Several species which were negative are listed in Table II A. Species of which less than ten specimens were examined and which were negative are Suncus murinus (muskrat), Mus mumculus, Macaca irus (Cynomolgus).

The animals collected from the central area had higher prevalence of infection with the third-stage larvae than those from other regions. Among newly discovered animals infected with the larvae, Gallus gallus domesticus (domestic chicken) and Anas platyrhynchus (domestic duck) are important sources of meat to Thai people. Chickens are frequently eaten uncooked in the form of a preparation called "Larbkai" by people in the north-eastern area; partially roasted chickens are a favorite dish for many people. The detailed findings of naturally infected animals are shown in Table II.

Experimental infection of certain animals with known numbers of the third-stage larvae of the helminth for determination of possible or potential second intermediate hosts showned that 24 species of animal in five classes had larvae in livers and or flesh . Of these experimentally infected animals, 3 species Tupaia glis (tree shrew), Gallus gallus domesticus (domestic chicken) and Anas platyrhynchus (domestic duck) were also found naturally infected with third-stage larvae. These larvae were found mostly in livers of the experimentally infected animals from 4 to 15 days after feedings. Thereafter they were commonly found singly in the flesh with formation of a fibrotic wall around each living larva about 30 days after the feeding. Larvae persisted as long as 222 days in one domestic pig and 172 days in

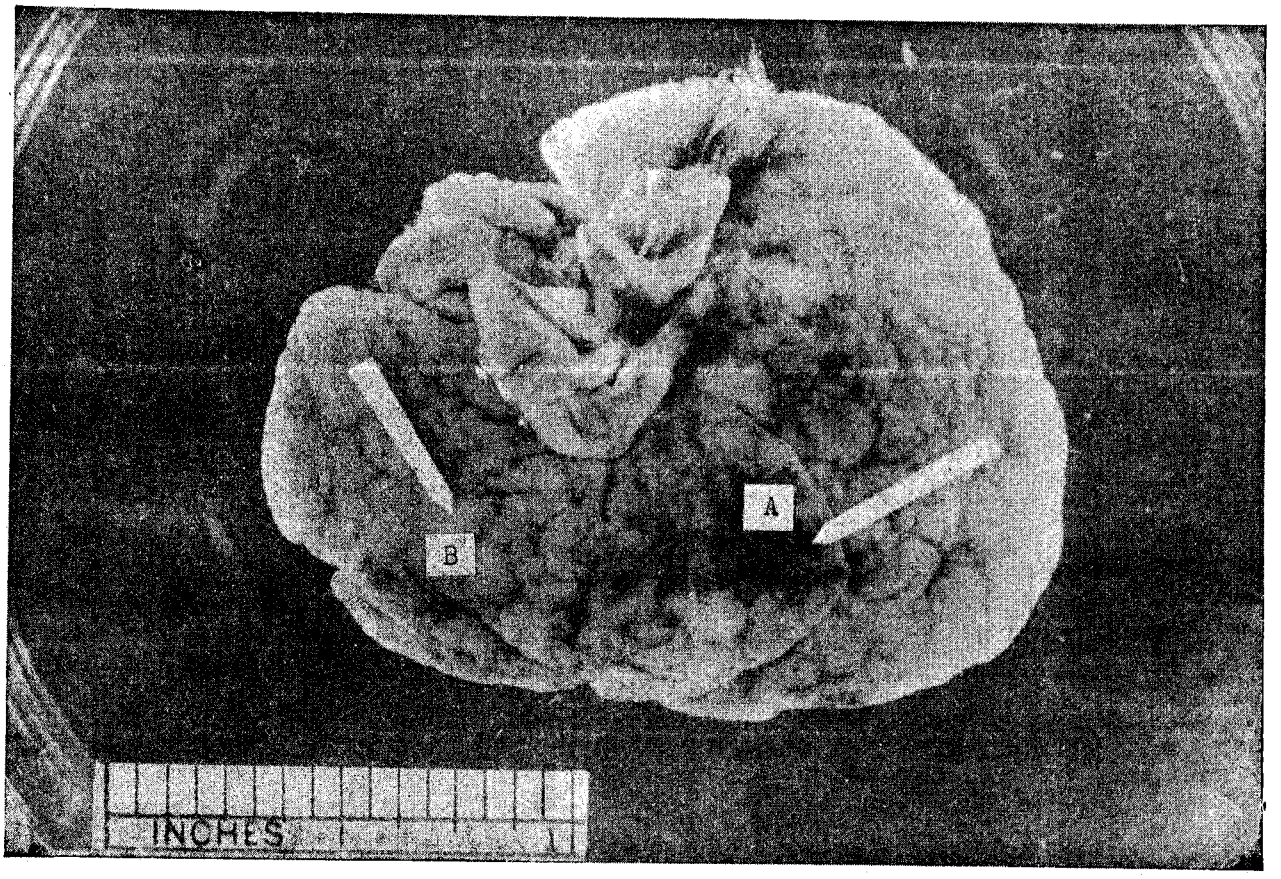


Fig 3. Tree shrew's liver experimentally infected by feeding with second-stage G. spinigerum larvae in cyclops showing scattered hemorrhagic areas and yellowish-white markings, presumably fibrotic reaction and resolution, caused by the development and the pressence of third-stage larvae twenty days after feeding. (A=a hemorrhagic area. B=a normal area).

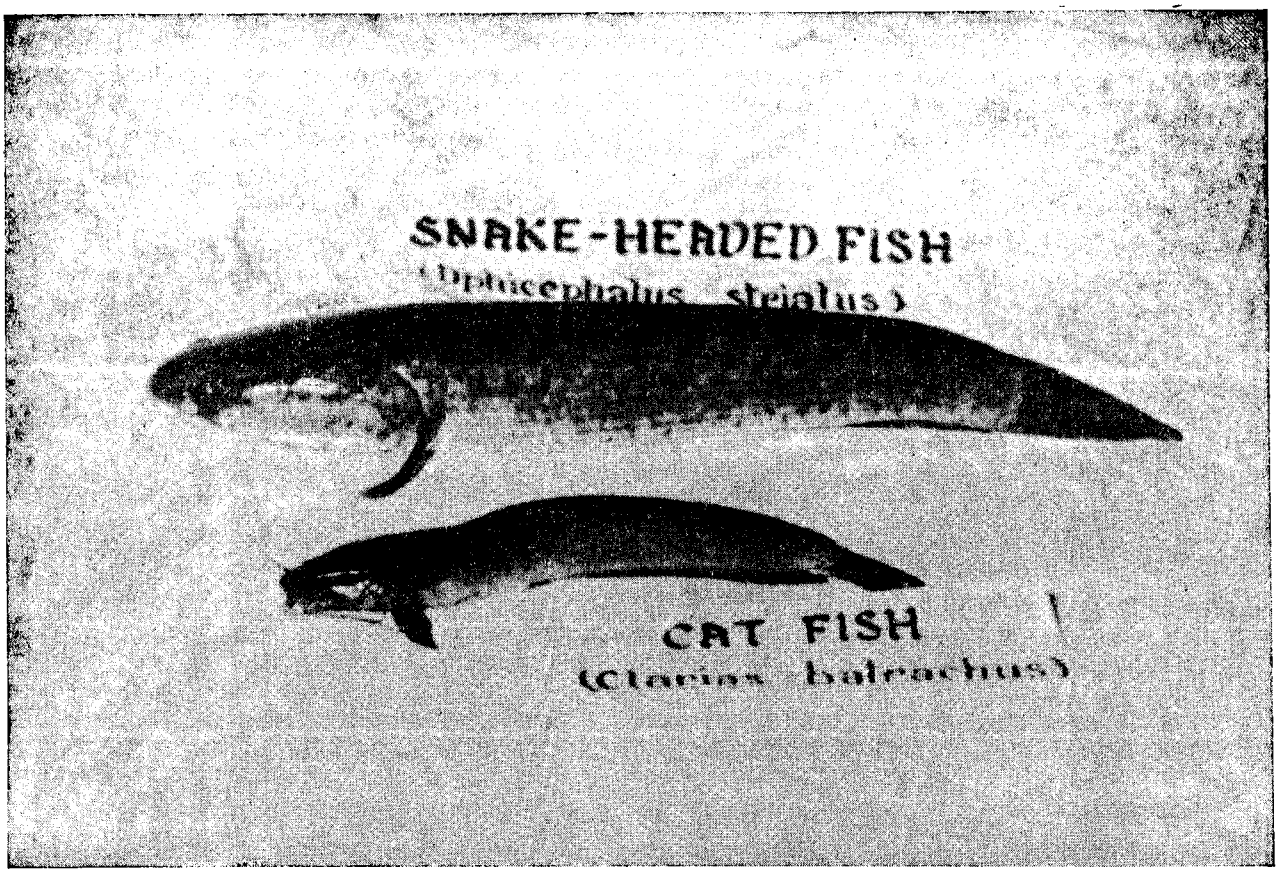


Fig 2. Snake-headed fish (Ophicephalus striatus) and cat fish (Clarias batrachus) showed the infection rates of 30.8% and 2.8% with G. spinigerum respectively. They probably constitute one of the important sources of human gnathostomiasis.

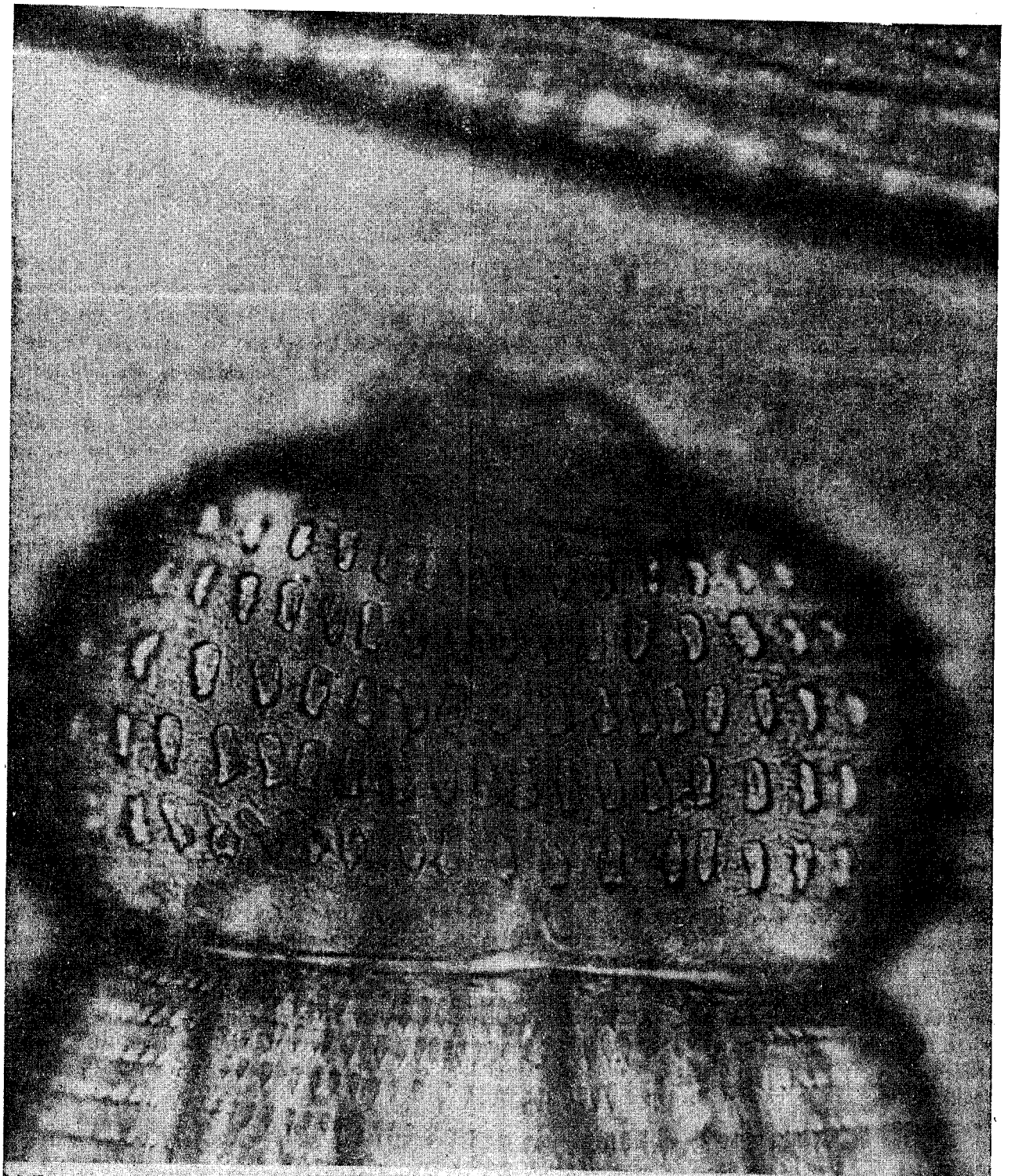


Fig.4. The only third-stage larva of G.spinigerum with 5 rows of cephalic spines developed from the second-stage in liver of a tree shrew.

one hamster at which times the animals were sacrificed. An interesting finding is that one living third-stage larvae without a cyst wall was found in cardiac muscle of one turtle (Domonia subtrijuga) sacrificed 14 days after feeding on third-stage larvae removed from snakes. There were exceptions in that 2 ground lizards, 1 tree lizard, 1 small fighting fish, 1 tiny frog, and 1 bull frog did show the larvae in their flesh at much earlier dates; 6, 7, 12, 8 and 4 days respectively.

Some experimental feedings were undertaken to determine if species of animal other than fresh-water fish acts as second intermediate hosts. Of 16 hamsters (Mesocricetus auratus), 11 (68.7%) were positive for thirds-tage larvae in the liver from 8 to 26 days. Of 18 white rats (Rattus norvegicus var albinus), 12 (66.6%) were positive with third-stage larvae in the livers from 7 to 30 days and in their flesh at 25 to 90 days. Of 13 white mice (Mus musculus musculus), 12 (92.3%) showed third-stage larvae in the livers from 7 to 31 days and in the flesh from 16 to 60 days. Also 5 of 7 experimented Norway rats (Rattus norvegicus) showed third-stage larvae in livers from 15 to 30 days and in their flesh from 30 to 45 days. Tree shrews (Tupaia glis) and small frogs (Rana limnocharis) showed similar results but many chickens, ducks and two tree and monitor lizards were negative. Pathologically, the infected livers showed many scattered macroscopic hemorrhagic areas of various sizes together with yellowish-white markings, presumably fibrotic changes and resolution, similar to the changes in livers caused by experimental feeding with third-stage larvae (Fig 3). Further detailed study to determine pathological changes of infected organs caused by various stages of the worm is planned.

Studies of the morphological development of third-stage larvae in livers of cyclops showed the typical four rows of cephalic spines. One third-stage larvae of G. spinigerum in the liver of a tree shrew killed 30 days after infection, showed five rows of cephalic spines (Fig 4).

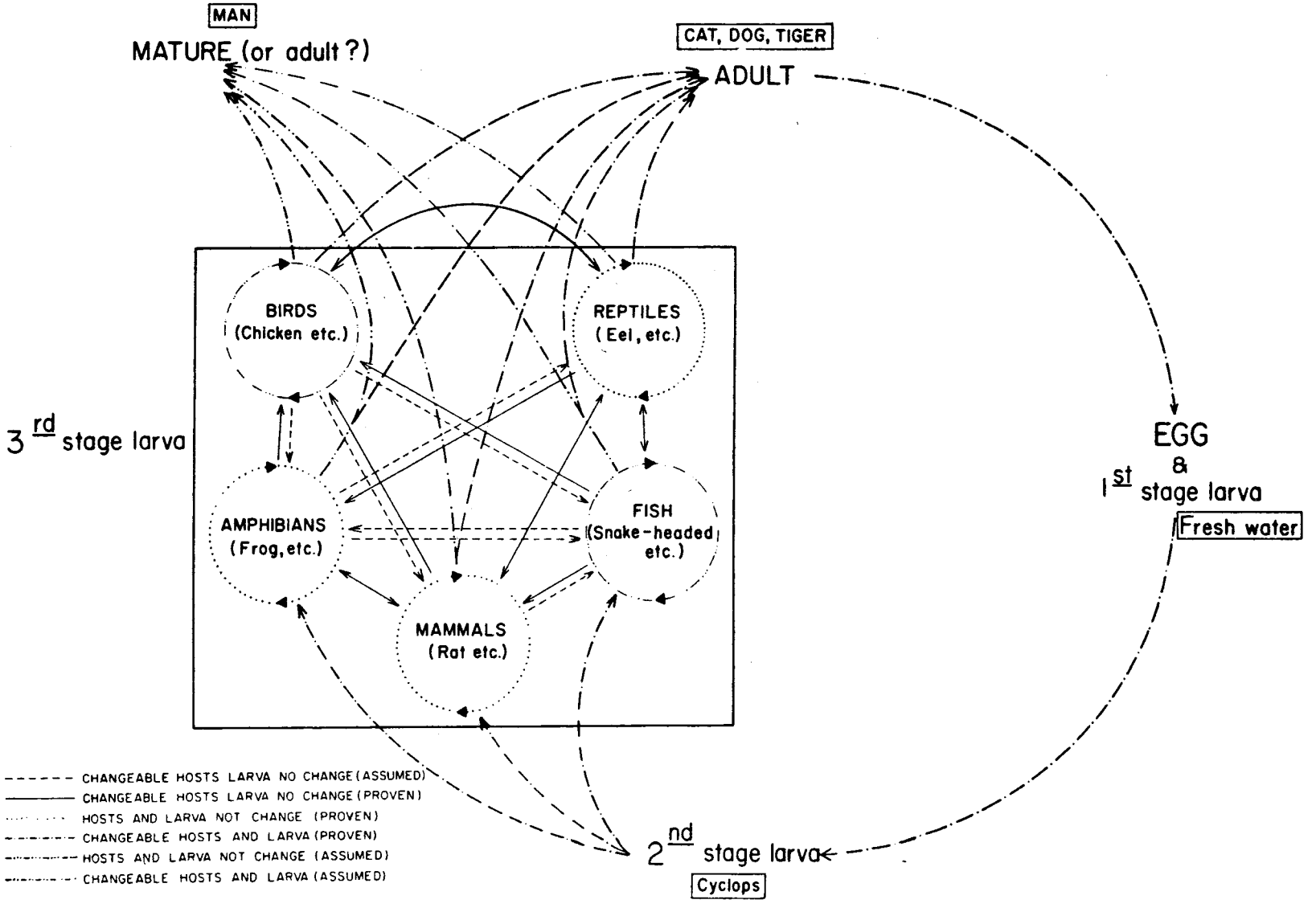
This study has clearly shown that hamsters, domestic rats, white mice, white rats, tree shrews, and small frogs can act as additional second intermediate hosts.

In summary, many species of animals other than the previously proved freshwater fishes have been shown to be second intermediate hosts. Moreover, they have been found among animals of five different classes; namely Pisces, Amphibia, Reptilia, Aves and Mammalia. 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 colour.

These experimental infections on certain animals with the third-stage and second-stage larvae have shown that many common laboratory animals namely hamster, Norway rat, white rat, white mouse, guinea pig, domestic pig, domestic chicken, domestic duck, monkey etc. were susceptible to infection with the third-stage larvae. Some animals (hamster, Norway rat, white rat, white mouse, tree shrew, and frog) were found to be second intermediate hosts, thus indicating that this helminth has more natural hosts previously reported. Domestic pigs which were positive after feeding with infective larvae may constitute one of the important sources of infection to human beings if it is found naturally infected. In many parts of Thailand, people eat pickled or fermented raw pork. A program was developed with the co-operation of Bangkok Slaughter House to examine at random about 100 kilogram of pork from 1000 pigs of central Thailand. Also some pickled or fermented pork collected at random from local shops in Chiengmai town by the Municipality Health Officer are to be investigated.

The life cycle of the worm, as it is presently known, is shown in Fig 5. (Second intermediate host means the animal in which the second-stage larvae can survive and develope into third-stage; the carrying or parathinic second intermediate host means the animal in which the third-stage larvae can survived, usually without further morphological development but slight growth). A study was initiated to determine whether third-stage larvae would migrate into chicken eggs. If these experiments prove to be positive, the infected hen's eggs may constitute another important source of human gnathostomiasis. In

FIGURE 5. A DIAGRAM OF LIFE CYCLE OF GNATHOSTOMA SPINIGERUM IN THAILAND



this connection 6 of 7 hens at the egg-laying stage which were kindly supplied together with properly prepared food by the University of Agriculture, were fed with third-stage larvae. One served as control. Up to now 241 eggs were found negative, but more eggs need to be studied.

A study on the number of eggs produced per day by female G. spinigerum was planned. Twenty cats and twenty dogs were infected, eggs passed in their stools were counted by Stoll's method. Also 15 naturally infected cats and dogs were included for comparison with those infected experimentally. Egg counts in 3 cats fed with third-stage larvae removed from snakes and other infected animals were 25169, 36671, and 52420 eggs per female worm per day. Egg counts in two naturally infected cats were 39109 and 28402 eggs per female worm per day. The infectivity rates of the larvae in the three experimantal cats were 90%, 60% and 43%. The duration of egg-laying by the female worms was 185 days, 37 days and 59 days respectively. An interesting finding was the spontaneous disappearance of the infection in one cat about 195 days after feeding or 37 days after the first finding of ova by stool examination. Cat#6 showed ova in its stool 81 days after feeding and the stool was negative at 185 days. This cat was autopsied 10 days later at which time 2 adult male G. spinigerum were found in a gastric tumor. On the other hand, the naturally infected cat had ova in its stool on 10 June 1965 and became negative on 2 September but on autopsy 4 days later, 4 adult worms (2 male and 2 female worms) were found in a gastric tumor. Another naturally infected cat showed ova for a short period of about 50 days and on autopsy 17 days later there were only 3 adult male G. spinigerum found in a gastric tumor. These preliminary findings showed spontaneous cure of infection in one cat and the other two had short egg-production periods and the last cat showed only three male worms in a gastric tumor a few days after positive stool egg-count.

Epidemiologically it ir desirable to determine whether cyclops which has already been infected experimentally, is a natural first intermediate host. 483 and 189 freshwater cyclops collected from shallow wells in Supanburi and Bangkok areas respectively were examined. All were negative. Further study will be done.

The effect of infection with G. spinigerum third-stage larvae on peripheral white blood cell counts was studied in 10 adult monkeys (9 macaca and 1 Presbytis). Three Macaca were fed with known number of larvae (60, 25, 70) removed from snakes and WBC and differential counts done at one to two week internals. One of these was sacrified for further study on the pathological change of the infected liver and the Presbytis sp. monkey died of unknown cause 114 days after the infection at which time there were 25 encysted third-stage larvae of G. spinigerum in the flesh. One monkey showed a great increase in total white blood cell counts from 7 days to 33 days and also eosinophilic cells started to increase from 7 days up to 97 days. Monkey (Macaca #2 showed a slight increase in number of white blood cells from preinfection period of 12, 450 to 17,600 cells 13 days after infection. Eosinophilic cells began to increase at 7 days. The Presbytis monkey (M#3) showed no marked increase in number of white cells but a moderate increase in number of eosinophilic cells between 32 to 96 days of infection. This monkey had 25 encysted larvae in its flesh when sacrified at 114 days of infection.

A plan for preliminary determination of skin sensitivity of some laboratory animals infected with the third-stage larvae by intradermal testing with unfractionated lyophilized antigens prepared from adults and third-stage larvae of G. spinigerum was developed. Four types of antigens made in modified Coca's solution (0.5% Nacl, 0.275% $NaHCO_3$ containing 1:5000 thiomersal) namely antigen A prepared from the whole third-stage larvae removed from snakes, antigen B prepared from the anterior parts including esophagus and cephalic glands of adult worms, antigen C prepared from posterior parts and antigen D made from the whole adult worms. All adult worms were removed from gastric tumors of dogs. Modified Coca's solution was used as control.

The preparation of antigens was as described in Bulletin of WHO. Vol 25, No. 4-5, 611-624.

Technique of intradermal test:

Inject intradermally at about one week intervals 0.10 ml. of antigens A.B.C.D. (1:10000 dilutions w/v), and an equal amount of modified Coca's solution as control in the abdominal skin of white rats, rabbits and monkeys a few weeks after being infected with a known number of third-stage larvae by oral feeding and intraspinal infection. The reactions of the injected skin were observed every five minutes for one hour. Positive reactions occurred about ten to fifteen minutes after injection (immediate type) by showing a small hyperemic area in more or less center of an elevated pale skin which had irregular border and larger than the control which gradually reduced in size. This positive reaction began to fade away about 20 minutes after the test and disappeared about 10 minutes later. Results are inadequate for conclusions to be made (Table VII).

Five rabbits were fed with third-stage larvae removed from snakes and a white rat after which they were similarly tested. Two rabbits died a few days after infection due to unknown causes at autopsy only two third-stage larvae were found in the flesh of one rabbit. The skin test on other rabbits is still in progress. 4 monkeys were skin tested, of which one was done some days after oral infection and three after intraspinal inoculation with the third-stage larvae from snakes. No conclusions can be drawn yet.

Summary: Human gnathostomiasis in Thailand is highly edemic and it appears that the incidence is tending to increase. Its potential seriousness as a possible cause of death has been recently recognized. 2786 suspected human cases during a three-year period or about 900 cases yearly were diagnosed by 92 hospitals and one rual health center of which about 80% were in central area of the country while only 6 cases were recognized in Southern part. It can be reasonably assumed as a result of personal communication that about 10 times as many cases as are reported may occur. With regard to animal definite hosts acting as reservoirs of the infection, 32 (2.0%) of 1623 dogs and 10 (3.0%) of 306 cats examined were positive. These animals may be considered as important reservoirs of human gnathostomiasis. The first reported occurrence of infection is a leopard cat (felis bengalensis) in Thailand is made.

Examination for natural infection of animals with G. spinigerum third-stage larvae showed more species of animals collected from central and north-eastern regions acting as second intermediate hosts than previously reported. Among them, Gallus gallus domesticus (domestic duck) are important sources of meat. Experimentally 21 species of animals were proved to be infected with third-stage larvae in the liver usually 4-15 days after feeding and remained infected for considerable periods.

Additionally hamster, rat, mouse, tree shrew and frog were also shown to be second intermediate hosts. Many of these experimentally infected animals were common laboratory animals and can be used effectively as experimental hosts.

The present findings of new natural and experimental second intermediate hosts are expected to contribute to the existing knowledge on life cycles and some epidemiological aspects of G. spinigerum. Preliminary investigations on egg production and spontaneous disappearance of adult worms from infected cats has given new knowledge on the spread of infection. Infectivity rates of the third-stage larvae in cats and macroscopical changes of infected livers caused by the third-stage larvae were presented. These experiments are still in progress.

Preliminary determination of skin sensitivity on experimentally infected white rats and rabbits were initiated. A study was made on white blood cell changes in the peripheral blood of monkeys fed or inoculated with third-stage larvae and the presence of short periods of leucocytosis and eosinaphilia demonstrated in some.

Publications: "Further Investigations on the Animals Infected with third-stage or Infective Larvae of Gnathostoma spinigerum in Thailand" by Svasti Daengsvang, Praja Thienprasitthi and Pasoog Chomcherngpat was accepted for publication in the forthcoming September 1966, of the American Journal of Tropical Medicine.

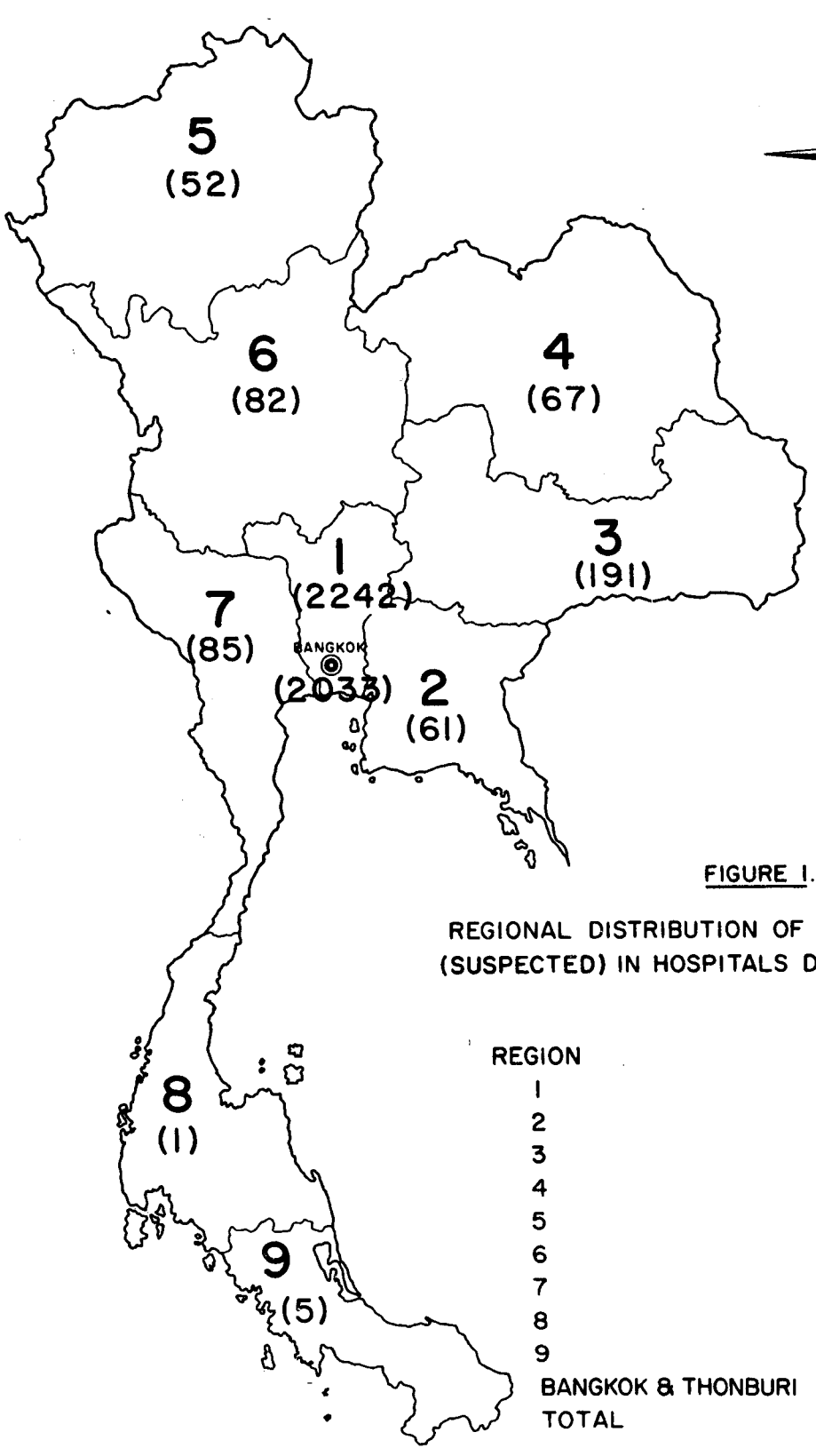


FIGURE 1.

REGIONAL DISTRIBUTION OF HUMAN GNATHOSTOMIASIS (SUSPECTED) IN HOSPITALS DURING 1961-1963

REGION	
1	2242
2	61
3	191
4	67
5	52
6	82
7	85
8	1
9	5
BANGKOK & THONBURI	2033
TOTAL	2786