

BODY OF REPORT

SEATO Medic Study No. 32 Studies on Gnathostomiasis in Thailand

Project No. 3A 025601 A 811 Military Medical Research Program
S. E. Asia

Task 01: Military Medical Research Program
S. E. Asia

Subtask 01: Military Medical Research Program
SEASIA (Thailand)

Reporting Installation: US Army-SEATO Medical Research Laboratory
APO San Francisco 96346

 Division of Medical Research Laboratories

 Department of Medical Zoology

Period Covered by Report: 1 December 1964 to 31 March 1965

Principal Investigator: Prof. Svasti Daengsvang, M.D.

Assistant Investigator: Mr. Pasoog Chomcherngpat

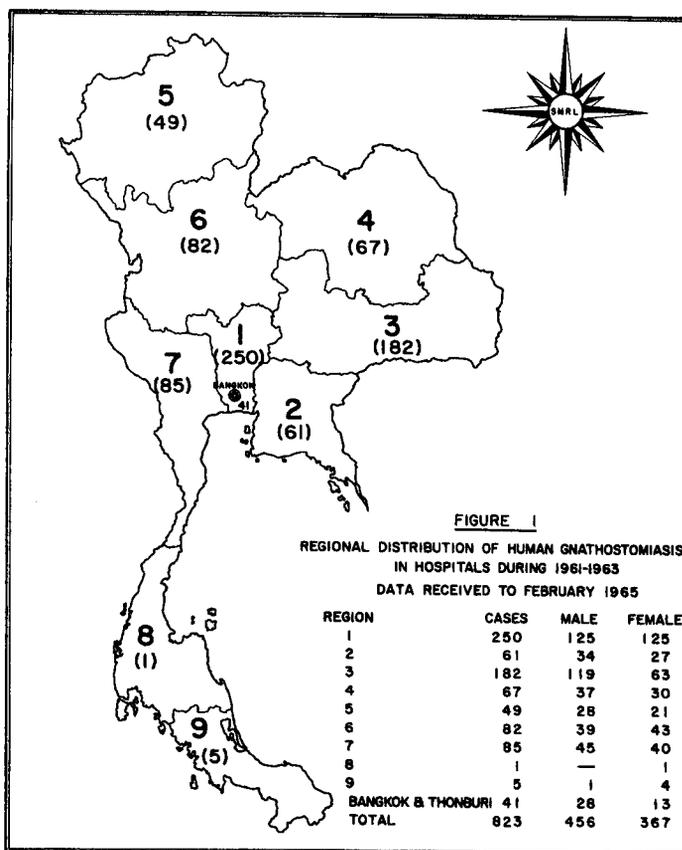
Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

Objective: To determine the prevalence of *Gnathostoma spinigerum* in Thailand and to carry out an epidemiological study of this parasite.

Description: This parasite was first described from a gastric tumor of a tiger 129 years ago. Since that time it has also been reported from domestic and wild cats, leopards, dogs, mink and raccoons, (in India, Thailand, Malaysia, China, Japan, U.S.A. and Australia). Human cases have been reported from Thailand, Malaysia, China, Japan, India, Java, Palestine, Burma, Indochina and the Philippines. Practically all human infections have been with the mature worm in the subcutaneous tissues. Thailand is the country with the heaviest infection rate in humans.

Progress: Through cooperation with the Rabies Control Unit of the Bangkok and Dhonburi Municipalities, a total of 277 dogs have been examined. Of these, 3 were shown to be positive on the basis of 3 simple smear preparations on each stool specimen. In all, 288 dogs GI tracts were examined, of which 4 revealed the presence of adult *G. spinigerum*. The total of these observations is 7 posi-



tives among 565 dogs (1.24%). In addition, 29 cats were studied of which 4 (13.9%) were positive. One adult Cynomolgus monkey and two civet cats (Felis viverrina) were negative on the basis of fecal or GI tract examinations.

It is now generally believed that not only is gnathostomiasis highly endemic in Thailand, but the prevalence is increasing. In order to assay the number of cases admitted to hospitals, a data-report form was prepared. Information has been received from 85 provincial hospitals and 5 hospitals in Bangkok. A total of 823 cases were reported during the past three years. Over 300 of these were from central Thailand while only 6 cases were from the southern part of the country (Fig. 1). These data not only indicate the high prevalence in central Thailand, but it can be assumed that the data from the remaining three teaching hospitals will reveal that the prevalence is even higher.

Concerning the second intermediate hosts, fresh water fish have been collected from Bangkok, Ayuthya, Suphunburi, and Sakon Nakhon Provinces. Animals examined have included 6 adult tree shrews (Tupaia glis), 2 birds (Pelargopsis capensis and Sturnia sinensis), 95 snakes (of 6 species) from the snake farm of the Souwabha Institute, 144 adult rats (of 5 species) from Choburi, Pathum-ani, Phitsanuloke and Bangkok. All were examined for third stage (infective) larvae in the flesh or visceral organs by use of a newly devised illumination box (Fig. 2). This piece of equipment has proved to be convenient and afford accurate examinations of large quantities of fish and animal flesh.

The results of the above examinations are presented in Table 1. The highest rate of infection with third-stage larvae occurred in two species of Ophicephalus fish from Suphanburi (100%) and Ayuthya (62%). Only an average of 32% of this species from Bangkok was infected. It should be noted, however, that the numbers of fish were small in each case and further observations are required. The snakes proved to have a larger average number of larvae per animal than did the fish; 9 - 17 for snakes and 2 - 6 for fish. None of the 144 rats were found to be infected.

A new study was initiated to determine additional second intermediate hosts, to ascertain which animals could be used as laboratory hosts, and to make observations on the changes which occur as a result of experimental infection. Two 6-week old pigs were killed 8 and 10 days after feeding with 10 and 25 encysted third stage larvae obtained from snakes and fresh water fish. The livers showed many macroscopic hemorrhagic areas caused by the larvae. These areas measured about 1-2 mm x 0.4-0.6 mm. In one liver two infective third stage larvae of G. spinigerum were found. The second liver was preserved for subsequent study. Hamsters were experimentally infected for the first time with this parasite. The source of larvae were fish O. striatus and snakes. From hamsters sacrificed 3-10 days after feeding it was found that some 70-80% of the third-stage larvae could be recovered from the livers and that there was little change in the morphology. Macroscopically the livers showed the same patchy hemorrhagic areas similar to those seen in pig livers. Infective larvae were also found in the muscles of two hamsters examined 15-26 days after feeding. A slight increase in size was noted but no change in the morphology of the larvae was noted (Figs. 3 and 4). White and domestic rats (Rattus norvegicus - Wistar strain, R. norvegicus and R. exulans) also proved to be suitable experimental hosts. Larvae from O. striatus were fed to these animals and third-stage larvae were recovered from livers 3 to 7 days after feeding. The macroscopic change of the infected liver was similar to that described for pigs and hamsters (Fig. 5). One white mouse (Mus musculus - Bagg swiss) was found to be negative 24 days after feeding with 10 encysted third-stage larvae obtained from a snake. Two domestic chickens (Gallus gallus domesticus) each fed 6 larvae from fish were found to be free of infection 3 and 7 days later, but one other chicken fed 7 third-stage larvae from an infected cat's liver showed, after 10 days, one third-stage larvae and the typical small patchy hemorrhagic lesions described in the experimentally infected animals mentioned above. Two more chickens were sacrificed 15 and 30 days after feeding with 10 and 15 third-stage larvae. One of them revealed 7 G. spinigerum larvae in the muscles of the breast and legs and the other had 3 larvae in the muscles of the wing (Table II).

The cyclops have been identified for the first time. They are Mesocyclops leckarti Claus, identified by Dr. Harry C. Yeatman, Department of Biology, University of the South, Tennessee.

Summary: Data concerning the prevalence of gnathostomiasis have been collected from hospitals in Thailand for the first time. A total of 90 or the 94 hospitals

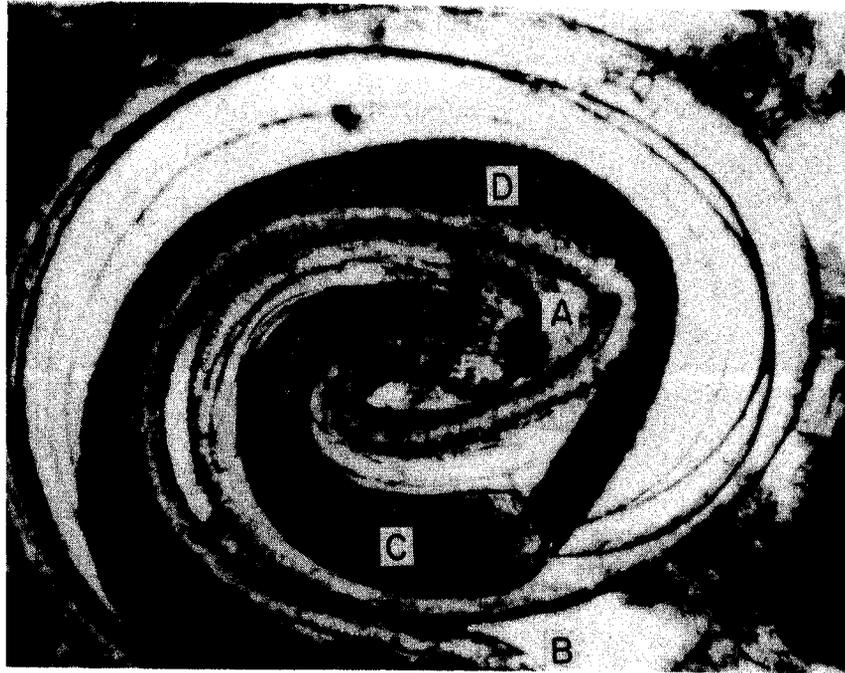


Figure 3. Living third-stage *Gnathostoma spinigerum* larva from hamster muscle 15 days post infection. (A = Cephalic part; B = Tail; C = Esophagus; D = Intestine) (Cyst diameter = 1.0 - 1.2 mm).

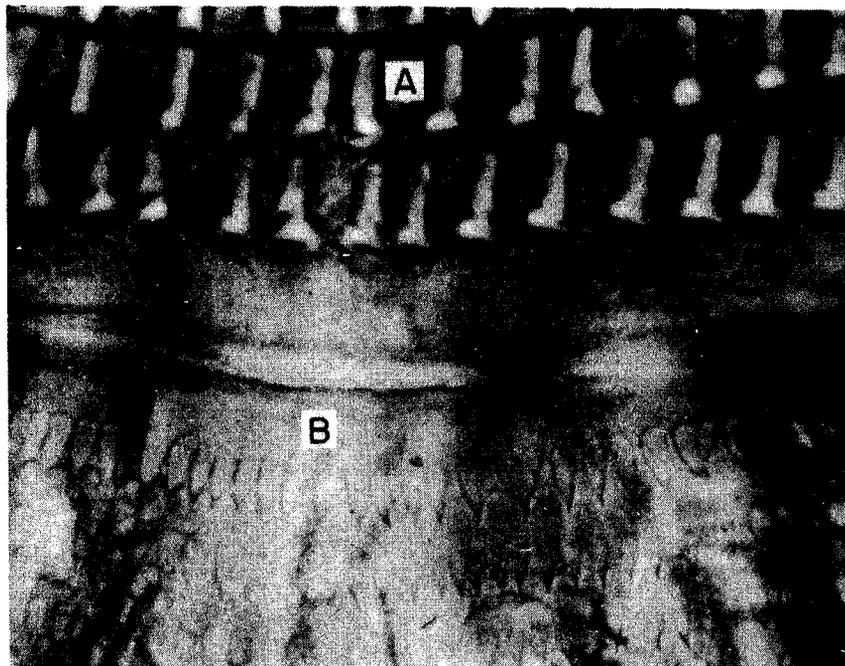


Figure 4. Anterior detail of living third stage *Gnathostoma spinigerum* larva from hamster muscle, showing parts of the cephalic and body surfaces. (A = Cephalic spines with oblong base and small hook, about $16 \times 6 \mu$; B = Anterior body spines, about $10 \times 5 \mu$).

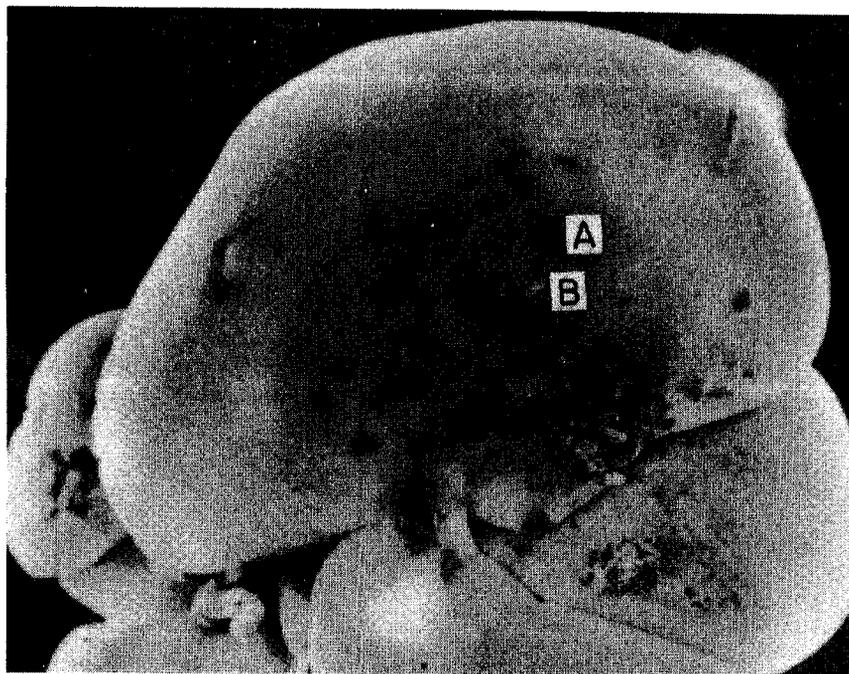


Figure 5. Rat liver experimentally infected with *G. spinigerum* larvae showing scattered hemorrhagic areas and yellowish-white markings, presumably fibrotic changes, caused by the presence of these third stage-stage larvae. (A = Hemorrhagic area 1.0 - 2.0 mm x 0.4 - 0.6 mm; B = Presumably fibrotic reaction).

Table II

EXPERIMENTAL INFECTION OF CERTAIN ANIMALS WITH THIRD STAGE LARVAE OF GNATHSTOMA SPINIGERUM

| Animal | Source and number of infective larvae | | | | Days after feeding | Number larvae recovered | | Remarks |
|--|---------------------------------------|-------|-----|-------|--------------------|-------------------------|-----------|---|
| | Fish | Snake | Cat | Liver | | in liver | in muscle | |
| Domestic pig | - | 25 | - | | 8 | * | Neg. | Liver preserved for study To be repeated |
| | 10 | - | - | | 10 | 2 | Neg. | |
| <i>Cricetus cricetus</i> | 5 | - | - | | 3 | 4 | Neg. | Liver preserved |
| | 5 | - | - | | 5 | 4 | Neg. | |
| | 12 | 3 | - | | 7 | * | Neg. | |
| | - | 10 | - | | 10 | 7 | Neg. | |
| | 5 | - | - | | 15 | Neg. | 4 | |
| | - | 30 | - | | 26 | Neg. | 19 | |
| <i>Rattus norvegicus</i> var <i>albinus</i> | 5 | - | - | | 3 | 2 | Neg. | To be repeated |
| | 5 | - | - | | 7 | 2 | Neg. | To be repeated |
| | 5 | - | - | | 15 | Neg. | Neg. | |
| <i>R. norvegicus</i> | - | - | 5 | | 5 | 3 | Neg. | |
| | 5 | - | - | | 6 | 1 | Neg. | |
| <i>R. exulans</i> | 5 | - | - | | 5 | Neg. | Neg. | To be repeated |
| <i>Mus bactrainus</i> <i>albula</i> | - | 10 | - | | 24 | Neg. | Neg. | To be repeated |
| <i>Gallus gallus</i> domesticus | 6 | - | - | | 3 | Neg. | Neg. | To be repeated |
| | 6 | - | - | | 7 | Neg. | Neg. | To be repeated |
| | - | - | 7 | | 10 | 1 | Neg. | |
| | 15 | - | - | | 15 | Neg. | 7 | |
| | 10 | - | - | | 30 | Neg. | 3 | |

* Larvae not counted.

have submitted information so far. In all, 823 cases have been reported, indicating a high level of endemicity, especially in the central area of the country. The study of natural and experimental infection in definitive hosts was undertaken by examining stools and the GI tracts of 565 stray dogs trapped in Bangkok and Dhonburi. Seven (1.2%) were positive.

Two young domestic pigs were sacrificed 8 and 10 days after feeding with larvae from fish and snakes. Infective larvae were recovered from their livers. Whether or not the pig can act as a definitive host (adult worm recoverable from gastric tumors) remains to be investigated.

Hamsters have been experimentally infected for the first time. Tree shrews have been found to be naturally infected. White and domestic rats were found to be suitable for experimental infection, and (of considerable importance) domestic chickens have been found to be suitable hosts. This last may constitute an important source of infection to humans.

An efficient new apparatus for examining tissues and organs of animals has been devised.

Conclusion: The data recorded thus far indicate that G. spinigerum is widespread in animals and that over 800 human cases have occurred in Thailand. Further studies will be undertaken along the lines of investigation mentioned above.