

SEATO Medical Research Study On

Eosinophilic Meningoencephalitis In Man

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Period of Report: 1 April 1966—31 March 1967

The studies so far completed show that eosinophilic meningitis is the most common parasitic disease of the central nervous system known in Thailand. It is a disease which is due to human ingestion of infective third stage larvae of A. cantonensis. Analysis of the more than 500 cases collected by Major Sompone suggests that the eating of raw Pila snails is the most common route of infection. However observations by Capt. Crook clearly show that infected pulmonate snails, upon drowning may shed into the water, larvae which are infective for animals and presumably man. Further, it has been shown that the standard chemical means of purifying water in the field will leave many of the shed larvae still infective. Thus the disease is already a problem among those who eat raw food and could possibly become a major problem to an army operating in the field, exposed to contaminated water.

A less common form of eosinophilic meningitis, the so called myeloencephalitic type, is described by Major Sompone. This differs from typical eosinophilic meningitis probably in etiology and certainly in its clinical manifestations and prognosis. Much more work needs to be done on this variety of the disease. The etiology is uncertain but at present it does not appear to be due to A. cantonensis. One of the great problems encountered in studying eosinophilic meningitis in whatever form, is the relative paucity of autopsy material in which an identifiable parasite can be found.

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1. Title: Studies on the Animal-Parasite Phase  
of Angiostrongylus cantonensis in Thailand.  
(Intermediate Host Studies; Definitive Host Studies)

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Period of Report: 1 April 1966—31 March 1967

**OBJECTIVE:** The objectives of this study are to evaluate potential, intermediate and definitive host species of Angiostrongylus cantonensis seasonally, in distinct areas of Thailand with regard to frequency of natural infection, ecology, distribution and the possible methods of either direct or indirect passage of the parasite to man.

**DESCRIPTION:** The areas selected for study are Bangkok-Dhonburi near the mouth of the Chao Phya River; Nakornrajsima (Korat), Ubol and Udorn in the Northeast; Chiangmai, Sukothai and Nakhon Sawan in the North; Kanchanaburi and small villages up the Khwae Rivers in the West; Narathiwat, Yala, Haad Yai, Trang, Nakhon Sri Thammarat, Krabi, Ranong, Chumphon and Prachuab Khiri Khan in the South Peninsula; and Trat, Chanthaburi and Rayong in the Southeast. Conduct of the study was divided into two efforts. One effort concerned the definitive hosts, the second concerned the intermediate hosts. Collections of definitive host mammals were conducted by transect live trapping through habitats offering suitable conditions for both definitive and intermediate hosts. Rodents so obtained were dissected and examined for the presence of adult A. cantonensis worms in the pulmonary arteries. The remainder of the rodent was used in connection with other laboratory projects. The infected mammals were recorded as noted in previous reports. Intermediate host studies were begun along the above mentioned transects through comprehensive gastropod collections to approximately five meters on either side of the transect along its length. The resulting gastropod material was recorded, as previously outlined, and shipped to the Bangkok laboratory for processing. The individual species were identified and grouped in aquaria. Prior to examination for A. cantonensis third stage larvae, the gastropods were examined for trematode intermediate stages by the Medical Malacology staff. The individual gastropods were then homogenized, digested and examined for the presence of A. cantonensis third stage larvae. All resulting metastrongylid larvae were fed by stomach tube into laboratory white Rattus norvegicus, WRAIR Strain. In 21 days the rat was sacrificed and the brain examined for the presence of fifth stage A. cantonensis larvae. Only after this definite proof, was a snail listed as positive. Natural epidemiological observations were conducted in the field during all studies to include possible gastropod-mammal and gastropod-human associations.

**PROGRESS:** South Peninsular Thailand was the first area studied during the period covered by this report. Throughout this generally tropical monsoon climatic area, a brief period, approximately from the last of February to mid April, is comparatively dry. To investigate the negative results obtained during the previous year between October and December, a comprehensive study was carried out during the relatively dry period. This season was chosen for ecological considerations. The dry period would allow rodent fecal material to collect in quantity and, provided there was some infection, increase the chance that suitable coprophagic

snails would come into contact with the material and become infected. Near the end of the dry period, the optimum conditions existed for a complete cycle of the parasite. The coprophagous gastropods had an increased opportunity for infection of sufficient duration to be infective. The possibility of finding mammals recently infected by eating infective snails, was correspondingly better. The remainder of the year the rains are of such a profuse nature as to prohibit the accumulation of infective fecal matter, allowing a minimum chance for gastropod infection and life cycle accomplishment. The study of those locations mentioned in the "Description" yielded 1,213 mammals of seven species. Twenty five positive Rattus norvegicus were found and one each Rattus rattus and Rattus exulans. Simultaneously 2,192 gastropods were collected. Examinations revealed 16 definitely positive Achatina fulica and four possible positives. The latter were metastrongylid larvae recovered dead which could not be fed to rats for proof of the species. Previous findings support the assumption that the dead larvae were A. cantonensis. This finding of only 20 infected gastropods, seemingly insignificant, serves to nullify the previous indication of a natural infection void in the Southern Peninsula of Thailand.

The positive findings in the south were so widely scattered that the infection, at the present time, can be regarded as insignificant. The finding of the infection only in A. fulica, an introduced species, and not in snails proven to be natural intermediate hosts elsewhere, indicates the possibility that the parasite was introduced into the peninsular region with A. fulica in late 1941. The low infection level presently indicated could be due to an interrelationship between the climatic interference in the life cycle and the present low level of the scattered A. fulica populations.

Studies in the northeast were conducted in the listed locations at the end of the hot season, May-June 1966, resulting in 327 rodents collected. Of that number, six were found to be positive. During the same period of time, 1,893 gastropods were collected with no positive isolates. The severe conditions of the Northeast hot season resulted in the majority of gastropods being collected from sub-surface or deep sub-humus layers in a state of estivation. This condition appears to have existed for at least one month. The estivating factor demonstrates climatic influence on gastropod vagility reducing the chances of infection to a minimum during this season.

Studies in the north were conducted at the listed sites during both the hot and cold seasons. During the hot season, April-June 1966, 241 mammals were collected of which 11 were positive. Among the 1,360 gastropods collected there were no positive isolates. These findings are commensurate with the findings in the northeast under the same climatic influences and are judged to be due to the same ecological factors. Mammal collections in the cold season totalled 541 animals of which 35 were positive. Ecological conditions, primarily moisture, were more favorable for pulmonate gastropod activity and infection as indicated by the result of eight positive and three probable positive isolates from 1,774 gastropods. The positive intermediate host fluctuation is notable in view of the relatively stable mammal infection rate.

Investigations in west Thailand commenced on 4 August 1966 during the rainy season. Among the 709 mammals collected, 59 were infected with A. cantonensis. The species Bandicota bengalensis accounted for all of the positive findings. Quantula striata, a new intermediate host, was found to be the only positive gastropod among the seven species examined during that season. Dry season collections in the area commenced on 1 December 1966. Live trapping yielded 676 mammals of which 66 were positive. With one exception, R. rattus, the positive animals were B. bengalensis. An unseasonal downpour brought large numbers of slugs (Veronicella sp.) to the near surface of the jungle litter and, among other gastropods, 930 of the slugs were collected. Approximately eight percent of the slugs were positive as well as the consistent one to two percent infection in Q. striata. The slugs had been collected in small numbers during the rainy season, however, none were positive then.

Studies in southeast Thailand were begun during the rainy season, September 1966. The region was divided into two ecologic sectors one with approximately 4,000 mm of rain per year in Trad and Chanthaburi, and the second with approximately 1,500 mm of rain per year in Rayong. In the former sector, 1,415

mammals were collected yielding 121 positives. Simultaneous gastropod collections yielded 1,741 specimens. Both Hemiplecta distincta and A. fulica were positive with the latter species almost 25% infected. Dry season studies in the 4,000 mm rainfall sector yielded 1,043 mammals of which 75 were positive. The reason, January and February 1967, was extremely dry and the 672 gastropods collected required considerable effort. Again, A. fulica was infected but the prevalence had fallen to about 15%. No other species were positive in this collection. Rayong, the 1,500 mm rainfall sector, yielded 363 mammals during the October 1966 rainy season work. In spite of comprehensive coverage there were no positive isolates. Gastropod collections included two known intermediate hosts; however, no positive material was found. It is noted that this rainy season was, according to climatological data over a 30 year period, "average" in all measured respects. The climate is similar to that of the Ubol area in spite of Rayong's proximity to the sea. The resulting impact on the mammal population is wide dispersal without indication of notable concentrations of colonies. This situation, only slightly condensed, is exhibited in the town of Rayong itself. Under the present conditions it is questionable whether or not a significant population of A. cantonensis can be supported in the natural host populations. Due to the comprehensive nature of the first study in this area and the fact that the dry season would offer even less opportunity for finding the parasite and the firm indication, based on two years experience, that no meaningful result could come from such an expenditure of personnel and money, no further seasonal work was done in this 1,500 mm rainfall sector of Southeast Thailand.

Collections in selected localities of Bangkok and Dhonburi were continued in such a manner as to monitor the level of the parasite population and determine the cause of fluctuations should such occur. Seasonal variations were observed as in previous years. A primary factor in the parasitic population fluctuations was the vagility of the intermediate hosts which was most influenced by seasonal moisture and temperatures. The definitive host infection rate, as in previous reports, fluctuated between approximately 18 and 6 percent with the higher figure during the late rainy season and the lower at the end of the hot season. During this study, a new intermediate host, Melanoides tuberculata, was found.

Studies on larval shedding from drowned intermediate host gastropods were emphasized. Although these studies are not complete, it is clear that a drowned pulmonate gastropod will shed up to 33% of its infecting larval burden if it has had an A. cantonensis infection of sufficient duration and there is a large larval burden. Due to the fact that the only gastropods found to be of importance in the life cycle of A. cantonensis in Thailand are pulmonate, this response to drowning assumes important proportions. Observations of proven intermediate host gastropods contaminating culinary water, reported at the Second Medical Conference on Parasitic Diseases, March 7-11 1966, further added importance to this shedding response. Experiments to determine the effect of chemical water treatment on the infectivity of shed larvae were conducted in view of the indicated epidemiological importance of water contamination. Chlorine treatment was of no measurable value, Iodine water purification tablets, routinely issued by the U.S. military, were used as directed for extreme water pollution and resulted in the killing of about 66% of the larvae. It is noted, in spite of the 66% control, the remaining approximately 33% were infective as demonstrated by rat inoculation. Filtration through coarse material such as sand removed all larvae from the water.

**SUMMARY:** Collections continued throughout this year with emphasis on the ecological and epidemiological conditions prevailing in each major region of Thailand. A light infection rate was again demonstrated in the Northeast. The infection rate in the north also remained much as it had been described during the previous year. Studies in the west, conducted for the first time, revealed a high rate of infection and a previously unreported intermediate host. Studies in the south revealed a few positive animals indicating very small pockets of infection generally corresponding to small isolated populations of A. fulica. The latter species was introduced by Japanese occupation forces between 1941 and 1945. Local residents in the South Peninsula state that the snails were brought from Taiwan, the location of the first reported human case of infection by A. cantonensis. The possibility that the observed infection among mammals in the south was imported with the snails is presented for consideration. Studies in the southeast conducted for the first time, revealed very high levels of infection in the high rainfall areas of Trat and Chanthaburi; however, no infection was found

in the dry region of Rayong. Studies concerning the infection levels in the Bangkok-Dhonburi area continued with seasonal fluctuations commensurate with earlier observations. The observed fluctuations were due to the seasonal influences on pulmonate gastropod intermediate hosts. During this year's examinations, no fresh or brackish water snails were found to be infected in spite of the thousands processed from areas exhibiting high infection rates among other gastropods.

The study emphasized the importance of intra-area evaluation of all ecological, life cycle, and epidemiological factors due to the fact that each region exhibited some degree of life cycle difference. The main difference in life cycles concerns the intermediate hosts involved in a given area and the influence of the various ecological conditions on those intermediate hosts. No two regions exhibited the same collection of proven intermediate hosts and since it is from a suitable intermediate host that mammalian infective third stage larvae must originate, epidemiological hypotheses obviously must be formulated from findings in the area in question.

Studies concerning the larval shedding from drowned pulmonate snails continue to emphasize the importance of this phenomenon in epidemiological considerations. This assumes proper perspective when it is noted that to this date, only pulmonate land snails have been shown to be of importance in the natural life cycle of the parasite throughout the Kingdom of Thailand. Further importance is directed to this situation with the realization that recognized chemical treatment does not kill all of the larvae in the water and those remaining are infective.