

(Annual report April 1966 — March 1967)

STUDY REPORT

Title: Clinical Manifestations and Epidemiological Studies of Eosinophilic Meningoencephalitis in Man.

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OBJECTIVES: To continue the complete epidemiological and clinical studies and laboratory evaluations on cases of eosinophilic meningoencephalitis in Thai subjects and perform such special animal investigations as are indicated. Based on last year's study, it is certain that this disease is the most common and important parasitic disease of the central nervous system in Thailand. Many aspects of the disease are still not known.

DESCRIPTION: During the period, 4 provinces were studied (namely Bangkok, Korat, Ubol and Pranchinburi). The first two provinces are the endemic areas of so called "typical type" of eosinophilic meningitis while "myeloencephalitis type" is commonly seen in the latter two provinces. In every proven positive case, a complete epidemiological evaluation, and clinical and laboratory investigations were performed. In all fatal cases, efforts were made to obtain autopsies in order to study the pathological changes.

Research studies in the laboratory were done to confirm the importance of Pila snails as the main source of human infection. Experimental animal studies were done to compare the local Angiostrongylus cantonensis to the Honolulu strain. The studies on the biochemical changes in the cerebrospinal fluid of eosinophilic meningitis cases were made in cooperation with Cpt., R.A. Rasmusser, Dept of Clinical Chemistry Division of Biochemistry, WRAIR.

PROGRESS: During this year, 166 cases of eosinophilic meningitis were studied, which makes a total of 512 cases for the two year study. Of these 166 cases, 150 belong to the "typical" Eosinophilic Meningitis and 16 are classified as myeloencephalitis.

TABLE 1

I. Epidemiology: Eosinophilic meningitis has been reported in 33 provinces; 18 central, 13 in the northeast and one each in the north and south.

TABLE 2

1. Age and sex distribution. The disease occurred at all ages but is most prevalent in the second and third decades. The youngest in this study was a two year old girl. Males were involved twice as often as females.

2. Seasonal Variations. The number of cases diagnosed and the rainfall registered in three provinces (Bangkok, Korat and Ubol) are presented in graph 1. The rainfall pattern in these areas was more or less the same, starting in April and continuing through November. In Bangkok the disease, mainly typical Eosinophilic Meningitis, was most prevalent during July and December. In Korat, however, the disease, also typical Eosinophilic Meningitis, occurred as a year round disease but the peaks were found to rise later than Bangkok. In Ubol, where the majority of cases were myeloencephalitis type the peak of the disease seems to occur sooner than in Bangkok. In these areas, there seems to be a correlation between the availability of the *Pila* snails and the peak of the disease. The snails are amphibious and they will start breeding soon after the rainy season. It takes a few months for snails to grow big enough to be eaten. They are abundant everywhere in the rainy season. In the dry season they are found only occasionally in ponds. Another explanation for the seasonal variation of this disease is the ease with which patients can come to the hospital. This is important in Korat which is a big province and has poor communications during the rainy period. In addition the rainy season is the harvesting time and unless they are very sick, patients will try not to come to the hospital.

3. Incubation period. The incubation period was not known in about 35 percent of cases of Eosinophilic Meningitis, because of either multiple possible sources of infection or an uncertain history. However in the cases who gave a definite history of ingesting only a single raw food within one month prior to the onset of symptoms, the incubation period varies from as short as one day to as long as 47 days. The majority of cases, however, have an incubation period of 3 to 20 days.

The incubation period can be determined more reliably by studying patients who came in groups having once joined in eating the same dish of raw food and having become sick within 1-2 weeks of each other. There were 34 such groups and a total of 132 cases. The incubation period varies from 3-36 days with the average of 16.5 days.

4. Sources of Human Infection. Eighty-five percent of typical EM cases gave a definite history of eating raw food only once during the one month prior to the onset of symptoms. Out of these, 84% gave a definite history of eating raw *Pila* snails. Fresh water shrimp and fresh water fish were eaten by 8.2 and 3.5 percent respectively. The rest ate other snails, crabs and beef. In myeloencephalitis type, 73% gave a definite history of eating raw food. 50% ate *Pila* snails, 26% fresh water shrimp, 7% water fish, 11% raw beef. Of the 34 "groups" of patients, all gave a definite history of eating only raw *Pila* together.

TABLE 3

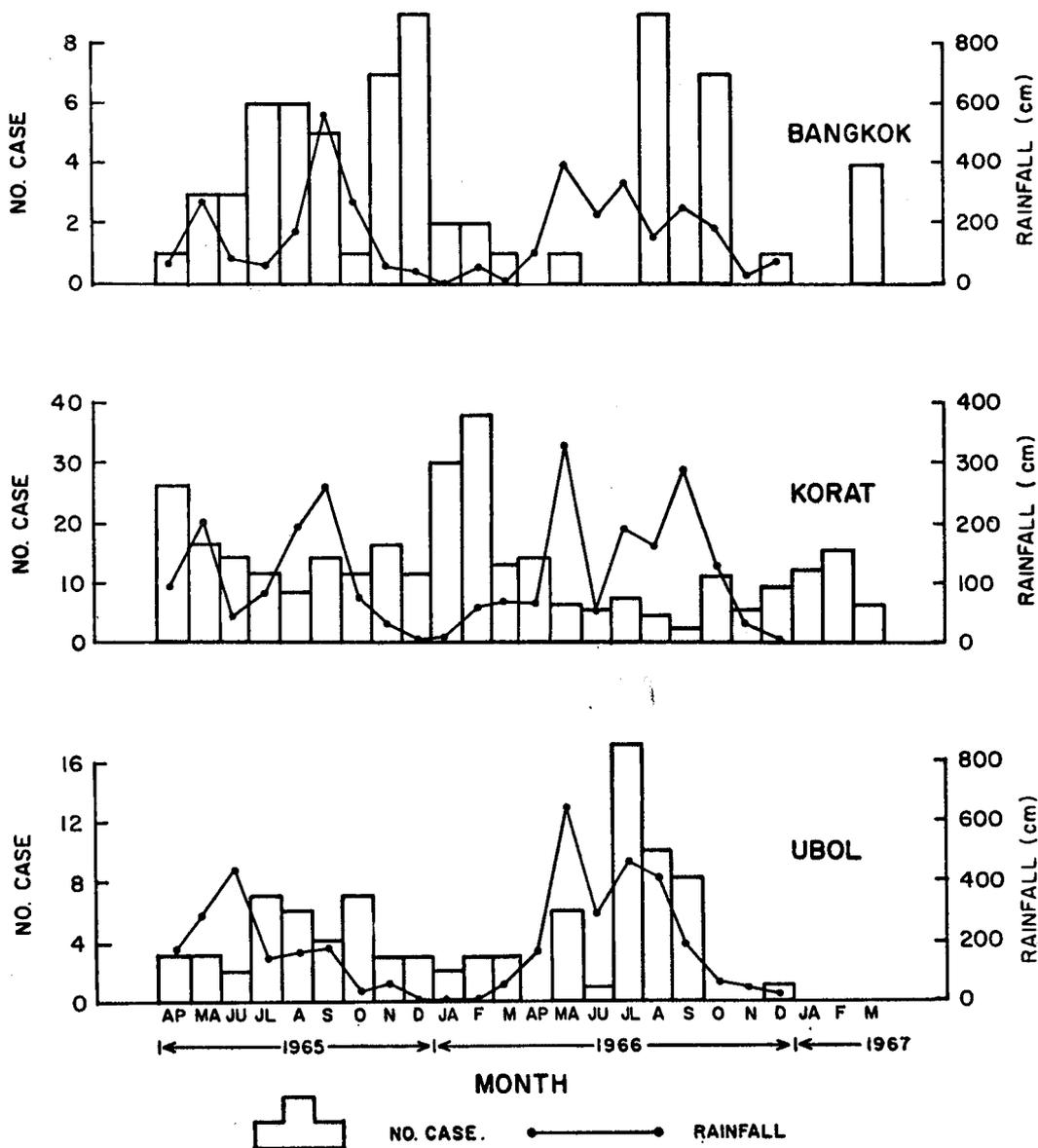
To confirm this epidemiological fact, *Pila* snails were collected from those areas where patients obtained the snails for eating. They were carefully studied for the presence of any parasites. *Pila* snails from 10 out of 27 areas examined were found to harbour infective *A. cantonensis* larvae, 16% of 236 snails examined were positive. The rate of infection in snails in each area varies from 1.8 to 72.7 percent. The number of infective third stage larvae found in positive snails varied from 1 to 732 with an average of 68.6 No infective *Gnathostoma* larvae were found.

TABLE 4

II Clinical Manifestations:

There were 478 cases of typical EM and 54 cases of the myeloencephalitis type. Headache is a constant finding in the typical EM but not in myeloencephalitis. Limb paralysis was not found in typical EM

GRAPH. I SEASONAL VARIATION OF EOSINOPHILIC MENINGOENCEPHALITIS
AND
THE RAINFALL IN BANGKOK, KORAT AND UBOL IN 1965-1967



while it was a constant finding in myeloencephalitis. Vision impairment occurred in both types. 11% in typical EM and 9% in myeloencephalitis. Facial Paralysis occurs in about 4% in both types. 2% of typical EM cases and 6% of myeloencephalitis cases experienced lateral rectus paralysis. Eye edema was noted in 6% myeloencephalitis cases and only 0.8% in typical EM cases. Death occurred in 13 cases or 22.2% of myeloencephalitis and 6 cases of typical EM or 1.2%.

TABLE 5, 6

III. Laboratory Studies

A leucocytosis over 10,000/cumm. was found in 52% of both types. Eosinophilia in the peripheral blood of over 10% was found in 72% of typical EM cases while only 11% of myelitis case showed this. The times of the highest cell counts in the spinal fluid fell within the first two weeks in 81.3%. The highest cell count in 83.8% of cases was between 200-4999 cells/cm,mm. 81% of typical EM and 64% of the myelitis type had an eosinophilia in the CSF of over 25%. Protein content of the CSF was over 50 mg percent in 72% to typical EM and 78% of myelitis cases. There have been no significant changes in the sugar and chloride content of the CSF. The opening pressure of the CSF during the acute stage was found to be increased in 45% of typical EM and 28% of myeloencephalitis cases.

IV. Special CSF Study

The spinal fluids of 44 cases were sent to Capt. Rasmussen at WRAIR for the special electrophoresis and enzyme studies. The following interesting points were noted.

1. 91% of cases studied had an abnormally high gamma globulin in the CSF.
2. LDH enzyme in the CSF was abnormal in 72% of cases particularly in the 5th and 4th fraction.
3. There is an interesting reverse relationship in enzyme GOT between the sera and the CSF in some instances: that is the GOT level in the CSF was higher than its level in the serum.
4. Using an electrophoretic technic with higher resolving power remarkable multiple bands in the gamma globulin fraction of the CSF were noted while no such bands were found in the sera.

Further studies are being done.

V. Pathology

Out of 13 deaths in the myeloencephalitis type only 6 autopsies were performed. No etiologic agent was found. The detailed pathological findings will be presented elsewhere. However Dr. Prasan Tangchai, a neuropathologist at Chulalongkorn Hospital, reported finding A. cantonensis in the brains of two Thai patients who died of meningitis. Comparing his histologic findings and the findings in the brains of our patients who died with the myelitis type of disease, it seems that the etiologic agent in our myeloencephalitis cases is not A. cantonensis. Histologically one may find microscopic necrotic, hemorrhagic areas in cases typical E.M. and grossly visible tracts in the myelitis type.

VI. Experimental Studies

1. Comparative studies of Thai and Honolulu Strains of A. cantonensis in Rats and Hamsters.

The infective larvae of local A. cantonensis (Nakornsawan) and Honolulu strain (Dr. Rosen's Lab) were given to white rats and hamsters. These animals were sacrificed at intervals and were examined for parasites. It was found that the Honolulu strain reached the heart and lungs 4 days sooner than the local

strain (29th vs. 33 day). It also appears that after the 10th day the local strain seems to have grown faster and bigger than Honolulu strain. The same findings are seen in the Hamsters. There have been no differences in the rate of larvae recovery between these two strains.

#2 Studies on Pila snails as intermediate hosts.

First stage larvae of local A. cantonensis were fed into naturally collected P. ampullacea and also to laboratory bred P. polita. In naturally collected snails, 25-42 percent of the larvae were recovered during the first week after infection; thereafter the percentage of larvae recovered fell. The third stage infective larvae were first recognized on the 21st day. On 47th day after infection only 3% of larvae were recovered from the snail. In the young, small (about 1 1/2 cm in size) laboratory bred P. polita the recovery rate in the first week was much higher, 60-93 percent. However the recovery rate fell thereafter to about 20% and remained at this level to the 47th day. The infective larvae were found in the foot of the snail. This experiment confirms that Pila snails are an intermediate host of A. cantonensis. The younger and smaller snails seem to be a much better host. The larvae can develop to the infective stage in as little as 18 days. There has been no explanation yet for the spontaneous reduction in the number of infective larvae in snails.

SUMMARY:

From this evidence certain conclusions may be drawn:

1. Eosinophilic meningitis is a significant disease and an important public health problem in the studied areas. The disease occurs at all ages but is more common in the second and third decades. It involves males twice as often as females. It can be found at any time of the year but the highest attack rate is in the rainy season.
2. Two distinct types of eosinophilic meningitis were recognized; one was called typical eosinophilic meningitis and another one was myeloencephalitis. Impairment of vision is an important complication in the disease.
3. The mortality rate was 1.2% in typical form and 12.9% in the myeloencephalitis form.
4. A. cantonensis is a causative agent in typical eosinophilic meningitis. No agent can be found as a cause of the myeloencephalitis type and there is evidence it is not A. cantonensis.
5. Eating raw Pila snails containing the infective larvae of Angiostrongylus cantonensis is the main source of typical E.M. in man, in Thailand. The consumption of other raw animals such as fresh water shrimp or fish may be of significance. In about 15% of the cases, no single source or route infection can be found.
6. The number of A. cantonensis larvae needed to produce the clinical disease in man probably varies a great deal, from as few as 1 or 2 to as many as hundreds. The difference in the number of larvae ingested may account for the wide range of the incubation periods as well as the varying severity and clinical manifestations.

TABLE 1:

Eosinophilic Meningoencephalitis Studied During April 1966—March 1967

Province	First year	Second year	Total
Bangkok	46	22	68
Korat	208	96	304
Ubol	46	43	89
Udorn	31	—	31
Prachinburi	16	4	20
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Total	347	165	512
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TABLE 2

List of Provinces where Eosinophilic Meningoencephalitis was reported.

CENTRAL THAILAND

Bangkok and Dhonburi
Chacherngsao
Pathoomthani
Nakornnayoke
Saraburi
Singburi
Chainat
Nakornsawan
Pisnuloke

Samutprakarn
Prachinburi
Ayudhaya
Suphanburi
Angthong
Lopburi
Uthaihani
Kampangpetch
Sukothai

NORTHEAST

Nakornrajsima
Srisakate
Chaiyaphoom
Mahasarakam
Kalasin
Nongkai
Nakornpanom

Bureerum
Ubol
Royed
Konkaen
Udorn
Sakolnakorn

NORTH

Chiangrai

SOUTH

Prachuabkirikan

TABLE 3

Number and Percent of 305 Patients with Eosinophilic Meningoencephalitis who consumed only one raw food within 4 weeks prior to the onset of first symptom according to 2 major types.

	Typical EM		Myeloencephalitis	
	Number	Percent	Number	Percent
Pila snails	237	84%	13	50%
Other snails	3	1.1	—	—
Small fresh water shrimp	21	7.5	7	26.9
Large fresh water shrimp	2	0.7	—	—
Small fresh water fish	9	3.2	1	3.8
Large fresh water fish	1	0.4	1	3.8
Crabs	1	0.4	1	3.8
Somfuk, Pla Som Raw Fish Dish	1	0.4	—	—
Koi Pla Ra Row Fish Dishes	4	1.4	3	11.5
Total	279		26	

TABLE 4

The Result on Examining Pila Snails From 27 Infective Areas For the Infective Larvae of A. cantonensis.

Location	No. Areas examined	No. Areas positive	Type of snails	No. snail examined	Percent of positive snail	No. larvae in each positive snail		
						Minimum	Maximum	Mean
Bangkoo	4	2	<u>P. polita</u>	17	0	—	—	—
			<u>P. ampullacea</u>	56	1.8	5	5	5
Korat	19	4	<u>P. polita</u>	105	8.5	1	12	2.8
Chaiyaphoom	2	2	<u>P. palita</u>	18	16.7	2	5	3.3
Lopburi	1	1	<u>P. polita</u>	7	14.2	2	2	2
Nakornsawan	1	1	<u>P. scutata</u>	33	72.7	1	732	107
Total	27	10	-----	236	16.1	1-5	2-732	68.6

Table 5

Summary on the Clinical Manifestations of 532 Cases of Eosinophilic Meningitis in Thailand According to Major Clinical Types

Clinical Manifestations	Typical Eosinophilic Meningitis	Myeloencephalitis
Headache	98	74
Temperature over 37°C	38	68
Facial palsy	4	4
Lateral rectus paralysis	2	6
Vision impairment	11	9
Signs of meningeal irritation	23	31
Eye edema	0.4	6
Coma	0.8	6
Paralysis of limb	—	100
Xanthochromic spinal fluid	5	24
Mortality rate	1.2	22.2
Total Cases	478	54

TABLE 6

**The difference between Typical Eosinophilic Meningoencephalitis
and Myeloencephalitis in 5 Provinces of Thailand.**

	Typical Eosinophilic Meningoencephalitis	Eosinophilic Myeloencephalitis
I. Epidemiology		
Location	Bangkok, Korat and Udorn	Ubol and Prachinburi
Seasonal Variations	3-4 Months after heavy rain or year round	Mid-rainy season
Suspected food	Mainly Pila snails	Uncertain
II. Clinical		
Severe Headache	Constant	Uncommon
Eye Edema	Rare	Common
Limb Paralysis	Not seen	Constant
Xanthochromia spinal fluid	Rare	Common
III. Mortality	Rare (1.2%)	High (13%)
IV. Pathology	Brain only No macroscopic parasitic tracts	Cord and brain hemorrhagic and necrotic macroscopic parasitic tracts
V. Etiologic Agent	<u>Angiostrongylus</u> <u>Cantonensis</u>	Unknown