

ANNUAL PROGRESS REPORT

SEATO Medic Study No. 2 Clinical Disease Accompanying Thai Hemorrhagic Fever Virus Infections

Project No. 3A Q25601 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 146, San Francisco, California

Division of Medical Research Laboratories Department of Virology

Period Covered by Report: 1 April 1963 to 31 March 1964

Principal Investigator: Major Scott B. Halstead, MC

Associate Investigators: Dr. Suchitra Nimmanitya*

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Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

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ABSTRACT

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The objective of this study is to determine the etiology of Thai hemorrhagic fever and describe the nature and relative frequency of inapparent mild and atypical syndromes which accompany infection with Thai hemorrhagic fever viruses in Asians. This is an etiologic study of all disease caused

by dengue and chikungunya viruses in Asians. Patients admitted to study largely come from Children's Hospital medical wards or Out-Patient Departments. In addition, some cases submitted for serologic study from other hospitals in Bangkok are included. Preliminary typing of dengue viruses recovered from humans in the 1962 outbreaks has been completed. Approximately 1/3 of all viruses successfully typed belonged to the dengue 1-TH Sman complex, and the dengue 2-TH-36 complex, respectively. Dengue 3 and 4 were also recovered. Dengue viruses were recovered from the blood of 20% of serologically positive dengue HF infections. In contrast, dengue virus was recovered in only 4% of 46 fatal cases studied, even though the collection of acute blood specimen was obtained at a similar stage of illness in each group. Chikungunya patients were admitted to hospital earlier and virus recoveries in this disease were very high.

Etiologic identification of fatal Thai hemorrhagic fever continues to be elusive. Of 46 autopsies processed in suckling mice only 1 dengue virus was recovered from heart tissue. It appears that dengue virus disappears from the blood of severely ill patients more rapidly than from patients who survive hospitalization or patients with mild disease. At the time of death, apparently, virus is all destroyed.

BODY OF REPORT

SEATO Medic Study No. 2

Clinical Disease Accompanying Thai Hemorrhagic Fever Virus Infections

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Objectives: To determine the etiology of Thai hemorrhagic fever and describe the nature and relative frequency of inapparent, mild and atypical syndromes which accompany infection with Thai hemorrhagic fever viruses in Asians.

Descriptions: Four categories of patients are studied at Children's Hospital by similar clinical and virologic methods:

1. Out-patients with febrile syndromes (PUO).
2. In-patients with admission diagnosis of hemorrhagic fever (HFI).
3. In-patients with febrile disease other than hemorrhagic fever (NH).
4. Surgical patients.

All patients, residents of Bangkok and Thonburi, are selected randomly within 24 hours of admission to Out-Patient Department or hospital. Virologic materials include acute and convalescent sera, throat and rectal swabs.

Autopsy materials and other specimens from fatal cases of Thai hemorrhagic fever are collected whenever possible. Infectious and serologic specimens submitted voluntarily from other hospitals in Bangkok are included in certain aspects of this study.

Progress: During the report period considerable progress has been attained in the work up of materials collected in 1962-1963.

Etiologic Studies

Table 11 shows the total dengue viruses isolated from human sera in 1962 and 1963. Of 154 patients studied in 1962, 28 viruses were recovered. In 1963, 7 viruses have been recovered from 68 cases. Virus recoveries from other study groups are shown. Data for chikungunya virus is shown in Table 12. Arranging virus recoveries by day of illness of acute specimen the expected pattern of decreasing virus recovery with day of illness is observed (Tables 13 and 14). The data stresses a significant difference between dengue and chikungunya disease---the latter usually presents for hospital admission on the 1st or 2nd day of fever, while the former presents on the 3rd to 6th day of fever. Curiously, virus recovery rates from patients with dengue illness not sick enough to be admitted were consistently higher than from hemorrhagic fever cases even when specimens were obtained on the same day of illness (Tables 15 and 16). This observation is consistent with the hypothesis discussed below that hemorrhagic fever patients get rid of dengue virus rapidly.

By analysing the percentage of confirmed dengue and chikungunya cases among the hemorrhagic fever patients studied at Children's Hospital

and projecting these figures to total admissions for hemorrhagic fever among Bangkok-Thonburi residents, an etiologic estimate of hemorrhagic fever is obtained for 1962. This is shown at a logarithmic scale at the bottom of Figure 2. The upper curves are an estimate of milder disease. This data was obtained by projecting serologically confirmed dengue and chikungunya infections in the Out-Patient Department sample, Children's Hospital, to the total OPD population for 1962 in all pediatric clinics in Bangkok. These data in turn were multiplied by 1.5, a figure derived from the interview data of the Bangkok Area Study (SMRL 8) representing the ratio of private MD visits to OPD visits for pediatric illnesses. It is obvious that these are crude estimates. In the vicinity of 180,000 illnesses due to dengue or chikungunya viruses are estimated for the period April through December, 1962.

Preliminary data are available from typing dengue viruses isolated in 1962. These are presented in Table 17. These results are obtained by testing mouse immune sera prepared to each isolate by 2 or 3 inoculations against prototype antigens by CF. This technique has provided ready separation of virus types, but further intensive studies of dengue virus groups are in progress. It can be seen that the dengue 1 and 2 complexes and dengue 3 predominated. There is a suggestion that among the untyped viruses new dengue types may exist.

Fatal Hemorrhagic Fever

Virologic study of fatal cases is a special problem of importance in the study of Thai hemorrhagic fever. Successful isolation of the pathologic agent would give solid evidence of the etiology of death, would enable the analysis of the virus types responsible for death and give clues to the pathogenesis of Thai hemorrhagic fever. Unfortunately, the recovery of viruses from Thai hemorrhagic fever has not proved simple. In 1961, Dr. Ambhan Dasaneyavaja reported the isolation of dengue virus from the liver of 4 year old Chinese girl dying of Thai hemorrhagic fever. Until the present study no other virus isolation from autopsy material has been reported.

Virus isolation and testing was done on autopsy specimens that were collected from many hospitals in Bangkok and Thonburi in 1962-1963. When the patient expired, the body was moved into a refrigerator awaiting autopsy permission. In most cases autopsy was performed within 24 hours after death. Tissues from different organs were transferred to the Virus Laboratory on wet ice. Venous blood from patients who subsequently died were obtained at Children's Hospital as part of the routine study. In some instances heart blood was drawn immediately after the patient expired.

Each organ was weighed. Then it was ground with purified aluminum and phosphate buffered saline with 0.75% bovine albumin (BAPS) with 500 units penicillin and kanamycin, 500 ug streptomycin and 25 ug mycostatin per ml were added to make a 10% suspension. The mixture was centrifuged at 10,000 rpm for 30 minutes in Servall refrigerated centrifuge. Supernatant fluid was inoculated into 1 day old suckling mice, 0.01 ml intracranially and 0.02 ml intraperitoneally.

The serum from venous blood or heart blood was diluted 1:4 with BAPS and was inoculated into the suckling mice in the same manner as autopsy materials.

The isolation process in mice is as follows:

Specimen $\xrightarrow{10\% \text{ mbr.}}$ 1st mouse passage $\xrightarrow{10\% \text{ mbr.}}$ 2nd mouse passage $\xrightarrow{10\% \text{ mbr.}}$ 3rd mouse passage
(10 days observation) (10 days observation) (21 days observation)

Specimens were obtained from 82 fatal cases of Thai hemorrhagic fever. The age and sex breakdown of these cases is shown in Table 18. Isolation attempts from autopsy materials is summarized in Tables 19 and 20. Virus isolation attempts in venous or heart blood samples is shown in Table 21.

From these data it is evident that virus isolation from autopsy specimens is very rare. Several explanations may be invoked for this failure to isolate virus:

1. Virus in tissue is destroyed rapidly after death of patient. To the contrary, it is well known that dengue virus survives for many days in human blood specimens or dead mice kept at 4°C.

2. High antibody titers in patient's blood may destroy available virus. Table 22 shows the distribution of HI antibody in blood specimens taken at the time of death of randomly selected cases. These data show that some but not all patients die with high antibody titers.

3. HFI patients with severe disease may overreact in some poorly understood way to destroy their virus more rapidly than patients surviving infection. This would explain the infrequent isolation of virus in venous blood obtained before death.

4. Finally, Thai hemorrhagic fever deaths may be caused by a virus type which is not isolated readily. Isolations to date from fatal cases may be virus types which are exceptions to this "lethal" virus which still has not been identified.

Summary and Conclusions: Preliminary typing of dengue viruses from the 1962 outbreak has been accomplished. Nearly 1/3 of viruses recovered were dengue 1-TH Sman complex, 1/3 were dengue 2-TH-36 complex and 1/5 were dengue 3. Dengue 4 was also recovered. Multiple serologic types predominantly dengue 1 and 2 were recovered from hemorrhagic fever, while proportionately more dengue 3 was recovered from milder disease. Thus, dengue 3 may result in milder disease than dengue 2 or 1. In any event, evidence obtained does not support the concept that there is a single hemorrhagic dengue type. Of serologically confirmed dengue hemorrhagic fever infections, virus was recovered in 20%. In contrast, in 46 cases with fatal outcome virus was recovered in only 4% even though day of collection of acute specimen was the same in both groups. Dengue viruses apparently disappear from the blood of mortally ill patients more rapidly than from patients who survive hospitalization or milder illness. At the time of autopsy virus has disappeared. Only 1 dengue virus was recovered from a single organ (heart) in 46 autopsies processed in suckling mice.

Table 11. Dengue virus isolations from patient groups studied at Children's Hospital, 1962-1963.

Patient group	1962			1963		
	No. tested	Isolation	%	No. tested	Isolation	%
HFI	154	28	18.1	68	7	10.3
NH	128	10	7.7	17	1	5.9
PUO	116	17	14.6	32	4	12.5
HFS	46	2	4.3	-	-	-

Table 12. Chikungunya virus isolations from patient groups studied at Children's Hospital, 1962-1963.

Patient group	1962			1963		
	No. tested	Isolation	%	No. tested	Isolation	%
HFI	154	9	5.8	68	5	7.3
NH	128	4	3.1	17	-	-
PUO	116	10	8.6	32	-	-
HFS	46	3	6.5	-	-	-

Table 13. Day of illness of dengue virus isolation in 151 HFI cases confirmed as dengue by serological (HI) technique.

Day of illness	No. cases of HI positive	No. virus isolates	%
1	3	0	-
2	16	7	43.8
3	42	11	26.2
4	40	9	21.4
5	25	3	12.0
6	21	1	4.7
7	4	0	-
	151	31	20.5

Table 14. Day of illness of chikungunya virus isolation in 20 HFI cases confirmed by serological (HI) technique as chikungunya positive.

Day of illness	No. cases of HI positive	No. virus isolates	%
1	6	6	100
2	7	6	85.7
3	2	1	50
4	2	1	50
5	2	0	-
6	1	0	-
	20	14	70

Table 15. Day of illness of dengue virus isolation from 35 PUO cases confirmed as dengue positive by serological (HI) technique.

Day of illness	No. cases of HI positive	No. virus isolates	%
1	14	8	57.1
2	5	2	40.0
3	6	4	66.6
4	8	5	62.5
6	2	0	0
	35	19	49.7

Table 16. Day of illness of chikungunya virus isolation from 9 PUO patients with chikungunya infection confirmed by serological (HI) technique.

Day of illness	No. cases of HI positive	No. virus isolates	%
1	8	8	100
2	-	-	-
3	1	1	100
4	-	-	-
	9	9	100

Table 17. Tentative type designations for dengue viruses recovered from humans in Bangkok, 1962. Based upon CF test of mouse immune serum to each isolate vs. prototype dengue antigens.

Patient Category	d1 - TH Sman	d2 - TH 36	d3	d4	Not complete
Hemorrhagic fever	9	10	3	2	3
Mild undifferentiated fever	10	7	7	1	2
Hospitalized febrile syndromes	2	3	3		1

Table 18. Age and sex of fatal Thai hemorrhagic fever cases submitted to laboratory for virus isolation. Bangkok, 1962-1963.

Age in year	Sex		Total
	Male	Female	
Under 1	4	8	12
1 - 2	-	5	5
2 - 3	4	6	10
3 - 4	5	4	9
4 - 5	4	5	9
5 - 6	7	8	15
6 - 8	4	3	7
8 - 10	6	4	10
10 - 15	1	0	1
15 - 20	0	1	1
20+	0	1	1
	35	47	82

Table 19. Day of death of 46 fatal cases of THF with autopsy organs submitted for virus isolation attempt.

Day of death	No. of patients	Isolation from organs
1	3	Neg.
2	5	Neg.
3	9	1 Pos. (dengue)
4	17	Neg.
5	5	Neg.
6	5	Neg.
7	1	Neg.
8	1	Neg.
	46	

Table 20. Results of virologic study of autopsy materials from 46 fatal cases of THF.

Organs	Numbers	Result
Brain	21	Neg.
Heart	24	Pos. (1 denguevirus)
Liver	45	Neg.
Lung	23	Neg.
Spleen	40	Neg.
Kidney	35	Neg.
Pancreas	4	Neg.
Thymus	3	Neg.
Adrenal gland	2	Neg.
Stomach	1	Neg.
Total organs	194	Positive 1 (0.53%)

Table 21. Isolation attempts on venous or heart blood samples obtained from patients dying of hemorrhagic fever, 1962-1963.

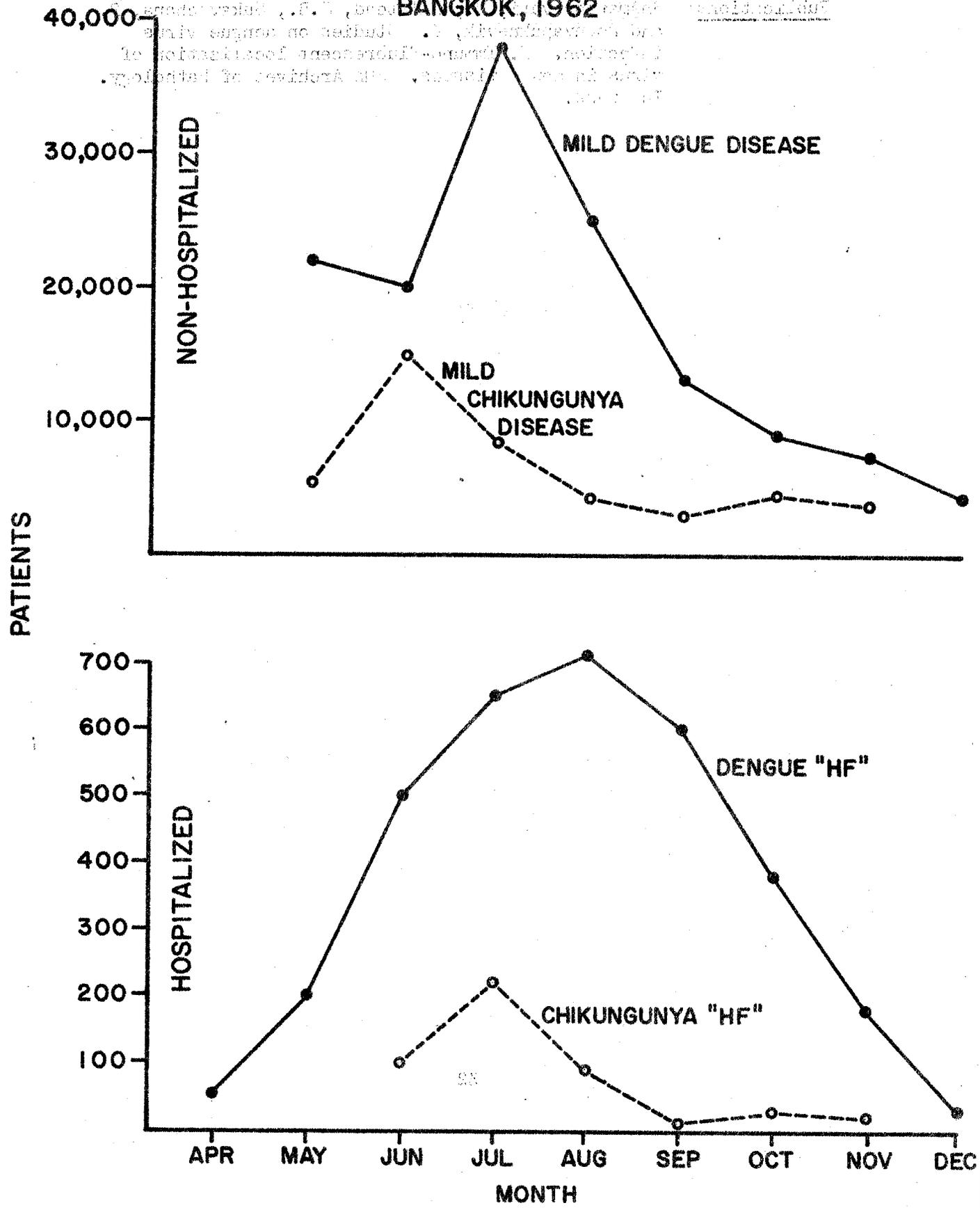
Day of illness	No. specimens	Isolation
1	3	1 (dengue)
2	1	Neg.
3	10	1 (dengue)
4	14	Neg.
5	13	Neg.
6	3	Neg.
7	2	Neg.
	46	2

Table 22. Hemagglutination-inhibition antibody titers for chikungunya and dengue 1 viruses in 13 hemorrhagic fever patients at day of death.

Patient number	Age (yrs.)	Sex	HI Titer	
			Chik	Dengue 1
1	4	F	80	640
2	6	M	40	160
3	21	F	320	163,840
4	4	F	40	2,560
5	5	M	20	20,480
6	2	F	640	10,240
7	6	F	0	1,280
8	3	F	20	160
9	1½	F	0	160
10	1	F	40	640
11	4	M	0	20,480
12	3	F	1,280	2,560
13	1 8/12	F	1,280	5,120

FIGURE 2. MONTHLY ATTACK RATES OF HEMORRHAGIC FEVER AND MILD DISEASE CAUSED BY CHIKUNGUNYA AND DENGUE VIRUSES (ESTIMATES)

BANGKOK, 1962



Publications: Bhamarapavati, N., Halstead, S.B., Sukavachana, P. and Boonyapaknevik, V. Studies on dengue virus infection. I. Immuno-fluorescent localization of virus in mouse tissues. AMA Archives of Pathology. In Press.