

## Combined Investigation of Dengue Hemorrhagic Fever in an Urban Population

**Project Coordinator:** Thomas J. Smith, LTC, MC

**Principal Investigators:** Ananda Nisalak, M.D.  
Boonsri Phuvichit\*, M.D.  
Curtis Bourgeois, Jr., LTC, MC  
Douglas Gould, Ph.D.  
Pethai Mansuwan,\*\* M.D.  
Sophana Kanchanapilant,\* M.D.  
Richard Grossman, MAJ, MC  
Thomas Smith, LTC, MC  
Suchinda Udomsakdi, M.D.

**Associate Investigators:** Hilary Evans, MAJ, MC  
Phanu Sittisomwongse, D.D.S.  
Salvadore Arellano, SP5  
Samuel Fulton, SFC

**Assistant Investigators:** Aree Boriharnvanakett, B.S.  
Chumpan Chavachati, B.S.  
Nonglak Khananuraksa, B.S.  
Panor Srisongkram, B.S.  
Phuangthong Puengkaew, R.N.  
Supatra Chulachumbok, R.N.  
Suvana Vithanomsat, R.N.

**OBJECTIVE:** To study clinical and biochemical abnormalities associated with dengue hemorrhagic fever, and investigate more efficient and specific virological methods of determining the etiology. In addition, to investigate ecological conditions which potentially contribute to favorable virus transmission by mosquito vectors.

**DESCRIPTION:** Previous investigations of dengue hemorrhagic fever have provided an epidemiological description of the disease as it occurs in a relatively isolated (insular) population. In this situation the fact of ecologic isolation apparently restricted annual epidemic disease to a single serotype (Annual Reports 1968, 1969). In a large urban population such as Bangkok, however, a multiplicity of ecologic situations with a large human population would seem to potentially offer totally different conditions.

The coordinated investigation by a number of different disciplines of all children admitted with dengue hemorrhagic fever over the course of a year offered the opportunity to study such urban dengue hemorrhagic fever. During the year an attempt was made to include all children admitted to Children's Hospital, Bangkok. Upon admission a physician recorded a complete history and all abnormal physical findings. Specimens were collected for hematology, clinical chemistry, serology and virus isolation. Complete blood counts, urinalyses, platelet count, bleeding time and prothrombin and partial thromboplastin times were measured. The battery of chemistry determinations included BUN, sodium, chloride, CO<sub>2</sub>, glucose, SGOT, SGPT, LDH and isozymes, bilirubin, total protein and protein electrophoresis.

Home environment, family history, epidemiologic information and mosquito populations were assessed on the day of admission by a home visit. This team consisted of public health nurses and entomology technicians. Background information concerning the present illness, past medical history, family history and a general description of pertinent features of the home environment was recorded, and interview and sera collected

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\* Staff Physician, Children's Hospital.

\*\* Director, Children's Hospital.

from family members. Indoor daytime collections of Aedes aegypti mosquitoes resting or biting and by pyrethrum spray knock-down were done, identified, and then frozen for virus isolation attempts. Artificial water containers in and around homes were enumerated and the number containing larvae recorded, and a representative sample collection recorded for identification. Mosquito evaluations included both the patient's home and the 10-20 immediately surrounding homes, in as many instances as possible.

A specific virus etiology was sought in each case. Acute sera were cultured for virus, and acute and convalescent sera from the patient and members of his family were tested serologically by HI, CF, and in some cases, neutralizing antibody.

Available evidence, obtained in this laboratory during the past several years, indicates that the lesion of dengue hemorrhagic fever is the result of host hypersensitivity or immunity to dengue virus antigen. Immunologic data indicate that children with severe hemorrhagic fever have had previous experience with members of the dengue virus complex. This experience is reflected in the early appearance of high-titered, broadly-reactive group B antibody, and the rapid clearing of dengue virus from the circulation. The latter has been interpreted to mean that the virus is bound by antibody, and that "immune-complexes" of antigen, antibody and complement reacting on the surface of endothelial cells may result in cellular injury. As part of the present study, direct evidence for the presence of cytophilic antibody in hemorrhagic fever was sought utilizing specimens obtained from hospitalized children. In addition the persistence of dengue virus otherwise obscured by antidengue antibody was sought in cells of the reticuloendothelial and lymphoid systems.

**PROGRESS:** During the study period April 1969 to March 1970, 273 patients were admitted to the study. This represented 68% of the 406 total cases clinically diagnosed at Children's Hospital during this period, most of those not studied having been private patients. Of these 273 patients, 210 were confirmed in the laboratory as dengue infections (see below). The monthly admission rates are shown in Table 1, along with the age distribution of cases. For comparison, the number of cases admitted to 16 other hospitals in Bangkok-Thonburi during 1968-1969 is also shown.

Of the 273 total cases 118 were male and 155 female, 31 of the cases were fatal, or 11.4%. Of the fatal cases, mean age was 6.5 years and median age 5.0 years, with the patients dying an average 3.7 days after hospitalization.

Cases admitted were diagnosed serologically according to previously described criteria (see Annual Report 1968). A summary of serological results are shown in Tables II and III. Of cases with dengue shock syndrome all 32 showed a secondary type serological response, whereas 4 of hemorrhagic fever cases showed a primary, and 165 a secondary, response.

A summary of clinical features is shown in Table IV with special emphasis on hemorrhagic and hematological features. Table V shows results of fibrinogen, and lactic dehydrogenase (LDH) isoenzyme determinations. The homes of 189 patients were visited as soon after admission as possible by public health nurses for family histories and blood sample collection from family members, and 147 homes were visited by an entomological field team. As shown in Table VI, 14% of family members on whom paired sera were available had evidence of concomitant dengue virus infection.

Virus isolations were attempted from four sources:

1. mosquitoes collected in and around the patient's house;
2. from acute phase sera of the patient;
3. autopsy specimens in fatal cases;
4. bone marrow aspirates during the acute phase.

A summary of virus isolates is presented in Table VII. Dengue 2 virus was most commonly isolated with 23 isolates, followed by 11 strains of dengue 3, 3 Chikungunya and one each dengue 1, Sindbis and unknown. At the time virus was present in the bone marrow it was also recovered from the blood of two patients.

Additional mosquito collection data is presented in the SEATO Medical Project on Mosquitoes report.

Table I. Monthly admission rates and age of patients.

Month	No. Patients		Age
April 1969	17	6	< 1 yr
May	28	7	1
June	36	15	2
July	43	26	3
Aug	36	22	4
Sept	20	26	5
Oct	16	29	6
Nov	10	13	7
Dec	4	15	8
		13	9
		15	10
		23	> 11

16 other Bangkok-Thonburi hospitals:

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
1968	62	32	38	44	68	104	209	214	357	272	242	252
(1,894)												
1969	278	174	156	170	241	310	497	516	500	358	141	48
(3,342)												

Table II. HI Serology Summary (N = 273 Cases)

Dengue Status	No. Cases	% of Grand Total	Dengue Isolates	CHIKUNGUNYA	
				No. Sero-Positive	CHIK Isolate
Primary Response	4	1.6	I	—	—
Secondary Response	197	81.4	—	5	—
a) 2° rise or fall	157	64.9	III (2)	4	—
b) Fixed Positive	40	16.5	—	1	—
Dengue Unclassifiable	9	3.7	III	—	—
<b>Total Dengue Positive</b>	<b>210</b>	<b>86.8</b>	<b>4</b>	<b>5</b>	<b>—</b>
Total Dengue Negative	32	13.2	I	5	3
a) True Negative	17	7.0	—	3	2
b) Fixed Negative	15	6.2	II	2	1
<b>GRAND TOTAL</b>	<b>242*</b>	<b>100.0</b>	<b>5</b>	<b>10</b>	<b>3</b>

\*Of the 31 cases with inadequate specimens, 30 were rapidly fatal cases.

Notes:

- (1) Of Dengue positive 1.9% had 1° response  
93.8% had 2° response
- (2) Proportion of case with CHIK diagnosis =  $5/242 = 4.1\%$
- (3) Proportion with CHIK and Dengue diagnosis =  $5/242 = 2.1\%$
- (4) Proportion of non-fatal study cases with serologic diagnosis of Dengue and/or CHIK =  $215/242 = 88.8\%$   
Thus the % nonfatal cases undiagnosed = 11.2%

Table III. HI Serologic DX by Age

Age (Yrs)	Tested		1° No.	2° No.	%	Fixed (+)		Dengue Unclass No.	Total Dengue		True Neg No.	Fix Neg No.	Tot Neg No.	CHIK No.
	No.	% of Total				No.	%		No.	Positives %				
<1	7	2.9	—	—	—	—	—	5	6	2.8	—	1	—	
1—	26	10.7	2	17	10.8	2	5.0	—	21	10.0	3	2	5	1
3—	49	20.2	—	37	23.6	10	25.0	1	48	22.8	1	—	1	—
5—	58	24.0	—	42	26.8	11	27.5	2	55	26.2	1	2	3	2
7—	35	14.5	—	23	14.6	5	12.5	—	28	13.3	4	3	7	1
9—	35	14.5	1	20	12.7	6	15.0	1	28	13.3	5	2	7	2
11—14	32	13.2	—	18	11.5	6	15.0	—	24	11.4	3	5	8	4
Total	242	100.0	4	157	100.0	40	100.0	9	210	99.8	17	15	32	10
Mean Age		6.80	—	6.70		7.2		—	6.6		—	—	8.2	9.4
Median Age		6.6	1—	6.21		6.5		<1	6.3		8—	8—	8.8	10
% Male		43.0	75.0	40:1		40.0		66.7	41.9%	or				
									M:F	1:2.4				

TABLE IV. Clinical and Laboratory Data, THF

	Dengue Serologic Diagnosis						Fatal Cases N = 31	
	Shock N = 32		Non-Shock N = 178		Total N = 210		No.	%
	No.	%	No.	%	No.	%		
Shock Cases	—	—	—	—	—	—	6/26	23.1
(+) Tourniquet Test	16/29	55.2	106/161	65.8	122/190	64.2	10/21	47.6
Rec. Plasma/(+) T.T.	15/16	93.8	61/106	57.5	76/122	62.3	9/10	90.0
Received Plasma	31	96.9	103	57.9	134	63.8	21	67.7
Adm. Temp. > 37.5°C	5/32	15.6	47/178	26.4	52/210	24.8	11/27	40.7
Petechiae	11/32	34.4	92/178	51.7	103/210	49.0	10/21	47.6
Liver	25/32	78.1	139/178	78.1	164/210	78.1	19/21	90.5
Lymphadenopathy	27/32	84.4	142/178	79.8	169/210	80.5	16/21	76.2
Pharyngitis	25/32	78.1	126/178	70.8	151/210	71.9	9/21	42.8
Conjunctivitis	4/32	12.5	18/178	10.1	22/210	10.5	0/21	0
History of Bleeding	6/32	18.7	77/178	43.2	83/210	39.5	12/21	57.1
Hemoconc. Index $\geq 0.2$	24/25	96.0	82/119	68.9	106/144	73.6	4/7	57.1
Platelet Ct. $\leq 60,000$	18/30	60.0	66/146	45.2	84/176	47.7	7/17	41.2
Hct $\geq 45\%$	25/31	80.6	74/169	43.8	99/200	49.5	13/21	61.9
SGOT > 125 SF Unit	8/22	36.4	42/138	30.4	50/160	31.2	9/12	75.0
TSP $\leq 5.5$ G%	14/26	53.8	49/150	32.7	63/176	35.8	6/14	42.8
WBC < 4,500	2/24	8.3	14/149	9.4	16/173	9.2	1/13	7.7
WBC $\geq 10,000$	7/24	29.2	65/149	43.6	72/173	41.6	7/13	53.8
Hemoglobin G% (Average)	13.21	n = 21	11.91	n = 113	12.11	n = 134	12.36	n = 5
BUN > 20 Mg%	10/26	38.5	46/154	29.9	56/180	31.1	12/15	80.0
Na < 135	15/30	50.0	60/89	67.4	75/119	63.0	4/7	57.1
Co <sub>2</sub> $\leq 15$	11/19	57.9	37/113	32.7	48/132	36.4	5/8	62.5

TABLE V. LDH and Fibrinogen Data

Test	Dengue Serologic Diagnosis			Fatal Cases n = 31	CHIK Diagnosis n = 10	No. Sero-Dx (non-fatal) n = 32	U.S. Normal
	Shock n = 32	Non-Shock n = 178	Total n = 210				
Fibrinogen } No. Test } Mean } Median }	8	34	42	4	—	9	400-700
	150.0 125	246.1 225	227.8 216.5	262.5 250	— —	480.1 375	
Total LDH } (Units) } No. Test } Mean } Median }	15	100	115	12	9	15	<250
	303.3 288.5	337.1 328	332.7 322.8	592.2 275	286.1 300	305.0 270	
LDH Components No. % Test	15	97	112	12	10	15	
LDH 1 Median	7.8	7.2	7.3	20.0	4.2	4.0	<5%
LDH 2 Median	12.5	12.4	12.4	12.5	9.2	9.2	<8%
LDH 3 Median	24.6	25.4	25.3	20.0	24.5	21.7	<25%
LDH 4 Median	31.2	29.7	29.9	24.4	33.8	35.0	<45%
LDH 5 Median	24.3	24.8	24.7	23.1	29.5	29.0	<30%

TABLE VI. Family Sera From Dengue — Diagnosed Cases

Age (Yrs)	True Neg.	Fixed Neg.	Total Neg.	Primary 1° Response	Secondary 2° Response			Dengue Unclassi- fied	Total Dengue (+)	Total
					↑	↓	Fix (+)			
0-4	5	3	8	2	3	—	1	6	14	
5-9	8	9	17	—	3	1	3	8	25	
10-14	4	3	7	—	2	—	—	2	9	
15-19	1	7	8	—	—	—	—	—	8	
20-29	1	35	36	—	2	1	1	6	42	
30-39	1	70	71	—	2	2	—	5	76	
40-49	1	14	15	—	—	—	—	1	16	
50—	2	7	9	—	—	—	—	—	9	
TOTALS	23	148	171	2	12	4	5	28	199	

Notes: (1) Average No. paired sera per family =  $\frac{199}{164} = 1.2$

(2) 0/23 paired sera from 19 non-dengue-diagnosed cases were Dengue (+)

(3) 164/210 (78.1%) Cases had one or more family sera drawn.

TABLE VII. Virus Isolates

Source	Virus	No. Strains
<u>A. aegypti</u>	dengue 2	13
	dengue 3	8
<u>C. quinguefasciatus</u>	dengue 2	1
	Sindbis	1
	unknown*	1
		1
Acute sera	dengue 1	1
	dengue 2	3
	dengue 3	3
	Chinkungunya	3
		3
Lymph node	dengue 2	3
Bone marrow	dengue 2	3

\*not neutralized with dengue 1-4, JE, Chik, Sindbis, Batai or Wesselsbron antisera.