

THE AFRIMS - BANGKOK CHILDREN'S HOSPITAL DENGUE HEMORRHAGIC  
FEVER PYREXIA OF UNKNOWN ORIGIN (DHF-PUO)  
STUDY, 1979

Principal Investigators : Donald S. Burke, LTC, MC  
Ananda Nisalak, M.D.  
Suchitra Nimmanitya, M.D.  
U-Sa Titsayakarn, M.D.  
Douglas M. Watts, Ph.D.

OBJECTIVES : To identify and obtain historical, clinical and laboratory data from children with dengue hemorrhagic fever (DHF), dengue fever (DF), and other febrile illnesses (non-dengue Pyrexia of undetermined origin, NDPVO) from a single hospital, in order to obtain comparisons of these groups of children regarding :

1. Racial and socioeconomic background
2. Nutritional status
3. Serologic evidence of previous flavivirus infection
4. Infecting dengue virus type.

BACKGROUND : A heated debate persists regarding the importance of sequential infections in determining the severity of dengue virus infections. Data previously reported from Thailand showing an association of DHF with secondary type antibody responses have been criticised on the basis that control population were inadequately defined, and studies from other countries have not shown such an association.

We therefore undertook a study of children with DHF, DF, and NDPVO's presenting to a single hospital in Bangkok. The study was designed so that virological factors (evidence of previous flavivirus exposure, infecting virus type) could be compared between these three groups where all clinical diagnoses and grading were made and reviewed without knowledge of serologic or isolation results.

History, physical examination, and clinical laboratory :

Data sheet : For all DHF and PUO patients a data sheet was completed by specially trained Department of Virology Nurses who interviewed and measured the patient, interviewed the patient's parents, and extracted the physicians physical examination data from the patient's chart.

Serologic diagnosis of dengue infections : An attempt was made to obtain acute and 2 to 4 week convalescent serum samples from all patients. This was done by giving the patient an appointment for a follow-up visit; if the patient failed to keep the appointment, then a phone call or more often a letter was sent to the patient as a reminder, and a second appointment made. If the patient still did not return, then one of the department of Virology nurses visited the patient's home. (Approximately 70% of patients returned for follow-up on the appointed day; an additional 10-15% in response to a letter reminder, and

an additional 5-10% were seen at a home visit. Overall paired acute and 2 to 4 week convalescent serum specimens were available from 91% of the DHF patients and 85% of the PUO patients.

All acute and convalescent sera were tested by the HAI technique against prototype D1, 2, 3 and 4 and JEV antigens by standard SEATO Lab/AFRIMS laboratory techniques. A serum sample was considered positive for HAI antibodies if a titer of  $\geq 1:10$  was obtained against any antigen. Acute dengue was diagnosed by the finding of a four fold rise in antibody titer to one or more antigens or by the finding of a high fixed titer ( $> 2560$  against any antigen) in either the acute or convalescent sera.

Virus isolation : From all patients, a sample of buffy coat suspended in plasma was inoculated into monolayers of LLC-Mk2 cells by the delayed and direct plaque technique according to standard AFRIMS laboratory procedures. Virus isolates were identified by plaque reduction neutralization with reference monkey anti-sera.

RESULTS : Diagnostic breakdown of the DHF and PUO groups is presented in Tables 1 - 8; the tables are self explanatory.

1979 appears to have been an unusual year for two reasons (1) the almost exclusive isolation of D2 from patients. Despite the fact that no isolate was obtained in 76% of the DHF patients, the near exclusive role of D2 is strongly reinforced by data from two other groups where higher isolation rates were obtained; among 14 children with DHF less than one year old, 8 dengue virus isolates, all type 2, were obtained; and among ten children with PUO, 9 dengue virus isolates, 8 types 2 and 1 type 1, were obtained. (2) The almost exclusive finding of secondary type antibody responses among children over 12 months of age (215/219, 98%). As shown in Table 9, even among one to two year old children, 13/14 or 93% had secondary type antibody responses. This contrasts ( $p = 10^{-7}$ ) with the finding that only 3/32 or 9% of age matches ND PUO patients (children with fevers proven serologically not be due to dengue) had detectable serum dengue antibodies. The proportion of children with serologic evidence of a prior flavivirus infection (detectable antibodies in ND PUO patients; secondary type Ab response in DHF patients) for children 0-12 years old is shown in Table 10.

Differences are significant ( $p < 0.05$ ) for all groups except children less than one year old and children older than 10 years.

Racial and socioeconomic data are presented in Table 9, and anthropometric data in Table 11.

Table 1. Dengue Virus Infections Among Patients with an Initial Diagnosis of Hemorrhagic Fever at Children's Hospital, Bangkok, 1 January 1979 - 31 December 1979 (299 Patients, 91% 2 week follow-up): Final Clinical and Serologic Diagnosis

	<u>Final Clinical Diagnosis</u>		<u>Total</u>
	<u>Not DHF</u>	<u>(+) DHF</u>	
<u>Final Serologic Diagnosis</u> Not Flavivirus	24 <sup>a</sup>	10 <sup>b</sup>	34
Incomplete	9 <sup>c</sup>	19 <sup>d</sup>	28
Flavivirus (±)	9 <sup>e</sup>	228	237
Total	42	257	299

(a) Final Clinical Diagnosis :

Viral infection	12	(11, 37, 56, 101, 107, 110, 116, 138, 167, 173, 195, 271)
Gastritis	1	(12)
Herpangina	2	(29, 249)
Pyrexia cause ?	1	(65)
Dengue Fever	1	(94)
Thalassemia	1	(95)
Mycoplasma pneumonia	1	(117)
Paratyphoid Fever	1	(140)
URI	2	(145, 189)
Enteric Fever	1	(184)
Pharyngitis	1	(186)

(b) 2/10 patients positive isolate of dengue 2 from acute blood despite lack of seroconversion (141, 270)

(c) Final Clinical Diagnosis :

Viral hepatitis	1	(30)
Pyrexia ? etiol	2	(41, 182)
Malaria	1	(60)
URI	2	(122, 197)
Influenza	1	(130)
Viral infection*	1	(188)
Pharyngitis	1	(200)

\* D2 isolate from acute blood

(d) 11 with HAI  $\geq$  2560 in acute blood (1 pos D2 isolate)  
8 with HAI  $\leq$  1280 in acute blood (2 pos D2 isolates)

(e) Final Clinical Diagnosis :

Viral infection	6*	(44, 85, 97, 202, 222, 268)
Encephalopathy	1	(86)
Myocarditis	1	(100)
Hepatitis	1	(153)

\* Include 2 patients with D2 isolates from acute blood

Table 2. Final clinical and serologic diagnoses among patients with initial clinical diagnosis of DHF.

Initial Clinical Dx DHF

299

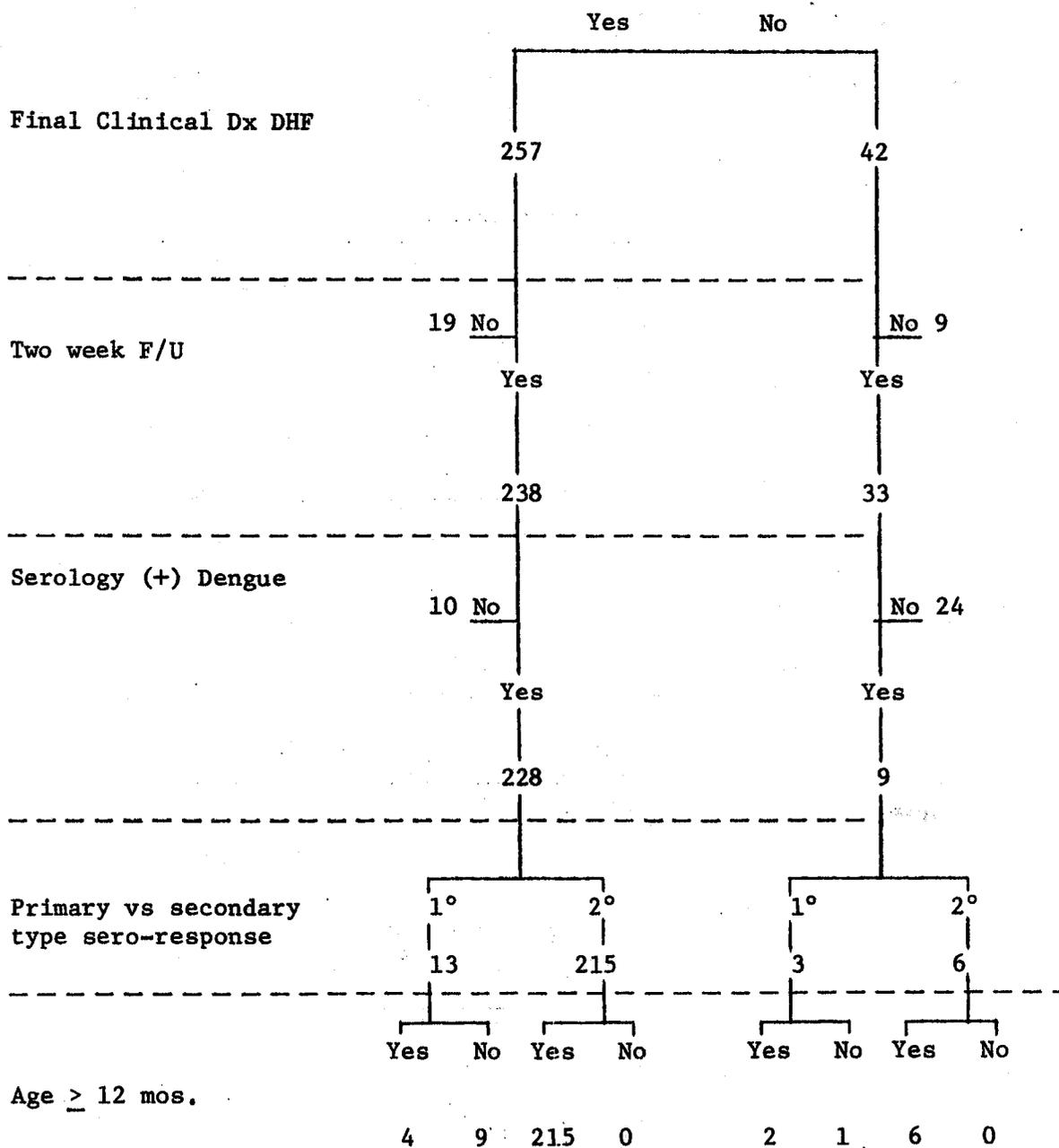


Table 3. Children with initial clinical diagnosis of DHF and serological confirmation of dengue infection,  $\geq 1$  year old, Bangkok Children's Hospital, 1979, primary versus secondary infections.

<u>Final clinical diagnosis and grade</u>	<u>Type of sero-response</u>	
	$\frac{1^\circ}{2}$	$\frac{2^\circ}{6}$
Not DHF		
<hr/>		
DHF Gr I	3 (146,129,241)	17
Gr II (+ I-II)	0	59
Gr III (+ II-III)	1 (005)	128
Gr IV (+ III-IV)	0	8
?	0	3
<hr/>		
Total DHF	4	215
<hr/>		

Table 4. Isolation of dengue viruses from patients with initial clinical diagnosis of DHF at Bangkok Children's Hospital, 1979

<u>Final clinical diagnosis</u>	<u>Serology (HAI)</u>	<u># positive / # tested</u>	<u>Types</u>
DHF	+	55/220	1 D1 53 D2 1 D4
DHF	No F/U	3/19 (229, 297, 299)	3 D2
DHF	0	2/10 (141 <sup>a</sup> , 270 <sup>b</sup> )	2 D2
Not DHF	+	2/9 (202, 222)	2 D2
Not DHF	No F/U	2/9 (188, 220)	1 D1 1 D2
Not DHF	0	0/24	-
<hr/>			
Total		64/299	2 D1 61 D2 1 D4

<sup>a</sup> Antibody titer < 10 to all dengue antigens by HAI in both acute and two week convalescent sera of a 3 year old child.

<sup>b</sup> No rise in titer between acute and two week convalescent sera from 3 month old child with pre-existing (transplacental) antibody in acute sera; patients mother had high titered (2560) anti-dengue HAI antibodies in her sera at the time of the two week convalescent blood specimen.

Table 5. Infants less than 1 year old with initial clinical diagnosis of hemorrhagic fever, Bangkok Children's Hospital, 1979

<u>Age (mos)</u>	<u>Case #</u>	<u>Final Clinical Dx</u>	<u>HAI serology</u>	<u>Isolation</u>
5	(029)	Herpangina	Neg	-
11	(116)	Viral infection	Neg	-
4	(122)	Viral URI	No F/U	-
5	(279)	DHF Gr II	No F/U	-
11	(44)	Viral infection	1°	-
2	(099)	DHF Gr IV	1° (mother seroconv)	-
3	(270)	DHF Gr II	Neg (mother seroconv)	D2
6	(299)	DHF Gr II	No F/U	D2
6	(090)	DHF Gr I	1°	D2
6	(180)	DHF Gr I	1°	D2
7	(246)	DHF Gr II	1°	-
7	(269)	DHF Gr II	1°	D2
7	(229)	DHF Fatal	No F/U	D2
7	(299)	DHF Gr II	No F/U	D2
8	(159)	DHF Gr II	1°	-
8	(228)	DHF Gr II	1°	-
9	(120)	DHF Gr II	1°	D2
11	(073)	DHF Gr II-III	1°	-

---

Total clinical DHF cases less than 1 year old :	<u>N</u> 14
With serologic confirmation :	9
With isolation confirmation :	8
With either serologic or isolation confirmation :	13

Table 6. Dengue Virus Infections Among PUO Patients at Children's Hospital, Bangkok, 10 May 1979 - 31 December 1979 (189 Patients; 85% 2 weeks follow-up) : Final Clinical and Serological Diagnoses.

	Final Clinical Diagnosis			Total
	Not DHF	Incomplete	DHF or R/O DHF	
Final Serologic Diagnosis Not Flavivirus	140 <sup>(a)</sup>	7	1 <sup>(b)</sup>	148
Incomplete	27 <sup>(c)</sup>	0	1 <sup>(d)</sup>	28
Flavivirus (+)	8 <sup>(e)</sup>	0	5 <sup>(f)</sup>	13
Total	175	7	7	189

(a) Includes following clinical diagnoses :

URI	49
Viral infection	30
PUO	13
Pharyngitis-tonsillitis	13
Measles	6
Enteric fever	5
Bronchitis	3
Influenza	3
Dengue fever	2 (007, 027)
Others	16*

\* (Two each of Hepatitis, Pneumonia, Herpetic stomatits, Kawasaki disease, viral exanthem; one each of roseola, drug rash, pertussis, parotitis, herpangina, gastroenteritis).

(b) One case of "viral infection (113) R/O DHF," no isolate.

(c) Includes 2 cases of dengue virus infection documented by isolation (both D2) on which no follow-up sera were obtained (091, URI; 155, Viral (infection))

(d) Includes one case of DHF Gr I referred to another hospital and lost to follow-up, no isolate.

(e) Includes clinical diagnoses of :

Viral infection :	3 (005, 061, 140)
URI :	2 (047, 125)
Pyrexia :	1 (100)
Pyrexia R/O hepatitis:	1 (173)
Enteric fever :	1 (150)

(f) Includes cases of DHF graded as follows :

DHF R/O :	1 (175)
" Gr I :	1 (028)*
" Gr 2 :	2 (105, 80)*
" Gr 2-3 :	1 (109)*

\* pos isolate of D2 (3/5)

Table 7. Dengue PUO's, 1979, Bangkok Children's Hospital

Case #	Age	Sex	Type serology	Virus type
005	5	M	2°	D2
061	3	M	2°	D2
140	6	F	2°	D2
047	4	F	2°	D2
125	4	M	2°	D2
100	9	M	2°	D2
173	8	F	2°	D2
150	4	F	1°	(JEV?)
091	4	M	?1°	D1
155	4	M	?2°	D2

Table 8. Dengue Fever (not including DHF) Infections Among PUO Patients in 1979 Virus Types Isolated in Primary and Secondary Infections, 1979.

Serologic response	Virus Type Isolated					
	D1	D2	D3	D4	JEV	None
Definite 1°	0	0	0	0	0	1 <sup>b</sup>
Probable 1°	1 <sup>c</sup>	0	0	0	0	0
Definite 2°	0	7	0	0	0	0
Probable 2°	0	1 <sup>d</sup>	0	0	0	0
Total <sup>a</sup>	1	8	0	0	0	1

(a)	1°	2°	
D2	0	8	$\chi^2 = 10.0$
Not 2	2	0	$P < .002$

(b) HAI response monospecific against JEV

(c) Four year old with no dengue HAI antibodies in acute serum specimen; no convalescent serum specimen available

(d) Four year old with acute serum HAI titers of D1-40; D2-20; D3-40; D4-20; JE-10; no convalescent serum specimen available

Table 9. Demography of DHF and PUO cases at Children's Hospital, 1979  
 (DHF cases D49 → D299 and PUO cases 1 → 189, May 10 → Dec. 31, 1979)

	<u>Confirmed Dengue</u>		<u>Proved Not Dengue</u>	
	<u>HF (192)</u>	<u>HF (27)</u>	<u>PUO (148)</u>	
Grand parents speak Chinese (N of 4)	1.44 ± 1.76(181)	1.24 ± 1.64(25)	0.58 ± 1.16(142)	
Family income (Baht/month X10)	2.90 ± 1.60(165)	3.15 ± 1.72(24)	2.95 ± 1.89(143)	
Mother's education (years)	4.53 ± 3.21(148)	5.20 ± 3.44(20)	6.00 ± 3.69(126)	
Father's education (years)	5.67 ± 3.83(146)	7.11 ± 4.97(19)	8.42 ± 4.00(122)	

Table 10. Evidence of pre-existing antibody in NDPVO and DHF patients

<u>Age (years)</u>	<u>Non-Dengue PUO</u> (#Ab(+) > 1:10/#total) (N = 148)	<u>DHF</u> (# secondary/#total) (N = 228)
< 1	2/13 (15%)	0/9 (0%)
1-2	3/32 (9%)	13/14 (93%)
3-4	12/32 (38%)	37/38 (97%)
5-6	12/25 (48%)	45/46 (95%)
7-8	16/23 (70%)	49/50 (98%)
9/10	12/15 (80%)	29/29 (100%)
11-12	5/7 (71%)	25/25 (100%)
13 +	1/1 (100%)	17/17 (100%)

Table 11. Anthropometrics of DHF and UDPVO patients

Age	Height (cm)		Weight (kilo)		Upper arm circumference (cm)	
	DHF	NDPUO	DHF	NDPUO	DHF	NDPUO
<1	67 ± 7 (8)	71 ± 8 (12)	6.7 ± 1.3 (8)	8.3 ± 2.4 (14)	13.0 ± 1.0 (7)	14.0 ± 1.0 (13)
1	94 ± 16 (2)	80 ± 5 (9)	13.5 ± 3.5 (2)	10.7 ± 2.9 (10)	15.5 ± 0.7 (2)	14.8 ± 1.1 (9)
2	88 ± 5 (8)	86 ± 8 (21)	11.3 ± 1.3 (8)	11.6 ± 2.0 (22)	14.5 ± 1.1 (8)	15.3 ± 1.4 (21)
3	94 ± 6 (19)	95 ± 6 (17)	12.6 ± 2.3 (19)	13.1 ± 1.7 (17)	14.8 ± 1.1 (19)	15.2 ± 1.1 (17)
4	103 ± 8 (15)	102 ± 7 (15)	14.6 ± 1.9 (10)	14.2 ± 2.4 (15)	15.2 ± 0.7 (15)	15.0 ± 0.9 (15)
5	105 ± 5 (19)	110 ± 8 (17)	14.7 ± 2.1 (19)	16.1 ± 1.5 (16)	15.0 ± 1.3 (19)	15.8 ± 1.1 (16)
6	114 ± 6 (15)	115 ± 4 (8)	18.7 ± 4.0 (17)	17.2 ± 1.6 (9)	15.6 ± 2.0 (18)	15.5 ± 1.0 (9)
7	118 ± 6 (27)	121 ± 4 (9)	18.3 ± 2.5 (25)	18.8 ± 2.3 (11)	15.6 ± 0.9 (24)	15.6 ± 1.1 (10)
8	122 ± 9 (19)	121 ± 7 (11)	19.4 ± 3.7 (19)	18.8 ± 3.1 (9)	16.1 ± 1.6 (19)	16.5 ± 1.0 (10)
9	129 ± 10 (14)	123 ± 6 (9)	21.7 ± 4.4 (14)	20.0 ± 2.6 (9)	16.4 ± 1.8 (14)	15.8 ± 1.6 (9)
10	132 ± 11 (13)	132 ± 6 (6)	24.7 ± 4.6 (12)	24.3 ± 4.9 (6)	17.0 ± 1.5 (13)	16.7 ± 2.1 (6)