DENGUE IN BANGKOK - 1980

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BACKGROUND : Clinical manifestations of dengue virus infections in humans range from an asymptomatic illness to shock, hemorrhage, and death. An integrated study of the entire spectrum (all clinical syndromes produced by dengue) has not been done in Bangkok since the early 1960's. A critical question, still incompletely resolved, is whether pre-existing immunity to flavivirus can sensitize on individual so that more severe disease manifestations occur when he becomes infected with dengue. One approach to this question is to prospectively study large groups of children. Another approach is to carefully examine groups of dengue patients with different well defined disease manifestations, in order to detect differences in age-corrected seroresponse patterns and virus isolations between patients with different disease manifestations.

MATERIALS AND METHODS : As shown in Figure 1, three groups of children were studied. Criteria for admission into each of the groups, and study outlines follow :

I. The D-80 study : All children who presented to the public ward or outpatient department of the Bangkok Children's Hospital (BCH) during the calendar year 1980 with a clinical diagnosis of DHF, and whose current home was listed as within the city limits of the greater Bangkok area (Krungthep Mahanakorn) were enrolled in the D-80 study. All children were evaluated by three different sets of criteria.

A. Clinical diagnosis and grading according to WHO criteria (Figure 2) as verified by S.N. accompanied by a detailed clinical check list.

B. Acute and day 10 to 14 convalescent HAI serology by the standard AFRIMS microtiter technique using acetone extracted sera and D1, D2, D3, D4, JEV, and chikungunya antigen (convalescent sera were obtained from 90% of D-80 cases). HAI seroresponses were categorized as primary or secondary as shown in Figure 3. The relatively high cut-off of 1:2560 was chosen (1) to ensure that secondary seroresponses were diagnosed only in those cases with distinctly vigorous antibody production and (2) because the distribution of all convalescent titers (maximum against any of the flaviviruses tested) of all febrile patients showed a distinct nadir at 1:1280 (see Figure 4).

C. Virus isolation according to the algorithm shown in Figure 5.

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This approach was previously determined to be the most efficient approach for isolating dengue viruses for acute DHF blood in Bangkok (Watts, unpublished data).

It must be strongly emphasized that all clinical evaluation, serologic evaluations, and isolation results were obtained by separate workers without knowledge of data derived by other members of the research team.

II. The PUO-80 study : Children who presented to the BCH OPD with undifferentiated pyrexia during the period 1 May - 31 December 1980 (coinciding with the peak of the DHF season) and who lived in metropolitan Bangkok were considered to be candidates for the study. An effort was made to study 2 or 3 patients per day during the 5 day work week. A history of recent acute illness with fever was a minimum requirement; physical examination evidence of definite focal purulence (cellulitis, UTI) constituted grounds for study non-admission. Focal symptoms (cough, sore throat, arthralgia) were specifically not used as selection criteria. All patients were examined and questioned using the "D-80" evaluation form (Appendix A).

A. Clinical diagnosis was defined by SN on the basis of chart review of all cases. In these cases which later were diagnosed as DHF, a grade was also assigned.

B. Acute and convalescent HAI serologies were performed as above for the D-80 study. Convalescent follow-up specimens were obtained from 91% of cases.

C. Virus isolation was performed according to the algorithm shown in Figure 6.

III. The Phibun School - 1980 study : During the year 1980 the Phibun school study (see AFRIMS Annual Report 1978-79, pp 118-122) was enlarged and continued. 1756 children age 4 to 16 were bled in June 1980 and January 1981, and their sera tested for HAI antibodies to DEN 1, 2, 3, 4, and JE. For the purposes of this report only serologic data are presented here; clinical data are presented in another section of this annual report.

RESULTS : Figures 7 and 8 show the final diagnostic categories of all patients entered in the D-80 and PUO-80 studies, along with the proportion with primary and secondary HAI seroresponse patterns. Figure 9 shows the statistical association of more severe forms of dengue with the secondary seroresponse pattern. To determine if this association was simply due to the possibility that DSS occurred in older children, we compared the proportions of children with secondary seroresponse patterns at each year of age with the corresponding age-specific flavivirus antibody prevalence in two control groups : (1) the Phibun school and (2) all those children in the PUO-80 study who were found to have illnesses other than dengue ("non-dengue" PUO). This latter groups represents a control group matched for age, sex, race, and socioeconomic status with the D-80 and dengue positive PUO patients (see Figure 21). The age specific antibody curves are presented in Figure 10. From this data we derived the convenient formula (mnemonic) that the age

specific HAI antibody prevalence at any age level in Bangkok in 1980 could be approximated by multiplying ten times the age in years :

Age 1 year : 10% HAI positive
Age 2 years : 20% HAI positive
Age 3 years : 30% HAI positive

Etc.

Thus one would expect that 10% of Bangkok one year old children should experience a secondary HAI response to a dengue infection, as should 20% of two year olds, 30% of three year olds, etc.

The actual observed proportions of secondary seroresponses in uncomplicated DF, DHF-NS, and DSS are shown in Figures 11, 12, and 13 respectively. Although the proportions of observed secondary responses in DF do not differ from those expected based on the seroprevalence data, the proportions of secondary seroresponses in all age groups are higher than expected in the DHF-NS and DSS patients. For all age groups except infants under 1 year, more severe disease is associated with the 2° seroresponse pattern (Figure 14).

One possible explanation for these observed differences could be that different virus types affected different age groups, and that the association of seroresponse patterns with disease severity was coincidentally due to the fact that the serotype causing severe disease occurred in older age groups.

Figure 15 shows the distribution of convalescent HAI titers of all D-80 and PUO-80 cases (all age groups including neonates) from which a virus was isolated and typed. Roughly equal numbers of cases with DEN-1 isolates had 1° and 2° seroresponses, while, the vast majority of DEN-2 isolates positive cases had a secondary response pattern. The disease severity of these cases is analyzed in Figure 16. For both DEN-1 and DEN-2 there is an increase in the proportion of secondary cases in more severe illness, although the trend is more striking with DEN-2: 57 DEN-2 isolates were made from DSS cases in children 1 year or older; all of whom had a secondary seroresponse pattern. Another obvious trend is that D1 was more likely to be isolated from mild cases (DF or DHF-NS) while DEN-2 was the usual isolate from DSS patients. In Figures 17, 18 and 19 these associations are shown to be independent of age.

Additional information, gathered by questionnaire, was analyzed to retrospectively determine if there had been some inadvertent bias in defining the study groups that might account for the observed differences in the clinical groups. Figure 20 shows that the associations between DEN-1 and a 1° seroresponse, and DEN-2 and a 2° seroresponse, were found in all the 7 most populated amphurs (districts) in Bangkok, and were not confined to only one sector. Figure 21 shows that there were no definite ethnic, gender, or socioeconomic differences between patients with various degrees of disease severity.

Figure 22 shows that in Bangkok children with DHF with a primary seroresponse pattern in 1980, Dengue 2 was the virus type most often isolated from neonates (age ≤ 12 months) while DEN-1 was isolated from older children (see elsewhere in this annual report). A striking statistic is that of 86 dengue 2 viruses isolated from DHF patients older than 12 months, only one had a primary seroresponse pattern. This is perplexing in light of the Phibun school seroconversion data for 1980 which showed that DEN-2 accounted for 38% of primary asymptomatic seroconversions, almost as many as did DEN-1 (52%).

The overall findings of this study are summarized in Figure 23.

Figure 1

AFRIMS STUDIES OF DENGUE IN BANGKOK (1980)

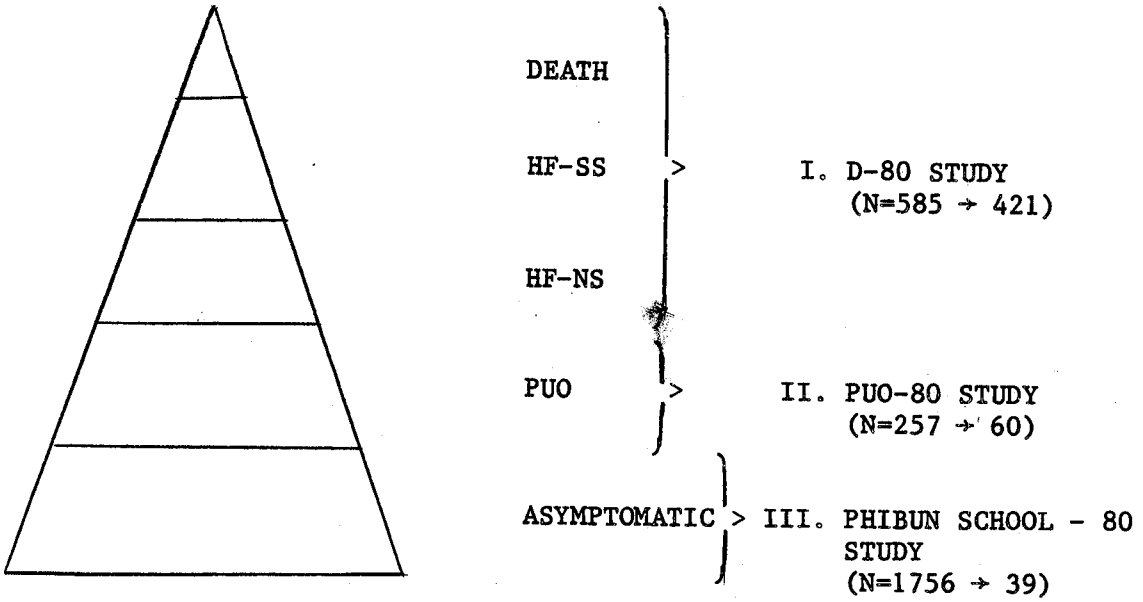


Figure 2

CLINICAL DIAGNOSIS AND GRADING: DEFINITIONS[a]

	FEVER $\geq$ 38.5°C	TOURNIQUET TEST	SPONTANEOUS BLEEDING	DECREASED PLATELETS	HEMO-CONCENTRATION	SHOCK
DF	+	+ or -	+ or -	+ or -	-	-
DHF-NS	+	+	+ or -	+	+	-
DSS	+	+	+	+	+	+

a. IN ACCORDANCE WITH THE "GUIDE FOR DIAGNOSIS, TREATMENT, AND CONTROL OF DENGUE HEMORRHAGIC FEVER, WHO, SECOND EDITION, 1980."

Figure 3

HAI SEROLOGY: DEFINITIONS

(1) FLAVIVIRUS ANTIBODY NEGATIVE :

HAI < 1:10 TO DEN 1, 2, 3, 4, JE

(2) FLAVIVIRUS ANTIBODY POSITIVE :

HAI ≥ 1:10 TO AT LEAST ONE ANTIGEN

(3) PRIMARY SERORESPONSE :

ACUTE TO CONVALESCENT HAI RISE OF ≥ 4 X TO AT LEAST ONE ANTIGEN

AND

CONVALESCENT HAI ≤ 1:1280 TO ALL FIVE ANTIGENS

(4) SECONDARY SERORESPONSE :

CONVALESCENT HAI ≥ 1:2560 TO AT LEAST ONE ANTIGEN

Fig. 4 Distribution of convalescent (day 14-28) HAI titers, Isolate positive dengue cases, D-80 and PUO-80 Studies, Bangkok, 1980.

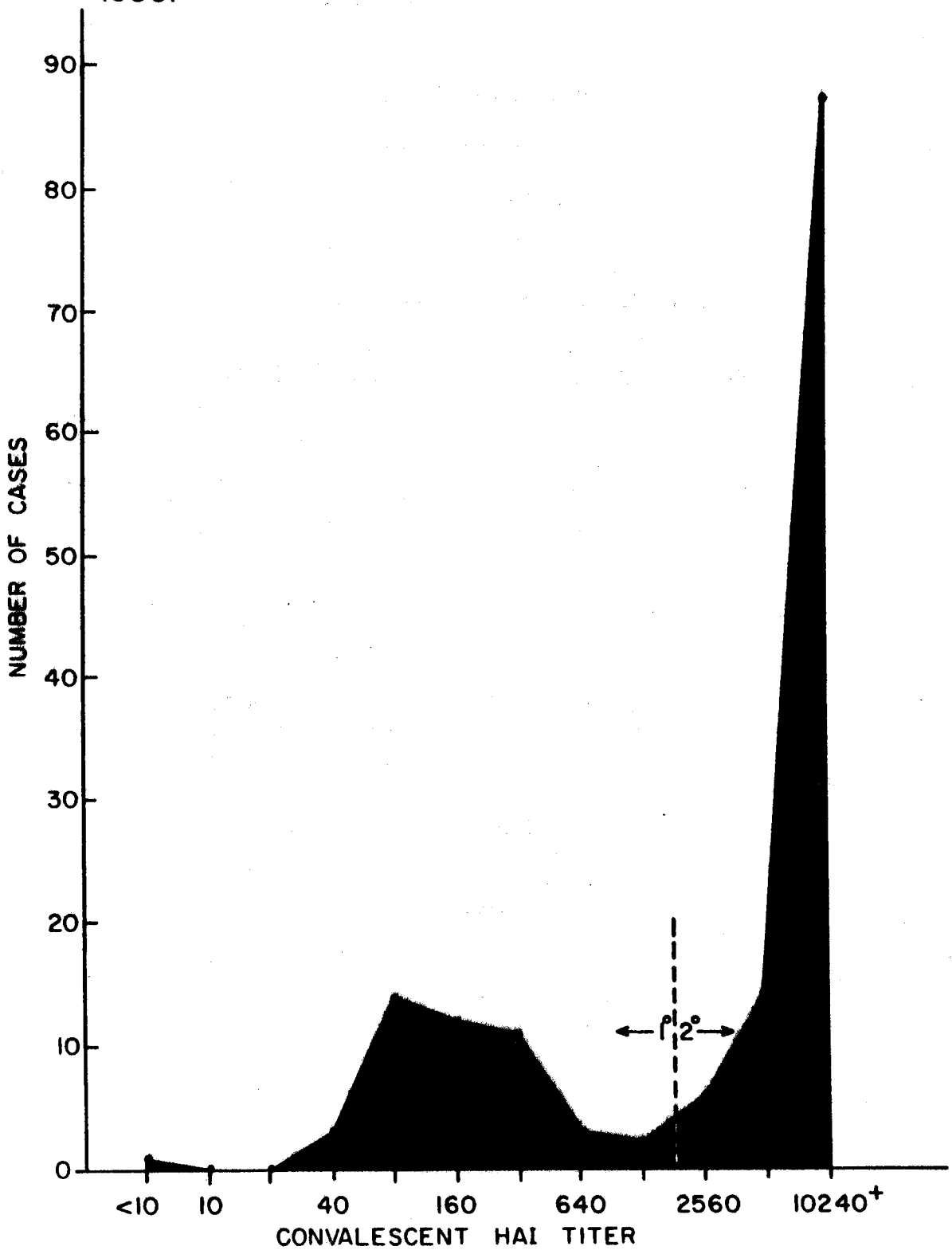


Figure 5

D-80 VIRUS ISOLATION ALGORITHM

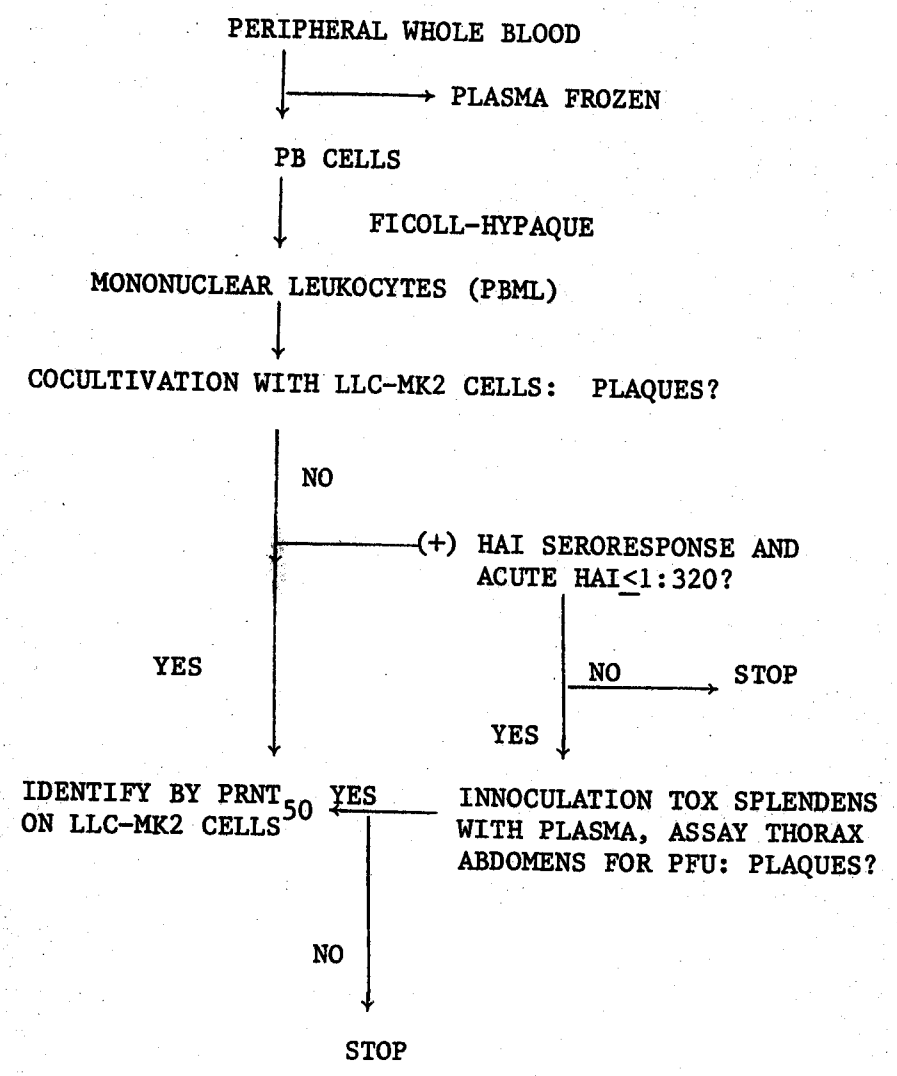


Figure 6

PUO-80 ISOLATION ALGORITHM

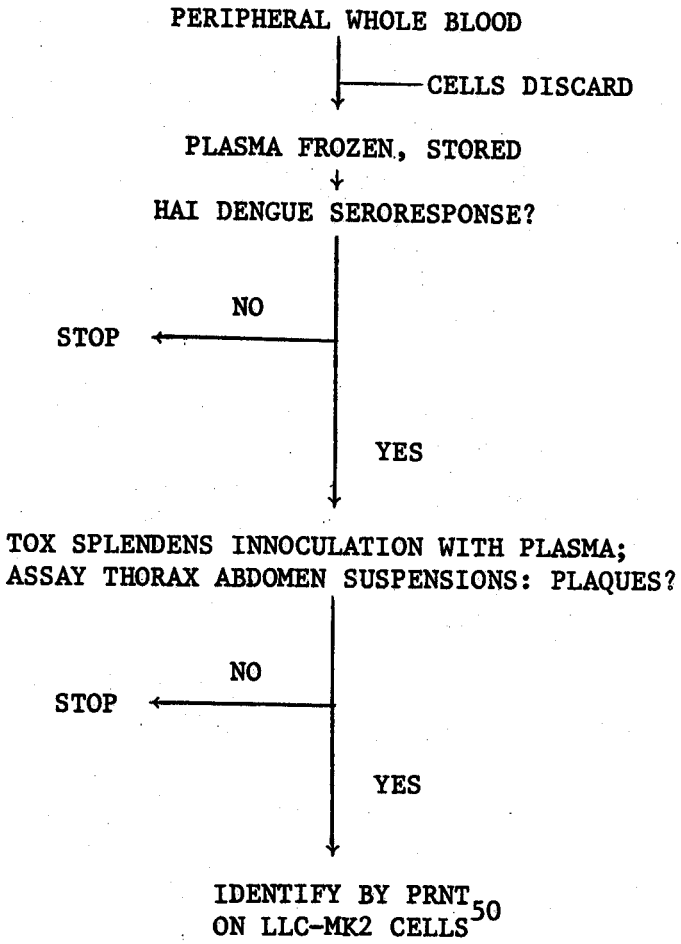


Figure 7

DETECTION AND CONFIRMATION OF CASES OF DENGUE HEMORRHAGIC FEVER (INCLUDING BOTH DSS AND DHF-NS), BANGKOK, 1980

585 ADMISSION CLINICAL DX = DHF

→ 97 FINAL CLINICAL DX = NOT DHF (44 DENGUE BY HAI SEROLOGY)

488 FINAL CLINICAL DX = DHF

(+) 4 x HAI RISE OR CONV HAI ≥1:2560	(-) 4 x HAI RISE AND CONV HAI<1:2560	NO SEROLOGIC F/U
415	23	50

% SEROLOGIC F/U = 90% (438/488)

% SEROLOGIC CONFIRMATION OF CLINICAL DIAGNOSES OF DHF = 95% (415/438)

Figure 8

DETECTION AND CONFIRMATION OF CASES OF UNCOMPLICATED DENGUE FEVER (DF), BANGKOK, 1980

257 INITIAL CLINICAL DX = PUO

→ 27 FINAL CLINICAL DX = DHF (24 CONFIRMED BY HAI)

230 FINAL CLINICAL DX = PUO

(+) 4 x HAI RISE OR CONV HAI≥1:2560	(-) 4 x HAI RISE AND CONV HAI<1:2560	NO SEROLOGIC F/U
60	150	20

% SEROLOGIC FOLLOW-UP = 91% (210/230)

% SEROLOGIC CONFIRMATION OF PUO CASES AS DENGUE = 29% (60/210)

Figure 9

HAI SERORESPONSE PATTERNS IN ACUTE DENGUE VIRUS INFECTIONS, CHILDREN AGES 0-15 YEARS, BANGKOK, 1980

CLINICAL DIAGNOSIS	HAI RESPONSE			
	1°	2°	TOTAL	% SECONDARY[a]
DSS	12	222	234	95%
DHF-NS	45	136	181	76%
DF	33	27	60	45%

a. BY CHI-SQUARE ANALYSIS :

DSS vs DF → $p<10^{-9}$

DHF-NS vs DF → $p<10^{-4}$

DSS vs DHF-NS → $p<10^{-7}$

Fig. 10 Age specific prevalence of anti-flavivirus antibodies in two Bangkok populations, 1980.

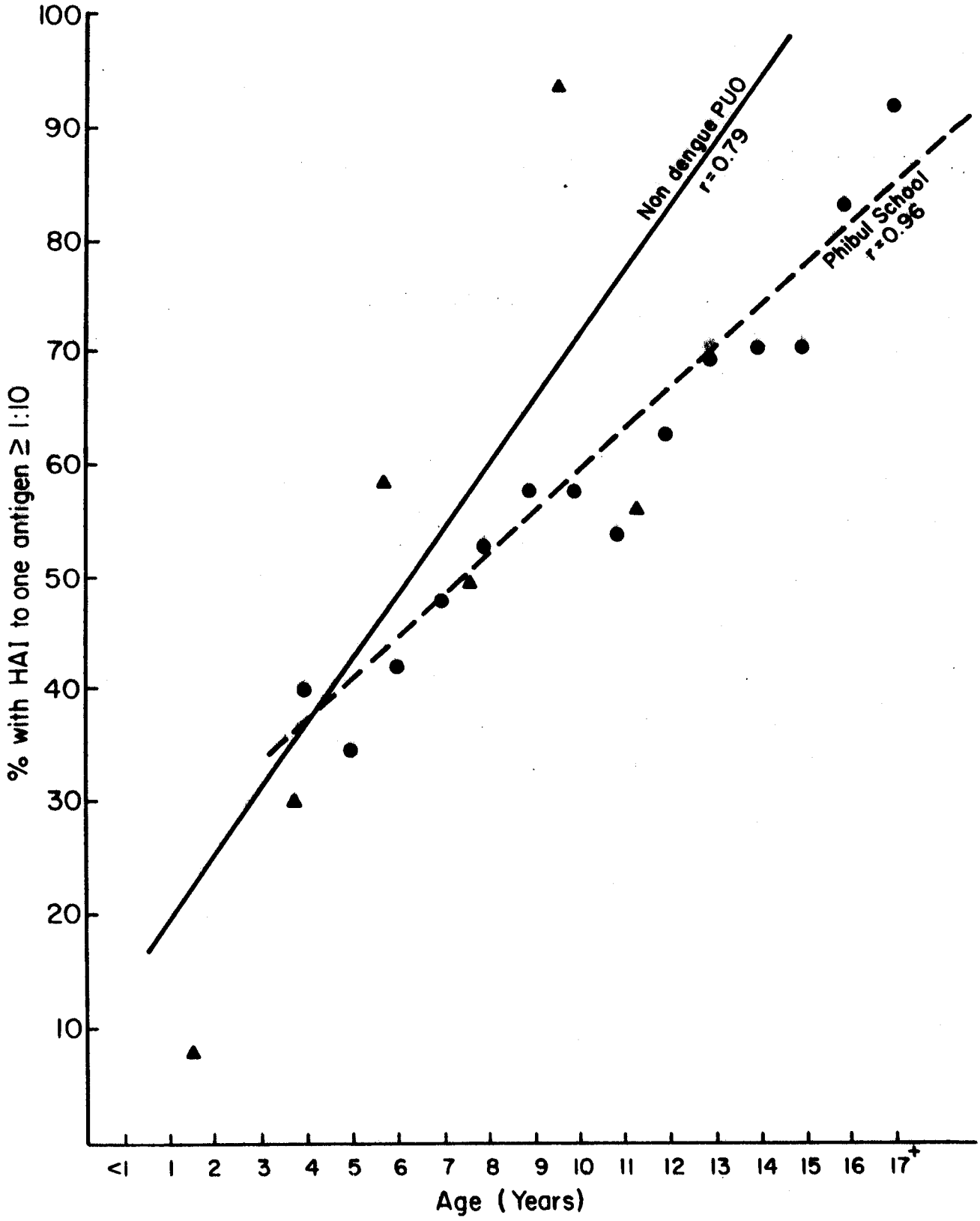


Figure 11
HAI SERORESPONSE PATTERNS IN UNCOMPLICATED DENGUE FEVER (DF)
BANGKOK, 1980

	HAI RESPONSE			% 2° HAI	
AGE	1°	2°	TOTAL	OBS	EXP
<1	1	0	1	0%	0%
1	3	0	3	0	10
2	2	1	3	33	20
3	5	1	6	17	30
4	4	0	4	0	40
5	6	2	6	25	50
6	2	2	4	50	60
7	1	3	4	75	70
8	3	2	5	40	80
9	2	5	7	71	90
10+	4	11	15	73	100
ALL	33	27	60		

Figure 12
HAI SERORESPONSE PATTERNS IN NON-SHOCK DENGUE
HEMORRHAGIC FEVER (DHF-NS), BANGKOK-1980

	HAI RESPONSE			% 2° HAI	
AGE	1°	2°	TOTAL	OBS	EXP
<1	19	0	19	0%	0%
1	1	0	1[a]	0	10
2	1	5	6	83	20
3	1	8	9	89	30
4	1	8	9	89	40
5	4	11	15	73	50
6	3	11	14	79	60
7	5	14	19	74	70
8	1	11	12	92	80
9	2	18	20	90	90
10+	7	50	57	88	100
ALL	45	136	181		

a. 12/12 MONTH OLD INFANT.

Figure 13

HAI SERORESPONSE PATTERNS IN DENGUE SHOCK SYNDROME (DSS) BANGKOK, 1980

	HAI RESPONSE			% 2° HAI	
AGE	1°	2°	TOTAL	OBS	EXP
<1	8	0	8	0%	0%
1	0	3	3	100	10
2	0	4	4	100	20
3	0	13	13	100	30
4	0	21	21	100	40
5	0	32	32	100	50
6	1	26	27	96	60
7	0	19	19	100	70
8	0	24	24	100	80
9	1	15	16	94	90
10+	2	65	67	97	100
ALL	12	222	234		

Figure 14

HAI SERORESPONSE PATTERNS IN DSS, DHF-NS, AND DF, BANGKOK, 1980

		CLINICAL DX			P VALUE[a]	
AGE	HAI RESPONSE	DSS	DHF-NS	DF	ALL DHF vs DF	DSS vs NS
<1	1°	8	19	1	NS	NS
	2°	0	0	0		
1-2	1°	0	1	5	.002	NS
	2°	7	6	1		
3-4	1°	0	2	9	3×10^{-8}	.11
	2°	34	16	1		
5-6	1°	1	7	8	3×10^{-7}	.001
	2°	58	22	4		
7-8	1°	0	6	4	.002	.004
	2°	43	25	5		
9-10+	1°	3	9	6	.006	.06
	2°	79	68	17		

a. FISHER'S EXACT TEST OR CHI-SQUARE TEST.

Fig. 15 Distribution of convalescent (day 14 – 28) HAI titers by dengue virus type isolated, D-80 and PUO-80 Studies, Bangkok, 1980.

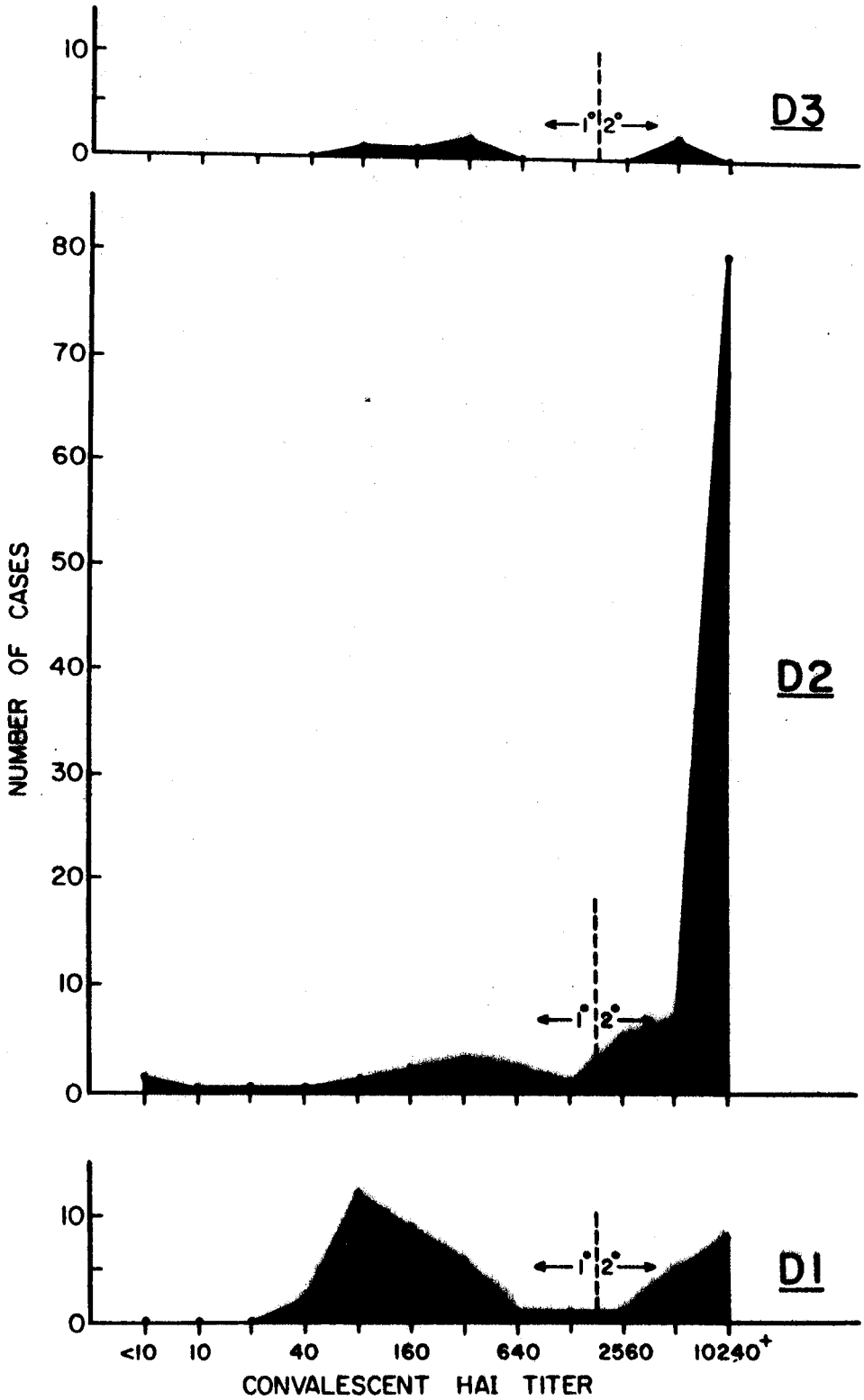


Figure 16

ALL DENGUE CASES≥1 Y.O., BANGKOK CHILDREN'S HOSPITAL, 1980

CLINICAL DIAGNOSIS	TOTAL PATIENTS	VIRUS ISOLATED (1°/2° HAI)			
		D1	D2	D3	NONE
DSS	226	2/2	0/57	0/1	2/162
DHF-NS	162	10/6	2/28	1/0	13/102
DF	59	15/4	3/9	2/0	12/14
ALL	447	27/12	5/94	3/1	27/278

Figure 17

UNCOMPLICATED DENGUE FEVER (DF)

BANGKOK, 1980

	D1 (N=20)		D2 (N=12)		D3 (N=2)		NO ISOL (N=26)	
AGE	1°	2°	1°	2°	1°	2°	1°	2°
<1	1	0	0	0	0	0	0	0
1	1	0	1	0	1	0	0	0
2	1	0	0	0	0	0	1	1
3	3	0	0	1	0	0	2	0
4	1	0	1	0	1	0	1	0
5	3	0	0	1	0	0	3	1
6	1	0	0	0	0	0	1	2
7	0	1	1	1	0	0	0	1
8	1	0	0	0	0	0	2	2
9	2	1	0	1	0	0	0	3
10+	2	2	0	5	0	0	2	4
ALL	16	4	3	9	2	0	12	14
≥1	15	4	3	9	2	0	12	14

Figure 18
DENGUE HEMORRHAGIC FEVER, NON-SHOCK (DHF-NS), BANGKOK, 1980

	D1 (N=18)		D2 (N=34)		D3 (N=1)		NO ISOL (N=128)	
AGE	1°	2°	1°	2°	1°	2°	1°	2°
<1	2	0	4	0	0	0	13	0
1	0	0	1	0	0	0	0	0
2	1	1	0	0	0	0	0	4
3	0	0	0	3	1	0	0	5
4	1	0	0	1	0	0	0	7
5	1	1	0	1	0	0	3	9
6	0	1	0	1	0	0	3	9
7	2	1	1	2	0	0	2	11
8	0	0	0	1	0	0	1	10
9	2	0	0	3	0	0	0	15
10+	3	2	0	16	0	0	4	32
ALL	12	6	6	28	1	0	26	102
≥1	10	6	2	28	1	0	13	102

Figure 19
DENGUE SHOCK SYNDROME (DSS)
BANGKOK, 1980

	D1 (N=5)		D2 (N=57)		D3 (N=1)		NO ISOL (N=171)	
AGE	1°	2°	1°	2°	1°	2°	1°	2°
<1	1	0	0	0	0	0	7	0
1	0	0	0	0	0	0	0	3
2	0	0	0	2	0	0	0	2
3	0	0	0	2	0	0	0	11
4	0	0	0	4	0	0	0	17
5	0	0	0	4	0	0	0	28
6	0	0	0	5	0	0	1	21
7	0	0	0	3	0	0	0	16
8	0	0	0	9	0	0	0	15
9	0	0	0	7	0	0	1	8
10+	2	2	0	21	0	1	0	41
ALL	3	2	0	57	0	1	9	162
≥1	2	2	0	57	0	1	2	162

Fig. 20

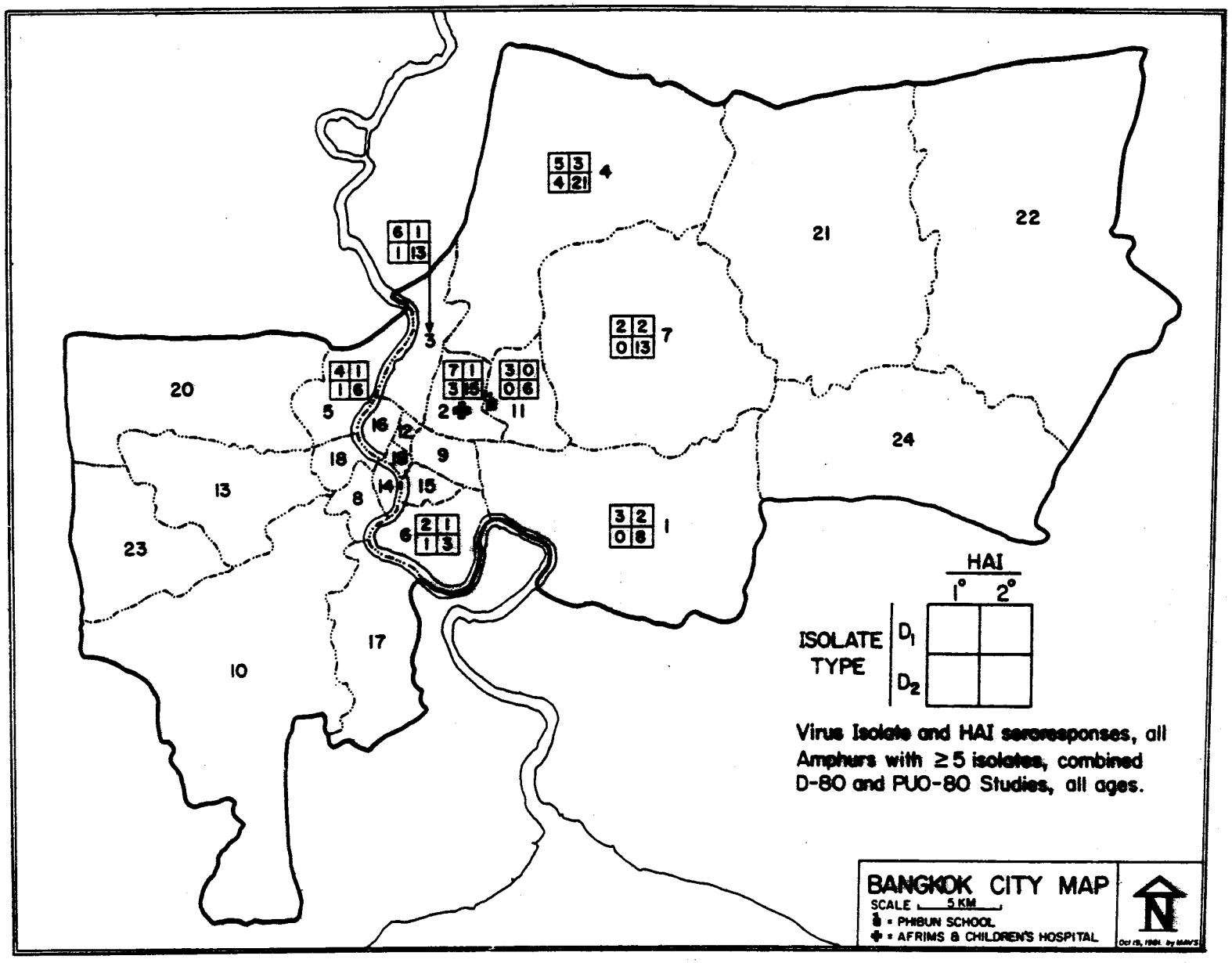

HAI
1° 2°
ISOLATE TYPE
D1
D2
Virus Isolate and HAI seroresponses, all Amphurs with ≥5 isolates, combined D-80 and PUO-80 Studies, all ages.
BANGKOK CITY MAP
SCALE 5 KM
= PHIBUN SCHOOL
= AFRIMS & CHILDREN'S HOSPITAL
N

Figure 21

CLINICAL GRADE	RACE (CHINESE)	SEX (FEMALE)	FAMILY INCOME (BAHT/MONTH X 10^{-3})	FATHER'S EDUCATION (YEARS)
PUO NOT DENGUE (N=150)	31%	45%	3.3 ± 1.9	5.4 ± 4.2
DF (N=60)	33%	42%	3.1 ± 1.9	6.2 ± 3.7
DHF (N-182)	33%	54%	3.3 ± 2.6	5.8 ± 3.6
DSS (N=234)	26%	56%	3.2 ± 1.2	5.6 ± 3.6
FATAL (N=6)	16%	83%	2.1 ± 0.9	3.0 ± 2.0

Figure 22

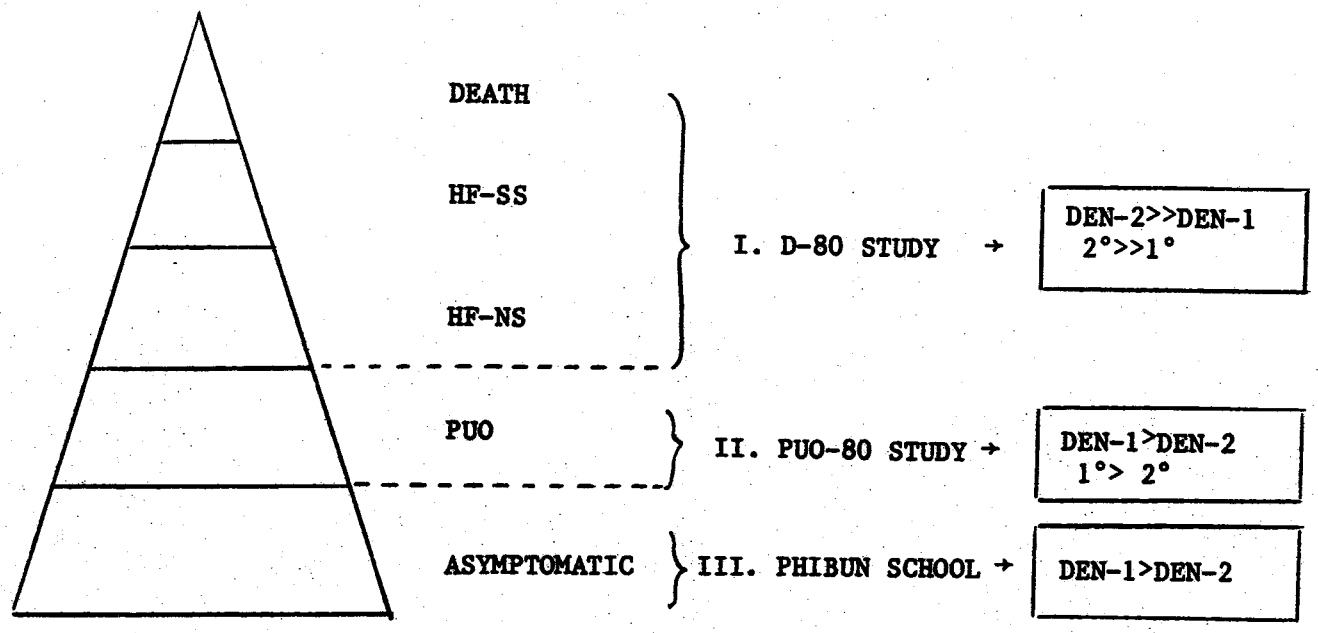
DENGUE ISOLATES IN PRIMARY DHF

	AGE	
	≤ 12 MOS	> 12 MOS
DEN 1	3	12
DEN 2	5	1

FET p = .01

- DEN 1 MOST COMMON ISOLATE IN PRIMARY DHF IN OLDER CHILDREN

- DEN 2 MOST COMMON ISOLATE IN PRIMARY DHF IN INFANTS

Figure 23

AFRIMS STUDIES OF DENGUE IN BANGKOK (1980): RESULTS