A PROSPECTIVE STUDY OF DENGUE INFECTIONS IN A BANGKOK SCHOOL, 1980

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OBJECTIVE : To determine if pre-existing immunity to dengue (as detected by conventional serologic techniques) is a major risk factor for development of DHF.

BACKGROUND : A continuing longitudinal prospective study of dengue virus infections among children at the Phibunprachasan school in the Din Dang section of Bangkok has been conducted by the U.S. Component of AFRIMS since May 1977 (AFRIMS Annual Progress Report, 1976-1977, pp. 50-54; AFRIMS Annual Progress Report, 1977-1978, pp 96-108; AFRIMS Annual Progress Report, 1978-1979, pp. 118-122; AFRIMS Annual Progress Report 1979-1980, pp 135-141, pp 165-167, and 201-202). The main objective of this study has been to define the epidemiology of dengue virus infections by serological methods. Six biannual surveys were obtained from the study cohort students between June 1977 and January 1980.

In early 1980 the incidence of cases of DHF in Bangkok reported to the Ministry of Health remained unusually high throughout the cool season. Therefore, at that time we predicted that a large epidemic of DHF would occur in the subsequent rainy season (AFRIMS Annual Progress Report, 1978-1979, pp 123-129), and designed a clinical study of school absenteeism to be superimposed onto the existing serological study. We predicted that in 1980 a sufficiently large number of DHF cases would occur among the Phibun school children to allow for rigorous statistical analysis of the role of pre-existing sero-immunity to dengue as risk factor for DHF.

MATERIALS AND METHODS :

Study Patients : Two groups of school children were studied. "ABC" Group (N=803). This group was the remainder of the cohort of 1,975 children originally studied in June 1977 and from whom a serum sample had been obtained every six months since that time. Only 41% (803/1,975) of this original "ABC" cohort were available for study in June 1980. Most of the drop-out from this cohort was among children in the older age groups who had completed the compulsory six years of education; of children 9 years old or younger in 1977, 60% remained in the study in 1980; of children 10 years old or older in 1977, only 23% remained. "Sub-group A" (N=304) were those students who were sero-negative in 1977 and remained sero-negative until June 1980. "Sub-group B" (N=91) were those students who had documented primary seroconversion sometime during the longitudinal study period (May 1977 and June 1980). "Sub-group C" (N-408) were those students who were sero-positive in 1977.

"Pre-bleed" group (N=1030). We estimated that the overall attack rate of DHF cases among the school children would be most 5 per 1000 students, and predicted that a study population of at least 1500 students would be required to detect statistically significant ($p < .05$) differences in attack rates between immunes and non-immunes even if the relative risk for immunes was infinitely greater than non-immunes. We therefore expanded the potential study group to include all students at the school by soliciting the participation of students who were not in the ABC cohort. This group of new study subjects is here in after referred to as the "Pre-bleed" group (N=1030). Signed parental consent was obtained from the parents or guardians of all "ABC" group and "pre-bleed" group subjects. Overall, a blood specimen was obtained in June 1980 from 62% of the total of 2979 children enrolled at the school. Specific reasons for refusal to participate were not solicited or analyzed.

Serologic sampling : A five milliliter sample of venous blood was obtained from the antecubital vein using syringe and 21 gauge needle. Blood was allowed to clot for four hours at room temperature, centrifuged, and the serum stored frozen at -20°C until testing. Samples were obtained in June 1980 (7th survey) and again in January 1981 (8th survey).

HAI and PRNT serologic testing : All sera from all study patients from the 7th and 8th bleeds were tested by HAI (after acetone extraction and goose red blood cell absorption) for antibodies to D1, 2, 3, 4, and JEV. Both sera from a given patient were tested simultaneously on the same microtiter plate. $PRNT_{50}$ antibody titers to prototype D1, 2, 3, and 4 viruses were measured on LLC-Mk2 cells on all specimen pairs which showed (1) a dengue HAI seroconversion from negative to positive (primary HAI seroconversion) or (2) a rise in HAI antibodies to 3 or more of the antigens tested (secondary HAI seroconversion). Table 1, Parts 1 and 2, define the HAI and $PRNT_{50}$ serologic criteria employed.

Absenteeism surveillance : All 70 classrooms at the Phibun school were visited daily by an AFRIMS nurse or nurses aid, and an attendence check made and recorded by the AFRIMS employee.

Detection of symptomatic dengue cases : All study children who were absent for two or more consecutive school days were interviewed by the AFRIMS nurse on the day the child returned to school, to determine the reason for absence. When necessary the child's parents were contacted to verify the child's story. If the reason for school absence was unrelated to febrile illness, e.g. an accident, a family problem, or truancy, then no further action was taken. If, however, the reason for school absence was a febrile illness, then a 5 ml venous blood sample was obtained on the day of return to school (first convalescent specimen) again 2 weeks later ("second convalescent specimen"). Also, if the child had been examined by a physician or had been hospitalized, permission was requested from the child's parents to review any clinical notes or records pertaining to the illness.

Both the first convalescent and second convalescent serum samples in each case were examined for HAI antibodies to D1, 2, 3, 4, and JEV and for IgM antibodies to a polyvalent dengue antigen (10 HA units each of D1, 2, 3, and 4) by antibody capture radioimmunoassay. Patients with a four fold HAI titer rise between the first and second convalescent specimens and/or a

positive (P/N 3.0 at a 10^{-2} dilution of serum) dengue IgM antibody capture RIA in either specimen were defined as confirmed dengue. All sera from children with symptomatic dengue infections were also tested for $PRNT_{50}$ antibodies to dengue 1, 2, 3, and 4.

No attempt was made to isolate dengue viruses from ill children. No attempt was made to retrospectively judge the accuracy of the clinical diagnoses of DHF made by private physicians, other than by serologic confirmation. Cases diagnosed as "DHF" were not grouped according to clinical grade because in several cases an accurate assessment was not possible on retrospective record review alone. Instead cases diagnosed as "DHF" were grouped simply according to whether or not the child was admitted to a hospital overnight or longer.

Detection of DHF cases among children not participating in the prospective study : In order to detect possible bias in the selection of children who formed the study group, we also monitored absenteeism among children not enrolled in the study group (study "non-participants"). As was done for study subjects, the absent child or the child's parents were interviewed, and permission was obtained to collect convalescent blood specimens from children who gave DHF as the reason for school absence. Sera were examined for HAI antibodies and IgM anti-dengue RIA antibodies.

RESULTS : The Phibunprachasan School reopened after the annual 2 month hot season vacation on 1 June 1980. After teachers and school administrators were consulted about the conduct of the study and parental consent forms were sent home and returned, the seventh Phibunprachasan school serologic survey was conducted from 16 to 24 June 1980. Serum specimens were obtained from 1833 students. Later (eight survey, January 1981) a second specimen was obtained from 1757 (96%) of these students.

Ideally the study period was intended to encompass the peak of the 1980 dengue epidemic season in Bangkok. In fact, the relatively late starting date (dictated by the date of school opening) of the study was at a time already well into the epidemic season. Of the total of 781 DHF cases admitted to Children's Hospital in the calendar year 1980, 350 (44%) had been admitted before June 16, the first day of the prospective study. Similarly of the total of 4,231 DHF cases reported to the Ministry of Health from the Bangkok metropolitan area in 1980, 31% were reported before the end of June.

Table 2 shows the age-specific prevalence of flavivirus HAI antibodies in sera from the study population seventh survey. The age of 50% antibody prevalence is 8 years. The curve is quite similar (only slightly lower) to that obtained in 1977 (first survey).

Table 3 is a summary of the pre-existing serologic status and subsequent serum antibody response in all study children who were absent for two or more days with a febrile illness during the study period.

Table 4 is similar to Table 3 but instead shows the serum antibody response of Phibun students who were absent with an illness clinically

diagnosed as "dengue hemorrhagic fever" but who had not volunteered to be part of the prospective study.

Table 5 is a summary of the dengue age specific attack rates for the spectrum of disease manifestations from asymptomatic to undifferentiated fever, "DHF-non-hospitalized" and "DHF-hospitalized."

Table 6 (parts 1, 2, 3, and 4) is a tabulation of all HAI and $PRNT_{50}$ results obtained on testing sera from all subjects with evidence of a dengue infection during the study period as determined by screening HAI serology on seventh and eighth surveys and/or by HAI and IgM ACRIA serology on convalescent illness samples. Parts 1, 2, 3, and 4 show asymptomatic primary infections, symptomatic primary infections, asymptomatic secondary infections and symptomatic secondary infections, respectively.

Table 7 (parts 1 and 2) is a summary of the $PRNT_{50}$ data presented in Table 6. Part 1 shows the identity of the infecting virus serotype (as determined by PRNT) in primary dengue infections. Because it is impossible to determine the infecting virus serotype in secondary dengue virus infections by PRNT, no attempt is made to do so here. Instead, Part 2 shows the pre-existing immunity in patients with secondary infections.

Table 8 is a tabular analysis of the specificity of the PRNT response in subjects with homotypic pre-existing sero immunity i.e., a determination if "the original antigenic sin" occurs with dengue.

Table 9 is a tabular analysis of the duration of pre-existing immunity prior to secondary dengue infections in those students who were part of the ABC cohort.

Table 1.1 Diagnostic criteria and definitions for dengue virus infection by HAI serology.

Term	Definition*	Survey	Example HAI titer D1	D2	D3	D4	JEV
HAI flavivirus sero-negative	HAI titer < 1:10 for all antigens tested	7	<10	<10	<10	<10	<10
HAI flavivirus sero-positive	HAI titer ≥ 1:10 for at least one antigen	7	<10	<10	<10	10	<10
HAI primary sero-conversion	Sero negative on 7th survey sample,	7	<10	<10	<10	<10	<10
	Sero positive on 8th survey sample.	8	<10	20	20	40	<10
HAI secondary sero-conversion	Sero positive in 7th survey sample	7	20	40	20	40	<10
	Sero positive on 8th survey sample	8	80	160	80	40	<10
	≥4 fold rise in titer to ≥ 3 antigens*						

* See AFRIMS Annual Progress Report, 1979-1980, pp. 135-141, "Assessment of Diagnostic Criteria for Dengue Virus Infection by HAI Serology on Sera Collected at Six Month Intervals."

Table 1.2 Diagnostic criteria and definitions for dengue virus infection by $PRNT_{50}$ serology.

Term	Definition	Survey	Example PRNT 1	2	3	4	Interpretation of example
Dengue neutralizing antibody (PRNT) sero-negative	$PRNT_{50}$ <1:20 for all 4 dengue serotypes	N	0	12	0	0	$PRNT_{50}$ sero-negative (Case #3275, 7th survey)
Dengue neutralizing antibody (PRNT) sero-positive	$PRNT_{50}$ >1:20 for at least one serotype	N	0	24	0	0	$PRNT_{50}$ sero-positive (to DEN 2, Case #929, 8th survey)
$PRNT_{50}$ primary seroconversion	4 fold or greater rise from PRNT sero-negative to sero-positive to at least one serotype	7 8	0 0	0 0	0 16	0 140	Primary PRNT seroconversion (to Den 4, Case #2544)
$PRNT_{50}$ secondary sero-conversion	4 fold or greater rise from PRNT sero-positive to sero-positive to at least one serotype	7 8	0 1,500	0 34	0 80	72 400	Secondary PRNT seroconversion (infection with ? serotype in DEN 4 immune, Case #1220)
Type specific sero-immunity	If only one serotype $PRNT_{50}$ $\geq$ 1:20, then sero-immune to that type	N	0	24	0	0	Den 2 immune (Case #929, 8th survey)
	or						
	If more than one serotype $PRNT_{50}$ $\geq$ 1:20, then sero-immune to all types with $PRNT_{50}$ $\geq$ 1:80	N	47	600	340	74	Den 2 and Den 3 immune (Case #1371, 7th survey)

Table 2. Age specific prevalence of HAI antibodies of flaviviruses, Phibun School, June 1980.

	Age (years)													
	4	5	6	7	8	9	10	11	12	13	14	15	≥16	Total
Number with specimen obtained in June 80 (7th survey)	65	89	82	201	168	204	221	200	174	147	121	95	66	1833
Number with no 8th survey specimen obtained (study drop-outs)	5	3	10	14	3	7	6	6	5	11	1	2	3	76
Number with both 7th and 8th survey specimens obtained	60	86	72	187	165	197	215	194	169	136	120	93	63	1757
HAI sero-positive in 7th survey (%) specimen	25 (42%)	30 (35%)	30 (42%)	89 (48%)	87 (53%)	114 (58%)	124 (58%)	105 (54%)	106 (63%)	95 (70%)	85 (71%)	66 (71%)	54 (86%)	1010 (57%)
HAI sero-negative in 7th survey specimen	35	56	42	98	78	83	91	89	163	41	35	27	9	747

Table 3. School absences due to febrile illness, prospectively studied cases, Phibun School, 1980.

	Study Number	Age/Sex		Hospital	Days Absent	Pre-existing HAI antibody status	Sero-response pattern
Diagnosed "DHF" Hospitalized							
	3295	4	M	Children's	9	+	2°
	1935	7	F	Children's	11	+	2°
	2494	7	F	Children's	8	+	2°
	2354	9	F	Mission	11	+	2°
	892	11	F	Vachira	13	+	2°
	433	12	F	Children's	13	+	2°
	408	14	F	Mission	5	+	2°
Diagnosed "DHF" Not Hospitalized							
	2733	7	M	Children's	9	0	Not dengue[a]
	2757	7	M	Bangkorlaem	9	0	Not dengue
	2548	7	F	Private clinic	2	+	Not dengue[b]
	2779	7	F	Private clinic	13	0	Not dengue
	2586	8	M	Children's	6	0	1°
	2335	9	F	Private clinic	4	0	Not dengue
	279	10	F	Private clinic	5	0	Not dengue
	818	10	M	Children's	7	+	2°
Undifferentiated febrile illness (not diagnosed as DHF)							
	2554	6	F	NA[c]	5	+	Not dengue
	2758	7	M	NA	6	0	Not dengue
	1863	7	F	NA	8	0	Not dengue
	2474	7	F	NA	4	0	Not dengue
	2576	7	F	NA	4	0	Not dengue
	2642	7	F	NA	9	0	Not dengue
	2770	7	F	NA	3	0	Not dengue
	2773	7	F	NA	3	+	Not dengue
	2774	7	F	NA	4	0	Not dengue
	2776	7	F	NA	9	+	Not dengue
	1874	8	M	NA	5	+	Not dengue
	1880	8	M	NA	4	+	Not dengue
	2457	8	M	NA	4	0	Not dengue
	2482	8	M	NA	2	0	Not dengue
	1862 (1)	8	F	NA	5	0	Not dengue
	1862 (2)	8	F	NA	4	0	Not dengue
	2448	8	F	NA	5	0	Not dengue
	2471 (1)	8	F	NA	5	+	Not dengue
	2471 (2)	8	F	NA	3	+	Not dengue
	2573	8	F	NA	12	+	Not dengue
	1911	9	M	NA	9	0	Not dengue
	1975	9	M	NA	8	0	Not dengue
	2336	9	F	NA	4	0	Not dengue
	262	10	M	NA	2	0	Not dengue
	532	10	M	NA	2	0	1°
	535	10	M	NA	4	0	Not dengue
	923	10	M	NA	6	+	Not dengue
	935	10	M	NA	5	+	Not dengue
	2254	10	M	NA	7	+	Not dengue
	560	10	F	NA	5	0	1°
	2255	10	F	NA	2	+	Not dengue
	123	11	M	NA	5	0	Not dengue
	2804	11	M	NA	6	+	Not dengue
	275	11	F	NA	4	0	Not dengue
	381	11	F	NA	5	+	Not dengue
	586	11	F	NA	3	0	Not dengue
	882	11	F	NA	3	+	Not dengue
	029	12	M	NA	8	+	2°
	357	12	M	NA	4	0	Not dengue
	208	12	F	NA	5	0	Not dengue
	001	13	M	NA	6	0	Not dengue
	005	13	F	NA	7	0	Not dengue
	351	13	F	NA	2	+	Not dengue
	977	14	M	NA	4	0	Not dengue
	1307	14	M	NA	5	0	1°

a. HAI negative in both first and second convalescent serum specimens.
b. HAI positive in both first and second convalescent serum specimens; no change in titer specimens; both specimens negative for IgM anti-dengue by radioimmunoassay.
c. NA = not applicable.

Table 4. Diagnoses of DHF among study non-participants, Phibun School, 1980.

Code number of cases hospitalized	Age/Sex		Hospital	Days Absent	Pre-existing antibody status	Sero-response pattern
SV 91480	7	M	Central	13	Unknown	2°
SA 82480	7	F	Children's	9	"	1°
NS 82380	8	F	Children's	11	"	2°
AC 82380	13	F	Chulalongkorn	14	"	2°
SS 103080	14	F	Children's	11	"	2°
Code number of cases not hospitalized						
OS 71880	7	M	Vachira	16	"	2°
SC 82580	7	F	Ramathibodi	2	"	2°
KS 62780	7	F	Ramathibodi	6	"	Not dengue
SN 91780	7	F	Private clinic	6	"	Not dengue
AS 91280	8	F	Private clinic	5	"	Not dengue

Table 5. Summary of Dengue by age.

	AGE													
	4	5	6	7	8	9	10	11	12	13	14	15	≥16	Total
Total number of subjects in age group	60	86	72	187	165	197	215	194	169	136	120	93	63	1757
Total dengue infections	4	2	3	14	10	7	15	12	12	9	5	8	2	103
Total symptomatic dengue infections	1	0	0	2	1	1	3	1	2	0	2	0	0	13
Fever	-	-	-	-	-	-	2	-	1	-	1	-	-	4
Non-hospitalized DHF	-	-	-	-	1	-	1	-	-	-	-	-	-	2
Hospitalized DHF	1	-	-	2	-	1	-	1	1	-	1	-	-	7
Total sero-negative on 7th survey	35	56	42	98	78	83	91	89	63	41	35	27	9	747
Total 1° dengue infection	3	2	3	5	8	2	9	6	2	4	2	1	0	47
Total symptomatic 1° dengue infections	-	-	-	-	1	-	2	-	-	-	1	-	-	4
Fever	-	-	-	-	-	-	2	-	-	-	1	-	-	3
Non-hospitalized DHF	-	-	-	-	1	-	-	-	-	-	-	-	-	1
Hospitalized DHF	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Total sero-positive on 7th survey	25	30	30	89	87	114	124	105	106	95	85	66	54	1010
Total 2° dengue infections	1	-	-	9	2	5	6	6	10	5	3	7	2	56
Total symptomatic 2° dengue infections	1	-	-	2	-	1	1	1	2	-	1	-	-	9
Fever	-	-	-	-	-	-	-	-	1	-	-	-	-	1
Non-hospitalized DHF	-	-	-	-	-	-	1	-	-	-	-	-	-	1
Hospitalized DHF	1	-	-	2	-	1	-	1	1	-	1	-	-	7

Table 6.1 Asymptomatic Primary Sero-conversions

Age	Case #	Serum #	HAI D1	HAI D2	HAI D3	HAI D4	HAI JE	$FRNT_{50}$ D1	$FRNT_{50}$ D2	$FRNT_{50}$ D3	$FRNT_{50}$ D4	$FRNT_{50}$ JE	Interpretation of PRNT Serology
4	3254	69243	0	0	0	0	0	0	19	10	180	X	2° infection in Den 4 immune
		1809	40	20	40	10	320	52	26	26	80	X	
	3257	69244	0	0	0	0	0	0	0	0	0	X	2° infection in Den 4 immune
		1661	80	40	80	320	40	320	42	59	130	X	
	3275	69261	0	0	0	0	0	0	12	0	0	X	1° Den 4 infection
		1678	0	0	0	40	0	0	14	14	240	X	
5	3218	69212	0	0	0	0	0	0	0	0	0	X	1° Den 2 infection
		1630	10	10	0	0	0	0	490	0	0	X	
	3232	69225	0	0	0	0	0	0	0	0	0	X	1° Den infection, ? Den 1 vs Den 2
		1753	20	10	20	10	0	40	50	10	0	X	
6	2605	68199	0	0	0	0	0	0	0	0	0	X	1° Den 2 infection
		1362	0	10	0	0	0	0	250	0	0	X	
	2692	68272	0	0	0	0	0	0	15	0	90	X	2° infection in Den 4 immune
		1430	10	0	10	40	10	0	22	0	700	X	
	3126	69130	0	0	0	0	0	12	16	0	0	X	1° Den 1 infection
		1549	40	20	40	40	10	320	60	28	10	X	
7	2666	68251	0	0	0	0	0	0	29	0	0	X	2° infection in Den 2 immune
		1415	10	10	20	80	0	0	82	0	130	X	
	2572	68171	0	0	0	0	0	0	0	0	0	X	1° Den 1 infection
		1335	40	20	40	80	10	176	37	28	20	X	
	2698	68277	0	0	0	0	0	0	0	0	0	X	1° Den 1 infection
		1443	80	20	40	80	40	1750	100	18	0	120	
	2737	68315	0	0	0	0	0	0	0	0	0	X	1° Den 1 infection
		1472	10	0	0	0	0	0	0	0	0	X	
	2947	68365	0	0	0	0	0	0	400	0	0	X	No infection, Den 2 immune
		1519	0	10	0	0	0	10	450	0	0	X	
8	1866	68010	0	0	0	0	0	0	0	0	0	0	No infection
		1171	10	0	10	10	40	0	10	0	0	0	
	1895	68030	0	0	0	0	0	0	0	0	400	X	2° infection in Den 4 immune
		1191	80	80	320	640	80	160	94	66	350	X	
	1899	68033	0	0	0	0	0	0	0	0	0	X	1° Den 1 infection
		1194	40	20	40	40	20	1600	67	35	18	X	
	2440	68044	0	0	0	0	0	0	14	0	55	0	No infection, Den 4 immune
		1205	0	0	0	10	0	0	0	0	21	0	
	2514	68113	0	0	0	0	0	0	0	0	0	0	1° Den 1 infection
		1276	40	10	20	20	20	800	20	28	26	0	
	2542	68139	0	0	0	0	0	400	200	0	0	X	No infection; Den 1, 2, immune
		1304	10	10	10	0	0	490	160	15	0	X	
	2544	68141	0	0	0	0	0	0	0	0	0	X	1° Den 4 infection
		1306	10	10	20	160	10	0	0	16	140	X	
	2565	68162	0	0	0	0	0	0	0	0	10	X	No infection; Den 4 immune
		1326	0	0	0	10	0	0	10	0	20	X	
9	2356	67897	0	0	0	0	0	0	0	0	0	X	1° Den 1 infection
		0351	40	10	40	80	20	780	23	44	22	X	
	2420	67959	0	0	0	0	0	0	0	0	0	X	1° Den 1 infection
		0410	40	0	20	10	0	400	0	0	10	X	
10	316	68507	0	0	0	0	0	0	0	0	0	X	1° Den 2 infection
		0603	0	20	10	10	0	0	1180	16	16	X	
	363	68529	0	0	0	0	0	0	0	0	0	0	1° JE infection
		0624	10	0	20	20	160	0	12	0	0	280	
	832	07647	0	0	0	0	0	86	0	0	0	X	No infection; Den 1 immune
		0113	20	0	10	0	0	77	0	0	0	X	
	887	69289	0	0	0	0	0	0	0	0	0	X	1° Den 2 infection
		0148	10	40	20	40	20	0	1380	0	0	X	
	902	67695	0	0	0	0	0	0	0	0	0	X	1° Den 2 infection
		0156	10	80	20	10	0	0	250	0	0	X	
	929	67708	0	0	0	0	0	0	0	0	0	X	1° Den 2 infection
		0171	0	20	0	0	0	0	24	0	0	X	
10	2257	68707	0	0	0	0	0	50	20	0	0	X	2° infection ∴ Den 1 immune
		1725	20	80	40	80	20	208	20	0	0	X	
	2369	67908	0	0	0	0	0	0	0	0	0	X	1° Den 2 infection
		0363	0	20	0	10	0	0	860	0	0	X	
11	249	69333	0	0	0	0	0	0	0	0	0	X	1° Den 1 infection
		0569	40	0	20	10	0	290	47	15	0	X	
	410	67579	0	0	0	0	0	0	0	0	11	X	1° Den 2 infection
		0048	0	20	0	0	0	0	860	0	0	X	
	432	68563	0	0	0	0	0	0	0	0	0	X	1° Den 1 infection
		0658	40	0	20	10	0	215	0	0	0	X	
	435	67582	0	0	0	0	0	150	0	0	0	X	No infection, Den 1 immune
		0051	10	0	0	0	0	120	0	0	0	X	
	2823	68638	0	0	0	0	0	0	0	0	0	X	1° Den 2 infection
		0755	0	20	10	10	0	0	3200	0	0	X	
	2840	68655	0	0	0	0	0	0	430	0	0	X	2° infection in Den 2 immune
		0770	0	20	0	0	0	0	2050	0	0	X	
12	009	68700	0	0	0	0	0	0	0	0	0	X	1° JE infection
		0437	0	0	10	10	40	0	23	0	0	145	
	089	68387	0	0	0	0	0	0	0	0	0	0	1° JE infection
		0480	10	20	10	20	40	0	0	0	0	310	
	430	68562	0	0	0	0	0	0	0	0	0	X	1° Den 4 infection
		0657	0	0	10	80	10	0	0	0	840	X	
	2869	68674	0	0	0	0	0	0	480	14	0	X	2° infection in Den 2 immune
		0797	40	40	80	80	40	150	3400	72	44	X	
13	022	68366	0	0	0	0	0	0	0	0	0	X	1° Den 1 infection
		0445	20	0	10	0	0	1200	0	18	0	X	
	621	68725	0	0	0	0	0	0	44	0	11	X	2° infection in Den 2 immune
		1791	40	40	80	80	40	25	120	28	28	X	
	2938	68919	0	0	0	0	0	0	290	17	16	X	No infection, Den 2 immune
		0823	10	10	20	20	10	16	700	23	37	X	
	2951	68928	0	0	0	0	0	0	120	0	0	X	No infection, Den 2 immune
		0832	10	10	10	40	0	0	40	0	0	X	
14	2997	69094	0	0	0	0	0	1250	42	40	13	X	No infection, Den 1 immune
		1100	80	10	20	40	20	1280	72	40	50	X	
15	1661	68866	0	0	0	0	0	40	10	0	0	X	No infection, Den 1 immune
		1038	0	0	10	10	0	108	0	0	0	X	

Table 6.2. Symptomatic primary dengue cases

Age/Sex		Study Number	Serum Number	HAI D1	HAI D2	HAI D3	HAI D4	HAI JE	$PRNT_{50}$ D1	$PRNT_{50}$ D2	$PRNT_{50}$ D3	$PRNT_{50}$ D4	$PRNT_{50}$ JE
10	M	532	69335	0	0	0	0	0	0	0	0	0	0
			69891	20	80	40	40	10	0	50	10	17	0
			70039	10	40	20	40	10	0	0	0	0	0
			0575	0	0	0	0	0	0	0	0	0	0
10	F	560	66653	0	0	0	0	0	0	0	0	0	0
			Ac 59538	0	0	0	0	0	0	0	0	0	0
			Conv 59559	40	20	80	40	20	640	205	120	0	0
			No 8th (resigned)										
14	M		69057	0	0	0	0	0	0	0	0	0	0
			69548	10	80	20	20	20	0	32	0	14	0
			69770	20	40	40	20	20	35	620	20	0	0
			0971	0	20	0	0	0	0	1200	10	18	0
8	M		68182	0	0	0	0	0	0	0	0	0	0
			69521	40	20	40	40	20	100	0	15	12	0
			69560	40	20	40	40	20	88	20	15	10	0
			1346	20	10	10	10	0	435	46	43	10	0

Table 6.3 Asymptomatic 2° Dengue (N=47).

Age	Case #	Sub-Group	Serum #	HAI 1	HAI 2	HAI 3	HAI 4	HAI J	PRNT 1	PRNT 2	PRNT 3	PRNT 4	PRNT J	Interpretation of PRNT Serology
7	2604	Pre	68198	80	80	160	160	160	0	13	750	178	X	2° infection in Den 3,4immune
			1361	1,280	1,280	2,560	2,560	1,280	1,100	1,020	11,900	600	X	
	2635	Pre	68223	10	0	20	10	0	0	0	375	0	X	2° infection in Den 3 immune
			1387	80	80	320	160	80	130	190	8,000	40	X	
	2703	Pre	68282	20	10	80	40	10	0	18	550	240	X	2° infection in Den 3,4immune
			1447	320	640	1,280	640	320	1,680	1,050	32,000	250	X	
	2739	Pre	68345	0	0	0	0	10	0	0	0	0	X	2° infection in JE immune
			1473	80	20	160	160	160	40	9,800	50	68	X	
	2771	Pre	6834	80	40	160	160	40	210	60	3,900	16	X	2° infection in Den 1,3immune
			1498	640	320	1,280	640	320	6,050	1,300	15,600	74	X	
	2772	Pre	68346	0	0	10	0	0	0	0	0	0	X	1° Den 1 infection
			1499	40	20	40	40	40	3,400	77	50	25	X	
	3163	Pre	69163	10	0	80	10	0	0	15	5,000	0	X	2° infection in Den 3 immune
			1581	160	80	320	160	80	700	198	20,000	53	X	
8	2537	Pre	68134	40	40	80	80	40	225	55	1,450	20	X	2° infection in Den1,2immune
			1299	160	160	320	320	80	640	2,000	4,700	45	X	
	2570	Pre	69386	20	20	20	40	10	290	37	25	375	X	No infection; Den 1,4 immune
			1739	160	80	80	160	40	265	35	64	600	X	
9	2312	Pre	67859	0	0	0	40	0	0	0	0	45	X	2° infection 4 immune
			0312	1,280	640	2,560	2,560	640	640	0	190	550	X	
	2340	Pre	67884	0	0	0	40	0	15	10	12	640	X	2° infection in Den 4 immune
			0336	320	640	1,280	1,280	640	780	40	155	640	X	
	2352	Pre	67893	0	0	10	40	0	0	0	0	350	X	2° infection in Den 4 immune
			0348	80	80	160	320	160	350	15	62	344	X	
	2392	Pre	67931	0	0	10	20	0	0	0	0	173	X	2° infection in Den 4 immune
			1734	80	160	320	640	160	500	40	175	320	X	
10	573	C	67633	40	20	10	10	0	700	12	0	0	X	2° infection in Den 1 immune
			0100	640	320	320	320	80	20,000	7,000	2,100	720	X	
	806	C	67638	40	40	80	40	20	60	100	110	0	X	2° infection in Den2,3immune
			0105	320	80	320	640	80	640	250	425	150	X	
	880	C	67686	40	80	80	160	40	60	1,280	18	210	X	2° infection in Den3,4immune
			0150	80	320	320	320	160	1,000	2,000	110	200	X	
	2202	Pre	67757	10	0	40	20	0	150	35	680	41	X	2° infection Den 1,3 immune
			0218	160	40	160	160	80	250	650	2,900	280	X	
	2501	Pre	68100	0	0	0	10	0	0	0	16	43	X	2° infection in Den 4 immune
			1263	40	40	40	160	40	700	14	100	100	X	
11	418	C	68555	20	10	80	40	10	15	13	2,100	0	X	2° infection in Den 3 immune
			0650	320	160	1,280	640	160	750	165	15,000	34	X	
	2263	Pre	67813	80	80	160	160	80	130	0	1,300	110	X	2° infection in Den1,3,4immune
			0270	320	320	640	640	320	700	17	2,500	600	X	
	2272	Pre	67820	40	40	40	80	20	800	80	200	10	X	2° infection in Den1,3immune
			0277	160	320	320	320	320	2,000	57	460	55	X	
	2781	Pre	68605	20	0	0	0	0	2,000	15	0	0	0	2° infection in Den 1 immune
			0429	40	20	40	80	80	210	50	110	130	0	
	2814	Pre	68629	0	40	10	10	0	0	750	12	0	X	2° infection in Den 2 immune
			0746	80	320	320	320	80	80	6,200	7,000	51	X	
12	005	C	68697	0	20	0	0	0	0	300	0	0	X	2° infection in Den 2 immune
			0434	20	160	80	40	40	0	10,200	50	0	X	
	056	A	68717	10	40	0	0	0	0	0	0	0	X	1° Den 1 infection
			0464	80	40	80	80	40	1,100	32	40	10	X	
	137	C	68415	40	20	40	40	40	90	85	0	0	X	2° infection Den 1,2 immune
			0506	320	320	1,280	1,280	1,280	740	4,500	200	155	X	
	147	B	69000	10	0	20	80	0	0	0	0	215	X	2° infection in Den 4 immune
			0515	160	80	320	640	160	1,500	90	45	335	X	
12	181	C*	68440	20	10	20	160	10	17	22	130	1,500	X	2° infection in Den3,4immune
			0537	160	160	320	640	80	300	180	500	1,200	X	
	215	C	68460	40	80	40	40	10	21	550	0	0	X	2° infection in Den 2,immune
			0555	320	320	160	640	80	800	1,900	75	35	X	
	498	C	68588	20	10	10	10	0	700	400	100	42	X	No infection; Den1,2,3immune
			0682	80	20	40	40	0	750	200	38	11	X	
	775	A	68760	0	20	0	0	0	0	0	0	0	X	No infection; non immune
			0713	40	160	80	20	0	0	15	0	0	X	
	2881	Pre	68686	0	0	0	20	0	0	0	0	160	X	2° infection in Den 4 immune
			0808	80	80	160	640	80	550	18	95	1,750	X	
13	057	C	68718	80	80	80	320	80	600	400	200	56	X	2° infection in Den1,2,3immune
			0465	320	640	640	320	320	10,000	8,200	3,000	18	X	
	125	C	68411	40	20	80	40	20	10	50	640	0	X	2° infection in Den 3 immune
			0500	320	80	640	320	160	350	280	1,000	40	X	
	344	A	68518	0	10	0	0	0	0	70	0	0	X	No infection; Den 2 immune
			0613	40	20	40	80	80	18	70	0	0	X	
	797	C	68766	80	40	80	80	40	375	40	0	10	X	2° infection in Den 1 immune
			0875	5,120	2,560	10,240	2,560	1,280	29,000	6,400	4,500	64	X	
	846	C	69288	20	20	40	320	10	14	30	0	680	X	2° infection in Den 4 immune
			0126	320	160	640	5,120	320	600	75	55	1,850	X	
14	124	B	68410	80	20	40	20	10	770	60	13	12	X	2° infection in Den 1 immune
			0853	1,280	320	640	640	320	18,000	4,000	700	110	X	
	1073	C	69030	0	20	0	10	0	0	10	0	15	X	1° Den 1 infection
			0913	320	320	640	1,280	320	1,600	140	225	183	X	
15	626	C*	68728	80	40	160	160	40	11	26	550	58	X	2° infection in Den 3 immune
			1761	160	160	640	640	160	48	1,000	850	88	X	
	1203	C	68784	40	40	160	80	40	70	83	525	37	X	2° infection in Den 3 immune
			0948	640	1,280	1,280	1,280	1,280	5,500	6,500	11,800	182	X	
15	1220	B	68788	0	0	0	20	0	0	0	0	72	X	2° infection in Den 4 immune
			0952	40	80	320	640	80	1,500	34	80	400	X	
	1267	C	68799	20	10	20	40	40	124	17	10	46	X	2° infection in Den 1 immune
			0961	160	160	160	320	320	570	510	38	155	X	
	1371	C	68827	20	20	40	40	20	47	600	340	74	X	2° infection in Den2,3immune
			0991	80	80	320	320	160	175	10,240	1,600	102	X	
	1485	C	69063	0	10	0	10	0	0	570	0	0	X	2° infection in Den 2 immune
			1020	160	160	1,280	640	640	320	5,120	450	200	X	
	1511	B	69064	0	0	0	10	0	0	0	0	44	X	2° infection in Den 4 immune
			1023	40	40	160	80	160	28	620	52	285	X	
16	1101	C	69037	40	40	80	40	20	200	600	850	112	X	No infection;Den1,2,3,4immune
			0922	80	80	320	320	160	170	300	700	64	X	
	1397	C	68843	40	40	40	40	20	32	135	350	0	X	2° infection,Den2,3 immune
			1005	320	320	640	640	160	3,000	12,500	11,800	280	X	

C* = Seropositive in first survey (June 1977) with serologic evidence of a previous "secondary" infection before 7th survey (June 1980).

Table 6.4 Symptomatic 2° Dengue

Age/Sex		Case #	Sub-Group	Serum #	HAI D1	HAI D2	HAI D3	HAI D4	HAI JE	$PRNT_{50}$ D1	$PRNT_{50}$ D2	$PRNT_{50}$ D3	$PRNT_{50}$ D4	Interpretation of PRNT Data
4	M	3295	Pre	69278	40	20	80	80	20	20	10	10	50	2° infect. in Den 3 immune
				1864/80(C1)	1,280	640	5,120	5,120	640	ND	ND	ND	ND	
				2173/80(C2)	1,280	2,560			2,560	1,600	2,800	13,000	900	
				ND	X	X	X	X	X	X	X	X	X	
7	F	1935		67729	40	10	20	40	10	700	50	110	0	2° infect. in Den 1, 3 immune
				69859		5,120			2,560	10^5	25,000	22,000	670	
				70015		5,120	5,120		1,280	10^5	10^5	37,000	4,000	
				0191	5,120	640	1,280	2,560	640	55,000	40,000	5,600	145	
7	F	2494	Pre	68094	40	10	20	20	0	1,620	20	29	0	2° infect. in Den 1 immune
				69453				2,560	0	10^5	10^5	25,000	2,300	
				69459					0	10^5	10^5	30,000	5,000	
				1256	160	160	80	40	20	4,400	450	1,200	320	
9	F	2354	Pre	67895	40	5,120		20	0	520	600	88	10	2° infect. in Den 1,2,3 immune
				69768	2,560	1,280	1,280	5,160	1,280	3,500	1,000	400	160	
				69835	320	80	160	1,280	160	55,000	17,000	8,500	500	
				0350				160	40	8,000	3,800	1,280	100	
10	M	818	C	67645	10	40	20	0	0	0	640	0	11	2° infect. in Den 2 immune
				69410					1,280	1,700	15,000	1,800	1,300	
				69456	1,280	5,120	2,560	2,560	640	4,000	8,500	4,800	70	
				0111	80	80	80	160	40	230	1,000	200	0	
11	F	892	C	67688	10	0	10	20	10	1,130	0	11	21	2° infect. in Den 1 immune
				70154	80	80	40	20	10	114	0	1,500	180	
				ND	X	X	X	X	X	X	X	X	X	
				1720	640	640	1,280	1,280	640	6,000	2,560	1,050	600	
12		029	C	68369	0	0	0	20	0	0	0	0	20	2° infect. in Den 4 immune
				69492		2,560			2,560	370	340	2,000	1,000	
				69561	1,280	2,560	2,560		640	1,100	500	1,600	320	
				0449	80	80	160	640	160	180	75	155	150	
12	F	433	C	68564	10	0	0	0	0	150	11	11	0	2° infect. in Den 1 immune
				69491					320	10^5	43,000	20,000	200	
				2693/80					640	10^5	10^5	28,000	400	
				0659	320	80	320	160	80	1,240	11,750	530	31	
14	F	408	B	67578	80	10	40	20	10	1,380	45	70	0	2° infect. in Den 1 immune
				69800	2,560	1,280	2,560	1,280	640	28,000	1,300	1,800	210	
				69844	2,560	1,280	2,560	2,560	640	35,000	43,000	14,000	1,000	
				0047	640	160	640	320	160	27,000	7,600	2,350	200	

Table 7.1 Infecting serotypes as determined by PRNT in 1° dengue infections

	Acute 1° infection with					2° Flavivirus infection	No infection
	D1	D2	D3	D4	?		
Hospitalized, DHF (N=0)	-	-	-	-	-	0	-
Not hospitalized, DHF (N=1)	1	-	-	-	-	0	-
Fever (N=3)	1	2	-	-	-	0	-
Asymptomatic (N=43)*	10	9	-	3	1	9**	11

* Does not include three 1° JE seroconversions
** Infection in Den 1 immune (1 case); Den 2 immune (4 cases); Den 4 immune (4 cases).

Table 7.2 Pre-existing immunity as determined by PRNT in 2° flavivirus infections

	Acute 2° infection in subject previously immune to:				Multiple types	No infection	1° infection
	D1	D2	D3	D4			
Hospitalized, "DHF" (N=7)	4	0	1	0	2**	0	0
Not hospitalized, "DHF" (N=1)	0	1	0	0	0	0	0
Fever (N=1)	0	0	0	1	0	0	0
Asymptomatic (N=46)*	5	4	6	10	14***	5	3

* Does not include one probable secondary infection in JE immune
** Den 1, 3, (1 case); Den 1, 2, 3, (1 case)
*** Den 1, 2 (1 case); Den 1, 3 (4 cases); Den 2, 3 (3 cases); Den 2, 4 (1 case); Den 3, 4 (3 cases); Den 1, 2, 3 (1 case); Den 1, 3, 4 (1 case).

Table 8. "Original antigenic sin" in secondary dengue virus infections in monotypic immune subjects (include both symptomatic and asymptomatic secondary infections, N=32).

Monotypic $PRNT_{50}$ antibodies in 7th survey specimens	DEN 1	DEN 2	DEN 3	DEN 4
DEN 1	8	1	-	-
DEN 2	-	4	1	-
DEN 3	-	1	6	-
DEN 4	8	1	-	2

= Cases in which "Doctrine of original antigenic sin" is upheld.

Table 9. Duration of pre-existing immunity in cases with symptomatic and asymptomatic secondary infections, ABC group.

Clinical Manifestation	Sub-Group C (Immune≥3 yrs)	Sub-Group B (Immune<3 yrs)	Total
Hospitalized DHF	3	1	4
Not hospitalized DHF	1	-	1
Fever	1	-	1
Asymptomatic	21	4	25
Total	26	5	31