A Longitudinal Serological Study of A Lower Socioeconomic School Population

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OBJECTIVES :

1. To describe the prevalence of serologic evidence of previous arboviral and hepatitis B viral infections in a susceptible school population.

2. To determine the incidence of clinical and subclinical infections with the above agents during the "disease season" and during the period of the year without a large amount of clinical illness.

3. To investigate socioeconomic parameters possibly related to infection occurrence.

BACKGROUND :

Arboviral Infections : A pre-and post-dengue transmission season study of 1131 Bangkok children, ages 1-14 years, and residing in 19 different study areas, was carried out in 1962 (1). During the one-year study period, five children (0.4%) had serologic evidence of recent Dengue Type 1 infection (HI titer $\geq$ 1: 640) and had been hospitalized with hemorrhagic fever. Seventy children (6.2%) had such serologic evidence without overt hemorrhagic fever. The percentage of children with Dengue Type 1 antibody titers of $\geq$ 1:640 ranged from 3% to 9% at the various ages. Over 40% of all children had detectable HI antibody to this virus, the percentage rising with increasing age and decreasing socioeconomic status.

Six hundred and twenty-eight children with a diagnosis of hemorrhagic fever were admitted to a clinical, virologic, and serologic study carried out from 1962 to 1965 (2). Over 80% of these children had dengue infections proven by virus isolation or serology; a secondary type antibody response occurred in 85% of these. The data suggested the hypothesis that the relative risk of dengue hemorrhagic fever and dengue shock syndrome is higher in children with secondary dengue infections. To date, dengue hemorrhagic fever remains the greatest cause of hospitalization and death among children in Thailand under the age of 15 years.

Hepatitis B Viral Infections : Study of a well-defined population of lower socioeconomic Bangkok residents (Huay Khwang) (3) has shown that 8.2% of the population have Hepatitis B surface antigen (HB_sAg) in their blood. Children in the 1-4 year age group have a 4.5% rate of antigen positivity. There was no significant difference in antigen prevalence between age groups. Antibody prevalence, on the other hand, was noted to increase rapidly from 15.4% to 48.9% between the ages of one and 19 years. These data suggested that the risk of acquiring hepatitis B antigen, but not antibody, was associated with the family unit.

METHODS : Students attending the Phibunprachasan School in the Din Daeng area of Bangkok were studied. A blood sample was collected in June 1977 prior to the "dengue season" (i.e. June-October) from 1988 students. One thousand nine hundred and three of these students had a repeat sample drawn after the "dengue season" in January 1978. A third blood sample was collected from 1249 students in June 1978. Basic demographic data on family size, family income, age and sex of family members, and the rank of the index child in the family were collected along with a medical history of the school child. Children who were absent from school for three or more days were visited, in their homes if necessary, by a public health nurse throughout the school year and acute and 14 day convalescent blood specimens were collected on children ill with symptoms possibly referable to a dengue or hepatitis viral infection. If the child was hospitalized, the treating physician was interviewed, acute and convalescent blood samples were collected, and a copy of the child's hospital record was obtained.

All blood samples obtained either were or will be tested for dengue, JEV and Group A arboviral antibodies by hemagglutination-inhibition. Chikungunya virus antigen was used to test for Group A antibodies. Virus isolation was attempted on all specimens obtained during the acute phase of a fefrile disease. Sera obtained were also tested for the presence of HB_sAg and anti-HB_s by radioimmune assay. Those with HB_sAg were subtyped and examined for the presence of HB_eAg and anti-HB_e. Serum specimens were collected from the families of index children who were HB_sAg positive and were tested in the same manner as that of the school child.

RESULTS :

Arboviral Infections : Table 1 presents the prevalence of positive titers ($\geq$1:20) by age and sex of the school children. Medical history of the child, family income, and number of children in the family did not appear to have a significant association with the distribution of antibody to either Group A or Group B arboviruses.

The group of children with one or more positive titers averaged approximately 16 months older than those without, but male/female differences were not found. Children, other than the eldest or only child, all have approximately the same risk of antibody acquisition. However, the eldest child's experience is moderately different from children with a lower rank in their family. [By "rank" is meant the order of the child in the family (e.g. the eldest child is ranked 1 and the fifth child in the family is ranked 5)]. The eldest child appears to have a slightly increased risk of antibody acquisition over children

with older siblings. Table 2 presents the data on the prevalence of Dengue Type 2. This finding is consistant in greater or lesser degree in all the Group B arboviruses tested.

Approximately 16% of the children at Phibunprachasan School showed evidence of an infection with either a Group A or Group B arboviral agent during the 1977 "dengue season". Of 824 students who were seronegative for any Group B agent, 110 (13.3%) sustained a primary type titer rise (maximum: 1:640), and an additional nine (1.1%) sustained a secondary type titer rise (minimum: 1:1280). Of the 1065 students with at least a 1:20 titer to one or more of the Group B arboviruses prior to the "dengue season", 175 (16.4%) sustained at least one fourfold or greater titer rise between the June 1977 and January 1978 blood specimens. The significance of a four fold "secondary" hemagglutination inhibition antibody titer rise to fewer than all five antigens is currently being evaluated by retesting selected specimens by the plaque reduction neutralization method. The corresponding values for Group A arboviral agents was 22 primary and two secondary responses out of 1540 susceptibles (1.6%) and 10 infections out of the 349 students with pre-existing antibody (2.9%). The distribution of four fold or greater HI antibody titer rises to the one group A and five group B antigens tested is presented in Tables 3 and 4.

Neither family income, geographic location of the family, number of children in the family, sex of the child nor the rank of the child within the family appeared to play a significant role in the distribution of infections. The age distribution of cases of infection, and to a lesser extent the sex distribution, was a reflection of the Prathom or school class in which the child was enrolled. For example, Table 5 presents the data on Group A infections. Since Prathom 6 is predominately aged 11-13, this focus of infection is represented in the age distribution. Similar mini-epidemics can be documented for Dengue Types 1, 3 and 4.

As can be seen from Table 4, the majority of secondary infections demonstrate fourfold or greater titer rises to multiple closely related Group B arboviruses. A minority however can be labeled as to infecting agent based on a single specific titer rise. The sum of primary and secondary infections with an isolated titer rise provide the basis for an age and sex distribution pattern for infections with the individual viral agents. Table 6 presents an example of this pattern method. The effect of isolated outbreaks of clinical and subclinical infection in Prathoms 5 (10-12 year old children) and 1 (7 and 8 year old children) can be seen. The total numbers of cases in these outbreaks are larger than depicted by tables such as Table 6 as most of the secondary infections will be among the indistinguishable combination groups.

Sixteen children were clinically diagnosed as having hemorrhagic fever during the course of the school year. Eleven of these showed no serologic evidence of arboviral infections when acute and convalescent HI antibody titers were compared. Of the 328 sero conversions (including 23 students who sero converted to both Groups A and B arboviruses) which occurred during the school year, only 5 (1.5%) were accompanied by clinical evidence of hemorrhagic fever. One of these was chikungunya for a hemorrhagic fever; total seroconversion ratio of 1:33 and 4 were dengue for a hemorrhagic fever : total seroconversion ratio of 1:72. All sixteen cases are currently undergoing further laboratory serologic evaluation.

Hepatitis B Viral Infections : Evidence of Hepatitis B Virus (HBV) infection was detected in sera of 38.9% of males and 33.3% of females in the population of the Phibunprachasan School sampled during June 1977 (Tables 7 and 8). Considerable variation was observed in the age specific prevalence rates of HBV infection with a gradual increase with age noted in both sexes.

The HBV antibody acquisition rates subsequent to a previous hepatitis B antigenemia for the entire population was 10.3% (Table 9). The difference between males and females was not significant. Antibody acquisition was observed in the age range 7 to 15 for males and 5 to 15 for females. The age-sex incidence of HBV infection, based on the acquisition of either HB_sAg or anti-HB_s is presented in Table 10. Overall, 4.3% of the population acquired HBV infection (either HB_sAg or anti-HB_s) during the period of seven months (June 1977 through January 1978). The acquisition of HB_sAg was 1.87% in males compared to a lower rate in females, 0.56%. No apparent difference was noted between sexes in the acquisition of antibody to HBV infection.

Among the study population of this lower socioeconomic group, 17.4% of males and 20.2% of females who were antigenemic in June 1977 spontaneously lost their antigen positivity by January 1978, while 5.5% of males and 7.3% of females lost their antibody during the study period (Table 11). The decline in detectable HB_sAg and anti-HB_s was greater in females than in males; however this difference was not statistically significant.

Study of the hepatitis B e antigen (HB_eAg), in students with HB_s antigenemia, demonstrated an apparent trend for students antigenemic for both HB_sAg and HB_eAg to have a greater chance of having household contacts with evidence of previous Hepatitis B infection (Table 12), but the number of families was too small for demonstration of statistical significance. Further work on this point will be necessary.

Determination of the arboviral and hepatitis B virus antibody status of the third blood sample collected in June 1978 is pending.

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Table 1. Prevalence of antibody to Group A and B arboviruses, Phibunprachasan School, Bangkok, Thailand, June 1977

GROUP A

Age	No. of males in the study	No. of pos. males	No. of females in the study	No. of pos. females	Total children in study	Total pos. children	% pos. children
4	14	-	25	-	39	-	-
5	31	4	37	3	68	7	10
6	57	-	31	3	88	3	3
7	75	5	113	10	188	15	8
8	115	11	90	4	205	15	7
9	116	9	113	10	229	19	8
10	123	16	126	22	249	38	15
11	110	19	110	19	220	38	17
12	120	22	128	29	248	51	21
13	105	27	157	44	262	71	27
14	45	13	82	22	127	35	28
15	17	7	28	3	45	10	22
16	2	-	6	4	8	4	50
17	-	-	2	-	2	-	-

GROUP B

4	14	4	25	3	39	7	18
5	31	15	37	14	68	29	43
6	57	23	36	15	93	38	41
7	75	32	113	50	188	82	44
8	115	63	90	40	205	103	50
9	116	66	113	62	229	128	56
10	123	74	126	73	249	147	59
11	110	57	110	60	220	117	53
12	120	76	128	76	248	152	61
13	105	76	157	115	262	191	73
14	45	34	82	53	127	87	69
15	17	15	28	17	45	32	71
16	2	2	6	5	8	7	88
17	-	-	2	2	2	2	100

Table 2. Prevalence of dengue type 2 antibody by family rank order

Rank of child	Antibody Titer	
	≤1:20	≤1:20
1	205	173
2+	956	611

df = 1

X^2_y = 5.53

$p < .025$

Relative risk = 1.08

Table 3. Primary cases of Group A and Group B arbovirus infections in children of Phibunprachasan School, Bangkok, Thailand Jun. 77 - Jan. 78

			Number of Infections
Group A			22
Group B			110
	Dengue 1	6	
	Dengue 2	10	
	Dengue 3	6	
	Dengue 4	61	
	JEV	1	
	Indistinguishable Combinations of 2 Group B		
	Dengue 1,2	1	
	Dengue 1,3	1	
	Dengue 2,3	2	
	Dengue 2,4	1	
	Dengue 3,4	11	
	Dengue 4, JEV	6	
	Indistinguishable Combinations of 3 Group B		
	Dengue 1,3,4	1	
	Dengue 2,4, JEV	1	
	Dengue 3,4, JEV	2	
			132

Table 4. Secondary cases of Group A and Group B arbovirus infections in children of Phibunprachasan School, Bangkok, Thailand, June 77 - Jan. 78

Virus		Number of infections by the case definition of 2°	
		Pre-existing antibody	Size of titer rise alone (1:1280 as minimum)
Group A		10	2
Group B		175	9
	Dengue 1	4	-
	Dengue 2	9	-
	Dengue 3	14	-
	Dengue 4	20	4
	JEV	2	-
Indistinguishable Combinations of 2 Group B			
	Dengue 1,2	2	-
	Dengue 1,3	1	1
	Dengue 1,4	1	-
	Dengue 2,3	1	-
	Dengue 3,4	4	-
	Dengue 4, JEV	-	2
Indistinguishable Combinations of 3 or more Group B		117	2
		185	11

Table 5. Comparison of Prathom 6 and the remainder of Phibunprachasan School with regard to infection with Group A arbovirus

		Prathom	
		6	Not 6
Infection with Group A Arbovirus	+	13	21
	-	211	1658

df = 1

X^2_y = 20.82

$p < .0005$

Table 6. Age and sex distribution of primary and secondary infections with dengue type 4 arbovirus, Phibunprachasan School, Bangkok, Thailand, Jun. 77 - Jan. 78

Age	Primary Infection		Secondary Infection		Total	
	M	F	M	F	M	F
4	-	-	-	-	-	-
5	1	2	-	-	1	2
6	-	1	-	-	-	1
7	3	9	1	1	4	10
8	4	5	1	-	5	5
9	4	3	1	-	5	3
10	7	4	4	8	11	12
11	-	3	1	4	1	7
12	3	3	1	1	4	4
13	4	4	1	-	5	4
14	-	1	-	-	-	1
15	-	-	-	-	-	-
16	-	-	-	-	-	-
	26	35	10	14	36	49

Table 7. The prevalence of hepatitis B virus surface antigen in students of the Phibunprachasan School, Bangkok, Thailand, June 1977

Age	Male			Female			Total		
	No. tested	HB_sAg*		No. tested	HB_sAg*		No. tested	HB_sAg*	
		No.	%		No.	%		No.	%
4	15	2	13.3	31	4	12.9	46	6	13.0
5	30	4	13.3	33	3	9.0	63	7	11.1
6	52	4	7.7	35	1	2.8	87	5	5.7
7	76	9	11.8	102	6	5.9	178	15	8.4
8	113	9	8.0	88	7	7.9	201	16	7.9
9	116	13	11.2	110	7	6.4	226	20	8.8
10	123	15	12.2	127	19	15.0	250	34	13.6
11	104	10	9.6	105	5	4.8	209	15	7.2
12	115	4	3.5	131	7	5.3	246	11	4.5
13	106	10	9.4	151	7	4.6	257	17	6.6
14	42	4	9.5	78	7	9.0	120	11	9.2
15	20	2	10.0	28	6	21.4	48	8	16.7
Total	912	86	9.4	1019	79	7.75	1931	165	8.5

* Based on the radioimmune assay (RIA) and immunoelectrophoresis (IEOP) tests.

Table 8. The prevalence of hepatitis B surface antigen (anti-HB_s) students of the Phibunprachasan School, Bangkok, Thailand, June 1977

Age	Male			Female			Total		
	No. tested	Anti-HB_s* No.	Anti-HB_s* %	No. tested	Anti-HB_s* No.	Anti-HB_s* %	No. tested	Anti-HB_s* No.	Anti-HB_s* %
4	15	3	20.0	31	2	6.5	46	5	10.9
5	30	3	10.0	33	3	9.1	63	6	9.5
6	52	5	9.6	35	7	20.0	87	12	13.8
7	76	13	17.3	102	29	28.4	178	42	23.6
8	113	28	24.8	88	13	14.8	201	41	20.4
9	116	30	25.9	110	25	22.7	226	55	24.3
10	123	38	30.9	127	24	18.9	250	62	24.8
11	104	32	30.8	105	24	22.9	209	56	26.8
12	115	43	37.4	131	31	23.7	246	74	30.1
13	106	49	46.2	151	55	36.4	257	104	40.5
14	42	15	35.7	78	28	35.9	120	43	35.8
15	20	10	50.0	28	16	57.1	48	26	54.2
Total	912	269	29.5	1019	257	25.2	1931	526	27.2

* Based on the RIA and IEOP tests.

Table 9. Evidence of HBV antibody acquisition of HBV* infection during study period. Phibunprachasan School, June 1977 - Jan. 1978

Age	Male	Female	Total
4	0	0	0
5	0	1	1
6	0	0	0
7	1	1	2
8	1	1	2
9	2	2	4
10	1	0	1
11	1	0	1
12	1	0	1
13	0	1	1
14	0	1	1
15	1	2	3
Total	8	9	17
Percentage of antibody acquisition	9.3	11.4	10.3

* Detection of HB_sAg in 1st bleeding and anti-HB_s in 2nd bleeding.

Table 10. The incidence of HBV infection during study period, Phibunprachasan School, June 1977 - Jan. 1978

Age	Male			Female			Total		
	HB_sAg	Anti-HB_s	Total	HB_sAg	Anti-HB_s	Total	HB_sAg	Anti-HB_s	Total
4	0	1	1	0	1	1	0	2	2
5	1	1	2	0	1	1	1	2	3
6	6	1	1	2	0	1	1	2	3
7	3	0	3	0	3	3	3	3	6
8	0	4	4	1	2	3	1	6	7
9	1	1	2	0	2	2	1	3	4
10	0	2	2	1	3	4	1	5	6
11	1	2	3	0	2	2	1	4	5
12	3	2	5	0	6	6	3	8	11
13	0	1	1	2	2	4	2	3	5
14	1	0	1	0	2	2	1	2	3
15	0	0	0	0	1	1	0	1	1
Total	11	15	26	4	26	30	15	41	56
Rate	11/588	15/588	26/588	4/710	26/710	30/710	15/1298	41/1298	56/1298
Percent	1.87	2.55	4.42	0.56	3.66	4.23	1.16	3.16	4.31

Table 11. Rate of disappearance of HBV surface antigenemia and of HBV antibody in students of Phibunprachasan School during study period, June 1977 - January 1978

Age	Male				Female				Total			
	HB_sAg		Anti-HB_s		HB_sAg		Anti-HB_s		HB_sAg		Anti-HB_s	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
4	0	(-)	1	(33)	3	(75)	0	(-)	3	(50)	1	(20)
5	1	(25)	0	(-)	0	(-)	0	(-)	1	(14)	0	(-)
6	1	(25)	1	(20)	0	(-)	2	(29)	1	(20)	3	(25)
7	1	(11)	2	(15)	1	(17)	3	(10)	2	(13)	5	(12)
8	0	(-)	2	(7)	0	(-)	1	(8)	0	(-)	3	(7)
9	2	(15)	1	(3)	1	(14)	1	(4)	3	(15)	2	(4)
10	5	(33)	2	(5)	4	(21)	0	(-)	9	(26)	2	(3)
11	3	(30)	0	(-)	2	(40)	3	(13)	5	(33)	3	(5)
12	1	(25)	1	(2)	2	(29)	3	(10)	3	(27)	4	(5)
13	0	(-)	3	(6)	1	(14)	1	(2)	1	(6)	4	(4)
14	1	(25)	1	(7)	2	(29)	4	(14)	3	(27)	5	(12)
15	0	(-)	0	(-)	0	(-)	0	(-)	0	(-)	0	(-)
Total	15		14		16		18		31		32	
Rate(%)	17.4		5.2		20.3		7.0		18.8		6.1	

Table 12. Household contacts of Hepatitis B antigenemic students; relation of HB_eAg, Phibunprachasan School

HB_eAg	No. of students	No. of families with* presence of HBV marker	
		No.	%
Students with HB_eAg	26	21	80.76
Students without HB_eAg	13	9	69.23

* Detection of HBV markers in one or more family members

P = 0.22 (Fisher's exact test)