

DENGUE NEUTRALIZING ANTIBODY SERO-CONVERSIONS
IN A POPULATION OF HEALTHY SCHOOL CHILDREN

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OBJECTIVE : To determine the proportion of flavivirus seroconversions due to each dengue virus serotype in a population of healthy school children shown to have seroconverted by HAI.

BACKGROUND : A longitudinal study of dengue virus infections among children attending a lower to middle class Bangkok school (the Phibunprachasan School in the Din Daeng area) was initiated in June 1977. (See AFRIMS Annual Report, Oct. 1977 to Sept. 1978, pp. 96-108). 825 children who were HAI sero-negative in June 1977 and subsequently rebled and tested in January 1978, 113 (14.9%) seroconverted to at least one dengue antigen. By HAI, most of these seroconversions appeared to be relatively monospecific to dengue type 4, but as reported elsewhere in this annual report, a "monospecific" HAI response to dengue 4 can occur in primary infections with D1, 2, or 3. We therefore, sought to determine the proportion of total seroconversion due to each dengue serotype by plaque reduction neutralization serology.

METHODS : Of the total of 113 paired serum specimens which showed seroconversions by HAI serology, sufficient sera remained to do PRNT₅₀ determinations on 67 pairs. PRNT₅₀ assays were done in LLC-Mk2 cells according to the method of Russell and Nisalak as modified for micro-cultures. Cells were grown in 24 well polystyrene plates and incubated in 5% CO₂.

Virus controls were diluted to give approximately 20 to 50 PFU/well. High mouse brain passage prototype virus strains were used for D1, 2, 3 and 4 and JEV.

RESULTS : Results are summarized in Figure 1. Eighteen of the 67 HAI "seroconversions" were shown to have unchanging PRNT₅₀ antibodies in both first and second serum. This data should not be extrapolated to assume that 28% of all HAI negative children have PRNT₅₀ antibodies; among sera from another 18 children who did not seroconvert by HAI none (0%) developed detectable PRNT₅₀ antibodies. (See elsewhere in this annual report).

It is probable that the relatively high percentage of pre-existing PRNT₅₀ antibodies among HAI seroconverters is due to fact that these children had antibodies at or near the threshold of detection by HAI; in the first specimens they were "negative" and on the second "positive". These false seroconversions are probably "biologic" false positives.

In 10 cases both the first and second sera lacked PRNT₅₀ antibodies. These probably represent laboratory errors (faulty extraction, faulty antigen dose, etc). When compared to the total of 825 children found to be sero negative in the first serum specimen, an error rate of approximately 10/825 (1.2%) is acceptable.

Of the 39 HAI seroconversions also documented as PRNT₅₀ seroconversions, the majority (67%) were due to D4 infections, while D1, D2 and D3 accounted for only a few percent each. This data contrasts with the proportion of dengue viruses of each type isolated from patients at Children's Hospital during the same epidemic season (D1 0%, D2 55%, D3 16%, D4 28%).

This data can be taken as preliminary evidence that the serotype of the infecting virus may be a critical factor in determining the severity of the clinical manifestations of a dengue virus infection. It also suggests that although only D2, D3 and D4 were isolated from DHF patients in 1977, all four virus types (including D1) were present in the metropolitan Bangkok area during that year.

Similar data will be derived for the 1978 and 1979 Phibunprachasan school serum collections.

Figure 1. Summary of PRNT₅₀ tests on serum for Phibunprachasan School, June 1977 and Jan. 1978 - Infection typing

Good paired sera

(-) HI → (+) HI

67

Sufficient amount

First serum PRNT₅₀ positive



18

Remaining

49

First serum PRNT₅₀ negative

but second serum PRNT₅₀

negative also

10

Good PRNT₅₀ seroconversion

39

D1	D2	D3	D4	JEV	?
3	5	3	26	0	2**
(8)	(13)	(8)	(67)	(0)	(5)

Number of seroconversions

to each type* and # of

total

* Virus type assigned based on finding of monospecific serum PRNT₅₀ (> 2 x nearest titer)

** Sero response not monospecific in two specimens