

Isolation of Dengue Viruses from Patients in Provincial Hospitals

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OBJECTIVE : To isolate dengue viruses from dengue hemorrhagic fever patients admitted to provincial hospitals of Thailand and to compare the virus serotypes with those isolated in Bangkok.

BACKGROUND : Since 1962 the personnel of the Medical Research Laboratory, Armed Forces Research Institute of Medical Sciences, have isolated dengue viruses from patients of the Children's Hospital, Bangkok. During this period the dengue virus serotypes that were isolated varied considerably. Dengue-2 virus was isolated from patients in all years that isolations were attempted. In the early 1960's, dengue-1, 3 and 4 were also present. Dengue-4 was isolated in 1965, but from 1969 through 1975 this serotype was not isolated from Bangkok patients (surveillance of dengue virus types by isolation was not conducted in Bangkok during the period 1966-1968). Approximately 50% of the viruses isolated from 1972 through 1975 were dengue-1 and 3 and the remainder were dengue-2 viruses. In 1976 dengue-4 was isolated much more frequently than dengue-1 and 3 viruses. The incidence of human dengue virus infections in Thailand increased earlier than expected and to unprecedented levels during 1977. The increase may have been due to the re-introduction of dengue-4 virus.

The purpose of this study was to isolate dengue virus from patients admitted to provincial hospitals and to compare the virus serotypes to those isolated from patients admitted to the Children's Hospital, Bangkok.

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METHODS : After consultation with the Director, Division of Epidemiology of the Ministry of Public Health, studies were conducted at the provincial hospitals at Khon Kaen, Phrae, Ubol, and Udorn. From August through October, 1977, blood specimens were obtained from clinically diagnosed DHF patients who had been ill for four days or less. A second specimen was collected from the same patients two to five days later. A short history and physical examination were recorded for each patient.

Serum and plasma specimens were stored and transported to AFRIMS in liquid nitrogen. Virus isolation attempts were carried out on acute plasma specimens using the direct and delayed plaque techniques on LLC-Mk₂ cells. Viruses were identified by the standard plaque reduction neutralization technique using prototype dengue virus anti-sera prepared in monkeys. Acute and convalescent sera were assayed for antibody by the hemagglutination inhibition (HI) technique.

RESULTS : Applying standard laboratory criteria for the serologic diagnosis of DHF (four fold titer rise with convalescent titer \geq 640 or fixed high titer $>$ 1280 by HI serology), 109 of the 139 clinical diagnoses of DHF in the provinces were confirmed; 15 were not confirmed; and 5 lacked a convalescent serum specimen. As shown in Table 1, thirty-six dengue virus strains were isolated from patients of four provincial hospitals. The results of dengue virus isolations from Bangkok Children's Hospital for the year 1977 are presented in the same table for comparison. Dengue virus serotypes 2, 3, and 4 were isolated; however, dengue-4 viruses were detected most frequently. Isolates of dengue-3 virus were relatively more common in specimens from the provinces than from Bangkok.

Table 1. Virus isolations from patients admitted to provincial hospitals (Aug.-Oct. 1977) and Bangkok Children's Hospital (Jan.-Dec. 1977) with the diagnosis of DHF

Place	No. Studied	Confirmed DHF	Probable DHF**	No. of Isolations	Identification					Unidentified
					D-1	D-2	D-3	D-4	CHICK	
Khon Kaen	42	31	1	14	0	1	6	6	0	1
Phrae	22	10	1	6	0	0	2	1	1	2
Ubol	35	33	1	5	0	1	3	0	0	1
Udorn	40	35	2	11	0	7	3	1	0	0
Provincial Hospitals Total	139	109	5	36	0	9	14	8	1	4
Children's Hospital (Bangkok)	493	233	103	82	0	37	11	19	2	13

* Diagnosis confirmed by HI serology.

** No convalescent serum specimen.