SURVEILLANCE OF DENGUE HEMORRHAGIC FEVER CASES IN THAILAND

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INTRODUCTION

Hemorrhagic fever transmitted by *Aedes aegypti* mosquitoes is one of the leading cause of illness and deaths in children under 15 years in Thailand. The epidemic in Bangkok metropolis and near-by provinces was documented in 1958 (Hammon, 1960; Halstead, 1966). Cases have been reported from every province of Thailand throughout the year with its peak during July and August (Min. Health 1958-1980). The trend of disease shows increase in severity and the morbidity reached the highest to 88.3 per 100,000 total population or 206 per 100,000 population under 14 years of age (Epidemiological Surveillance, 1977). Currently, cases of hemorrhagic fever reported to the Ministry of Public Health are based entirely on clinical information. The presumptive diagnosis may not be accurate resulting from wide diversity of clinical manifestations from shock, hemorrhagic signs to fever of unknown origin. In the majority of mild cases it is difficult to differentiate from other diseases.

The present study was carried out by the use of hemagglutination - inhibition (HI) test to confirm the clinical diagnosis of dengue hemorrhagic fever cases reported from the 72 provinces of Thailand. The results of this laboratory surveillance of dengue hemorrhagic fever (DHF) cases will assist the Ministry of Public Health in monitoring of DHF cases and in the planning of *Aedes aegypti* control programme.

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MATERIALS AND METHODS

Acute and convalescent blood specimens were obtained from patients admitted to 72 provincial hospitals of Thailand. The acute specimens were obtained on the day of admission and the convalescent specimens were taken on the day patients were discharged from the hospitals. Blood specimens were absorbed onto filter - paper discs (Carls Schleicher and Scheull Co. Keene, New Hompshire, No. 740 -E, 12.7 mm in diameter), air dried at ambient temperature. The discs and clinical history of the patients were submitted to the Epidemiology Division, Ministry of Public Health for recording and then forwarded to the Faculty of Public Health for laboratory confirmation.

The hemagglutination inhibition test (HI) of Clarke and Casals (1958) adapted to microsystem was used for serological diagnosis (Sever, 1962). The blood was eluted from the discs by soaking overnight at 4°C in 0.4 ml of borate saline, pH 9.0. On the following day, 0.5 ml of a 25 % acid washed kaolin - borate saline mixture was added to each eluate. After incubating at room temperature for 20 minutes with occasional agitation, the mixtures were centrifuged at 2500 rpm for 30 minutes. The supernatant was transferred to a tube containing 0.2 ml of packed goose erythrocytes and allowed to incubate at 4°C

for 30 minutes with occasional agitation. Mixtures were centrifuged and the supernatant (eluate) were removed for HI testing. The treated blood eluates were tested with dengue 2 virus antigen. The sera of patients with no dengue infection were retested with chikungunya virus antigen. The method of serological diagnosis of dengue hemorrhagic fever using filter - paper discs and one dengue antigen is applicable for extensive survey for mosquito -borne viral diseases in hyperendemic areas such as Thailand (Top *et al.*, 1975). Dengue infection were recorded in hemorrhagic fever patients if paired - sera yielded either a four - fold rise in antibody titer regardless of the titer of the initial serum, or a titer equal to or greater than 1:640 in both sera. Paired sera that had antibody titer that did not meet these criteria came from cases considered to be old infection from other group B viruses that exist in Thailand. The criteria used for hemorrhagic fever caused from chikungunya infection was recorded if the sera show a four-fold rise in antibody titer to chikungunya antigen.

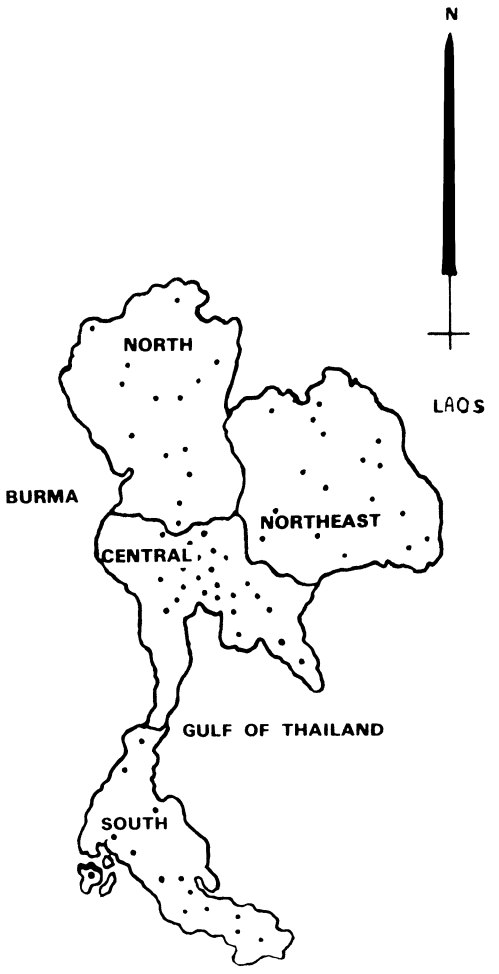

Fig. 1—Provinces in Thailand submitting blood specimen of patients for Dengue virus serological examination.

RESULTS

The location of the provinces submitting blood samples from hemorrhagic fever patients is shown in Fig. 1. As seen in Table 1 the percentage of blood sampling in 1974, 1975 and 1976 were 36.0, 30.4 and 41.6 of the total number of cases reported. The results of HI test for DHF cases are shown in Fig. 2 and the percentage of dengue infection are 42, 54 and 52 in 1974, 1975 and 1976 respectively. There is a variation in the percentage of DHF in central, northeast, north and south region of Thailand.

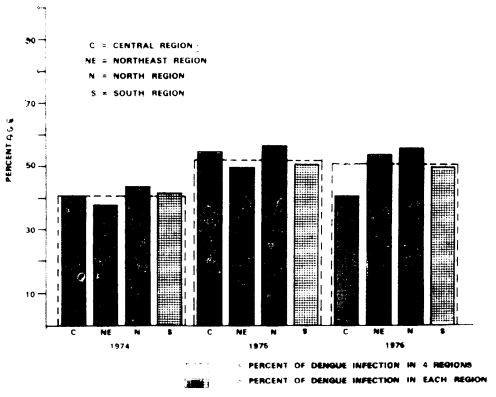

Fig. 2—Percentage of dengue virus infection in hemorrhagic fever cases from 72 provinces of Thailand.

Fig. 3 shows dengue hemorrhagic fever cases confirmed by HI test distribution every month. A few cases were reported in December through February with its peak in July to August. In general the number of total cases and dengue infected cases in the 3 years is similar. The same pattern of DHF occurrence in the 4 regions: north,

Table 1

Comparison of number of blood samples submitted for laboratory confirmation and total hemorrhagic fever cases in Thailand.

Region	Total morbidity+			Total examination			Percent of examination		
	1974	1975	1976	1974	1975	1976	1974	1975	1976
Central	2442	5653	1251	1132	1653	738	46.4	29.2	59.0
Northeast	2149	5995	2923	645	1891	1411	30.0	31.5	48.3
North	1887	2728	2298	419	514	566	22.2	18.8	24.6
South	613	1005	645	354	624	246	57.7	62.1	38.1
Thailand	7092*	15421	7117	2551*	4682	2961	36.0	30.4	41.6

+ Morbidity was obtained from the Epidemiological Division, Ministry of Public Health.
* The indicated incidence excluding Bangkok Metropolis.

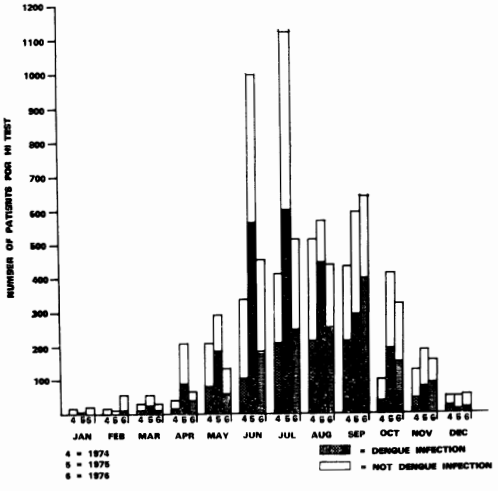

Fig. 3—Result of HI test for dengue virus infection in hemorrhagic fever cases in 1974, 1975 and 1976.

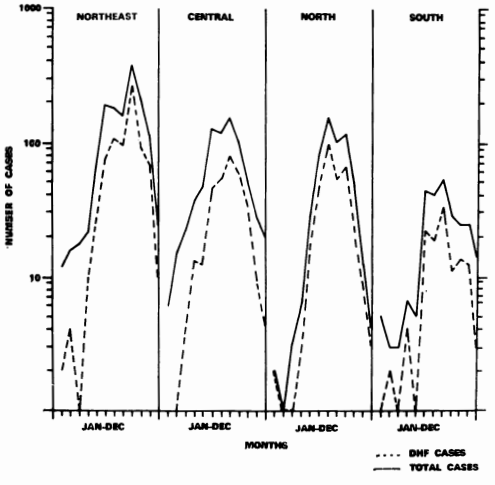

Fig. 4—Laboratory confirmation (HI test) for dengue infection in hemorrhagic fever cases from 4 regions of Thailand.

central, northeast and south is demonstrated in Fig. 4.

The total number of patients in 1974, 1975 and 1976 according to age groups are shown in Table 2. A high number of cases were from age groups between age 1 to 10 years, and low morbidity in older ages. Fig. 5 shows the results of laboratory confirmation for dengue hemorrhagic fever in different age groups. The percentage of HI positive for dengue virus infection is high in ages 4 to 10 years. In 1974 the percentage of DHF was at the lowest level, not more than 50% but increased to 65% and 78% in 1975 and 1976 respectively. In age 1 to 3 years, the percentage of DHF was 40.

As seen in Table 3 there were 42 cases of hemorrhagic fever caused by chikungunya infection from the total of 2,379 patients. It is found in northeast, central and north but none in the south. There are 145 cases

in DHF cases. In view of potential source of dengue virus transmission in Thailand, the estimation of case specific DHF will be 23 and 51 per 100,000 children age 15 years in 1975 and 1976. These infection rates do not include other mild DHF who were not hospitalized and this portion is the majority of dengue virus infected cases. Hemorrhagic fever caused by chikungunya virus was only 1.18% in non - dengue virus infection or 0.19% in total serological examination.

In view of *Aedes aegypti* control for reduction of dengue hemorrhagic fever, the study of chikungunya virus infection would be of little value to the national surveillance programme because the two infections are transmitted by the same mosquito vectors in Thailand. The study of age specific DHF cases reveals that the majority cases are in age 4 to 10 years group. Although it was reported by Nimmannitaya *et al.*, (1969) to be between 3-6 years. Thus there is a shift of DHF morbidity to older groups. An interesting point in this study is that a high proportion of DHF occurs in age 11 to 15 years. The background supporting this view point is very complicating to the pathogenesis and epidemiology of dengue hemorrhagic fever with causative dengue viruses type 1,2,3, and 4 in Thailand. The results of HI test reveal dengue virus infection in hemorrhagic fever cases from every province of Thailand. The trend of disease severity in term of morbidity and mortality of unspecified hemorrhagic fever have increased (Epidemiological Surveillance of the Ministry of Public Health). The changing pattern of dengue hemorrhagic fever in the cities is challenging for further studies which may be the outcome of changes in ecological system. This epidemiological surveillance should be operated in every community for mosquito - vector control and prevention of dengue hemorrhagic fever in Thailand.

SUMMARY

A long-term surveillance system is necessary for planning and evaluation of hemorrhagic fever control. Dry blood collection and using one dengue 2 antigen have been proved to be an efficient method to detect low and high level of HI antibody, determined to prove dengue infection. The percentage of dengue infection serologically proved from cases reported throughout a 3 year study was 52%. Dengue infected cases were reported outside epidemic period with lowest incidence in January. The majority of dengue proven cases occur at age 6 years. For chikungunya infection, studied in Bangkok metropolis and in this study in 72 provinces shows similar result indicating that chikungunya virus shows no significance in clinical and laboratory study in the surveillance programme. This study provides definite information for the planning and evaluation of hemorrhagic fever control.

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REFERENCES

CLARKE, D.H. and CASALS, J., (1958). Techniques for hemagglutination and hemagglutination inhibition with arthropod-borne virus. *Amer. J. Trop. Med. Hyg.*, *7* : 561.

EPIDEMIOLOGICAL SURVEILLANCE (1958-1980). Ministry of Public Health, Thailand.

EPIDEMIOLOGICAL SURVEILLANCE, (1977). Ministry of Public Health, Thailand.

Table 2

Number of patients examined serologically for DHF infection from 72 provinces of Thailand.

Age group (years)	Number of patients		
	1974	1975	1976
0- 2	408	349	393
2- 4	519	587	504
4- 6	552	956	698
6- 8	401	1003	513
8-10	308	640	319
10-12	170	439	216
12-14	80	256	71
14	4	32	109

Table 3

Hemagglutination inhibition test for Chikungunya infection in hemorrhagic cases from 4 regions of Thailand in 1976.

Region	Total cases tested	Chikungunya positive		
		Recent infection	Old infection	No HI titer
Northeast	1108	28 (1.18)	105 (4.41)	975 (40.9)
Central	571	7 (0.3)	15 (0.63)	549 (23.1)
North	495	7 (0.3)	17 (0.71)	471 (19.8)
South	205	0	8 (0.33)	197 (7.4)
Thailand	2379	42 (1.8)	145 (6.1)	2192 (92.1)

Percentage shown in parenthesis.

that showed no rise of antibody titer to chikungunya virus.

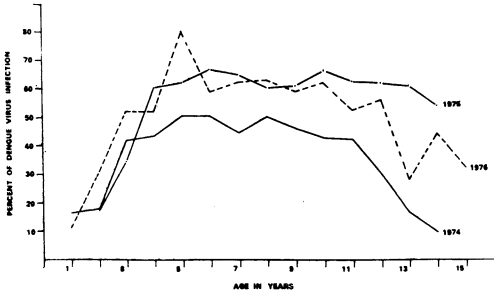

Fig. 5—Percentage of dengue virus infection by age group in hemorrhagic fever cases in 1974, 1975 and 1976.

DISCUSSION

The clinical diagnosis of dengue hemorrhagic fever with serological confirmation is the most accurate parameter to record the morbidity and mortality of DHF. The laboratory monitoring of DHF is part of the plan of the dengue hemorrhagic fever surveillance. It is confirmed in this study hemorrhagic fever in childeren is caused by dengue viruses as reported in every province of Thailand. The increase in percentage of serologic diagnosis in 1975 and 1976 may reflect the importance the physicians give to serological determination

HAMMON, W. McD., RUDNICK, A. and SATHER, G.E., (1960). Virus with epidemic hemorrhagic fever of the Philippines and Thailand. *Science, 131* : 1102.

NIMMANNITAYA, S., HALSTEAD, S.B., COHEN, S. N. and MARGIOTTA, M.R., (1969). Dengue and chikungunya infection in man in Thailand. 1. Observations on hospitalized patients with hemorrhagic fever. *Amer. J. Trop. Med. Hyg., 18* :954.

SEVER, J.L., (1962). Application of a microtechnique to viral serological investigations. *J. Immunol., 88* : 320.

TOP, H. JR., GUNAKASEM, P., CHANTRASRI, C. and SUPAVADEE, J., (1975). Serological diagnosis of dengue hemorrhagic fever using filter paper discs and one dengue antigen. *Southeast Asian J. Trop. Med. Pub. Hlth*; *6* : 18.