DENGUE HEMORRHAGIC FEVER: KNOWLEDGE, ATTITUDE AND PRACTICE IN ANG THONG PROVINCE, THAILAND

Leera Kittigul[1], Kedsuda Suankeow[1], Dusit Sujirarat[2] and Sutee Yoksan[3]

[1]Department of Microbiology, [2]Department of Epidemiology, Faculty of Public Health, Mahidol University, Bangkok; [3]Center for Vaccine Development, Institute of Science and Technology for Research and Development, Mahidol University, Salaya, Nakhon Pathom, Thailand

Abstract. A cross-sectional study was carried out between July 1998 and June 1999 to identify dengue virus-infected patients under age 15 admitted to seven government hospitals in Ang Thong Province, a central region of Thailand, and to assess the knowledge, attitude, and practice (KAP) of their care takers. To differentiate dengue cases, clinical evaluation and laboratory diagnosis were used. Serum samples were collected from 90 admitted children and also from 80 healthy students. The dengue cases were classified as dengue fever (9 cases, 12.2%) and dengue hemorrhagic fever (DHF: 65 cases, 87.8%). Nine patients had dengue shock syndrome, but no death occurred. With serological confirmation, primary antibody response was observed in 8 (11.3%) and definite secondary infection in 49 (69%). Out of 41 serum samples, 14 (34.1%) were positive for dengue virus isolation: dengue serotypes 1, 2 or 3. A total of 131 care takers of enrolled children were interviewed in the context of KAP in DHF. The majority of them were mothers with primary school education level. Half of the care takers were workers. DHF knowledge of the care takers of the dengue cases, non-cases, and healthy students was almost the same. However, the care takers of dengue cases recognized petechiae as a danger sign, p-value of 0.006. They had a higher response in prevention, control and treatment of DHF than the other two groups after their children were admitted to hospital, p-value of 0.000. The results indicated that DHF remains a public health problem in this area and the people need more understanding of the disease. Continuous campaigns are required for community participation so as to prevent and control DHF successfully.

INTRODUCTION

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are global problems affecting tens of millions of people (Monath, 1994). The incidence and geographical distribution of dengue have greatly increased in recent years. In Thailand, since 1990, the number of reported DHF cases has been increasing although the mortality rate has decreased (Division of Epidemiology, 1990-1999). The emergence of epidemic DHF as a global public health problem in the past 15 years is associated closely with demographic and societal changes. The factors involved have been unprecedented population growth and, with that, unplanned and uncontrolled urbanization, especially in tropical developing countries, increased air travel, climate changes, the lack of effective mosquito control, and the deterioration of public health infrastructure (Gubler and Clark, 1995). There are four dengue serotypes; DEN-1, DEN-2, DEN-3, and DEN-4, which are maintained in an urban transmission cycle in tropical and subtropical areas by the mosquito *Aedes aegypti* (WHO, 1997). The risk for DHF is higher where two or more virus serotypes are circulating simultaneously (Sangkawibha *et al*, 1984; Rico-Hesse *et al*, 1997). Other risk factors for individual susceptibility to DHF and dengue shock syndrome (DSS) are secondary dengue infection, good nutritional status, Caucasians and Chinese (Halstead, 1980; Kittigul *et al*, 1997; Thein *et al*, 1997).

Dengue vaccine developments have been under clinical trial and vaccines are as yet not available for public use. Many of the fundamental re-

Correspondence: Dr Leera Kittigul, Department of Microbiology, Faculty of Public Health, Mahidol University, 420/1 Rajvithi Road, Bangkok 10400, Thailand.
Tel: 66 (0) 2245 5525; Fax: 66 (0) 2245 8351
E-mail: phlkt@mahidol.ac.th

search questions for the prevention and control of dengue disease can be answered only by well-designed prospective epidemiological studies (Gubler, 1989). Environmental sanitation strategies were emphasized in the survey of knowledge, attitude and practice (KAP) in dengue prevention and control (Rosenbaum *et al*, 1995). Health education on DHF was required for the *Aedes* control program and the main effective mass media for public health education were radio and television (Swaddiwudhipong *et al*, 1992). Previous KAP surveys were conducted in the general population of household respondents. Here, DF and DHF patients admitted to government hospitals in Ang Thong Province, Thailand, were described. The KAP of their care takers were interviewed and compared with that of care takers of non-cases and healthy students, to assess the association with DHF.

MATERIALS AND METHODS

Study design

A cross-sectional study of DHF surveillance was carried out in seven government hospitals in Ang Thong Province, 107 km from Bangkok, in the central region of Thailand. DHF surveillance was conducted for one year, between July 1998 and June 1999. The care takers of patients and healthy students were interviewed about their knowledge, attitude and practice of DHF.

Serum samples

Blood samples were collected from patients less than 15 years of age after informed consent was obtained. They were clinically diagnosed and laboratory-confirmed as dengue virus-infected. All patients were classified by the case definition of WHO as DF, DHF or DSS (WHO, 1997). Control blood samples were collected from patients aged under 15 years who were diagnosed as non-dengue infected and also from healthy students. A blood sample (3-5 ml) was obtained from each patient by venipuncture and the serum was separated and preserved at -70ºC until used. The acute sera were collected from the patients within a day of hospital admission and the convalescent sera were collected shortly before discharge from the hospital and, if possible, 7-10 days following admission.

Hemagglutination inhibition test

The sera were extracted with acetone and tested for hemagglutination inhibition (HI) antibodies using a method modified from Clarke and Casals (1958), adapted for use in a microtiter system. Dengue virus and Japanese encephalitis (JE) virus antigens were produced by sucrose acetone extraction of the brain of suckling mice infected with the following prototype virus strains; DEN-1 (Hawaii), DEN-2 (New Guinea), DEN-3 (H-87), DEN-4 (H-241) and JE (Nakayama). The interpretation of dengue antibody responses in the HI test, following the WHO criteria, was recorded.

IgM/IgG-capture enzyme-linked immunosorbent assay

The immunoglobulin M (IgM) and immunoglobulin G (IgG) against dengue virus in serum samples were detected by IgM/IgG-capture enzyme-linked immunosorbent assay (MAC/GAC ELISA) as described elsewhere (Innis *et al*, 1989). An IgM:IgG ratio equal to or greater than 1.8:1 was defined as a primary dengue virus infection, whereas a ratio of less than 1.8:1 was defined as a secondary infection.

Virus isolation

Virus isolation was performed by intrathoracic inoculation of patients' sera into groups of *Toxorhynchites splendens* mosquitos (Rosen and Gubler, 1974; Watts *et al*, 1982). Dengue virus infection of mosquitos was determined by the direct fluorescent test, using anti-dengue FITC conjugate on the mosquito head-squash preparation (Kuberski, 1979). Detected dengue virus was further serotyped by the indirect fluorescent antibody test, using dengue type-specific monoclonal antibody (Henchal *et al*, 1982).

Data analysis

The data were analyzed by the SPSS program and presented in terms of percentages, means, and standard deviations. Associations among the studied groups were analyzed using the Kruskal-Wallis one-way analysis of variance by ranks. Differences giving a p-value <0.05 were considered statistically significant.

RESULTS

Subject demographics

The total enrollment of children in Ang Thong Province, between July 1998 and June 1999, was 167 volunteers. With clinical and laboratory findings, the patients admitted to 7 hospitals included 74 dengue virus infected cases (DF; 9 cases and DHF; 65 cases), and 13 non-cases. Eighty control students participated in this study. In DHF children, the attack rate of boys was higher than girls, with a male-to-female ratio of 1.5:1. The mean age was 9.3 ± 3.2 years. The high numbers of DHF patients (52.3%) belonged to the 10-14 year age group. In DF patients, the 5-9 year age group was dominant and more boys were attacked with dengue viruses than girls, as shown in Table 1. Half of the cases (54%, 40/74 cases) occurred during the rainy season, from July to September, 1998.

Clinical manifestations and laboratory findings

Fever was documented in all cases. Most of the DHF patients had tourniquet-test positive (86.1%), nausea (84.6%), and vomiting (69.2%). Half of them presented with abdominal pain. Hepatomegaly was found in 29.2%. Shock syndrome accounted for 13.8% with no deaths. Most of the DHF patients had thrombocytopenia (64.1%) and hemoconcentration (86.2%). The number of platelets decreased to less than 100,000, starting from the third day after onset of fever. The sixth day of illness presented lower platelet numbers than the other days. On the ninth day of illness, the platelet counts had risen in to the normal range.

Diagnosis of dengue virus infection

All 74 serum samples were confirmed for dengue virus infection using laboratory methods.

Table 1
Characteristics of study subjects.

Characteristics	DF No. (%)	DHF No. (%)	Non-dengue infection No. (%)	Healthy students No. (%)
Age (years)	0	8 (12.3)	6 (46.2)	4 (5.0)
0-4	6 (66.7)	23 (35.4)	3 (23.1)	41 (51.2)
5-9	3 (33.3)	34 (52.3)	4 (30.7)	35 (43.8)
10-14	8.3 ± 2.4	9.3 ± 3.2	4.8 ± 4.3	9.4 ± 0.6
Mean ± SD	7	11	10	12
Range (years)				
Sex				
Male	8 (88.9)	39 (60)	6 (46.2)	44 (55)
Female	1 (11.1)	26 (40)	7 (53.8)	36 (45)
Male:Female	8 : 1	1.5 : 1	1 : 1.2	1.2 : 1

Table 2
Serological diagnosis of antibody response in dengue patients.

Clinical syndrome	Primary No. (%)	Secondary No. (%)	Primary/secondary No. (%)	Total No. (%)
DF	1	6	1	8 (11.3)
DHF	7	43	13	63 (88.7)
Total	8 (11.3)	49 (69)	14 (19.7)	71 (100)

Based on the HI test, 62 serum samples showed recent dengue virus infection. The mean ± SD of days after onset of fever were 4.5 ± 1.3 days for acute sera and 8.4 ± 3.1 days for convalescent sera. The duration between those two sera was 3.95 ± 2.95 days. Dengue IgM antibodies were detected in nine serum samples using MAC ELISA whereas one serum was positive for flavivirus infection. The other two samples were positive for dengue viruses by isolation in the mosquitos (*Toxorhynchites splendens)*.

Antibody response in dengue virus-infected patients

Seventy-one cases with serologically confirmed dengue virus infection were analyzed for primary or secondary infection. Primary antibody response was found in 8 (11.3%) and definite secondary infection in 49 (69%) of the cases. Either primary or secondary antibody response was notified in 14 (19.7%) of the cases, as shown in Table 2.

Dengue virus isolation

Dengue virus was isolated from 14 out of the 41 acute screen samples (34.1%) tested. Two isolates (DEN-1, n=1; DEN-3, n=1) were recovered from primary DHF cases. Nine isolates were obtained from secondary DHF cases (DEN-1, n=1; DEN-2, n=5; DEN-3, n=3). The other 3 isolates could not be assigned a definite serologic diagnosis because of having low HI or ELISA titers and the inavailability of a convalescent blood sample. They were recovered from the serum collected during the acute stage, 2-6 days after onset of symptoms (mean ± SD = 3.8 ± 1.4 days).

Table 3
Characteristics of care takers.

Characteristics	Dengue cases, No.(%) n=57	Non-cases, No.(%) n=13	Healthy students, No.(%) n=61
Sex			
Male	6 (10.5)	2 (15.4)	6 (9.8)
Female	51 (89.5)	11 (84.6)	55 (90.2)
Male:Female	1 : 8.5	1 : 5.5	1 : 9.2
Age; mean ± SD (years)	38.7 ± 11.3	41.2 ± 10	37.8 ± 9.8
Status			
Mother	39 (68.4)	10 (76.9)	42 (68.9)
Father	5 (8.8)	0	2 (3.3)
Other	13 (22.8)	3 (23.1)	17 (27.8)
Education			
No education	3 (5.3)	0	3 (4.9)
Primary school	36 (63.1)	10 (76.9)	37 (60.7)
Junior high school or equal	6 (10.5)	1 (7.7)	10 (16.4)
Senior high school or equal	7 (12.3)	1 (7.7)	8 (13.1)
Academic degree or higher	5 (8.8)	1 (7.7)	3 (4.9)
Income (Baht/month)			
< 2,000	5 (8.8)	0	10 (16.4)
2,001-4,000	18 (31.6)	4 (30.8)	11 (18)
4,001-6,000	17 (29.8)	6 (46.1)	24 (39.4)
> 6,000	17 (29.8)	3 (23.1)	16 (26.2)
Occupation			
Farmer	15 (26.3)	0	2 (3.3)
Government official	5 (8.8)	1 (7.7)	0
Commerce	4 (7)	4 (30.8)	19 (31.1)
Worker	25 (43.9)	6 (46.1)	33 (54.1)
Unemployed	8 (14)	2 (15.4)	7 (11.5)

Determination of dengue virus infection by MAC ELISA

Of 30 patients, 17 cases were positive for dengue IgM by MAC ELISA. Most of them (10/17 cases; 59%) had secondary antibody response. Either primary or secondary antibody response were observed in 3 patients. Those positive IgM serum samples were collected between day 3 and day 6 (mean ± SD = 3.8 ± 0.8 days) after symptom onset.

Dengue shock syndrome patients

Among 9 DSS patients, more than half were female within the 5-9 year age group. Secondary dengue infection was found in 5 cases, whereas only 1 case had definite primary infection.

KAP of DHF in care takers

A survey interview was carried out with 131 care takers of enrolled subjects. The mean age of the care takers was in the range 38-41 years. The majority of them were mothers, and their educational level was mainly primary school. The distribution of family income per month was no different in proportion. Nearly half of them were workers (Table 3).

Most of the care takers realized that DHF was a disease transmitted by mosquito bites and occured in children. The most common mosquito breeding places were water containers and reservoirs. Approximately 80% of care takers knew that high fever, muscle pain, vomiting and petechiae were symptoms of DHF. Paracetamol was the first drug of choice. Using a tepid sponge to reduce fever was well-known. There was no marked difference between the knowledge of care takers in the three groups (dengue cases, non-cases, and healthy subjects). However, the care takers of dengue cases had better knowledge than the non-dengue cases and healthy subjects about mosquito biting time, bleeding phenomena, serial blood examination and destruction of mosquito breeding sites (data not shown).

Attitudes to transmission, important signs and symptoms, treatment, and prevention of DHF were investigated. Most of the care takers agreed that dengue virus transmission was by mosquitos, about using a tepid sponge to reduce fever for treatment, and destroying of mosquito breeding sites helped prevent DHF. Incorrect attitudes about signs and symptoms were noticed in three groups. Half of them did not prefer to drink wa-

Table 4
Knowledge, attitude and practice for dengue hemorrhagic fever in three groups of care takers.

DHF	Dengue cases' care takers n=57	Non-cases' care takers n=13	Healthy students' care takers n=61	p-value[a]
Knowledge				
Disease	62.86[b]	76.42	66.71	0.450
Signs and symptoms	68.31	76.42	62.34	0.432
Treatment	69.03	66.35	63.10	0.660
Prevention	65.16	64.08	67.20	0.937
Attitude				
Transmission	64.18	69.04	67.05	0.837
Signs and symptoms	55.45	73.81	74.20	0.006
Treatment	67.02	75.23	63.08	0.543
Prevention	68.22	79.54	61.04	0.222
Practice				
Prevention and control	81.97	58.69	52.63	0.000
Treatment	81.97	55.38	53.34	0.000
KAP	82.44	64.62	50.93	0.000

[a]Kruskal-Wallis test.
[b]means of scores: total score = 100.

ter containing temephos sand or granules.

Practices in treatment, prevention and control of DHF were addressed. Although the destruction of mosquito breeding sites by covering water containers, and changing stored water frequently was the most common practice to prevent mosquito breeding sites as compared to other control methods the actual practice of the care takers in the three groups was low. Vector control, by adding temephos sand or granules to water containers was more frequently employed, particularly for bathing-water containers, than destruction of mosquito breeding sites.

The KAP of the care takers of dengue cases, non-dengue cases and healthy students were compared and are presented as means of scores in Table 4. DHF knowledge was almost the same and not satisfactory. The wrong attitude to petechiae as a danger sign of the care takers in the dengue group was significantly different from the non-dengue and healthy student groups, p-value of 0.006. Regarding practice of prevention, control and treatment of DHF, the care takers in the dengue group had significantly higher scores for practice than other two groups, p-value of 0.000.

DISCUSSION

During the period July 1998 to June 1999, a total of 87 suspected dengue patients attended seven government hospitals in Ang Thong Province, and 74 patients were confirmed as having recent dengue virus infection. The present study gave lower numbers of dengue infected cases than the annual reports (Epidemiology Division, 1990-1999) since all cases were laboratory-diagnosed as having dengue infection by virological and serological methods, clinical findings and hematologic profile, whereas the annual reports were based only on clinical diagnosis. Moreover, some dengue patients may be lost because physicians diagnosed dengue during early screening prior to laboratory confirmation. Therefore, the frequency of dengue cases would be expected to exceed the observed rate.

Males were more infected with dengue viruses than females. The high numbers of DHF patients belonged to the 10-14 year age group, although previous studies reported the peak age for contracting dengue infection to be 5-9 years (Halstead, 1980; Kittigul *et al*, 1997). It is possible that the trend of dengue disease increases in relation to increased age.

Of the 73 subjects who had serologic and/or virologic evidence of recent dengue virus infection, 69% of DF and DHF patients had secondary antibody response compared with 93% in the study by Vaughn *et al* (1997). A high frequency of secondary infection is a warning signal for physicians that patients may develop DSS (Sangkawibha *et al*, 1984).

One serum sample from a DSS patient collected on day 3 was positive for DEN-2 virus isolation but negative with serological tests. It is possible that on day 3, dengue virus was still present in serum, whereas the level of antibody was not high enough to be interpreted as recent dengue virus infection (Kalayanarooj, 1997). Some patients could not be identified as primary or secondary infection because the time interval between acute- and convalescent-phase sera was less than 7 days, and their acute sera were delayed in collection.

The duration of viremia in the enrolled patients was 2-6 days. The virus isolation rate (34.1%) was moderate and three serotypes could be isolated (DEN-1, DEN-2, and DEN-3). The virus types identified were DEN-1 (n=4), DEN-2 (n=6) and DEN-3 (n=4). Serotype prevalence was similar to our study done in acute dengue patients at the Siriraj Medical School in Bangkok, during the years 1998-1999, where DEN-1, DEN-2 and DEN-3 occurred in around 96-97% of the total isolates (n=186) (unpublished observation). DEN-4 was the least prevalent serotype and could not be detected in this study due to the low number of samples. One reason for the moderate isolation rate is improper collection and storage of blood specimens. Dengue virus isolation rate in serum or plasma sample was reported at 8-98% (Waterman *et al*, 1985, Chanyasanha *et al*, 1995; Kalayanarooj *et al*, 1997).

MAC-ELISA was an appropriate method for detecting IgM to dengue antigens in patients who lacked convalescent sera. Acute serum samples collected between day 3 and day 6 (mean ± SD =

3.8 ± 0.8 days) were positive for IgM. Most dengue virus-infected patients had IgM detectable on day 5 (Vaughn *et al*, 1997; Kittigul *et al*, 1998).

The study of KAP in the prevention and control of DHF found that DHF information had penetrated well into communities, although knowledge of the practices for preventing DHF did not have full coverage. Social mobilization using the mass media, community talks and public relations, and the King's project campaigns in 1999, had raised DHF knowledge. Radio and television were the main public channels for distributing health education and information. A KAP survey of household respondents in an urban community in the western region of Thailand, mentioned health education by health personnel being a major role in providing DHF information and prevention methods (Swaddiwudhipong *et al*, 1992). In Trinidad and Tobago, respondents' knowledge of dengue was not correlated with levels of *Ae. aegypti* abundance. Therefore, the present study found a need to provide both DHF education and practice in mosquito control (Rosenbaum *et al*, 1995).

Since the public health promotion about DHF by the mass media emphasized that DHF was an important problem, and case fatalities were reported, the people in the communities had good knowledge, but bad attitudes towards DHF. They thought that DHF was a dangerous disease and also recognized petechiae as a danger sign. Therefore, careful mass media campaigns are needed that will build greater awareness of communication, to help ensure successful DHF understanding and appropriate attitudes.

When all information from the interview about KAP was analyzed, the care takers of the dengue cases were different from the non-cases and the healthy subjects in their attitude to signs and symptoms, prevention practices, and control and treatment of DHF. Although the opinions about signs and symptoms were not satisfoctory, they had better responses for control measures than the other two groups. This may be due to their children having become sick from dengue virus infection and being admitted to hospital. Thus, they had some experience of dengue disease and counselling by medical staff to abtain knowledge of DHF prevention.

This study had limitations because the results of the KAP investigation, when considered in the context of the case-control study that was conducted concurrently, revealed potential limitations in the recommended control measures. It was not known what DHF prevention practices were being used just prior to the occurrence of dengue cases. A prospective study, in which prevention practices should be determined prior to data collection about DHF incidence, would be better for detecting differences in dengue disease rates. However, this study describes the extent to which the dengue cases had learned and adopted DHF prevention measures.

ACKNOWLEDGEMENTS

The authors thank the nursing staff of the Pediatrics Wards in the government hospitals of Ang Thong Province for their co-operation and assistance in specimen collection and with patient records.

REFERENCES

Chanyasanha C, Kalayanarooj S, Morita K, *et al*. Dengue virus isolation and viral genome detection by reverse transcriptase-polymerase chain reaction from serum specimens of dengue patients. *Southeast Asian J Trop Med Public Health* 1995; 26: 495-502.

Clarke DH, Casals J. Techniques for hemagglutination and hemagglutination inhibition with arthropod-borne viruses. *Am J Trop Med Hyg* 1958; 7: 561-73.

Division of Epidemiology, Ministry of Public Health, Thailand. Annual surveillance report. Bangkok: War Veterans Organization, 1990-1999 (in Thai).

Gubler DJ. Surveillance for dengue and dengue hemorrhagic fever. *Bull PAHO* 1989; 23: 397-404.

Gubler DJ, Clark GG. Dengue/dengue hemorrhagic fever: the emergence of a global health problem. *Emerg Infect Dis* 1995; 1: 55-7.

Halstead SB. Dengue hemorrhagic fever–a public health problem and a field for research. *Bull WHO* 1980; 58: 1-21.

Henchal EA, Gentry MK, McCown JM, Brandt WE. Dengue virus-specific and flavivirus group determinants identified with monoclonal antibodies by indirect immunofluorescence. *Am J Trop Med Hyg* 1982; 31: 830-6.

Innis BL, Nisalak A, Nimmannitya S, *et al*. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. *Am J Trop Med Hyg* 1989; 40: 418-27.

Kalayanarooj S, Vaughn DW, Nimmannitya S, *et al*. Early clinical and laboratory indicators of acute dengue illness. *J Infect Dis* 1997; 176 : 313-21.

Kittigul L, Meethien N, Sujirarat D, Vasanavat S. Meungin S. Factors aftecting dengue shock syndrome in Khon Kaen hospital. *Thai J Epidemiol* 1997; 5: 139-46.

Kittigul L, Suthachana S, Kittigul C, Pengruangrojanachai V. Immunoglobulin M-capture biotin-streptavidin enzyme-linked immunosorbent assay for detection of antibodies to dengue viruses. *Am J Trop Med Hyg* 1998; 59: 352-6.

Kuberski T. Fluorescent antibody studies on the development of dengue-2 virus in *Aedes albopictus* (*Diptera: Culicidae*). *J Med Entomol* 1979; 16: 343-9.

Monath TP. Dengue: the risk to developed and developing countries. *Proc Natl Acad Sci USA* 1994; 91: 2395-400.

Rico-Hesse R, Harrison LM, Salas RA. Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. *Virology* 1997; 230: 244-51.

Rosen L, Gubler DJ. The use of mosquito to detect and propagate dengue viruses. *Am J Trop Med Hyg* 1974; 23: 1153-60.

Rosenbaum J, Nathan MB, Ragoonanansingh R, *et al*. Community participation in dengue prevention and control: a survey of knowledge, attitudes and practice in Trinidad and Tobago. *Am J Trop Med Hyg* 1995; 53: 111-7.

Sangkawibha N, Rojanasuphot S, Ahandrik S, *et al*. Risk factors in dengue shock syndrome: a prospective epidemiologic study in Rayong, Thailand. I. The 1980 outbreak. *Am J Epidemiol* 1984; 120: 653-69.

Swaddiwudhipong W, Lerdlukanavonge P, Khumklam P, Koonchote S, Nguntra P, Chaovakiratipong C. A survey of knowledge, attitude and practice of the prevention of dengue hemorrhagic fever in an urban community of Thailand. *Southeast Asian J Trop Med Public Health* 1992; 23: 207-11.

Thein S, Aung MM, Shwe TN, *et al*. Risk factors in dengue shock syndrome. *Am J Trop Med Hyg* 1997; 56: 566-72.

Vaughn DW, Green S, Kalayanarooj S, *et al*. Dengue in the early febrile phase: viremia and antibody responses. *J Infect Dis* 1997; 176: 322-30.

Waterman SH, Kuno G, Gubler DJ, Sather GE. Low rates of antigen detection and virus isolation from the peripheral blood leukocytes of dengue fever patients. *Am J Trop Med Hyg* 1985; 34: 380-4.

Watts DM, Harrison BA, Nisalak A, Scott RM, Burke DS. Evaluation of *Toxorhynchites splendens* (*Diptera: Culicidae*) as a bioassay host for dengue viruses. *J Med Entomol* 1982; 19: 54-9.

World Health Organization. Dengue hemorrhagic fever. Diagnosis, treatment, prevention and control. 2nd ed. Geneva: WHO, 1997.