American Journal of Epidemiology
Copyright © 2002 by the Johns Hopkins Bloomberg School of Public Health
All rights reserved

Vol. 156, No. 1
Printed in U.S.A.
DOI: 10.1093/aje/kwf006

Spatial and Temporal Circulation of Dengue Virus Serotypes: A Prospective Study of Primary School Children in Kamphaeng Phet, Thailand

Timothy P. Endy[1], Ananda Nisalak[1], Supamit Chunsuttiwat[2], Daniel H. Libraty[3], Sharone Green[3], Alan L. Rothman[3], David W. Vaughn[4], and Francis A. Ennis[3]

[1] Department of Virology, United States Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.
[2] Division of General Communicable Diseases, Department of Communicable Disease Control, Ministry of Public Health, Nonthaburi, Thailand.
[3] Center for Infectious Disease and Vaccine Research, University of Massachusetts Medical School, Worcester, MA.
[4] Department of Virus Diseases, Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Silver Spring, MD.

Received for publication October 1, 2001; accepted for publication February 25, 2002.

Dengue virus occurs as four distinct serotypes, each of which causes epidemics throughout the tropical and subtropical regions of the world. Few studies have examined co-circulation of multiple dengue virus serotypes in a well-defined cohort population over time and their capacity to produce severe dengue disease. In this paper, the authors report the details and findings of the first 3 years (1998–2000) of an ongoing prospective study of dengue virus transmission and disease severity in a cohort of children in northern Thailand. A total of 108 dengue virus isolates were obtained from 167 acute dengue virus infections; 23% were DEN-1, 35% were DEN-2, 41% were DEN-3, and 1% were DEN-4. Despite the proximity of the schools, there was marked spatial and temporal clustering of transmission of each dengue serotype. Serotype-specific antibody levels prior to the dengue transmission season were not predictive of the incidence of dengue virus infections or the predominant serotype transmitted at individual schools. All dengue serotypes produced severe dengue illness, although DEN-3 produced more severe symptoms than the other dengue serotypes. The authors' findings emphasize the complexity of dengue serotype-specific virus transmission and severe dengue disease and have important implications for dengue control and vaccine development. *Am J Epidemiol* 2002;156:52–9.

dengue hemorrhagic fever; dengue virus; disease attributes; epidemiologic factors; infection; serotyping

Abbreviations: EIA, enzyme immunoassay; HAI, hemagglutination inhibition; Ig, immunoglobulin; PRNT, plaque reduction neutralization titer.

Dengue virus, family Flaviviridae, genus *Flavivirus,* is comprised of four distinct serotypes (DEN-1, DEN-2, DEN-3, and DEN-4) and has been recognized as a cause of human illness and worldwide pandemics for over 200 years (1–3). DEN-1 and DEN-2 were first isolated from US soldiers during World War II and DEN-3 and DEN-4 from patients with dengue hemorrhagic fever in the Philippines and Thailand in 1954 (4, 5). The co-circulation of all four dengue serotypes and their capacity to produce severe dengue disease was demonstrated as early as 1960 in Bangkok, Thailand (6).

Dengue has emerged as a global health problem, as evidenced by a series of epidemics throughout the tropical and subtropical regions of the world (7). DEN-2 was present in the Americas during the 1970s, and DEN-1 was introduced in 1977, DEN-4 in 1981, and DEN-3 in 1994 (7). The propensity of specific dengue serotypes to produce more severe disease was initially observed by Siler and Simmons during human clinical studies on dengue infection (8, 9).

Few studies have examined the long-term circulation of dengue serotypes in one population over time and their capacity to induce epidemics or severe dengue disease (10,

Reprint requests to Dr. Timothy P. Endy, Division of Virology, United States Army Medical Research Institute for Infectious Disease (USAMRIID), 1425 Porter Street, Building 1425, Fort Detrick, MD 21702–5011 (e-mail: Timothy.Endy@det.amedd.army.mil).

11). The Prospective Study of Dengue Virus Infection in Primary School Children in Kamphaeng Phet, Thailand, is a prospective cohort study designed to answer these and other fundamental questions about why children develop severe dengue disease. This study, initiated in January 1998, follows approximately 2,200 primary school children from grades 2 through 6 for development of subclinical or severe dengue disease. In this paper, we report data from the first 3 years of this study (1998–2000) on the circulation of dengue serotypes within this population over time and their association with severe dengue disease.

MATERIALS AND METHODS

Details regarding the study site, study design, and methods are reported separately (12).

Study site

The study site and field laboratory have been described previously (13, 14). Briefly, the site is located in the province of Kamphaeng Phet, an agrarian area of 8,608 km^2 located approximately 358 km northwest of Bangkok, Thailand. This study is being conducted in subdistrict Muang, which, according to the year 2000 census, has a population of 198,943 and 49,593 households.

Study design

Children were enrolled from 12 elementary schools in the district (figure 1). Baseline demographic information, height and weight, and a blood sample for plasma and peripheral blood mononuclear cells were obtained every January to evaluate participating students. Each year, all participants were evaluated on June 1, August 15, and November 15, when a blood sample was obtained for dengue serology. Case surveillance of study participants for active acute illness occurred from June 1 to November 15, the peak dengue transmission season in Thailand.

Acute illness from dengue infection was identified by using school absence as the indicator for evaluation. Absent students are identified by their teachers and are evaluated by a village health worker by using a symptom questionnaire and obtaining an oral temperature with a digital thermometer. Students who have a history of fever within 7 days of school absence or an oral temperature of 38°C (100.4°F) or more are brought to the public health clinic and evaluated by a public health nurse. A physical examination is conducted, and an acute-illness blood sample is obtained. A convalescent blood sample is obtained 14 days later. Acutely ill children are also identified if they report to the school nurse as feeling ill or when they are admitted to the hospital.

Laboratory assays

Details regarding serologic assays are being reported separately (12). Hemagglutination inhibition (HAI) assays and antidengue immunoglobulin (Ig)M/IgG enzyme immunoassays (EIA) were performed as described previously (15, 16). Plaque reduction neutralization titers (PRNT) against

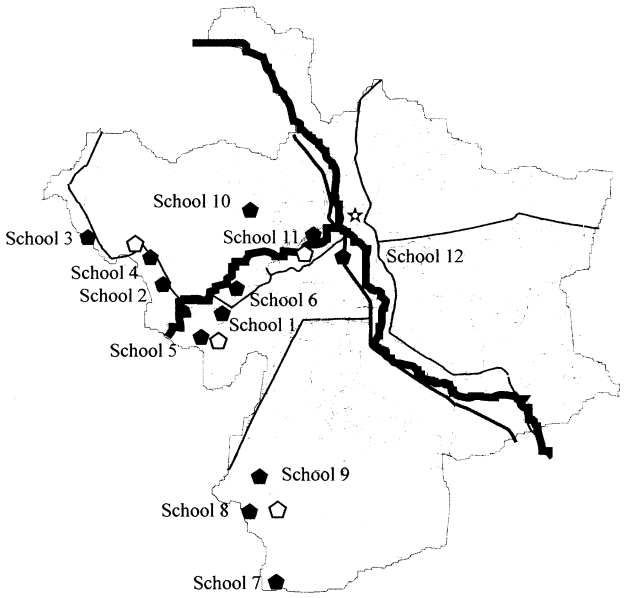

FIGURE 1. Participating study schools in Kamphaeng Phet Province, Thailand, 1998–2000. Black pentagons, schools; white pentagons, public health clinics; star, Kamphaeng Phet Provincial Hospital and location of field laboratory. Thick black line, Ping River and its tributaries; thin black lines, roads. The distance from the Provincial Hospital to school 11 is approximately 1.5 km.

DEN-1, DEN-2, DEN-3, and DEN-4 were measured by using the method of Russell et al. (17) and Yuill et al. (18). Japanese encephalitis infection as a source of dengue antibody cross-reactivity was excluded by performing Japanese encephalitis-specific HAI assay and IgM/IgG by EIA concurrently with all test sera.

Virus isolation and typing EIA to identify dengue serotypes. Dengue viruses were isolated in *Toxorhynchites splendens* mosquitoes, as described previously, and were amplified in C6/36 cell cultures (19). Dengue serotypes were identified by using an antigen-capture EIA, also described previously (20, 21).

Detection of dengue virus RNA by reverse-transcriptase polymerase chain reaction. Detection of dengue virus RNA and serotype identification were performed by using the Lanciotti procedure (22).

Serologic definitions of acute dengue virus infection

Dengue virus infection was defined as isolation of a dengue virus or detection of dengue virus RNA by reverse-transcriptase polymerase chain reaction from serum or plasma during an acute febrile illness, with serologic evidence of acute dengue infection. Dengue virus infection by serology was defined as a fourfold or greater rise in HAI antibody against any dengue virus serotype between the acute and convalescent specimens or in paired sera. Dengue virus-specific IgM levels of 40 units or more by IgM capture EIA were also considered diagnostic of an acute dengue virus infection. Primary dengue infection was defined, as described previously (16), as an acute dengue infection with

an IgM-to-IgG ratio of 1.8 or greater by IgM capture EIA in the acute or convalescent specimen (16). A ratio of less than 1.8 was defined as an acute secondary dengue infection.

Clinical definitions of serologically confirmed dengue virus infection

Inapparent dengue virus infection. This condition was defined as a fourfold or greater rise in HAI antibody against any dengue virus serotype between paired sera obtained during the surveillance months (June, August, and November), without an associated febrile illness being identified.

Acute dengue fever. This condition was defined as a school absence of a child with a history of fever or fever on examination and serologic evidence of acute dengue virus infection with no evidence of dengue hemorrhagic fever according to World Health Organization criteria (23).

Acute dengue hemorrhagic fever and dengue hemorrhagic fever grade. These conditions were defined as a school absence of a child with a history of fever or fever on examination and serologic evidence of acute dengue virus infection with evidence of dengue hemorrhagic fever and dengue hemorrhagic fever grade according to World Health Organization criteria (23). Charts of hospitalized children are reviewed independently and their dengue illness determined to be either dengue fever or dengue hemorrhagic fever; if dengue hemorrhagic fever is identified, it is assigned a severity grade by an expert in the field (Dr. Suchitra Nimmannitya, Queen Sirikit National Institute of Child Health, Bangkok, Thailand).

Statistical analysis

Statistical analysis was performed by using SPSS software for Windows (version 10.0; SPSS Inc., Chicago, Illinois). Incidence rates were determined by using the total study population at the time of surveillance as the denominator. Student's *t* test, analysis of variance, Pearson's correlation, or linear regression was used to determine differences or associations among continuous variables; chi-square tests were used for proportions.

Human use review and approval

The study protocol was reviewed and approved by the Human Use Review and Regulatory Agency of the Office of the Army Surgeon General, the Institutional Review Board of the University of Massachusetts School of Medicine, and the Thai Ethical Review Board of the Ministry of Public Health, Thailand.

RESULTS

Study population characteristics

The characteristics of the study population have been described previously. For details, refer to Endy et al. (12).

Dengue virus infection

Incidence of dengue virus infection. The average incidence of dengue virus infection was 5.8 percent per 6-month period of observation during the dengue transmission season (henceforth referred to as per year), which was comprised of 3.1 percent per year of inapparent dengue infection and 2.7 percent per year of acute symptomatic dengue. The average incidence of symptomatic nonhospitalized dengue virus infection was 2.1 percent per year. The incidences of hospitalized dengue fever and hospitalized dengue hemorrhagic fever were 0.2 percent and 0.3 percent per year, respectively. Of the symptomatic cases of dengue infection, 3.9 percent were primary dengue virus infection and the remainder were secondary dengue infection. Dengue incidence varied by year. The incidence in 1998 was 7.9 percent, consisting of 4.3 percent inapparent dengue infection and 3.6 percent symptomatic dengue virus infection. In 1999, the incidence was 6.5 percent, consisting of 3.2 percent inapparent and 3.3 percent symptomatic dengue virus infection. The incidence in 2000 was 2.2 percent: 1.4 percent inapparent and 0.8 percent symptomatic dengue virus infection. Dengue incidence varied by school during each season and from year to year. Peak incidence occurred in school 4 (20.3 percent) during 1998, school 2 (12.8 percent) during 1999, and school 9 (11.5 percent) during 2000. School 1 was the only school with no evidence of dengue transmission during this 3-year period.

Population characteristics and dengue serotype-specific virus isolation. For the first 3 years of this study, a total of 167 children had an acute symptomatic dengue virus infection. Viral isolation was attempted from serum samples obtained from these children and yielded dengue viruses from 108 children; the viral isolation rate was 65 percent. DEN-3 was the most common serotype isolated (41 percent of all isolates), followed by DEN-2 (35 percent), DEN-1 (23 percent), and DEN-4 (1 percent) (table 1). No significant age or sex differences were noted between the groups infected with each serotype. Of the 108 children from whom a virus was isolated, three had primary dengue infections (two DEN-1 and one DEN-2), and the remainder had secondary dengue virus infections. None of the three subjects with symptomatic primary infections was hospitalized. Primary dengue in children may be milder than secondary dengue virus infections (24); therefore, these children were excluded from the analysis of dengue serotype and disease severity.

Viral isolation rates (number of viral isolates divided by number of children with acute dengue virus infection) and detection of each dengue virus serotype varied between schools and between years. Viral isolation rates were 64 percent in 1998, 78 percent in 1999, and 62 percent in 2000 ($p < 0.05$ only for comparison of 1999 and 2000 by chi-square test).

Spatial and temporal occurrence of dengue serotypes. Despite the geographic proximity of the schools in the study area (figure 1), occurrence of infection with each of the dengue serotypes clustered within schools and varied both spatially and temporally (figure 2). The majority of acute dengue infections and isolations occurred during June, July,

TABLE 1. Characteristics of dengue-infected children, by specific dengue serotype, Kamphaeng Phet Province, Thailand, 1998–2000

Characteristic	Symptomatic dengue serotype			
	DEN-1 ($n = 25$)	DEN-2 ($n = 38$)	DEN-3 ($n = 44$)	DEN-4 ($n = 1$)
Mean age (years) (1 standard deviation)	10 (1.60)	10 (1.4)	10 (1.6)	10
Male/female sex (ratio)	14/11 (1.3)	15/23 (0.7)	20/24 (0.8)	

and August each study year, corresponding to the peak dengue season.

DEN-3 was the predominant dengue virus serotype isolated during 1998 (78 percent of all isolates) and was found primarily in schools 4 and 7. DEN-1 was the next most frequent serotype (16 percent) and occurred in schools 3, 4, and 6–9. DEN-2 was the least frequent serotype isolated (6 percent of all isolates) and was found in schools 5 and 6. DEN-4 was not isolated from any study subject during 1998. In schools 3 and 5, only a single dengue serotype was isolated (DEN-1 and DEN-2, respectively). Two dengue serotypes were isolated during 1998 from children at each of four other schools (DEN-1 and DEN-3 or DEN-2 and DEN-3). At schools 6 and 8, there were single isolates of each of two serotypes (DEN-1 and DEN-2, and DEN-1 and DEN-3, respectively); at schools 4 and 7, there was a single isolate of DEN-1 but multiple isolates of DEN-3.

All four dengue virus serotypes were isolated in 1999. DEN-2 was the predominant serotype (55 percent of isolates) and occurred in schools 2–4 and 9–12. DEN-1 was the next most frequently isolated serotype (33 percent) and was found in schools 6–9. DEN-3 comprised 12 percent of all viral isolates and occurred in schools 5, 6, and 12. Only one DEN-4 serotype was isolated from school 4. In schools 2, 3, 5, 7, 8, 10, and 11, only a single serotype was isolated. Two dengue serotypes were isolated during 1999 from children at each of four other schools. At schools 4 and 12, there were single isolates of each of two serotypes; at schools 6 and 9, DEN-1 predominated.

DEN-2 was the only dengue serotype isolated during 2000, a year in which dengue incidence was comparatively low. This serotype was isolated from schools 9 and 11.

Dengue incidence and preexisting dengue serotype-specific antibodies. The spatial and temporal diversity of detection of each dengue serotype within this well-defined population suggests that preexisting dengue serotype-specific antibodies may influence the circulation and occurrence of dengue virus serotypes and illness. For most schools that had a high incidence of infections with one dengue serotype, the same serotype was not detected the following year (data not shown). For example, school 4 experienced a large outbreak of DEN-3/DEN-1 in 1998 followed by DEN-2 and DEN-4 in 1999. Similarly, school 3 had a year with DEN-1 virus infections followed by DEN-2, and school 5 had DEN-2 and then DEN-3 infections. Schools 6 and 7 had an occurrence of DEN-1 and school 11 DEN-2 over 2 consecutive years, and dengue incidence declined each year.

To explore this pattern further, dengue serotype-specific HAI antibody titers were determined for each child prior to the dengue season during January of each year. The reciprocal geometric mean antibody titer by dengue virus serotype was 17 for DEN-1, 15 for DEN-2, 21 for DEN-3, 21 for DEN-4, and 15 for Japanese encephalitis. The population distribution of HAI antibody titers was also examined, and 90 percent of the population had a reciprocal antibody titer of 80 or less to each dengue virus serotype. We calculated the proportion of the population in each school with an HAI dengue serotype-specific antibody titer greater than 1/80 (10 percent of the population, approximately 4–5 times the reciprocal geometric mean titer) and looked for a correlation with dengue incidence by schools during the year. When we used Pearson's correlation or linear regression, no association was found between predengue-season population antibody and dengue incidence. School 1, for example, experienced no dengue virus infections during the 3 years of the study, although the proportion of students with high dengue HAI antibody levels was low. This finding suggests that serologic immunity was not the only factor influencing dengue transmission in a given school and that other factors not measured in this study, such as local environment and vector breeding, are also important determinants.

Disease severity by serotype-specific infection. Hospitalization rates per 100 dengue infections were determined for each study year and school. The hospitalization rate for acute dengue infection was 8.9 percent in 1998, 12.7 percent in 1999, and 2.7 percent in 2000 ($p > 0.05$ between years by chi-square test). The proportion of children hospitalized was 3/23 (13 percent) for DEN-1, 7/29 (24 percent) for DEN-2, and 14/44 (32 percent) for DEN-3. The proportion of children with dengue hemorrhagic fever was 2/23 (9 percent) for DEN-1, 4/29 (14 percent) for DEN-2, and 7/44 (16 percent) for DEN-3. A greater proportion of children with acute DEN-3 virus infection was hospitalized and developed dengue hemorrhagic fever than the other dengue virus serotypes, although these differences were not statistically significant ($p > 0.05$ by chi-square test).

Symptoms were elicited from each child with acute dengue virus infection on the first day of school absence. Symptom frequency as a marker of disease severity was determined for each dengue virus serotype. For all dengue serotypes, headache was the symptom reported most frequently. Headache, lethargy, and muscle pain were reported more frequently (84 percent, 40 percent, and 24 percent, respectively) by children with DEN-3 virus infection. Cough was reported more frequently in DEN-1 infections (56 percent).

TABLE 2. Sequence of dengue serotype-specific virus infection and disease severity in children with monotypic preillness neutralizing antibody response, Kamphaeng Phet Province, Thailand, 1998–2000

Dengue virus serotype sequence	No. of children	Symptomatic acute, nonhospitalized dengue infection (no.)	Symptomatic hospitalized dengue fever (no.)	Symptomatic hospitalized dengue hemorrhagic fever (no.)
DEN-1–DEN-3	3	1	1	1
DEN-2–DEN-1	4	3		1
DEN-2–DEN-3	4	3		1
DEN-3–DEN-1	1	1		
DEN-3–DEN-2	3	2	1	
DEN-4–DEN-1	12	12		
DEN-4–DEN-2	2	1		1

For all children, dengue serotype-specific $PRNT_{50}$ was determined in January (preillness), preceding acute dengue virus infection, to determine the sequence of infection with different dengue serotypes in primary and secondary infections (table 2). A monotypic $PRNT_{50}$ pattern was evident for 29 of 108 children, enabling us to determine the sequence of infection based on the preillness serotype-specific antibody titer and the virus isolated during the secondary infection. Dengue hemorrhagic fever occurred during secondary infection in the following sequences: DEN-1–DEN-3, DEN-2–DEN-1, DEN-2–DEN-3, and DEN-4–DEN-2. None of the 12 children with symptomatic DEN-1 infection after primary DEN-4 infection required hospitalization ($p < 0.05$, Fisher's exact test, two tailed compared with all other sequences).

DISCUSSION

Our 1998–2000 results demonstrate that all four dengue virus serotypes circulated in our northern Thailand study population. However, despite the close proximity of the study schools, occurrence of infection with each dengue serotype varied markedly between schools and over time. Results show that, for schools with a high incidence of dengue infection from one dengue virus serotype, it is unlikely that the same serotype will cause the outbreak the following year but that a different dengue virus serotype may cause acute dengue infection. This finding suggests that serotype-specific protective herd immunity may prevent recurrence of the same serotype but that, 1 year later, cross-protective immunity is minimal, allowing another dengue virus serotype to cause an outbreak. On the other hand, our findings suggest that preexistent dengue antibody, as measured by dengue virus serotype-specific HAI antibody, does not predict either dengue incidence or the most likely serotype to emerge subsequently. This information emphasizes that dengue transmission is dependent on a number of environmental and mosquito vector factors. Our findings also demonstrate that each of the dengue serotypes differs in disease severity, as measured by hospitalization, and early symptoms. Both dengue virus serotype and sequence of dengue virus infections were determinants of disease severity.

Understanding the epidemiology of how dengue virus serotypes circulate has important public health implications for understanding virus transmission, disease severity, vector control, and development of a safe and efficacious vaccine. Identifying the determinants of how dengue virus serotypes are circulated and transmitted in a population and their association with dengue disease severity will enable accurate models to be developed to predict dengue outbreaks of severe disease and to identify potential interventions by using vector control and a potential vaccine. Our results emphasize the need for a vaccine that includes all four dengue virus serotypes to induce protective immunity.

Much has been written about the spread of dengue virus throughout the subtropical and tropical areas of the world and the virus's potential to produce outbreaks and severe dengue disease (3, 24, 25). Previous studies that have examined the circulation of dengue serotypes in a well-defined population over time and the virus's capacity to produce localized outbreaks and severe dengue disease have demonstrated the importance of long-term, serotype-specific surveillance over time in understanding the pathogenesis of this virus (26, 27). In our own studies on the circulation of each dengue serotype in Thailand from 1973 to 1999, we have demonstrated that all four dengue serotypes circulate in any given year in both northern Thailand and Bangkok, with one serotype predominating as the cause of the outbreak (Nisalak et al., unpublished manuscript). Our localized population is a microcosm of the situation observed nationally and offers a different perspective on dengue virus circulation. In our population, one serotype emerges as the predominant dengue serotype in any given year as the cause of the outbreak. On closer inspection, this outbreak actually consists of many localized minioutbreaks of different dengue virus serotypes, with one dengue virus serotype emerging as the predominant virus.

Our study implies that most virus transmission occurs within the community or the schools. Occurrence of multiple dengue serotypes in a mobile society such as Thailand could also result from a serotype being introduced or an infection occurring in an urban area such as Bangkok or a nearby city that is then spread to a rural area such as Kamphaeng Phet. Previous studies have described the circulation of all four dengue serotypes within a population. Virus isolation in children with dengue hemorrhagic fever in Jakarta, Indonesia, demonstrated the temporal occurrence of all four dengue serotypes in an urban area, with DEN-3 as the predominant

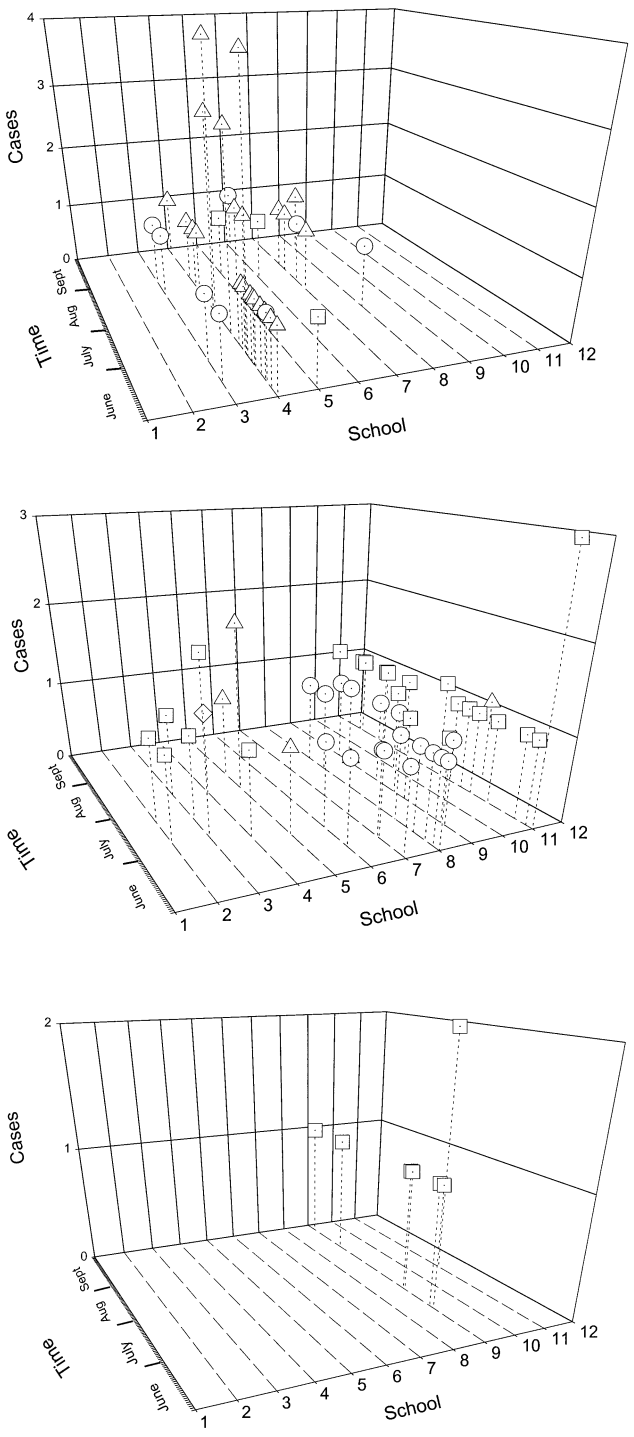

FIGURE 2. Isolation of dengue virus serotypes in patients with symptomatic acute dengue virus infection, by school and time, during the 1998 (top), 1999 (middle), and 2000 (bottom) surveillance seasons, Kamphaeng Phet Province, Thailand. Circles, cases of DEN-1 serotype; squares, cases of DEN-2 serotype; triangles, cases of DEN-3 serotype; diamond, cases of DEN-4 serotype.

one (28). Early studies in Bangkok during the 1960s of children with dengue hemorrhagic fever also showed that all four dengue serotypes occurred (29, 30). The observation that some dengue virus strains produce more severe disease than others was inferred from experiments using human volunteers and from epidemiologic studies of dengue occurring in the Pacific Islands and Indonesia (4, 26, 31, 32). In addition, dengue viruses have been attenuated in the laboratory and used as experimental vaccines; several induced antibody and T-lymphocyte responses in human volunteers, with minimal reactogenicity (33–35). Recently, DEN-2 strains of the Southeast Asian genotype have been found to be more pathogenic than the American genotype of DEN-2, providing a molecular basis from which to begin to understand some aspects of dengue virulence and pathogenicity (36–38).

Our findings that hospitalization rates and symptom severity are greater for certain dengue serotypes are consistent with the current understanding of dengue virus biology, in that some strains and serotypes in combination with host determinants are more capable than others of producing more disease. These determinants include host and viral factors as well as the immunologic history of infection with other dengue serotypes. Secondary dengue is more severe than primary dengue and is associated with greater severity of constitutional symptoms as well as development of dengue hemorrhagic fever (39, 40). Immunologic history also relates to the sequence of dengue serotypes in primary and secondary infections. Previous studies in Thailand have reported that the sequence DEN-1–DEN-2 resulted in a higher proportion of dengue hemorrhagic fever cases (11). However, in Indonesia, DEN-2 followed by DEN-1 resulted in severe disease (41). We found no cases with the DEN-1–DEN-2 sequence. Of the four children with DEN-2–DEN-1, one had dengue hemorrhagic fever. In contrast, none of the 12 children with the sequence DEN-4–DEN-1 required hospitalization, implying that this sequence may induce a milder host immune response resulting in less severe dengue disease.

Our observations on the dengue sequence of infection should be kept in context because of difficulty in interpreting PRNTs and the effects of flavivirus antibody cross-reactivity on these titers. The sequences of infection and disease severity presented here are for children with clear monotypic titers to only one dengue serotype followed by the virus serotype determined by viral isolation. For the majority of children, preillness neutralizing titers were difficult to interpret for one dengue serotype, and information on these cases is not presented here.

Our preliminary results based on 3 years of surveillance provide observations on the complicated process involved in dengue virus circulation and the characteristics of each serotype with regard to producing severe dengue disease. Our findings emphasize the complexity of dengue virus transmission and pathogenicity involved in producing severe dengue disease. Our results emphasize the need for a dengue vaccine to prevent dengue illness, and they underscore the importance of developing a tetravalent vaccine as the most effective way to prevent dengue disease. Our study design provides the framework in which to evaluate the effectiveness of a tetravalent dengue vaccine in preventing severe dengue disease and in preventing transmission of dengue virus serotypes in a population.

ACKNOWLEDGMENTS

This work was funded by National Institutes of Health grant P01 AI34533 and the United States Army Medical Research and Materiel Command, Fort Detrick, Maryland.

The authors thank the staff at the Department of Virology, Armed Forces Research Institute of Medical Sciences (AFRIMS) for their carefully performed diagnostic testing, data collection, and data entry. They acknowledge the support of Dr. Choorat Koosakulrat and his staff, Office of the Provincial Public Health, Kamphaeng Phet Province, and the efforts of the clinical research nurses at AFRIMS and the support staff at the Kamphaeng Phet Field Station.

REFERENCES

1. Chambers TJ, Hahn CS, Galler R, et al. Flavivirus genome organization, expression, and replication. Annu Rev Microbiol 1990;44:649–88.
2. Calisher CH, Karabatsos N, Dalrymple JM, et al. Antigenic relationships between flaviviruses as determined by cross-neutralization tests with polyclonal antisera. J Gen Virol 1989;70:37–43.
3. Halstead SB. The XXth century dengue pandemic: need for surveillance and research. World Health Stat Q 1992;45:292–8.
4. Sabin AB. Research on dengue during World War II. Am J Trop Med Hyg 1952;1:30–50.
5. Hammon WM, Rudnick A, Sather GE. Viruses associated with epidemic hemorrhagic fevers of the Philippines and Thailand. Science 1960;131:1102–3.
6. Halstead SB, Scanlon J, Umpaivit P, et al. Dengue and chikungunya virus infection in man in Thailand, 1962–1964: IV. Epidemiologic studies in the Bangkok metropolitan area. Am J Trop Med Hyg 1969;18:997–1021.
7. Gubler DJ, Clark GG. Dengue/dengue hemorrhagic fever: the emergence of a global health problem. Emerg Infect Dis 1995; 1:55–7.
8. Siler JF, Hall MW, Hitchens AP. Dengue: its history, epidemiology, mechanism of transmission, etiology, clinical manifestations, immunity, and prevention. Philippine J Sci 1926;29:1–304.
9. Halstead SB. Etiologies of the experimental dengues of Siler and Simmons. Am J Trop Med Hyg 1974;23:974–82.
10. Burke DS, Nisalak A, Johnson DE, et al. A prospective study of dengue infections in Bangkok. Am J Trop Med Hyg 1988; 38:172–80.
11. 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.
12. Endy TP, Chunsuttiwat S, Nisalak A, et al. Inapparent and symptomatic acute dengue virus infection: a prospective study of primary school children in Kamphaeng Phet, Thailand. Am J Epidemiol 2002;156:40–51.
13. Hoke CH Jr, Nisalak A, Sangawhipa N, et al. Protection against Japanese encephalitis by inactivated vaccines. N Engl J Med 1988;319:608–14.
14. Innis BL, Snitbhan R, Kunasol P, et al. Protection against hepatitis A by an inactivated vaccine. JAMA 1994;271:1328–34.
15. Clarke DH, Casals J. Techniques for hemagglutination and hemagglutination inhibition with arthropod-borne viruses. Am J Trop Med Hyg 1958;7:561–73.
16. 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.
17. Russell PK, Nisalak A, Sukhavachana P, et al. A plaque reduction test for dengue virus neutralization antibodies. J Immunol 1967;99:285–90.
18. Yuill TM, Sukhavachana P, Nisalak A, et al. Dengue-virus recovery by direct and delayed plaques in LLC-MK2 cells. Am J Trop Med Hyg 1968;17:441–8.
19. Rosen L. The use of *Toxorhynchites* mosquitoes to detect and propagate dengue and other arboviruses. Am J Trop Med Hyg 1981;30:177–83.
20. Henchal EA, McCown JM, Seguin MC, et al. Rapid identification of dengue virus isolates by using monoclonal antibodies in an indirect immunofluorescence assay. Am J Trop Med Hyg 1983;32:164–9.
21. Kuno G, Gubler DJ, Santiago de Weil NS. Antigen capture ELISA for the identification of dengue viruses. J Virol Methods 1985;12:93–103.
22. Lanciotti RS, Calisher CH, Gubler DJ, et al. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. J Clin Microbiol 1992;30:545–51.
23. Guidelines for treatment of dengue fever/dengue haemorrhagic fever in small hospitals. New Delhi, India: World Health Organization, Regional Office for South-East Asia, 1999:5.
24. Innis BL. Dengue and dengue hemorrhagic fever. In: Porterfield JS, ed. Kass handbook of infectious diseases: exotic virus infections. London, United Kingdom: Chapman & Hall Medical, 1995:103–46.
25. Gubler DJ, Trent DW. Emergence of epidemic dengue/dengue hemorrhagic fever as a public health problem in the Americas. Infect Agents Dis 1993;2:383–93.
26. Gubler DJ, Suharyono W, Lubis I, et al. Epidemic dengue hemorrhagic fever in rural Indonesia. I. Virological and epidemiological studies. Am J Trop Med Hyg 1979;28:701–10.
27. Gubler D, Suharyono W, Sumarmo, et al. Virological surveillance for dengue haemorrhagic fever in Indonesia using the mosquito inoculation technique. Bull World Health Organ 1979;57:931–6.
28. Suharyono W, Gubler D, Lubis I, et al. Dengue virus isolation in Indonesia, 1975–1978. Asian J Infect Dis 1979;3:27–32.
29. Halstead SB, Udomsakdi S, Singharaj P, et al. Dengue and chikungunya virus infection in man in Thailand, 1962–1964. III. Clinical, epidemiology, and virologic observations on disease in non-indigenous white persons. Am J Trop Med Hyg 1969;18:984–96.
30. Nimmannitya S, Halstead SB, Cohen S, et al. Dengue and chikungunya virus infection in man in Thailand, 1962–1964. I. Observations on hospitalized patients with hemorrhagic fever. Am J Trop Med Hyg 1969;18:954–71.
31. Fagbami AH, Mataika JU, Shrestha M, et al. Dengue type 1 epidemic with haemorrhagic manifestations in Fiji, 1989–90. Bull World Health Organ 1995;73:291–7.
32. Gubler DJ, Reed D, Rosen L, et al. Epidemiological, clinical, and virologic observations on dengue in The Kingdom of Tonga. Am J Trop Med Hyg 1978;27:581–9.
33. Bhamarapravati N, Yoksan S, Chayaniyayothin T, et al. Immunization with a live attenuated dengue-2-virus candidate vaccine (16681-PDK 53): clinical, immunological and biological responses in adult volunteers. Bull World Health Organ 1987;65:189–95.
34. Bhamarapravati N, Yoksan S. Study of bivalent dengue vaccine in volunteers. (Letter). Lancet 1989;1:1077.

35. Vaughn DW, Hoke CH Jr, Yoksan S, et al. Testing of a dengue 2 live-attenuated vaccine (strain 16681 PDK 53) in ten American volunteers. Vaccine 1996;14:329–36.
36. Watts D, Porter K, Putvatana P, et al. Failure of secondary infection with American genotype dengue 2 to cause dengue haemorrhagic fever. Lancet 1999;354:1431–4. (Comments published in Lancet 1999;354:1401–2).
37. Rico-Hesse R, Harrison LM, Salas RA, et al. Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. Virology 1997;230:244–51.
38. Leitmeyer K, Vaughn D, Watts D, et al. Dengue virus structural differences that correlate with pathogenesis. J Virol 1999;73: 4738–47.
39. Cobra C, Rigau-Perez JG, Kuno G, et al. Symptoms of dengue fever in relation to host immunologic response and virus serotype, Puerto Rico, 1990–1991. Am J Epidemiol 1995;142: 1204–11.
40. Halstead SB, Nimmannitya S, Cohen SN. Observations related to pathogenesis of dengue hemorrhagic fever. IV. Relation of disease severity to antibody response and virus recovered. Yale J Biol Med 1970;42:311–28.
41. Graham R, Juffrie M, Tan R, et al. A prospective seroepidemiologic study on dengue in children four to nine years of age in Yogyakarta, Indonesia. I. Studies in 1995–1996. Am J Trop Med Hyg 1999;61:412–19.