



Epidemiology of Inapparent and Symptomatic Acute Dengue Virus Infection: A Prospective Study of Primary School Children in Kamphaeng Phet, Thailand

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

Dengue viruses are a major cause of morbidity in tropical and subtropical regions of the world. Knowledge about the epidemiology and host determinants of inapparent and severe dengue virus infections is limited. In this paper, the authors report findings from the first 3 years of a prospective study of dengue virus transmission and disease severity conducted in a cohort of 2,119 elementary school children in northern Thailand. A total of 717,106 person-school days were observed from 1998 to 2000. The incidence of inapparent and of symptomatic dengue virus infection was 4.3% and 3.6% in 1998, 3.2% and 3.3% in 1999, and 1.4% and 0.8% in 2000, respectively. Symptomatic dengue virus infection was responsible for 3.2%, 7.1%, and 1.1% of acute-illness school absences in 1998, 1999, and 2000, respectively. The early symptom complex of acute dengue virus infection is protean and difficult to distinguish from other causes of febrile childhood illnesses. The authors' results illustrate the spatial and temporal diversity of dengue virus infection and the burden of dengue disease in schoolchildren in Thailand. Their findings increase understanding of dengue virus transmission and disease severity in a well-defined cohort population and offer a study design in which to test the efficacy of potential dengue vaccines. *Am J Epidemiol* 2002;156:40–51.

dengue virus; disease attributes; epidemiologic factors; infection

Abbreviations: ANOVA, analysis of variance; CI, confidence interval; EIA, enzyme immunoassay; HAI, hemagglutination inhibition; Ig, immunoglobulin; RR, relative risk.

The clinical manifestation of dengue virus infection as dengue fever has been recognized for over 200 years (1–3). Dengue hemorrhagic fever, the potentially fatal form of dengue virus infection, became generally recognized in the 1950s following outbreaks in the Philippines and Thailand (4, 5). Dengue fever and dengue hemorrhagic fever are now considered major causes of morbidity and mortality in the subtropical and tropical regions of the world (6). Dengue viruses, the causative agent of dengue fever and dengue hemorrhagic fever, are comprised of four distinct serotypes (DEN-1, DEN-2, DEN-3, and DEN-4) and are members of the family Flaviviridae, genus *Flavivirus* (7, 8).

Over the last 40 years, the incidence of dengue virus transmission and disease in Thailand has increased from a reported annual rate of 9/100,000 in 1958 to 189/100,000 in 1998 (9). The largest reported outbreak of dengue occurred in 1987, with an incidence rate of 325/100,000 (9).

Dengue virus infection can manifest as clinically inapparent, an undifferentiated febrile illness, classic dengue fever, or dengue hemorrhagic fever (3). The majority of dengue infections in children are thought to be subclinical and can present atypically as an undifferentiated febrile illness (10, 11). The pathogenesis of severe dengue disease, dengue hemorrhagic fever, has been reviewed previously

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and is thought to be a consequence of a heightened immune response due to cross-reactive T-cell responses and/or enhancing dengue antibody during secondary dengue virus infection (12–14).

The rates of subclinical and of severe dengue virus infection were estimated in a prospective cohort study of students conducted in Bangkok, Thailand (15). The majority of dengue virus infections, 87 percent, were subclinical. Preexistent dengue virus exposure, as determined by the presence of dengue virus antibody, was a significant risk factor for development of dengue hemorrhagic fever.

The Prospective Study of Dengue Virus Infection in Primary School Children in Kamphaeng Phet, Thailand, is a cohort study initiated in January 1998. Its purpose is to study the epidemiology and immunology of inapparent to severe dengue disease and to identify risk factors for developing severe dengue disease after acquiring a secondary dengue infection. Here, we report on the study design, epidemiology of inapparent and symptomatic dengue virus infections, and clinical presentation of dengue virus infection during the first 3 years of this study.

MATERIALS AND METHODS

Study site

This study is being conducted in subdistrict Muang, Kamphaeng Phet Province, Thailand, located 358 km north-west of Bangkok. According to the year 2000 census, subdistrict Muang has a total population of 198,943. Kamphaeng Phet city is the provincial capital and has a 332-bed public health hospital that is the major source of health care for the province.

School-based study

Subdistrict Muang has 92 public schools and, according to the year 2000 census, a total of 22,131 school-aged children. Twelve primary schools were selected to participate in this study on the basis of their reliable road access, desire to participate in the study, and location within a 3-hour driving radius from the field station laboratory at the Kamphaeng Phet Provincial Hospital (figure 1).

Children were recruited during January 1998 from grades 1–5 (they were expected to be in grades 2–6 at the start of the school year in May). Children were eligible to remain in the study until graduation from sixth grade. During each subsequent year, new first-grade students were enrolled in the cohort each January. Enrollment criteria for study participation were attendance at a study school, enrollment in grades 1–6 thereafter, and informed parental consent. Exclusion criteria included planning to move outside of the study area within the first 12 months of the study and having a history of thalassemia requiring blood transfusion.

Study design

Baseline demographic information, height and weight, and a blood sample were obtained every January. Volunteers were evaluated (height, weight, blood sample for dengue

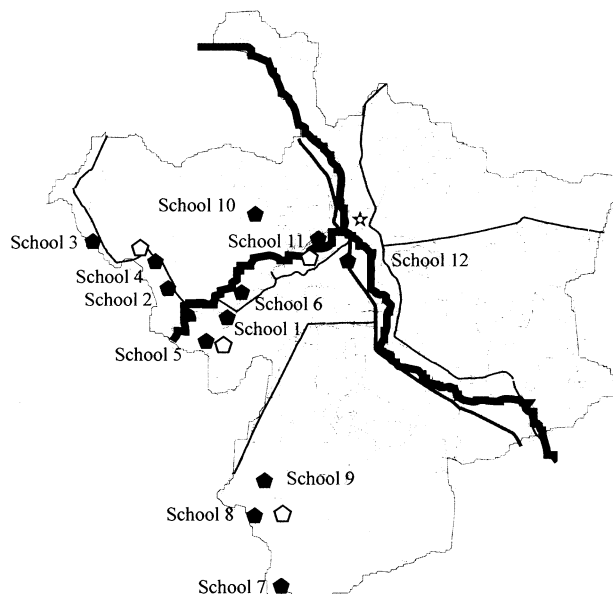


FIGURE 1. Participating study schools in Kamphaeng Phet Province, Thailand, 1998–2000. Black pentagons, schools; white pentagons, public health clinics; star, Kamphaeng Phet Provincial Hospital and location of field laboratory. Thick black line, Ping River and its tributaries; thin black lines, roads.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

serology) three times during the surveillance period (June 1, August 15, and November 15) of each year. Case surveillance of study participants for active acute illness occurred during the dengue season from June 1 to November 15.

Active case surveillance. Acute illness due to dengue virus infection was identified on the basis of absence from school or a visit to the school nurse. Teachers at study schools were provided with a roster of student participants, and school absences were recorded during the morning of each school day; this information was then given to one of four participating public health clinics (figure 1). Absent students were visited by village health workers that afternoon and were evaluated by using a symptom questionnaire and by obtaining an oral temperature with a digital thermometer. Students who had a history of fever within 7 days of school absence or an oral temperature of 38°C (100.4°F) or more were brought to the public health clinic and were evaluated by a public health nurse; a physical examination was conducted, and an acute-illness blood sample was obtained. A convalescent blood sample was obtained 14 days later. Children who were dehydrated; had prolonged diarrhea or vomiting, a high temperature, or a change in sensorium; or appeared severely ill were referred for further evaluation and possible admission to the Kamphaeng Phet Provincial Hospital. Children who visited the school nurse were evaluated in the same manner as those who were absent from school. A student who was absent from school and had no history of fever on examination was followed each school-absence day until a fever or history of fever was documented or the student returned to school.

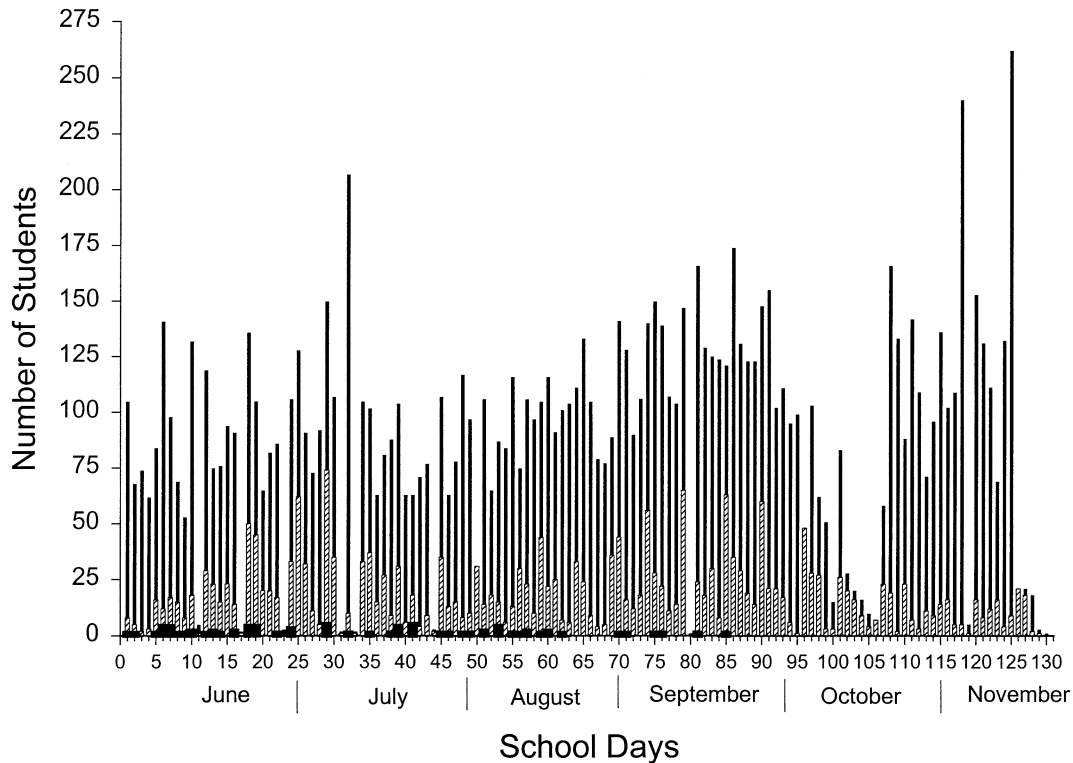


FIGURE 2. Daily numbers of all students absent (thin black bars), students absent-ill (hatched bars), and students absent-ill with acute symptomatic dengue (thick black bars), by school day during 1998, Kamphaeng Phet, Thailand.

Hospital/clinic surveillance. Throughout June–November, and including weekends and holidays, clinical research nurses tracked children who reported to the public health clinic with an illness or were admitted to the hospital. Students were evaluated by using the same methods and criteria as those for active surveillance.

Subject identification, database, and quality control

Each volunteer was given a unique identification number upon enrolling in the study. Quality assurance regarding student absences, follow-up, and blood samples was monitored daily by the field station manager to ensure that all student absences were evaluated within 24 hours.

Specimen handling

Each January, venous blood was drawn from each volunteer into Vacutainer CPT tubes (Becton-Dickinson (BD), Franklin Lakes, New Jersey) and was processed according to the manufacturer's instructions. Specimens arrived and were processed within 3 hours of collection. Plasma was stored at -70°C , and peripheral blood mononuclear cells were cryopreserved in an isopropanol-cooling chamber at -70°C and were stored in liquid nitrogen following transport to Bangkok. All other blood specimens obtained were drawn into serum separator tubes, and sera were stored at -70°C .

Laboratory assays

Preparation and testing of dengue antigens. Dengue virus antigens were produced as described previously (16–19) and were used in the hemagglutination inhibition (HAI) assay and the immunoglobulin (Ig)M/IgG enzyme immunoassay (EIA). HAI was performed by using the method of Clarke and Casals adapted to microtiter plates (20). The antidengue IgM/IgG EIA method used was described previously (21).

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 (22). Dengue serotypes were identified by using an antigen-capture EIA, also described previously (23, 24).

Detection of dengue viral RNA by reverse-transcriptase polymerase chain reaction. Dengue virus RNA was detected by adopting a modification of the primers used in the Lanciotti procedure (25).

Serologic definitions of dengue virus infection

No evidence of recent dengue virus infection. This condition was defined as no detectable dengue virus antibody in acute and convalescent sera by EIA or HAI, or stable antibody titers by HAI with titers of less than 1:2,560 to all dengue antigens without a fourfold rise in titer.

Dengue virus infection. Dengue virus infection was defined as isolation of a dengue virus or detection of dengue

TABLE 1. Student-absent days, absent-ill days, and absent-acute-dengue-illness days,* by study year, Kamphaeng Phet, Thailand, 1998–2000

Year	No. of school days during the surveillance period	Students absent	Students absent-ill	Students absent-ill with acute dengue infection
1998	130	90	18	0.6
1999	126	64	7	0.5
2000	116	63	7	0.1

* All values in the last three columns are expressed as mean number of students per school day.

virus RNA by reverse-transcriptase polymerase chain reaction from serum or plasma and serologic evidence of dengue virus infection by HAI or EIA. HAI criteria for dengue virus infection were a fourfold rise in antibody titers against any dengue virus serotype between the acute and convalescent specimens or in paired sera. By EIA, acute dengue virus infection was defined as a dengue virus-specific IgM level of 40 units or more. To exclude Japanese encephalitis virus infection and antibody cross-reactivity, sera were tested concurrently for Japanese encephalitis-specific HAI antibody and Japanese encephalitis IgM by EIA (21).

Primary or secondary dengue infection. Primary dengue infection was defined as an acute dengue infection with an IgM-to-IgG ratio of 1.8 or greater by EIA in the acute or convalescent specimen (21). Infection with an IgM-to-IgG 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 rise in HAI antibody against any dengue virus serotype between two sequential sera samples obtained during the surveillance months (June, August, or November), without a febrile illness identified during active surveillance in the time period in which seroconversion occurred. Sera were tested concurrently for Japanese encephalitis-specific HAI antibody to exclude Japanese encephalitis infection and antibody cross-reactivity as a cause for a fourfold rise in dengue antibody.

Acute dengue fever. This condition was defined as an identified febrile illness with laboratory confirmation of acute dengue virus infection and no evidence of dengue hemorrhagic fever according to World Health Organization criteria (26). Acute dengue fever was further classified as symptomatic nonhospitalized or symptomatic hospitalized.

Acute dengue hemorrhagic fever and dengue hemorrhagic fever grade. These conditions were defined as an identified febrile illness with laboratory confirmation of acute dengue virus infection and evidence of dengue hemorrhagic fever according to World Health Organization criteria (26). 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).

TABLE 2. Numbers of cases and incidence of inapparent and symptomatic acute dengue in students, by school and year, Kamphaeng Phet, Thailand, 1998–2000

School	1998					1999					2000				
	Inapp.*	Symptomatic			Incidence (%)	Inapp.	Symptomatic			Incidence (%)	Inapp.	Symptomatic			Incidence (%)
		NH*	HDF*	HDHF*			NH	HDF	HDHF			NH	HDF	HDHF	
1	0	0	0	0	0.0	0	0	0	0	0.0	0	0	0	0	0.0
2	1	0	0	0	1.0	6	4	0	0	12.8	0	0	0	0	0.0
3	10	6	0	0	7.9	5	3	3	0	6.2	1	0	0	0	0.7
4	35	36	5	9	20.3	13	2	0	0	4.1	5	0	0	0	1.9
5	5	8	0	0	4.5	6	2	2	3	4.7	1	0	0	0	0.4
6	6	2	0	0	10.1	2	2	0	1	7.5	0	1	0	0	2.1
7	4	5	0	0	10.8	0	3	0	1	5.5	0	0	0	0	0.0
8	16	4	1	0	8.5	4	8	1	0	5.7	5	2	0	0	3.1
9	3	0	0	0	2.2	5	7	0	1	10.2	7	7	0	1	11.5
10	2	0	0	0	2.0	0	1	0	0	1.1	0	0	0	0	0.0
11	8	2	0	0	3.3	12	16	1	2	10.8	4	2	0	0	2.1
12	2	0	0	0	1.7	8	1	0	1	9.3	1	0	0	0	1.0
Total	92	61	6	9	7.9	61	49	7	9	6.5	24	12	0	1	2.2

*Inapp., inapparent; NH, nonhospitalized; HDF, hospitalized dengue fever; HDHF, hospitalized dengue hemorrhagic fever.

Statistical analysis

Statistical analyses were 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 or analysis of variance (ANOVA) was used to determine differences in continuous variables, and 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

In January 1998, 2,214 students were enrolled in the study, with 2,119 remaining for the start of surveillance. In subsequent years, loss of students from January to June was a result of the graduation of sixth graders. The mean dropout rate over the 3 years, 1998–2000, was 5 percent primarily because families moved out of the surveillance schools. The gradual decline in the study population at the start of active surveillance from 2,119 in 1998 to 1,928 in 1999 to 1,713 in 2000 reflected the changing demographics of the surveillance schools, with smaller second-grade classes and waning community interest in the program. No differences in sex distribution were noted from year to year or between schools (data not shown). Median age shifted from 10 years in 1998 and 1999 to 9.3 years in 2000, although the distribution of students by school grade did not vary (data not shown).

The percentage of children who received the Japanese encephalitis vaccine, and their mean age on receiving it, varied between schools and study years. The percentage of students vaccinated was 70 percent in 1998, 61 percent in 1999, and 58 percent in 2000. Mean age on receiving the vaccine declined from 7.3 years (standard deviation, 0.8) in 1998 to 6.6 years (standard deviation, 1.6) in 1999 to 5.7 years (standard deviation, 2.4) in 2000 ($p < 0.001$ by ANOVA). This decline in the percentage of children receiving the Japanese encephalitis vaccine and in their mean age on receiving it may reflect the national Thai policy on Japanese encephalitis vaccination. Such vaccination became part of the Expanded Program of Immunization in Kamphaeng Phet Province in 1992, with vaccination at 12 and 18 months of age. After 1992, an effort was made in Kamphaeng Phet to immunize all children to Japanese encephalitis who were not covered by this program. The reported national vaccination rate for Japanese encephalitis is 80 percent, although it may be lower in Kamphaeng Phet (27).

Symptomatic dengue infection

Student-absent days. Mean per-school-day numbers of students absent, absent-ill, and absent-ill with acute dengue virus infection were then calculated for each study surveillance period (table 1). During 1998, a total of 11,777 student-absent days occurred; 20 percent of the students had a fever on examination or a history of fever, and 0.6 percent of all students contributing to student-absent days were laboratory positive for acute dengue virus infection, accounting for 3.2 percent of all acute illnesses. The daily number of absent-ill students displayed a bimodal peak in early July and mid-September, with all symptomatic acute dengue virus infections occurring from June to September (figure 2). During 1999, a total of 8,034 student-absent days occurred; 11 percent of the students were acutely ill, and 0.8 percent of all students contributing to absent-student days were laboratory positive for acute dengue virus infection (7.1 percent of all acute illnesses). The daily number of absent-ill students was constant throughout the surveillance period, with acute dengue cases occurring from June to September (figure 3). During 2000, a total of 7,302 student-absent days occurred; 12 percent of the students were acutely ill, and 0.2 percent of all students contributing to school-absent days were laboratory positive for acute dengue virus infection (1.5 percent of all acute illnesses). The daily number of absent-ill students displayed a bimodal peak in early July and mid-September, with acute dengue infections occurring sporadically until October (figure 4).

Inapparent and symptomatic dengue virus infection. For the 3 years of the study, the overall incidence of dengue virus infection was 5.8 percent. The incidence of inapparent dengue virus infection and symptomatic dengue was 3.1 percent and 2.7 percent, respectively. The incidence of symptomatic nonhospitalized dengue, hospitalized dengue fever, and hospitalized dengue hemorrhagic fever was 2.1 percent, 0.2 percent, and 0.3 percent, respectively. There were no fatal cases of dengue. Of the symptomatic cases of dengue infection, 6/154 (3.9 percent) were primary dengue virus infections.

In 1998, the overall incidence of dengue virus infection was 7.9 percent: 4.3 percent for inapparent and 3.6 percent for symptomatic (table 2). The incidence of nonhospitalized symptomatic dengue virus infection was 2.9 percent and of hospitalized dengue infection was 0.7 percent (0.4 percent for dengue hemorrhagic fever). School 4 had the highest incidence, with 20.3 percent of the study population experiencing dengue virus infection during the 6 months of surveillance, followed by school 7 (10.8 percent) and school 6 (10.1 percent). There was marked spatial variation in dengue incidence among the study schools (table 2 and figure 1). School 1 was the only one with no serologic evidence of dengue virus infection. The majority of dengue virus infections occurred during June and July, with no cases of symptomatic dengue infection after September (figure 5). The majority of cases of inapparent dengue occurred during the June 1–August 15 interval, although seroconversions did occur during August 16–November 30 despite a decline in symptomatic dengue (figure 5).

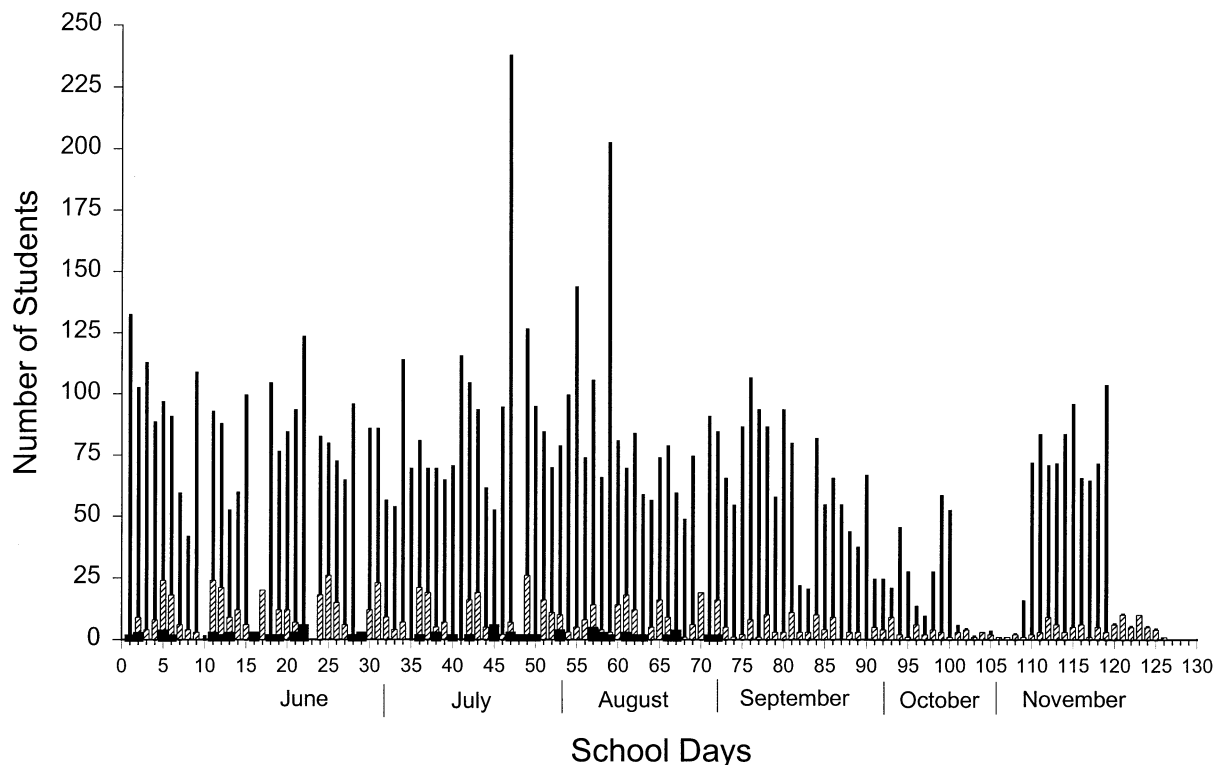


FIGURE 3. Daily numbers of all students absent (thin black bars), students absent-ill (hatched bars), and students absent-ill with acute symptomatic dengue (thick black bars), by school day during 1999, Kamphaeng Phet, Thailand.

In 1999, the overall incidence of dengue virus infection was 6.5 percent: 3.2 percent for inapparent and 3.3 percent for symptomatic (table 2). The incidence of symptomatic and severe dengue disease was 2.5 percent for nonhospitalized symptomatic dengue virus infection and 0.8 percent for hospitalized dengue infection (0.5 percent for dengue hemorrhagic fever). School 2 had the highest incidence, with 12.8 percent of the study population experiencing dengue virus infection during the 6 months of surveillance, followed by school 11 (10.8 percent) and school 9 (10.2 percent). Spatial variation in dengue incidence among the schools was observed, with school 1 again having no serologic evidence of inapparent or symptomatic dengue (table 2). The majority of dengue infections occurred during June and July, with no cases of symptomatic dengue infection occurring after October (figure 6). The majority of cases of inapparent dengue occurred during the June 1–August 15 time period.

In 2000, the overall incidence of dengue virus infection was 2.2 percent: 1.4 percent for inapparent and 0.8 percent for symptomatic (table 2). The incidence of nonhospitalized symptomatic dengue virus infection was 0.7 percent and of hospitalized dengue infection was 0.06 percent. School 9 had the highest incidence, with 11.5 percent of the study population experiencing dengue virus infection during the 6 months of surveillance, followed by school 8 (3.1 percent) and schools 6 and 11 (2.1 percent each). Four schools (1, 2, 7, and 10) had no dengue virus transmission. Only school 1 had

no evidence of dengue transmission during the first 3 years of this study. Dengue infections occurred sporadically during the surveillance months (figure 7). The majority of cases of inapparent dengue occurred during the June 1–August 15 interval.

Disease severity of acute symptomatic dengue virus infection. Hospitalization rates for acute dengue infection were determined for each study year and school as a measurement of dengue disease severity. The hospitalization rate for acute symptomatic dengue virus infections 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 ratio of inapparent to symptomatic dengue virus infection was 1.2 in 1998, 0.9 in 1999, and 1.8 in 2000. There was marked variation in dengue virus infection incidence rates, dengue hospitalization rates, and inapparent to symptomatic dengue ratios between schools and study years (figure 8). Total hospitalization rates for all years correlated with total dengue incidence ($r = 0.36$, $p = 0.03$, Pearson's correlation, two tailed). The ratio of inapparent to symptomatic dengue infection was not correlated to total dengue incidence nor to hospitalization rates ($r = -0.03$, $p = 0.9$, Pearson's correlation, two tailed and $r = 0.3$, $p = 0.08$, Pearson's correlation, two tailed, respectively). Other factors that did not differ among children with inapparent or more severe dengue infections were mean age, sex, and history of Japanese encephalitis vaccination (data not shown).

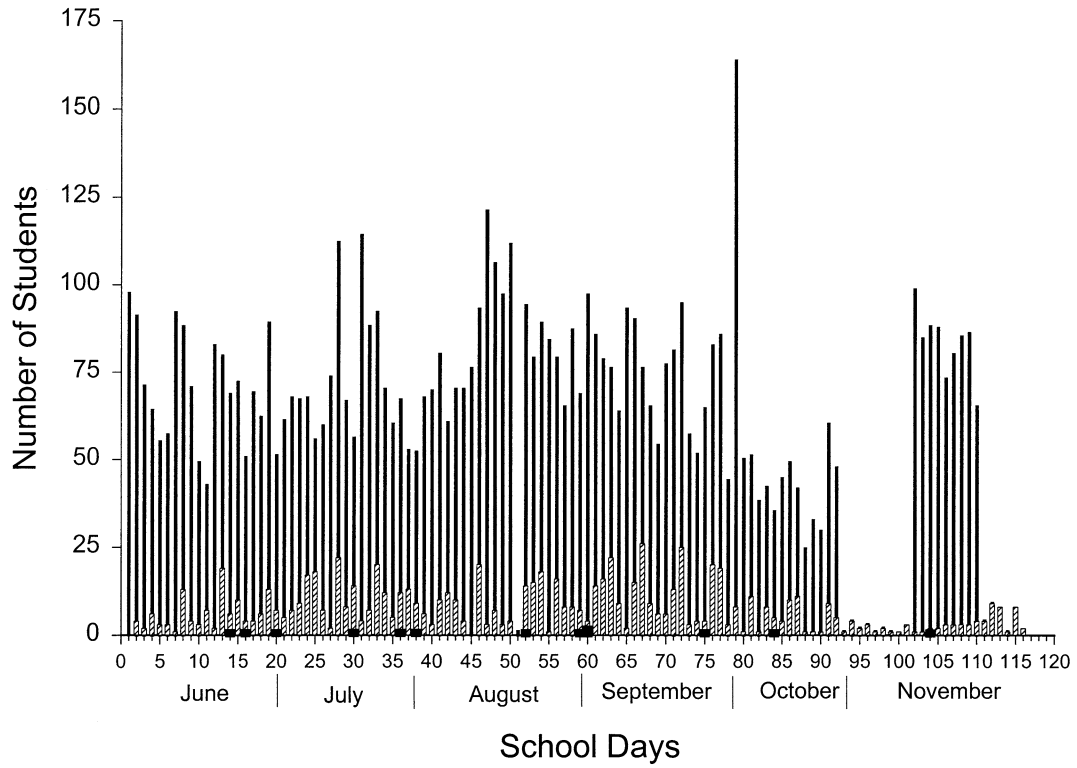


FIGURE 4. Daily numbers of all students absent (thin black bars), students absent-ill (hatched bars), and students absent-ill with acute symptomatic dengue (thick black bars), by school day during 2000, Kamphaeng Phet, Thailand.

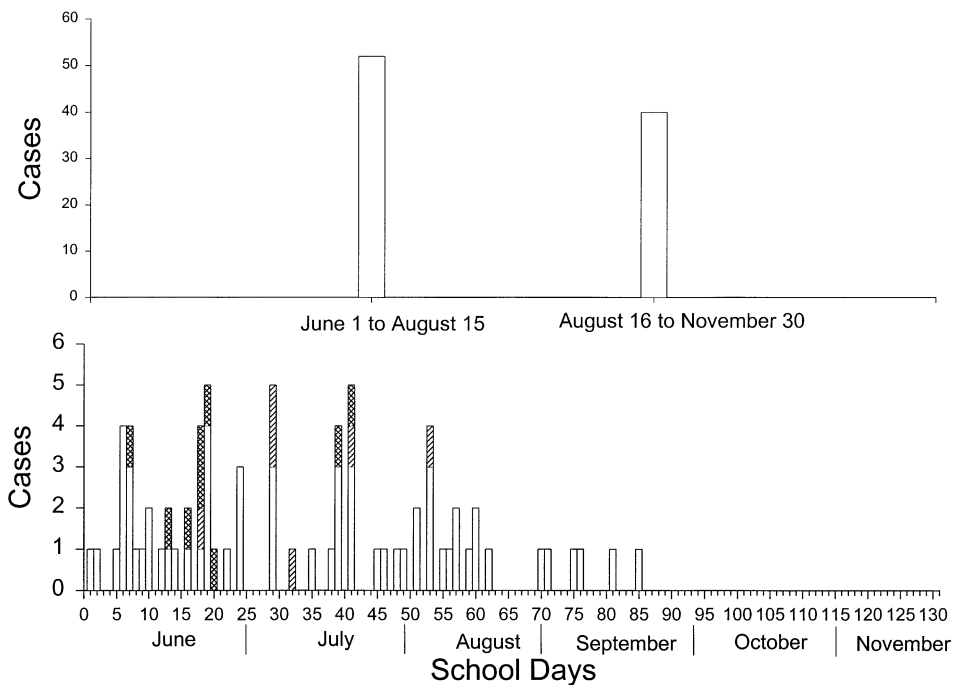


FIGURE 5. Numbers of students with acute inapparent dengue infection (top, white bars) and symptomatic acute dengue virus infection (bottom) during the 1998 surveillance season, Kamphaeng Phet, Thailand. Symptomatic acute dengue virus infection was further divided into symptomatic nonhospitalized acute dengue (white bars), hospitalized dengue fever (single-hatched bars), and hospitalized dengue hemorrhagic fever (double-hatched bars).

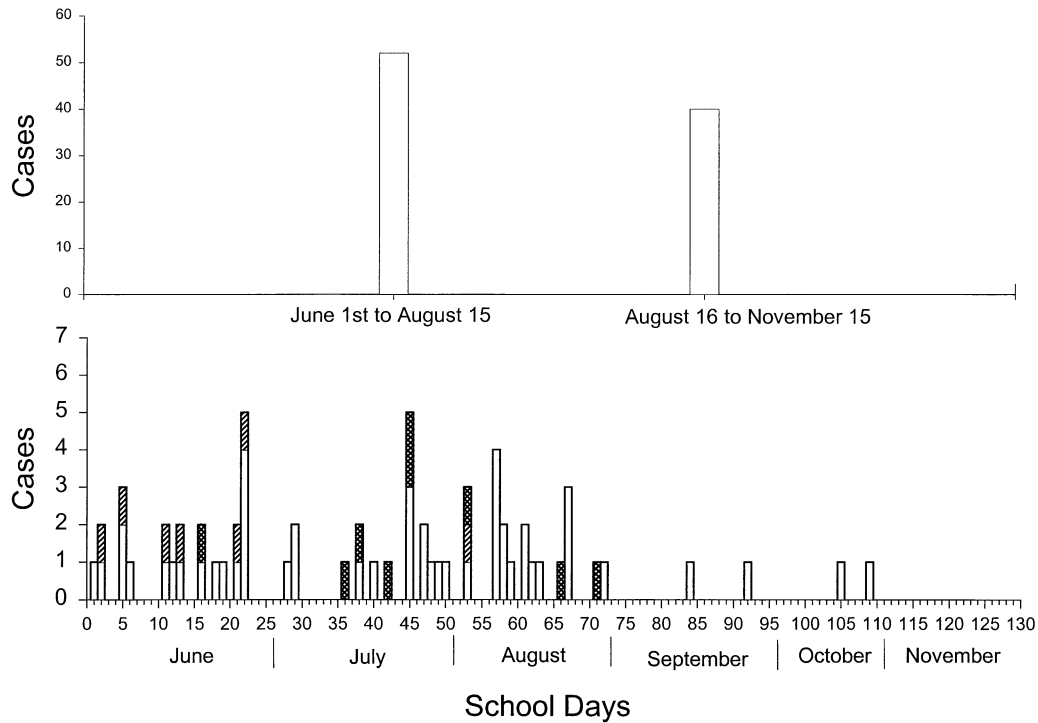


FIGURE 6. Numbers of students with acute inapparent dengue infection (top, white bars) and symptomatic acute dengue virus infection (bottom) during the 1999 surveillance season, Kamphaeng Phet, Thailand. Symptomatic acute dengue virus infection was further divided into symptomatic nonhospitalized acute dengue (white bars), hospitalized dengue fever (single-hatched bars), and hospitalized dengue hemorrhagic fever (double-hatched bars).

Symptom complex and predictive value of early symptoms to diagnose acute dengue virus infection. The symptoms of acute dengue and nondengue febrile illnesses were determined on the first day of school absence (table 3). Headache was the most common presenting symptom in children with acute dengue virus infection (64 percent), followed by cough, rhinorrhea, lethargy, anorexia, muscle pain, vomiting, and nausea. Joint pain, rash, and bleeding manifestations were uncommon on the first day of illness. The classic symptoms of dengue fever (headache, anorexia, lethargy, rash, myalgia or arthralgia) were observed infrequently in children on the first day of school absence (mean number of reported symptoms within this complex, 1.6). These findings did not differ from those for children with an acute nondengue illness (mean number of reported symptoms, 1.7; $p > 0.05$ by Student's t test). There was a trend toward an increased number of symptoms within the dengue fever symptom complex in children with more severe dengue disease, with a mean of 1.5 symptoms for children with nonhospitalized dengue fever, 1.8 symptoms for children with hospitalized dengue fever, and 2.2 symptoms for children with dengue hemorrhagic fever ($p > 0.05$ by ANOVA).

A characteristic that distinguished acute dengue virus infection from other febrile illnesses was the relative absence of common symptoms associated with childhood febrile illness. Headache, although the most common symptom reported for acute dengue infection, was reported less frequently than in children with other febrile illnesses (rela-

tive risk (RR) = 0.8, 95 percent confidence interval (CI): 0.7, 0.9; $p < 0.001$ by chi-square test). Similarly, rhinorrhea was reported less frequently for acute dengue compared with other febrile illnesses (RR = 0.6, 95 percent CI: 0.4, 0.8; $p < 0.001$ by chi-square test), as was cough (RR = 0.5, 95 percent CI: 0.3, 0.7; $p < 0.0001$ by chi-square test) and diarrhea (RR = 0.4, 95 percent CI: 0.1, 0.8; $p < 0.05$ by chi-square test). The presence of rash on the first school-absence day was an infrequently reported symptom for acute dengue virus infection (5 percent) but was more common for acute dengue than for other febrile illnesses (RR = 2.6, 95 percent CI: 1.01, 6.12; $p < 0.05$ by chi-square test).

DISCUSSION

Understanding the epidemiology of the entire spectrum of dengue virus infection has important public health implications for understanding virus transmission, determining disease burden during an outbreak, and controlling vectors. To our knowledge, our study is unique among other previously reported studies on dengue epidemiology because of its 1) long-term follow-up of a well-defined school cohort population, 2) ability to distinguish the entire spectrum of dengue virus infections, and 3) determination of spatial and temporal patterns associated with dengue virus transmission. Our results demonstrated the high burden of dengue virus transmission and dengue disease in children residing in northern Thailand. Dengue incidence showed marked spatial

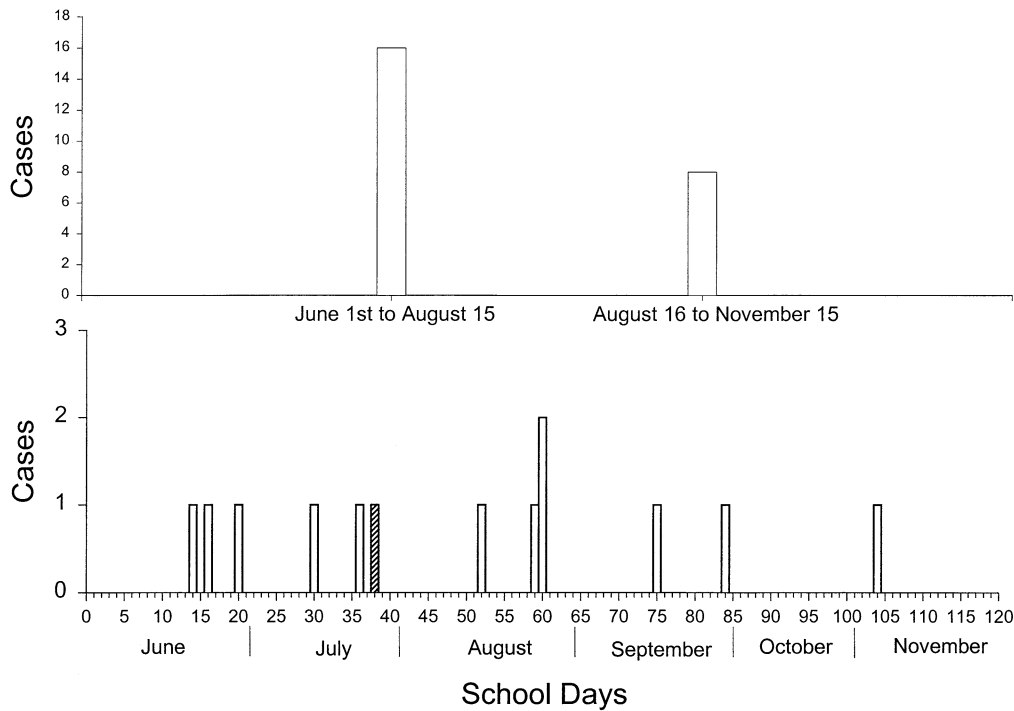


FIGURE 7. Numbers of students with acute inapparent dengue infection (top, white bars) and symptomatic acute dengue virus infection (bottom) during the 2000 surveillance season, Kamphaeng Phet, Thailand. Symptomatic acute dengue virus infection was further divided into symptomatic nonhospitalized acute dengue (white bars) and hospitalized dengue fever (hatched bar).

TABLE 3. Clinical presentation of symptomatic dengue infection in students in Kamphaeng Phet, Thailand, 1998–2000*

Symptom	Symptomatic dengue infection				Nondengue acute illness (<i>n</i> = 1,882) (% positive)
	Nonhospitalized (<i>n</i> = 122) (% positive)	Hospitalized dengue fever (<i>n</i> = 13) (% positive)	Hospitalized dengue hemorrhagic fever (<i>n</i> = 19) (% positive)	Total (<i>n</i> = 154) (% positive)	
Headache	73/119 (61)	9/13 (69)	14/19 (74)	96/151 (64)	1,442/1,872 (77)
Anorexia	25/120 (21)	5/13 (39)	8/19 (42)	38/152 (25)	475/1,855 (26)
Drowsiness/lethargy	39/119 (33)	4/13 (31)	6/19 (32)	49/151 (32)	534/1,862 (29)
Rash	4/118 (3.0)	2/13 (15)	1/19 (5.0)	7/150 (5.0)	35/1,855 (2.0)
Rhinorrhoea	44/119 (37)	3/13 (23)	6/19 (32)	53/151 (35)	923/1,866 (50)
Cough	51/118 (43)	7/13 (54)	6/19 (32)	64/150 (43)	1,159/1,872 (62)
Nausea	16/118 (14)	5/13 (39)	7/19 (37)	28/150 (19)	472/1,865 (25)
Vomiting	18/120 (15)	5/13 (39)	7/19 (37)	30/152 (20)	440/1,863 (24)
Abdominal pain	16/118 (14)	4/13 (31)	6/19 (32)	26/150 (17)	389/1,867 (21)
Diarrhea	4/119 (3.0)	1/13 (8.0)	1/19 (5.0)	6/151 (4.0)	92/1,860 (5.0)
Muscle pain	27/119 (23)	2/13 (15)	5/19 (26)	34/151 (23)	417/1,863 (22)
Joint pain	19/119 (16)	1/13 (8.0)	3/19 (16)	22/151 (15)	229/1,866 (12)
Bleeding	2/119 (2)	0/13 (0.0)	1/19 (5.0)	3/151 (2.0)	22/1,859 (1.0)
Dengue fever symptoms† (mean (standard deviation))	1.5 (1.4)	1.8 (1.4)	2.2 (1.8)	1.6 (1.4)	1.7 (1.3)

* All values represent the number of students reporting the presence of the symptom divided by the number of students completing the questionnaire for symptomatic acute dengue virus infection (columns 2–5) or for nondengue febrile illnesses (column 6).

† Number positive for the classic symptoms of dengue fever: headache, anorexia, lethargy, rash, myalgia or arthralgia.

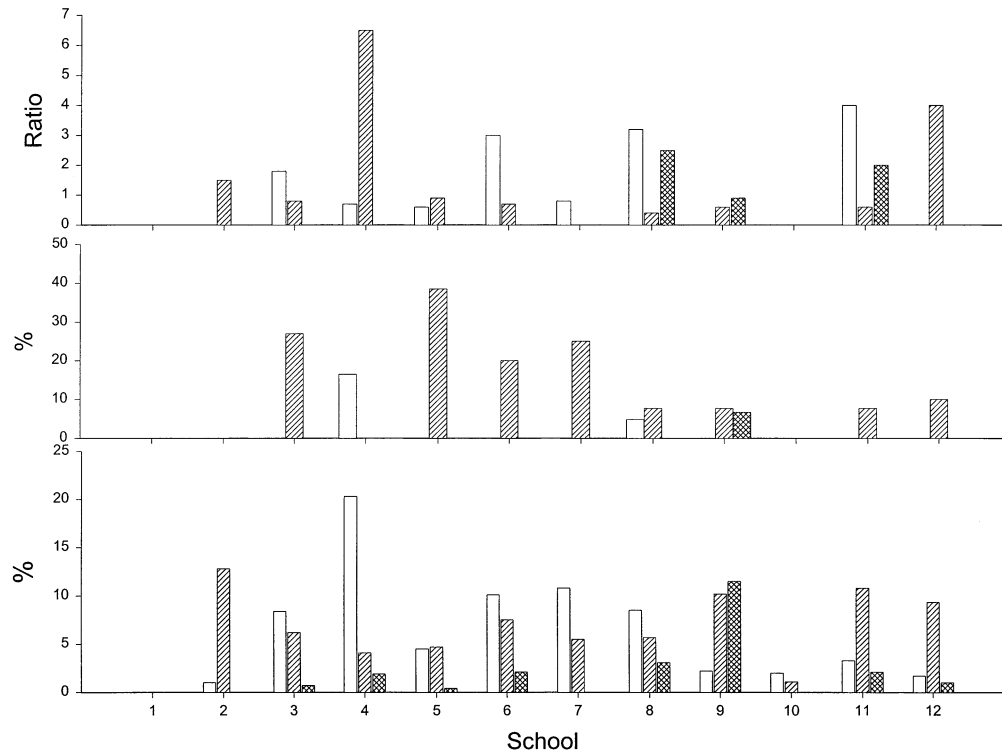


FIGURE 8. Dengue disease severity by school and study year, Kamphaeng Phet, Thailand. Data on surveillance months for 1998 (white bars), 1999 (single-hatched bars), and 2000 (double-hatched bars). Bottom: dengue incidence per 100 students; middle: hospitalizations per 100 dengue infections; top: ratio of the incidence of inapparent to symptomatic dengue. The absence of a bar for a given year indicates that no dengue cases were associated with that school (bottom), no hospitalizations were associated with dengue infection during that year (middle), or the ratio of inapparent to symptomatic dengue was either zero or infinity (top).

and temporal diversity within the school population; some schools experienced high attack rates, and schools in close proximity had little or no dengue virus infections. This finding illustrates the complexity of dengue virus transmission in a population and the potential factors that influence transmission and dengue disease, such as local environment, preexisting immunity, and vector burden. In our population, the incidence of symptomatic dengue infection underestimated the overall incidence of infection by approximately 50 percent.

Our results on the early symptoms of acute dengue infection suggest that, in its early manifestation, dengue is protean and difficult to distinguish from other febrile childhood illnesses in Thailand. Symptoms not usually associated with acute dengue infection, such as rhinorrhea and cough, were common, making acute dengue difficult to distinguish from other febrile childhood illnesses.

There is a paucity of data on the epidemiology of inapparent dengue disease and dengue disease severity. Burke et al. performed a prospective cohort study of 1,757 school students aged 4–16 years living in Bangkok, Thailand (15). These authors found an acute illness rate of 3.4 percent, a symptomatic dengue attack rate of 0.7 percent, and a subclinical infection incidence of 5.1 percent. The rate of total dengue virus infection was 5.9 percent, with the

majority (87 percent) either asymptomatic or minimally symptomatic.

In our study, inapparent and symptomatic secondary dengue infection occurred with nearly equal incidence and contrary to opinions that most dengue infections are silent (3). Our method of evaluating students on the first day of school absence may explain our higher-than-expected rate of identifying symptomatic infections. Other explanations for this observation may relate to viral and host determinants in our population, the time period during which our study was conducted, and the location of the study. Previous studies in Bangkok were conducted during a DEN-1 and DEN-2 outbreak (28). In our study, DEN-3 was the primary virus isolated (44 percent of all isolates) (29). The severity of disease observed in our study could be related to a number of factors, such as the dengue serotype circulating in the population, viral genetic factors associated with severe disease, and the host's preexisting immunity from a prior dengue virus infection to another serotype leading to antibody enhancement and cross-reactive memory T cells. This possibility would be consistent with previous observations of dengue epidemics in the Pacific Islands and in Taiwan, where certain dengue serotypes occurred as subclinical dengue infections and others were associated with major epidemics of severe disease (30–33).

Sabin and Siler first described acute dengue illness as being comprised of fever, headache, bone pain, muscle pain, and rash (34, 35). "Atypical" presentations of acute dengue illness were reported in studies conducted in Bangkok in 1962: 18 percent of hospitalized children were originally diagnosed with an upper respiratory infection, 5 percent with influenza, and 3 percent with bronchitis (10). Our results also demonstrate that children with acute dengue fever manifest without its classic features and that their illnesses can be difficult to distinguish from other childhood febrile illnesses. This finding is important for clinicians to realize, because early identification of dengue hemorrhagic fever and proper treatment with fluid management have been demonstrated to significantly reduce mortality (36).

Dengue is a global health concern, and no effective vaccine is available to prevent infection. Our findings increase understanding of this disease and provide an important model in which public health strategies can be devised to control virus transmission and test the safety and efficacy of a candidate dengue virus vaccine.

ACKNOWLEDGMENTS

This project and publication were made possible by National Institutes of Health grant 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.

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