Am. J. Trop. Med. Hyg., 68(2), 2003, pp. 191–202

SEROTYPE-SPECIFIC DENGUE VIRUS CIRCULATION AND DENGUE DISEASE IN BANGKOK, THAILAND FROM 1973 TO 1999

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Abstract. Dengue virus circulation and association with epidemics and severe dengue disease were studied in hospitalized children with suspected dengue at the Queen Sirikit National Institute of Child Health in Bangkok, Thailand, from 1973 to 1999. Dengue serology was performed on all patients and viral isolation attempted on laboratory-confirmed patients. Acute dengue was diagnosed in 15,569 children and virus isolated from 4,846. DEN-3 was the most frequent serotype in primary dengue (49% of all isolates), DEN-2 in secondary and in dengue hemorrhagic fever (37% and 35%, respectively). The predominant dengue serotype varied by year: DEN-1 from 1990–92, DEN-2 from 1973–86 and 1988–89; DEN-3 in 1987 and 1995–99; and DEN-4 from 1993–94. Only DEN-3 was associated with severe outbreak years. Our findings illustrate the uniqueness of each serotype in producing epidemics and severe disease and underscore the importance of long-term surveillance of dengue serotypes in understanding the epidemiology of these viruses.

INTRODUCTION

Dengue virus (DV) is the causative agent of dengue fever (DF) and dengue hemorrhagic fever (DHF) and consists of four distinct serotypes (DEN-1, DEN-2, DEN-3, and DEN-4).[1,2] DHF, the potentially fatal form of dengue virus infection, was first recognized in Bangkok, Thailand, in 1958.[3–5] Since then, dengue disease incidence has increased from 9/100,000 in 1958 to 189/100,000 in 1998, with the largest reported incidence of 325/100,000 in 1987.[6] Dengue has thus become a severe and intractable public health problem in Thailand.[7]

Bangkok is the epicenter of dengue in Thailand and the site where the original clinical descriptions of DHF and its association with immunologic priming by a previous dengue infection (secondary dengue infection) were made.[3,8–11] The Queen Sirikit National Institute of Child Health (QSNICH), formerly Bangkok Children's Hospital, has admitted the greatest number of dengue cases for 40 years because it is government-financed, accessible to all, and a widely recognized center of excellence. Since 1973, the QSNICH and the Armed Forces Research Institute of Medical Sciences have collaborated to conduct longitudinal surveillance of dengue in Bangkok. In this report, we present the results of that collaboration to illustrate the long-term circulation and associations with disease severity and large epidemics of the four dengue virus serotypes.

MATERIALS AND METHODS

Study design and sites. The QSNICH is located in Bangkok, Thailand, and is a Ministry of Public Health tertiary care facility with 538 beds. There is a hemorrhagic fever unit with 15–20 beds and a short-term observation unit with approximately 20 beds.

From 1973, children younger than 18 admitted with suspected dengue had acute and convalescent sera obtained for serology and virus isolation. The attending physician assigned DHF grade at the time of discharge using World Health Organization (WHO) criteria.[12] Statistical analysis was performed using SPSS for Windows version 10.0 (SPSS Inc.).

Moderate to severe annual dengue epidemics. The national annual dengue incidence surveillance was used to classify each year's epidemic as moderate, or severe[6] (Table 1). Dengue incidence per 100,000 was ranked into percentiles; the 50th was 71, the 75th was 134, and 90th was 175. Annual epidemics with an incidence ranging from the 75th to 90th percentile were classified as moderately severe, and those greater than the 90th percentile as severe. Accordingly, there were moderately severe epidemics in 1984, 1985, 1989, 1990, and 1997, and severe epidemics in 1987 and 1998.

Changes in laboratory methodology over time. Dengue and DHF cases were diagnosed by a number of different virus isolations and/or serologic methods from 1973 to 1999 (Table 2).

Serologic definitions of acute dengue virus infection. A case without dengue antibody in acute and convalescent sera or stable antibody titers without a 4-fold rise was classified as having no evidence of recent dengue virus infection. Acute

TABLE 1

Reported total dengue cases and incidence in Thailand, from 1973 to 1999

Year	Total Number of Cases	Incidence per 100,000	Year	Total Number of Cases	Incidence per 100,000
1973	8,280	21	1987†	174,285	325
1974	8,160	20	1988	26,926	49
1975	17,767	42	1989*	74,391	134
1976	9,616	22	1990*	92,005	163
1977	38,768	88	1991	43,511	77
1978	12,547	28	1992	41,125	71
1979	11,478	25	1993	67,017	115
1980	43,328	92	1994	51,688	88
1981	25,670	54	1995	60,330	102
1982	22,250	46	1996	37,929	63
1983	30,025	61	1997*	101,689	167
1984*	69,101	137	1998†	129,954	211
1985*	80,076	155	1999	24,826	40
1986	27,837	53			

* Moderately severe dengue year. †Severe dengue year. (See Methods for definition.)
Source: [6]

TABLE 2
Dengue diagnostic tests at armed forces research institute of medical sciences during study period

Methodology	Years Used During Study Period
Viral isolation and identification	
Direct viral plaque on LLC-MK2 cell line.*	1973 to 1977.
Mosquito inoculation in *Toxorhynchites splendens.*†	1977 to present.
Immunofluorescent antibody identification of serotypes.‡	1973 to 1983
Antigen capture enzyme immunoassay for serotype identification.§	1981 to present.
Nested serotype-specific polymerase chain reaction.‖	1995 to present.
Serologic characterization	
Hemagglutination inhibition assay.¶	1973 to present.
Plaque reduction neutralization test.**	1973 to present.
Fluorescent antibody assay.††	1973 1987.
IgM/IgG isotype-capture enzyme immunoassay.‡‡	1987 to present.

* [28], † [29], ‡ [30], § [31,32], ‖ [33], ¶ [34], ** [35,28], †† [36], ‡‡ [13].

primary or secondary dengue virus infection was based on previously published serologic criteria.[13] Acute dengue virus infections unclassifiable as primary or secondary were classified as indeterminate.

Statistical analysis. Statistical analysis was performed using SPSS for Windows version 10.0 (SPSS Inc.). Student's *t* test, analysis of variance, and Pearson's correlation or linear regression are used to determine differences or associations among continuous variables, and χ^2 or binomial test for proportions. Backward logistic regression was used to assess the independent influence of a number of factors on dengue disease severity.

RESULTS

From 1973 to 1999, there were 15,376 patients (773 infants and 14,603 older children) with laboratory-confirmed acute dengue virus infection. Infants represent a unique population at risk for severe dengue disease.[14,15] Therefore, their demographics and disease outcome are discussed separately, and unless stipulated, subsequent data refer to results from patients 12 months or older.

Specificity of clinical diagnosis. There was no correlation between year and the number of laboratory-confirmed acute dengue cases of all hospitalized suspected dengue, suggesting that changes over time were not due to changes in hospital admission criteria. There was a correlation between the reported national annual disease incidence and the specificity of diagnosis of children diagnosed as suspected dengue by clinical criteria in our series ($r = 0.424$, $P = 0.028$). This suggests that the clinical criteria for acute dengue infection were unchanged and that the likelihood of a child's having acute dengue depended on the incidence of disease in the population.

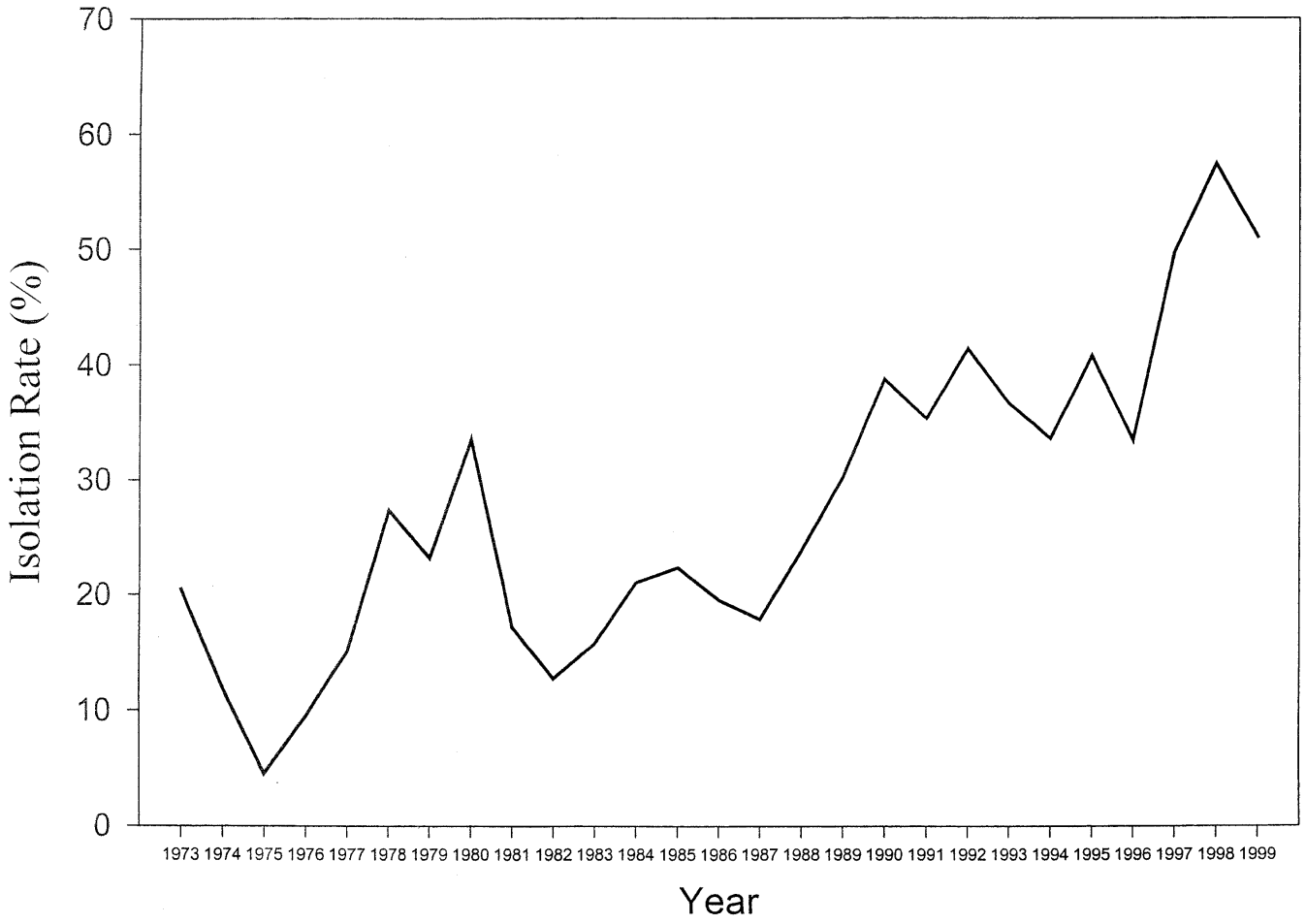

FIGURE 1. Viral isolation rate of serologically confirmed acute dengue infections during study period.

Dengue virus isolation rates. The dengue virus isolation rate was calculated as the number of isolates divided by the number of dengue cases. No more than one dengue serotype was recovered per patient, in contrast to other reports.[16] The overall isolation rate in children was 33% and increased during the study period ($\beta = 0.84$, $P < 0.0001$ by linear regression) from 21% in 1973 to a peak of 57% in 1998 (Figure 1).

Annual and seasonal occurrence of dengue and dengue serotypes. There was a strong correlation between the total number of QSNICH dengue cases and the dengue incidence in the metropolitan Bangkok area ($r = 0.90$, $P < 0.0001$, Figure 2) and the nation ($r = 0.852$, $P < 0.0001$).[6]

Dengue in Bangkok peaks during the monsoon months of June to October (Figure 3). Before 1979, few dengue cases occurred during the cool, dry winter months of December and January. Since 1979, dengue cases have occurred year-round. From 1973 to 1986, DEN-2 was the dominant dengue isolate. In 1987, for the first time, DEN-3 became the most frequent isolate. DEN-2 regained predominance in 1988–89, followed by DEN-1 in 1990–92, DEN-4 in 1993–94, and DEN-3 in 1995–9. Each dengue serotype appears to have caused several clearly distinguishable outbreaks at 7- to 9-year intervals.

Patient demographics. The mean age of patients with dengue was 7.6 years (range 1.1 to 17 years, SD 3.4 years). Mean age for any year varied less than ½ SD from the overall mean, with a small increase in mean age over time ($\beta = 0.49$, $P = 0.01$ by linear regression). The overall modal age was 6 years, which also increased over time from 5 years during 1973–79 to 8 years during 1990–99 ($\beta = 0.50$, $P = 0.008$ by linear regression). There were proportionately more males (52%) than females (48%) ($P < 0.001$ by binomial test).

Antibody response patterns. Of all dengue virus infections, 16% were primary dengue infection (Table 3). The proportion with primary dengue increased over time with a 3- to 4-year cyclical oscillation in the rate of primary dengue (Figure 4) ($\beta = 0.67$ by linear regression, $P < 0.0001$). The mean age of children with primary dengue was 6.6 years (SD 3.5) compared with 7.8 years (SD 3.3) for secondary dengue ($P < 0.001$, 2-tailed Student's t test).

Dengue virus serotypes by antibody patterns. DEN-3 was the most frequent isolate in primary dengue (49%), followed by DEN-1 (44%), DEN-2 (5%), and DEN-4 (2%) (Table 3). There was a correlation between the yearly proportion of primary dengue cases and the isolation of DEN-1 ($R = 0.5$, $P = 0.0007$ by Pearson correlation) but no other type, suggesting that Bangkok DEN-1 strains were especially pathogenic among children without immune priming from prior flavivirus infection.

DEN-2 was the most frequent isolate in secondary dengue

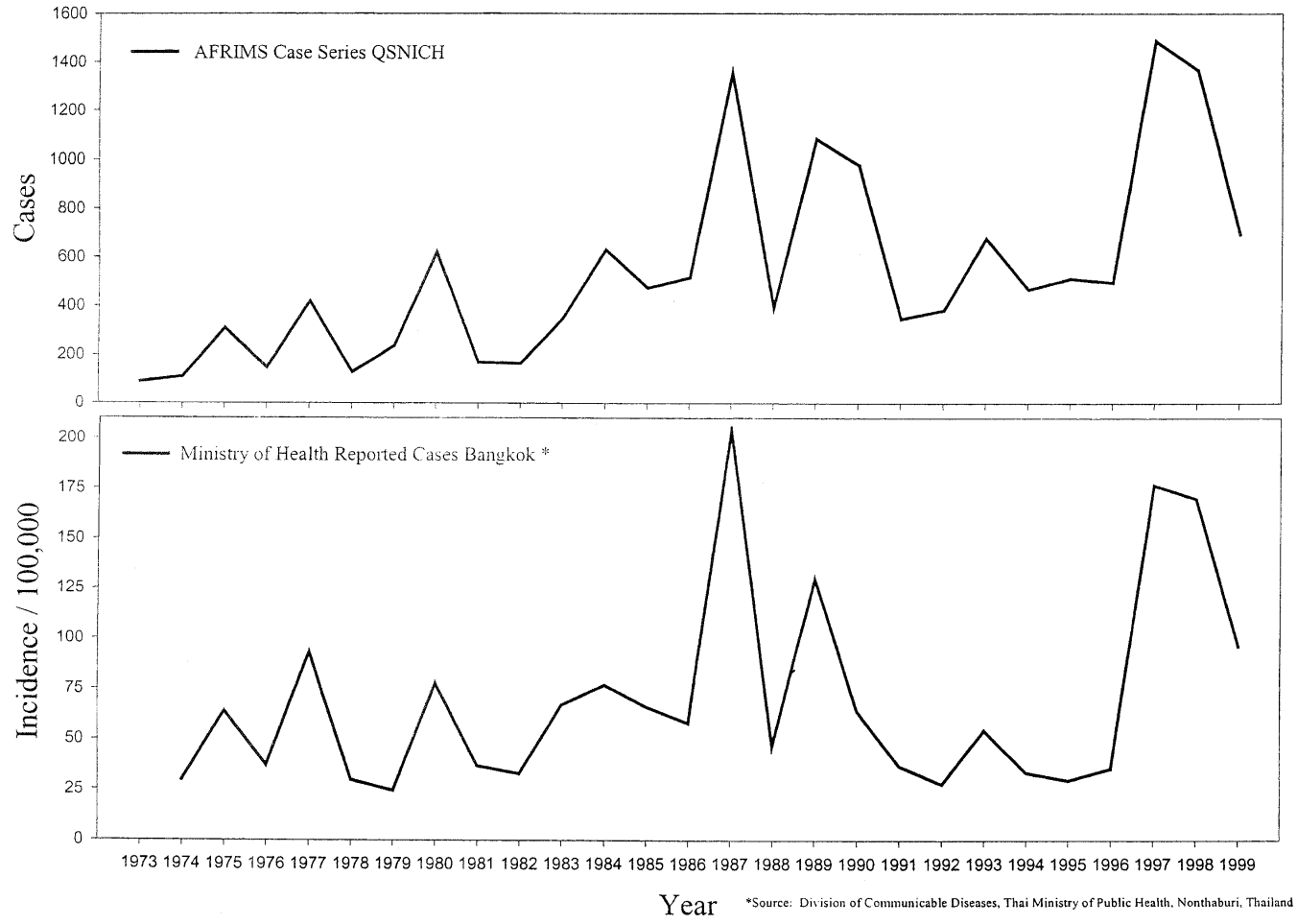

FIGURE 2. Armed Forces Research Institued of Medical Sciences serologically confirmed dengue cases and ministry of public health reported incidence for Bangkok, Thailand

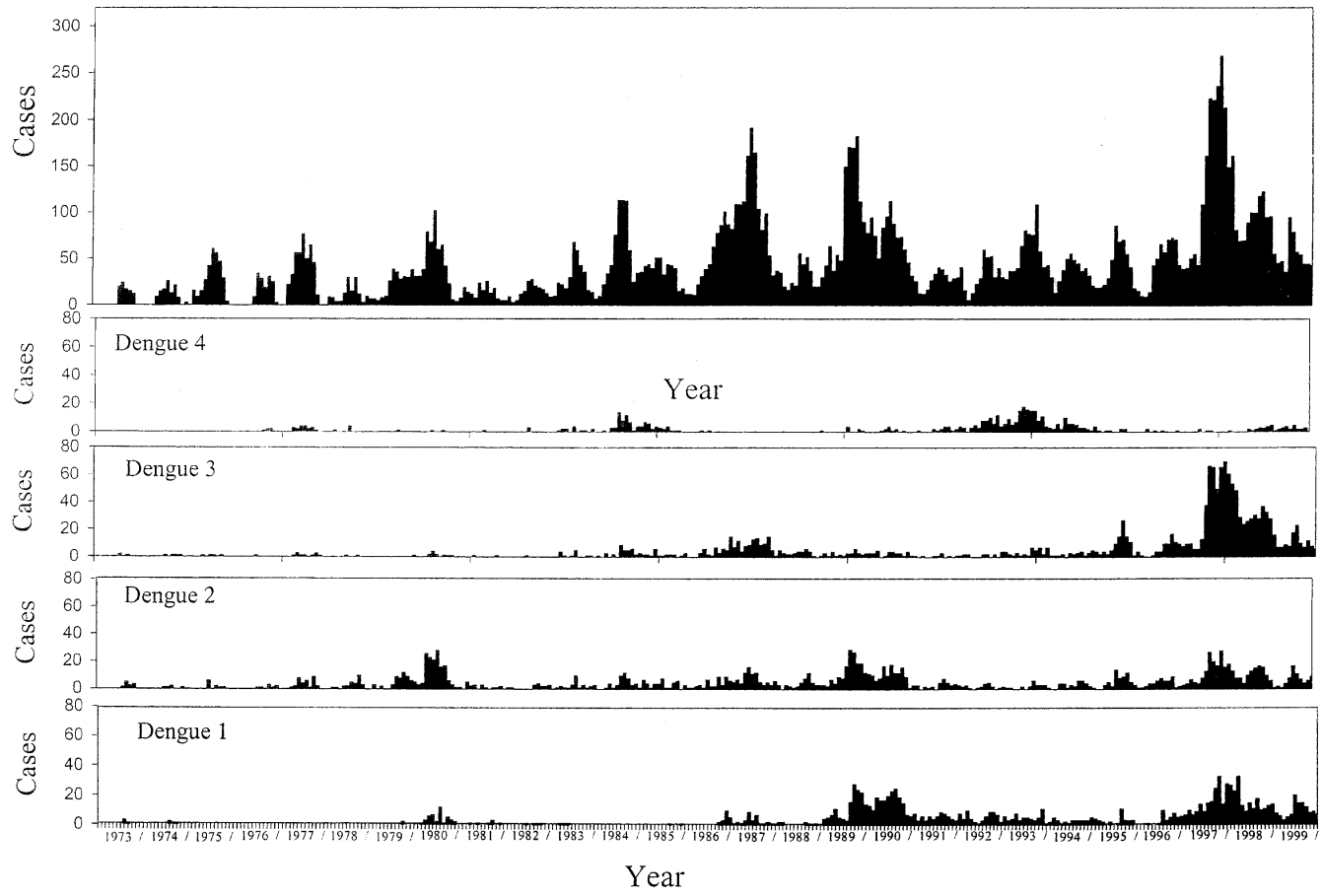

FIGURE 3. Seasonal occurrence of serologically condirmed dengue virus infection and dengue serotypes at the Queen Sirikit National Institute for Child Health from 1973–1999.

(37%) followed by DEN-3 (29%), DEN-1 (22%), and DEN-4 (12%). There was a correlation between the yearly proportion of secondary dengue cases and the isolation of DEN-2 ($R = 0.5$, $P = 0.004$ by Pearson correlation) but no other type, suggesting that Bangkok DEN-2 strains were more pathogenic among children with immune priming from prior flavivirus infection. Overall, DEN-4 was isolated more often from secondary dengue cases (12%) than primary (2%) ($P < 0.0001$, χ^2). DEN-3 was the most frequent isolate among combined primary and secondary cases, suggesting that Bangkok strains combined pathogenicity attributes of DEN-1 and DEN-2.

Factors associated with severe dengue outbreak years. DEN-1, DEN-2, or DEN-3 was the dominant isolate during moderately severe dengue outbreak years. In contrast, only DEN-3 was the dominant isolate in the two severe outbreaks. These data further suggest that Bangkok dengue strains have differing ability to elicit severe disease and that DEN-3 has been the most successful.

Dengue antibody pattern and dengue disease severity. There were 2,396 children (16%) hospitalized with a diagnosis of DF and 11,731 (80%) with DHF (Table 4). Of those with DHF, 16% were diagnosed with DHF grade I, 32% with DHF grade II, 45% with DHF grade III and 7% with DHF grade IV.

DHF was more likely to result from a secondary than a primary infection (odds ratio, 5.0; 95% confidence interval 4.5–5.6). DHF resulting from primary infection was milder: grade I (30% of all primary DHF), grade II (47%), grade III (22%), and grade IV (1.1%) ($P < 0.0001$ by χ^2 for linear trend). Secondary dengue DHF was associated with more-severe DHF: grade I (15% of all secondary DHF), grade II (31%), grade III (47%), and grade IV (7%) ($P < 0.0001$ by χ^2 for linear trend). The proportion of DHF due to primary infection varied yearly from a low of 1% in 1978 to a high of 14% in 1997 and has increased over time ($\beta = 0.61$, $P = 0.001$ by linear regression).

Dengue serotypes and dengue disease severity. The frequency of isolated dengue serotypes was examined by clinical severity (Table 4). DEN-3 was the most frequent isolate in DF patients (39% of all DF viral isolates) followed by DEN-1 (35%), DEN-2 (17%), and DEN-4 (9%). The frequency of isolated dengue serotypes differed between primary and secondary DF. DEN-3 and DEN-1 were the most frequent isolated dengue serotypes in primary DF (46% each of all primary DF viral isolates) followed by DEN-2 (6%), and DEN-4 (2%). DEN-3 was the most frequent isolated dengue serotype in secondary DF (34% of all secondary DF viral isolates) followed by DEN-1 (29%), DEN-2 (24%), and DEN-4 (13%).

Among DHF cases in children, DEN-2 was the most frequent isolate (35% of all DHF viral isolates) followed by DEN-3 (31%), DEN-1 (24%), and DEN-4 (10%). The frequency of isolated dengue serotypes differed between pri-

TABLE 3

Summary of the yearly occurrence of dengue serotypes by primary or secondary dengue infection in children

Year	Suspected Dengue Cases	Confirmed Dengue by Serology				Viral Isolation by Serotype															
						DEN-1				DEN-2				DEN-3				DEN-4			
		1°	2°	Indeterminate	Total	1°	2°	Ind.	Total	1°	2°	Ind.	Total	1°	2°	Ind.	Total	1°	2°	Ind.	Total
1973	131	10	77	1	88	2	1	1	4	2	9	0	**11**	2	1	0	3	0	0	0	0
1974	142	13	95	1	109	3	1	0	4	0	4	1	**5**	3	1	0	4	0	0	0	0
1975	385	23	285	1	309	0	0	0	0	0	9	1	**10**	3	1	0	4	0	0	0	0
1976	171	6	140	1	147	0	0	0	0	0	8	0	**8**	1	0	0	1	0	4	1	5
1977	486	20	392	7	419	0	0	0	0	1	28	4	**33**	3	8	0	11	2	14	3	19
1978	174	7	115	6	128	0	0	0	0	1	22	5	**28**	0	2	0	2	0	4	1	5
1979	281	7	227	3	237	0	1	1	2	1	49	2	**52**	0	0	0	0	0	1	0	1
1980	717	59	543	19	621	20	19	3	42	7	137	9	**153**	2	4	5	11	0	0	2	2
1981	184	10	158	1	169	4	3	0	7	0	18	1	**19**	0	2	0	2	0	1	0	1
1982	185	16	146	3	165	2	1	1	4	0	11	2	**13**	1	0	0	1	0	3	0	3
1983	380	9	337	2	348	1	2	0	3	0	27	1	**28**	4	8	1	13	0	11	0	11
1984	707	27	590	14	631	0	0	0	0	3	42	4	**49**	8	19	6	33	2	45	4	51
1985	531	18	452	4	474	0	2	0	2	0	42	0	**42**	5	18	0	23	3	32	4	39
1986	577	26	482	9	517	0	17	3	20	2	40	1	**43**	7	24	5	36	0	2	0	2
1987	1457	106	1248	0	1354	14	18	0	32	5	84	0	89	25	96	0	**121**	0	0	0	0
1988	477	41	352	1	394	4	5	0	9	1	48	1	**50**	6	29	0	35	0	0	0	0
1989	1190	134	933	15	1082	47	90	6	143	5	136	6	**147**	8	19	3	30	1	6	0	7
1990	1143	145	831	0	976	75	116	0	**191**	3	138	0	141	10	24	0	34	0	12	0	12
1991	538	59	287	0	346	30	36	0	**66**	0	33	0	33	4	5	0	9	0	14	0	14
1992	471	47	335	0	382	21	44	0	**65**	1	26	0	27	5	8	0	13	2	51	0	53
1993	774	62	614	1	677	21	36	1	58	1	20	0	21	14	25	0	39	2	128	0	**130**
1994	584	47	409	12	468	5	23	6	34	2	30	2	34	8	18	2	28	2	57	2	**61**
1995	624	85	419	9	513	7	24	2	33	1	60	0	61	23	71	7	**101**	0	14	0	14
1996	631	85	412	0	497	8	31	0	39	0	51	0	51	20	52	0	**72**	0	4	0	4
1997	1690	287	1170	31	1488	44	131	12	187	1	133	4	138	118	277	15	**410**	1	4	0	4
1998	1668	213	1110	45	1368	36	152	9	197	1	141	6	148	96	303	29	**428**	0	12	0	12
1999	1079	115	560	21	696	22	89	4	115	2	72	2	76	30	91	10	**131**	0	28	5	33
Total	17377	1677	12719	207	14603	366	842	49	1257	40	1418	52	1510	406	1106	83	**1595**	15	447	22	484

* Bold in years indicates moderate to severe dengue hemorrhagic fever years based on national reported rates of dengue. (See Methods.) Bold in serotypes indicates the predominant isolated serotype for that year.

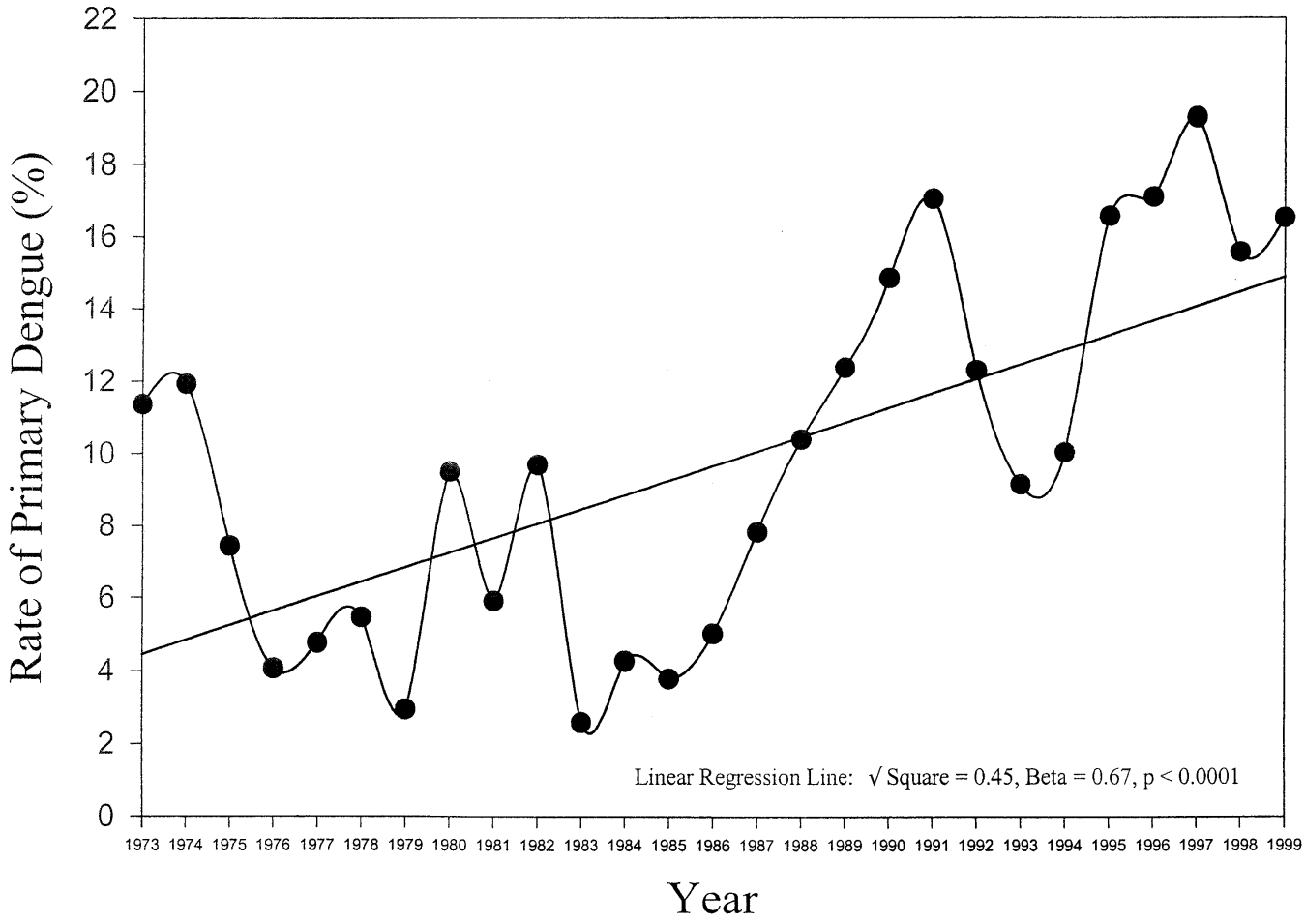

FIGURE 4. Percentage of primary dengue of all diagnosed dengue cases by year

mary and secondary DHF. DEN-3 was the most frequently isolated dengue serotype in primary DHF (50% of all primary DHF viral isolates) followed by DEN-1 (44%), DEN-2 (4%), and DEN-4 (2%). DEN-2 was the most frequently isolated dengue serotype in secondary DHF (40% of all secondary DHF viral isolates) followed by DEN-3 (28%), DEN-1 (20%), and DEN-4 (12%).

To eliminate the interactions among serologic status, severe dengue years, and dengue serotypes on the presence of DHF, backward logistic regression analysis was used to assess the independent influence of each variable. Secondary dengue (log odds ratio [LOR] 3.8, 95% confidence interval [C.I.] 2.8, 5.1) and dengue serotype (DEN-1 [LOR 1.7, C.I. 1.5, 1.9], DEN-3 [LOR 1.5, C.I. 1.3, 1.7], and DEN-4 [LOR 1.5, C.I. 1.2, 1.9]) each independently contributed to the presence of DHF when the other factors were controlled. Severe dengue year independently contributed to the absence of DHF (LOR 0.8, C.I 0.8, 0.9) and DEN-2 did not independently contribute to DHF (LOR 0.8, 0.7, 1.0).

The overall case-fatality for all years was 0.5%. The peak case-fatality rate was 1.6% during the 1987 severe outbreak. Overall, there was a gradual but not significant decline in case-fatality, with a rate of 0.2% during the last 5 years of the study ($\beta = -0.19$, $P = 0.33$ by linear regression). Case-fatality for primary and secondary dengue did not differ (0.6% and 0.4% respectively, $P = 0.27$ by χ^2) and was similar also among serotypes.

Dengue in infants. From 1973 to 1999, there were a total of 773 infants with acute dengue virus infection (Table 5). The mean age was 7 months (range 1–12 months, SD 2.4 months) with a modal age of 6 months and an equal representation of males and females (51% and 49%, respectively). The peak number of cases occurred from 6–8 months of age (Figure 5). The total case-fatality among infants with confirmed acute dengue was 11/773 (1.4%) and was greater than the 0.5% case-fatality for all years in children (odds ratio 3.1, 1.5<OR<6.1, $P < 0.0001$). No trend was noted between year and case-fatality, with only 8 years having deaths from acute dengue.

Dengue virus was isolated in 384 infants (total viral isolation rate of 50%), with DEN-2 the most frequently isolated virus (37% of all isolates) followed by DEN-3 (33%), DEN-1 (26%), and DEN-4 (4%). DEN-1 was the most frequent isolate in DF (41% of DF viral isolates) followed by DEN-2 (28%), DEN-3 (28%), and DEN-4 (3%). DEN-2 was the most frequent isolate in DHF (38% of DHF viral isolates) followed by DEN-3 (32%), DEN-1 (26%) and DEN-4 (4%). Among infants with fatal dengue infection, all four serotypes were isolated: DEN-2 (nine cases), DEN-1 (six), DEN-3 (five), and DEN-4 (three).

TABLE 4
Summary of Dengue Fever and Dengue Hemorrhagic Fever by Year and Dengue Serotype from 1973–1999

Year and dengue grade	Confirmed dengue by serology				Viral Isolation by Serotype															
					DEN-1				DEN-2				DEN-3				DEN-4			
	1°	2°	Ind.	Total	1°	2°	Ind.	Total	1°	2°	Ind.	Total	1°	2°	Ind.	Total	1°	2°	Ind.	Total
1973																				
DF*	2	0	0	2	1	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0
DHF†	7	77	1	85	1	1	1	3	2	9	0	11	1	1	0	2	0	0	0	0
Fatal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unclass.	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1974																				
DF	–	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DHF	6	63	1	70	1	1	0	2	0	4	1	5	2	1	0	3	0	0	0	0
Fatal	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Unclass.	6	31	0	37	1	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0
1975																				
DF	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DHF	20	199	1	220	0	0	0	0	0	9	1	10	3	1	0	4	0	0	0	0
Fatal	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unclass.	3	83	0	86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1976																				
DF	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DHF	4	119	1	124	0	0	0	0	0	8	0	8	1	0	0	1	0	4	1	5
Fatal	1	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unclass.	1	20	0	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1977																				
DF	9	28	1	38	0	0	0	0	0	0	0	0	0	0	0	0	1	3	1	5
DHF	11	364	6	381	0	0	0	0	1	28	4	33	3	8	0	11	1	11	2	14
Fatal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unclass.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1978																				
DF	6	3	3	12	0	0	0	0	0	0	3	3	0	0	0	0	0	0	0	0
DHF	1	112	3	116	0	0	0	0	1	22	2	25	0	2	0	2	0	4	1	5
Fatal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unclass.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1979																				
DF	3	6	1	10	0	0	1	1	1	1	0	2	0	0	0	0	0	0	0	0
DHF	4	219	2	225	0	1	0	1	0	48	2	50	0	0	0	0	0	1	0	1
Fatal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unclass.	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
1980																				
DF	26	38	3	67	7	3	0	10	4	9	3	16	0	0	0	0	0	0	0	0
DHF	33	502	16	551	13	16	3	32	3	126	6	135	2	4	5	11	0	0	2	2
Fatal	0	2	0	2	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Unclass.	0	1	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
1981																				
DF	0	10	0	10	0	1	0	1	0	2	0	2	0	0	0	0	0	0	0	0
DHF	10	146	1	157	4	2	0	6	0	16	1	17	0	2	0	2	0	1	0	1
Fatal	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unclass.	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1982																				
DF	12	21	1	34	2	0	1	3	0	1	0	1	1	0	0	1	0	1	0	1
DHF	4	125	2	131	0	1	0	1	0	10	2	12	0	0	0	0	0	2	0	2
Fatal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unclass.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1983																				
DF	3	34	1	38	1	1	0	2	0	2	0	2	0	2	1	3	0	2	0	2
DHF	5	301	1	307	0	1	0	1	0	25	1	26	3	6	0	9	0	9	0	9
Fatal	1	2	0	3	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
Unclass.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1984																				
DF	7	51	3	61	0	0	0	0	1	7	0	8	3	4	1	8	1	6	2	9
DHF	19	536	10	565	0	0	0	0	2	35	3	40	4	15	5	24	1	38	2	41
Fatal	1	3	1	5	0	0	0	0	0	0	1	1	1	0	0	1	0	1	0	1
Unclass.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1985																				
DF	8	46	1	55	0	0	0	0	0	5	0	5	1	3	0	4	2	3	1	6
DHF	9	404	2	415	0	2	0	2	0	37	0	37	4	15	0	19	1	29	2	32
Fatal	1	2	1	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Unclass.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Dengue fever
† Dengue hemorrhagic fever

TABLE 4
Continued

Year and dengue grade	Confirmed dengue by serology				Viral Isolation by Serotype															
					DEN-1				DEN-2				DEN-3				DEN-4			
	1°	2°	Ind.	Total	1°	2°	Ind.	Total	1°	2°	Ind.	Total	1°	2°	Ind.	Total	1°	2°	Ind.	Total
1986																				
DF	9	143	5	157	0	8	2	10	1	11	0	12	2	5	3	10	0	1	0	1
DHF	17	336	4	357	0	9	1	10	1	29	1	31	5	19	2	26	0	1	0	1
Fatal	0	3	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unclass.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1987																				
DF	44	94	0	138	7	5	0	12	3	7	0	10	10	16	0	26	0	0	0	0
DHF	59	1136	0	1195	7	12	0	19	2	75	0	77	13	80	0	93	0	0	0	0
Fatal	3	18	0	21	0	1	0	1	0	2	0	2	2	0	0	2	0	0	0	0
Unclass.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1988																				
DF	19	54	0	73	1	3	0	4	0	9	0	9	2	7	0	9	0	0	0	0
DHF	21	296	0	317	3	2	0	5	1	39	0	40	4	22	0	26	0	0	0	0
Fatal	1	2	1	4	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Unclass.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1989																				
DF	65	107	3	175	24	19	1	44	3	11	0	14	4	4	2	10	1	3	0	4
DHF	68	812	12	892	22	67	5	94	2	121	6	129	4	15	1	20	0	3	0	3
Fatal	1	3	0	4	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0
Unclass.	0	11	0	11	0	4	0	4	0	3	0	3	0	0	0	0	0	0	0	0
1990																				
DF	68	132	0	200	35	31	0	66	2	17	0	19	4	8	0	12	0	4	0	4
DHF	76	695	0	771	39	85	0	124	1	121	0	122	6	16	0	22	0	6	0	6
Fatal	0	3	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Unclass.	1	1	0	2	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1
1991																				
DF	25	29	0	54	11	7	0	18	0	1	0	1	2	2	0	4	0	2	0	2
DHF	31	243	0	274	18	28	0	46	0	31	0	31	2	3	0	5	0	11	0	11
Fatal	0	3	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unclass.	3	12	0	15	1	1	0	2	0	1	0	1	0	0	0	0	0	1	0	1
1992																				
DF	26	77	0	103	11	13	0	24	0	4	0	4	4	2	0	6	1	19	0	20
DHF	21	254	0	275	10	31	0	41	1	20	0	21	1	6	0	7	1	32	0	33
Fatal	0	1	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Unclass.	0	3	0	3	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
1993																				
DF	22	79	1	102	11	9	1	21	0	5	0	5	3	7	0	10	0	18	0	18
DHF	39	527	0	566	10	27	0	37	1	15	0	16	11	18	0	29	2	110	0	112
Fatal	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unclass.	1	7	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1994																				
DF	18	51	7	76	4	2	4	10	1	4	2	7	6	2	0	8	0	9	1	10
DHF	29	347	5	381	1	21	2	24	1	26	0	27	2	16	2	20	2	47	1	50
Fatal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unclass.	0	11	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
1995																				
DF	45	91	8	144	5	5	2	12	1	8	0	9	12	16	6	34	0	1	0	1
DHF	36	313	1	350	2	19	0	21	0	48	0	48	11	53	1	65	0	13	0	13
Fatal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unclass.	4	15	0	19	0	0	0	0	0	4	0	4	0	0	0	0	0	0	0	0
1996																				
DF	49	79	0	128	2	7	0	9	0	5	0	5	15	10	0	25	0	1	0	1
DHF	34	307	0	341	6	23	0	29	0	40	0	40	5	39	0	44	0	3	0	3
Fatal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unclass.	2	26	0	28	0	1	0	1	0	6	0	6	0	3	0	3	0	0	0	0
1997																				
DF	108	188	12	308	17	24	4	45	0	12	0	12	46	56	8	110	0	1	0	1
DHF	153	904	12	1069	23	94	4	121	1	112	3	116	60	205	5	270	1	2	0	3
Fatal	0	4	1	5	0	3	0	3	0	1	1	2	0	0	0	0	0	0	0	0
Unclass.	26	74	6	106	4	10	4	18	0	8	0	8	12	16	2	30	0	1	0	1
1998																				
DF	61	154	18	233	5	21	2	28	1	15	2	18	27	35	14	76	0	2	0	2
DHF	149	927	24	1100	31	128	6	165	0	119	4	123	68	261	13	342	0	10	0	10
Fatal	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unclass.	3	27	3	33	0	3	1	4	0	7	0	7	1	7	2	10	0	0	0	0

TABLE 4
Continued

Year and dengue grade	Confirmed dengue by serology				Viral Isolation by Serotype															
					DEN-1				DEN-2				DEN-3				DEN-4			
	1°	2°	Ind.	Total	1°	2°	Ind.	Total	1°	2°	Ind.	Total	1°	2°	Ind.	Total	1°	2°	Ind.	Total
1999																				
DF	61	104	10	175	12	18	2	32	2	9	1	12	15	21	4	40	0	5	3	8
DHF	52	437	7	496	10	68	2	80	0	61	1	62	15	66	2	83	0	22	2	24
Fatal	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
Unclass.	2	19	3	24	0	3	0	3	0	2	0	2	0	4	3	7	0	1	0	1
All Years																				
DF	696	1622	78	2396	156	177	20	353	20	145	11	176	158	200	39	397	6	81	8	95
DHF I	272	1608	29	1909	86	184	9	279	4	168	10	182	64	193	8	265	1	69	2	72
DHF II	434	3260	57	3751	85	287	13	385	7	322	14	343	122	350	23	495	5	146	6	157
DHF III	201	5045	25	5271	30	154	2	186	7	644	14	665	42	290	4	336	2	134	5	141
DHF IV	11	788	1	800	0	14	0	14	2	100	0	102	2	41	1	44	1	10	0	11
DHF Total	918	10701	112	11731	201	639	24	864	20	1234	38	1292	230	874	36	1140	9	359	13	381
Fatal	10	52	5	67	2	4	0	6	0	6	3	9	4	0	1	5	0	2	1	3
Unclassified	53	344	12	409	7	22	5	34	0	33	0	33	14	32	7	53	0	5	0	5

DISCUSSION

Based on our results, we have demonstrated the following: All four dengue serotypes circulate continuously in Thailand, with one serotype emerging as the cause of each periodic moderate-to-severe epidemic. Each dengue virus serotype has characteristics that influence the nature of dengue epidemic and disease severity. DEN-1, 2, and 3 are associated with moderately severe dengue epidemic years but only DEN-3 with severe dengue years, suggesting that DEN-3 may possess viral characteristics different from those of the other dengue serotypes that result in large epidemics. DEN-2 and DEN-3 are associated with severe dengue disease, DHF, whereas DEN-4 is found primarily in secondary dengue. DEN-4, unlike the other dengue serotypes, may require preexisting heterotypic dengue antibody for replication or to produce clinically apparent disease. Severe dengue disease in our series results from a combination of several independent contributing factors including serologic status, severe outbreak years, and the circulating dengue serotype.

Our findings extend the early observations of dengue transmission in Thailand and illustrate the constancy in dengue disease severity and serotypes over time despite an increase in overall disease incidence and virus transmission. In 1962, all four dengue serotypes were isolated in the Bangkok area and associated with the occurrence of severe dengue disease.[17–19] Our observations extend these observations by demonstrating the dramatic periodic shifts in the occurrence of the predominant dengue serotype over time.

Multiple factors influence dengue transmission, the cycle of epidemics, and the emergence of new dominant serotypes. Vector density and infection rates reflect access to breeding sources and environmental temperature changes due to urbanization and the El Nino phase of the Southern Oscillation.[20–23] Herd susceptibility/immunity and other intrinsic population characteristics are additional factors that presumably influence the emergence of new serotypes and drive high disease rates that create epidemics.[24,25]

The finding that the yearly proportion of primary dengue cases is correlated to the isolation of DEN-1 and no other dengue serotype suggests that Bangkok DEN-1 strains were more pathogenic among children without immune priming from prior flavivirus infection. The occurrence of more DEN-1 over time may in part explain why the proportion of hospitalized children with primary dengue has increased in our population. Our findings with DEN-1 illustrate the fact that each serotype has unique characteristics manifested in how the disease presents in the population.

Infants represent a unique susceptible population for severe dengue disease. The study of dengue in this group has

TABLE 5
Summary of Dengue Fever, Dengue Hemorrhagic Fever, and Dengue Serotypes Among Infants for All Years

Disease severity	# Viral isolates/total dengue	% of total viral isolation by serotype			
		DEN-1	DEN-2	DEN-3	DEN-4
DF*	32/77	41	28	28	3
DHF† I	67/133	19	39	36	6
DHF II	168/363	28	37	30	5
DHF III	82/141	26	39	33	2
DHF IV	12/23	25	42	33	0
DHF Total	329/660	26	38	32	4
Fatal	9/11	0	67	33	0
Unclassified	14/25	29	14	50	7
Total	384/773	26	37	33	4

* Dengue fever.
† Dengue hemorrhagic fever.

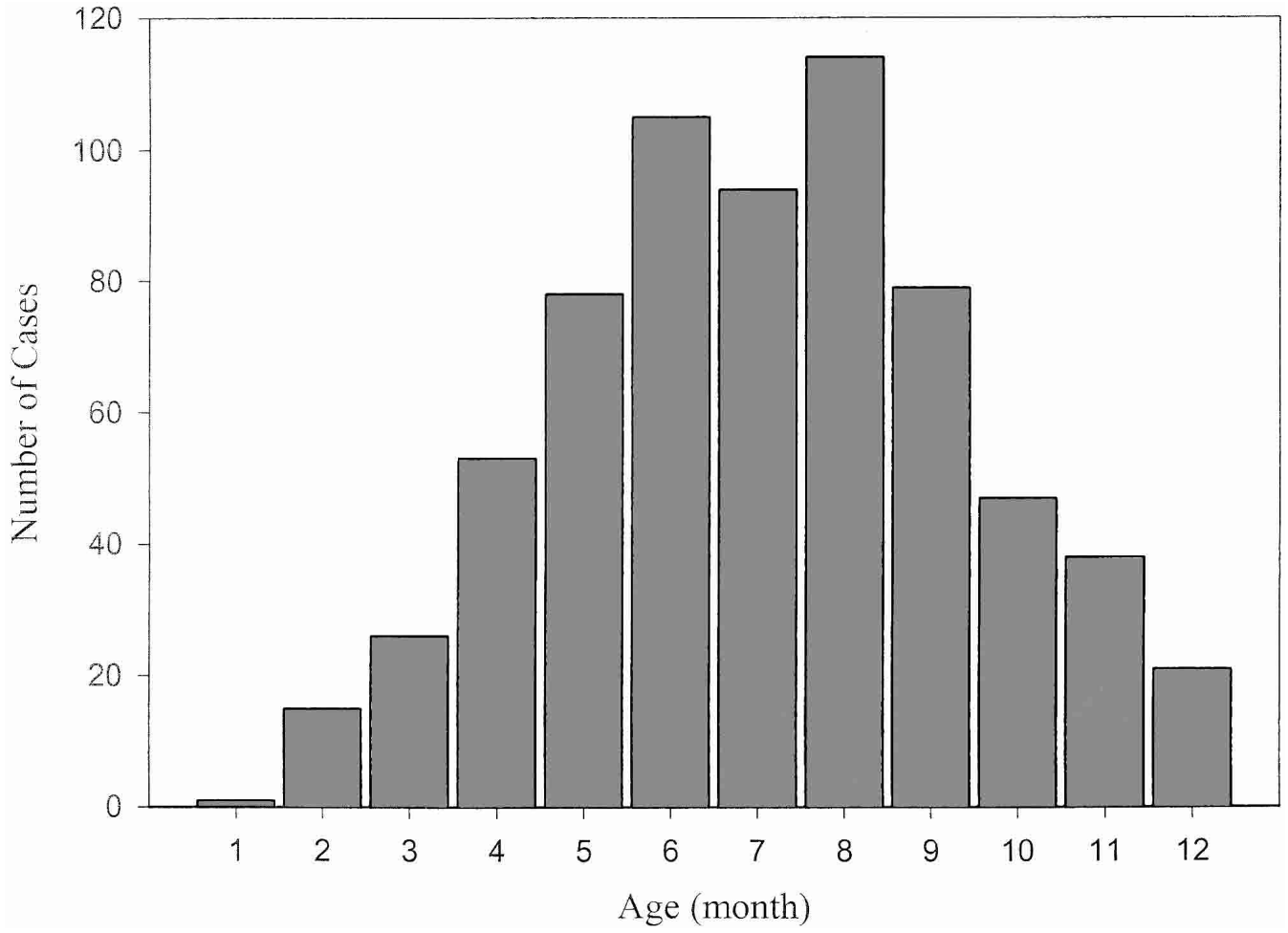

FIGURE 5. Age distribution of infants with dengue hemorrhagic fever and fatal dengue

led to many of our current theories on dengue pathogenesis, maternal antibody, and dengue antibody enhancement.[14,15,26,27] Our series demonstrated that case-fatality among infants with confirmed acute dengue was three times greater than that of other children. This may reflect the susceptibility of this population toward complications that arise from plasma leakage and shock syndrome. This may be due to the small amount of volume reserve that exists in infants when plasma leakage occurs, under-recognition of potential DHF in febrile infants, or overtreatment with aggressive volume replacement when volume depletion occurs. The isolation of DEN-2 and DEN-3 as the most frequently isolated virus in infants, as it is for secondary dengue infection in other children, suggests that the characteristics of these viruses in producing severe dengue disease in the host relies more on the presence of heterotypic dengue antibody than immunologic priming of cellular or T-cell immunity.

This frequency of dengue serotype isolation is summarized for all years as DEN-3 > DEN-2 > DEN-1 > DEN-4; for primary dengue, as DEN-3 > DEN-1 > DEN-2 > DEN-4; for secondary dengue, as DEN-2 > DEN-3 > DEN-1 > DEN-4; for all DF patients, as DEN-3 > DEN-1 > DEN-2 > DEN-4; and for all DHF, as DEN-2 > DEN-3 > DEN-1. The high degree of correlation of DEN-1 and DEN-2 isolation rates, and its absence with DEN-3, with primary and secondary dengue suggests a propensity of these serotypes to associate with these serologic responses. Our results also indicate that DEN-4 is unusual in that it is the least frequently isolated serotype in any dengue disease state and is not associated with severe dengue outbreaks, and that 97% of isolates are from secondary dengue infections. These observations underscore the importance of viewing each dengue serotype as a separate virus that has evolved with its own unique viral characteristics and propensity to produce disease in the presence or absence of heterotypic dengue antibody.

Examination of severe dengue disease, DHF, in our series demonstrates that it is primarily a condition of secondary dengue infection, with primary DHF in children associated with milder disease. Dengue serotype isolation and dengue disease severity demonstrate that for children with DF, DEN-3 > DEN-1 > DEN-2 > DEN-4, and for children with DHF, DEN-2 > DEN-3 > DEN-1 > DEN-4.

Over time, there was a subtle change in the demographics of children with hospitalized dengue, with a slight increase in mean age from 7.4–7.8 years and an increase in the modal age from 5 years in 1973 to 8 years in 1999. There also was an increase in the percentage of hospitalized primary dengue over time. These findings suggest that children are getting dengue infection at a later age. These observed changes may be due to the overall improvement in the standard of living in

Thailand. Improved housing standards, screens on windows, and reduction of mosquito breeding containers around homes may all contribute to decreasing the intensity of potential exposure to dengue-infected mosquitoes in young children. Dengue infection occurs at an older age, though, as intensity of exposure to dengue-infected mosquitoes increases with greater exposure to the outside environment at home or school. Symptomatic dengue virus infection may be age-dependant and produce more-severe disease in older children and thus might explain the observation that the proportion of hospitalized children with primary dengue is increasing.

Our results suggest that dengue serotypes and their isolation from a spectrum of serologic responses to dengue infection and disease severity is not a random event, and that specific dengue serotypes are more frequently isolated in specific dengue disease states. Our results demonstrate the complexity of the dengue virus transmission and its interaction with the host response, and have important implications in identifying factors to predict severe dengue years, developing interventions to control dengue outbreaks, and developing an effective dengue vaccine.

This longitudinal serotype-specific study of dengue virus over 27 years is unique for its long-term characterization of dengue serology, disease severity, and circulating dengue serotypes and serves as a surveillance model to be instituted by other countries faced with the growing threat of dengue virus infection.

Received January 17, 2002. Accepted for publication August 29, 2002.

Acknowledgments: We thank the doctors and nurses at the Queen Sirikit National Institute of Child Health for their outstanding patient care and participation in this study, and the staff members at the Department of Virology, Armed Forces Research Institute of Medical Sciences, over the past 27 years who have carefully performed diagnostic testing, data entry, and specimen archival. We also thank Dr. Daniel Libraty for his critical reading of this manuscript and Dr. Scott Halstead for sharing his thoughts and vast knowledge base, and giving his comments and suggestions on improving the manuscript.

Financial support: This work was funded by the U.S. Army Medical Research and Materiel Command, Fort Detrick, MD.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as reflecting the official views of the United States Army or the Department of Defense.

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