

## Forty Years of Dengue Surveillance at a Tertiary Pediatric Hospital in Bangkok, Thailand, 1973–2012

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**Abstract.** Long-term observational studies can provide valuable insights into overall dengue epidemiology. Here, we present analysis of dengue cases at a pediatric hospital in Bangkok, Thailand, during a 40-year period from 1973 to 2012. Data were analyzed from 25,715 hospitalized patients with laboratory-confirmed dengue virus (DENV) infection. Several long-term trends in dengue disease were identified including an increase in mean age of hospitalized cases from an average of 7–8 years, an increase after 1990 in the proportion of post-primary cases for DENV-1 and DENV-3, and a decrease in the proportion of dengue hemorrhagic fever and dengue shock syndrome cases in primary and post-primary cases over time. Exploratory mechanistic analysis of these observed trends considered changes in diagnostic methods, demography, force of infection, and Japanese encephalitis vaccination as possible explanations. Thailand is an important setting for studying DENV transmission as it has a “mature” dengue epidemiology with a strong surveillance system in place since the early 1970s. We characterized changes in dengue epidemiology over four decades, and possible impact of demographic and other changes in the human population. These results may inform other countries where similar changes in transmission and population demographics may now or may soon be occurring.

### INTRODUCTION

Dengue virus (DENV) is the causative agent of dengue disease. DENV has four antigenically distinct serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). DENV infection results in a broad spectrum of clinical presentations from asymptomatic infection to undifferentiated febrile illness, dengue fever (DF), and dengue hemorrhagic fever (DHF) including dengue shock syndrome (DSS).<sup>1</sup> Severe clinical manifestations are more likely to occur with repeated infections with a different serotype from the initial infecting serotype.<sup>2–4</sup> There is no specific treatment, although with proper diagnosis and management, fatality rates in Thailand are less than 1%.<sup>5,6</sup> Thailand has had sustained transmission of all four DENV serotypes since 1958.<sup>2</sup>

The epidemiological and clinical manifestations of DENV infections are the result of complex interactions among virologic, immunologic, and ecologic factors. Many questions remain about how these factors interact to result in dengue disease patterns. Small but important differences in these contributing factors in different environments and years make it difficult to draw general conclusions from short-term studies. Thus, long-term observational studies can provide invaluable insights into overall dengue behavior.

Since 1962, Queen Sirikit National Institute of Child Health (QSNICH) and the Armed Forces Research Institute of Medical Sciences (AFRIMS) have engaged in collaborative studies on dengue. An earlier report from this collaboration presented findings from 27 years of dengue surveillance from 1973 to 1999, highlighting serotype-specific epidemiological and clinical patterns of disease.<sup>2</sup> Here, we analyze an additional 13 years covering 40 continuous years of dengue sur-

veillance from 1973 to 2012. This unique dataset reveals several long-term trends in dengue disease including changes in age distribution of hospitalized cases, relative proportions of primary and post-primary infections, and disease severity.

### METHODS

**Ethics statement.** The retrieval and analysis of coded preexisting data in this study was approved by the Institutional Review Boards of Walter Reed Army Institute of Research and QSNICH. Blood samples from passive surveillance were originally collected at QSNICH for public health purposes. All data were analyzed anonymously.

**Description of the dataset.** The analyzed data were from public health samples collected during passive surveillance of hospital-attended dengue cases from 1973 to 2012 at QSNICH, a 420-bed tertiary care pediatric hospital located in Bangkok, Thailand, which serves as a Thailand Ministry of Public Health (MOPH) dengue referral center for Bangkok. Acute and convalescent blood samples from clinically suspected dengue patients at QSNICH were tested for evidence of DENV infection at AFRIMS. Dengue testing was performed using virologic and serologic techniques varying during different time periods, as listed in Table 1. Acute blood samples were tested by viral isolation and/or hemi-nested reverse transcriptase polymerase chain reaction (RT-PCR) as previously described.<sup>7–11</sup> Acute and convalescent blood samples were tested by dengue serological assays as previously described.<sup>12–15</sup> Primary infection refers to the first DENV infection in an individual and was determined serologically by dengue hemagglutination inhibition assay and/or dengue IgM/IgG capture enzyme-linked immunosorbent assay (ELISA) according to published criteria.<sup>15</sup> Post-primary infection refers to any DENV infection subsequent to primary infection and was also determined serologically.<sup>15</sup> Clinical classification was based on World Health Organization guidelines applicable at the time of hospitalization.<sup>16</sup>

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TABLE 1  
Dengue diagnostic methods used during study period

Methodology	Year
Viral isolation and identification by direct viral plaque on LLC-MK2 cell line	1973 to 1977
Hemagglutination inhibition assay	1973 to present
Plaque reduction neutralization test	1973 to present
Fluorescent antibody assay	1973 to 1987
Immunofluorescent antibody identification of serotypes	1973 to 1983
Mosquito inoculation in <i>Toxorhynchites splendens</i>	1977 to present
Antigen-capture ELISA for serotype identification	1981 to present
IgM/IgG-capture ELISA	1987 to present
Hemi-nested serotype-specific PCR assay	1995 to present

ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction.

The QSNICH data were compared with reported dengue cases collected from Bangkok by the MOPH from 1973 to 2012. The MOPH passive sentinel surveillance system collected information on clinically confirmed dengue cases of all ages, of which the majority, but not all, presented to hospitals. Infections among these patients were generally not serotyped, although age and some disease severity information were available. The QSNICH cases will be included in this MOPH total, although all the QSNICH cases have been laboratory confirmed.

**Statistical analysis.** Descriptive statistics were used to summarize dengue case data and trends over time. Statistical analyses were performed using SPSS for Windows version 19.0 (SPSS Inc., Chicago, IL) and R version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

To evaluate mechanisms for the changes in transmission trends over time, we used an age-stratified catalytic model of DENV infection to simulate the impact of changes in birth rate and force of infection (FOI) on the primary/post-primary distributions of cases (see Supplemental Information). The model uses Bangkok birth rates and previous estimates of decreasing FOI during this time period,<sup>17</sup> assumes two infections, and presumes that post-primary (here second) infections were 1.5 times more likely to result in hos-

pitalization than primary infections (the trends are insensitive to this proportion—more detail in the Supplemental Information). We modeled primary and post-primary infections in this time period.

## RESULTS

Data were analyzed from 25,715 patients with laboratory-confirmed DENV infection admitted to QSNICH from 1973 to 2012. Ninety-nine percent of the patients in this dataset were 15 years old or younger.

**Case numbers and serotype distributions.** Of 25,715 dengue cases, both primary/post-primary infection status and serotype determination were available for 12,090 (47%) patients. The majority of the 53% of the cases did not have a serotype, with only 2.7% missing primary secondary classification. Serotype detection rates increased with the use of diagnostic PCR (since 1995) from 30% to 70–80%. Figure 1 shows the number of cases of each serotype, untyped cases, and total cases over time. Of the cases with serotype information, 38.5% were DENV-1, 26.9% were DENV-2, 24.6% were DENV-3, and 10% were DENV-4. From 1973 to 1986, DENV-2 was generally the predominant serotype. In 1987, a large DENV-3 epidemic occurred. DENV-1 was predominant for much of the period from 1988 to 2012 except in 1993–1994, 1995–1999, and 2010–2011 when DENV-4, DENV-3, and DENV-2 predominated, respectively. DENV-4 incidence appeared to peak every 8–12 years, although with generally much smaller epidemics compared with the other serotypes.

There was an increase in the number of cases over the entire study period ( $P < 0.01$ ), but no significant increase from 1987 onwards (when IgG/IgM ELISA was introduced). Figure 2 shows the ratio (with binomial 95% confidence intervals [CIs]) of QSNICH to MOPH dengue cases for each year. This ratio was around 0.1 in the 1970s, 0.15 in the 1980s, peaked at 0.25–0.3 in the 1990s, then steadily decreased until 2012 when it was approximately 0.06. No relationship was seen between this ratio and the proportion of cases of each serotype in each year.

**Age distribution of cases.** The mean age of cases by serotype and primary/post-primary status over 40 years is shown

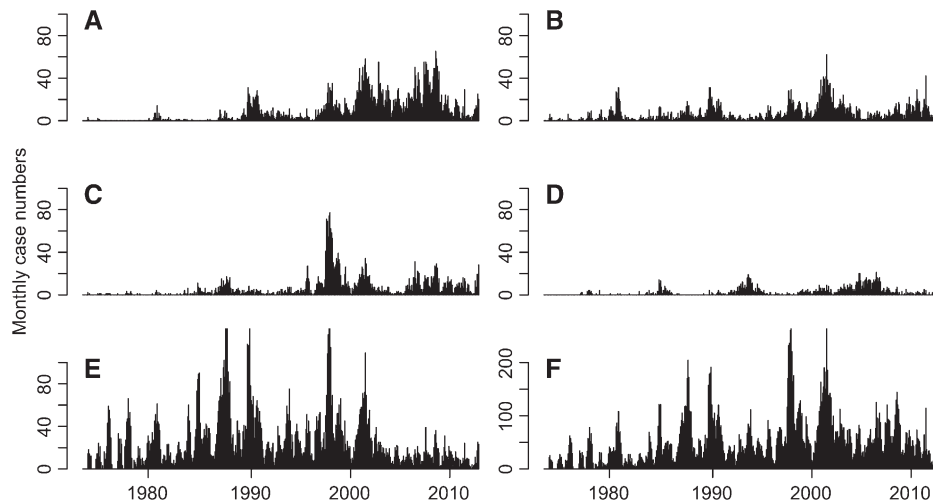


FIGURE 1. Time series of monthly dengue cases. (A–F) DENV-1, DENV-2, DENV-3, DENV-4, serotype unavailable and all cases. DENV = dengue virus.

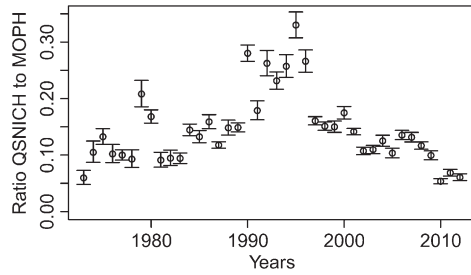


FIGURE 2. Ratio of Queen Sirikit National Institute of Child Health (QSNICH) and Thailand Ministry of Public Health (MOPH) dengue cases.

in Figure 3. The CIs have become smaller in recent years due to the increase in the proportion of cases with known serotype. There were fluctuations in this mean age over time, particularly in recent years. However, an increase in the mean age of cases occurred for most serotypes and for all serotypes combined for both primary and post-primary infections. The increase in mean age is clear through 2005 for primary cases (linear association of age with year,  $P$  values < 0.01) and until the end of the data for post-primary ( $P$  value < 0.01). For primary cases, we observe a decrease from 2005 until 2012, with a suggestion of this downturn in the post-primary cases.

If the 40-year study period is divided into 10-year intervals, the mean age (with 95% CI) for each successive decade was 5.13 (4.49, 5.73), 4.31 (3.93, 4.61), 5.26 (4.98, 5.47), and 5.95 (5.45, 6.17) years for primary infections; 7.34 (7.19, 7.47), 7.55 (7.46, 7.63), 8.18 (8.09, 8.24), and 8.53 (8.39, 8.59) years for post-primary infections; and 7.10 (6.97, 7.25), 7.09 (7.00, 7.17), 7.62 (7.55, 7.70), and 8.10 (8.00, 8.18) for all infections. For comparison, we looked at the mean age of dengue cases under 15 years of age in the MOPH system for

the last three decades (for which MOPH data were available). The mean age (with 95% CI) was 7.04 (7.00, 7.08), 8.04 (8.00, 8.08), and 8.84 (8.80, 8.88), respectively. These ages were similar to the mean ages of the QSNICH post-primary cases for the last three decades, and showed the same increasing trend.

**Primary and post-primary infections.** Only 15.2% of dengue cases were primary whereas 82.1% were post-primary (2.7% could not be determined due to lack of appropriate convalescent sample). The distribution of primary and post-primary infections differed by serotype. DENV-1 and DENV-3 were more likely to be primary cases than DENV-2 and DENV-4 (Figure 4). Over the course of the 40-year study period, the proportion of DENV-2 and DENV-4 cases that were post-primary remained consistently high. Interestingly, the proportion of DENV-1 and DENV-3 cases that were post-primary increased over the entire study period and from 1987 onwards (linear associations, all  $P$  values < 0.001). Given the smaller proportion of cases that were typed in the early years, this increase was particularly obvious from 1990 onwards, until the mid-2000s. After this time, there was a possible decrease/stabilization in the proportion of post-primary cases (Figure 4).

**Exploring the mean age and increase in post-primary proportion trends.** The catalytic model showed that with only changes in births and a decrease in the FOI as simulated here, it is possible to recreate an increase in the proportion of cases that are secondary from 1990 to 2000 followed by a leveling off/decrease (Figure 5). This trend is consistent with what was actually observed in recent years for DENV-1 and DENV-3 (Figure 4); however, the modeled increase is smaller than that actually observed. With Japanese encephalitis (JE) vaccination included in the model, the increase in the proportion of post-primary cases was predicted to last until 2005, followed by a similar decrease.

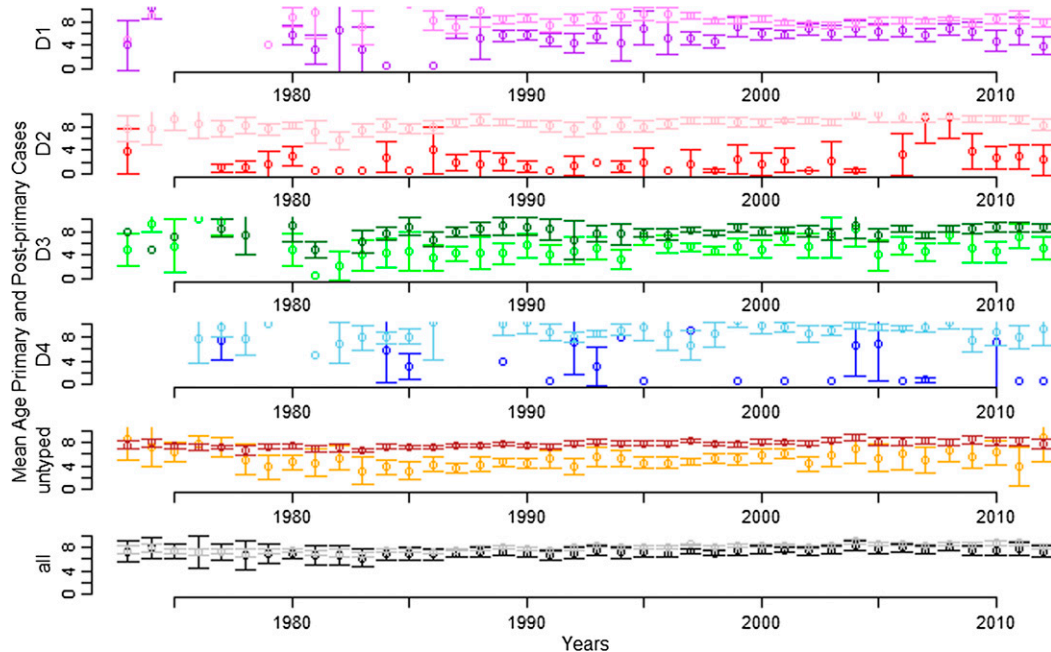


FIGURE 3. Mean age of primary and post-primary cases by serotype, and for untyped and all cases. Primary mean age for each group is shown in dark purple, red, light green, dark blue, orange, and black, respectively. Secondary mean age for each group is shown in light purple, light pink, dark green, light blue, brown, and gray, respectively. D1 = DENV-1; D2 = DENV-2; D3 = DENV-3; D4 = DENV-4.

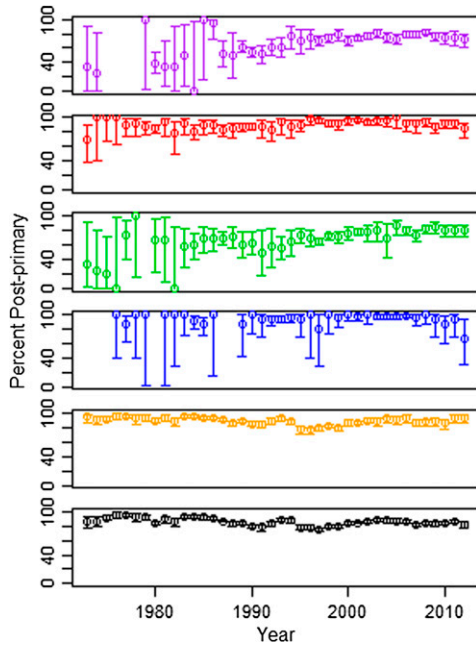


FIGURE 4. Percentage of dengue cases those were post-primary over time by serotype. DENV-1 to DENV-4, serotype unavailable and all cases.

**Disease severity over time.** Figure 6 shows the proportion of all primary and post-primary dengue cases classified as DHF or DSS over time. For primary infections, despite large CIs, it appears there was a decrease in this percentage over 40 years from almost all cases being DHF or DSS in 1973 to around 50% of cases in the 2000s. For post-primary infections, the percentage of DHF/DSS cases was also close to 100% initially, remained fairly steady at around 80% throughout the 1980s, 1990s, and early 2000s, and then sharply decreased in 2005 with an increase again in the most recent years. Figure 7 shows the proportion of DHF/DSS for primary and post-primary infections by age. The decreasing linear relationship of disease severity with time is significant for both primary and secondary cases ( $P$  values < 0.001). In primary infections, no clear relationship with age was apparent. In post-primary infections, there were also large CIs, but the highest proportion of DHF/DSS cases occurred in the

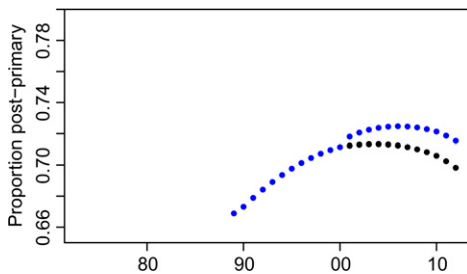


FIGURE 5. Catalytic model output of post-primary (second infections) proportion under a decrease in force of infection (FOI), known birth rates in Bangkok, and three times as many post-primary than primary infections being observed. Black dots reflect the model without Japanese encephalitis (JE) vaccination and blue dots reflect the model with JE vaccination. Estimates are shown from 1988 onward since estimates for FOI for the population  $\leq 15$  years old were possible for their whole lifespan.

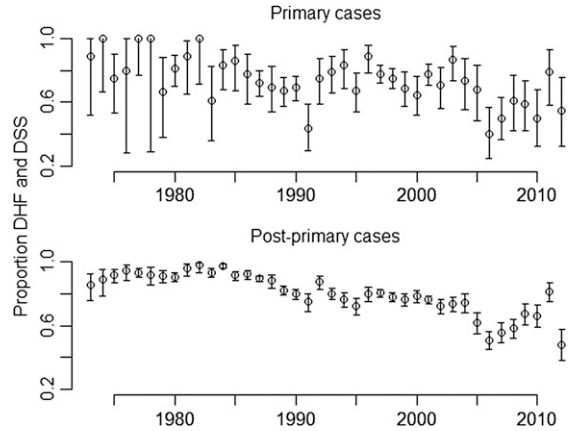


FIGURE 6. Proportion of primary and post-primary dengue cases that were dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) over time.

5–9 year age group, with a nonsignificant suggestion of a slight decrease thereafter.

The proportion of cases of each serotype (DENV-1, DENV-2, DENV-3, DENV-4 and untyped) which are DHF/DSS is 0.66 (95% CI: 0.62, 0.69), 0.75 (0.68, 0.75), 0.71 (0.66, 0.75), 0.77 (0.58, 0.90), and 0.77 (0.74, 0.79) for primary cases and 0.69 (0.66, 0.70), 0.80 (0.79, 0.82), 0.73 (0.70, 0.75), 0.74 (0.71, 0.77), and 0.85 (0.84, 0.86) for secondary cases.

DISCUSSION

All four DENV serotypes circulated continuously in Bangkok from 1973 to 2012, causing both DF and DHF in children. Our evaluation of serotype-specific dengue disease patterns over a 40-year period furthers our understanding of the relationships among serotype, severity, and epidemiology of dengue.

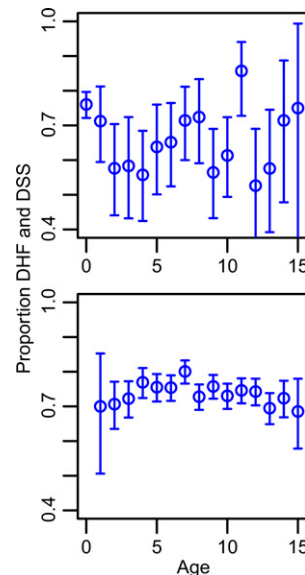


FIGURE 7. Proportion of dengue cases that were dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) for primary and post-primary cases in different age groups.

Since 1973, the mean age of dengue cases increased from 7.12 to 8.14 years. A similar increase was noted in cases under 15 years of age from the MOPH, and is consistent with an increase seen throughout Thailand.<sup>17</sup> The observed increase in mean age is consistent with a decrease in the FOI as has been shown in previous works.<sup>17,18</sup> During the time of mean age increase (and implied decrease in FOI), there was also an increase in the proportion of cases that were post-primary for DENV-1 and DENV-3. This increase is counterintuitive to what one would expect in children with a decreasing FOI. One would expect to see a decrease in post-primary proportion, as both primary and post-primary infections would occur at an older age, and therefore, a greater proportion of post-primary than primary cases would be too old for a pediatric hospital. In fact, the opposite was observed. However, we demonstrated that a catalytic model accounting for the differential population level susceptibility to infection over time due to changing FOI and birth rates predicted a period of around 10 years (1990–2000) during which a disproportionate number of people were primed for a post-primary infection compared with the number of people newly entering the population as susceptible to a primary infection. This may have led temporarily to an increase in the post-primary proportion; however, the model does not show an increase as large as that observed, suggesting other processes might be at play, an area that warrants further exploration. In the model, as this population aged and the birth rates stabilized, the effect diminished (around 2000) and the post-primary proportion stabilized as would be expected with a decrease in FOI. The simulation of JE vaccination in the model continued this increase for another 5 years also consistent with observation. The consistency of our model with observed data highlights the importance of understanding all facets of demography and other possible contributing factors when considering trends in case counts. The suggestion of a decrease in mean age in the last few years was not explored here, and will be of interest to follow with data from subsequent years.

An overall decrease in disease severity occurred during the 40-year study period. There are several plausible, non-mutually exclusive explanations for the decreasing severity over time. A difference in disease severity with age is a phenomenon that has been noted previously, for example, in Cuba, Thailand, and Singapore,<sup>19–23</sup> which could be consistent with the observed trends; however, we do not observe such a clear relationship with age and severity here and observe a fairly small shift in age of cases. Secondly, in 1999, a new handbook was published with recommendations for hospitalization of suspected dengue cases.<sup>16</sup> These changes in hospitalization criteria could have led to a greater number of less severe cases being hospitalized, and could therefore account for the observed trends in disease severity. In addition, the decrease in disease severity in post-primary cases could have been the result of some primary cases that are more likely to be less severe, being categorized as post-primary in the era of JE vaccination. Finally, our findings and previous literatures show that DENV-1 (and DENV-3 to some extent) is less associated with severe disease than the other serotypes. The decrease in severity over time would be consistent with the increase in cases of these serotypes. The factors associated with disease severity deserve continued focus, so we are able to fully understand observed changes, particularly after the introduction of a vaccination.

This study demonstrated serotype cycling over the years in Bangkok as has been well documented previously.<sup>2,24,25</sup> DENV-1 and DENV-2 predominated for long periods of time, and conversely, DENV-4 has never reached the same peaks in case numbers as the other serotypes. It is not clear whether differences in the predominant serotypes were due to differences in FOI or to differences in their ability to cause disease upon infection. In recent years, all four serotypes have more consistently circulated at the same time. However, the ability to determine serotype has increased, which may have influenced the observed trend toward more serotypes circulating at one time.

There was a slight increase in overall case numbers over time. However, this becomes nonsignificant when we consider only the time period with consistent serological testing. This suggests that this trend was due to reporting/testing changes rather than a true increase in cases numbers. In the last decade, QSNICH dengue cases have been around 6% of the MOPH cases. Over time, this proportion has fluctuated, with a particularly high proportion during the 1990s. This does not appear to be related to the serotype distributions at each time, but could be due to changes in the surveillance system. This is important to bear in mind when using each dataset to make conclusions about trends in dengue epidemiology.

An updated analysis of trends of dengue in Bangkok is timely for understanding what may happen in the future, with or without the introduction of a dengue vaccine. Thailand is an important setting for studying DENV transmission as it has a “mature” dengue epidemiology with a strong surveillance system in place since the early 1970s. Description and understanding of how this epidemiology has developed over the years and interacted with other changes in the population such as demography is important for other countries where similar changes in transmission and population demographics may now or may soon be occurring.

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