Prospective Cohort Studies of Dengue Viral Transmission and Severity of Disease

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Abstract As the four serotypes of dengue virus (DENV) systematically spread throughout the tropical and subtropical regions globally, dengue is increasingly contributing to the overall morbidity and mortality sustained by populations and thereby challenging the health infrastructures of most endemic countries. DENV-human host-mosquito vector interactions are complex and cause in humans either asymptomatic or subclinical DENV infection, mild to severe dengue fever (DF), severe dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS). Over the past decade, we have seen an increase in research funding and public health efforts to offset the effects of this pandemic. Though multiple vaccine...
development efforts are underway, the need remains to further characterize the
determinants of varying severities of clinical outcomes. Several long-term prospec-
tive studies on DENV transmission and dengue severity have sought to define the
epidemiology and pathogenesis of this disease. Yet, more studies are required to
quantify the disease burden on different populations, explore the impact of DENV
serotype-specific transmission on host-responses and dengue severity and measure
the economic impact of dengue on a population. In this section, we will review
the critical past and recent findings of dengue prospective studies on our
understanding of the disease and the potential role of future prospective cohort
studies in advancing issues required for vaccine field evaluations.

1 Introduction

The global dengue pandemic and its associated morbidity and mortality have drawn
in additional research funding to energize the scientific community to pursue a
greater understanding of dengue, its virology, pathogenesis, epidemiology and
transmission factors. However, there exist only eight published, long-term dengue
prospective cohort studies since 1984 (Table 1) that studies disease severity and
virus transmission.

Prospective studies offer the advantage of determining the true incidence of
disease within a defined cohort to ascertain absolute and relative risk, the spectrum
of clinical outcomes (asymptomatic infection to severe hospitalized disease), anal-
ysis of risk factors of disease severity and the spatial and temporal diversity
of serotype-specific dengue virus (DENV) transmission. Long-term prospective
studies can examine the impact of year-to-year variation in dengue incidence,
disease severity and serotype-specific transmission. Additionally, prospective-
cohort studies and their examination of the full burden of disease can be used to
determine the economic burden of DENV infection, essential information for
countries in evaluating health priorities and the resources required for vector
control and in determining the cost-effectiveness of a DENV vaccine to prevent
infection. DENV vaccine developers rely on prospective studies to provide accurate
information on dengue incidence for sample-size estimation for efficacy studies and
in determining the spatial and temporal transmission of different DENV serotypes
for serotype-specific vaccine efficacy. Furthermore, prospective studies develop the
field site infrastructure and community awareness necessary to conduct phase III
dengue vaccine efficacy studies. The limited number of prospective dengue studies
may be a reflection of the associated time and cost necessary for study execution.
Additional limitations include: (a) the potential introduction of bias if every mem-
ber of the cohort is not followed or if the surveillance is limited in identifying all
infection or disease, (b) the length of the study may be less than the latency period
of the disease such as onset of dengue hemorrhagic fever (DHF) and (c) prospective
studies are inherently inefficient for studying rare complications of the disease such
as encephalitis.
<table>
<thead>
<tr>
<th>Study Site</th>
<th>Population Size</th>
<th>Age Range</th>
<th>Study Period</th>
<th>Dengue Infection</th>
<th>Symptomatic Dengue</th>
<th>Incidence (Average)</th>
<th>Symptomatic: Asymptomatic Ratio</th>
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<tr>
<td>Rayong, Thailand Sangkawibha et al. (1984)</td>
<td>1,056</td>
<td>4–14 years</td>
<td>1980–1981</td>
<td>9.4%</td>
<td>n/a</td>
<td>0.1%</td>
<td>1:9</td>
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<tr>
<td>Bangkok, Thailand Burke et al. (1988)</td>
<td>1,757</td>
<td>4–16 years</td>
<td>1980–1981</td>
<td>11.8%</td>
<td>0.7%</td>
<td>0.4%</td>
<td>1:8</td>
</tr>
<tr>
<td>Yangon, Myanmar Thein et al. (1997)</td>
<td>12,489</td>
<td>1–9 years</td>
<td>1984–1988</td>
<td>5.1%</td>
<td>n/a</td>
<td>0.0%</td>
<td>1.8</td>
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<tr>
<td>Yogyakarta, Indonesia Graham et al. (1999)</td>
<td>1,837</td>
<td>4–9 years</td>
<td>1995–1996</td>
<td>29.2%</td>
<td>0.6%</td>
<td>0.0%</td>
<td>n/a</td>
</tr>
<tr>
<td>Kamphaeng Phet I, Thailand Endy et al. (2002a)</td>
<td>2,119</td>
<td>7–11 years</td>
<td>1998–2002</td>
<td>7.3%</td>
<td>3.9%</td>
<td>1.0%</td>
<td>1.0:19</td>
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<tr>
<td>West Java, Indonesia Porter et al. (2005)</td>
<td>2,536</td>
<td>18–66 years</td>
<td>2000–2002</td>
<td>7.4%</td>
<td>1.8%</td>
<td>0.0%</td>
<td>1:3</td>
</tr>
<tr>
<td>Managua, Nicaragua Balmaseda et al. (2006)</td>
<td>1,186</td>
<td>4–16 years</td>
<td>2001–2002</td>
<td>9.0%</td>
<td>0.85%</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Kamphaeng Phet II, Thailand (14 and unpublished data)</td>
<td>2,095</td>
<td>4–13 years</td>
<td>2004–2006</td>
<td>6.7%</td>
<td>2.2%</td>
<td>0.5%</td>
<td>1:3.0</td>
</tr>
</tbody>
</table>

\( ^a \text{number in cohort tested for dengue antibody (incidence denominator)} \\
\( ^b \text{n/a = not available; not provided in the published paper} \)
We review in this section the published prospective dengue cohort studies, explore the information acquired from recent studies and discuss research needs for the future as we continue to understand this important disease.

2 Previous Prospective Cohort Studies

Table 1 summarizes the published, prospective dengue cohort studies to date. The first study was conducted in Rayong, Thailand starting in January 1980 among 3,185 children who were randomly sampled from schools and households (Sangkawibha et al. 1984). The population prevalence of neutralizing antibody to the four dengue serotypes was estimated and incidence of infection with each DENV serotype was determined in first grade children who were rebled a year later. Examination of pre- and post-epidemic cohort blood samples revealed that the incidence of dengue infection in 251 seronegative children was 39.4%. Of the 22 shock syndrome cases admitted to the hospital, all had secondary antibody responses based on acute and convalescent serology. The risk factors for dengue shock syndrome (DSS) in Rayong were secondary infections with DENV-2, which followed primary infections with DENV-1, DENV-3 or DENV-4. The Rayong study confirmed the high burden of dengue illness in this population, the association of secondary dengue infections with severe dengue illness and the importance of sequential dengue serotypes in producing shock syndrome.

The second study was a 2 year (1980–1981) school-based study involving 1,757 children, ages 4–16 years, in Bangkok, Thailand (Burke et al. 1988). The children were followed using active surveillance to identify children absent from school for 2 or more consecutive school days. Upon evaluation, if the child had a febrile illness, acute and convalescent serum samples were obtained for serologic testing. Antibody titer revealed that 50% of the enrolled students had evidence of dengue antibody, likely indicative of a DENV infection experienced prior to the age of 7 years. Most (87%) of the students who became infected during the study period were asymptomatic as determined by lack of clinical illness; 53% of the symptomatic DENV infections were recognized as dengue DHF requiring hospitalization. Significant study findings were an incidence in dengue-naïve participants of 6.3%, an incidence of 5.5% in dengue-experienced participants, a hospitalization rate among symptomatic infected children of 53% and a symptomatic-to-asymptomatic ratio of 1:8. The odds ratio for developing DHF in participants with preexisting dengue immunity was ≥ 6.5. This was the first study to determine the full-burden of DENV infection within a cohort and the relationship of preexisting immunity, secondary dengue infection, to dengue disease severity.

A 5 year (1984–1988) study was performed in two townships in Yangon, Myanmar (Thein et al. 1997). A study population of approximately 12,500 children was sampled pre- and post-monsoon season in age-specific cohorts: ages 2, 3, 5 and 6 years. Cohorts in each age group combined varied by year: 1,283 in 1984; 1,513 in 1985; 1,947 in 1986; 1,978 in 1987; and 1,239 in 1988. The total number sampled
and tested for dengue antibody was 3,579. Surveillance for severe disease was performed by monitoring hospital admissions at Yangon Children’s Hospital. Over 5 years there were 50 hospital admissions diagnosed as dengue fever (DF) (incidence of 0.08% per year) and 145 with DHF or DSS (incidence of 0.02% per year) for a total incidence of symptomatic hospitalized dengue of 0.1% per year. Over 5 years, there were 920 participants who had serologic evidence of DENV infection for an average incidence of 5.1%. The authors concluded that severe DHF/DSS was associated with having serologic evidence of a preexisting DENV infection (secondary dengue).

A 1 year (1995–1996) study was initiated in 1995 in Yogyakarta, Indonesia (Graham et al. 1999). The cohort study involved children ages 4–9 years with blood samples collected at the start of the study and 1 year later. Passive surveillance was performed identifying febrile children presenting to a participating study clinic or admitted to a hospital with suspected dengue. The overall dengue incidence for 1 year was 29.2% with a symptomatic and severe hospitalized dengue illness incidence of 0.6% and 0.4%, respectively.

The Bangkok, Rayong, Yangon and Yogyakarta studies formed the basis of our understanding that secondary DENV infections predispose to risk for subsequent severe dengue upon re-exposure to DENV. Additionally, attempts were made to understand the full burden of DENV infection in a population and the role of serotype-specific DENV transmission. The limitations of these studies were the relatively short periods of observation, mixed ages of cohort populations making incidence determinations difficult and lack of serotype-specific assays to identify the true infecting serotype and subclinical infections. The Bangkok study was the first to determine the full-burden of DENV infection in a well-defined cohort population by using active surveillance to evaluate fevers in children absent from school with acute DENV infections. That study was limited to 1 year and thus variations in serotype exposure and host response from year-to-year could not be determined.

3 Recent Prospective Cohort Studies

Four studies have been carried out in the last decade, one in Nicaragua (the first in the Americas), one in Indonesia (the first in adults) and two in Thailand. The two Thai studies were conducted in sequence for over 7 years of a planned 10 years of continuous observation of dengue disease severity in the province of Kamphaeng Phet. Each study will be discussed in turn, describing the key research advances that have contributed to recent gains in our knowledge of dengue.

A 2 year study was conducted in 1,186 schoolchildren, ages 4–16 years, in Managua, Nicaragua during 2001–2002 (Balmaseda et al. 2006). Blood was drawn in March or May of every year prior to the dengue season. Children who had 3 consecutive days of school absence were evaluated by the school nurse or public health clinic for fever and/or dengue-like illness. The incidence of DENV infection
(symptomatic and asymptomatic) in this first cohort study in the Americas was 12% during the first year of the study and 6% during the second year (average incidence of 9%). The incidence of symptomatic dengue was 0.85% and the ratio of symptomatic to asymptomatic infections was 1:13 during the first year of the study and 1:6 during the second year of the study.

A 3 year study was conducted in 2,536 adults, ages 18–66 years, in West Java, Indonesia from 2000–2002 (Porter et al. 2005). Volunteers were bled every 3 months and actively followed at work for acute illness. The first 2 years of the study demonstrated a symptomatic dengue incidence of 18 per 1,000 person-years and an estimated asymptomatic rate of 56 per 1,000 person-years for a symptomatic to asymptomatic ratio of 1:3.

Two studies, Kamphaeng Phet Study I (KPSI) in 1998–2002 and KPSII in 2004–2008, were performed in Kamphaeng Phet, Thailand. Both studies were conducted in subdistrict Muang, which, as per a year 2000 census, had a population of 198,943 with 49,593 households. Both studies were initiated under a combined National Institutes of Health, United States Army and Thai Ministry of Health-funded grant in collaboration with the University of Massachusetts Medical School, University of California, Davis and the Armed Forces Research Institute of Medical Sciences (AFRIMS). The basis for both Kamphaeng Phet studies was the enrollment of primary school children. Children in KPSI were enrolled as they entered second grade and were followed continuously for up to 5 years or until they graduated at the end of 6th grade. Participating students were evaluated every January with baseline demographic information, height and weight and a blood sample obtained for plasma and peripheral blood mononuclear cells (PBMC’s). All participants were evaluated during the first part of June, August and November of each year, when a blood sample was obtained for dengue serology (Endy et al. 2002a; b). In KPSII, children were enrolled from kindergarten to grade 5 and remained in the study for up to a 5 year period; routine blood samples were obtained every June and January to assess for dengue antibody conversion.

For both studies, active acute illness case surveillance of the study participants occurred from June to mid-November, the peak DENV transmission season in Thailand. Acute illness from DENV infection was identified using school absence as the indicator for evaluation. Absent students were identified by their teacher and evaluated by a village health worker with a symptom questionnaire and an oral temperature obtained with a digital thermometer. Students who had a history of fever within 7 days of school absence or an oral temperature ≥38°C (100.4°F) were brought to a public health clinic and evaluated by a nurse. A physical examination was conducted. Acute and convalescent (14 days later) blood samples were obtained. Acutely ill children were also identified if they reported ill to the school nurse or were admitted to the hospital. At the end of each year, based on comparison of dengue antibody responses between blood sampling times in KPSI (June–August, August–November or January–January) and in KPSII (January–January) and evaluation of febrile school absences, acute DENV infections were categorized as: (1) asymptomatic (fourfold rise in dengue antibody titer between sequential blood samples without a reported febrile school absence); (2) symptomatic dengue
not requiring hospitalization; (3) symptomatic DF requiring hospitalization; and (4) symptomatic DHF requiring hospitalization. Dengue reverse transcriptase-polymerase chain reaction (RT-PCR) and occasionally, viral isolation, were performed on all acute dengue samples and thus evaluation of the full-burden of DENV infections by virus serotype was attempted. The January blood sampling and PBMC collection provided a valuable archive in which to determine preillness host factors that determine risk for development of severe DENV infection. The results from these studies, summarized below, provide important insights into the dynamics of DENV transmission in a population, the diversity of serotype-specific virus transmission and the host dynamics that produce subclinical to severe dengue illness.

3.1 Dengue Incidence Diversity in Time and Space

For KPS I and II, the overall average annual incidences of DENV infection were 7.3% and 6.7%, respectively; those of symptomatic dengue were 3.9% and 2.2%, those of hospitalized dengue illness 1% and 0.5% and those of severe dengue (DHF) 0.6% and 0.1% respectively. Figure 1 displays the heterogeneity of DENV incidence in a subset of schools that participated in KPS I. In general, dengue incidence was cyclical in each school, with relatively mild years followed by more severe years. Some schools had a severe dengue year, e.g., school 4 in 1998 with an incidence of nearly 20%, while other schools a short distance away had less severe dengue, e.g., school 5 during 1998 with an incidence of less than 5%. The cyclical nature of dengue illness on a temporal and spatial scale is an important concept from these studies and is reflected in the national occurrence of reported dengue illness in Thailand, where relatively mild years are followed by more severe years (Nisalak et al. 2003).

On average, all schools experienced a significant burden of dengue illness during the 4–5 year period (Fig. 1). The importance of understanding dengue incidence temporally and spatially is its utility in understanding the pathogenesis of dengue illness and disease severity. Important questions generated from this information are the role of herd immunity from the previous year’s dengue transmission in modifying dengue disease in the subsequent year and how serotype-specific DENV transmission and infection rates are affected. Lastly, dengue incidence and its temporal and spatial diversity in a population is important in designing dengue vaccine efficacy studies and estimating the population and geographic location required to assess statistical efficacy.

3.2 Dengue Serotype and Strain Diversity in Time and Space

In Kamphaeng Phet Province, all four DENV serotypes are known to cocirculate. The spatial and temporal diversity of serotype-specific DENV circulation was not appreciated until the prospective studies were performed. In KPSI, for example, one
school had an outbreak with a single DENV serotype while another school a short distance away had a completely different DENV serotype present. Figure 2 demonstrates the spatial and temporal diversity for 1998 through 2001. In 1998, school 4 had a relatively pure DENV-3 outbreak, while a short distance away school 3 had predominantly DENV-1 and school 5 predominantly DENV-2 transmission. The following year, school 4 exhibited predominantly DENV-2 transmission while other schools experienced predominantly DENV-1 transmission. During KPSII
from 2004–2007, DENV-1 and DENV-4 were the predominant serotypes. Analysis of isolated viruses from KPSI demonstrated viral genetic variation in both time and space, with multiple viral lineages circulating within individual schools (Jarman et al. 2008). This suggests that there is frequent gene flow of DENV into this microenvironment. Analyses of DENV-2 samples demonstrated clustering of viral isolates within individual schools and evidence of frequent viral gene flow among schools closely related in space. These results suggest that a combination of frequent viral migration into Kamphaeng Phet coupled with population (school)
3.3 The Changing Ratio of Subclinical to Clinical Dengue Illness

One value of the prospective studies of dengue disease and virus transmission is to examine the changing relationship between subclinical and clinical DENV infections. Asymptomatic (or subclinical) DENV infection, though a significant component of overall DENV infection, is likely to go unreported despite its potential contribution to the ongoing DENV transmission cycle. Understanding the pathogenetic basis of subclinical infection is important as it reflects a complex interaction between the virus, preexisting DENV antibody and T-lymphocytes and other host factors that determine disease severity and outcome. During KPSI, the changing nature of subclinical to clinical dengue was examined and is illustrated in Fig. 1. In this figure, the ratio of asymptomatic to symptomatic DENV infection was calculated for each school by year. The horizontal line represents a ratio of one, signifying one asymptomatic dengue infection for each case of symptomatic infection. A ratio above this line represents more asymptomatic infections and a ratio below the line represents more symptomatic infections. As illustrated, this ratio was fluid in KPSI, with some schools experiencing more symptomatic disease than other schools and shifting the following year, with others experiencing greater or fewer symptomatic infections. School 4, for example, experienced a ratio of 6.5 during 1999, a ratio near equivalent to what was observed in the prospective cohort study in Bangkok during 1980. The following year this ratio was lower and in 2002 this school experienced a much more severe year than the previous years. Similar patterns were seen for other schools with a cyclic variation in disease severity over time. This suggests that asymptomatic to symptomatic disease ratios at single time points may not reflect the experience of the population over time and that there is an undercurrent of protective immunity that may not prevent infection but may modify disease severity. This was suggested in a study of preillness sera of children who later developed hospitalized dengue; pre-illness heterotypic neutralizing antibody to the child’s own DENV isolate was associated with modification of the disease severity (Endy et al. 2004).

3.4 Economic Burden of Dengue Disease

KPSI has provided unique information on the full burden of DENV infection over a 5 year period of time. This information was used to calculate the Disability Adjust Life-Years (DALYs) lost to DENV infections in order to determine the economic impact of dengue in this population (Anderson et al. 2007). The mean cost of dengue was 465.3 DALYs per million population per year, which accounted for subdivision shape the genetic diversity of DENV at a local scale. Over 5 years of the KPSI study, nearly all schools experienced transmission of three or more DENV serotypes (Fig. 2).
15% of DALYs lost to all febrile illnesses. Non-hospitalized patients with dengue illness represented a substantial proportion of the overall disease burden, 44–73% of the total DALYs lost to dengue each year. The infecting DENV serotype was an important determinant of DALYs lost with DENV-1 responsible for 9% of total DALYs lost, DENV-2 for 30%, DENV-3 for 29% and DENV-4 for 1%. During large outbreak years, DALYs lost to dengue was greater than that calculated for the tropical diseases, meningitis and hepatitis B and three times greater than reported by the World Health Report for 2003 (The world health report 2003). This study demonstrated the under-reporting of DALYs based on reported dengue illness, which focuses on severe hospitalized illness and the value of prospective cohort studies to understand the full economic burden of DENV infection.

3.5 Cluster Investigation

In addition to evaluating the burden of dengue infection, the KPSII study provided unique information on the spatial spread of DENV in the household of dengue-infected children (Mammen et al. 2008). Cluster investigations were conducted within 100 meters of homes where febrile index children with and without DENV infection lived. Information on both human infection and mosquito density and infection were collected to define the spatial and temporal dimensions of DENV transmission. During the first 2 years of the KPSII study, 556 village children were enrolled as neighbors of the index cases. All DENV infections found in these neighbors occurred around DENV-infected index cases, with 12.4% of enrollees becoming infected in a 15 day period. This study demonstrated the focal nature of DENV transmission and the value of cluster investigations as an adjunct to prospective cohort studies in determining the full scope of DENV transmission.

4 Summary

Despite the diversity in the study techniques and populations studied, several recurrent themes emerge from the published prospective dengue cohort studies. First, the incidence of DENV infection in the countries studied is significant, with a range of 5%–29% per year with most studies establishing an annual incidence rate between 5% and 10%. Second, symptomatic DENV infections represent only a fraction of the full burden of DENV infection with incidence ranging from 0.6% to 4% per year. Third, the ratio of symptomatic to asymptomatic DENV infection ranges from 1:1 to 1:8, suggesting variability in subclinical infections contributing to the overall dengue burden. This variability, however, may be attributed to differences in surveillance approaches, prior DENV exposure and/or host genetics. From the Kamphaeng Phet studies, this variability was observed even within a small geographic area over time and was dependent on heterotypic protective
immunity from prior DENV exposure and the varying predominance of circulating DENV serotypes each year. Lastly, important information was gathered to evaluate the economic burden of dengue on a population and the potential cost-effectiveness of a vaccine in alleviating morbidity and the burden on the health infrastructure.

Prospective cohort dengue studies provide invaluable reagents (pre-illness sera and peripheral blood mononuclear cells) for further scientific discovery of patho-genetic determinants. Critical questions remain regarding the mediators of severe dengue and the correlates of protection. Additional research is needed to address these areas that are critical to vaccine testing and evaluation. Expanding cohort studies, to include those countries (especially in the Americas and South Asia) where dengue is inadequately characterized, will enable us to further understand the unique host factors that may contribute to differences in dengue risk and may thereby underlie potential differences in vaccine response. Investing in long-term follow-up of cohort populations is important to understand the spatial and temporal variability in DENV circulation and the impact of preexisting dengue antibody on dengue disease severity.

The prospective cohort study is an important method to understand the full-burden of DENV infection, the effects of serotype-specific DENV transmission and the effects of the virus-host interactions that result in mild to severe dengue illness.

References


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