The WHO Regional Office for South-East Asia publish the annual Dengue Bulletin.

The objective of the Bulletin is to disseminate updated information on the current status of dengue fever/dengue haemorrhagic fever infection, changing epidemiological patterns, new attempted control strategies, clinical management, information about circulating DENV strains and all other related aspects. The Bulletin also accepts review articles, short notes, book reviews and letters to the editor on DF/DHF-related subjects. To provide information for research workers and programme managers, proceedings of national/international meetings are also published.

All manuscripts received for publication are subjected to in-house review by professional experts and are peer-reviewed by international experts in the respective disciplines.
From the Editor’s Desk

Dengue has become one of the fastest spreading diseases with half of the world population being at risk and millions of infections and taking thousands of lives every year. Dengue is endemic in ten out of eleven countries in WHO South-East Asia Region, with the exception of Democratic Republic of Korea. In the year 2015, a total of 428,287 cases of dengue were reported from WHO SEA Region. This amounts to more than 50% increase compared to the previous year. With large outbreaks in several countries, the year 2015 saw the largest number of reported cases in the region. However, the case fatality rate went down from 0.47 to 0.36 compared to the previous year, reflecting sustained efforts of the countries to strengthen the capacity to manage clinical cases of dengue.

Considering the dramatic increase in incidence and ability to cause large outbreaks affecting mass population, especially in bigger cities, dengue has already been identified as a major public health problem in many countries. The frequency and increasing magnitude of dengue outbreaks have gained much attention of media, general public and research community. The research community has been engaged on several research activities covering wide range of dengue spectrum and transmission dynamics. In line with this priority, every year Dengue Bulletin is published encouraging researchers to explore different aspects of the disease and contribute to the knowledge gap and evidence base for combating the rapid spreading of this deadly disease.

The 39th volume of Dengue Bulletin has captured the recent researches on clinical management, possible association with genetics and pathological factors, surveillance system, software application, role of dengue vaccine and a book review. We hope these peer reviewed articles will help the policy makers, programme managers, physicians and researchers to make scientifically proven, cost-effective efficient decisions in future.

We now invite contributions for Volume 40. The deadline for receipt of contributions is 30 June 2017. Contributors are requested to please pursue the instructions given at the end of the Bulletin while preparing the manuscripts. Contributions should either be sent accompanied by flash drives to the Editor, Dengue Bulletin, WHO Regional office for South East Asia, Mahatma Gandhi Road, I.P Estate, Ring Road, New Delhi 110002, India, or by email as a file attachment to the Editor at se_denguebulletin@searo.who.int. Readers who want copies of the Dengue Bulletin may write to the same address or the WHO Country representative in their country of residence. The pdf version will be available on the WHO SEARO website.
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The quality and scientific standing of the *Dengue Bulletin* is largely due to the conscious efforts of the experts and also to the positive response of contributors to comments and suggestions.

The manuscripts were reviewed by Dr Aditya P Dash and Dr Mohamed A Jamshed, with respect to format; content; conclusions drawn, including review of tabular and illustrative materials for clear, concise and focused presentation; and bibliographic references.
Genetics of susceptibility to severe dengue virus infections: an update and implications for prophylaxis, prognosis and therapeutics

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Abstract

The clinical outcome of dengue virus (DENV) infection is influenced by variation in the human and viral genes. Genetic susceptibility to severe forms of dengue involves multiple genes that contribute to the innate immune recognition of the virus (genes coding for entry receptors and pattern recognition receptors), recognition of DENV by the adaptive immune system involving products of human leukocyte antigen genes and effector responses of the immune system (genes affecting cytokines, chemokines and immunomodulators). Genetic susceptibility to severe dengue is further complicated by the genetics of the virus involving multiple serotypes/genotypes, gene–environment and gene–gene interactions. A series of prospective immunogenetic studies with complete data on infecting serotype and immune status are warranted to delineate the complex host–virus interactions. This might identify the genetic factors that are definitely associated with severe dengue and could lead to the development of novel therapeutic and prophylactic measures. This review updates the status of immunogenetics of severe dengue infections, and attempts to identify the gaps and evolve more sensible approaches to study the genetics of susceptibility to severe dengue.

Keywords: Dengue, severe dengue, immune response.

Introduction

Dengue and its severe forms, namely, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), caused by four serotypes of dengue virus (DENV), have become a major public health concern that is straining the health systems of both developing and developed countries. More than 50% of the people at risk of getting infected are living in the South East Asian Region (SEAR) of the World Health Organization (WHO). Lack of approved vaccines

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and antivirals for prevention and treatment of the disease and the failure of vector control programmes to combat the disease-carrying mosquitoes of *Aedes* species had contributed to the spread and increased incidence of dengue.\(^1\)

The reason why only some infected individuals progress to more severe forms of the disease while others develop a mild form of the disease or remain asymptomatic is still elusive. Epidemiological studies carried out during various major outbreaks and studies based on continued surveillance have suggested the role of immune status (primary or secondary); infecting serotypes/genotypes; sequence of infecting serotype during primary and secondary infections; time interval between primary and secondary infections; host immune response; and the host genetics in determining dengue disease severity\(^2\) (see Figure 1). The present review is an attempt to compile and update the role of host genetics in determining dengue disease severity, identify the gaps and evolve more sensible approaches to study the genetics of susceptibility to severe forms of dengue, which will lead to findings having implications in the prediction and treatment of severe forms of dengue.

*Figure 1: Genetic factors influencing clinical outcome of dengue virus infection*
Genetic factors influencing clinical outcomes of DENV infection operate at different levels of immune response (see Figure 1). They include:

1. Entry of the virus into cells of the immune system;
2. Recognition of the virus by the innate immune system and associated response;
3. Recognition of viral epitopes by CD4+ and CD8+ T-cells in the context of human leukocyte antigen (HLA) class I and class II molecules presented by antigen presenting cells; and
4. The effector phase of the immune response during which different effector cells and the molecules secreted by them mediate the immune response against the virus and its elimination.

**Polymorphisms in the genes related to viral entry and severe dengue**

Dendritic cell specific intercellular adhesion molecule grabbing 3 non integrin (DC-SIGN) helps the DENV to infect DCs while Fcγ Receptor II A (FcγRIIA) augments DENV infection of monocytes during secondary infection through antibody dependent enhancement (ADE) process. Single nucleotide polymorphisms (SNPs) in the genes coding for these receptors are known to influence the expression and function of the receptors. An SNP (rs4804803) in the promoter region of the gene CD209 that codes for DC-SIGN has been shown to be associated with DHF in Thai and Taiwanese populations while the same SNP showed no association with DHF in Brazilian and Indian populations. An SNP (rs1801274) in the FCGR2A gene, which substitutes arginine with histidine in 131st position of FcγRIIa, is known to affect binding of IgG antibodies to FcγRIIa and might influence ADE. The arginine variant of the gene has been shown to be associated with protection to DHF in Vietnamese children and a Cuban population. The histidine variant of the gene has been shown to be associated with bleeding symptoms and persistence of clinical symptoms in the Cuban population. In contrast, the same histidine variant was shown to be associated with protection to symptomatic infection in a Mexican population.

**Polymorphisms in the genes related to innate immunity and severe dengue**

After its entry, DENV virus is recognized by pattern recognition receptors (PRR). PRRs such as toll like receptor (TLR) -3, -7 and -8 are known to recognize RNA genome of the virus in the endosomes and initiate the expression of antiviral response. Apart from TLRs, retinoic acid
Genetics of susceptibility to severe dengue

Inducible gene (RIG)-1 family of receptors (RLRs) such as RIG-1 and myeloid differentiation factor are known to recognize viral RNA in the cytosol and initiate the innate immune response. Innate immune response culminates in the production of type I interferons (IFN), which in turn induces the expression of antiviral proteins, including oligoadenylsynthetases (OAS). OAS activates the enzymes that degrade the viral RNA. Genetic variations in the genes coding for TLRs, RLRs, associated signaling proteins and OAS are known to affect innate immune response and hence susceptibility to severe dengue. The so-called T allele of rs377529, a coding region SNP in the TLR3 gene has been shown to be associated with protection to DHF in an Indian population. The C/T genotype of rs669260, an intronic SNP in DDX58 gene, which codes for RIG-1, has been shown to be associated with susceptibility to DHF in an Indian population. Haplotypes of OAS (OAS1-OAS3-OAS2) gene cluster was found to be associated with clinical outcomes of DENV infection in an Indian population. A recent study on OAS gene polymorphisms in a Thai population has revealed that OAS3 S381R variant, which demonstrated strong antiviral activity in cell culture, was associated with dominant protection to shock in patients infected with DENV-2. In a Brazilian population, SNPs in the gene coding for janus kinase (JAK)-1, which is a signaling protein associated with type I IFN receptor, were found to be associated with DHF. In an Indian population, an SNP (rs8177374 C/T genotype) in the coding region of the gene coding for toll-interleukin-1 receptor domain containing adapter protein (TIRAP) was found to be associated with DHF.

Various C type lectins such as mannose binding lectin (MBL) and C type lectin domain family member 5 A bind to DENV to initiate an inflammatory response. Deficiency of MBL, a complement component, has shown to be associated with DHF in an Indian population. The wild type genotype of a coding region SNP in the MBL2 gene, associated with higher levels of MBL, was observed to be associated with protection against thrombocytopenia in dengue infection in a Brazilian population. Interaction of DENV with a CLEC5A stimulates the release of pro-inflammatory cytokines. Blockade of CLEC5A suppresses pro-inflammatory cytokine response and DENV induced plasma leakage in a mouse model. In a Brazilian paediatric population, T/T genotype of an SNP (rs1285933) in the CLEC5A gene was associated with severe dengue. It was also demonstrated that CLEC5A SNPs regulated tumor necrosis factor (TNF)-α production in severe dengue disease.

Complement system, which is composed of about 30 proteins, has been reported to be an important player in the innate defence against DENV. Altered regulation of complement activation has been implicated in dengue disease pathogenesis. Complement factor H (CFH) is an important regulator of complement activation. CFH levels are influenced by SNPs in the gene coding for CFH. In a Brazilian population, T allele of rs375334, a promoter region SNP, correlated with higher levels of CFH, has been shown to be associated with protection against severe dengue. In contrast, complotype involving SNPs in the genes coding for factor B, C3 and CFH was not associated with dengue in a Thai population.
Genetic variations in natural killer cell receptors and its association with dengue

Natural killer (NK) cells play an important role in the innate immunity against infectious diseases. NK cells have an array of receptors to help them in discriminating between self and non-self and regulate NK cell response. Killer cell immunoglobulin like receptors (KIR) and major histocompatibility class I related molecules (MIC) are important among them and are polymorphic. MICA*008 and MICB*008 alleles were reported to be associated with symptomatic dengue in a Cuban population and the association was stronger in DF cases versus asymptomatic controls. In a Brazilian population, presence of KIR2DL5 and KIR2DS1 genes were associated with DF. The AA genotype of the KIR gene complex was associated with protection to DF. A study from India has shown that KIR3DL1/KIR3DS1 loci influence the risk of developing mild DF.

Human leukocyte antigens and severe dengue

Human leukocyte antigen (HLA) class I and class II molecules expressed by antigen presenting cells are involved in presenting viral peptides (epitopes) to CD4+ and CD8+ T-cells and activating them to act against virus infected cells. Humans differ in the composition of the HLA alleles they possess and this difference contributes to the interindividual variation in the repertoire of viral epitopes presented to the T-cells and hence the quality of the immune response generated and susceptibility to clinical outcomes of viral infection. HLA alleles have been shown to be associated with susceptibility to many infectious diseases. Numerous studies in dengue also have implicated the association of HLA class I and class II alleles with dengue disease severity (see Table 1).

In a Vietnamese population, HLA-A*24 allele was found to be associated with susceptibility to DHF while HLA-A*33 was associated with protection to DHF. Specifically, HLA-A*24 with histidine at codon70 was associated with severe forms of dengue while HLA-DRB1*09:01 was associated with protection to severe dengue. A preliminary study on HLA antigens in DHF cases revealed the association of HLA-A1 and HLA-A9 with susceptibility to primary DHF and HLA-B13 and HLA-B15 with protection to grade II DHF in secondary infections. In an ethnic Thai population, in secondary infections, HLA-A*02:03 was associated with DF while HLA-A*02:07 was associated with DHF in DENV-1 and DENV-2 infections. Among the HLA-B alleles, HLA-B*51 was associated with DHF while HLA-B*52 was associated with DF in secondary infections. A recent study on the analysis of HLA supertypes in dengue cases from the Thai population revealed the association of HLA-B*44 supertype with protection to DHF and HLA-A*02 and HLA-A*01/*03 supertypes with susceptibility to DF in secondary infections. Moreover, HLA-B*07 supertype was associated with susceptibility to DHF in secondary infection. In a Sri Lankan population, HLA-B*31 and HLA-DRB1*08 alleles were associated with DSS in secondary infection while HLA-A*24 was associated with DHF in
**Table 1:** Association between HLA alleles and clinical outcomes of dengue reported in different populations

<table>
<thead>
<tr>
<th>Population</th>
<th>Associated HLA allele/genotype/supertype</th>
<th>Nature of dengue disease severity</th>
<th>Nature of association</th>
<th>Immune status</th>
<th>Dengue virus serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thailand</td>
<td>HLA-A1 and HLA-A9</td>
<td>DHF</td>
<td>Susceptibility</td>
<td>Primary</td>
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<tr>
<td></td>
<td>HLA-A2 and HLA-B blank</td>
<td>DHF</td>
<td>Susceptibility</td>
<td>Secondary</td>
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<tr>
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<td>DHF grade II</td>
<td>Protection</td>
<td>Secondary</td>
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</tr>
<tr>
<td></td>
<td>HLA-A*24</td>
<td>DHF</td>
<td>Susceptibility</td>
<td>Secondary</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>HLA-A*33</td>
<td>DHF</td>
<td>Protection</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Vietnam</td>
<td>HLA-A*24</td>
<td>Symptomatic disease</td>
<td>Protection</td>
<td>Secondary</td>
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</tr>
<tr>
<td>Thailand</td>
<td>HLA-A*02:03</td>
<td>DF</td>
<td>Susceptibility</td>
<td>Secondary</td>
<td>All</td>
</tr>
<tr>
<td></td>
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<td>DHF</td>
<td>Susceptibility</td>
<td>Secondary</td>
<td>1 &amp; 2</td>
</tr>
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<td>Secondary</td>
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</tr>
<tr>
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<td>HLA-B*52</td>
<td>DF</td>
<td>Susceptibility</td>
<td>Secondary</td>
<td>1 &amp; 2</td>
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<td>Brazil</td>
<td>HLA-DQ1</td>
<td>DF</td>
<td>Susceptibility</td>
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<td>Unknown</td>
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<td>Mexico</td>
<td>HLA-DRB1*04</td>
<td>DHF</td>
<td>Protection</td>
<td>Overall and Secondary</td>
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<tr>
<td>Cuba</td>
<td>HLA-A<em>31 and HLA-B</em>15</td>
<td>DHF</td>
<td>Susceptibility</td>
<td>Secondary</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>HLA-DRB1*07</td>
<td>DHF</td>
<td>Protection</td>
<td>Secondary</td>
<td>2</td>
</tr>
<tr>
<td>Jamaica</td>
<td>HLA-A<em>24 and HLA-DRB5</em>01/02</td>
<td>DF</td>
<td>Susceptibility</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>HLA-A<em>23, HLA-Cw</em>04, HLA-DQ81<em>02, HLA-DQ81</em>03 and HLA-DQ81*06</td>
<td>DF</td>
<td>Protection</td>
<td>Unknown</td>
<td>Unknown</td>
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<tr>
<td>Population</td>
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<td>Nature of dengue disease severity</td>
<td>Nature of association</td>
<td>Immune status</td>
<td>Dengue virus serotype</td>
</tr>
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<tr>
<td>Venezuela</td>
<td>HLA-B<em>15 and HLA-B</em>37</td>
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<td>Vietnam</td>
<td>HLA-A<em>2402/02/13 and HLA-B</em>35</td>
<td>DF and DSS</td>
<td>Protection</td>
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<td>Unknown</td>
</tr>
<tr>
<td>Mexico</td>
<td>HLA-DQB1*09:01</td>
<td>DF</td>
<td>Protection</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>HLA-A<em>02:02,03:02,05:01 and HLA-DRB1</em>08</td>
<td>DSS</td>
<td>Susceptibility</td>
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<td>Unknown</td>
</tr>
<tr>
<td>Brazil</td>
<td>HLA-A*01</td>
<td>DHF</td>
<td>Protection</td>
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<td>Unknown</td>
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<tr>
<td>Brazil</td>
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<td>DF</td>
<td>Protection</td>
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<td>Unknown</td>
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<tr>
<td>Brazil</td>
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<td>Protection</td>
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<td>Unknown</td>
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<tr>
<td>Brazil</td>
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<td>Symptomatic dengue</td>
<td>Protection</td>
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<td>Malaysia</td>
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<td>Overall</td>
<td>Unknown</td>
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<tr>
<td>Malaysia</td>
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<td>DGH</td>
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<td>HLA-B*48</td>
<td>DGH</td>
<td>Susceptibility</td>
<td>Secondary</td>
<td>Unknown</td>
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</tbody>
</table>
### Table: Genetics of Susceptibility to Severe Dengue

<table>
<thead>
<tr>
<th>Population</th>
<th>Associated HLA allele/genotype/supertype</th>
<th>Nature of Dengue Disease Severity</th>
<th>Nature of Association</th>
<th>Immune Status</th>
<th>Dengue Virus Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>HLA-DRB1<em>07 allele and HLA-DRB1</em>07/*15 genotype</td>
<td>DHF</td>
<td>Susceptibility</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>HLA-A*33</td>
<td>DF</td>
<td>Susceptibility</td>
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<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>HLA-B<em>18 and HLA-Cw</em>07</td>
<td>Symptomatic dengue</td>
<td>Susceptibility</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Thailand</td>
<td>HLA-B*44 supertype</td>
<td>DHF</td>
<td>Protection</td>
<td>Secondary</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>HLA_A<em>02 and HLA-A</em>01/*03 supertype</td>
<td>DF</td>
<td>Susceptibility</td>
<td>Secondary</td>
<td>Unknown</td>
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</tbody>
</table>

References: 27-44.
primary infection. In a Malaysian population, HLA-B*13 was associated with severe dengue while HLA-B*18 was associated with protection to progression against severe dengue. In an Indian population, HLA-A*33 was associated with DF while HLA-B*18 and HLA-C*07 were associated with symptomatic dengue. Among the HLA class II alleles, HLA-DRB1*07 allele and HLA-DRB1*07/*15 genotype were associated with DHF. In a Philippines paediatric population, HLA-A*33:01 was found to be associated with protection from severe dengue.

Studies carried out in Brazilian dengue cases reported the association of HLA-A*01 with susceptibility to dengue. HLA-B*44 was found to be associated with susceptibility to DHF in secondary infections with DENV-3. Among the HLA class II alleles, a study reported higher frequency of HLA-DQ1 in Brazilian DF and symptomatic dengue cases. In a Mexican population, HLA-DRB1*04 was associated with protection to DHF. Another study from Mexico reported the association of HLA-DQB1*03:02 with DHF and HLA-DQB1*02:02 with DF. Studies carried out in Cuba reported the association of HLA-A*31 and HLA-B*15 with susceptibility to DHF while HLA-DRB1*04 and HLA-DRB1*07 were associated with protection to DHF. The association of HLA-B*15, HLA-DRB1*04 and HLA-DRB1*07 in secondary infections were significant.

Apart from HLA alleles, genetic variants in the gene coding for molecules involved in the processing and presentation of antigens are also known to influence susceptibility to infectious diseases. A study from southern India has reported the association of polymorphisms in the genes coding for transporters associated with antigen presentation (TAP) with severe dengue. Coding region polymorphisms at the 333rd and 637th positions of TAP1 protein and at the 379th position of TAP2 protein were found to be associated with severe dengue in primary infection.

### Cytokine and chemokine gene polymorphisms and their association with dengue disease outcomes

Cytokines are immune mediators produced by the immune cells that contribute to the immune response. Cytokines have been reported to be the major contributors in the pathogenesis of dengue. Elevated levels of pro-inflammatory cytokines such as TNF-α and IFN-γ and anti-inflammatory cytokines such as interleukin (IL)-10 and tumour growth factor (TGF)-β have been reported in DHF cases by multiple studies. SNPs in the cytokine genes affect their production and function. Numerous studies have investigated the association of cytokine gene polymorphisms with dengue disease severity (see Table 2).

Multiple studies reported the association of genotype/alleles of TNF gene, correlated with higher TNF-α levels, with DHF or bleeding manifestations in Thai, Cuban, Mexican and Venezuelan dengue cases. TNF gene haplotypes involving rs1800629, rs361525 and rs1800610 were found to be differentially associated with DHF in the context of immune
### Table 2: Cytokine gene polymorphisms and their association with dengue disease severity in different populations

<table>
<thead>
<tr>
<th>Population</th>
<th>Gene</th>
<th>SNP</th>
<th>Associated allele/genotype/haplotype</th>
<th>Form of dengue and nature of association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venezuela and Cuba</td>
<td>TNF</td>
<td>rs1800629</td>
<td>A allele</td>
<td>Susceptibility to DHF</td>
</tr>
<tr>
<td>Malaysia</td>
<td>TNF</td>
<td>rs1800629</td>
<td>A allele and GA genotype</td>
<td>Protection to DHF/DSS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs361525</td>
<td>A allele and GA genotype</td>
<td>Susceptibility to DHF/DSS</td>
</tr>
<tr>
<td>Thailand</td>
<td>TNF</td>
<td>rs1800629</td>
<td>A allele</td>
<td>Susceptibility to risk of bleeding</td>
</tr>
<tr>
<td>India</td>
<td>TNF</td>
<td>rs1799964</td>
<td>CC genotype</td>
<td>Susceptibility to symptomatic dengue</td>
</tr>
<tr>
<td></td>
<td>IL8</td>
<td>rs4973</td>
<td>AT genotype</td>
<td>Susceptibility to DHF</td>
</tr>
<tr>
<td></td>
<td>IL10</td>
<td>rs1800871</td>
<td>AG genotype</td>
<td>Susceptibility to DHF</td>
</tr>
<tr>
<td></td>
<td>IL17F</td>
<td>rs763780</td>
<td>TC genotype</td>
<td>Susceptibility to symptomatic dengue</td>
</tr>
<tr>
<td>Cuba</td>
<td>TGFB1</td>
<td>rs1800471</td>
<td>G allele and GG genotype</td>
<td>Protection to DHF</td>
</tr>
<tr>
<td>Brazil</td>
<td>IL6</td>
<td>rs1800795</td>
<td>GC genotype</td>
<td>Protection to DF</td>
</tr>
<tr>
<td>Taiwan</td>
<td>TGFB1</td>
<td>rs1800469</td>
<td>CC genotype</td>
<td>Susceptibility to DHF</td>
</tr>
<tr>
<td>Thailand</td>
<td>TNF</td>
<td>rs1800629,</td>
<td>G-G-A haplotype (TNF-3)</td>
<td>Susceptibility to primary DHF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs361525 and</td>
<td>G-A-G haplotype (TNF-4)</td>
<td>Susceptibility to secondary DHF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs1800610</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thailand</td>
<td>ILB</td>
<td>rs1143627</td>
<td>C allele carriage</td>
<td>Susceptibility to DSS</td>
</tr>
<tr>
<td></td>
<td>IL1RA</td>
<td>86 bp VNTR</td>
<td>2/4 genotype</td>
<td>Susceptibility to DSS</td>
</tr>
</tbody>
</table>

References 46-53.
status. In contrast to earlier studies, the allele/genotype of TNF gene, correlated with higher TNF-α levels, was associated with protection to DHF in a Malaysian population. In an Indian population, combinations of high TNF-α producing genotypes and HLA class I and class II alleles were associated with DHF. Apart from TNF-α, IL-1β, another pro-inflammatory cytokine, has also been implicated in dengue disease pathogenesis. A study from Thailand reported the association of IL1B rs1143627 C allele carriage with susceptibility to DSS. The same study also reported the association of 86 base pair repeat polymorphisms (2/4 genotype) in the gene coding for IL-1 receptor antagonist with susceptibility to DSS. IL-8, another inflammatory cytokine, levels have been constantly reported to be elevated in DHF cases. The heterozygous genotype of IL8 rs4973 was found to be associated with protection to DHF in an Indian population.

SNPs in the anti-inflammatory cytokine genes such as IL10 and TGFB have also been studied in dengue disease pathogenesis. Alleles/genotypes of promoter region polymorphism (rs1800871) in IL10 gene, correlated with low levels of IL-10, have been shown to be associated with susceptibility to DHF in a Cuban population. In an Indian population, heterozygous genotype of IL10 rs1800871 polymorphism was found to be associated with protection to DHF. The CC genotype of rs1800469, a promoter region SNP in the TGFB gene, was found to be associated with DHF and DENV-2 viral load in a Taiwanese population. In a Cuban population, the GG genotype and ‘G’ allele of rs1800471, a coding region SNP at the 25th codon of TGFB gene, was observed to be associated with protection to DHF.

When the combinations of cytokine gene polymorphisms were analysed, combinations of genotypes correlating with higher pro-inflammatory cytokine levels and lower anti-inflammatory cytokines were observed to be associated with susceptibility to DHF in Cuban and Indian populations.

Vitamin D is an immunomodulator known to enhance innate immune response and suppress adaptive immune response. Vitamin D acts through the vitamin D receptor (VDR). Many studies have related vitamin D and its related molecules in the pathogenesis of dengue. VDR gene polymorphisms have been shown to be associated with many infectious diseases. A study from Vietnam reported the association of VDR rs731236 C allele with protection to severe dengue. In Western India, VDR rs222870 T allele in a dominant mode was associated with susceptibility to DHF.

Genome-wide association studies and severe dengue

In the era of genomics, genome-wide association studies (GWAS) investigating thousands of SNPs have been successful in identifying the disease loci and subsequently help in pinpointing the causal variants by fine mapping studies. A GWAS was carried out in 2008 paediatric DSS cases and 2018 controls from Vietnam. A replication study involving the most significantly associated markers was independently carried out in 1737 DSS cases and 2934 controls from Vietnam. These studies identified a susceptibility locus at MICB in the chromosome 6.
involving an SNP (rs3132468) that is located outside the HLA class I and class II loci. Another variant (rs3765524) within phospholipase C, epsilon 1 (PLCE1) gene located in chromosome 10 was also found to be associated with DSS. An extended study on the identified SNPs in a larger cohort of dengue cases and controls revealed that these SNPs were not only associated with DSS but also with less severe clinical phenotypes.58

Challenges in studying immunogenetics of dengue

Many of the studies dissecting the genetics of susceptibility to severe dengue are underpowered. Further, these studies are complicated by the circulating serotypes/genotypes in different geographical regions; varying disease profile in the Americas, South East Asia and the Indian subcontinent; immune status of the studied patients; and the dilemma as to whom to use as healthy controls. Genotypes of the circulating serotype vary from place to place and, in many endemic regions, all the four serotypes are circulating. Disease profile in South East Asia is characterized by a large number of DSS cases and mainly children are the most affected. In the Americas and India, the disease profile is characterized by a large number of DF cases, DHF among severe cases and young adults are the most affected.59–60 In most of the studies, data is not available for infecting serotype and immune status, which might contribute to inconsistencies observed between different studies.

Many studies have used cord blood samples or dengue negative healthy controls while a few have used healthy controls dominantly consisting of seropositive individuals without a history of hospitalization. A small number of studies have used asymptomatic healthy controls. However, the definition of asymptomatic itself might have issues since there could be a recall bias related to very mild symptoms among the asymptomatic subjects. Using seronegative healthy controls might reduce the power of study since there is a possibility that the seronegative subjects, if infected, might progress to DF or DHF. Hence a uniform criterion should be defined for selection of controls used in the dengue immunogenetic studies. The appropriate way is to use cases with mild disease as controls. Care must be taken to match these controls with severe cases for immune status and infecting serotype. Matching for immune status between controls (mild disease) and cases (severe disease) is possible if the subjects are sampled during the acute phase of the disease. However, matching for serotype might be an issue in places where multiple serotypes are circulating. Unless the blood samples are obtained within four to five days of onset of disease, detecting the serotype may not be possible. However, recent studies have suggested that patients secrete viruses in their urine samples after the appearance of IgM antibodies in blood.61 This strategy of detecting infecting serotype using urine sample can be utilized to enhance the number of study subjects with serotype data. Moreover, delineating T-cell response against serotype specific epitopes might help in determining the infecting serotypes and those serotypes that are involved in previous infections.
Hence, more prospective studies with sufficiently powered sample size in different populations integrating data on immune status, infecting serotype and the number of serotypes the patients are exposed to might help in delineating the susceptibility factors against severe dengue.

**Immunogenetics of dengue: implications for vaccine design, prognosis and therapeutics**

Studies carried out in different populations have reported the HLA alleles that are associated with protection and those that are associated with susceptibility to severe dengue. HLA alleles have also been reported to be associated with vaccine response in other viral diseases and might have relevance for dengue vaccine studies. Investigation of CD8+ T-cell response in dengue cases has demonstrated that HLA class I alleles, associated with protection to severe dengue, restrict epitopes, which induce polyfunctional T-cell response of higher magnitude. HLA alleles, associated with susceptibility to severe dengue, restrict epitopes, which induce a weak T-cell response that lacks multifunctionality. A recent study on DENV-specific CD4+ T-cells revealed that the virus-specific cells were highly polarized, with a strong bias towards a CD4 cytolytic phenotype, and these cells correlated with a protective HLA DR allele. The epitopes restricted by protective HLA alleles include both conserved and serotype specific epitopes. Thus, identification and characterization of conserved and serotype specific epitopes which are restricted by multiple HLA alleles prevalent in different populations, might be useful in vaccine design. Recent vaccine trials based on recombinant live attenuated tetravalent chimeric yellow fever dengue vaccine (CYD) reported varying vaccine efficacy for different serotypes. Whether host genetic factors also contribute to varying vaccine efficacy observed in the vaccine trials needs further investigations. CYD lacks non structural proteins that are targeted by T-cells and hence the vaccinees lack T-cell immune response against dengue. Serotype specific and conserved epitopes restricted by multiple HLA alleles can supplement envelope protein based dengue vaccines.

Moreover, various non-HLA gene polymorphisms identified to be associated with severe dengue, if replicated in multiple prospective cohort studies, can serve as markers to predict severe dengue and help in the clinical management of dengue cases. Genetic studies have identified the role of genes coding for virus entry receptors, PRRs, innate immune and inflammatory mediators in the pathogenesis of severe dengue. Hence, it is possible to identify the mechanism of association of genetic variants with severe dengue, which might have implications for developing therapeutics for severe dengue targeting these gene products.

**Conclusion**

Susceptibility to severe dengue is a polygenic phenomenon and is influenced by gene–gene and gene environment (infecting serotype, immune status and other factors) interactions.
Multiple prospective cohort studies with data on infecting serotype and immune status might lead to understanding the pathogenesis of severe dengue. Such results might have implications for vaccine design, prognosis and therapeutics against severe dengue.

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References


Genetics of susceptibility to severe dengue


Temporal spatial distribution of dengue and implications on control in Hulu Langat, Selangor, Malaysia

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\textsuperscript{*}Petaling District Health Office, Selangor, Malaysia

Abstract

Dengue has continued to be one of the most important public health problems in Malaysia. Dengue infections and mortalities have remained at a high level. By combining Geographic Information System (GIS) and ovitrap surveillance, this study aims to discover the spatial patterns of dengue infections and also the association between ovitrap monitoring. This is a retrospective review involving all confirmed dengue cases and ovitrap data from 2009 to epidemiology week 10 in 2012, from Hulu Langat, Selangor and a total of 6907 cases were analysed using ArcGIS version 10.0 and SPSS version 19.0. Results showed age group of 21 to 30 had the highest number of cases at 1866 (27%) cases, majority were male with 3948 (57.2%) cases and Malay with 3908 (56.6%) cases. Kajang had the highest number of ovitrap placement at 257 (39.0%) and has higher level of indexes. The majority of cases were located in Ampang, followed by Cheras and Kajang. Ovitrap Index did not have a significant relationship between low or high risk areas for dengue ($\chi^2=2.847$, p=0.092). Spatial global pattern analysis by average nearest neighbour resulted in nearest neighbour ratio of less than 1 with Z-score ranging between 68.17 to -14.51 and p value of <0.001. When mapping clusters with hotspot analysis (Getis-Ord Gi), hotspots were identified. Hotspot areas fell on the northwest, west, southwest and the biggest area at the northeast side of the Hulu Langat district. This study demonstrated significant spatial patterns of clustering of dengue cases in Hulu Langat district. Integration of GIS application with dengue cases and ovitraps in the Hulu Langat district can provide a new insight regarding incidence of dengue and vector populations.

Keywords: Dengue, cluster, ovitrap, spatial analysis

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Introduction

In 2012, report by World Health Organization (WHO) showed dengue ranks at the top as the world’s most important mosquito born viral disease. The emergence and spread of all four serotypes (DEN 1, DEN 2, DEN 3 and DEN 4) represent a global pandemic threat. The incidence of dengue has also increased 30-fold during the past five decades. Yearly, around 50-100 million new reported infections occur in more than 100 endemic countries. Western Pacific Region of WHO ranks the top in both cases reporting and frequency of epidemics. Over 90% of the total dengue cases reported in this region are contributed by Cambodia, Malaysia, Philippines and Vietnam. Malaysia alongside Thailand, Vietnam, Laos and Singapore are presumably known as the clusters for dengue infections in the Asia-Pacific region.

Since the first reported case of dengue in 1901 and dengue haemorrhagic fever in 1962, dengue has remained as one of the most important public health problems in Malaysia. In a decade, dengue infections and mortalities have increased with the second half of the decade show overall incidence have stabilized but remained at a high level. Malaysia is the only country which has this trend compared to other countries in the Western Pacific region. Recently in 2014, Malaysia had the worse epidemic with a historical 240% increase in cases and almost similar increase in numbers of deaths compared to the same period in 2013. Until the end of November 2014, Malaysia had total cases of 93 402 and 178 deaths. The predominance of dengue cases has also shifted from children to adults.

Given the unique biological characteristic of Aedes populations, trapping eggs via the ovitrap method has become the easiest and cost effective surveillance for Aedes. Many studies have proven that ovitrap surveillance could be used for the prediction of dengue outbreak and is highly recommended as a surveillance tool. In fact, ovitrap allows better assessment of infestation densities as compared to the conventional larvae search methods which do not provide information regarding mosquito population density.

Geographic Information System (GIS) has allowed the integration of environmental and disease elements associated with mosquito breeding patterns. By using GIS, areas of dengue incidence can be detected with great accuracy and urgent prevention measures can be taken to tackle this disease. GIS is beneficial in improving the cases prediction capability and strengthening preventive measures through good resources allocation.

This research can contribute useful information regarding vector control programmes to reduce the burden of this disease. The Hulu Langat district reports a high number of dengue cases yearly and contributes significantly to the total cases in Malaysia and the state of Selangor. The benefit of this analysis will be better planning of preventive actions that can be more focused on the affected areas. This study also highlights the role of ovitraps. By combining GIS and ovitrap surveillance, this study aims to discover the spatial patterns of
dengue infections and also the association between ovitrap monitoring. Correct identification of the pattern and its significance will help the district in effectively planning and implementing prevention and control programmes.

Methodology

Hulu Langat is the fifth largest district in the state of Selangor on the west coast of Peninsular Malaysia, which is located between Putrajaya and Kuala Lumpur. It is made up of seven mukim (subdivisions), which are Ulu Langat, Ampang, Cheras, Ulu Semenyih, Kajang, Semenyih and Beranang. Ulu Langat and Ulu Semenyih are partly covered by forest and hilly areas. Ampang and Cheras have more infrastructures involving high human activities like shopping centres, offices and residential areas. Kajang has abundant residential areas, schools, higher learning centres and small scale industries. Semenyih and Beranang have growing township area with some areas of small industries.

According to the 2010 census, Hulu Langat district has a total population of 1,138,198. Ampang and Kajang are among the highest populated subdivisions in Hulu Langat with populations of 342,676 and 342,657 respectively. This district has a mixed population of urban and rural settlements. The majority of the population is centred in towns near Kuala Lumpur. Hulu Langat has more cases of dengue than almost any other district in the country. Vector control programme is currently done by the Hulu Langat District Health Office located in Kajang.

This is a retrospective review involving all confirmed dengue cases and ovitrap data from 2009 to epidemiology week 10 in 2012, from Hulu Langat district. Approval for research has been obtained from the Hulu Langat District Health Office.

Vector density estimation is based on ovitrap index (OI). OI is the number of positive *Aedes* ovitraps over the total number of recovered ovitraps determined from each ovitrap surveillance done in percentage. Ovitrap index was divided into 4 levels. Level 1 is OI less than 5%, Level 2 is between 5 to 19%, Level 3 is between 20 to 39% and Level 4 is above 40%.

Data analysis for descriptive and analytical purposes was done by using *Statistical Package for Social Sciences* (SPSS) Version 19.0. GIS spatial data was analysed using ArcGIS software version 10. Spatial analyses included mean centre, average nearest neighbour and hotspot analysis (Getis-Ord Gi). Mean centre is calculated by finding the average of the x-coordinate of all the features, then finding the average of all the y-coordinate values. The resulting x, y coordinate pair is the mean centre. Mean centre indicates a coordinate that has the shortest total distance from other case coordinates. This can be useful for determining the movement of a set of data over time. In this study, mean centre can show movement of dengue cases over a period of time.
Average Nearest Neighbour is an index calculated to reveal the average distance of a case to all its neighbours compared to the average distance for a random distribution. This technique for pattern analysis will answer the question whether the data is assumed to be clustered, dispersed or occurred due to random chance. Nearest neighbour ratio of less than 1 points towards a more clustering pattern, while ratio of more than 1 points towards a more dispersed pattern. Getis-Ord Gi statistic will give a Z score value that will reveal spatial features with either high or low value cluster. The greater the Z score value, the more extreme the clustering of hotspot, while with lesser Z score value, the clustering of low values or coldspot will be more concentrated. Utilization of the Average Nearest Neighbour analysis in GIS can show a clustered or dispersed pattern of dengue cases in the studied area and determine whether it is significant or not. Dengue cases hotspot areas can also be revealed by using the Getis-Ord Gi statistic in GIS.

Results

Dengue cases in the district showed age distribution more at the younger adult age group. Age group of 21 to 30 had the highest number of cases which contributed to 1866 cases, which was 27.0% of all the cases. The oldest case was found to be at the age of 84 years old. There were more male cases compared to females, which contributed to 3948 cases and was 57.2% of all cases. Malay holds the majority cases with 3908, which was 56.6% of all the dengue cases in Hulu Langat (Table 1).

Kajang had the highest number of ovitrap settled up in a particular locality at a given time, which was 257 (39.0%) and has higher level of indexes. The lowest number of ovitrap was in mukim Ulu Semenyih. According to the level of Ovitrap index % (OI), Level 3 had the highest number with 252 (38.2%). Level 1 was the least found (Table 2).

There were not many changes in numbers over the years in dengue cases trend. From 2009 to 2011, there were 2263, 2404 and 2043 cases respectively with incidence rate of 198.82, 211.21 and 179.49 per 100 000 population. Number of deaths in those years was 12, 3 and 7 respectively from the year 2009 to 2011.

In general, by looking at the trend of cases, there would appear to be more cases during start of the year compared to at the end, with minor dengue cases fluctuation between weeks. Ovitrap index level seems to follow the trend of dengue cases except at some particular weeks. For example, week 33-35 and 50-51 had high number of ovitrap indexes but cases do not appear to be as high as expected. However, there is a limitation in this aspect because the ovitraps are not consistently placed at a particular area every week and might not represent the real trend between dengue cases and ovitrap index.

The top three dengue cases were notified from Ampang with 2571 (37.2%), followed by Cheras with 1631 (23.6%) and Kajang with 1290 (18.6%). Higher ovitrap indexes and
Temporal spatial distribution of dengue and implications on control in Hulu Langat, Selangor, Malaysia

Table 1: Characteristics of dengue cases’ distribution from 2009 to March 2012

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of cases (N= 6907) n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age in years</strong></td>
<td></td>
</tr>
<tr>
<td>Under 5 years old</td>
<td>207 (3.0)</td>
</tr>
<tr>
<td>5 to 10 years old</td>
<td>462 (6.7)</td>
</tr>
<tr>
<td>11 to 20 years old</td>
<td>1622 (23.4)</td>
</tr>
<tr>
<td>21 to 30 years old</td>
<td>2055 (29.8)</td>
</tr>
<tr>
<td>31 to 40 years old</td>
<td>1241 (18.0)</td>
</tr>
<tr>
<td>41 to 50 years old</td>
<td>758 (11.0)</td>
</tr>
<tr>
<td>51 to 60 years old</td>
<td>414 (6.0)</td>
</tr>
<tr>
<td>61 years old and above</td>
<td>148 (2.1)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
</tr>
<tr>
<td>Malay</td>
<td>3908 (56.6)</td>
</tr>
<tr>
<td>Chinese</td>
<td>1741 (25.2)</td>
</tr>
<tr>
<td>Indian</td>
<td>775 (11.2)</td>
</tr>
<tr>
<td>Others</td>
<td>483 (7.0)</td>
</tr>
<tr>
<td><strong>Mukim (Subdivisions)</strong></td>
<td></td>
</tr>
<tr>
<td>Ampang</td>
<td>2571 (37.2)</td>
</tr>
<tr>
<td>Cheras</td>
<td>1631 (23.6)</td>
</tr>
<tr>
<td>Kajang</td>
<td>1285 (18.6)</td>
</tr>
<tr>
<td>Semenyih</td>
<td>869 (12.6)</td>
</tr>
<tr>
<td>Beranang</td>
<td>332 (4.8)</td>
</tr>
<tr>
<td>Ulu Langat</td>
<td>219 (3.2)</td>
</tr>
<tr>
<td>Ulu Semenyih</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

numbers were noticed in Kajang area. Ampang showed less ovitrap monitoring done even though it has the highest number of notified dengue cases.

Ovitraps were placed at all the mukim but only minimal ovitraps were located at the Ulu Langat and Ulu Semenyih area (Figure 1). There was a positive significant correlation between the dengue cases and Ovitrap Index of < 5% with calculated \( r = 0.295, p = <0.05 \), with a \( R^2 = 0.087 \). Regarding Ovitrap Index and dengue risk area, no significant relationship was noted \( (x^2=2.847, p=0.092) \). Low dengue risk area is defined as an area which has less than 10 dengue cases reported while high dengue risk area is defined as an area which has 10 or more dengue cases reported.\(^{15} \)
### Table 2: Ovitrap data distribution from 2009 to 2012

<table>
<thead>
<tr>
<th>Ovitrap</th>
<th>No. of ovitrap at one locality at a given time (N= 659) n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mukim</strong></td>
<td></td>
</tr>
<tr>
<td>Kajang</td>
<td>257 (39.0)</td>
</tr>
<tr>
<td>Semenyih</td>
<td>193 (29.3)</td>
</tr>
<tr>
<td>Cheras</td>
<td>147 (22.3)</td>
</tr>
<tr>
<td>Ampang</td>
<td>39 (5.9)</td>
</tr>
<tr>
<td>Beranang</td>
<td>20 (3.0)</td>
</tr>
<tr>
<td>Ulu Langat</td>
<td>3 (0.5)</td>
</tr>
<tr>
<td>Ulu Semenyih</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Ovitrap index (O.I) level</strong></td>
<td></td>
</tr>
<tr>
<td>Level 1 (OI &lt;5%)</td>
<td>66 (10.0)</td>
</tr>
<tr>
<td>Level 2 (OI 5%-19%)</td>
<td>215 (32.6)</td>
</tr>
<tr>
<td>Level 3 (OI 20%-39%)</td>
<td>252 (38.2)</td>
</tr>
<tr>
<td>Level 4 (OI &gt;40%)</td>
<td>126 (19.1)</td>
</tr>
</tbody>
</table>

**Figure 1:** Ovitrap locations in Hulu Langat from 2009–2012
Temporal spatial distribution of dengue and implications on control in Hulu Langat, Selangor, Malaysia

**Figure 2:** Mean centre and standard distance for dengue cases in 2009 for dengue cases in 2009

**Figure 3:** Mean centre and standard distance for dengue cases in 2010

**Figure 4:** Mean centre and standard distance for dengue cases in 2011

**Figure 5:** Mean centre and standard distance for dengue cases in 2012
From the year 2009 until March 2012, the majority of cases can be clearly seen located in Ampang, followed by Cheras and Kajang (Figure 2 to Figure 5). In this situation, the mean centre was noted to move from north to south from 2009 to 2012. It was located in Cheras in 2009 and moved southwards to Kajang in 2012.

From 2009 to 2012, the z-score ranges from -68.17 to -14.51 and p-values were all significant, which was < 0.001. All the nearest neighbour ratios were less than 1 and this indicates that all the cases are trending towards clustering. From 2009 to 2012, several hotspot locations can be identified. Hotspot locations are situated at Ampang, Cheras, Kajang and the biggest area at the northeast of Hulu Langat district (Figure 6). The majority of the areas in the Hulu Langat district have a high population density which includes places like Ampang, Beranang and Kajang. An area which has a low population density includes a forest reserve, located in mukim Ulu Langat (Figure 7).

**Figure 6:** Hotspot analysis for dengue cases from 2009–2012

Note: Hotspot locations in Hulu Langat district are red-coloured areas in the dashed-red box
In general, Ampang revealed plenty of hotspot areas and also has high ovitrap index scattered at that area. Two areas that did not have any ovitrap monitoring are hotspots. Those places are located at the northeast (Ulu Langat subdivision) and southwest (Kajang subdivision) areas of Hulu Langat district (Figure 8).

**Discussion**

Spatial analysis demonstrated that all the dengue cases are found to be distributed in clusters. It also revealed hotspot locations in the Hulu Langat district. In addition, there was a positive significant correlation between the dengue cases and Ovitrap Index of < 5%.

Figure 7: Population density of Hulu Langat district

[Map showing population density with notes: A – Ampang subdivision, B – Beranang subdivision, C – Kajang subdivision]
Figure 8: Ovitrap Index and hotspot areas in Hulu Langat

It is documented that incidence of dengue will increase in areas with high human activity. Large housing areas, industrial areas and school areas are some of the examples of places where there will be more humans performing their daily activities. In this study, the subdivisions of Ampang, Cheras and Kajang consist of many housing areas, schools and shops. This can certainly contribute to high incidence of dengue cases, as shown by the high trend from 2009 to 2012 at those areas. The local effects of human mobility in an urban area, such as going to the office or school, need better understanding and can be applied to the spatial dynamics of dengue transmission.
Besides places with high human activity, places which are abandoned can also be a breeding ground for mosquitoes. Cemeteries, which can have containers such as flower vases, can be a source for Aedes breeding.\(^2\) Spatial analysis revealed two cemeteries located at hotspot areas in the Hulu Langat district. Therefore, surveillance by ovitrap or control measures such as fogging and placement of larvicides should not be forgotten at these cemeteries.

Other locations that might be of interest for control and prevention of dengue include areas located at the south of Kajang. These places were noted to be hotspots for dengue cases and must be monitored closely. High human activity in these areas and the large area of land that is located close to natural forest make it an ideal place for mosquito breeding. However, we did not include surrounding environmental exposure risks in our research. In areas where clusters of cases appear to be high, they may have an environmental risk pattern which surely will be beneficial to explore such as temperature and rainfall.

Population density also plays a role where highly populated areas can be a high risk area for dengue.\(^{11,17}\) In the Hulu Langat district, subdivisions like Ampang, Beranang and Kajang in particular, showed a high population density. There will be more susceptible patients that will be exposed to Aedes mosquitoes in a highly populated area and this will increase the chances of a person to develop dengue.\(^{18}\)

Concerning vector populations, there was a weak but positive correlation between presence of dengue cases and ovitrap index. Places with high ovitrap indexes located in the Ampang subdivision and Cheras subdivision also recorded high number of dengue cases. As these areas are located in an urban area, cleanliness must be given priority. If garbage disposal management is not done correctly, accumulation of rubbish thrown at the housing areas that can include containers such as cans or tyres will provide a perfect breeding ground for Aedes mosquitoes.

Regarding locations for ovitrap placements, more ovitraps should be placed at the subdivision of Ulu Langat (northeast of Hulu Langat district) and Kajang (southwest of Hulu Langat district) as these are hotspots. Subdivisions of Beranang and south of Kajang do not need to have a lot of ovitraps as these are not found to be hotspots. Lastly, analytical analysis has shown that the Ovitrap Index does not have a significant relationship between low or high risk areas for dengue. Therefore, the placements of ovitraps in the district of Hulu Langat should be revised or changed accordingly from time to time.

Integration of GIS application with dengue cases and ovitraps in the Hulu Langat district can provide a new insight regarding incidence of dengue and vector populations. Spatial analysis done from this study will be useful to health authorities to implement surveillance, control and prevention of dengue. A more focused effort, targeted at hotspot areas identified by the GIS software, can save time and budget in order to reduce incidence of dengue in this district. Future studies that integrate GIS can look into other variables such as socioeconomic factors, rainfall and temperature which may reveal new information regarding dengue. The
next step in this area of research would be to expand the study area to other districts which are known to have persistent dengue hotspots and eventually looking at the national level.

Conclusion

GIS is a tool that can integrate maps of a particular area and data of medical diseases. Therefore, for future use, GIS can be effectively utilized to identify targeted areas for dengue control programmes and benefit the population’s health.

Acknowledgements

The authors would like to thank the Director General of Health Malaysia for permission to publish this paper. We also would like to acknowledge with thanks the assistance of officers from the Hulu Langat District Office for data release to be used in this research.

References


Pathogenesis of dengue associated haematological dysfunction

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Abstract

Dengue virus infection is rapidly emerging to be an important challenge to global public health as the geographical regions affected by the virus as well disease severity have shown significant changes in the recent past. The clinical disease caused by dengue virus infection remains broad spectrum but haematological dysfunctions constitute a major hallmark. Thrombocytopenia, bleeding disorders and peripheral blood leukopenia are common features and severe bleeding with frank capillary leakage and shock develops into dengue haemorrhagic fever/shock syndrome (DHF/DSS) in a subset of individuals. The pathophysiology of the dysfunctional haematology remains incompletely understood. While earlier studies have indicated that several factors like immunopathology and high viremia are important triggers of disease severity, recent studies have revealed out-of-box evidence of dengue virus directly affecting platelets and endothelial cells that could institute early but crucial triggers for tipping the disease equilibrium towards a severe form. These events, primarily, direct binding of dengue virus to human platelets leading to activation and inflammatory responses, including possible support of virus replication by platelet environment, have been dramatic recent findings.\textsuperscript{1,2,3} Moreover, the susceptibility of vascular endothelium to dengue virus and alteration of subsequent endothelial physiology, especially cell adhesion biology and transport could be important in the genesis of capillary dysfunction seen in DSS cases. Collectively, in view of these recent findings, the paradigm shift in potential for developing novel clinical intervention and therapeutic approaches in combating the severe clinical haematological dysfunction associated with dengue infection is rapidly gaining credence as future strategies.

Keywords: Dengue, haematological dysfunction, platelet abnormalities

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Introduction

Dengue is a flavivirus of significant public health importance as is evident from the evolution of the recent outbreaks spanning over a decade that has witnessed more severe disease and haemorrhagic manifestations. According to a few recent studies, approximately 3.6 billion people are at risk of acquiring dengue infections; with approximately 230 million new infections every year, including over 2 million cases of severe disease (DHF/DSS) and 21 000 deaths.\(^4\) Haematological abnormalities are frequently encountered in patients with dengue infections. These include platelet abnormalities such as thrombocytopenia, leukopenia, disseminating intravascular coagulopathies, vascular leakage and other bleeding diathesis that may show varied case presentations. Besides thrombocytopenia, hypofibrinogenemia is also a prominent finding in DHF cases. Although the pathology remains unclear, increased intravascular clotting and low levels of factor II, V, VII, VIII, IX, X and XII have also been documented.\(^5\) The possibility of direct and indirect virus mediated injury to the vascular endothelium is also becoming significant in light of recent findings. The present review explores the current knowledge on our understanding of the pathophysiologic mechanisms involved in the etiology of dengue associated haematological dysfunction.

Dengue associated haematological abnormalities

A typical case of classical dengue fever (DF) presents with a febrile onset associated with severe retro-orbital pain, myalgia, vomiting and fatigue. The fever and related symptoms are usually self-limiting in nature and the patient recovers within a span of a week. The presentation of myopathy can differ from mild to severe deep muscle and joint pain but not radiculopathies. Laboratory function tests usually show a haemogram typical of a viral infection with elevated monocytes but the differential diagnosis lies with thrombocytopenia and mild to severe leukopenia. Serum alanine transferase levels are found to be elevated commonly, but more serious complications such as fulminant hepatic failure or encephalitis are rare.\(^6,7,8\) However, in a fraction of dengue virus infected cases severe haematological abnormalities with life threatening bleeding and vascular leakage are observed, a syndrome collectively termed dengue haemorrhagic fever with shock syndrome (DHF/DSS).

Normal haemostasis is maintained through a fine balance between coagulation and fibrinolysis. The nature of alteration in coagulation factors in cases of DHF in comparison with DF have been studied in detail and the findings suggest that activation of coagulation and fibrinolysis is more acute in DHF/DSS cases when compared with classical DF cases.\(^9\) Frank cases of disseminated intravascular coagulopathy (DIC) are rarely observed in dengue virus infection and in a study involving 40 paediatric cases with different stages of disease, mild to moderate degree of prothrombin deficiency was noted in 15–50% of the cases in grade II and III, respectively, with laboratory confirmed evidence of consumption coagulopathy in 30% of the cases with DSS.\(^10\) Although a study on other coagulation related factors, especially the effect on von Willibrand factor, showed a positive correlation with thrombocytopenia in
a group of paediatric cases with DHF, however, more case controlled studies are required before a conclusive profile on the effect of dengue associated changes in coagulation factor biology can be summarized.

The origin of the thrombocytopenia, leukopenia and transient neutrophil deficiency in dengue disease has been attributed to possible suppression of haematopoiesis directly by DENV inhibiting megakaryocytic differentiation; combined with indirect immune injury this has been suggested to play an important role in the origin of dengue associated thrombocytopenia.1,12,13,14,15

**Platelet abnormalities in dengue disease**

Human blood platelets are anucleated so-called packets of cytoplasm fragmented from megakaryocytes formed from the bone marrow through the process of thrombopoiesis. These organelles are crucial for normal haemostasis and their physiology is carefully orchestrated via a fine balance between ligand activation and intra-platelet signalling events. Interestingly, platelets have mitochondria to suffice their energy requirement but they have a limited life span of 120 days. Platelet dysfunction is very common in DENV infection and is clinically presented as thrombocytopenia, aggregation abnormalities and other dysfunctional behaviour.5,16 While the mechanisms remain incompletely understood, antiplatelet antibodies, abnormalities in coagulation factors and suppression of thrombopoiesis in the bone marrow directly by DENV or by disease pathology have been implicated as major causes.13,17,18 Interestingly, early studies had also shown evidence of direct suppression of thrombopoiesis and haematopoetic suppression in bone marrow of fatal DHF cases.19 In a landmark study carried out in 1989, platelet functions were profiled in 35 paediatric cases with serologically confirmed dengue virus infection. Findings from this study showed abnormal platelet aggregation responses to agonists, increased plasma betathromboglobulin and platelet factor 4. Moreover, the ADP induced aggregation was also seen to be compromised significantly in these cases.20 Direct effects of dengue virus binding to platelets leading to functional abnormalities have not been explored in detail and need further study. In 1995, Wang et al. showed that dengue 2 virus could bind to human blood platelets in presence of virus specific antibodies.21 This observation opened a new vista in exploring the mechanisms of dengue-associated thrombocytopenia that was further supported from the direct evidence of dengue 2 virus binding and physiologically activating platelets.1

Immunopathogenic mechanisms, especially presence of anti-platelet cross reactive antibodies against both viral and platelet antigens, have been postulated, and also experimentally demonstrated to some extent but conclusive evidence is still lacking.22,23 It has been demonstrated that DENV infected vascular endothelial cells attract both platelets and neutrophils to them and through both apoptosis and autoimmune mechanisms lead to destruction of these cells.24 Although evidence of platelet associated antibodies, especially anti-DENV IgM and immune complex deposits, has been shown in patients with severe
dengue disease, the pathophysiology of this process is not clear as patients with very low platelet counts may not present with any bleeding diathesis. The early evidence of immunopathogenic mechanisms as an important component of platelet lesions in dengue disease came from the studies of Funahara, who showed three fundamental observations: (i) DENV antigens could directly associate with platelets; (ii) anti DENV antibody binding to platelet induces thrombocyte destruction; and (iii) modulation of endothelial cell function by DENV. Subsequent studies attempting to characterize the nature of anti-platelet immune response showed evidence of anti-platelet IgM autoantibodies that could be detected even eight to nine months after the initial illness. In 2003, Oishi et al. studied 53 hospitalized patients with confirmed diagnosis of secondary dengue infection and studied the pattern of platelet-associated IgG and thrombocytopenia. Their findings were consistent with the earlier studies of Funahara et al. that anti-platelet antibodies could play an important role in dengue-associated thrombocytopenia. Interestingly, in a subsequent study involving a larger study population of 78 patients, both platelet associated IgG and IgM were studied in confirmed secondary dengue infections with a study design to correlate the incidence of thrombocytopenia. An inverse correlation between platelet counts and pAIgG and pAIgM were found in these patients and the pAIg levels were significantly higher in the patients with clinical presentation of DHF compared with those of DF, further confirming the role of anti-platelet immunglobins in the origin of thrombocytopenia.

The hunt for the elusive cross reactive epitope(s) between DENV proteins and platelets remains controversial. A study in mice model suggested that DENV NS1 protein may have shared epitopes, especially in the C-terminal region in a 251-372 amino acid stretch, the findings need more in-depth studies as definite animal models for DENV are still lacking. The possibility of anti DENV NS1 antibody reacting to a platelet surface enzyme-disulfide isomerase has also been shown.

Paradigm shifts in pathophysiology of dengue associated thrombocytopenia were found in two studies. The first by Anderson et al. who showed that non homologous antibodies to dengue serotypes could enhance platelet binding by the virus immune complex but not affected by presaturating the system with excess purified Fc, suggesting that presence of antibody was essential. The second paper by Ghosh et al. was the first to demonstrate that dengue 2 virus could directly interact and activate normal donor platelets in the absence of any antibodies. These observations have been subsequently supplemented with new data and confirm the direct binding of dengue virus to human platelet as a fundamental event in the pathophysiology of thrombocytopenia.

**Dengue and vascular endothelial cells**

Human vascular endothelial cells are probably one of the most physiologically unique and functionally complicated cells. These cells make up the inner lining of all blood vessels and constitute the major functional barrier between blood and organ parenchyma at the tissue...
level. Although originally thought to be having passive barrier functions, current knowledge on endothelial cell physiology is beginning to understand its manifold functionalities, especially in immune responses and inflammation. Rapid developments in the field of cell culture techniques have further enriched our understanding of vascular endothelial cell biology.

Endothelial cells have been shown to be involved in the pathogenesis of a variety of infectious diseases ranging from tuberculosis, septicemic shock states to severe viral haemorrhagic fevers such as Ebola, Marbug and Hantaan. In the case of severe dengue infections such as DHF and DSS, the role of vascular endothelial cells is gaining prominent ground. Many studies ranging from human autopsies and in vitro experiments suggest that endothelial cells are susceptible to DENV both in vivo and in vitro. Several studies have also suggested that DENV infection of endothelial cells can lead to release of cytokine like IL6 and IL8, induce apoptosis and alter the transcriptome of the cell and affect gene expression. Interestingly, a more heterogenous and differential gene expression profile was observed after DENV infection of microvascular endothelial cell lines like ECV 304 and HMEC. Importantly, the molecular and cellular events that lead to the major physiological alterations of endothelial cells causing haemorrhage and vascular leak still remain incompletely understood. Several novel findings from recent research have suggested that ubiquitine-proteasome pathways, engagement of late endosomal events by the virus and cellular endosomal membrane reorganization, especially through autophagic induction, could constitute several key endothelial stress reactions to DENV. Moreover, alterations in expression of haemostatically important molecules on endothelial cell surface has also been shown but its significance in vivo remains to be elucidated.

The main pathophysiology of the capillary leak in DSS is due to compromised integrity of inter-endothelial cell junctional barrier. Under normal physiological situations, the junctions between endothelial cells are precisely regulated through a well-orchestrated network of signalling pathways involving, Fyk, Syn, FAK kinases and ion channel activities. In DENV infection, in vitro studies have shown that alterations in the beta integrin biology of endothelial cell line HMEC-1 could breach the paracellular integrity. Further showed that the so-called cytokine tsunami associated with severe DEN disease could also lead to increased trans-endothelial electrical resistance and enhanced permeability of endothelial cells. Interestingly, Cardier et al. showed that sera from cases of severe dengue infection, DHF and DSS cases could also activate vascular endothelial cells in vitro, suggesting possible presence of soluble vasoactive factors that might be involved in barrier disruption and haemorrhage. Although DENV can infect different cell types, cells of the monocytic lineage such as Langerhans cells and interstitial DCs are thought to be the primary targets of DENV. Immature monocyte-derived DCs are also susceptible to DV with subsequent activation and release of β3-integrin, which is required for DV entry into human HMEC-1. Permeability alteration of microvascular endothelium is a factor in the plasma leakage produced by DENV infection, and β3-integrin plays a central role in maintaining capillary integrity and regulating vascular permeability vasoactive cytokines such as IL-8 and TNF-alpha.
An injury causes transendothelial migration of the DCs and expression of matrix metalloproteinases that can have deleterious effects on endothelial cell integrity. In vitro, DV suppresses soluble VEGFR2 production by endothelial cells but upregulates surface VEGFR2 expression and promotes response to VEGF stimulation. Luplertlop et al. showed that DV-infected DCs overproduced soluble gelatinolytic matrix metalloproteinase MM9 and MMP2 with loss of the platelet endothelial adhesion molecule (PECAM) expression and increase in vascular permeability. Angiopoetin-2 has also been shown to be upregulated in human umbilical vein endothelial cells, which by itself can induce endothelial hyper-permeability and also further augment inflammation through sensitization to TNF-α. These studies have initiated the need to examine in more detail the possible role of host factors in the origin of dengue-associated vascular pathophysiology.

In summary, the recent findings of dengue virus interacting with both platelets and vascular endothelium leading to altered physiological responses in both might constitute critical and irreversible events that have potential to evolve by engaging other pre-existing pathology of the infection process towards severe bleeding diathesis and capillary leakage characterized by DHF/DSS. More in-depth understanding of these processes poses an immediate future challenge in developing newer intervention strategies and therapeutics.

References


Pathogenesis of dengue associated haematological dysfunction


Pathogenesis of dengue associated haematological dysfunction


Dengue has been primarily an infection of the tropical and subtropical countries, mainly Southeast and South Asia, Central and South America, and the Caribbean, but in the last few years, it is emerging in the United States of America and also making its entry into Europe. Phylogenetic analyses suggest that currently circulating serotypes diverged from their sylvatic ancestors approximately 1000 years ago, while the transmission to humans occurred independently for all the four dengue virus (DENV) types only a few hundred years ago. Recent phylogeography studies attempted identifying the geographic origins of DENV types and their genotypes as well as mapping the dispersion of the viruses globally. Southeast Asia, where all four serotypes have been co-circulating since the Second World War, has been identified as a source population for the rapid and dramatic re-emergence of DENV globally over the last half-century. India’s role during the Second World War and its strategic location at the tip of the Indian Ocean facilitating global movements in the aftermath of the war have been stated to be significant for the propagation of the virus. In recent years, India has further contributed to the dissemination of DENV viruses due to various reasons. With this background, the present review aims at compiling the phylodynamics of the four serotypes of dengue viruses in India vis-à-vis the global scenario, the understanding of which is important in the management and control of the spread of these viruses.

**Keywords:** Dengue types 1-4, India, molecular clock, phylogeography, migration links.

**Introduction**

Dengue is the most prevalent flavivirus infection of the tropical and subtropical countries where the mosquito vectors *Aedes aegypti* and *Aedes albopictus* thrive. The dengue virus (DENV) is a single stranded, positive-sense RNA virus and its genome is composed of three structural genes encoding the nucleocapsid (C) protein, a membrane (M) protein, an envelope glycoprotein (E) and seven non-structural (NS) proteins. There are four serotypes of the virus referred to as DENV-1, DENV-2, DENV-3 and DENV-4, which are antigenically and phylogenetically distinct. The four serotypes are further subdivided into numerous phylogenetically distinct genotypes. All four serotypes can cause the full spectrum of the
disease from a subclinical infection to a mild self-limiting disease, the dengue fever (DF), and a severe disease that may be fatal, the dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS).4

Southeast Asia, where all four serotypes have been co-circulating since the Second World War, has been recognized as a main source-sink for the dispersion of DENV globally over the last half-century.5 The spread of the virus was initially facilitated during the Second World War, by the movement of ships and transport of goods.4 However, the recent past evidenced by remarkable growth in human population, globalization, extensive and unrestrained urbanization, international trade and commerce, and mass human movements in the form of pilgrimages, international travel and tourism etc,6–9 further contributed to the virus dissemination. The World Health Organization estimates a global annual incidence of around 50–100 million cases of DF and 500 000 cases of DHF in tropical and subtropical countries, mainly Southeast and South Asia, Central and South America, and the Caribbean.10 In the last few years, dengue has also emerged in the United States of America and has also made its entry into Europe.11 Based on high spatial-resolution estimates of contemporary global dengue risk and burden in 2010, it was estimated that India accounts for one third of the global dengue infections.12

The first isolation of DENV was in 1943,13 while the first isolation in India at Kolkata was in 1944, from serum samples of US soldiers.14 The disease is endemic in most major cities and all four serotypes are known to circulate in India. Recent reviews elaborate on the epidemiology, circulation of serotypes in different parts of the country and changes in the circulating serotypes in consecutive years as well as the current status of DENV in the country.15,16 Several reports are also available with regard to the evolution of DENV at a local level and in different states in the country,17–20 and likewise also at a global level, the majority of existing studies have focused on the evolution of individual DENV types at regional or local scales.21–29 A recent study summarized the 70-year global distribution of dengue types in a series of global maps.30 However, a comprehensive picture of the evolutionary dynamics of the four DENV types in India vis-à-vis the global dynamics would serve the purpose of comprehending the establishment and endemicity of DENV in the country and subsequent dissemination of the virus. This review is an overview of studies carried out with respect to the phylogeny, molecular clock, rates and dates of evolution and phylogeography of all the four serotypes utilizing Indian as well as representative global sequence data over a period of about 50–60 years.

**Phylogeny and molecular clock analyses**

Phylogeny based molecular clock analysis involves the determination of rates of nucleotide substitution and root ages viz. divergence times for different genotypes as well as the time to the most recent common ancestor (tMRCA) of various sub-groups. The inputs required are the dated nucleotide sequences of known sampling time and output is seen as calibrated
Phylodynamics of dengue viruses in India vis-à-vis the global scenario

Molecular clock analyses of DEN viruses are usually based on full-length E-gene sequences. Most studies apply the BEAST1.5.3,31 which uses the Bayesian Markov Chain Monte Carlo (MCMC) approach and allows implementation of both strict as well as relaxed clock models.32 The Tree Annotator program also available in BEAST is used to generate the maximum clade credibility (MCC) tree while tools such as Fig Tree 1.2.3 (http://tree.bio.ed.ac.uk/) are used for visualization of the annotated tree. The 95% Highest Posterior Density (HPD) intervals are used to ascertain the uncertainty in the parameter estimates.

DENV-1: DENV-1 has been circulating in India since the 1940s,33,34 the time frame coinciding with the first reports of DENV-1 in 1943 in Japan and French Polynesia; and Hawaii in 1944 and 1945.30 Over the period since then, the disease profile has changed from mild to severe forms.35 DENV-1 was associated with a DHF outbreak in Delhi in 1997.36 DENV-1 was also implicated during recent outbreaks in Delhi in 2006 and 2008.37–39

Phylogeny of DENV-1 sequences reveals the delineation of this serotype into five genotypes described based on their geographical distribution.40,34 These genotypes are also designated as I–V.41 An MCC tree (Figure 1a) of 269 DENV-1 sequences of isolates over the time span 1944-2011, with the earliest Indian isolates from India of 1962, showed that the majority of the Indian strains belong to the Cosmopolitan genotype (Genotype V) while a single Indian strain of 2001 was noted to belong to the Asian genotype (Genotype I).42 Within the Cosmopolitan genotype, Indian strains are distributed in three main sub-lineages viz. India I, II and Afro-India.40,34 An earlier report by Anoop et al.19 had also reported the categorization of Indian viruses into four Indian lineages on the basis of core/prM region. Three Indian isolates of 1962 and 1963, notably including isolates from mosquitoes, clustered with the American and India I sub-lineages. The estimated rate of nucleotide substitution (Table 1) showed the 95% confidence interval (CI) to be 6.8x10⁻⁴ - 9.1x10⁻⁴ substitutions/site/year (subs/site/year).42 Based on these rates, the inferred mean root age was ~108 years (HPD: 78, 150 years), indicating the existence of DENV-1 since ~1903 (beginning of the 20th century). As per one of the earliest reports considered as a first report of DHF in medical literature, dengue epidemics were noted in 1897.43 Similar rates as well as tMRCA for DENV-1 have been reported.34,44,45,23 Further, molecular clock studies showed that the different genotypes of DENV-1 emerged in the 20th century with Thailand and Malaysian genotypes being the more recent ones. The Cosmopolitan genotype and the South Pacific genotype emerged around the same time period (~65 years ago, 1940s) while the Asia genotype was estimated to have emerged earlier (~92 years ago, 1920s). The tMRCA for the Asia genotype was earlier estimated to be ~1937.45,23 However, the discrepancy could be explained as inclusion of the earliest isolate from Japan (1943) in our dataset, as against the earliest isolate of this genotype being considered as a Hawaii isolate of 1944 in the former study while a Thailand (Asian genotype) isolate of 1980 was considered in the latter study.

The existence of phylogenetically distinct lineages in India suggests that there may have been independent entries of the virus into the country. The India III lineage containing...
**Table 1**: Rates of evolution*, estimated age and probable ancestral locations for the root and genotypes of the 4 serotypes of DENV

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Most likely ancestral location (probability)</th>
<th>tMRCA (Time to the Most Recent Common Ancestor) with 95% HPD (Highest Posterior Density) interval</th>
<th>Node age</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>DENV-1</strong> [6.8 x 10^{-4} – 9.1 x 10^{-4} subs/site/year]**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root</td>
<td>Southeast Asia (0.5)</td>
<td>108.3 (77.7, 150)</td>
<td>1902.7 (1861, 1933.3)</td>
<td></td>
</tr>
<tr>
<td>Cosmopolitan (V)</td>
<td>India (0.86)</td>
<td>64.9 (59.2, 71.3)</td>
<td>1946.1 (1939.7, 1951.8)</td>
<td></td>
</tr>
<tr>
<td>Malaysia (III)</td>
<td>Southeast Asia (0.93)</td>
<td>47 (39.7, 56.6)</td>
<td>1964 (1954.4, 1971.3)</td>
<td></td>
</tr>
<tr>
<td>South Pacific (IV)</td>
<td>Southeast Asia (0.66)</td>
<td>63.8 (49.3, 81.1)</td>
<td>1947.2 (1929.9, 1961.7)</td>
<td></td>
</tr>
<tr>
<td>Asia (I)</td>
<td>Southeast Asia (0.45)</td>
<td>91.6 (75, 111.6)</td>
<td>1919.4 (1899.4, 1936)</td>
<td></td>
</tr>
<tr>
<td>Thailand (II)</td>
<td>Southeast Asia (0.98)</td>
<td>53.1 (48.4, 62.3)</td>
<td>1957.9 (1948.7, 1962.6)</td>
<td></td>
</tr>
<tr>
<td><strong>DENV-2</strong> [5.9 x 10^{-4} – 8.7 x 10^{-4} subs/site/year]**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root</td>
<td>Southeast Asia (0.12)</td>
<td>325.2 (159.3, 567.4)</td>
<td>1685.8 (1443.6, 1851.7)</td>
<td></td>
</tr>
<tr>
<td>Sylvatic</td>
<td>West Africa (0.23)</td>
<td>158.4 (78.4, 267.6)</td>
<td>1852.6 (1743.4, 1932.6)</td>
<td></td>
</tr>
<tr>
<td>Cosmopolitan (IV)</td>
<td>Southeast Asia (0.36)</td>
<td>67.9 (49, 92.5)</td>
<td>1943.1 (1918.5, 1962)</td>
<td></td>
</tr>
<tr>
<td>Asian II</td>
<td>Southeast Asia (0.61)</td>
<td>83.3 (73.5, 97.4)</td>
<td>1927.7 (1913.6, 1937.5)</td>
<td></td>
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<tr>
<td>Asian I</td>
<td>Southeast Asia (0.98)</td>
<td>57.6 (48.2, 69.7)</td>
<td>1953.4 (1941.3, 1962.8)</td>
<td></td>
</tr>
<tr>
<td>Asian/American (III)</td>
<td>Southeast Asia (0.88)</td>
<td>44.4 (34.3, 56.8)</td>
<td>1966.6 (1954.2, 1976.7)</td>
<td></td>
</tr>
<tr>
<td>American (V)</td>
<td>India (0.3)</td>
<td>103.3 (73.4, 143.7)</td>
<td>1907.7 (1867.3, 1937.6)</td>
<td></td>
</tr>
<tr>
<td><strong>DENV-3</strong> [6.9 x 10^{-4} – 1.0 x 10^{-3} subs/site/year]**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root</td>
<td>Philippines (0.08)</td>
<td>127 (74, 199)</td>
<td>1883 (1811, 1936)</td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>Indonesia (0.3)</td>
<td>49 (41, 60)</td>
<td>1961 (1950, 1969)</td>
<td></td>
</tr>
<tr>
<td>GII</td>
<td>Thailand (0.93)</td>
<td>32 (29, 38)</td>
<td>1978 (1972, 1981)</td>
<td></td>
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<tr>
<td>GIII</td>
<td>India (0.7)</td>
<td>56 (47, 68)</td>
<td>1954 (1942, 1963)</td>
<td></td>
</tr>
<tr>
<td>GIV</td>
<td>Caribbean (0.2)</td>
<td>80 (56, 112)</td>
<td>1930 (1898, 1954)</td>
<td></td>
</tr>
<tr>
<td>GV</td>
<td>Philippines (0.7)</td>
<td>57 (54, 64)</td>
<td>1953 (1946, 1956)</td>
<td></td>
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</tbody>
</table>
isolates exclusively from the 1960s and the Afro-India lineage with isolates from 1970 and 1971 are representative of extinct lineages. Indian isolates of lineage I from 2003-2006 were closely related with isolates from East Africa (Reunion, 2004) and an isolate from Singapore of 1990, which is a major economic and travel hub in Southeast Asia. Lineage India II had viruses from 1962, 1982 and 2005 along with isolates from West Asia, East Africa, East Asia and Southeast Asia and, therefore, represents an evolving lineage. This change in the lineage of DENV-1 was suggested to be the cause for disease severity of DENV-1 in India. The 2005 isolates from Pune belonging to lineages I and II indicate that there was co-circulation of two lineages during a single outbreak.

**DENV-2:** DENV-2 was first reported in 1944 in Papua New Guinea and Indonesia. In 1944 itself, DENV-2 was first isolated in India from the sera of American soldiers during the Second World War. Since then, epidemics of DENV have been recorded from almost all over India at different times. Limited phylogenetic studies of DENV-2, utilizing a small fragment of either the E/NS1 gene or the capsid–premembrane (C–prM) gene, have been reported, showing a change in the circulating genotype of DENV-2 in India. Our earlier studies for DENV-2 had indicated transmission of DENV-2 among India, Caribbean Islands and the Americas while later, to further elucidate the direction of dispersal, we carried out phylogeography analyses utilizing a larger and more temporally spread data. The MCC tree for 307 E-gene isolates of DENV-2 (1944–2011) in this study represents the six genotypes including the sylvatic genotype as described previously (Figure 1b). The Indian isolates from 1956 to 1971 belonged to the American genotype previously designated as genotype V, which also contains the Caribbean isolates (1953–1981), isolates from South America (1967–1986), Central America (1984–1994), Oceania (1971–1974) and Trinidad (1953). Indian isolates from 1974 to 2010 fall in the Cosmopolitan genotype previously designated as genotype IV, which includes strains with a wide geographical distribution.
**Figure 1:** Maximum Clade Credibility (MCC) tree for (a) DENV-1 (b) DENV-2. The collapsed branches correspond to the genotypes/major sub-clades. The countries (ISO 3166-1-alpha-2 Codes) and sampling times of the isolates are also indicated. The numbers correspond to the tMRCA (Time to the Most Recent Common Ancestor) estimates of key nodes, the ancestral states with their probabilities. (Figure based on Ref. 42)
Almost all the isolates in this genotype are dated beyond 1970 and comprise isolates from South-Central Asia, East Asia, East Africa, West Asia, Southeast Asia and Oceania.

Similar estimates of rate (mean 7.2x10^{-4} subs/site/year and 95% HPD, 5.9x10^{-4} - 8.7x10^{-4}, Table 1) and age are reported in previous studies as also estimated in our study. Similar evolutionary rates are thus inferred for DENV-1 and DENV-2 as also shown by Twiddy et al. Moreover, our results indicate an earlier existence of DENV-2 as compared to that of DENV-1 as also reported earlier. The tMRCA of the cosmopolitan genotype was ~1940s.
This genotype was divided into two subgroups, indicated by node A and B (Figure 1b). A majority of Indian isolates belong to one subgroup of the Cosmopolitan genotype along with isolates of Sri Lanka, Bangladesh, Bhutan, Saudi Arabia, Seychelles and a few of Australia, Uganda and Singapore, having an ancestral time around 1960s.

**DENV-3:** DENV-3 was first reported in 1953 in the Philippines and Thailand, and has been reported in Asia every year since 1962 with Thailand reporting several outbreaks. DENV-3 isolates are distributed into five genotypes, I–V, with six lineages (A–F) in GIII. Re-emergence of DENV-3 was reported in the 2005 outbreak in Northern India. The same genotype was implicated in causing DHF outbreaks in the Americas. The data from India is fragmentary with no representation from 1967 to 2005 except for a single 1984 isolate. Bayesian analyses of a total of 95 E-gene sequences of Indian DENV-3 isolates (two of 1966, and the majority from the period 2005–2010) analysed with global representative sequences of DENV-3 revealed that the all Indian isolates grouped into GIII (Figure 2a). The Indian isolates of 1966, including one mosquito and one human, are the oldest representative isolates of GIII, and formed the new lineage F. Lineage E, which was not a tight cluster, consisted of isolates from Sri Lanka (pre-DHF era), India (1984) and Samoa (1986). Lineage D included one Indian isolate of 2009, from Kerala, along with isolates from Sri Lanka (1993–2000, post-DHF) and Taiwan. Lineage C included Indian isolates of 2003–2010 along with isolates from Cambodia, Bhutan, Saudi Arabia, Abidjan and Tanzania. Lineage B consisted of isolates from Sri Lanka (post-DHF era) and Somalia while lineage A was represented by American isolates. Under the relaxed uncorrelated exponential clock with constant growth population model, the mean substitution rate was $9.0 \times 10^{-4}$ subs/site/year (95% HPD limits: $6.9 \times 10^{-4} – 1.0 \times 10^{-3}$, Table 1) similar to that reported by Araujo et al. DENV-3 is the only serotype for which no sylvatic isolates are available. The estimate of the tMRCA for all genotypes of DENV-3 was about 127 years ago (95% HPD: 74 199 years) with respect to the Indian isolates of 2010, correlating to the period around 1883. DENV-3, at the oldest node, diversified into two main branches, one that seeded Pacific region (GIV) and became extinct and the second that diverged to populate South Asia/South Pacific (GI/ GV), Thailand (GI) and the Indian subcontinent (GIII). Within genotype III, there was a sequential emergence of lineages E, D, A and B. The tMRCA of lineage C, wherein the majority of the Indian isolates clustered, dated to the period 1991–1999.

**DENV-4:** DENV-4 has five genotypes, including sylvatic as GIV and GI with three lineages (A–C). Though this serotype was rarely reported after the 1970s in India, it was reported in Delhi in 2003. A similar re-emergence of DENV-4, GI in Brazil in 2008 after three decades was reported by Melo et al. However, genotypes I, II, and III of DENV-4 have been detected yearly in Thailand since 1963. The MCC tree based on E gene sequences (n=54) of DENV-4 isolates from India (period 1961 to 1965, 1979 and 2009) and global representatives showed that the Indian isolates were distributed into two genotypes, GI and GV (Figure 2b). Recent Indian DENV-4 isolates belonged to genotype GI. The old Indian isolates from the 1960s along with one Thailand isolate from 1963 clustered independently, and based on the high diversity from the other DENV-4 genotypes, the cluster was assigned.
**Figure 2:** Maximum clade credibility (MCC) tree of E-gene sequences under the relaxed uncorrelated exponential clock with constant population size. The names of DENV isolates include GenBank accession number, reference to country origin and year of sampling. The branches are coloured according to the respective geographical region. Ancestral states with their probabilities are shown at key nodes (labelled) in brackets and the scale bar indicates time in years (a) DENV-3 (b) DENV-4. (Ref. 56)
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(b)
the new genotype, GV. The mean substitution rate was 6.9 x10^{-4} subs/site/year (95% HPD limits: 4.1x10^{-4} – 1x10^{-3}, Table 1). The estimated tMRCA for all genotypes of DENV-4 was about 158 years with respect to the latest isolates of 2009 (1851 with 95% HPD: 1718–1930) (Figure 2b), which matched with earlier reports. GI and GV, which contained Indian isolates, had a tMRCA 1847–1935. The tMRCA for GV, which was populated exclusively with Indian isolates from 1960s and one isolate from Thailand (1963) was 1863–1944. The absence of recent isolates suggests that the GV is an extinct genotype. GI (tMRCA 1868–1943) branched into the three lineages, A, B and C. All the Indian isolates from 1979 to 2009 clustered within lineage A (tMRCA dating to 1929–1956) along with Sri Lankan isolates.

Phylogeography and migration pattern analyses

Phylogeography studies allow us to reconstruct the spatiotemporal spread of the virus and thus they can be used to uncover the factors that influence the patterns of population dynamics and spatial diffusion. In such studies, the spatial information of the sequences is used to infer the most probable ancestral geographic region/country at different internal nodes of the tree by fitting a standard continuous-time Markov chain (CTMC) model with the Bayesian stochastic search variable selection (BSSVS) in BEAST. The MCMC tree thus obtained is used as an input in software such as SPREAD 1.0.3 to visualize and analyse the dispersion pathways. The Bayes factor (BF) test available in SPREAD can be further applied to identify well-supported rates of transitions between the different geo-regions.

DENV-1: Currently, knowledge about the phylogeography of DENV-1 is mainly limited to studies done for a particular country or region (America by Allicock et al., Brazil by Drumond et al., and Asia by Sun and Meng). Therefore, the knowledge about DENV-1 phylogeography is mainly elaborative for those regions. A work based on data from 45 distinct geographic locations isolated during the period 1944 to 2009 analysed the worldwide spread of DENV-1. Though the study revealed the demographic history of the major DENV-1 genotypes circulating in the Asian and South Pacific regions, the source of dispersal into the Caribbean could not be ascertained unanimously probably because of missing temporal data from certain countries. Several studies as well as our studies based on E-gene sequences of DENV-1 isolates of global and Indian representatives covering a broader time span (1944–2011) revealed that the most probable ancestral geographical location for DENV-1 was Southeast Asia (Figure 3a). The ancestral state for the different genotypes except the Cosmopolitan genotype was also found to be from Southeast Asia. This result inferring Southeast Asia as a main source population for the dispersion of the virus, corroborates with earlier reports that suggested that the Southeast Asian region is the setting that began the global dengue pandemic, as a consequence of the ecological disruption during and after the Second World War. India was determined to be the ancestral state of the Cosmopolitan genotype, which was in support to a result by Sun and Meng. Further, the ancestral state for all the major nodes in the Cosmopolitan genotype was also found to be India. The America lineage within the Cosmopolitan genotype is seen to have two clusters (Figure 1a),
which had India as the ancestral state, indicative that there may have been at least two separate introductions of the virus from India to the American region. The earlier introduction to the Caribbean region was estimated to have happened around 1971, while the latter reintroduction was estimated to be around 1981. The tMRCA estimate obtained by Allicock et al.\textsuperscript{21} based on 109 DENV-1 genotype V American isolates corroborates our estimate of the first introduction into the Caribbean region. India seems to have played a central role in dispersion of the virus to the Caribbean region as also indicated by significant Bayes factor (Figure 3a). The role of mosquitoes in the propagation and dissemination of dengue viruses can be emphasized considering that the outer branches of the cluster of Indian and America lineages include two mosquito isolates. Within the Cosmopolitan genotype, several migration events corresponding to the India II lineage were found to have occurred from India to other Southeast and East Asian countries (that is, Thailand, Singapore, South Korea, etc.) since the 1970s. This implies that these countries as popular tourist destinations may be playing an important role in virus dissemination. Export of the virus was also observed earlier from India to Africa in the Afro-India lineage. The estimated time frame being around 1952 most likely reflects the movements between Africa and Asia after the Second World War.
The earliest possible introduction of the virus to the American region appears to be from East Asia ~1940s in the form of the Asian genotype. The Asian genotype virus also entered India from East Asia around 2000, but does not seem to have got established in the country. Introduction of the virus to Sri Lanka also appears to be from West Asia as supported by significant migration link between these regions. A previous study suggested that the dengue virus was introduced in West Asia due to pilgrimage, tourism or by migrant labour from Southeast Asia and Africa. Our results confirm this conclusion with strong supports to the links between these regions. Geographical proximity is one of the factors that play an important role in viral dispersal, as indicated by significant transitional pathways between North, Central, South America and Caribbean islands (Figure 3a). The Caribbean region spread the virus to South and Central America, which further spread it to North America. The same is true for Oceania, Asian and African regions.

**DENV-2:** In case of DENV-2, the only available reports have investigated the phylogeography in a restricted geographic area (for Caribbean basin by Foster et al., Vietnam by Rabaa et al., Brazil by Drumond et al., and Peru by Cruz et al.). From our own phylogeography studies of DENV-2 with a global dataset, West Africa was estimated to be the most probable ancestral location for the sylvatic strains of DENV-2. This result is supported by the fact that the African region is considered as the original habitat of Aedes sp. mosquitoes which are the principle vectors in dengue transmission. Based on the available data, it was not possible to accurately pinpoint the ancestral location for human DENV-2, though Southeast Asia bears the highest probability in our study. The geographical ancestor for the Asian and Asian/American genotypes was found to be Southeast Asia (Figure 1b). The ancestral strain for the Cosmopolitan genotype was also most probably located in Southeast Asia. Notably, the ancestral state for the American genotype was determined to be India, though with a lower state probability. The BSSVS analysis indicated that Southeast Asia, East Asia, Oceania and India have played a central role in the dispersal of DENV-2 (Figure 3b). These regions showed significant transmission links with the other regions, irrespective of their geographical proximity. This is supported by earlier reports, which suggested global epidemiology and that the transmission dynamics of dengue viruses were changed dramatically in Southeast Asia during the Second World War. This led to geographical expansion of the disease, as epidemics expanded westward from Southeast Asian countries to India and Sri Lanka, and eastward to China. India is at the core of the viral diffusion process showing significant migration links with regions like South-Central/East Asia, Oceania, West Asia, East Africa and even South/Central America. Significant links between India and South/Central America as well as the maximum probability of India as the ancestral location of the American genotype, suggests a spread from India to America rather than what was previously thought. This hypothesis is strongly supported by the very high ancestral state probability for India at the node with mean root age in the 1940s (Figure 1b). This time frame coincides with the period of the Second World War, supporting the conclusion that transmission dynamics of dengue viruses were changed dramatically in Southeast Asia during and after the Second World War. The links with highly significant Bayes factors viz. Oceania-India/Caribbean, Central America-South/North America and East...
Asia-West Africa-South America is also important in this regard (Figure 3b). Further, frequent migration of DENV-2 from Caribbean islands to the American mainland had been reported by Pires Neto et al.\textsuperscript{73} The same is reflected in significant links between Caribbean North/ Central/ South America. One subgroup of the Cosmopolitan genotype had Southeast Asia as the ancestral location while the other subgroup had India as the geographical origin with very high probability. This shows that the virus has dispersed from India to countries in regions including South-Central Asia, East Asia, West Asia, and Oceania since 1950s and 1960s. Specifically, two separate introductions into West Asia were noted from India in the 1990s and in 2004, most likely by pilgrims. Movement of the virus from India to the Southeast Asian countries was also noted in the 2010s. Increased urbanization and movement of people has caused hyperendemicity of dengue virus in various countries.\textsuperscript{74} This is evident from shared significant links between different regions over the globe (Figure 3b).

**DENV-3:** Global phylogeography for DENV-3 is not well understood. Araújo et al.\textsuperscript{28} investigated the phylogeography of three main genotypes separately. Their migration pattern analysis of the main DENV-3 genotypes showed that genotype I was mainly confined to the maritime portion of Southeast Asia and South Pacific, genotype II stayed within continental areas in Southeast Asia, while genotype III spread across Asia, East Africa and into the Americas. Reconstruction of the ancestral states by phylogeographic analysis using sequences representative of all five genotypes in our study\textsuperscript{56} could not resolve the common ancestral state of all DENV-3 genotypes as the state probability was very low (Figure 2a). On the other hand, the most parsimonious ancestral state was determined to be Indonesia for GI, Thailand for GII, Caribbean (CAR) for GIV and Philippines for GV. The ancestral state of GIII, which was predicted to be India, differed from the earlier prediction of Sri Lanka (1967–1979).\textsuperscript{28} Within GIII, there was a sequential emergence of lineages E, D, A and B with Sri Lanka as the ancestral state, while India was the suggested ancestral state for lineages F and C. During 1981–1988 an introduction into India from Sri Lanka, which evolved into lineage C during 1991–1999 was suggested. Therefore, two events, one exportation from India (pre-DHF period) and one importation from Sri Lanka (post-DHF period), occurred. Movement of DENV-3 between the two countries was supported by a high BF value (Figure 3c). It is possible that additional events have been masked by the paucity of data from India during 1970–2000. During the latter period (1999–2003) the virus was likely to have been exported from India to Saudi Arabia, Africa and China. BF analysis provided significant evidence for movement of the virus between India and West Asia. It has been suggested that pilgrimage to Haj, which was subsidized by the Indian government since 1993, might be instrumental in the importation of viruses to West Asia.\textsuperscript{69} Investigations in Bhutan during the 2006–2007 outbreaks indicated introduction of lineage C from northern India.\textsuperscript{75} Broadcasting of the virus from India to Cambodia was also indicated with a significant BF value. On the other hand, Bangladesh and Myanmar were populated with GII viruses from Southeast Asia as suggested earlier.\textsuperscript{76} Further, dispersal of the virus from Sri Lanka to Africa and Central America was also supported by significant values of BF.
DENV-4: Limited studies exist on the global phylogeography of DENV-4 as well. In studies carried out by our group, India was predicted to be the ancestral state for the entire DENV-4 serotype albeit with low probability (Figure 2b). Earlier reports had placed GIV (sylvatic) at the root of DENV-4 based on phylogenetic analysis. The addition of data on Indian isolates from the 1960s and the use of phylogeography analysis placed India at the root of DENV-4 viruses during 1719–1931, a period that coincides with movement of people between Africa and Asia. Phylogeography by Villabano and Zanotta, which did not have the data from India, showed Malaysia/Thailand as the ancestor state for DENV-4. India was also the suggested common ancestor for GIII and GIV (sylvatic) genotypes and for GI/GV and GII genotypes. GI represented currently circulating viruses from India and Southeast Asia. India was predicted to be the ancestral state at almost all nodes within GI indicating that viruses from India possibly spread to Thailand, Sri Lanka, Cambodia and the Philippines. Lineage A of GI had the Philippines as the ancestral state while lineages B and C had India as the ancestor. The Philippines thus probably exported the virus to Japan and Thailand. GV shared a common ancestor (India, 1863–1944) with a Thailand isolate, implying movement of viruses from India to Thailand.

Further, Indonesia was suggested as the ancestral state of GII, containing viruses from the Pacific region, and Indonesia may also have spread the virus to the Americas via the Caribbean. Migration pattern analysis revealed fewer transitions and BF values much lower than that observed for DENV-3 (Figure 3d). The highest BF values were for the transitions between South America/Caribbean, India/Thailand and South America/Central America.

Concluding remarks and implications

Documentation of the serotype-specific record of DENV spread has important implications for understanding patterns in dengue hyperendemicity and disease severity as well as for vaccine design and deployment strategies. This review is an attempt to provide an overview of the global evolutionary dynamics of the four serotypes of DENV with emphasis on the role played by India. As noted in our studies, the population of dengue viruses in India has undergone major changes since the 1960s with either genotype or lineage changes. The viruses circulating in India in 1950s and causing mild disease were either replaced or evolved into lineages/genotypes with greater virulence and/or transmissibility. Genotype shifts for DENV-2 (American to Cosmopolitan) and DENV-4 (genotype V to I) and lineage changes for DENV-1 (India III/ Afro-India to India I/II of Cosmopolitan genotype) and DENV-3 (F to C/D of genotype III) were noted. Thus, new introductions, in situ evolution along with limited recombination have contributed to diversity and the dengue evolutionary dynamics in the country.

Estimates of the evolutionary rates revealed no significant differences among the DENV serotypes (Table 1). Phylogeography studies for DENV-1 inferred that the cosmopolitan genotype, circulating in India, West and East Africa, Caribbean region, East and Southeast...
Asia, originated in India around the 1940s. India was also estimated to be the probable geographical origin for the American genotype of DENV-2, circulating in India, South and Central America, and Caribbean regions. The introduction of the genotype into India was most likely to have occurred around the 1910s while the time span of the exportation of this genotype was around 1940s. Further, India was also determined to be the ancestral source for a subgroup of the Cosmopolitan genotype of DENV-2. The more recent dispersal between Southeast Asian (including India) and East Asian countries was noted for both DENV-1 and DENV-2 after the 1960s. On the other hand, for both DENV-3 and DENV-4, the transportation of viruses between Sri Lanka and India was evident during the 1970s and 1980s which correlated to human movements during the Sri Lankan civil war. The probable role of exchange of viruses between India and Sri Lanka in the evolution of virulent strains of DENV-3 genotype III, which originated in India, is also important. Though the origin of DENV-3 could not be ascertained, interestingly, our studies revealed that DENV-4 may have originated in India. This corroborates with a report that dengue virus separated into distinct serotypes independently because of geographical partitioning of different primate populations.

India thus continues to play a crucial role in the establishment, evolution and global dispersal of the four DENV serotypes. The existence of DENV in India and the major mosquito vector species for DENV transmission since early times is well known. The unprecedented population growth, increased population density, unplanned and uncontrolled expanding urbanization, climatic factors, water storage pattern in houses and waste management practices, coupled with increased density of the vector mosquito, infestation of new geographical areas by vector mosquitoes and inadequate mosquito control measures, have contributed to the establishment and evolution of the DENV serotypes in the country. India’s role during the Second World War and its strategic location at the tip of the Indian Ocean facilitating global movements in the aftermath of the war may have been significant for the propagation of the virus. In recent times increased air travel as a consequence of trade and tourism has further contributed to the dissemination of DENV viruses. So also, the emergence of diversity of the DENV genotypes in more recent times, coincides with human population growth, urbanization, massive human movement, and with the reports of the first cases of DHF in Asia. Overall several factors, other than geographic proximity, also affect the continual dispersion and reintroduction of new DENV variants in different regions and understanding of these should help in the management and control of DENV spread.

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References


Phylodynamics of dengue viruses in India vis-à-vis the global scenario


Phylodynamics of dengue viruses in India vis-à-vis the global scenario


Association of dengue symptoms with haematological parameters: a retrospective study of 10 hospitals in India

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Abstract

The clinical diagnosis of dengue is challenging because of dynamic clinical presentations. This study was designed to describe the clinico-haematological parameters by assessing (i) the incidence of thrombocytopenia in dengue infection, and (ii) the association between bleeding manifestations with platelet count and severity of dengue infection. A multicentre study was conducted at national level between 2010 and 2012 among 10 medical colleges cum tertiary care hospitals from 10 states across five regions in India. Data collected from these selected hospitals were compared. Platelet count of <100 000/l was observed in 881 (73.36%) dengue fever (DF) cases, 121 (85.82%) dengue haemorrhagic fever (DHF) cases and 66 (75.86%) dengue shock syndrome (DSS) cases, in which 52.53% was reported among children. There was not much difference in the severity level of thrombocytopenia in all age groups. The percentages of developing haemorrhagic manifestations in patients with DF, DHF and DSS were 26.69%, 68.60% and 50.65%, respectively; there were 38.70% occurring in patients with platelet count <50 000. The patients with a raised haematocrit of >40%
and thrombocytopenia (platelet count <50 000) were observed to be marginally at risk of getting haemorrhagic symptoms. There were patients with severe thrombocytopenia (platelet <50 000) who had no prior bleeding whereas some patients with normal platelet value (>100 000) had bleeding manifestations. The haemorrhagic manifestation was significantly related to the severity of thrombocytopenia in DF cases, but there was no significant association found between the bleeding symptoms and the degree of thrombocytopenia among the patients progressing to DHF/DSS as 57.14% and 35.29% of patients having platelet count >100 000/l had bleeding manifestations. The changing clinical pattern of disease shows the heterogeneity in severity of dengue infection as the clinical bleeding manifestations have no direct relationship with the degree of platelet count especially in case of DHF/DSS.

**Keywords:** Dengue, clinical presentation, haemorrhagic manifestation, thrombocytopenia, India.

**Introduction**

Dengue with its two deadly manifestations, that is, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), has emerged as one of the major arboviral infections in the world in terms of morbidity, mortality and economic burden. The infection is by now viewed as a global epidemic with recorded prevalence in more than 125 countries. Worldwide an estimated 3.6 billion people (over 50% of the world’s population) are at risk of infection with more than 50 million new infections occurring annually; between 250 000 and 500 000 had severe infection and required hospital admission and there were 20 000–25 000 deaths, mainly in children. Although the global burden of the disease is uncertain, the increasing incidence with geographical expansion and changing disease patterns is alarming both for public health and the global economy. India represents significantly a larger burden, accounting for nearly 34% of the global burden of apparent dengue infection, with regular reporting of dengue outbreaks compared to other countries. The cost of dengue illness was estimated as high as US$1.1 billion or US$0.88 per capita and the direct medical cost of care accounted for US$545 million or US$0.43 per capita in 2012 in India. There is currently no effective vaccine or antiviral drug for dengue but early detection and proper prognostication may reduce adverse complications and lower fatality rate.

Clinical presentation of dengue fever shows heterogeneity in severity along a wide spectrum of signs and symptoms. Typically, it may be subclinical or may manifest a range from a self-limiting fever to a severe form, DHF, which may progress to shock (DSS) and finally, death. Despite rapid advancement in dengue laboratory tests, the availability and accessibility of diagnostic facilities and adhering to universal diagnostic guidelines was limited in India. As per the guidelines of World Health Organization (WHO), the diagnosis and laboratory criteria including the isolation of dengue virus/demonstration of fourfold change/detection of viral genomic sequence is not being performed to confirm DF/DHF/DSS in India and most dengue diagnosis is primarily made based on clinical and other laboratory recognition. The
clinical diagnosis of DHF/DSS is not easy in the early phase of illness which requires the presence of four criteria, including haemorrhagic manifestations and thrombocytopenia as per WHO case definition. In recent decades, several studies have reported the conflicting association between the platelet count and the presence or the magnitude of bleeding manifestation and bleeding tendency that did not differentiate DF and DHF. Although thrombocytopenia and bleeding manifestation are the key diagnostic criteria of DHF, it is also commonly observed in mild DF and many patients progress to DHF/DSS without having any bleeding manifestations. The clinical diagnosis of dengue is challenging because of dynamic clinical presentations on the one hand and difficulty in reliability in terms of differentiating dengue from other febrile illness clinically, on the other hand. A retrospective study was conducted to understand the dengue infection in the population across different regions in India over six years (2006–2011). The present communication highlights the observation on the clinico-haematological parameters and their role in prediction of severity of dengue infections, especially with an intent to assess (i) the incidence of thrombocytopenia in dengue infection, and (ii) the association between bleeding manifestations with platelet count and severity of dengue infection.

Material and methods

Study design

This study was part of a cross-sectional, hospital-based retrospective study conducted at national level between 2010 and 2012 in India. The sample of 10 medical colleges cum tertiary care hospitals was selected from 10 states across five regions in India to understand the range of dengue illness in the concerned state and region. Out of the selected samples, two hospitals were paediatric hospitals and remaining were general hospitals. The selection of study sites, sampling procedures, study framework and methodology were published earlier.

The medical records of the patients who were hospitalized during January 2006 through December 2011 with a clinical discharge diagnosis of patients with dengue/DHF/DSS (ICD10 code A90) formed the sample frame. A systematic random sample of 1665 medical records of hospitalized patients discharged with diagnosis of dengue was reviewed and analysed. A data extraction instrument was developed to collect the required data from the medical records. The collection tool was focused on (1) socio-demographic details; (2) date of admission and discharge; (3) referral facility; (4) admission and discharge diagnosis; (5) patient’s report of ambulatory visit/hospitalization prior to hospitalization at study sites; (6) patient’s report of date of onset of illness; (7) setting (casualty/ICU/ward/private room); (8) length of stay (nights) and total duration of illness through discharge; (9) signs and symptoms; (10) patient’s temperature and blood pressure measurements at admission and discharge; (11) haematological and biochemical profile; and (12) reclassification of dengue cases as per the WHO classification.
Laboratory and haematological profile

The performance status of serological investigations such as IgM, IgG capture ELISA and NS1 antigen along with tourniquet test were retrospectively collected and recorded. Additionally, haematological parameters including haemoglobin (Hb), total leukocyte count (TLC), haematocrit (HCT), platelet count and liver function tests such as serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) were also recorded. Along with this details of those patients who underwent chest X-ray and ultrasound examination of the abdomen were also recorded.

Statistical analysis

The SPSS version 16.0 was used for statistical analysis. Chi-square or Fisher’s exact test were used to assess the statistical difference between categorical variables among dengue groups. Comparisons of means of continuous variables were performed using ANOVA and Kruskal-Wallis Tests. Relative Risk (RR) was calculated to evaluate the risk exposure between two groups. Correlations were analysed through Pearson’s coefficient. A p-value of less than 0.05 was considered as significant for all analyses.

Results

Disease presentation of dengue cases

A total of 1665 medical records were randomly sampled from 10 medical hospitals, in which data from 1541 medical records were reviewed and included for the analysis. Among 1541 cases, 1302 (84.49%), 147(9.54%) and 92 (5.97%) had provisional discharge diagnosis of DF, DHF and DSS, respectively, in which 0.7% of cases have reported co-infection with other febrile illnesses. A dengue serological test was done for 1182 (76.70%) patients, in which about 943 (79.78%) were found to be seropositive to dengue. The primary infection with IgM positive was 658 (55.67%) whereas secondary infection with IgM and IgG both were positive in 169 (14.30%) patients. Only IgG positive was detected in 85 (7.19%) patients, which contributed about 21.49% of secondary infections in our study. A case fatality risk (CFR) in our study was 4.61% with 71 patients’ deaths. The mortality appears greatest with 3.18% (49 patients) in the first 24–48 hours of their hospitalization, in which 28 patients (1.82%) died before they had the opportunity to undergo diagnostic investigations. Though geriatric population (age >60) contributed 36 (3.75%) cases of hospitalization, they understandably exhibited a high mortality rate of 14.29%. Thus the geriatric population was also proved to be at a risk equal to that of children in terms of dengue mortality.
Duration of illness and utilization of health care services

The infected patients normally do not seek professional help at tertiary hospital at onset of infection, but wait until the disease has worsened. About 311 (20.18%) patients were treated in primary or secondary care units and referred at the point where hospitalization is required for tertiary care management. About 21.64%, 59.71% and 18.65% patients sought professional help for hospitalization at a tertiary care study site at 0–3, 4–8 and >8 days respectively (Figure 1) with an average (SD) of 6.51(±5.24) days after the onset of illness, including an average of 2.81 nights of hospital stay and 1.38 ambulatory visits that may have taken place either at primary or secondary health care facilities. Only 174 (11.29%) cases sought health care facilities within 48 hours from onset of the disease. The average duration of hospitalization and total duration of illness until discharge was observed as 5.65 (±4.12) and 12.16 (±6.86) nights, respectively.

Socio-demographic characteristics

Table 1 summarizes the socio-demographic characteristics of the hospitalized dengue patients. Overall sex ratio (male: female) for dengue cases was 1.76:1 (64% and 36%, respectively). The data collected from two paediatric hospitals (n=581) indicated that number of cases in the age group of 0–1, 1–4 and 5 and above years were 76 (13.08%), 140 (24.10%) and 365 cases (62.82%), respectively, in which the high incidence of 10.53% of dengue death

Figure 1: Percentage of dengue patients admitted at study sites (n=1539)
was reported among the infants (0–1 years). The mean (±SD) age of the patients was 6.51 (±4.52) years. The data from the general hospitals revealed that a maximum number of 534 (55.63%) cases were in the age group of 15–44 years, followed by <15 years (29.48%). The age distribution was not statistically significant among dengue groups and mean age was observed as 24.79 (±17) years.

**Laboratory investigation**

The median platelet counts in DF, DHF, and DSS were 60 000, 47 000 and 40 000, respectively (p=0.001). The mean Hct values across the groups do not show a difference statistically (p=0.582). More than 40% of haematocrit was observed in 198 (31.58%) cases. The mean ALT in DF, DHF and DSS was 136.71U/L, 361.31U/L and 243.22U/L, respectively (p=0.032). About 59.75% and 15.98% of patients had elevation of ALT 40–200 and above 200 U/L, respectively. A statistically significant association was not found with regard to AST between the dengue groups. Elevation of AST >200 U/L was found in 25.63% of dengue patients. The basic characteristic of subjects is shown in Table 1.

A platelet count of <100 000/l was observed in 881 (73.36%) DF, 121 (85.82%) DHF and 66 (75.86%) DSS, in which 52.53% was reported among children. Of the 1068 (74.74%) patients with thrombocytopenia (platelet count <100 000), 184 (12.88%) had severe thrombocytopenia (platelet count <20 000), followed by 428 (29.95%) who had moderate thrombocytopenia (platelet count 20 000–50 000). Though the severity of thrombocytopenia was found to be high in age group <15 years, it was also equivalently reported in other age groups (>15 years). A statistically significant association was found between the age, haematocrit rise, serologically positive cases and platelet group except between the sex and platelet group (p-value 0.176).

**Clinical presentation**

The development of haemorrhagic manifestations as seen in patients with DF, DHF, and DSS were 26.69%, 68.60% and 50.65%, respectively, with 38.70% occurring in patients with platelet count <50 000. The patients with a raised haematocrit of >40% and thrombocytopenia (platelet count <50 000) were observed to be marginally at risk of getting haemorrhagic symptoms. There were 22 and 282 dengue patients who developed severe thrombocytopenia (platelet count <10 000 and platelet <50 000, respectively) without prior bleeding whereas some patients with normal platelet value (>100 000) had bleeding manifestations. The bleeding manifestation was not statistically associated with age groups (p-value 0.051) and was commonly reported in both groups (Table 3).

Table 2 and Figure 2 show the association between the platelet count and dengue classification with presence of bleeding symptom. Ironically, the haemorrhagic manifestation was significantly related to the severity of thrombocytopenia in DF cases, but there was
**Table 1:** Patient profiles by types of dengue infection: clinical dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS)

<table>
<thead>
<tr>
<th>Patient profiles</th>
<th>DF (n = 1302) Mean ± SD</th>
<th>DHF (n =147) Mean ± SD</th>
<th>DSS (n = 92) Mean ± SD</th>
<th>No. of patients</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age group (Year) ^ (n, %)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>52 (11.8)</td>
<td>13 (14.9)</td>
<td>11 (20.8)</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>1-4</td>
<td>100 (22.7)</td>
<td>31 (35.6)</td>
<td>9 (17.0)</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td>5 and above</td>
<td>289 (65.5)</td>
<td>43 (49.4)</td>
<td>33 (62.3)</td>
<td>365</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>6.81 ± 4.566</td>
<td>5.34 ± 4.204</td>
<td>5.91 ± 4.373</td>
<td>581</td>
<td>0.016*</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (n, %)</td>
<td>849 (65.2)</td>
<td>81 (55.1)</td>
<td>53 (57.6)</td>
<td>983</td>
<td>0.024*</td>
</tr>
<tr>
<td><strong>Haematological</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>11.427 ± 2.73</td>
<td>11.143 ± 2.69</td>
<td>11.901 ± 2.66</td>
<td>1397</td>
<td>0.138</td>
</tr>
<tr>
<td>Patient profiles</td>
<td>DF ( (n = 1302) ) Mean ± SD</td>
<td>DHF ( (n = 147) ) Mean ± SD</td>
<td>DSS ( (n = 92) ) Mean ± SD</td>
<td>No. of patients</td>
<td>( P )-value</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------------------------</td>
<td>--------------------------------</td>
<td>-----------------------------</td>
<td>----------------</td>
<td>-------------</td>
</tr>
<tr>
<td><strong>Platelet (/µL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50 000</td>
<td>492 (41.0)</td>
<td>73 (51.8)</td>
<td>47 (54.0)</td>
<td>612</td>
<td></td>
</tr>
<tr>
<td>50 000-100 000</td>
<td>389 (32.4)</td>
<td>48 (34.0)</td>
<td>19 (21.8)</td>
<td>456</td>
<td></td>
</tr>
<tr>
<td>&gt; 100 000</td>
<td>320 (26.6)</td>
<td>20 (14.2)</td>
<td>21 (24.1)</td>
<td>361</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>84 796 ± 79 313</td>
<td>66 450 ± 64 335</td>
<td>73 370 ± 73 779</td>
<td>1429</td>
<td>0.001**</td>
</tr>
<tr>
<td>Median</td>
<td>60 000</td>
<td>47 000</td>
<td>44 000</td>
<td>1429</td>
<td></td>
</tr>
<tr>
<td><strong>Haematocrit (PCV), (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-40 gm.</td>
<td>343 (67.4)</td>
<td>47 (69.1)</td>
<td>39 (78.0)</td>
<td>429</td>
<td></td>
</tr>
<tr>
<td>Above 40 gm.</td>
<td>166 (32.6)</td>
<td>21 (30.9)</td>
<td>11 (22.0)</td>
<td>198</td>
<td></td>
</tr>
<tr>
<td>Mean haematocrit(SD)</td>
<td>36.76 ± 7.55</td>
<td>36.09 ± 8.66</td>
<td>35.79 ± 6.72</td>
<td>627</td>
<td>0.582</td>
</tr>
<tr>
<td><strong>Biochemical</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ALT (U/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-40 units</td>
<td>141 (25.9)</td>
<td>9 (14.5)</td>
<td>8 (17.8)</td>
<td>158</td>
<td></td>
</tr>
<tr>
<td>&gt;40-200 units</td>
<td>321 (59.0)</td>
<td>44 (71.0)</td>
<td>24 (53.3)</td>
<td>389</td>
<td></td>
</tr>
<tr>
<td>&gt;200 units</td>
<td>82 (15.1)</td>
<td>9 (14.5)</td>
<td>13 (28.9)</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>Mean ALT (SD)</td>
<td>136.71 ± 249.66</td>
<td>361.31 ± 129.20</td>
<td>243.22 ± 567.81</td>
<td>651</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>71</td>
<td>93</td>
<td>88</td>
<td>651</td>
<td>0.032*</td>
</tr>
<tr>
<td><strong>AST (U/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-40 units</td>
<td>134 (23.6)</td>
<td>9 (14.1)</td>
<td>8 (16.7)</td>
<td>151</td>
<td></td>
</tr>
<tr>
<td>Patient profiles</td>
<td>DF (n = 1302) Mean ± SD</td>
<td>DHF (n = 147) Mean ± SD</td>
<td>DSS (n = 92) Mean ± SD</td>
<td>No. of patients</td>
<td>P-value</td>
</tr>
<tr>
<td>------------------</td>
<td>-------------------------</td>
<td>-------------------------</td>
<td>------------------------</td>
<td>-----------------</td>
<td>---------</td>
</tr>
<tr>
<td>&gt;40-200 units</td>
<td>297 (52.4)</td>
<td>34 (53.1)</td>
<td>23 (47.9)</td>
<td>354</td>
<td></td>
</tr>
<tr>
<td>&gt;200 units</td>
<td>136 (24.0)</td>
<td>21 (32.8)</td>
<td>17 (35.4)</td>
<td>174</td>
<td></td>
</tr>
<tr>
<td>Mean AST (SD)</td>
<td>169.93 ± 263.36</td>
<td>239.70 ± 467.98</td>
<td>397.73 ± 1140.4</td>
<td>679</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>87</td>
<td>85</td>
<td>97.5</td>
<td>679</td>
<td>0.259</td>
</tr>
<tr>
<td><strong>Case management</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dead (n, %)</td>
<td>38 (2.9)</td>
<td>12 (8.2)</td>
<td>21 (22.8)</td>
<td>71</td>
<td>0.0001***</td>
</tr>
</tbody>
</table>

^Paediatric hospital; ^ ^General hospital; *Significant at 5% level **Significant at 0.5% level ***Significant at 0.1% level
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Platelet count</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;20 000/µl</td>
<td>20 000/µl to 50 000/µl</td>
<td>50 000/µl to 100 000/µl</td>
</tr>
<tr>
<td>No. of cases of dengue infection</td>
<td>184 (12.88)</td>
<td>428 (29.95)</td>
<td>456 (31.91)</td>
</tr>
<tr>
<td>No. of patients with bleeding (%)</td>
<td>61/139 (43.88)</td>
<td>117/321 (36.45)</td>
<td>104/317 (32.81)</td>
</tr>
<tr>
<td>No. of DF cases</td>
<td>142 (11.82)</td>
<td>350 (29.14)</td>
<td>389 (32.39)</td>
</tr>
<tr>
<td>No. of DF cases with bleeding symptoms</td>
<td>39/108 (36.11)</td>
<td>72/252 (28.57)</td>
<td>72/260 (27.69)</td>
</tr>
<tr>
<td>No. of DHF cases</td>
<td>25 (17.73)</td>
<td>48 (34.04)</td>
<td>48 (34.04)</td>
</tr>
<tr>
<td>No. of DHF cases with bleeding symptoms</td>
<td>17/20 (85.00)</td>
<td>30/41 (73.17)</td>
<td>23/41 (56.10)</td>
</tr>
<tr>
<td>No. of DSS cases</td>
<td>17 (19.54)</td>
<td>30 (34.48)</td>
<td>19 (21.84)</td>
</tr>
<tr>
<td>No. of DSS cases with bleeding symptoms</td>
<td>5/11 (45.45)</td>
<td>15/28 (53.57)</td>
<td>9/16 (56.25)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (n, %)</td>
<td>115/184 (62.5)</td>
<td>281/428 (65.7)</td>
<td>294/456 (64.5)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15 years</td>
<td>87 (11.2)</td>
<td>239 (30.6)</td>
<td>235 (30.1)</td>
</tr>
<tr>
<td>15 and above</td>
<td>97 (14.95)</td>
<td>189 (29.12)</td>
<td>221 (34.05)</td>
</tr>
<tr>
<td>Haematocrit ≥ 40%</td>
<td>48/102 (47.06)</td>
<td>61/170 (35.88)</td>
<td>51/173 (29.48)</td>
</tr>
<tr>
<td>Dengue serology positive cases (n, %)</td>
<td>119/138 (86.23)</td>
<td>285/334 (85.33)</td>
<td>269/331 (81.27)</td>
</tr>
</tbody>
</table>

*Significant at 1% level **Significant at 0.5% level ***Significant at 0.1% level
Table 3: Association of bleeding to age, PCV, platelet count and dengue infection

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Bleeding</th>
<th>Non-bleeding</th>
<th>N</th>
<th>RR (95% of C.I.)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, n</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15 years</td>
<td>187</td>
<td>424</td>
<td>611</td>
<td>0.845 (0.714-1.001)</td>
<td>0.051</td>
</tr>
<tr>
<td>15 and above</td>
<td>172</td>
<td>303</td>
<td>475</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PCV, n</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 40.0 %</td>
<td>116</td>
<td>184</td>
<td>300</td>
<td>1.283 (0.965-1.706)</td>
<td>0.078</td>
</tr>
<tr>
<td>&lt; 40.0 %</td>
<td>44</td>
<td>102</td>
<td>146</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Platelet count, n</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50 000/μL</td>
<td>178</td>
<td>282</td>
<td>460</td>
<td>1.363 (1.142-1.627)</td>
<td>0.001*</td>
</tr>
<tr>
<td>&gt;50 000/μL</td>
<td>153</td>
<td>386</td>
<td>539</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Disease category, n</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DF</td>
<td>237</td>
<td>651</td>
<td>888</td>
<td></td>
<td>0.000**</td>
</tr>
<tr>
<td>DHF</td>
<td>83</td>
<td>38</td>
<td>121</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSS</td>
<td>39</td>
<td>38</td>
<td>77</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant at 0.5% level ** Significant at 0.1% level

no significant association found between the bleeding symptoms and the degree of thrombocytopenia among the patients progressing to DHF/DSS, as 57.14% and 35.29% of patients having platelet count >100 000/l had bleeding manifestations. Haematemesis, melena, epistaxis and gum bleeding except petechial rash were significantly more common in adults when compared to children (Table 4).

**Limitation**

This study has some unavoidable limitations, since the study was conducted at tertiary care units only, the frequency of thrombocytopenia was observed to be high as the clinicians typically treated patients whose condition was severe in nature and who had often been referred by primary/secondary care units. At the same time, the effectiveness of the study was directly influenced by the availability, completeness, accuracy and systematic recording of patient’s medical/laboratory investigations history in the medical records as the retrospectively collected data seemed to be inadequate, especially in documenting the course of pre-hospitalization, missing values/lack of notifying regarding clinical observations during hospital stay and the strategy of treating severe bleeding (platelet transfusion). Another likely
Figure 2: Association of bleeding manifestation with platelet counts by types of dengue infection

Discussion

This study highlights the association between the haematological parameters with bleeding manifestations and severity of dengue infection. The demographic profile of the patients revealed that the proportion of dengue cases for the age group of 15–44 was highest, which is consistent with other Indian studies. The geriatric population exhibited lowest infection incidence but registered highest mortality rate (14.29%), followed by paediatric (0–1 year) who contributed about 11.81% in accordance with other similar observations. The mean age of the dengue cases (admitted in general hospitals) was 24.79 (±17) years, indicating that the majority of cases are in productive working age. It indicates that the adults were infected eccentrically compared to that of children, which is corroborated by other studies.
### Table 4: Patients with clinical bleeding and laboratory features by comparing children and adults with dengue infection

<table>
<thead>
<tr>
<th>Bleeding disorder</th>
<th>Children</th>
<th>Adults</th>
<th>N</th>
<th>RR (95% of C.I.)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of patients with</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petechial rash, ecchymoses or purpura</td>
<td>94/359 (26.18)</td>
<td>46/264 (17.42)</td>
<td>140/623</td>
<td>1.503 (1.097-2.059)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Malena</td>
<td>59/325 (18.15)</td>
<td>63/276 (22.83)</td>
<td>122/601</td>
<td>0.795 (0.579-1.092)</td>
<td>0.156</td>
</tr>
<tr>
<td>Haematemesis/ Blood in vomits</td>
<td>28/286 (9.79)</td>
<td>63/290 (21.72)</td>
<td>91/576</td>
<td>0.451 (0.298-0.682)</td>
<td>0.000**</td>
</tr>
<tr>
<td>Other bleeding sites ^</td>
<td>48/447 (10.74)</td>
<td>37/321 (11.53)</td>
<td>85/768</td>
<td>0.932 (0.622-1.396)</td>
<td>0.731</td>
</tr>
<tr>
<td>Platelet count &lt; 100 000 cells/cum (%)</td>
<td>561/780 (71.92)</td>
<td>507/649 (78.12)</td>
<td>1068/1429</td>
<td>0.921 (0.867-0.977)</td>
<td>0.007*</td>
</tr>
<tr>
<td>Haematocrit ≥ 40%</td>
<td>71/343 (20.70)</td>
<td>127/284 (44.72)</td>
<td>198/627</td>
<td>1.434 (1.275-1.614)</td>
<td>0.000**</td>
</tr>
</tbody>
</table>

^Other bleeding sites include epistaxis, gum bleeding, haematuria etc; *Significant at 1% level **Significant at 0.1% level
Although the thrombocytopenia is one of the predicting components in measuring severity of disease or a bleeding tendency, it was also commonly observed in DF. As compared to DF patients, patient with DHF/DSS have lower platelet counts. The bleeding tendency was also significantly observed in DF, although it was characteristically observed in DHF patients. Statistical analysis showed a significant association between haemorrhagic manifestations and the degree of thrombocytopenia, but only in context with DF cases. As disease progresses to DHF/DSS, the relationship between the degree of thrombocytopenia and bleeding manifestations becomes less pronounced. There are some patients who developed severe thrombocytopenia (platelet level <10 000) without bleeding manifestations, whilst others had bleeding manifestations with a platelet count of >100 000.\textsuperscript{22,23} In our analysis, platelet counts were not very much supportive in predicting severity of dengue infection or a bleeding tendency, suggesting that there are likely other important factors, apart from the platelet count, which also play a role in determining severity and bleeding tendency in dengue infection.\textsuperscript{22,24} A high haematocrit value was significantly associated with the decreasing value of thrombocytopenia but only a weak correlation was observed.

In summary, although the severity of thrombocytopenia and development of bleeding tendency is characteristically demonstrated in patients with DHF/DSS, it has been also commonly observed at significant level in DF patients. Furthermore, the clinical bleeding manifestations have no direct relationship with the degree of platelet count, especially in case of DHF/DSS. The changing clinical pattern of disease shows the heterogeneity in severity, on the one hand, and the necessity for further clinico-haematological research to comprehend the role of platelets in pathogenesis of dengue infection, on the other.

**Acknowledgements**

Authors are thankful to the Secretary, Department of Health Research, Government of India, and the Director General, Indian Council of Medical Research, New Delhi, for permission and encouragement. Authors are also thankful to various medical and health institutions across the country for providing help at all levels of this project.

**Conflict of interest**

The authors declare that they don’t have any conflict of interest.

**References**


Association of dengue symptoms with haematological parameters: a retrospective study of 10 hospitals in India


Estimation of burden using active dengue sentinel surveillance and fever surveillance

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a Medical College, Thiruvananthapuram, Kerala, India

Abstract

The State of Kerala in India is now hyperendemic for dengue. Thiruvananthapuram district reports the maximum number of cases in the state. The present surveillance mechanisms rely mostly on passive surveillance. This paper tries to estimate the burden of dengue in the district and state using one component of active surveillance, namely sentinel surveillance, in a representative sample of hospitals along with the fever surveillance data.

The proportion of dengue among acute febrile illness obtained by two cross sectional studies in 2011 and 2012 in Thiruvananthapuram district were projected on the fever surveillance reports of 2006–2013 for the state and the district. The population reported in the Census 2011 was used as denominator to calculate the incidence per 100 000.

The average incidence of dengue is estimated to be 1369 per 100 000 in the district and 1514 per 100 000 in the state, if 20% of fevers are dengue. If a lower proportion of 2.8%, obtained from 2011 study, is applied, the average estimated incidence for the state and district will be 212 and 192/100 000, respectively. Only 1–10% of the estimated cases are being reported in the state. In the district the reported cases are only 5–40% of the real burden.

The estimated burden of disease in Kerala is high. The reported cases are only the tip of the iceberg. The paper tries to highlight the relevance and possibilities of using good quality data for making estimation of disease burden, citing the example of dengue.

Keywords: Dengue, incidence, iceberg, Kerala, surveillance, Thiruvananthapuram.

Introduction

Dengue is one of the most serious and fast emerging tropical mosquito-borne disease. The burden of this most important arboviral disease is 465 000 DALYs across the globe. India is heading to transform into a major hyperendemic niche for dengue infection. Kerala State in India, is now hyperendemic for dengue with presence of multiple serotypes, high rates of
coinfection and local genomic evolution of viral strains. The district of Thiruvananthapuram reports maximum number of cases in the state. Active disease surveillance based on strong health information systems is one of the salient features of the global strategy for control of dengue fever/dengue haemorrhagic fever (DF/DHF). The surveillance system comprises passive surveillance, active surveillance and event-based surveillance. The present policies in most health systems rely on passive surveillance or event-based surveillance. This paper tries to estimate the burden of dengue in the district and the state, using one component of active surveillance, namely sentinel surveillance, in a representative sample of hospitals along with the fever surveillance data.

Methods

Two cross sectional studies were conducted in the outpatient (OP) and casualty departments of primary, secondary and tertiary health care institutions of Thiruvananthapuram. The first study was conducted during the period February to July 2011 and covered pre-monsoon and monsoon seasons. The second study was conducted from January to December 2012. The details of these studies are available from previous publications. Patients more than 5 years old, with acute febrile illness were recruited. Among them, those who had a confirmatory evidence of the etiology of fever other than dengue (for instance, urinary tract infection) were excluded. RT-PCR was used as the confirmatory test for dengue in patients with fever of less than or equal to five days and serology was used when the patients had fever of more than five days. From these studies, the proportion of dengue among acute undifferentiated febrile illness was calculated. The number of fever cases during the years 2006–2013 for Thiruvananthapuram district and Kerala State were obtained from the fever surveillance data reports of the Directorate of Health Services and the State Disease Control and Monitoring Cell reports. The proportion of dengue from the two cross sectional studies was projected to the total fever cases in the district and the state, to obtain the number of dengue cases for the corresponding years. The population of the district and the state reported in the Census 2011 was used as denominator to calculate the incidence/100 000. Using the reported number of cases during these years, the percentage of estimated cases reported was calculated. The incidence of infection was calculated using an apparent to symptomatic dengue ratio of two and four.

Results

In the cross sectional study done in 2011, only 2.8% (95% CI 0.5-5.1), that is, 7/254 acute febrile illnesses were dengue. In the study conducted in 2012, among the 851 cases of acute febrile illness, 20.4% [95% CI: 17.8–23.3] were positive for dengue. The average estimated annual number of fever cases in Thiruvananthapuram and Kerala from 2006 to 2013 was 226 501 and 2 528 956, respectively. The average number of dengue cases estimated using the 2012 study findings is 505 791 for the state (Table 1) and 45 300 for the district (Table 2).
Table 1: The estimates of dengue for the State of Kerala using 2012 findings

<table>
<thead>
<tr>
<th>Year</th>
<th>Total fever</th>
<th>Estimated number of dengue</th>
<th>Reported number</th>
<th>Incidence of Dengue</th>
<th>Incidence of infection (I:S -2)</th>
<th>Incidence of infection (I:S -4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>3 029 138</td>
<td>605 827</td>
<td>7911</td>
<td>1814.52</td>
<td>3629.05</td>
<td>7258.10</td>
</tr>
<tr>
<td>2012</td>
<td>2 406 629</td>
<td>481 325</td>
<td>4056</td>
<td>1441.63</td>
<td>2883.25</td>
<td>5766.51</td>
</tr>
<tr>
<td>2011</td>
<td>1 927 684</td>
<td>385 536</td>
<td>1304</td>
<td>1154.73</td>
<td>2309.46</td>
<td>4618.91</td>
</tr>
<tr>
<td>2010</td>
<td>1 621 641</td>
<td>324 328</td>
<td>1019</td>
<td>971.40</td>
<td>1942.80</td>
<td>3885.60</td>
</tr>
<tr>
<td>2009</td>
<td>3 575 855</td>
<td>715 171</td>
<td>657</td>
<td>2142.02</td>
<td>4284.04</td>
<td>8568.08</td>
</tr>
<tr>
<td>2008</td>
<td>2 224 086</td>
<td>444 817</td>
<td>726</td>
<td>1332.28</td>
<td>2664.56</td>
<td>5329.12</td>
</tr>
<tr>
<td>2007</td>
<td>3 136 941</td>
<td>627 388</td>
<td>1417</td>
<td>1879.10</td>
<td>3758.20</td>
<td>7516.40</td>
</tr>
<tr>
<td>2006</td>
<td>2 309 672</td>
<td>461 934</td>
<td>2503</td>
<td>1383.55</td>
<td>2767.10</td>
<td>5534.19</td>
</tr>
</tbody>
</table>

Table 2: The estimates of dengue for Thiruvananthapuram district using 2012 findings

<table>
<thead>
<tr>
<th>Year</th>
<th>Total fever</th>
<th>Estimated number of dengue</th>
<th>Reported number</th>
<th>Incidence of Dengue</th>
<th>Incidence of infection (I:S -2)</th>
<th>Incidence of infection (I:S -4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>353 806</td>
<td>70 761</td>
<td>4188</td>
<td>2139.56</td>
<td>4279.11</td>
<td>8558.22</td>
</tr>
<tr>
<td>2012</td>
<td>269 570</td>
<td>53 914</td>
<td>2477</td>
<td>1630.16</td>
<td>3260.32</td>
<td>6520.64</td>
</tr>
<tr>
<td>2011</td>
<td>195 072</td>
<td>39 014</td>
<td>865</td>
<td>1179.65</td>
<td>2359.30</td>
<td>4718.60</td>
</tr>
<tr>
<td>2010</td>
<td>184 012</td>
<td>36 802</td>
<td>656</td>
<td>1112.77</td>
<td>2225.54</td>
<td>4451.07</td>
</tr>
<tr>
<td>2009</td>
<td>226 113</td>
<td>45 222</td>
<td>290</td>
<td>1367.36</td>
<td>2734.73</td>
<td>5469.45</td>
</tr>
<tr>
<td>2008</td>
<td>134 689</td>
<td>26 937</td>
<td>503</td>
<td>814.50</td>
<td>1629.00</td>
<td>3258.00</td>
</tr>
<tr>
<td>2007</td>
<td>270 697</td>
<td>54 139</td>
<td>800</td>
<td>1636.97</td>
<td>3273.95</td>
<td>6547.90</td>
</tr>
<tr>
<td>2006</td>
<td>178 050</td>
<td>35 610</td>
<td>1150</td>
<td>1076.71</td>
<td>2153.43</td>
<td>4306.86</td>
</tr>
</tbody>
</table>

It is 70 811 and 6342 for the state (Table 3) and the district (Table 4) if the 2011 study findings are applied. The average incidence of dengue is estimated to be 1369 per 100 000 in the district and 1514 per 100 000 in the state, if 20% of fevers are dengue. If the result of the 2011 findings is applied, the average estimated incidence for the state and district will be 212 and 192/100 000, respectively. The percentage of estimated cases reported is shown in Figures 1 and 2. The average incidence of dengue infections was estimated to
Table 3: The estimates of dengue for the State of Kerala using 2011 findings

<table>
<thead>
<tr>
<th>Year</th>
<th>Total fever</th>
<th>Estimated number of dengue</th>
<th>Reported number</th>
<th>Incidence of Dengue</th>
<th>Incidence of infection (I:S -2)</th>
<th>Incidence of infection (I:S -4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>3 029 138</td>
<td>84 815</td>
<td>7911</td>
<td>254.03</td>
<td>508.07</td>
<td>1016.13</td>
</tr>
<tr>
<td>2012</td>
<td>2 406 629</td>
<td>67 385</td>
<td>4056</td>
<td>201.83</td>
<td>403.66</td>
<td>807.31</td>
</tr>
<tr>
<td>2011</td>
<td>1 927 684</td>
<td>53 975</td>
<td>1304</td>
<td>161.66</td>
<td>323.32</td>
<td>646.65</td>
</tr>
<tr>
<td>2010</td>
<td>1 621 641</td>
<td>45 405</td>
<td>1019</td>
<td>136.00</td>
<td>271.99</td>
<td>543.98</td>
</tr>
<tr>
<td>2009</td>
<td>3 575 855</td>
<td>100 123</td>
<td>657</td>
<td>299.88</td>
<td>599.77</td>
<td>1199.53</td>
</tr>
<tr>
<td>2008</td>
<td>2 224 086</td>
<td>62 274</td>
<td>726</td>
<td>186.52</td>
<td>373.04</td>
<td>746.08</td>
</tr>
<tr>
<td>2007</td>
<td>3 136 941</td>
<td>87 834</td>
<td>1417</td>
<td>263.07</td>
<td>526.15</td>
<td>1052.30</td>
</tr>
<tr>
<td>2006</td>
<td>2 309 672</td>
<td>64 670</td>
<td>2503</td>
<td>193.70</td>
<td>387.39</td>
<td>774.79</td>
</tr>
</tbody>
</table>

Table 4: The estimates of dengue for Thiruvananthapuram using 2011 findings

<table>
<thead>
<tr>
<th>Year</th>
<th>Total fever</th>
<th>Estimated number of dengue</th>
<th>Reported number</th>
<th>Incidence of Dengue</th>
<th>Incidence of infection (I:S -2)</th>
<th>Incidence of infection (I:S -4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>353 806</td>
<td>9906</td>
<td>4188</td>
<td>299.54</td>
<td>599.08</td>
<td>1198.15</td>
</tr>
<tr>
<td>2012</td>
<td>269 570</td>
<td>7547</td>
<td>2477</td>
<td>228.22</td>
<td>456.44</td>
<td>912.89</td>
</tr>
<tr>
<td>2011</td>
<td>195 072</td>
<td>5462</td>
<td>865</td>
<td>165.15</td>
<td>330.30</td>
<td>660.60</td>
</tr>
<tr>
<td>2010</td>
<td>184 012</td>
<td>5152</td>
<td>656</td>
<td>155.79</td>
<td>311.58</td>
<td>623.15</td>
</tr>
<tr>
<td>2009</td>
<td>226 113</td>
<td>6331</td>
<td>290</td>
<td>191.43</td>
<td>382.86</td>
<td>765.72</td>
</tr>
<tr>
<td>2008</td>
<td>134 689</td>
<td>3771</td>
<td>503</td>
<td>114.03</td>
<td>228.06</td>
<td>456.12</td>
</tr>
<tr>
<td>2007</td>
<td>270 697</td>
<td>7579</td>
<td>800</td>
<td>229.18</td>
<td>458.35</td>
<td>916.71</td>
</tr>
<tr>
<td>2006</td>
<td>178 050</td>
<td>4985</td>
<td>1150</td>
<td>150.74</td>
<td>301.48</td>
<td>602.96</td>
</tr>
</tbody>
</table>
**Figure 1:** Percentage of estimated cases of dengue reported in the state

**Figure 2:** Percentage of estimated cases of dengue reported in the district
Table 5: Age distribution of study subjects and positive cases

<table>
<thead>
<tr>
<th>Age group</th>
<th>2011 study</th>
<th></th>
<th>2012 study</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dengue positive</td>
<td>N=7</td>
<td>Dengue negative</td>
<td>N=247</td>
</tr>
<tr>
<td>5–10</td>
<td>0 (0%)</td>
<td>5 (2%)</td>
<td>14 (8.0%)</td>
<td>52 (7.7%)</td>
</tr>
<tr>
<td>11–20</td>
<td>1 (14.3%)</td>
<td>55 (22.3%)</td>
<td>39 (22.4%)</td>
<td>67 (24.7%)</td>
</tr>
<tr>
<td>21–30</td>
<td>2 (28.6%)</td>
<td>57 (23.1%)</td>
<td>33 (19.0%)</td>
<td>31 (19.4%)</td>
</tr>
<tr>
<td>31–40</td>
<td>3 (42.9%)</td>
<td>55 (22.3%)</td>
<td>30 (17.2%)</td>
<td>123 (18.2%)</td>
</tr>
<tr>
<td>41–50</td>
<td>1 (14.3%)</td>
<td>38 (15.4%)</td>
<td>27 (15.5%)</td>
<td>87 (12.9%)</td>
</tr>
<tr>
<td>51–60</td>
<td>0 (0%)</td>
<td>21 (8.5%)</td>
<td>19 (10.9%)</td>
<td>74 (11.0%)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>0 (0%)</td>
<td>16 (6.5%)</td>
<td>12 (6.9%)</td>
<td>41 (6.1%)</td>
</tr>
</tbody>
</table>

be 3030-6060/100 000 for the state and 2739-5479/100 000 for the district in the worst case scenario. Using the better case scenario, it would be 424-848/100 000 for the state and 384-767/100 000 for the district. The age distribution of the cases is shown in Table 5. All age groups studied are affected in 2012 study, with the 11–20 year age group most afflicted.

Discussion

The proportion of dengue among acute febrile illness obtained as 20.4% and used for the estimation of incidence is comparable with other studies. The first global estimates of total dengue virus infections were based on an assumed constant annual infection rate among a crude approximation of the population at risk, 10% in 1 billion or 4% in 2 billion, yielding figures of 80–100 million infections per year worldwide in 1988. This was revised to 50–100 million infections, although larger estimates of 100–200 million have also been made. If 50–100 million cases occur worldwide, taking the denominator as 7 billion, the incidence of dengue globally will be 700- 1000/100 000 population. India contributes to 34% of the global burden, an estimated 47–97 million cases annually. Using these estimates, the incidence/100 000 population will be 3900–7000, for India. The estimated incidence of dengue from this study, although slightly higher than the estimated worldwide incidence, is close to the estimates for the country. Estimates of incidence in other parts of the world place the average annual risk of dengue at 612 cases per 100 000 (urban 727 and rural 217). A review of 38 cohort studies from 19 countries, which calculated the actual incidence, has obtained a mean incidence of 129.7/1000 person year (standard deviation ±132). In a major epidemic in Malaysia in 1998, the estimates were as low as 123/100 000 and in Indonesia it was 37/100 000 in 2012. Therefore, the estimates for India and that obtained in our
study are higher than many other nations. Infection with any of the four dengue serotypes can be asymptomatic in 65–90%. The number of asymptomatics is three times more than symptomatics. In Asians, the figures are 204.4 million (151.8–273.0) and 66.8 million (47.0–94.4), respectively, and thus the inapparent to symptomatic (I:S) ratio is 3. Hence, we chose to find the ratio of 2 and 4 for estimation in this study. A recent seroprevalence study in Singapore showed a ratio of 23 asymptomatic cases to each reported clinical case. The mean I:S obtained in a review of 38 cohort studies was 4.3 (standard deviation ± 2.8). Thus there exists a wide variation in the report ratios of inapparent to symptomatic infections.

Passive surveillance using case definitions is labour intensive. It is also limited by the low specificity, non-uniformity in the case definitions used and inconsistency in reporting standards. It is especially difficult in diseases such as dengue with nonspecific clinical manifestations. This results in underreporting/overreporting and it weakens the surveillance systems. Effective fever surveillance is easy to establish. A web-based reporting system may improve reporting completeness. It has collateral benefits for estimation of burden of other diseases that manifest as fever. The opportunities and challenges of acting beyond the traditional surveillance systems in favour of syndromic surveillance have been reviewed.

The limitations of the study are the following. Children less than 5 years were excluded from testing, but extrapolation is done to the entire population and it could be an overestimation. However, this is not a major concern because <5 years is a small percent of the population and does not include the peak age of symptomatic disease. Coexistence of dengue fever with other causes of acute febrile illness may not have been picked, since those with a definitive diagnosis of cause of fever were excluded. The estimations could be lower than actual, if such phenomenon exists. IgM remains positive for three months; therefore, the use of a single value might not always reflect that the present episode of fever is due to dengue. The time points of fever surveillance and active sentinel surveillance of dengue in this study are different. The number of samples collected was not stratified proportional to the number of fever cases during each month. Since the district of Thiruvananthapuram reports the maximum number of cases of dengue, the projection for the state may be a worst case scenario. The projections are dependent on the validity and quality of the fever surveillance in the state.

Conclusions

The estimated burden of dengue in the State of Kerala and the district of Thiruvananthapuram are high. The reported cases represent only the tip of the iceberg. The paper tries to highlight the relevance and possibilities of using good quality data for making estimation of disease burden, citing the example of dengue. It adds further to the need of good quality fever surveillance systems. Similar studies need to be done in different geographical regions to estimate the real burden of the disease.
References


Estimation of burden using active dengue sentinel surveillance and fever surveillance


Identification of key containers of Aedes breeding – a cornerstone to control strategies of dengue in Delhi, India


*National Institute of Malaria Research (NIMR), New Delhi

**Municipal Corporation of Delhi (MCD)

***New Delhi Municipal Corporation (NDMC)

Abstract

The present study is based on five years’ data (December 2007–November 2012) with the objective to study whether Aedes breeding takes place throughout the year and to identify so-called key containers for Aedes breeding in Delhi, India. Larvae collected from 250–300 randomly selected localities of Delhi were brought to the laboratory and habitat-wise rearing was done till emergence, to identify the key container preferred by Aedes aegypti. During non-transmission season, highest positivity was observed in overhead tanks (positivity 46.5%; Container Index: 3.21) and curing tanks (positivity 16.9%; Container Index: 4.96). There was only a threefold rise in container indices of overhead tanks and curing tanks from non-transmission to transmission season whereas in other containers the rise was more than fivefold. Independent ‘t’ test carried out on positivity of various breeding habitats during both seasons showed that overhead tanks, curing tanks and cemented pits were not significantly different (p>0.05). It was concluded that overhead tank acts as key container of Aedes breeding in Delhi as it shows a consistent breeding throughout the study period. The rest of the containers do play intermittent roles in supporting the breeding of Aedes mosquito and hence need separate attention while reforming control strategies.

Keywords: Dengue, Aedes aegypti, key container, container index.

Introduction

Dengue is the fastest spreading vector-borne viral disease and is now endemic in over 100 countries. At present half of the world’s population is living in areas at risk for dengue. In India, the first outbreak of dengue was observed from Vellore in 1956 and first dengue...
haemorrhagic fever (DHF) outbreak was observed from Kolkata in 1963. In 2013, more than 75,000 dengue cases were recorded from India, with 193 deaths. Besides this, dengue penetrated in states reporting sporadic cases earlier, such as Odisha (7132 cases, six deaths), Gujarat (6272 cases, 15 deaths), Karnataka (6408 cases, 12 deaths), Kerala (7938 cases, 29 deaths), Assam (4526 cases, two deaths) and Uttar Pradesh (1414 cases, five deaths). Almost all the states of the country are reporting dengue cases, including Delhi. Delhi witnessed dengue outbreaks during 1967, 1970, 1982, 1988, 1990, 1992, 1996 and 2003.

*Aedes aegypti*, the primary vector of dengue in most part of the world, is well adapted to urbanized areas, especially the surroundings of human dwellings, and breeds often in man-made containers. *Aedes aegypti* can breed in the lid of a small bottle or in big storage containers such as overhead tanks. On the basis of the proximity to human dwelling, source and the use of the water, *Aedes* breeding can be classified in two broad categories viz. domestic and peri-domestic. Domestic breeding habitats were defined as human-filled receptacles (in and around human dwellings), for instance, overhead tanks, coolers and water storage containers, whereas peri-domestic breeding habitats are discarded receptacles (far from human dwellings), such as solid waste (dump types, disposable plastic/thermacol glasses, broken earthen pots etc.), bird pots, cemented pits etc., normally filled by rain.

Entomological research has shown that in most areas there are a relatively small number of containers that consistently serve as the primary producers of *Aedes aegypti*, termed as key containers, with other containers playing minor roles in mosquito production, which may vary from place to place.

*Aedes aegypti* control programmes could be more cost effective and sustainable by concentrating efforts to control mosquito breeding in key containers and by rationalizing manpower and insecticide use in transmission season. *Aedes aegypti* population dynamics are influenced by social risk factors that vary by season and lagged climate variables that vary by locality.

National Institute of Malaria Research (NIMR), under the aegis of Indian Council of Medical Research (ICMR), has been conducting a longitudinal study in collaboration with Municipal Corporation of Delhi (MCD) and New Delhi Municipal Corporation (NDMC) on *Aedes* breeding survey in Delhi since 2007 with an underlying hypothesis that *Aedes* breeding is found throughout the year. The current study also aimed to identify key containers for breeding of *Aedes aegypti* in Delhi.
Materials and methods

Study area

The present study was carried out in Delhi, the national capital of India, that lies in the latitude 28.38 N and longitude 77.12 E on the bank of river Yamuna. As per 2011 Census, the population of Delhi is around 16 million spread over an area of around 1483 sq km. with population density of about 11 000 per sq. km. About 93% of the population resides in the urban area of state and all areas are likely to be urbanized soon in coming years.

Larval surveys

Aedes breeding survey was carried out by NIMR throughout the year in different localities of Delhi since 2006. The present study is based on five years’ data collected between December 2007 and November 2012. During the period, about 250–300 randomly selected localities of Delhi were surveyed by a team of five or six field workers from NIMR in collaboration with MCD and NDMC, using pipette or dipper depending on container type, as per WHO norms. Larvae collected from field sites were brought to the laboratory and habitat-wise rearing was done till emergence, to identify the key container preferred by the dengue vector, that is, *Aedes aegypti*. Houses were selected randomly from different income groups and each potential breeding site in the house was checked thoroughly. Besides houses, government offices, schools, nurseries, parks, picnic spots, police stations, bus depots, dispensaries, hospitals, etc. were also surveyed from time to time.

Overhead tanks (plastic and cemented tanks fixed on the terrace of house), water storage containers, curing tanks (cemented tanks built at construction sites), coolers and flower pots were identified as five major types of domestic containers, and bird pots, mud pots, pits (cemented) and solid waste (dump tyres, disposable plastic/thermocol glasses etc.) were identified as four major types of peri-domestic containers in Delhi. On the basis of *Aedes* breeding and number of cases recorded by MCD, June to November months were identified as transmission season and December to May months were identified as non-transmission season.

On an average four breeding sites were found in each house and 60 houses were surveyed per day. Working approximately 20 days per month, about 5000 containers were surveyed in a month. Over five years, a total of 284 248 containers were checked. Container-wise data was pooled on monthly basis and container index was calculated using following formula:

\[
\text{Container Index (CI)} = \frac{\text{Containers Positive}}{\text{Containers Checked}} \times 100
\]
Statistical analysis

The data were entered in Excel 2007 and statistical analysis was done by SPSS software package (Version 20).

Results

Out of 284,248 breeding habitats surveyed during the period (December 2007 to November 2012), 46% and 54% containers were checked during transmission and non-transmission season, respectively. A total of 23,593 habitats were found positive with container index of 8.30. During the five years of study period Container Index was observed in the range of 8.4–16.2 (average Container Index: 13.45) during transmission season whereas the same was in the range of 2–3 (average Container Index: 2.3) during non-transmission season (Table 1).

Seasonal prevalence of habitats

Month-wise container index of domestic containers, that is, overhead tanks, water storage containers, curing tanks, coolers and flower pots, and peri-domestic containers, that is, bird pots, mud pots, cemented pits and solid waste, are shown along with their images in Figure 1 (a-i). Variation in container indices is consistent with the variation observed in average rainfall (Figure 1 (a-i)) except the container indices of overhead tanks, curing tanks and cement pits. The variation observed in cemented pits was minor as compared to overhead tanks and curing tanks (Figure 1 a,b & h). Other containers did not vary significantly during non-transmission season as apparent by Figure 1 (c-g & i).

Table 1: Container Index during transmission and non-transmission season (2007–2012)

<table>
<thead>
<tr>
<th>Period</th>
<th>Checked</th>
<th>+ve</th>
<th>CI</th>
<th>Period</th>
<th>Checked</th>
<th>+ve</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jun’08–Nov’08</td>
<td>27 752</td>
<td>4414</td>
<td>15.9</td>
<td>Dec’07–May’08</td>
<td>25 882</td>
<td>528</td>
<td>2</td>
</tr>
<tr>
<td>Jun’09–Nov’09</td>
<td>29 454</td>
<td>3851</td>
<td>13.1</td>
<td>Dec’08–May’09</td>
<td>24 901</td>
<td>798</td>
<td>3.2</td>
</tr>
<tr>
<td>Jun’10–Nov’10</td>
<td>29 580</td>
<td>4790</td>
<td>16.2</td>
<td>Dec’09–May’10</td>
<td>25 071</td>
<td>632</td>
<td>2.5</td>
</tr>
<tr>
<td>Jun’11–Nov’11</td>
<td>36 450</td>
<td>4995</td>
<td>13.7</td>
<td>Dec’10–May’11</td>
<td>27 393</td>
<td>582</td>
<td>2.1</td>
</tr>
<tr>
<td>Jun’12–Nov’12</td>
<td>29 082</td>
<td>2441</td>
<td>8.39</td>
<td>Dec’11–May’12</td>
<td>28 683</td>
<td>562</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>152 318</td>
<td>20 491</td>
<td><strong>13.5</strong></td>
<td><strong>Total</strong></td>
<td>131 930</td>
<td>3102</td>
<td><strong>2.4</strong></td>
</tr>
<tr>
<td><strong>G. Total</strong></td>
<td>Checked</td>
<td>284 248</td>
<td>+ve</td>
<td><strong>23 593</strong></td>
<td>Cl 8.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

G. Total Checked 284 248 +ve 23 593 CI 8.3
Identification of key containers of Aedes breeding – A cornerstone to control strategies of dengue in Delhi, India

**Figure 1:** Seasonal prevalence in (a) overhead tanks (b) curing tanks (c) coolers (d) water storage containers (e) flower pots (f) mud pots (g) bird pots (h) cemented pits (i) solid waste
Transmission season

During transmission season a total of 152,318 containers were checked, 77.7% of which were domestic whereas remaining 22.3% containers were peri-domestic. Difference in domestic and peri-domestic containers during transmission season was tested by independent t test. The independent samples t test was associated with a statistically significant difference (p <0.05) for both the containers, t (8) = 3.12, p = 0.01. Among 20,491 containers found positive, domestic containers and peri-domestic containers contributed 64.9% and 35.1% positivity, respectively. Container Index was observed highest in solid waste (29.07%) followed by mud pots (18.25%) and cemented pits (16.61%). Maximum positivity was observed in water storage containers (26.2%) followed by overhead tanks (16.1%) among domestic containers whereas solid waste (18.1%) and cemented pits (6.5%) were two major positive peri-domestic containers (Table 2).

Non-transmission season

During non-transmission season a total of 131,930 containers were checked, 84.2% of which were domestic whereas remaining 15.8% containers were peri-domestic. The difference in domestic and peri-domestic containers during non-transmission season was tested by

Table 2: Number and proportion of habitats checked and positive with Container Index during transmission season

<table>
<thead>
<tr>
<th>Type of container</th>
<th>Habitat</th>
<th>Checked</th>
<th>Positive</th>
<th>Container Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Domestic</td>
<td>OHT</td>
<td>31,960</td>
<td>21%</td>
<td>3303</td>
</tr>
<tr>
<td></td>
<td>Curing tanks</td>
<td>7,837</td>
<td>5%</td>
<td>1,000</td>
</tr>
<tr>
<td></td>
<td>Cooler</td>
<td>27,975</td>
<td>18%</td>
<td>2,104</td>
</tr>
<tr>
<td></td>
<td>Containers</td>
<td>39,963</td>
<td>26%</td>
<td>5,375</td>
</tr>
<tr>
<td></td>
<td>Flower pot</td>
<td>10,684</td>
<td>7%</td>
<td>1,515</td>
</tr>
<tr>
<td></td>
<td>Mud pot</td>
<td>7,220</td>
<td>5%</td>
<td>1,318</td>
</tr>
<tr>
<td></td>
<td>Bird pot</td>
<td>5,842</td>
<td>4%</td>
<td>821</td>
</tr>
<tr>
<td></td>
<td>Cemented pits</td>
<td>8,042</td>
<td>5%</td>
<td>1,336</td>
</tr>
<tr>
<td></td>
<td>Solid waste</td>
<td>12,795</td>
<td>8%</td>
<td>3,719</td>
</tr>
<tr>
<td></td>
<td>G. total</td>
<td>152,318</td>
<td>100%</td>
<td>20,491</td>
</tr>
</tbody>
</table>
**Table 3:** Number and proportion of habitats checked and positive with Container Index during non-transmission season

<table>
<thead>
<tr>
<th>Type of container</th>
<th>Habitat</th>
<th>Checked</th>
<th>Positive</th>
<th>Container Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Domestic</td>
<td>Overhead tanks</td>
<td>44,936</td>
<td>34%</td>
<td>1441</td>
</tr>
<tr>
<td></td>
<td>Curing tanks</td>
<td>10,581</td>
<td>8%</td>
<td>525</td>
</tr>
<tr>
<td></td>
<td>Cooler</td>
<td>12,909</td>
<td>10%</td>
<td>204</td>
</tr>
<tr>
<td></td>
<td>Containers</td>
<td>30,945</td>
<td>24%</td>
<td>303</td>
</tr>
<tr>
<td></td>
<td>Flower pot</td>
<td>11,762</td>
<td>9%</td>
<td>16</td>
</tr>
<tr>
<td>Peri-domestic</td>
<td>Mud pot</td>
<td>4,608</td>
<td>4%</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>Bird pot</td>
<td>5,123</td>
<td>4%</td>
<td>136</td>
</tr>
<tr>
<td></td>
<td>Cemented pits</td>
<td>7,611</td>
<td>6%</td>
<td>263</td>
</tr>
<tr>
<td></td>
<td>Solid waste</td>
<td>3,455</td>
<td>3%</td>
<td>103</td>
</tr>
<tr>
<td><strong>G. total</strong></td>
<td></td>
<td>131,930</td>
<td>100%</td>
<td>3102</td>
</tr>
</tbody>
</table>

An independent t test. The independent samples t test was associated with a statistically significant difference (p < 0.05) for both the containers t (8) = 6.44, p = 0.001. Out of 3102 containers found positive, domestic and peri-domestic containers contributed 80.2% and 19.8% positivity, respectively. Among domestic containers, maximum positivity and Container Index was observed in domestic containers, that is, overhead tanks (positivity 46.5%; Container Index: 3.21) and curing tanks (positivity 16.9%; Container Index: 4.96) (Table 3). On the basis of highest positivity during non-transmission season, overhead tanks and curing tanks were identified as key containers for breeding of *Aedes aegypti* in Delhi.

**Comparison of breeding habitats during non-transmission and transmission season**

A t test was performed to compare individual containers in transmission and non-transmission season and p values were found significant for all the individual tests (Table 4). Analysis of Variance (ANOVA) was performed to ascertain any difference among container indices of various breeding habitats during transmission and non-transmission seasons and it was also found significant, F (17) = 11.23, p < 0.05.
Identification of key containers of Aedes breeding – A cornerstone to control strategies of dengue in Delhi, India

Among domestic containers, overhead tanks and curing tanks showed consistent breeding behaviour as there is only a threefold rise in container indices from non-transmission to transmission season whereas water storage containers, flower pots and coolers showed seasonal breeding behaviour as there is a more than fivefold rise in Container Index (Table 4, Figure 2). All peri-domestic containers, that is, mud-pots, bird pots, cemented pits and solid waste also show seasonal breeding behaviour as an increase is observed in Container Index of more than fivefold from non-transmission season to transmission season. On the basis of consistency in breeding behaviour, overhead tanks and curing tanks were re-identified as key containers.

To test the null hypothesis for difference in positivity of various breeding habitats checked during transmission and non-transmission seasons, an independent t test was performed. The independent samples t test was associated with a statistically significant difference (p <0.05) for coolers, water storage containers, flower pots, bird pots, mud pots and solid waste. The three containers, namely overhead tanks, curing tanks and cemented pits, were not found significantly different (p >0.05) for both the seasons (Table 5). As there is no significant difference in overhead tanks, curing tanks and cemented pits, it could be hence inferred that the breeding in these habitats does not change during transmission and non-transmission season and, therefore, can be considered as key containers.

Table 4: Habitat-wise container index during transmission and non-transmission season (2007–2012)

<table>
<thead>
<tr>
<th>Type of container</th>
<th>Breeding habitat</th>
<th>Container Index</th>
<th>Change (folds)</th>
<th>P value (significant if ≤ .05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Non-transmission</td>
<td>Transmission</td>
<td></td>
</tr>
<tr>
<td>Domestic</td>
<td>OHT</td>
<td>3.21</td>
<td>10.33</td>
<td>3↑</td>
</tr>
<tr>
<td>Curing tanks</td>
<td></td>
<td>4.96</td>
<td>12.76</td>
<td>3↑</td>
</tr>
<tr>
<td>Cooler</td>
<td></td>
<td>1.58</td>
<td>7.52</td>
<td>5↑</td>
</tr>
<tr>
<td>Containers</td>
<td></td>
<td>0.98</td>
<td>13.45</td>
<td>14↑</td>
</tr>
<tr>
<td>Flower pot</td>
<td></td>
<td>0.14</td>
<td>14.18</td>
<td>104↑</td>
</tr>
<tr>
<td>Peri-domestic</td>
<td>Mud pot</td>
<td>2.41</td>
<td>18.25</td>
<td>8↑</td>
</tr>
<tr>
<td></td>
<td>Bird pot</td>
<td>2.65</td>
<td>14.05</td>
<td>5↑</td>
</tr>
<tr>
<td></td>
<td>Cemented pits</td>
<td>3.46</td>
<td>16.61</td>
<td>5↑</td>
</tr>
<tr>
<td></td>
<td>Solid waste</td>
<td>2.98</td>
<td>29.07</td>
<td>10↑</td>
</tr>
</tbody>
</table>
**Table 5:** Independent t test for various breeding containers for *Aedes aegypti* checked during the study

<table>
<thead>
<tr>
<th>Habitat</th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
<th>95% Confidence Interval of the difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t</td>
<td>df</td>
<td>Sig. (2-tailed)</td>
<td>Lower</td>
</tr>
<tr>
<td>Overhead tanks</td>
<td>1.887</td>
<td>8</td>
<td>0.096</td>
<td>-82.644</td>
</tr>
<tr>
<td>Curing tanks</td>
<td>1.731</td>
<td>8</td>
<td>0.122</td>
<td>-31.551</td>
</tr>
<tr>
<td>Coolers</td>
<td>4.606</td>
<td>8</td>
<td>0.002</td>
<td>189.73</td>
</tr>
<tr>
<td>Water storage containers</td>
<td>3.756</td>
<td>8</td>
<td>0.006</td>
<td>391.66</td>
</tr>
<tr>
<td>Flower pots</td>
<td>2.58</td>
<td>8</td>
<td>0.033</td>
<td>31.817</td>
</tr>
<tr>
<td>Bird pots</td>
<td>3.265</td>
<td>8</td>
<td>0.011</td>
<td>40.231</td>
</tr>
<tr>
<td>Mud pots</td>
<td>2.415</td>
<td>8</td>
<td>0.042</td>
<td>10.918</td>
</tr>
<tr>
<td>Cemented pit</td>
<td>2.053</td>
<td>8</td>
<td>0.074</td>
<td>-26.477</td>
</tr>
<tr>
<td>Solid waste</td>
<td>5.623</td>
<td>8</td>
<td>0.000</td>
<td>426.62</td>
</tr>
</tbody>
</table>

**Figure 2:** Comparison in container index of breeding habitat during transmission and non-transmission season
It was also observed that during five years (30 months each in transmission and non-transmission season) overhead tanks were found positive almost every month (29 out of 30 months) whereas curing tanks (20 out of 30 months) and cemented pits (11 out of 30 months) were not found positive consistently (Figure 3). Non-consistent behaviour of cemented pits and curing tanks during non-transmission season revealed that overhead tank was the only key container of *Aedes* breeding in Delhi.

**Discussion**

In our study overhead tanks were identified as key container of *Aedes* in Delhi as breeding was observed in overhead tanks throughout the period of five years. A study carried out by Sharma et al., 2005, during the year 2000 in different localities of Delhi identifies overhead tanks and cement tanks as breeding foci for *Aedes aegypti*.\(^\text{19}\) Identification of key containers was also carried out in different parts of India and the world. Roof gutters were identified as key container for *Aedes aegypti* in Australia\(^\text{20}\) whereas plastic drums, metal drums and plastic containers were identified key sites for breeding of *Aedes aegypti* in the Philippines.\(^\text{21}\) In India, cement tanks were identified as key breeding habitats for *Aedes aegypti* in desert as well as semi-arid areas of Rajasthan.\(^\text{22}\) A study carried out in Delhi by Katyal et al. reported that cement tanks and non-removable clay jars acted as mother foci during winter months in 1964.\(^\text{23}\) Delhi has witnessed rapid unplanned urbanization, resulting in high-density population areas with inadequate systems of water and solid waste management which have provided excellent breeding places for *Aedes aegypti* mosquitoes.\(^\text{24}\) Population density

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**Figure 3:** No. of months breeding habitats found positive during Transmission and Non Transmission Season

![Figure 3](image-url)
Identification of key containers of Aedes breeding – A cornerstone to control strategies of dengue in Delhi, India

and water storage practices have a direct relationship with prevalence of Aedes aegypti. Further study is needed to know whether control of the breeding of Aedes aegypti in key habitats during non-transmission season would arrest the breeding in secondary habitats and dengue transmission.

**Conclusion**

Breeding of Aedes mosquitoes was observed round the year in overhead tanks, a domestic breeding site; it can hence be considered as a key container of its breeding in Delhi. Overhead tanks maintain breeding of Aedes in non-transmission season which amplifies multi-fold in secondary containers, especially solid wastes, during transmission season. Identification of key containers will help the authorities to take timely and focused interventions and reformulate control strategies of Aedes breeding in a sustainable and environment friendly manner.

**Acknowledgments**

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**Declaration**

Authors state that this article has not been published and will not be submitted for publication elsewhere if accepted for publication in the WHO Dengue Bulletin.

**References**


Identification of key containers of Aedes breeding – A cornerstone to control strategies of dengue in Delhi, India


A comprehensive report of dengue activity in Kolkata, India – a four-year profile

Tanuja Khatun, Shyamalendu Chatterjee

ICMR Virus Unit, Kolkata, West Bengal, India

Abstract

Dengue is a perpetual public health problem in Kolkata, India. Every year it strikes either in a portion or the whole of the city in the form of an outbreak of acute febrile illness. Two clinical forms of dengue infection have been recognized – dengue fever, a relatively mild, self-limiting febrile illness, and dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS), a severe infection with vascular and haemostatic abnormalities that can lead to death – which are not unknown in this city, especially in the rainy season. In Kolkata, although sporadic cases are reported round the year, mainly the infection flares during monsoon and persists up to autumn. Thus, the city has become an endemic zone of dengue. Blood samples from such patients are routinely referred to the ICMR Virus Unit, Kolkata, for the diagnosis of dengue infection as it is an Apex Referral Laboratory of National Vector Borne Disease Control Programme, Delhi, for the detection of dengue in eastern India. Here we report the status of dengue infection since 2011 to 2014 by performing serological detection of dengue specific IgM antibody by ELISA method and molecular diagnosis by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) test followed by nested PCR for the determination of serotype. During this period a total of 2686 samples were received from different medical colleges and hospitals of this city. Only 980 (36.48%) samples were positive to IgM antibody by ELISA method. A total of 573 dengue IgM negative acute samples having ≤4 days of illness were selected for isolation of virus through tissue culture system using C6/36 cell line. Subsequently these 573 samples, irrespective of the appearance of Cyto Pathic Effect (CPE) in tissue culture, were subjected to RNA extraction, RT-PCR followed by nested PCR for molecular detection of dengue serotype. Only 104 (18.15%) samples were RT-PCR positive of which 62 samples had monotypic infection with Dengue-1(D1), Dengue-2 (D2), Dengue-3 (D3); 38 samples had dual infection with any two of the four serotypes; three samples had infection with D1, D3 and Dengue-4 (D4); and only one sample had the infection with D1, D2 and D3. During the entire study period significant incidences of DHF were observed in the patients infected with multiple serotype. Females were much more affected than the males. The highly affected age group was 0–10 years, followed by 11–20 and 21–30 years. Although this four-year study establishes the concurrent circulation of all the dengue serotypes in Kolkata, with majority of D3, the epidemiology of dengue still remains unpredictable.

Keywords: Dengue, serotype, Kolkata.

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Introduction

The Indian scenario with mosquito-borne diseases such as dengue is interesting and intriguing. Dengue viruses are members of the genus Flavivirus of the family Flaviviridae, which consists of four antigenetically distinct serotypes named D1, D2, D3 and D4 that do not offer cross protection. Dengue viral infection can either cause dengue fever (DF), a relatively mild, self-limiting febrile illness that is known to be endemic in India, or dengue haemorrhagic fever (DHF)/dengue shock syndrome (DSS), which can be life threatening and responsible for a high mortality rate, especially in children. The epidemiology of DF and DHF is changing fast and has increased dramatically in recent decades. In India, dengue was first isolated in 1946 and since then many epidemics have been reported from many parts of the country. Dengue was first reported from Kolkata in 1963 and again in 1964. Subsequently, DHF cases have been reported from many parts of the country, including Delhi. Timely and correct diagnosis is very crucial for patient management as no definite vaccine has been developed against all dengue serotypes. Since 2000, Indian Council of Medical Research (ICMR) Virus Unit, Kolkata, is engaged for the systematic investigation for the detection of dengue infection in Kolkata and its suburbs; prior to that, no such systematic record is available. This paper deals with a comprehensive report from ICMR Virus Unit on dengue cases from 2011–2014 including the currently circulating serotype, and establishes dengue endemicity in this city.

Materials and method

Geographic and meteorological data

Kolkata (22°82’N, 88°80’E) is the capital of West Bengal and the seventh-largest metropolitan city in area and population in the world. Approximately 7.5% of the total population of India lives in this state. The city is served by an international airport and a seaport. It also has two big and busy railway stations that serve as the gateways of the city. The railroads cover almost all the districts, some of which situated at the border of Bangladesh. Frequent infiltration of citizens from both the countries takes place crossing the border, which may facilitate the emergence of dengue infection in the state along with Kolkata. The main seasons are summer, monsoon and winter. Summer begins in March with temperatures of 38–45°C, followed by monsoon which lasts up to September. A high humid temperature prevails in these two seasons, which is favourable for viral growth. The breteau index of the vector mosquito Aedes aegypti is moderately high in Kolkata, which serves as the principal/potential vector for dengue.
Patient and clinical samples

The ICMR Virus Unit Kolkata functions as an Apex Referral Laboratory for the detection of dengue infection in the eastern part of India. For this reason, blood samples from clinically suspected dengue cases along with a short history of illness and duly signed consent form of patients are routinely referred to the ICMR Virus Unit from outpatient departments (OPD) and indoor of different medical colleges as well as different hospitals and private practitioners for the detection of dengue infection, if any. The clinically suggestive features that are initially considered for the diagnosis of dengue infection are: high fever, usually sharp and sudden onset with or without chill, flushing of the face, suffused eyes/retro-orbital pain, nausea/vomiting, malaise/joint pain, generalised skin rash, prostration headache and enlarged lymph nodes. In this study, apart from fever, any two of these criteria were considered. The samples after excluding the possibilities of prokaryotic and bacterial infection by investigation at respective hospitals were referred to ICMR Virus Unit. This study was duly approved by the institutional ethical committee.

Sample collection

Approximately 5ml blood samples of clinically suspected cases were collected by venous puncture by the health workers and medical technicians. During these four years of study period, a total of 2686 samples were thus received along with a short history of illness and duly signed consent from the patient/ patient’s guardian maintaining the cold chain for the detection of dengue infection, if any. The sera/serum were separated by centrifugation at 1500 rpm for 10 minutes at 4°C and kept at -80°C in aliquots until tested.

Methods employed for the diagnosis of dengue virus infection were serological method by detecting dengue specific IgM antibody by enzyme linked immunosorbent assay (ELISA), virus isolation through tissue culture technique using C6/36 cell line and molecular methods by RNA extraction followed by RT-PCR and Nested PCR which is a very sensitive method.

Serology

All the 2886 samples were tested for the detection of dengue IgM antibody by dengue specific IgM capture ELISA (Mac ELISA) method, using a kit (prepared by National Institute of Virology, Pune, India). The prescribed protocol OD measured at 450nm using an ELISA reader (thermo scientific multi scan EX) was followed.
Virus isolation

For this purpose out of 2686 samples only 573 dengue IgM negative acute serum samples having ≤ 4 days of illness were screened. Virus isolation was carried out in the C6/36 cell line to observe CPE following the method described elsewhere.20 Uninfected cell line was used as negative control and cell lines infected with dengue virus 1 to 4 (obtained from the National Institute of Virology, Pune, India) were included as positive controls in each run.

RNA extraction

Attempts were made to isolate the viral RNA from the tissue culture fluid which produced CPE in tissue culture, negative control, and four positive controls and directly from the rest of the screened samples using a Qiagen viral isolation kit (Qiagen, GmbH, Hilden, Germany) with a slight modification of the manufacture’s protocol.

RT-PCR/Complementary DNA synthesis

Reverse transcription and amplification were conducted in a single reaction tube by the procedure describe by Lanciotti et al. using a highly conserved primer pair, D1 (forward) and D2 (reverse).20,21

Nested PCR

The nested PCR assay employed in this study could distinguish the four dengue serotypes by the size of the products as described by Lanciotti et al.20,21 This includes a step of second round PCR/nested PCR using the primer D1 and four serotype specific primers, TS1, TS2, TS3 and TS4.20,21

Agarose Gel Electrophoresis:

RT-PCR and Nested PCR products were analysed by running 1.5% agarose gel stained with ethidium bromide and examined under ultraviolet light using a digital gel documentation system. The expected size of the external RT-PCR products is 511bp. The expected size of nested PCR product is 482bp for D1, 119bp for D2, 290bp for D3 and 392bp for D4.

Result

During the four years of study period, out of 2686 total samples, highest number of cases were referred in the year 2012 followed by 2014, 2011 and 2013. A total of 2686 blood/serum samples were tested for dengue virus specific IgM antibody, of which 980 (36.48%)
were positive. A total of 573 dengue IgM negative acute samples having ≤4 days of illness were screened for RT-PCR test, of which 104 (18.15%) were positive by producing prominent band at 511bp in 1.5% agarose gel electrophoresis (Table 1).

**Table 1:** Year-wise distribution of dengue positive cases by ELISA and RT-PCR methods.

<table>
<thead>
<tr>
<th>Year</th>
<th>Elisa test</th>
<th>RT PCR test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number tested</td>
<td>Number positive</td>
</tr>
<tr>
<td>2011</td>
<td>350</td>
<td>13</td>
</tr>
<tr>
<td>2012</td>
<td>1278</td>
<td>488</td>
</tr>
<tr>
<td>2013</td>
<td>160</td>
<td>43</td>
</tr>
<tr>
<td>2014</td>
<td>898</td>
<td>436</td>
</tr>
<tr>
<td>Total</td>
<td>2686</td>
<td>980</td>
</tr>
</tbody>
</table>

Amongst 2686 samples, 1649 sera were from male and 1037 sera were from female patients, of which 411 (24.9%) male and 569 (54.9%) female were positive to dengue IgM antibody. Similarly, out of 573 screened samples, 104 were positive by RT-PCR of which 39 were male (6.81%) and 65 were female (11.34%). See Figure 1 (a) and (b).

All the 573 screened samples were subjected to tissue culture for the isolation of virus. Up till now only nine samples produced prominent CPE after fourth passage, which were identified by RT-PCR/Nested PCR method as DEN3-2, DEN2-3, DEN1+DEN2-2 and DEN1+DEN3-2. Others are still under passage.

**Figure 1(a) & (b):** Sex-wise distribution of dengue positive cases by ELISA and RT-PCR methods.
Highest number of positivity was observed in the age group of 0–10 years (48.09%), followed by the age group of 11–20 (43.15%), 21–30 (39.97%), 31–40 (38.38%), 41–50 (35.48%) and 51+ (24.01%). The age wise dengue positive cases are presented in Table 2.

For the detection of the serotype 104 RT-PCR positive samples were further subjected to nested PCR. The nested PCR revealed that 62 samples had monotypic infection with D1, D2, D3; 38 samples had dual infection with any two of the four serotypes; three samples had infection with D1, D3 and D4; and only one sample had the infection with D1, D2 and D3. Thus all the four serotypes are circulating in the city of Kolkata. See Table 3 and Figures 2 and 3.

\textit{Table 2:} Age- and year-wise distribution of dengue positive cases by ELISA and RT-PCR method

<table>
<thead>
<tr>
<th>Year</th>
<th>0–10</th>
<th>11–20</th>
<th>21–30</th>
<th>31–40</th>
<th>41–50</th>
<th>51+</th>
</tr>
</thead>
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<tr>
<td>2011</td>
<td>01</td>
<td>02</td>
<td>05</td>
<td>04</td>
<td>00</td>
<td>2</td>
</tr>
<tr>
<td>2012</td>
<td>122</td>
<td>92</td>
<td>127</td>
<td>73</td>
<td>47</td>
<td>31</td>
</tr>
<tr>
<td>2013</td>
<td>18</td>
<td>26</td>
<td>27</td>
<td>12</td>
<td>06</td>
<td>05</td>
</tr>
<tr>
<td>2014</td>
<td>36</td>
<td>107</td>
<td>170</td>
<td>96</td>
<td>46</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>177</td>
<td>227</td>
<td>329</td>
<td>185</td>
<td>99</td>
<td>67</td>
</tr>
</tbody>
</table>

\textit{Table 3 & Figure 2:} Serotype distribution shows monotypic and multiple infection during 2011–2014

<table>
<thead>
<tr>
<th>Table 3</th>
<th></th>
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<tbody>
<tr>
<td>Serotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>07</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D3</td>
<td>36</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D4</td>
<td>00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1+D2</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1+D3</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1+D4</td>
<td>07</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1+D2+D3</td>
<td>01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1+D3+D4</td>
<td>03</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Discussion

Over the years, dengue fever has become an important arboviral infection in different geographical regions of the world that support the growth of mosquitoes. Its range exceeds over 100 tropical and subtropical countries with more than 2.5 billion people at the risk of infection.\textsuperscript{22} In India, D1 and D4 was first was reported in 1964\textsuperscript{23,24} and D3 in 1968.\textsuperscript{25} DHF was first reported from Kolkata.\textsuperscript{26,27} Till date several reports of DF and DHF have been reported from different states in India.\textsuperscript{28–34} The city of Kolkata is now a known dengue endemic zone; frequent outbreaks of DF and DHF have been occurring since the last two centuries. In Kolkata, DF was first reported in 1824 and after that several epidemics of DF were recorded in 1836, 1906 and 1911.\textsuperscript{35} In this present four-year study, the detection of IgM antibody to dengue virus by ELISA test in 980 samples out of 2686 cases and molecular detection of dengue specific RNA in 104 samples out of 573 cases, proves the continuous circulation of dengue virus in Kolkata. According to the sex wise distribution of the suspected cases (Figure 1 (a) & (b)) there was a significant higher incidence of dengue IgM sero positivity.

\textbf{Figure 3:} Dengue specific RT-PCR followed by Nested PCR products were analysed by running 1.5% agarose gel electrophoresis stained with ethidium bromide. Lane L-100bp plus DNA ladder, Lane1-Dengue positive control, Lane 2-19: dengue positive samples. The expected size of nested PCR product is 482bp for D1, 119bp for D2, 290bp for D3 and 392bp for D4.
in females and not in males. This may be explained by the fact that the female individual usually resides at home at the day time and gets exposed to the mosquitoes (Aedes aegypti) as it is domestic and peri-domestic in nature.

A significant higher incidence of dengue IgM sero positivity was observed in the age group of 0–10 years, followed by 11–20 years (Table 2), which is similar to the observation made by earlier works at different times and from different parts of India. Out of 573 acute samples, only nine produced prominent CPE. RT-PCR test with 573 samples yielded only 104 (18.15%) prominent band, at par with the control. Out of 104 samples only 62 (59.6%) had mono typic infection with different dengue serotype and 38 (36.5%) had dual infection, while three samples had infection with D-1, D-3 and D-4 serotype. Only one sample had infection with D-1, D-2 and D-3. Patients with multiple serotype infection had the history of either biphasic or tri-phasic illness with fever and some of them had haemorrhage manifestation, particularly in the younger and young adult age group. In Kolkata, detection of multiple serotype in same patients has already been reported elsewhere.20,36 This may be due to the lack of any protective antibody in them.

In this study a total of \([2686-(980+104)] = 1602\) samples with a history of five to seven days’ febrile illness were truly dengue negative, possibly due to the unprotected transportation that might have damaged the IgM antibody/viral titre or due to the presence of other different etiological agents responsible for dengue-like illness. The present study establishes the co-concurrence of all the four dengue serotypes in the city of Kolkata. Such types of study involving year-round and decade-wide observation in Kolkata have established the same observation elsewhere.20 The highest incidence of dengue cases in the young and young adult population is possibly due to the lack of any immunity and protecting antibody. Although all the serotypes are in circulation in the city of Kolkata, D-3 proved to be the dominating serotype. The co-circulation of all the serotypes is a definite indication of impending outbreaks of DHF at any time in Kolkata, posing a major public health problem for the city.

**Conclusion**

This scenario on the dengue status over the four-year period reported from Kolkata may be no different from other parts of India, as the climatic and demographic features are the same and so is the risk. While concluding, the authors desire to convey that the proposed study has two fold interests. First, it is informative, because this work being in its infancy needs to be introduced to the profession and secondly, here is an invitation to those who feel inspired to help in this mission.
Author’s contribution

TK and SC participated in the conception and design of the study. TK carried out the clinical assessment, immune assays and molecular testing; all authors carried out analysis and interpretation of the data and drafted the manuscript. All authors read and approved the final manuscript. SC is guarantor of the paper.

Acknowledgement

The authors convey their sincere thanks to the staff members of ICMR virus unit for their help. They also gratefully acknowledge the help received from National Institute of Virology for providing the kit for the detection of IgM antibody and the virus strains of all the four serotype of dengue viruses. The co-operation and help received from the doctors of all the medical colleges and hospitals are thankfully acknowledged for sending the samples from suspected cases. The authors expressed their gratitude to the Officer-In-Charge, ICMR Virus Unit, for allowing them to carry out the work in this department.

Financial assistance

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Conflict of interest

The first author is a senior research fellow and the corresponding author is a Scientist D at ICMR Virus Unit. The authors have no financial and competing interests that are affected by the materials in the manuscript.

Reference


A comprehensive report of dengue activity in Kolkata, India – a four-year profile


Impact of dengue vaccination: a public health perspective

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Abstract

From a public health perspective, dengue control is an ongoing challenge and is now the most important mosquito-borne viral disease globally. The incidence of dengue has increased 30-fold in the past 50 years with nearly half of the world’s population at risk. The annual economic cost of dengue in Asia is estimated at US$ 2 billion (exclusive of costs for preventive and vector control efforts). Prevention, the key strategy for dengue management, remains a challenge due to lack of awareness and sustainable vector interventions. After decades of research for vaccine against dengue, one candidate – a chimeric tetravalent dengue vaccine – has completed all three phases of clinical trials and significantly reduced (by 80-90%) the incidence of severe dengue disease and number of hospitalizations. Other promising candidate vaccines are likely to be available in the near future. Much preparatory advocacy work is needed for their introduction in order to reach high coverage rates, equitable access and sustainable funding for a new vaccination programme. Sophisticated tools including mathematical modelling studies will also help determine the cost effectiveness and impact on dengue disease incidence in each epidemiological setting. The key drivers of success for any public health action are knowledge, social movement and political commitment. The Asian Dengue Vaccination Advocacy (ADVA) group is working to facilitate policy and implementation dialogues among dengue experts and public health leaders to support dengue vaccine introduction in Asian countries.

Keywords: Immunization programmes; dengue; vaccine; immunization; public health programme; health policy; dengue in Asia; ADVA.

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Impact of dengue vaccination: A public health perspective

Introduction

The incidence of dengue, caused by viruses spread by the *Aedes* mosquito vector, has increased 30-fold in the past 50 years. An estimated 390 million cases occur every year in more than 100 countries, mostly in the developing world, and 40% of the world’s population is at risk. Each year, an estimated half a million people contract severe dengue, often accompanied by bleeding and shock, and require hospitalization. The annual economic cost of dengue illness in Asia is estimated at US$ 2 billion, not including the costs of preventive and vector control efforts. Unfortunately, the situation is likely to remain grim in the coming years. Increased urbanization, travel and migration, the pressures of globalization, and global warming are likely to maintain dengue transmission at high levels and continue to result in major outbreaks in affected countries.

Control of dengue is a complex challenge

From a public health perspective, dengue control remains both a challenge and an enigma. For a start, it is widely accepted that the true burden of disease is significantly underestimated by 3- to 25-fold. There is a suggestion that in India the burden is underestimated 300-fold. In the absence of anti-viral treatment for severe dengue disease, the mainstays of dengue control have been to reduce the mosquito vectors that transmit the dengue virus, and a high level of public awareness and community involvement in the fight against dengue. While vector control remains a key strategy, it has not always been effective. Singapore, for example, spent about US$ 0.5 billion over the past decade on control programmes and has successfully reduced the *Aedes* premises index to very low levels, yet still experiences major outbreaks of dengue. To add to the challenges in controlling dengue, and after decades of research for an effective vaccine to prevent dengue, there is currently one vaccine candidate so far, which has completed all three phases of clinical trials. In spite of these challenges, what is clear is that we need to fight dengue on many fronts through an integrated and inter-sectoral approach, which includes good clinical case management, integrated surveillance (both entomological and disease monitoring), with effective field-laboratory-clinic coordination, sustained vector control and community participation, engagement and ownership.

Strategies to control dengue

Countries affected by dengue around the world are guided by a strategy developed by the World Health Organization (WHO), the WHO Global Strategy for Dengue Prevention & Control (2012-2020). The strategy is composed of five technical pillars: timely, accurate diagnosis and appropriate case management; integrated surveillance and outbreak preparedness; sustainable vector control; future vaccine implementation; and basic, operational and implementation research. It is hoped that such a strategy will help to achieve
three key objectives: reducing dengue mortality by $\geq 50\%$ by 2020, reducing dengue morbidity by $\geq 25\%$ by 2020 and obtaining a true estimate of disease burden by 2015. Arguably, and with regard to achieving the first two objectives, the availability of an effective vaccine to prevent dengue would be very valuable.

### Promising candidate vaccines on the horizon

Historically, the search for an effective dengue vaccine has been ongoing for more than 80 years, since the earliest attempt by Blanc and Caminopetros in 1929. More recently, vaccine research by Professors Natth Bhamapravati and Sutee Yoksan at Mahidol University in Thailand, catalysed research through their commitment and energy to develop a vaccine. Several promising candidate vaccines are now under investigation. Among them are live attenuated vaccines, chimeric, and inactivated viruses, DNA and subunit vaccines. The chimeric tetravalent dengue vaccine manufactured by Sanofi-Pasteur, which has a three-dose regimen, each dose given six months apart, has completed all phases of clinical trials and is now licensed or under review for licensure in several endemic countries. This vaccine has undergone extensive testing in 30,000 participants in 10 endemic countries in Asia and Latin America with good efficacy and safety data. Importantly, the vaccine significantly reduced (by 80-90%) the incidence of severe dengue disease and the number of hospitalizations. The cost-saving impact of this effect for the population and the health care delivery system is likely to be considerable. Sophisticated mathematical modelling studies have also indicated that the said vaccine will have a considerable impact on dengue disease incidence as well as being cost-effective. The vaccine has reached licensure or is under the review process by several national regulatory authorities.

Underscoring the importance of the problem, at least three to four other vaccine manufacturers also have dengue vaccines, which are being evaluated in various phases of testing, including Takeda GlaxoSmithKline/Fiocruz/Walter Reed, NIH/Butantan and Merck. These vaccines may become available in the coming years.

While promising candidate vaccines are likely to be available in the near future, much preparatory advocacy work is needed to prepare for their introduction in order to reach high coverage rates, equitable access and sustainable funding for the new vaccination programme. As an example, the Asian Dengue Vaccination Advocacy (ADVA) group is a regional initiative established in 2011 consisting of independent scientific and educational experts, which aims to disseminate information and make practical recommendations about how to prepare for dengue vaccine introduction in ASEAN countries. A new global alliance called Partnership for Dengue Control, established in 2013, will facilitate policymaking dialogue among dengue experts and public health leaders to support synergies and integration of innovative tools against dengue, ensuring the most effective strategies will be used.
The policy context – towards implementation of a dengue vaccine

If a vaccine to prevent dengue seems attractive as an intervention with potentially good public health impact, what information and knowledge would be needed before a policy is formulated and implemented?

In general terms, when a policy maker is faced with making a policy decision he/she is usually interested in getting answers to three questions: Can it work? Will it work? Is it worth it?’

To answer the first question, there is strong evidence from the clinical trials that the vaccine can work, particularly in reducing the severity of disease and in those who were previously exposed. The answer to the second question is a little problematic as the vaccine in question is a brand new intervention. Mathematical modelling studies, an important component of the Dengue Vaccine Initiative agenda, are helping to assess the projected impact from dengue vaccine immunization against infection, disease and transmission in different epidemiological settings. The modelling simulations also indicated that the effectiveness of the vaccine is significantly increased if initial routine vaccination is supplemented with ‘catch up’ campaigns with additional age groups. Economic analysis including mathematical modelling methods, will also help answer the third question, which relates to cost effectiveness. The economic burden of dengue is substantial. Under certain assumptions, including a certain threshold for the cost of completing the regimen, vaccination is expected to be cost effective.

In addition to the above, however, there are important additional considerations to be taken into account before a policy is developed and implemented: (1) there is a clear need for robust diagnostic tools to help in good case identification and management; (2) integrated surveillance and accurate burden estimates are crucial; (3) impact estimates by mathematical modelling and rigorous evaluation of an integrated vaccination programme should be carried out; (4) the vaccine should achieve adequate coverage, which includes roll out and targeted catch-up campaigns; (5) attention should be given to public acceptance and the potential of using the vaccine during epidemics; (6) integration with existing vector control programmes is crucial; (7) successful implementation is dependent on robust health systems within the country with the flexibility and capacity to integrate new technologies and/or adapt existing ones; (8) equitable, affordable and sustainable access to the vaccine is an important goal; and (9) policies must be supported by adequate and sustained programme financing, particularly for low-income countries.

With regard to programmatic considerations for the vaccine itself, there are additional technical features, which could also influence successful implementation. This includes the desirability of having a heat-stable vaccine with simple routes of administration, fewer doses and flexible dosing schedules.
What is really needed to make it happen?

From a public health perspective, however, the successful implementation of a dengue vaccine programme is also dependent on a set of higher-level factors, which goes beyond the technical and economic considerations. Articulated by Professor Prawase Wasi of Thailand as the concept of “the triangle that moves the mountain”\(^\text{17}\), it proposes that the key drivers of success, the three corners of the triangle, are knowledge, social movement and political commitment.

Knowledge plays a particularly important role, especially knowledge that informs policy-making. In the words of Julio Frenk, “There is a pathway from good science to publication to evidence, and to programmes that work. In this way research becomes an inherent part of problem-solving and policy implementation.” But without social mobilization and movement and political commitment, knowledge alone is unlikely to be sufficient.

Conclusion

A safe and effective dengue vaccine would be a major public health tool for dengue prevention and control, to be integrated with existing surveillance, vector control methods and other innovative tools under development. Implementation is a collective responsibility requiring political will and commitment, trust, community ownership, sound research, and sustained and dedicated resources. All stakeholders must play a stronger advocacy role to ensure that evidence-based knowledge and new public health tools are translated into policy and practice, and that programmes are monitored and evaluated for impact. Importantly, we must plan early and act soon to accelerate introduction of dengue vaccine(s) in countries with the highest disease burdens.\(^{15}\) The need to seize the moment is not simply a public health imperative but also a moral and ethical imperative.

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References


Dengue and dengue haemorrhagic fever

(2nd Edition; Edited by Duane J. Gubler, Eng Eong Ooi, Subash Vasudevan and Jeremy Farrar. 2014)

Dengue is one of the fastest transmitting arbovirus diseases; no longer confined to just one region, it has expanded from country to country and region to region, taking a significant number of lives every year. It is estimated that every year there are 390 million new infections and 29,000 deaths due to dengue in over 128 countries. Dengue is caused by dengue viruses (four serotypes-DENV-1, DENV-2, DENV-3 and DENV-4) belonging to the genus Flavivirus, family Flaviviridae. The disease pattern and prevalence of dengue is dynamic and has gone through continuous changes ever since its evolution. With the changing disease pattern and the advancement of technology, the prevention, management, and research are continuously changing and it is therefore essential to update knowledge about recent developments for clinicians, researchers and managers. The book Dengue and Dengue Haemorrhagic Fever, 2nd edition, edited by Duane J. Gubler, Eng Eong Ooi, Subash Vasudevan and Jeremy Farrar, which has nicely captured and compiled the updates from around the world and reports on recent advances, is useful for all categories of readers.

The book runs into 606 pages divided into five parts (history and epidemiology, the disease, the virus, virus-host interaction and dengue prevention). There are 29 excellent articles (chapters) contributed by a number of eminent experts.

The book was published in 2014 with the intention that the authors’ research efforts would deliver several promising prevention and control tools for dengue. The book was successful in this regard; articles on economic burden study, surveillance, immunological response, clinical management guidelines, and vector control help managers to analyse, plan and adopt effective strategies for dengue prevention.

The first six chapters in the book clearly explain the evolution of dengue viruses, their pattern of transmission and the disease burden. A nice comparison of the region to region, recent challenges of intercontinental movements of dengue holds the attention of the readers while the economic burden of the diseases should spur policy-makers to develop effective strategies to address the situation.

The second part of the book “deals with the clinical side of the disease and explains the pathogenesis, clinical features and management guidelines”. Here again, the regional experience has been shown and evidence from clinicians’ practical experiences has been
Dengue and dengue haemorrhagic fever compiled that guides the reader to make a decision while handling any outbreak or simply managing a case. The third and fourth parts are basically for the molecular scientist and microbiologist who can gain insights into the immunological reactions of the human and other hosts to the dengue viruses. At the same time, the final section makes a nice compilation for students and researchers, providing good reference and evidence elaborating the recent advancement on dengue preventions that is also another effective evidence for policy makers and implementers. Chapter 25 highlights the example of yellow fever, which is emerging again in recent days, and notes that yellow fever was controlled in Cuba by controlling the vectors and sources; this is also a relevant example in today’s scenario.

The research-based articles identify the research gaps in their own field and suggest further investigations to enable strong conclusions to be drawn. In the preface, Gubler quotes, “There are still a number of unresolved issues related to Dengue viruses …” The book identifies most of the areas where further work is required and advocates more research.

The chapters are distributed in an orderly manner; starting with the burden and epidemiology of the disease, the book takes the readers to the proper surveillance, case management, immunology, virus-host interaction and finally to the recent updates on dengue prevention.

Readers can easily find the main and related topics of their interest. There is a consistent flow of chapters following a common theme of generating more information about dengue. Though the authors belong to different parts of the world, uniformity in the writing style and the choice of simple wording can be seen throughout the book, and this makes it palatable for all sets of readers. The scientific articles explain the technical terms and elaborate the explanation in a brief way that helps a layman to understand the terms easily.

Most of the papers have used secondary sources and the authors did their analysis with existing papers and documents on the recent developments (up until 2014). In the preface, the editor describes the objective of the book and summarizes the key findings. The referencing style has followed the international standard (APA) and helps the reader find the references promptly. The graphs and charts used in the book are self-explanatory and relevant to the articles; editors have maintained this uniformity throughout the articles and this is much to be appreciated.

However, as the book makes clear, there are still many vague areas that need to be clarified; examples include clinical case definition, virus biology and pathogenesis, animal models and efficacy of existing vector control methods. As already pointed out by the editors, there may be a few conflicting statements in the book, due to the large number of authors
The editors have been very careful to include the most updated information on all aspects of dengue.

This is a wonderful book available on dengue. For sure, this book will be extremely useful to researchers, public health specialists, teachers and students as well as physicians.

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Instructions for contributors

*Dengue Bulletin* welcomes all original research papers, short notes, review articles, letters to the Editor and book reviews which have a direct or indirect bearing on dengue fever/dengue haemorrhagic fever prevention and control, including case management. Papers should not contain any political statement or reference.

Manuscripts should be typewritten in English in double space on one side of white A4-size paper, with a margin of at least one inch on either side of the text and should not exceed 15 pages. The title should be as short as possible. The name of the author(s) should appear after the title, followed by the name of the institution and complete address. The e-mail address of the corresponding author should also be included and indicated accordingly.

References to published works should be listed on a separate page at the end of the paper. References to periodicals should include the following elements: name and initials of author(s); title of paper or book in its original language; complete name of the journal, publishing house or institution concerned; and volume and issue number, relevant pages and date of publication, and place of publication (city and country). References should appear in the text in the same numerical order (Arabic numbers in parentheses) as at the end of the article. For example:


Figures and tables (Arabic numerals), with appropriate captions and titles, should be included on separate pages, numbered consecutively, and included at the end of the text with instructions as to where they belong. Abbreviations should be avoided or explained at the first mention. Graphs or figures should be clearly drawn and properly labelled, preferably using MS Excel, and all data clearly identified.
Instructions for contributors

Articles should include a self-explanatory abstract at the beginning of the paper of not more than 300 words explaining the need/gap in knowledge and stating very briefly the area and period of study. The outcome of the research should be complete, concise and focused, conveying the conclusions in totality. Appropriate keywords and a running title should also be provided.

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