Genotype shift of dengue virus (DENV1) during the 2017 outbreak of dengue fever in Thiruvananthapuram, Kerala, India

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An unprecedented outbreak of dengue occurred during 2017 in Kerala. Thiruvananthapuram was worst affected. We carried out a joint investigation with the Health department of the State, to understand the epidemiological and entomological factors involved in this outbreak, so as to develop strategies to contain it. Blood samples from suspected patients were collected from three worst affected areas and genomic analysis of the Dengue virus (DENV) was performed. Also, a cross sectional entomological survey was carried out in these areas. The data obtained was compared with the available secondary data of DENV in Kerala. The investigations revealed a genetic shift from the erstwhile predominant DENV2/DENV3 serotypes to the newly introduced DENV1 Asian genotype during the current outbreak. Breeding indices of Aedes aegypti, the predominant vector species was also found to be remarkably high. Asian genotype of DENV1 was detected in field collected Aedes aegypti also. The index cases of the Asian genotype of DENV1 in Kerala were detected from Erumeli village (gateway to the famous Sabarimala shrine) among two plantation workers migrated from the neighbouring Karnataka state, during 2013. This introduced virus strain attained an epidemic proportion in 2017 in Thiruvananthapuram, owing to immunologically naïve population and high receptivity.

Keywords: Aedes aegypti, DENV serotypes

Dengue is one of the major emerging arbo-viral diseases affecting about 390 million people every year globally. Among these, about 96 million manifest the infection with clinical symptoms. India is reported to have highest burden of the disease with 32.5 million clinical cases1 annually. However, the existing surveillance system in India severely under report the magnitude of dengue cases in the Country. The total number cases recorded during 2017 is only 0.188 million clinical cases (National Vector-Borne Disease Control Program, New Delhi). This figure mostly includes dengue fever patients treated as inpatients in Government hospitals across the Country. The large chunk of cases reported to private medical institutions remains generally unreported to the surveillance system. This ineffectual surveillance system remains to be the major factor which severely affects the formulation and implementation of efficient strategies for prevention of dengue outbreaks in the country.

An unprecedented outbreak of dengue fever occurred in Kerala state during March-August, 2017. A total of 21,993 confirmed cases and 165 deaths were reported during the year (Directorate of Health Services, Government of Kerala), contributing to 10.61% of the total cases recorded within the country during the year (https://www.nvbdcp.gov.in/index4.php?). This accounts for the maximum number of cases ever recorded annually, in the state during the last decade. Although cases were reported from all the 14 districts in the state, more than 50% were from Thiruvananthapuram District and particularly from Thiruvananthapuram Corporation area. We, in collaboration with Dept. of Health Services and Govt. Medical College, Thiruvananthapuram, investigated the epidemiological and entomological risk factors involved in the outbreak, so as to devise a suitable control strategy to contain the outbreak, and the findings are reported here.

Materials and Methods

Kerala, located in the southernmost region of India and on the western side of the Western Ghats, is a densely populated (3.34 crores) state with an area of 36863 sq. km. It enjoys a tropical climate with profuse rainfall during pre-monsoon months as well as from both south-west and northeast monsoon seasons. The temperature recorded never reaches extremes and ranges from 34°C (April-May) to 22°C (Dec-Jan). Average total annual rainfall in the state is about 3000 mm. Thiruvananthapuram city (corporation), located in Thiruvananthapuram District has an area of 214.86 sq. km. and a population of 957,730 distributed in 100 administrative wards.

Data on dengue cases reported in the state and Thiruvananthapuram District during 2008-2017 was...
obtained from Directorate of Health Services, Govt. of Kerala. Daily reporting data on the number of dengue cases recorded in the Thiruvananthapuram Corporation was also obtained.

A cross sectional entomological survey was carried out in three worst affected wards viz., Nettayam (Vattiyoorkavu, PHC), Pappanamcode Estate and Ponnumangalam (Nemom Taluk Hospital) of Thiruvananthapuram City (Fig. 1) to assess vector species prevalence and immature breeding indices. Indoor and outdoor resting adult collections were carried out in 24 houses and its surroundings in each study area. Also, two Biogents (BG) sentinel traps were set up in outdoor settings in the area for a period of 24 h in each area. Immature surveys were carried out in the same area and samples collected were reared to adults. Adults were identified, abdominal conditions recorded and were transferred to TRI reagent (Molecular Research, USA) towards processing for Dengue virus infection. The samples were processed as described earlier. Ethical clearance for the study incorporating the informed consent form from patients included in the study was obtained from the Institutional Human Ethics Committee of Government Medical College, Thiruvananthapuram (IEC. No.07/23/2017/MCT dated, 7th July 2107).

About 1.5 mL blood was drawn from patients after obtaining their consents, transferred to RNAse free 2 mL Eppendorf tubes, stored at room temperature for 5-10 min and were transported to VCRC in cold packs

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**Fig. 1 — Map of Thiruvananthapuram Corporation indicating the study areas (shaded)**
maintaining a temperature below 4°C. Serum from the samples were separated and viral RNA was extracted using QIAGEN Viral RNA extraction Kit (QIAGEN, Germany), following the kit protocols. The viral RNA was dissolved in about 30 µL of RNAse free deionized water and was stored at –80°C until further processing. The viral RNA extracted were subjected to RT-PCR towards amplification of the partial sequences of Capsid-pre Membrane (CprM) gene region using DNA primers already reported for detection of dengue viruses. The samples were also subjected to Real time RT-PCR for ZIKV infection using Real star Zika virus detection kit, following the kit protocol. The DENV positive samples were further subjected to nested PCR for detection of the serotypes using an ABI Fast PCR thermal cycler using ABI kit and a set of one forward and four reverse primers as described. The 511 bp of CprM gene fragments were amplified and custom sequenced with Scigenom, Kochi, Kerala. These sequences were analyzed for understanding its genotypes. Blood samples from suspected dengue cases received from Govt. Medical College, Thiruvananthapuram, District and Taluk hospitals of Kollam, Kottayam, Idukki, Pathanamthitta and Ernakulam Districts, were also processed and included in the study.

Secondary data

Dengue positive samples from Kerala, processed earlier for serotyping/genotyping during the period between 2008 and 2016 by us were compared with the isolates processed during 2017 to have an understanding on the evolutionary trends of the dengue virus. 28 DENV1 sequences, since the first record of DENV1 Asian genotype in Kerala during 2013, based on our studies, were subjected to phylogenetic analysis by Maximum Likelihood analysis in MEGA 7.0 software. Bootstrap analysis with 1000 replications were carried out to generate a consensus phylogenetic tree. All the DNA sequences used in this study were submitted to the GenBank.

Meteorological data for the Thiruvananthapuram Corporation was obtained from India Meteorological Department (IMD) website: (http://imdtn.gov.in/index.php?option=com_content&task=view&id=26&Itemid=40).

Results and Discussion

The incidence of dengue cases had been on an increasing trend since 2006 in the state as well as Thiruvananthapuram District (Fig. 2). Cases were recorded throughout the year. Maximum cases were reported during pre-monsoon season (May-July), as this period receive intermittent summer rains, supporting profuse vector breeding. The annual average incidence of dengue reported during 2006-2016 in Kerala State and Thiruvananthapuram District was 9.26 (range: 2.06-23.78) and 42.35 (range: 8.97-126.75), respectively. During 2017, the incidence of dengue cases rose tremendously to 59.50 and 270.77 in Kerala state and Thiruvananthapuram District, respectively. Thiruvananthapuram Corporation area, with about 28.96% of population of Thiruvananthapuram District contributes to about 70.69% (average for 2014-2016) of dengue cases recorded. Monthly dengue incidence in the Thiruvananthapuram Corporation in 2017 showed a sudden increase of cases from February, 2017 reaching a peak in the month of May (Fig. 3). The incidence of Dengue in the Corporation area during March-Aug 2017 outbreak was unprecedented and was 9.2 times greater than those recorded during the corresponding months in the past years (2014-2016).
Entomological surveys revealed that the Aedes indices were extraordinarily high in all three wards surveyed (Fig. 1 & Table 1). The pupal indices ranged from 23.81 to 120 per 100 houses surveyed. Emergence of immatures collected in the surveys produced 88 adults on emergence. Adult collections and BG sentinel trap collections yielded 116 specimens. *Ae. aegypti* was the predominant species (58.5%) followed by *Ae. albopictus*. From these collections *Ae. aegypti* (n=117) and *Ae. albopictus* (n=77) specimens were processed by RT-PCR for DENV/ZIKV infection in 47 and 37 pools, respectively. One pool (consisting of three specimens of semi-gravid *Ae. aegypti*, collected indoor) was found positive for DENV infection. No ZIKV infection was recorded. This lone positive pool was found infected with two serotypes, DENV1 & DENV2. Genomic analysis of the DENV1 CprM amplified fragment showed that it belonged to the Asian genotype.

During 2017, altogether 181 serum samples from various districts of Kerala including the corporation area were screened for arbo-viral infections and 121 were found positive for DENV. Among these, 62% were of DENV1 serotype. The percentage of other serotypes recorded were 23.9% (DENV2), 5.75% (DENV3) and DENV4 (1.81%). Eight samples positive for DENV1 were found co-infected with DENV2 or DENV3 (Fig. 4). Genetic analysis of 19 CprM gene sequences of DENV1 collected during 2017 showed that Asian genotype (Genotype I) to be the predominant (73.68%) strain. The American African genotype of DENV1 was recorded in only 26.32% of the samples (n=5). No ZIKV infection was recorded.

Our earlier analysis of 257 samples from six districts of Kerala during 2008-2016 showed that 71 were positive for dengue infection and DENV2 was predominant serotype (45.1%) followed by DENV3 (32.4%), DENV1 (9.8 %) and DENV4 (7.04%). Five positives for DENV1 were co-infected with DENV2, DENV3 or DENV4. Asian genotype of DENV1 was never detected from Kerala before 2013.

The phylogenetic analysis of 28 DENV1 isolates from Kerala including those collected from Thiruvananthapuram Corporation is provided in Fig. 5. Nineteen samples were collected during 2017 and among these 14 were found infected with Asian genotype and 5 with American African genotype. Nine cases recorded from 2013, since the first report of Asian genotype in the state were also included in the analysis. The Asian genotype of DENV1 were

![Table 1 — Entomological indices in the study villages of Thiruvananthapuram Corporation during July 2017](chart)

<table>
<thead>
<tr>
<th>Villages</th>
<th>House indices</th>
<th>Container indices</th>
<th>Breteau indices</th>
<th>Pupal indices (no. of pupae/100 houses)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nettayam</td>
<td>37.14</td>
<td>38.64</td>
<td>48.57</td>
<td>88.57</td>
</tr>
<tr>
<td>Pappanamcode Estate</td>
<td>23.81</td>
<td>60.0</td>
<td>28.57</td>
<td>23.81</td>
</tr>
<tr>
<td>Ponnumangalam</td>
<td>55.0</td>
<td>61.54</td>
<td>80.0</td>
<td>120.0</td>
</tr>
</tbody>
</table>

![Fig. 4 — Shift on the distribution of Dengue serotypes during 2017 and prior to 2017 (2008-2016)](chart)

![Fig. 5 — Phylogenetic analysis of CprM gene sequences of DENV1 isolates collected from Kerala (2013-2017)](chart)
recorded in 2013 among two migrant rubber plantation workers, from a tribal village (Gundattur) in Heggada Devan Kotte District of Karnataka state. Since then sporadic cases of this genotype were reported from Thrissur (2014), Kottayam (2016) and Idukki (2016) Districts. The GenBank Accession numbers for the sequences are MG003153 to MG003172.

Data collected from the IMD, Thiruvananthapuram showed that the total rainfall in the pre-monsoon season of 2017 in Thiruvananthapuram District (367.6 mm up to May 31\textsuperscript{st}, 2017) was as usual rainfall in the season during past years (% departure = 0). However, the month of March recorded a comparatively higher rainfall than usual.

Dengue virus circulating in the Country are of four different serotypes viz., DENV1, DENV2, DENV3 and DENV4. Each serotypes has about 4 to 6 genotypes described\textsuperscript{6}. The seasonal and geographic distribution of the serotypes circulating in the country is little understood, since facilities for serotyping is not available even with the District health authorities. DENV1 was first recorded in Japan and French Polynesia during 1943. During 1950s it spread to Asian Countries and its prevalence steadily increased in the region since then\textsuperscript{5}. Later DENV1 was also reported from American and African regions also. In India, DENV1 was recorded mainly from North India during 2002-2010\textsuperscript{8} and it belonged to American/African genotype i.e., the Genotype III\textsuperscript{7,9}. In Kerala also similar trend was noted\textsuperscript{2,4}. Four lineages were recorded for this genotype. The Asian genotype of DENV1 was mainly recorded in China, Thailand, Singapore and Sri Lanka. Asian genotype of a different genetic lineage had been reported, but rarely in India during 1978\textsuperscript{10} and in 1990s\textsuperscript{7}.

The most predominant serotypes reported in Kerala during 2008-2016 were DENV2 and DENV3 (77.46%), while DENV1 constituted only 9.86% of the cases. However, our investigations during 2017 revealed a shift in this trend, transforming genotype I to be the predominant serotype (61.98%). Two DENV1 genotypes viz., the newly introduced Asian (I) and American African (III) genotypes were found prevalent during 2017 outbreak. Among the DENV1 infected samples, only Genotype III was recorded in Kerala till 2012\textsuperscript{2}. This trend was similar to that recorded from other parts of the Country also, till 2012\textsuperscript{2,8}. An interesting observation recorded in our study is the prevalence of co-infections of all other serotypes with DENV1 (6.56%). Co-existence of different serotypes in a patient necessitates a rethinking of the theory of antibody dependent enhancement theory\textsuperscript{12} of dengue virulence.

In our earlier investigations, we recorded the Asian genotype of DENV1 in Kerala among two migrant rubber plantation laborers during 2013, from the village Erumeli, known as the gateway to the famous shrine temple of Sabarimala, located in the Western Ghats. Since then sporadic cases of Asian genotype of DENV1 were reported from Kottayam (2013), Ernakulam and Thrissur (2014) Districts also (Fig 5). It could be inferred that this genotype of the virus had been circulating in a sub optimal level in the state since its introduction in 2013. This eventually caused an epidemic situation during 2017, worse affecting the capital City of the State, Thiruvananthapuram, which had been the focus of Dengue transmission in Thiruvananthapuram District\textsuperscript{11}. Thiruvananthapuram District contributes more than 50.0% of Dengue cases in the state, every year. The genetic lineage of all the Asian genotype isolates during 2017 exactly matched with the isolates from Chennai, India and the Sri Lanka strains (Fig. 5). No amino acid variations in the CprM gene amplified was recorded among these isolates. Hence, it could be ascertained from the phylogenetic analysis, that the strains which caused the current outbreak in Kerala would have been introduced from neighboring Tamil Nadu state that was worst affected during 2012-2014.

A similar shift to DENV1 genotype, caused an unprecedented outbreak of Dengue affecting about 12,000 cases in Tamil Nadu during 2012\textsuperscript{6}. The genotype was postulated to be introduced from China\textsuperscript{31}, Sri Lanka\textsuperscript{14,15}, Singapore\textsuperscript{16} where a similar evolutionary change of DENV1 occurred during 2009-2014, causing epidemic resurgences of dengue fever. A pro-active vector control strategy, as suggested was initiated by the state health authorities, during mid-July, 2017 effectively containing the outbreak of Dengue by August-September 2017 (Fig. 3). However, a recent investigation recorded a similar increase of DENV1 serotype during the 2017 outbreak\textsuperscript{17} of Dengue in Thiruvananthapuram, Kerala.

**Conclusion**

From the current study, it could be concluded that the important epidemiological risk factors involved in the unprecedented outbreak of dengue in Thiruvananthapuram and Kerala during 2017 were the serotype/genotype shift of DENV1 and the high
receptivity in the region. The build-up of the high population density of Ae. aegypti could have been prevented by an efficient disease/vector surveillance system and an optimal source reduction activities during the pre-monsoon season.

**Conflict of Interest**

The authors declare no conflict of interest.

**References**


