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# Dengue infections and circulating serotypes in Mizoram-north eastern part of India, from 2016 to 2018

Authors
**Dr Lalhmingmawii[1], Dr RSC Vanlalruati[2*]**
[1,2]Department of Microbiology, Civil Hospital Aizawl: Mizoram, India
*Corresponding Author
**Dr RSC Vanlalruati**
Department of Microbiology, Civil Hospital Lunglei: Mizoram, India
Address: 207B, Near LCM Interiors, Chanmari I, Lunglei: Mizoram- 796701, India

**Abstract**
**Objectives:** *Dengue has emerged as a significant public health problem in Mizoram. In this study, we describe the Dengue fever infections and circulating serotypes for a period of three years from June 2016 to May 2018.*
**Material and Methods:** *Two Sentinel Surveillance hospitals in the state of Mizoram, which are the only two Sentinel Surveillance hospitals for Dengue in the whole state were selected for this study. Acute blood samples from each patient suspected of Dengue fever were tested by Dengue specific RT-PCR, $NS_1$ ELISA and IgM ELISA.*
**Results:** *Of 2596 febrile cases, 23.72% were NS1 and or RT-PCR positive for Dengue. Dengue serotype 2 was responsible for 87.17% of the cases, Dengue serotype 3 for 7.69% and co-infection of serotype 2&3 in 5.12% of the cases.*
**Conclusion:** *DENV-2, and to a lesser extent DENV-3, infection were responsible for a high proportion of febrile illnesses in Mizoram in the years 2016 to 2018.*
**Keywords:** *DENV-2, DENV-3, RT-PCR, Serotyping, Mizoram, India.*

## Introduction

Dengue fever is the most common mosquito spread viral disease and a major international public health concern. It is a self limiting disease found in tropical and sub-tropical regions around the world, predominantly in urban and semi-urban areas. The global prevalence of dengue has grown significantly in recent decades. The disease is now endemic in more than 100 countries in South-east Asia, Western Pacific, Eastern Mediterranean, Africa, and the Americas. South-east Asia and Western Pacific are most seriously affected. World Health Organization (WHO) currently estimates there may be around 50 million cases of dengue infection worldwide every year with around 24,000 deaths. India is also endemic for Dengue Fever (DF) and Dengue Haemorrhagic Fever (DHF). It is caused by dengue virus which belongs to genus Flavivirus family Flaviviridae and includes serotypes 1, 2, 3 & 4 (Den-1, Den-2, Den-3 and Den-4). When a person has had classic dengue (i.e. infection by one serotype), a second infection later by another serotype increases the likelihood of suffering from DHF[1]. The expansion of Dengue in India has been related to unplanned urbanization, changes in environmental factors,

host-pathogen interactions and population immunological factors. Inadequate vector control measures have also created favourable conditions for Dengue virus transmission and its mosquito vectors[2]. Both Aedes aegypti and Aedes albopictus are the main mosquito vectors[3]. There are currently no FDA-approved vaccines or antiviral drugs for Dengue virus (DENV) infection, and treatment is limited to fluid replacement and palliative care[4,5].

Directorate of National Vector Borne Diseases Control Programme (NVBDCP), Government of India has identified a network of laboratories (sentinel surveillance hospitals (SSHs) and apex referral laboratories) for surveillance of dengue fever cases across the country since 2007. These laboratories are also meant to augment the diagnostic facilities in all endemic areas. They are linked with Apex Referral Laboratories (ARLs) with advanced diagnostic facilities for backup support and serotyping of dengue samples[6]. In Mizoram, two district hospitals viz. Civil Hospital Aizawl and Civil Hospital Lunglei were identified as SSH for Dengue and Chikungunya and used to carry out various activities like testing blood samples from the suspected patients with viral syndrome, maintaining line-listing of positive cases of Dengue and Chikungunya and capacity building of Primary Health Centres/Community Health Centres within the state.

We report here the results of Dengue infections and circulating phenotypes for a period of three years from June 2016 to May 2018. This is the first time-series report on Dengue study in Mizoram, north-eastern part of India.

## Material and Methods

### Ethics Statement

This study was reviewed by the Institutional Ethics Committee of Civil Hospital Aizawl and Civil Hospital Lunglei, Mizoram and considered exempt because samples were routinely received at the two sentinel surveillance hospitals in the state. All patient samples used for this study were de-linked from personal identifiers and renumbered with a study identifier.

### Patients and Samples

This study included human serum samples sent to two Sentinel Surveillance Hospitals of the whole state viz. one district hospital in Aizawl and another in Lunglei. All human serum samples sent to these sites were for Dengue surveillance diagnosis, including all patients with clinically suspected DF. Clinical DF was based on the following symptoms: sudden onset of fever, headache, retro-orbital pain, body aches, nausea and vomiting, joint pains, weakness and rash[3]. For each patient, the Dengue Requisition Form– containing demographic characteristics, date of onset of fever, timing of sampling, other constitutional symptoms, travelling history was filled out at the time of sample collection. Samples were analyzed using $NS_1$ antigen detection and Dengue IgM Capture ELISA as well as reverse transcriptase Polymerase Chain Reaction (RT-PCR), depending on the days of onset of symptoms, as described below.

### Dengue Assays

World Health organization (WHO) and NVBDCP state that samples collected within five days of the onset of symptoms must be analyzed either by the $NS_1$ antigen detection assay followed by RT-PCR for confirmation (When $NS_1$ is positive) or directly submitted to viral isolation for confirmation and DENV serotyping. On the other hand, samples that are collected after six or more days of the onset of symptoms must be analyzed based on the serological method using Dengue IgM Capture ELISA[6].

### $NS_1$ Antigen Detection

NS1 antigen detection was performed with the Panbio Dengue Early ELISA Standard Diagnostic Inc. according to the manufacturer's instructions.

### DENV Serotyping by RT-PCR

NS1 positive serum samples were preserved and sent to Apex laboratory of NVBDCP, Dibrugarh: Assam, India for RNA extraction and RT-PCR. Strict control measures were adopted to prevent cross contamination between samples. The RT-

PCR assay employed in this study can distinguish the four DENV serotypes by the size of the amplicons, as described by Lanciotti and colleagues[7]. For first round PCR, 3 µL of cDNA was added to 1 × Taq buffer, 200 µM dNTPs (Promega), 0.4 µM primers (D1 and D2) and 1.5 U of Taq polymerase (Bangalore Genei, Bangalore, India) in 25 µL reaction mixture. The PCR conditions for the external PCR were: 94ºC for 2 minutes followed by 35 cycles of 94ºC for 30 seconds, 52ºC for 30 seconds and 72ºC for 60 seconds and a final extension at 72ºC for 10 minutes. The expected size of the PCR products was 511 bp. External PCR was followed by nested PCR using the primer D1 and four serotype-specific primers, TS1, TS2, TS3 and TS4. The amplified product of the external PCR was diluted in a ratio of 1:5. The 25 µL nested PCR mixture was prepared by adding 1 µL of the diluted external product to 1 × Taq buffer, 200 µM dNTPs (Promega), 0.4 µM primers (D1, TS1-TS4) and 1.5 U of Taq polymerase (Bangalore Genei), Nested PCR was run as follows: 94ºC for 1 minute followed by 25 cycles of 94ºC for 30 seconds, 54ºC for 30 seconds and 72ºC for 60 seconds and final extension at 72ºC for 10 minutes. The sizes of the nested PCR products were 482 bp for DENV-1, 119 bp for DENV-2, 290 bp for DENV-3 and 392 bp for DENV-4. The PCR products were electrophoresed through 2% agarose gel, stained with ethidium bromide and examined under ultraviolet light using a gel documentation system (Wealtec, Sparks, NV, USA).

**Antibody Tests (IgM)**

For serological tests, the Dengue IgM Capture ELISA kit (Panbio Standard Diagnostic Inc.) was used according to the manufacturer's instructions.

Statistical Analysis:

Surveillance data were entered into an Excel® (Microsoft Corporation, Redmond, WA) database by Sentinel Surveillance Hospital staffs over the study period. Statistical calculations were performed, and figures and tables produced in Excel® (Microsoft Corporation, Redmond, WA).

**Results**

A total of 2596 samples collected from suspected dengue fever were analyzed between June 2016 and May 2018, of which 616 (23.72%) were positive for dengue based on any of the tests used. From 2016 to 2018, samples collected within five days of the onset of symptoms were analyzed by the $NS_1$ antigen detection assay, and positive samples (39 cases) were confirmed by dengue specific RT-PCR. Samples collected after the fifth day of the onset of symptoms were analyzed by the dengue-IgM capture ELISA (Table 1).

In the state of Mizoram, dengue infection cases occurred during summer, displaying a peak in June to September, while few cases were reported from November to February. The monthly distribution of dengue-positive cases along the years (2016-2018) in Mizoram, is shown in Figure 1.

In 2016, a total of 1276 dengue suspected cases were reported and 35.42% (452) were positive based on $NS_1$ or IgM ELISA (Table 2), with highest prevalence in the age group 15 – 24 years old and there was no gender preponderance in this year. There is no information about the serotypes circulating in this year because collected samples were inappropriate for viral isolation.

During 2017, 971 dengue suspected cases were reported and 10.40% (101) were dengue positive by $NS_1$ or IgM ELISA. The prevalence was highest in the age group 25 – 34 years old and there was no gender preponderance. The DENV-2 serotype was detected in that year with a proportion of 90.47% and DENV-3 being 9.53%.

In 2018, a total of 349 suspected cases of dengue were reported in Mizoram, positivity was 18.05% (63) and the most affected age group was 15 - 24 years old. There was no gender preponderance in this year. DENV-2 was detected in 83.33% and DENV-3 in 5.55% of dengue cases serotyped in this year. Moreover, concomitant circulation of DENV-3 was first detected in Mizoram in two (11.11%) patients.

Table 3 resumes the DENV serotypes circulating in Mizoram in each year, from June 2016 to May 2018.

**Table 1:** Dengue cases reported in the state of Mizoram, from June 2016 to May 2018

| | | | Methodology | | |
|---|---|---|---|---|---|
| **Year** | **Total** | **Positive/Total (%)** | **$NS_1$** | **IgM** | **RT-P** |
| 2016 | 1276 | 452(35.42) | 362 | 90 | 0 |
| 2017 | 971 | 101(10.40) | 37 | 64 | 2 |
| 2018 | 349 | 63(18.05) | 28 | 35 | 18 |
| Total | 2596 | 616 | 417 | 189 | 39 |

Methods: Dengue NS1 ELISA, Dengue IgM ELISA, RT-PCR

Results: Out of 2596 samples between June 2016 and May 2018, 616 (23.72%) were positive for dengue based on any of the tests used, 39 cases were confirmed by dengue specific RT-PCR.

**Figure 1:** Seasonality of positive dengue cases in the state of Mizoram, North East India, according to months (2016 – 2018)

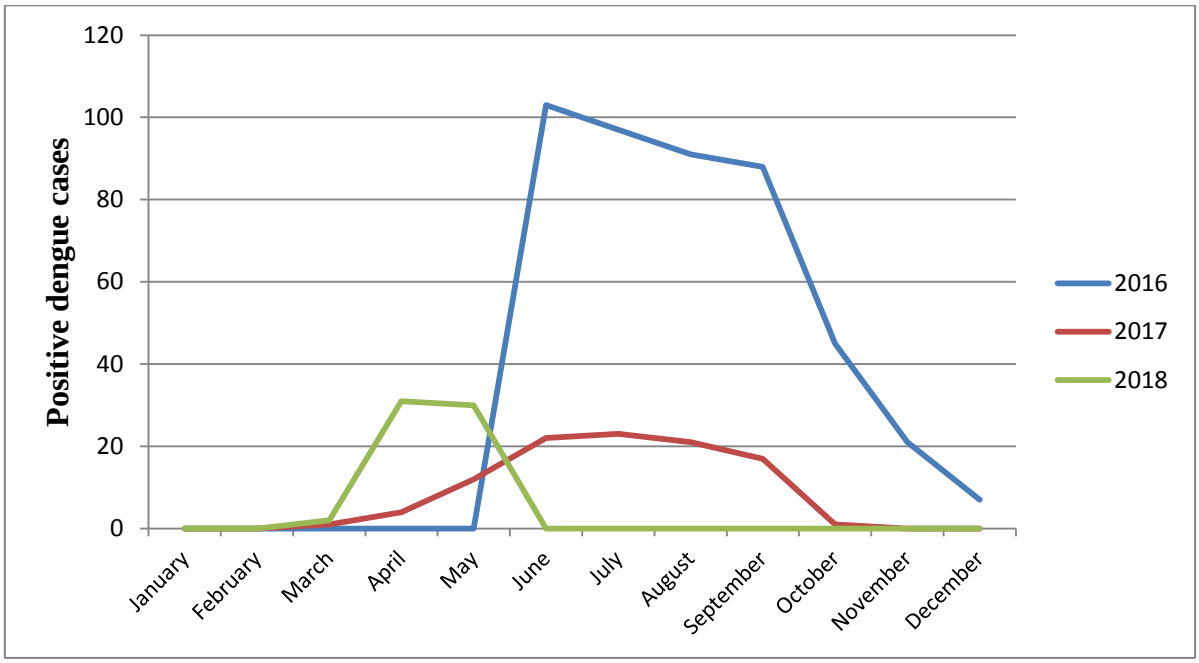


**Table 2:** Dengue cases in Mizoram according to age (June 2016 to May 2018)

| Year | 2016 | 2017 | 2018 | Total |
|---|---|---|---|---|
| Age (years) | n (%) | n (%) | n (%) | n (%) |
| 0 – 5 | 9 (1.99) | 7 (6.93) | 12 (19.04) | 28 (4.54) |
| 6 – 14 | 47 (10.39) | 19 (18.82) | 7 (11.11) | 73 (11.85) |
| 15 – 24 | 121 (26.77) | 18 (17.82) | 17 (26.99) | 156 (25.32) |
| 25 – 34 | 101 (22.35) | 21 (20.79) | 11 (17.46) | 133 (21.59) |
| 35 – 44 | 66 (14.60) | 18 (17.82) | 8 (12.70) | 92 (14.94) |
| 45 – 54 | 40 (8.85) | 10 (9.90) | 3 (4.76) | 53 (8.60) |
| 55 yrs & above | 68 (15.05) | 8 (7.92) | 5 (7.94) | 81 (13.15) |
| Total | 452 | 101 | 63 | 616 |

Methods: Percentage

Results:

(1) No gender preponderance during the study period ie from 2016 to 2018

(2) In 2016: highest prevalence in the age group 15 – 24 years old (26.77%)

(3) In 2017, highest prevalence in the age group 25 – 34 years old (20.79%)

(4) In 2018, the most affected age group was 15 - 24 years old (26.99%)

**Table 3:** Dengue cases in the state of Mizoram according to serotypes (June 2016 – May 2018)

| | Serotypes (%) | | |
|---|---|---|---|
| Year | DENV-2 | DENV-3 | Co-circulation of DENV-2 & 3 |
| 2016 | 0 | 0 | 0 |
| 2017 | 19 (90.47) | 2 (9.53) | 0 |
| 2018 | 15 (83.33) | 1 (5.55) | 2 (11.11) |
| Total | 34 (87.17) | 3 (7.69) | |

Methods: Percentage

Results:

(1) There is no information about the serotypes circulating in this year.
(2) In 2017, the DENV-2 serotype was detected with a proportion of 90.47% and DENV-3 being 9.53%.
(3) In 2018, DENV-2 was detected in 83.33% and DENV-3 in 5.55% of dengue cases serotyped in this year.
(4) Concomitant circulation of DENV-2 & DENV-3 detected in this year.

**Discussion**

This is the first health institution based study that provides evidence on seroprevalence of DENV infection in two cities of Mizoram. The results of this study showed a seroprevalence of 23.72% in the state of Mizoram. Several factors favour transmission of DENV in the study areas such as the climatic conditions (i.e high temperature) during summer, providing optimal environmental and biological circumstances for vector mosquito breeding and reproduction, and also increased urbanization in the study areas might favour the emergence and survival of DENV infected Aedes mosquitoes[8].

The most frequently affected age groups being 15-34 years old highlights that adults are mostly affected by dengue in Mizoram.

We were able to document the specific serotypes responsible for dengue infections in 2016 – 2018. All laboratory confirmed cases were due to either DENV-2 or DENV-3, with DENV-2 being the predominant serotype (87.17%).

We assess three diagnostic assays (RT-PCR, Dengue IgM ELISA and $NS_1$ ELISA) in a sentinel surveillance setting. During the viremic phase (up to day 5 of illness), molecular biological or virological approaches such as RT-PCR or $NS_1$ should be employed; after the viremic phase ( > 5 days), serological assays are indicated with dengue IgM being the most frequently used assay[9]. A combination of methods that target different time periods maximizes diagnostic sensitivity. Given the constraints of such large sentinel surveillance as ours, it was programmatically not feasible to take a convalescent serum at 14 – 21 days after discharge which would have helped in confirming the diagnosis – hence we lack a definitive "gold standard" diagnosis in those patients where we only have a single IgM result[9,10]

The proportion of $NS_1$ positive subjects in the first 5 days of illness was higher than that of RT-PCR. Higher sensitivity of $NS_1$ compared with RT-PCR has been documented in some studies[11,12,13] but not in others[14]. It is important to note that we tested $NS_1$ by ELISA and not with the cheaper rapid diagnostic (RDT) kits that are now widely available. Hunsperger et al showed that sensitivity of $NS_1$ by ELISA is higher (60-75%) compared with $NS_1$ RDT (38-71%)[13].

Although this is the first attempt to study a seroprevalence of DENV infection in Mizoram, the study has several limitations. A comparison of the acute serum with the convalescent serum from the same patients was not done due to the nature of a cross-sectional study design. The study was conducted in suspected dengue samples that may not necessarily reflect the true seroprevalence at the community level where some mild or asymptomatic infection might occur. There is also possibility of some false positive cases due to cross-reactivity of other anti- flavivirus antibodies with DENV. Despite these limitations, this study

ultimately provides the first baseline data on seroprevalence of DENV infection and the prevalent serotypes in this part of the country.

Molecular studies are also fundamental to understand viral evolution, viral dynamics and its interaction with hosts, and to predict the severity of the disease. DENV phylogenetics and phylodynamics in this region should be taken into account in future studies. This approach will contribute to improve the quality of surveillance data, to predict circulation of different dengue serotypes and genotypes as well as to evaluate the impact of preventive measures such as mosquito control or a future vaccine.

Finally, considering that dengue was recently introduced in this region, the control of further infections requires a network of well-trained and skilled health professionals, entomologists, environment surveillance agents, laboratory technicians and molecular biologists, as well as social education and government support.

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