E-ISSN:2320-8686
P-ISSN:2321-127X
Research Article
Dengue Serotypes

International Journal of Medical Research and Review

2023 Volume 11 Number 6 November-December

Publisher
MEDRESEARCH
www.medresearch.in

# Dengue Serotypes In Manipur – Findings From A Retrospective Analytical Study In A Tertiary Care Hospital

**Anand Singh L[1], Suson T[2*], Geeta Chanu C[3], Ranjana Devi K[4], Rebachandra Singh H[5]**

DOI: https://doi.org/10.17511/ijmrr.2023.i06.02

[1] Laitonjam Anand Singh, Scientist C Virus Research Diagnostic Laboratory VRDL, Department of Microbiology, Regional Institute of Medical Sciences, Imphal, Manipur, India.

[2*] Thingujam Suson, Scientist B Virus Research Diagnostic Laboratory VRDL, Department Of Microbiology, Regional Institute Of Medical Sciences, Imphal, Manipur, India.

[3] Chongtham Geeta Chanu, Research Assistant Virus Research Diagnostic Laboratory VRDL, Department Of Microbiology, Regional Institute of Medical Sciences, Imphal, Manipur, India.

[4] Khuraijam Ranjana Devi, Principal Investigator VRDL and Professor Head, Department Of Microbiology, Regional Institute of Medical Sciences, Imphal, Manipur, India.

[5] Heigrujam Rebachandra Singh, CoPrincipal Investigator VRDL Professor, Department Of Microbiology, Regional Institute of Medical Sciences, Imphal, Manipur, India.

**Objectives:** As a part of an ongoing research programme on vector-borne viral diseases especially Dengue, a retrospective analytical study on the occurrence and distribution of Dengue virus serotype(s) in the state of Manipur – a small state situated in the northeastern region of the Indian subcontinent was carried out at Viral Research & Diagnostic Laboratory (VRDL), Department of Microbiology, Regional Institute of Medical Sciences (RIMS), Imphal which is a tertiary care hospital. **Materials & Methods:** A total of 914 blood samples from clinically dengue-suspected patients were screened for the presence of Dengue infection by adopting the ELISA (IgM) technique during the period from 01/06/2022 to 02/12/2022. Further, anti-Dengue IgM antibody-positive samples having high Optical Density (OD) value(s) were selected and subjected to RT PCR to determine the serotype(s) of the Dengue virus. **Results:** Of the 914 blood samples examined for the presence of Dengue infection, 111 (12.14%) were found positive for anti-Dengue IgM antibody indicating acute infection of Dengue virus. Of the positive patients, there were 56 (50.45%) males and 55 (49.54%) females. Predominant clinical features observed among the Dengue-confirmed patients included – fever (74%), headache (19%), arthralgia / joint pain (9%), myalgia (6%) and vomiting (6%) respectively. The study revealed that while the circulation of three (03) Dengue virus serotypes, namely DENV – 1, DENV – 2 & DENV – 3 were observed in the Tengnoupal district, the circulation of two Dengue virus serotypes i.e., DENV – 1 & DENV – 2 was evident in Imphal West & Bishnupur districts respectively. The present study also reveals the occurrence of Dengue virus serotype – 1 (DENV - 1) in Churachandpur district. **Conclusion:** The present study reveals the circulation/distribution of three Dengue virus serotypes namely, DENV – 1, DENV – 2 and DENV – 3 among the studied samples.

**Keywords:** Dengue virus, Dengue IgM ELISA, Dengue serotype (RT PCR)

**Corresponding Author**

Thingujam Suson, Scientist B Virus Research Diagnostic Laboratory VRDL, Department Of Microbiology, Regional Institute Of Medical Sciences, Imphal, Manipur, India.
Email: suson93@gmail.com

**How to Cite this Article**

Anand Singh L, Suson T, Geeta Chanu C, Ranjana Devi K, Rebachandra Singh H, Dengue Serotypes In Manipur – Findings From A Retrospective Analytical Study In A Tertiary Care Hospital. Int J Med Res Rev. 2023;11(6):136-145.
Available From
https://ijmrr.medresearch.in/index.php/ijmrr/article/view/1452

**To Browse**

| Manuscript Received | Review Round 1 | Review Round 2 | Review Round 3 | Accepted |
|---|---|---|---|---|
| 2023-11-06 | 2023-11-08 | 2023-11-15 | 2023-11-22 | 2023-11-29 |

| Conflict of Interest | Funding | Ethical Approval | Plagiarism X-checker | Note |
|---|---|---|---|---|
| Nil | Nil | Yes | 18% | |

# Introduction

Dengue, a febrile illness, is a vector-borne viral disease caused by any one or more than one of the four serotypes of Dengue virus (DENV) and is transmitted by the bites of the female mosquitoes belonging to the genus *Aedes aegypti* and other *Aedes* species (*A. albopictus*). It is considered as one of the top ten potential threats to global health and is also one of the major causes of morbidity in endemic regions of the world [1]. It is estimated that a total of 2.5 billion people are living in Dengue endemic areas [2] and an additional 120 million people travel to these affected areas annually [3]. Dengue infects 50-100 million people each year in > 100 countries and is estimated to have caused at least 20,000 deaths annually [2,4]. WHO [5] has put on record that the incidence of dengue in recent years increased from 5,05,430 cases in 2000 to 5.2 million in 2019. Dengue is also one of the important mosquito-borne viral infections in India and India contributes about 34% (33 million) of the global burden of symptomatic Dengue infections.

Dengue virus belongs to the family Flaviviridae and under the genus Flavivirus there are four (04) antigenically similar but immunologically distinct serotypes, namely – Dengue virus (DENV) – 1, - 2, - 3 and – 4 respectively [6]. Dengue virus was not reported in the state of Manipur till October 2007, although a large outbreak/epidemic of suspected Dengue fever (DF) had started in September 2007. Subsequently, a large number of fever cases of unknown origin were reported from Moreh, a small border township of Manipur situated in the Indo-Myanmar international border area in November 2007. Later, it was confirmed as a Dengue outbreak [7,8]. Since then, dengue has been reported sporadically from this tiny state with increasing frequency either as a disease cluster or as an outbreak in various areas of the state in recent years.

It is generally agreed that the determination of the infecting serotype(s) through the use of molecular technique(s) provides relevant scientific data and specific information about the infecting serotype(s). Moreover, baseline information on circulating serotypes in a particular geographical area or region would help predict outbreaks if changes in the most prevalent serotypes are observed during the early phase of the Dengue season.

However, despite having a lacuna in this important aspect of dengue infection, the literature review reveals very few publication(s) [7,8] about dengue serotype studies in Manipur.

Therefore, visualizing the importance of conducting such type(s) of study, the present work is being taken up towards unravelling the circulating dengue serotypes prevalent among the human population of this state.

# Materials And Methods

A total of 914 blood samples were referred from patients admitted in the various clinical departments of RIMS hospital [ i) Outdoor patient department (OPD): 452 samples; ii) Indoor patient department (IPD / Ward): 287 samples; altogether 739 samples]; District hospital, Moreh, Tengnoupal district [17 samples]; State Health Department / Integrated Disease Surveillance Programme (IDSP) [81 samples]; over and above these samples, 77 samples were also collected by VRDL, RIMS from field visits at various villages situated in and around Moreh, a small township in the Indo-Myanmar border towards laboratory diagnosis of Dengue virus infection. This retrospective analytical study was conducted during the period from 01/06/2022 to 02/12/2022. A brief clinical history of the patient(s) with emphasis on DF / DHF associated signs & symptoms of the individual(s) as per WHO [9] / Anon [10] criteria/guidelines, age, sex and ethnicity along with concise information about their socio-economic status, knowledge of health and hygienic practices with particular reference to preventive and prophylactic measures against mosquito-borne viral diseases were obtained from the patient(s) / individual(s) while receiving the sample(s) either at VRDL Lab or collecting it during the field visits.

**Inclusion criteria:** Individual(s) who fulfil the clinical case definition/criteria as laid down by WHO [9] / Anon [10], as probable cases of Dengue fever – i.e., acute febrile illness, with two or more of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, rash or any haemorrhagic manifestation

**Exclusion criteria:** Individuals who do not fulfil the clinical case definition of Dengue fever.

All the Dengue-negative samples were also tested for other possible aetiologies like Malaria, Japanese Encephalitis (JE) and Zika virus.

The serological testing for Dengue and serotyping of the anti-dengue IgM antibody-positive samples are briefly summarized below:

**A. Anti-dengue IgM antibody detection:**

Detection of the IgM antibodies against Dengue virus was done by employing NIV Dengue IgM capture ELISA kit / MAC ELISA kit [Lot no. / Batch no. 22 – 069; Version: 2.4; Expiry date: 19/04/2023] supplied from Diagnostic Reagent Facility, National Institute of Virology (NIV), Pune, India. The test was done as per the SOP as described in the kit insert and by employing a bacteriological incubator (Make: Parex; Model no.: PSW144/14481813) and ELISA Washer [Make: Meril; Model no.: EIA Wash (Merilyzer); Sl. No. 090220]. The absorbance of the processed sample was measured at 450 nm by employing a Photometric ELISA Reader [Make: Meril; Model no.: EIA QUANT (Merilyzer); Sl. No. 081733].

The test result was interpreted as per the guidelines/instructions as described in the kit literature [In case, Sample OD £ Negative Control OD x 2.0, then the sample should be considered as Negative; if Sample OD is ≥ Negative Control OD x 3.0, then the sample should be considered as Positive; Sample OD > Negative Control OD x 2.0 but < Negative Control OD x 3.0, then Sample should be considered as Equivocal].

**B. Serotype determination:**

Of the 111 Dengue-positive samples, 11 samples having high OD values (on ELISA test) were selected under this pilot study/research program and subjected to Real-Time PCR (RT PCR) to determine the serotype of the Dengue virus. The serotype identification process involves the following three important steps:

I. Extraction of the viral nucleic acid (RNA)

II. Amplification of the viral RNA using RT PCR (by way of synthesizing cDNA through reverse transcription) and

III. Post-PCR analysis towards interpreting the test results

Viral RNA extraction was done from the blood samples by using a QIAamp viral RNA mini kit (Make: Qiagen, Germany; Lot no.: 166033751; Expiry date:04/03/2024).

Amplification of the viral RNA was done in RT PCR (Model: Rotor-Gene-Q; Instrument type: 5 Plex HRM; Instrument Sl no. R0916174; Make: Qiagen, Germany) by employing Real Star Dengue Type RT PCR kit 1.0 (Make: Altona Diagnostics; Lot no.: 037783; Manufacturing date: 03/08/2022; Expiry date: 28/04/2023).

Interpretation(s) of the test result(s) was done by observing the nature of amplification curve(s) and corresponding Critical threshold (Ct) values of the test sample(s) in relation to the amplification cycle or as per the guidelines furnished in the RT PCR multiplex kit insert.

This study was approved by the institutional Research Ethics Board [Vide Ref. no: A/206/REB/Prop (FP)/210/138/15/2023; dt. 25/10/2023] of the Regional Institute of Medical Sciences, Imphal.

# Results

Of the 914 blood samples screened for the presence of dengue infection, 111 (12.14%) were found positive for Dengue virus as Dengue virus-specific IgM antibodies were detected in them (IgM ELISA), thereby establishing and confirming acute infection of Dengue. Out of 111 positive patients, there were 56 (50.45%) males and 55 (49.54%) females. The sex difference was found to be statistically insignificant ($|z|CV = 0.016$; $z < 1.96$; $P < 0.05$). An age-wise break-up among the Dengue confirmed patients revealed 27.9% of the studied population to have belonged to the age group of ≥ 51 years, while 21.6% were in the age group of 21-30 years [Table - I]. The study also revealed that while those in the age group of 0-10 years constituted 14.4%, those in the age group of 31-40 years constituted the minimum infected age group with an incidence rate of 9.9% only.

The predominant clinical features observed in patients diagnosed with Dengue fever (DF) included fever (74%), headache (19%), joint pain (9%), myalgia (6%) and vomiting (6%). Non-specific clinical features like drowsiness, diarrhoea, malaise, sore throat, cough, lethargy, loss of appetite, rashes, nausea, general weakness and pain in the abdomen etc. were observed in 8 (7.2%) of the Dengue-positive patients. The haemorrhagic manifestation was observed in 1 (0.9%) patient only.

While concurrent clinical features (syndromes) like fever, headache, arthralgia, myalgia and retro-orbital pain were observed in 2 patients, Dengue haemorrhagic fever with severe thrombocytopenia was evident in only 1 patient. None of the patients were found positive for Malaria, Japanese encephalitis (JE) and Zika virus.

**Table – I: Age-wise distribution of Dengue-positive patients (N = 111)**

| Age (in yrs) | RIMS Hospital ♂ ♀ | State Health Department / IDSP ♂ ♀ | District Hospital, Moreh ♂ ♀ | Field Collection ♂ ♀ | Total ♂ ♀ | Grand Total (%) |
|---|---|---|---|---|---|---|
| 0 – 10 | 7 2 | 1 1 | 1 0 | 1 3 | 10 6 | 16 (14.4) |
| 11 – 20 | 8 5 | 0 0 | 1 1 | 1 1 | 10 7 | 17 (15.3) |
| 21 – 30 | 4 13 | 2 1 | 0 0 | 1 3 | 7 17 | 24 (21.6) |
| 31 – 40 | 4 2 | 2 3 | 0 0 | 0 0 | 6 5 | 11 (9.9) |
| 41 – 50 | 5 3 | 1 0 | 1 0 | 1 1 | 8 4 | 12 (10.8) |
| ≥ 51 | 8 10 | 5 2 | 0 0 | 2 4 | 15 16 | 31 (27.9) |
| Total | 36 35 | 11 7 | 3 1 | 6 12 | 56 55 | 111 (99.9) |

Of the 111 anti-Dengue IgM antibody-positive samples, 11 samples having high OD values were selected for the present pilot study programme towards determining the dengue serotype(s) and were subjected to Real-Time PCR.

Out of these 11 samples, each of 4 samples was from patients residing/living in Tengnoupal district and Imphal West district; 2 samples and 1 sample were from patients residing/living in Churachandpur and Bishnupur district respectively [Table - II]. RT PCR test revealed the circulation of 3 Dengue serotypes in the studied population/samples i.e., Dengue serotype – 1 (DENV – 1), Dengue serotype – 2 (DENV – 2) and Dengue serotype – 3 (DENV – 3) respectively [Table – II]. In the present study, while single infection with DENV – 1 and DENV – 2 were observed in 5 and 4 patients; concurrent infection with DENV – 1 & DENV – 3 and DENV – 1 & DENV – 2 were recorded in 1 patient each [Table – II & Fig. I].

In the present study, on overall assessment, the serotype DENV - 1 was found to be the predominant serotype as it was detected in 7 patients [as single infection in 5 patients & as concurrent infection with serotype DENV – 2 and serotype DENV - 3 in 1 patient each]; followed by serotype DENV- 2 in 5 [as single infection in 4 patients & as concurrent infection with serotype DENV- 1 in 1 patient] and serotype DENV- 3 in 1 [as concurrent infection with serotype DENV - 1 in 1 patient] patient respectively.

In the present study, single infection with serotype DENV - 3 was evident in none of the 11 patients being studied but as mixed infection with serotype DENV - 1 in only 1 patient.

**Table – II: Dengue serotypes observed in the studied samples/population**

| Sl No. | Patient ID | District to which the patient belongs to | Age (in years) | Sex | Ethnicity | Dengue serotype(s) detected | RT PCR (Amplification cycle = 45)Ct Value(s)of test sample(s) |
|---|---|---|---|---|---|---|---|
| 1 | RS-1 | Tengnoupal | 08 | M | Muslim | DENV - 1 | 29.63 |
| 2 | RS-2 | Tengnoupal | 17 | F | Muslim | DENV - 1 | 31.59 |
| 3 | RS-3 | Churachandpur | 80 | F | Kuki | DENV - 1 | 30.42 |
| 4 | RS-4 | Churachandpur | 45 | F | Kuki | DENV - 1 | 33.37 |
| 5 | RS-5 | Imphal West | 75 | F | Meitei | DENV - 1 | 34.00 |
| 6 | RS-6 | Imphal West | 72 | M | Nepali | DENV - 2 | 24.56 |
| 7 | RS-7 | Imphal West | 38 | F | Nepali | DENV - 2 | 36.39 |
| 8 | RS-8 | Imphal west | 55 | F | Nepali | DENV - 2 | 35.88 |
| 9 | RS-9 | Tengnoupal | 75 | F | Kuki | DENV - 2 | 38.20 |
| 10 | RS-10 | Tengnoupal | 42 | M | Meitei | DENV - 1 & - 3 | 12.1432.97 |
| 11 | RS-11 | Bishnupur | 25 | F | Meitei | DENV - 1 & - 2 | 29.4634.21 |

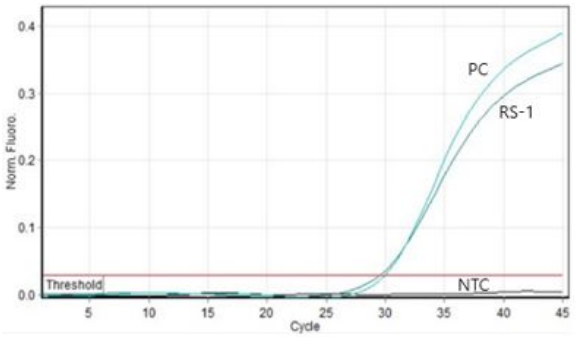
**Table– III: A comparative account on the occurrence of dengue serotype(s) in selected districts of Manipur**

| Name of the district(s) | Area / Locality | Dengue serotype(s) | Year of report | Nature of test | Reference / Authority |
|---|---|---|---|---|---|
| Tengnoupal (recently demarcated from the erstwhile Chandel district) | Moreh | DENV - 2 | 2012 | RT PCR | Sankari et. al., 2012 |
| Tengnoupal | Moreh | DENV - 1 | 2013 | RT PCR | Khan et. al., 2013 |
| | | DENV - 2 | | | |
| | | DENV - 3 | | | |
| | | DENV - 4 | | | |
| Tengnoupal | Moreh | DENV - 1# | 2023 | RT PCR | Present study |
| | | DENV - 2Ø | | | |
| | | DENV - 3© | | | |
| Imphal West | Imphal | DENV - 1* | 2023 | RT PCR | Present study |
| | | DENV - 2D | | | |
| Churachandpur | - | DENV - 1 | 2023 | RT PCR | Present study |
| Bishnupur | - | DENV - 1 | 2023 | RT PCR | Present study |
| | | DENV - 2 | | | |

Ø©D. Mounnaphai
* Kanglatongbi
DSagolband Tera
# Muslim Nagar

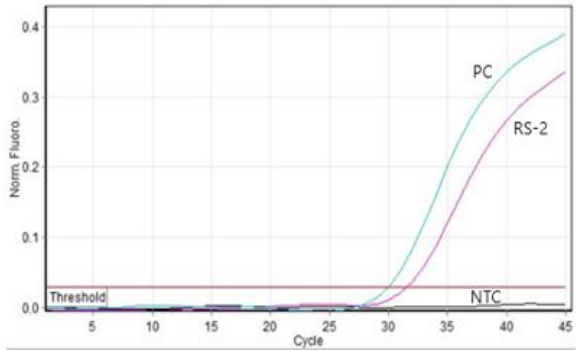
This study revealed the circulation of Dengue serotype DENV - 1 in Sagolband Tera, a locality within the Imphal Municipal area and serotype DENV - 2 at Kanglatongbi, a rural setting on the outskirts of Imphal City. These two areas fall under the administrative jurisdiction of Imphal West district although these two localities

Are 20 kilometres apart. On the other hand, in Moreh Town (under Tengnoupal district) while the occurrence of Dengue serotype DENV - 1 was established at Muslim nagar and its surrounding areas, that of serotype DENV- 2 and DENV - 3 were observed at D. Mounnaphai and nearby villages [Table-III]. This study also revealed that while circulation of Dengue serotype - DENV – 1 was observed in Churachandpur district, that of serotype DENV – 1 and DENV – 2 were evident in Bishnupur district [Table – III & Fig. II]



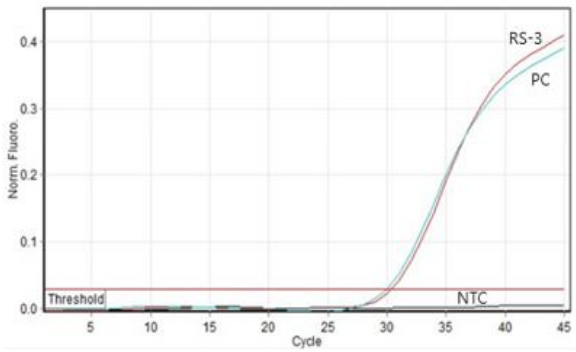

1(a): Amplification curve for RS-1

DENV-1 serotype (Ct = 29.63)



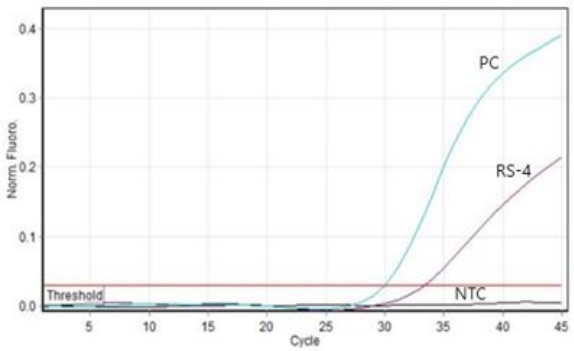

1(b): Amplification curve for RS-2

DENV-1 serotype (Ct = 31.59)



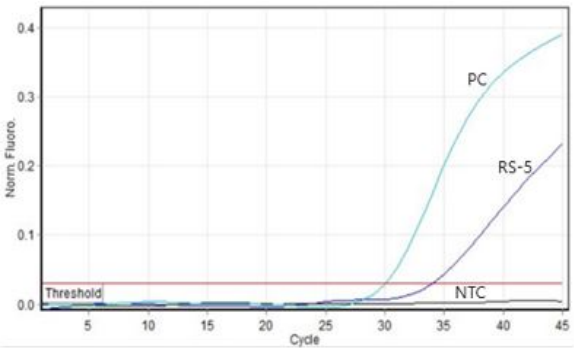

1(c): Amplification curve for RS-3

DENV-1 serotype (Ct = 30.42)



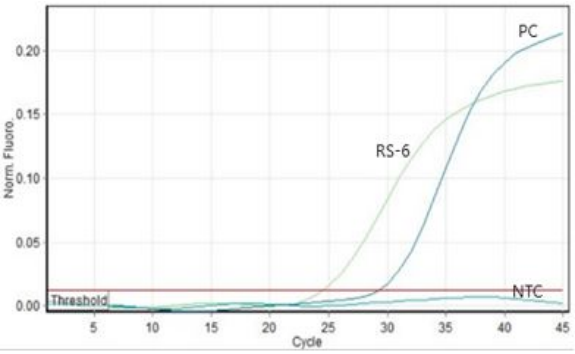

1(f): Amplification curve for RS-6

DENV-2 serotype (Ct = 24.56



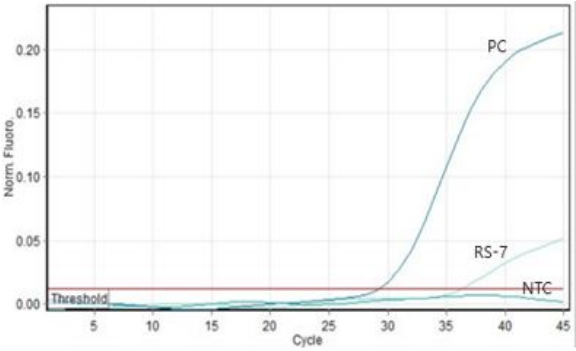

1(e): Amplification curve for RS-5

DENV-1 serotype (Ct = 34.00)



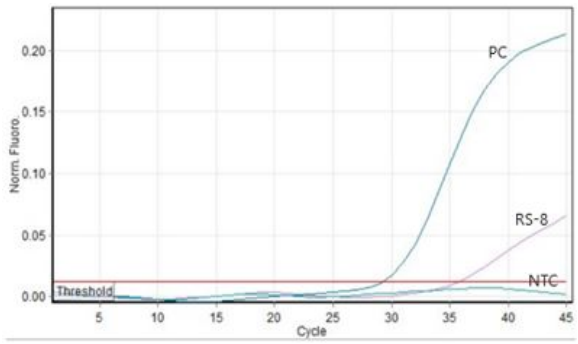

1(f): Amplification curve for RS-6

DENV-2 serotype (Ct = 24.56



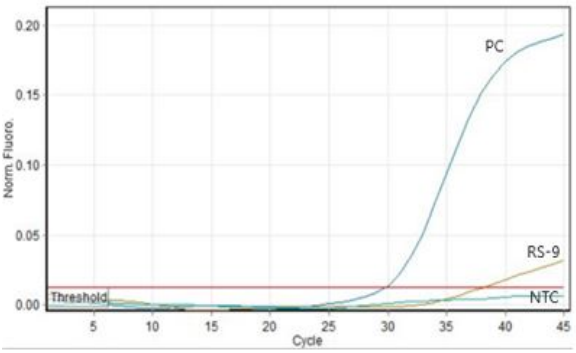

1(g): Amplification curve for RS-7

DENV-2 serotype (Ct = 36.39)



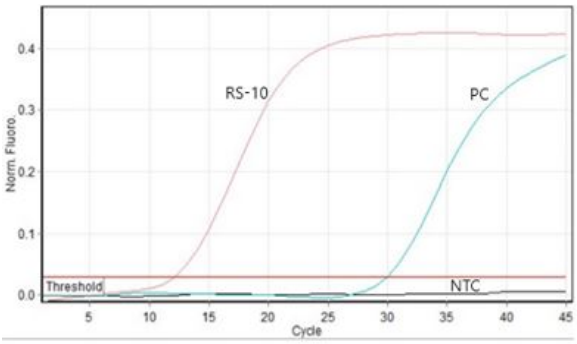

1(h): Amplification curve for RS-8

DENV-2 serotype (Ct = 35.88)



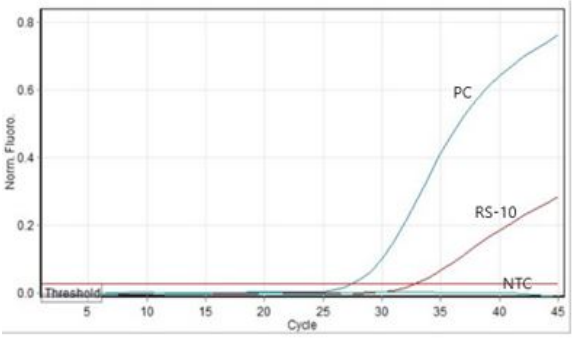

1(i): Amplification curve for RS-9

DENV-2 serotype (Ct = 38.20)



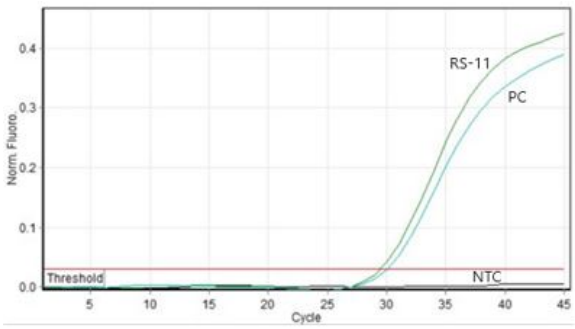

1(j): Amplification curve for RS-10

DENV-1 serotype (Ct = 12.14)




1(k): Amplification curve for RS-10

DENV-3 serotype (Ct = 32.97)




1(l): Amplification curve for RS-11

DENV-1 serotype (Ct = 29.46)

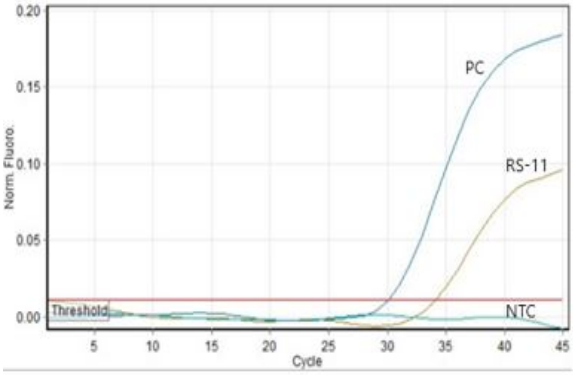



**Figure 1: Graph depicting RT-PCR amplification curves**

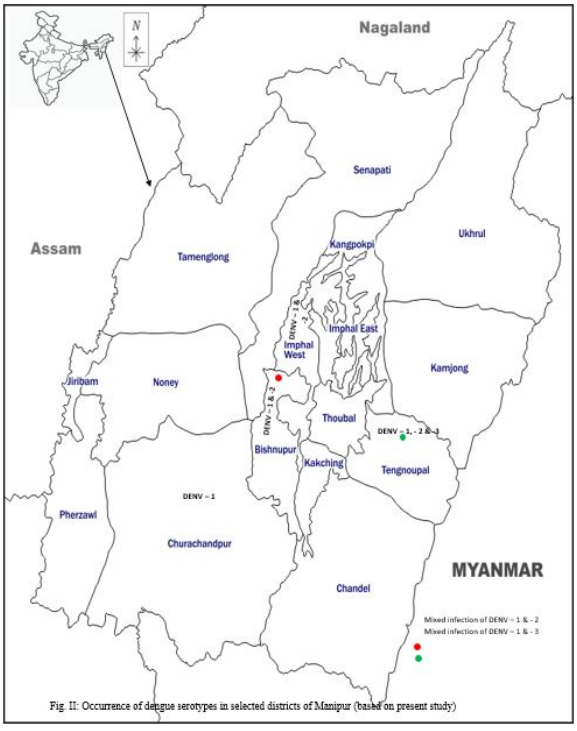



Fig. II: Occurrence of dengue serotypes in selected districts of Manipur (based on present study)

# Discussion

Dengue virus is a spherical 50 nm enveloped virion having an 11 kb positive sense-capped ssRNA genome. The ssRNA genome encodes three structural proteins, namely – Capsid (C), Pre-membrane / membrane (PrM / M), Envelope (E) and seven non-structural proteins. It also has a lipid envelope. Of the three structural proteins, the envelope (E) protein is the major surface protein on the virus and is therefore the primary antigen that elicits the neutralizing antibody response. The virus as already mentioned has four antigenically similar but immunologically distinct serotypes namely, – Dengue (DENV) virus DENV – 1, DENV - 2, DENV - 3 and DENV – 4 respectively. These four closely related viruses are called serotypes because each has different interactions with the antibodies in the human blood serum. Serotypes are groups within a single species of microorganisms such as bacteria or viruses which share distinctive surface structures (i.e., serotypes refer to separate groups within a single species of microorganisms that all have the same antigens on their surfaces but have distinct epitopes). The four serotypes present different symptoms and influence immune response to subsequent dengue infections rendering surveillance, risk assessments and disease control particularly challenging. RNA viruses including Dengue virus exhibit high mutation rates. The reason is that while DNA replication enzyme DNA polymerase has a special quality, i.e., the proofreading activity which prevents/eliminates the occurrence of mutation, RNA viruses lack DNA polymerase enzyme but have RNA polymerase enzyme. RNA polymerase enzyme lacks exonuclease proofreading activity and because of this, RNA viruses exhibit high mutation rates leading to the generation of mutant forms/varieties resulting in the production of different genotypes.

In Manipur, few workers had already done preliminary investigative study on the occurrence(s) of dengue serotypes based on blood samples collected from Moreh, a small township in the Indo-Myanmar border of the erstwhile Chandel district of Manipur, when the state witnessed its first sudden outbreak of fever and febrile illness from late 2007 through middle of 2008, during which several clinically dengue suspected cases were reported in the local hospital of Moreh town [7,8].

Concerning the occurrence of dengue serotypes in Manipur, Khan *et al*. [8] based on a study of 282 samples from Moreh, reported the circulation of all the four dengue serotypes i.e., DENV – 1, DENV – 2, DENV – 3 and DENV – 4 in this small border town of the state, however during the present pilot study, only three dengue serotypes namely, DENV – 1, DENV – 2 and DENV – 3 were observed in Moreh. This non-detection of DENV–4 in the present study might be due to the adoption of small sample size. While Khan *et al*. [8] reported dengue serotype DENV - 3 as the most prevalent dengue serotype during their study at Moreh, the present study reveals the dengue serotype DENV – 1 as the most prevalent serotype during the current study period. While Khan *et al*. [8] observed a single infection with DENV – 1 in only 1 patient, the present study revealed 5 individuals to have been infected with this particular serotype. The present finding of only one patient to have concurrent infection with dengue serotype DENV - 1 and DENV - 3 is much lower compared to that of the findings of Khan *et al*. [8], who reported the observation of this concurrent infection in 11 patients.

While Khan *et al*. [8] reported the co-circulation of all four dengue virus serotypes i.e., DENV – 1, DENV – 2, DENV – 3 and DENV – 4 in Moreh town among the studied population (N = 282); Sankari *et al*. [7] reported the occurrence of dengue virus serotype DENV - 2 only in their studied population (N = 58; 42 individuals in 2007 and 16 individuals in 2008) that also in the Moreh town of Manipur. None of the above two workers took up such type of studies in other districts of the state. However, during the present study occurrence of three dengue virus serotypes namely, DENV – 1, DENV – 2 and DENV – 3 were recorded among the human population residing in other districts of Manipur. Although Khan *et al*. [8] reported the circulation of dengue virus serotype DENV – 2 in the Moreh town of the state, no further study about phylogenetic analysis was done.

However, Sankari *et al*. [7] based on phylogenetic analysis using blast search and multiple alignment reported that the nucleotide sequence of the dengue virus serotype DENV - 2 recovered from Moreh town was found to be 100 % similar to the nucleotide sequence of the DENV – 2 of Cambodian origin, which confirms that the then DENV – 2 circulating in the Moreh town was that of Cambodian origin.

Co-circulation of multiple dengue virus serotypes has been reported from many parts of the world including India, however concurrent infection with more than one serotype of dengue virus in the same individual is of infrequent observation and rarely documented. Although in recent years, co-circulation of multiple dengue virus serotypes is being increasingly reported with concurrent infections [11,12], including in Manipur [8]; to date, concurrent infections with dengue serotypes had been reported only from Moreh town, a small border township in the erstwhile Chandel district of Manipur. The literature review reveals no authentic scientific work(s) or publication(s) about the occurrence of dengue virus serotype(s) in the other districts of Manipur. The present study is probably the first documented evidence of dengue serotyping studies being done in the other districts of the state other than the earlier studies that had been initiated by few workers in Moreh [7,8].

The present finding of the occurrence of dengue serotype(s) DENV - 1 and DENV - 2 in Imphal West and Bishnupur districts and that of dengue serotype DENV - 1 in Churachandpur district are the first reports of circulation of specific dengue serotypes in these three districts of Manipur. The present observation of co-circulation of three dengue serotypes (DENV – 1, DENV – 2 and DENV - 3) in the Moreh town of erstwhile Chandel district (now rechristened as Tengnoupal district) is more or less in close agreement with the findings of earlier workers like Sankari *et al*. [7] and Khan *et al*. [8], in which while the former reported the occurrence of DENV – 2 serotype only, the latter documented co-circulation of four dengue serotypes (DENV – 1, DENV - 2 DENV – 3 and DENV - 4) in Moreh.

In India, DENV – 2 and DENV – 3 were reported to be the most predominant serotypes during the period from 2003 to 2007, however, DENV – 1 replaced these strains in the year 2008 [13]. The present observation of concurrent infections of DENV – 1 and DENV – 2 and also that of DENV – 1 and DENV – 3 in some of the patients are also in agreement with the findings of earlier workers who had also reported similar observations [8,11,14]. Kurukumbi *et al.* [15] reported DENV – 1 to have been associated with dengue haemorrhagic fever (DHF) outbreak in Delhi in 1997 and this finding corroborates our present observation of a clinically DHF confirmed 80 yr old female tribal patient from Churachandpur district who was also found to have been infected with DENV – 1.

The possibility of concurrent infections with more than one serotype may be expected to occur in a population when there is a co-circulation of multiple serotypes of dengue virus in that particular area or region. This is also quite possible as the feeding behaviour of dengue vector mosquitoes characterized by multiple blood meals in a single gonotrophic cycle [16] enables them to become infected by multiple serotypes in each feeding and subsequently transmit the serotypes to a single host. It has also been postulated that concurrent infections by multiple dengue virus serotypes may lead to more severe forms of the disease [17]. Although the clinical and health impact of dengue virus infection on pregnancy is yet to be fully investigated and studied, findings from the earlier investigations suggested maternal mortality, vaginal bleeding and miscarriage to have been associated with pregnant women infected with the dengue virus [18,19,20,21]. While Khan *et al.* [8] reported the occurrence of four dengue serotypes (DENV – 1, DENV – 2, DENV – 3 and DENV - 4) at Moreh, the present study records the presence of only three dengue serotypes namely DENV – 1, DENV – 2 and DENV – 3. The possible reason for the non-detection ofthe DENV – 4 serotype in the present study might be due to the small sample size as this study was taken up as a pilot study programme. However, our present observation is markedly different compared to the findings of Sankari *et al*. [7] who based on a study at Moreh during 2007- 2008 reported only DENV – 2 to have been evident during their study period.

# Conclusion

This pilot study/research programme helps enrich and broaden our existing knowledge pertaining to dengue virus serotypes being circulated in the state of Manipur in general and the three districts of the state namely, Imphal West, Bishnupur and Churachandpur in particular as the literature review reveals no documented evidence of such studies in these three districts of the state in the past. Based on this study, it was observed that during the period of study, while two dengue serotypes namely, DENV – 1 & DENV – 2 were detected in Imphal West and Bishnupur district, DENV – 1 was observed in Churachandpur district. Further, the study also showed that while concurrent infection with DENV-1 & -3 was observed in a patient in Tengnoupal district, another patient living in Bishnupur district

Was also found to have dual infection with DENV-1 & DENV-2. Epidemiological studies by earlier workers revealed that dengue serotypes can vary by region and change over time. Moreover, the prevalence and incidence of each serotype may change from year to year.

Therefore, based on the above findings, it is strongly felt that further studies pertaining to DENV surveillance should be continued in other districts of the state towards unravelling a complete picture of the circulating serotypes as well as to note any shift in the dominance of these serotypes/genotypes.

Findings from such studies will help us in managing, preventing and controlling the dengue viral disease/outbreak considering the variability of the circulating dengue serotypes and its endemicity in the varied topographical features in the different districts of the state as it will provide baseline information on specific circulating serotypes endemic in a particular topographical area. This would help in predicting outbreaks if changes in the most prevalent serotypes are observed during the early phase of the dengue season as specific dengue serotypes play a key role in antibody-dependent enhancement (ADE) which contributes significantly to disease pathogenesis and severity.

**Author's contribution: Dr L. Anand Singh**: Study design, data analysis (Molecular), paper drafting & writing, proofreading **Thingujam Suson**: Data analysis (Molecular), typing, proofreading **Chongtham Geeta Chanu**: Data analysis (ELISA), proofreading **Dr Khuraijam Ranjana Devi**: Framework of the study design, proofreading, overall supervision **Dr Heigrujam Rebachandra Singh**: Proofreading, overall supervision.

**Sources of support in the form of grants, equipment, drugs or all of these:**

Department of Health Research (DHR) / Indian Council of Medical Research (ICMR), Ministry of Health & Family Welfare, Government of India, New Delhi for establishing VRDL, RIMS under the centrally sponsored scheme entitled "Setting up of Nation-Wide Network of Laboratories for Managing Epidemics and National Calamities". [Vide Sanction Order No. VIR/61/2013/ECD-1; Dt. 17/11/2016]

**Acknowledgement, if any:** The authors are grateful to the Director, Regional Institute of Medical Sciences (RIMS), Imphal (Manipur) & Head, Department of Microbiology, RIMS for providing necessary laboratory facilities. The authors are also thankful to the Medical Superintendent, RIMS for co-operation and support towards carrying out this study by way of encouraging the VRDL staff. The authors also wish to thank the staff of the IDSP / State Health Department towards providing cooperation and support while collecting samples from the field. The authors are also grateful to the Department of Health Research (DHR) / Indian Council of Medical Research (ICMR), Ministry of Health & Family Welfare, Government of India, New Delhi towards providing financial/technical support.

# References

1. WHO. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control, Geneva, Switzerland, WHO-TDR publication, 2009; http://www. who. libdoc. *who.int-publications 2009-9789241547871_eng pdf [Crossref][PubMed] [Google Scholar]*

2. WHO. Dengue and Dengue haemorrhagic fever. 2006; Fact sheet No. 117 http://www. *who.int/mediacentre/factsheets/fs117/en/print.html [Crossref][PubMed][Google Scholar]*

3. WTO. Tourism highlights. 2007; http://www. world-tourism. *org/facts/menu.html [Crossref] [PubMed][Google Scholar]*

4. CDC. Dengue: Epidemiology. 2016; http://www. cdc. *gov/dengue/epidemiology [Crossref][PubMed] [Google Scholar]*

5. WHO. Dengue and Severe dengue. 2023; https://www. who. *int/publications/i/items/9789241547871 [Crossref] [PubMed][Google Scholar]*

6. Halstead, S. B. Global perspective on Dengue research. Dengue Bul 2000; 24:77-82. *[Crossref] [PubMed][Google Scholar]*

7. Sankari T, Hoti SL, Singh TB, Shanmugavel J. Outbreak of dengue virus serotype-2 (DENV-2) of Cambodian origin in Manipur, India - association with meteorological factors. Indian J Med Res. 2012; 136(4):649-655. *[Crossref][PubMed][Google Scholar]*

8. Khan SA, Dutta P, Borah J, Chowdhury P, Doloi PK, Mahanta J. Dengue

Outbreak in an Indo-Myanmar boarder area: epidemiological aspects and risk factors. Trop Biomed. 2013; 30(3):451-458. *[Crossref][PubMed][Google Scholar]*

9. WHO. Dengue haemorrhagic fever: Diagnosis, Treatment, Prevention and Control. 1997; 2nd Edition, Geneva. . *[Crossref][PubMed][Google Scholar] [Crossref][PubMed][Google Scholar]*

10. Anon. Clinical case definition of Dengue/DHF/DSS (Appendix 4. 2); In Information booklet for the Network of Virus Research & Diagnostic Laboratories (VRDLs), Resource Centre, VRDLs, National Institute of Virology, Pune (India). 2019; P-33. *[Crossref][PubMed][Google Scholar]*

11. Bharaj P, Chahar HS, Pandey A, et al. Concurrent infections by all four dengue virus serotypes during an outbreak of dengue in 2006 in Delhi, India. Virol J. 2008; 5:1. *Published 2008 Jan 9. doi:10.1186/1743-422X-5-1 [Crossref][PubMed][Google Scholar]*

12. Sarkar A, Taraphdar D, Chatterjee S. Molecular typing of dengue virus circulating in Kolkata, India in 2010. J Trop Med. 2012; doi:10. *1155/2012/960329 [Crossref][PubMed][Google Scholar]*

13. Chakravarti A, Matlani M, Kashyap B, Kumar A. Awareness of changing trends in epidemiology of dengue fever is essential for epidemiological surveillance. Indian J Med Microbiol. 2012; 30(2):222-226. *doi:10.4103/0255-0857.96699 [Crossref][PubMed][Google Scholar]*

14. Anoop M, Issac A, Mathew T, et al. Genetic characterization of dengue virus serotypes causing concurrent infection in an outbreak in Ernakulam, Kerala, South India. Indian J Exp Biol. 2010; 48(8):849-857. *[Crossref][PubMed][Google Scholar]*

15. Kurukumbi, M. , Wali, J. P. , Broor, S. , *Aggarwal, P., Seth, P., Handa, R., Dhar, L. and Vajapayee, M. Seroepidemiology and active surveillance of dengue fever/dengue haemorrhagic fever in Delhi. Ind J Med Sc 2001; 55(3):149-156 [Crossref][PubMed][Google Scholar]*

16. Scott, T. W. , Naksathit, A. , Day, J. *F., Kittayapong, P. and Edman, J.D. A fitness advantages for Aedes aegypti and the viruses it transmits when females feed only On human blood. Am J Trop Med Hyg 1997; 57 (2):235-239 [Crossref][PubMed][Google Scholar]*

17. Lorono-Pino, M. A. , Cropp, C. B. , *Farfan, J.A., Vorndam, A.V., Rodriguez-Angulo, E.M., Rosado-Paredes, E.P., Flores-Flores, L.F., Beaty, B.J. and Gubler, D.J. Common occurrence of concurrent infections by multiple dengue virus serotypes. Am J Trop Med Hyg 1999; 61(5):725-730 [Crossref][PubMed][Google Scholar]*

18. Ismail, N. A. , Kampon, N. , Mahdy, Z. *A., Jamil, M.A. and Razi, Z.R. Dengue in pregnancy. Southeast Asian J Trop Med PublHlth 2006; 37 (4):681-683 [Crossref][PubMed][Google Scholar]*

19. Waduge, R. , Malavige, G. N. , Pradeepan, M. , *Wijeyaratne, C.N., Fernando, S. and Seneviratne, S.L. Dengue infections during pregnancy: A case series from Sri Lanka and review of the literature. J Clin Virol 2006; 37 (1):27-33 [Crossref][PubMed][Google Scholar]*

20. Adam I, Jumaa AM, Elbashir HM, Karsany MS. Maternal and perinatal outcomes of dengue in Port Sudan, Eastern Sudan. Virol J. 2010; 7:153. *Published 2010 Jul 13. doi:10.1186/1743-422X-7-153 [Crossref][PubMed][Google Scholar]*

21. Pouliot SH, Xiong X, Harville E, et al. Maternal dengue and pregnancy outcomes: a systematic review. Obst Gynaecol Surv. 2010; 65(2):107-118. *doi:10.1097/OGX.0b013e3181cb8fbc [Crossref][PubMed][Google Scholar]*