Original Research Article

A Study on Comparison of Dengue Infection in Two Consecutive Years in a Tertiary Care Hospital

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A B S T R A C T

Dengue viral infection is now recognized as one of the most important mosquito borne human infections of 21st century. About 40% of the world population has been living in areas where there is a risk of Dengue transmission. The epidemics in endemic countries are occurring more frequently with increasing magnitude. Dengue infection is a systemic and dynamic disease. Timely and correct diagnosis is very critical for patient management. This study was conducted among the PUO cases admitted in the Medical and Paediatric Medicine wards at Govt. Thiruvannamalai Medical College. Aim of this work is to study the Seroprevalance of Dengue infection in these cases by subjecting the serum samples with IgM capture ELISA technique, to categorize the Dengue cases as Dengue Fever, Dengue Haemorrhagic Fever and Dengue Shock Syndrome (according to WHO guidelines) and compare between 2 consecutive years. 1176 Adults were tested during 2015 and positives were 184 cases, giving a positivity rate of 15%. 112 children were tested of which 16 were positive, giving a positivity rate of 14% 900 Adults were tested during 2016 and positives were 22 cases, giving a positivity rate of 2.5%. 175 children were tested of which 2 were positive, giving a positivity rate of 1.1%. It was found that more number of cases was noticed from November to February than that of other months. Increased incidence of Dengue was found during monsoon and post monsoon period it was found that among the Dengue positive cases 164 cases (73%) were Dengue fever, 60 cases (27) were Dengue haemorrhagic fever and there was no case with Dengue shock syndrome. As per the present study Dengue cases were more during the month of November to February in the post monsoon season which can be useful to public health authorities to plan special preventive strategies. Dengue fever (72.5%) and Dengue haemorrhagic fever (27.5%) were present and there was no case with Dengue shock syndrome, as per WHO guidelines. Classification helps in knowing the type of infection prevalent in Thiruvannamalai. The Dengue IgM seropositivity among the suspected cases indicates active Dengue virus activity. Increase in the probable secondary infection especially in a country like ours where multiple serotypes are prevalent raises concern over probable increase in the incidence of the more serious DHF/DSS.

Introduction

Dengue viral infection is now recognized as one of the most important mosquito borne human infections of 21st century. According to World Health Organization (WHO) estimates, in the last 50 years its incidence has increased to 30 - fold with geographic expansion to new countries and in the present decade from urban to rural settings.

About 40% of the world population has been living in areas where there is a risk of Dengue transmission.[24] the epidemics in endemic countries are occurring more frequently with increasing magnitude. Dengue infection is a systemic and dynamic disease. Infection with Dengue virus causes spectrum of clinical illness ranging from in apparent infection to mild nonspecific viral syndrome to classical Dengue fever to severe and fatal haemorrhagic disease.

It occurs in tropical areas and affects up to 100 million people each year, including 500,000 cases of Dengue hemorrhagic fever and about 30,000 deaths, mostly among children.[10,25] Dengue fever is a viral disease transmitted by mosquitoes (arthropod-borne) and caused by four serotypes of Dengue virus (DEN-1, DEN-2, DEN-3, and DEN-4). After an incubation period of about 4 to 10 days the illness begins abruptly and is followed by three phases namely febrile, critical and recovery.

Dengue Haemorrhagic Fever is a severe form of Dengue viral infection characterized by sudden onset of fever, usually of 2 to 7 days duration with nonspecific signs and symptoms.

The critical stage of DHF occurs either 24 hours before or 24 hours after the temperature falls to normal or below normal. During this time haemorrhagic manifestations usually occur, and signs of circulatory failure may appear.[22,23] Classical DHF with a capillary leak syndrome that has a unique immuno pathological basis associated with heterologous antibody-dependent enhancement of viral infection of cells of the mononuclear phagocyte lineage. Infection of these cells stimulates the release of vasoactive mediators that apparently causes increased vascular permeability if not promptly detected and corrected, can lead to hypovolemia, shock

and death.[4,16] Dengue virus is an enveloped positive-sense RNA virus.

The genomic RNA is approximately 10.7 kb in length and is composed of three structural protein genes that encode for nucleocapsid or core protein (C), a membrane-associated protein (M), an envelope protein (E) and seven non-structural protein genes including NS1 protein.

NS1 is a highly conserved glycoprotein which appears essential for viral replication. Dengue virus attachment to the host cell surface is mediated by the viral attachment protein (VAP), which seems to be the glycoprotein E present on the viral membrane.[18] Dengue virus can infect its host cells through the binding of virus complexes to the Fc receptor or through the direct interaction of viral proteins with a specific host cell receptor.[12]

The acquired immune response following Dengue infection consists of the production of IgM and IgG antibodies primarily directed against the viral envelope proteins. The immune response varies depending on whether the individual has a primary (first Dengue or other *Flavivirus* infection) or a secondary (had Dengue or other *Flavivirus* infection in past) Dengue infection.[7]

Dengue infection induces a lifelong protective immunity to the homologous serotype only and there is no cross-reactive immunity to the heterologous serotype.

Instead it has been generally accepted that secondary or multiple Dengue virus infection is a major risk factor for Dengue Haemorrhagic Fever / Dengue Shock Syndrome, in addition to other factors, such as viral virulence and host genetic background.[5] Therefore, differentiation of primary versus secondary or multiple Dengue viral infection is essential in analyzing data

for epidemiological, pathological, clinical and immunological studies.[2]

Many factors including unprecedented population growth, increased population density, unplanned and uncontrolled urbanization, increased global travel, increased density of the vector mosquito, infestation of new geographical areas by vector mosquitoes, warm and humid climate and water storage pattern in houses that promote the spread of Dengue virus have all contributed to the remarkably increased incidence of Dengue infection in India during the past few decades.[1,2,22]

Timely and correct diagnosis is very critical for patient management. Dengue can be diagnosed by isolation of the virus, serological tests, or molecular methods.

Diagnosis of acute (on-going) or recent Dengue infection can be established by testing serum samples during the first 5 days of symptoms and/or early convalescent phase (more than 5 days of symptoms).[20]

Acute infections can also be laboratory confirmed by identification of Dengue viral antigen or RNA from the serum of the infected individual / autopsy tissue specimens by immunofluorescence or immunohistochemical analysis, or by seroconversion from negative to positive IgM antibody to Dengue or demonstration of a fourfold or greater increase in IgG antibody titers in paired (acute and convalescent) serum specimens. [8,21]

Aims and objectives

This study was conducted among the PUO cases admitted in the Medical and Paediatric wards at Govt. Thiruvannamalai medical college -To Study the Seroprevalance of Dengue infection in these cases by subjecting

the serum samples with IgM capture ELISA technique, to categorize the Dengue cases as Dengue Fever, Dengue Haemorrhagic Fever and Dengue Shock Syndrome (according to WHO guidelines) and compare between 2consecutive years.

Materials and Methods

Study place

This study was conducted among the PUO cases admitted in the Medical and Paediatric ward at Govt Thiruvannamalai medical college

Study period

The study period was 2 years from January'2014 to December'2016.

Study subjects

The study population includes suspected cases of Dengue Fever with 3-10 days duration.

Study design

Observational study.

Ethical considerations

Written consent to participate in the study was obtained from the subjects or from their guardians after the full explanation of the study. This study was reviewed and approved by Institutional Ethical Committee.

Inclusion criteria

All fever patients suspected for Dengue irrespective of age and sex. Acute febrile illness of 3-10 days duration with two or more of the following symptoms headache, myalgia, arthralgia, Retro orbital pain, rash, haemorrhagic manifestation and Leucopenia.

Exclusion criteria

Febrile cases without the above symptoms, Haemorrhagic conditions without symptoms of Dengue fever and Patients with clinical evidence of Respiratory tract infection/ Urinary tract infections/Injury/Sepsis /Gastro Intestinal Disorders or other apparent causes of fever were excluded.

Sample collection

Blood samples were collected, after getting written consent from the suspected cases of Dengue. 5 ml of whole blood was drawn from the intravenous route with aseptic precautions & transferred to sterile screw caped vials. The caps were fixed with adhesive tape to prevent leakage during transport. The blood samples were transported to the lab immediately in an ice box with proper labeling (name of the patient, identification number and date of collection)

Processing of samples

The samples were taken to the laboratory immediately and they were allowed to clot by placing it in a rack at room temperature (20-25°C) for at least 30 minutes. After that the serum was separated by centrifugation. The serum was then transferred to a sterile vial, and was stored at the required temperature depending upon the usage [Short term storage at +4°C and long term storage at -70°C].

IgM capture ELISA was done for all the suspected samples, at Department of Microbiology, Thiruvannamalai Medical College Thiruvannamalai.

Results and Discussion

This study was carried out among Dengue suspected cases presenting to a Tertiary care hospital (Thiruvannamalai Medical College Hospital) over a period of two years. 1176 Adults were tested during 2015 and positives were 184 cases (Chart 1), giving a positivity rate of 15%.112 children were tested of which 16 were positive, giving a positivity rate of 14% (Chart 2). 900 Adults were tested during 2016 and positives were 22 cases (Chart 3), giving a positivity rate of 2.5%.175 children were tested of which 2 were positive, giving a positivity rate of 1.1% (chart 4).

It was found that more number of cases was noticed from November to February than that of other months. Increased incidence of Dengue was found during monsoon and post monsoon period (Chart 5). It was also noted that there was more number of cases in 2015 as compared to 2016.

In the present study it was found that 137 out of 224(61%) of the affected population were males and 87 out of 224 (39%) were females. (Chart 6)

In the common age group of patient presenting with Dengue infection was 16 to 30 (38.75%) years followed by 31 to 45 (25%) years. Fever (100%) was the most common presenting symptoms followed by myalgia (87%) and headache (68%). Haemorrhagic manifestations were seen in 63 cases (27.5%). The commonest haemorrhagic manifestation seen in Dengue patients were gum bleeding and melena.

All the samples were further analysed according to the clinical manifestation and it was found that among the Dengue positive cases 164 cases (73%) were Dengue fever, 60 cases (27) were Dengue haemorrhagic fever and there was no case with Dengue shock syndrome.

The more cases were found with clinical manifestation of Dengue fever (Table 1).
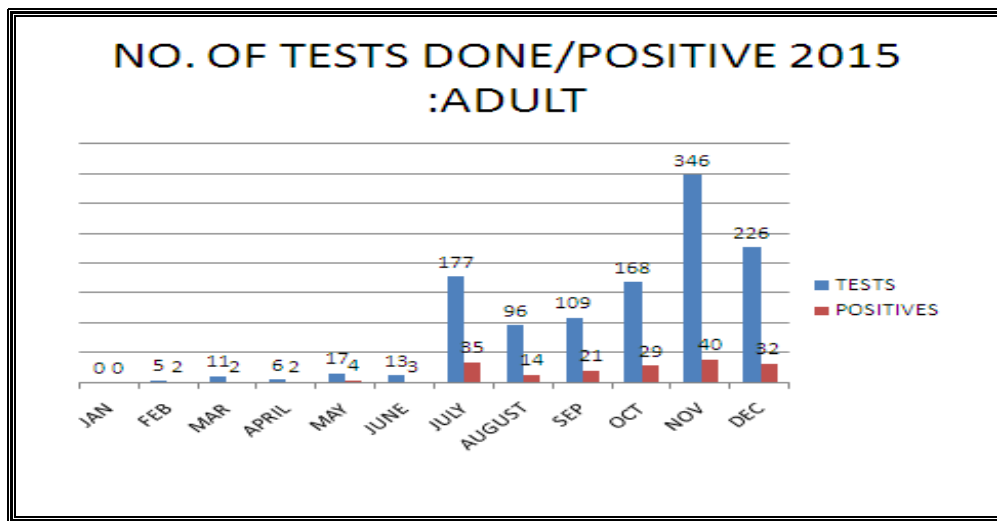
In India the recent outbreak of Dengue infection has caused an alarmingly high morbidity and mortality. Rapid and accurate diagnosis is therefore vital to ensure prompt treatment of those critically ill patients
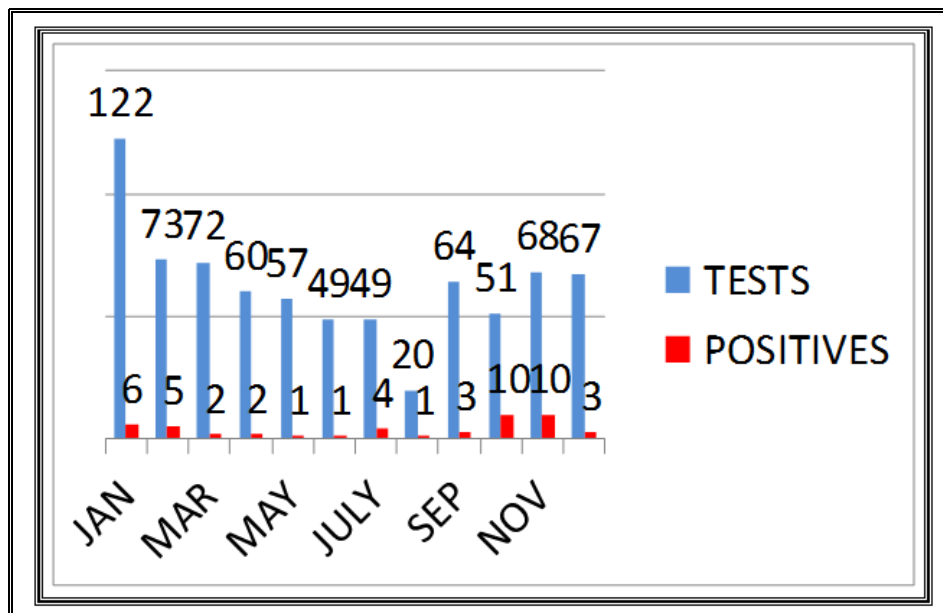
This study was done with serum samples from patients with clinical symptoms suggestive of Dengue in 2016 and 2017. The high prevalence of Dengue cases at

Thiruvannamalai in the recent years makes it necessary to evaluate the sero positivity of Dengue in our hospital. The Dengue infection was most prevalent during post-monsoon season and is more of male preponderance.

The Dengue fever and Dengue Haemorrhagic Fever were identified in Thiruvannamalai and no Dengue Shock Syndrome cases were documented.
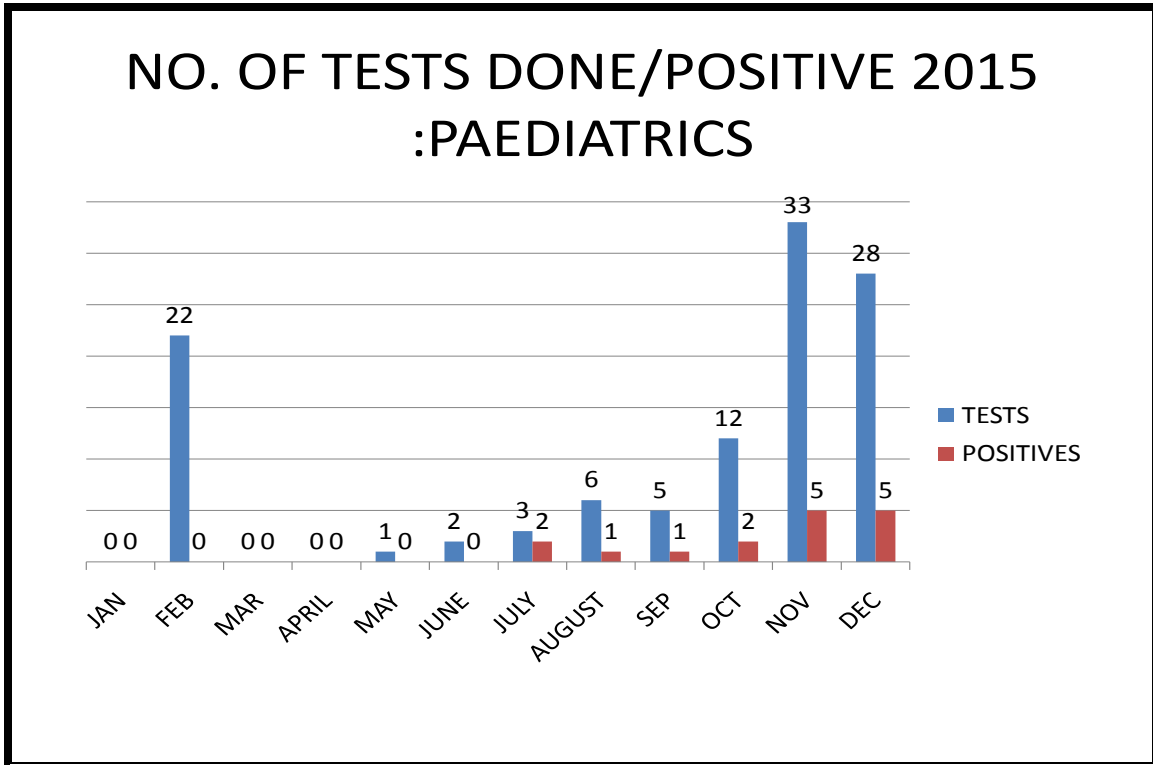
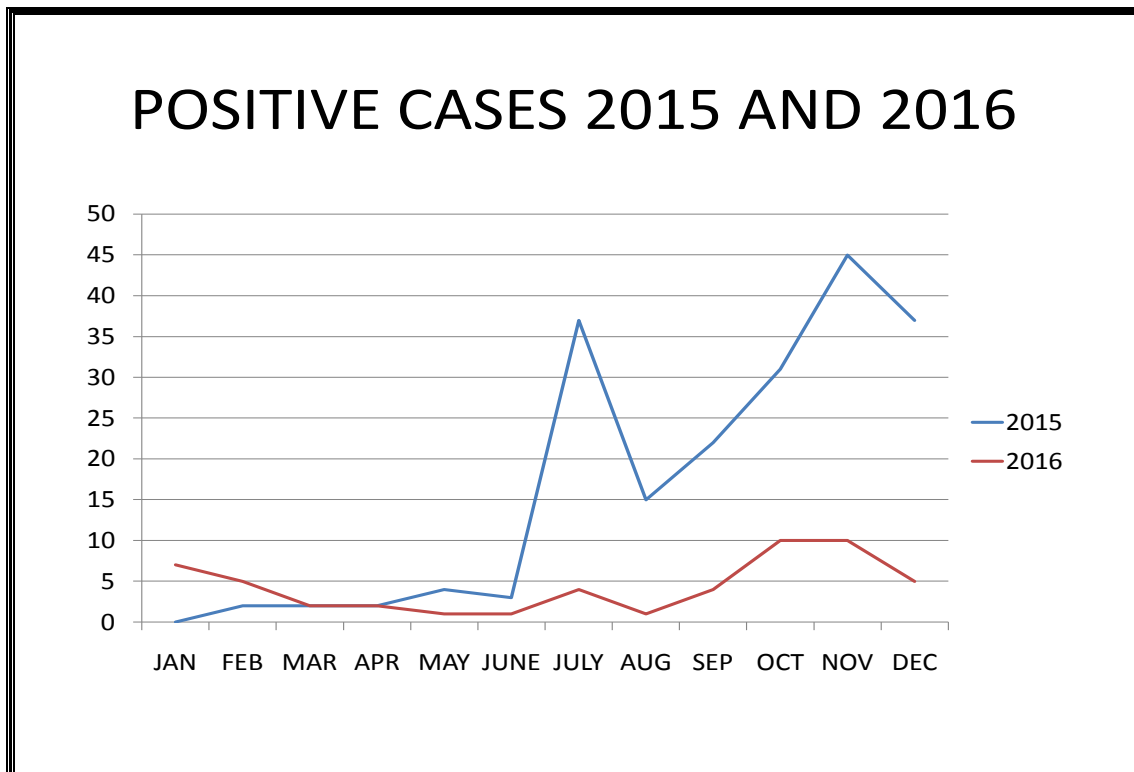**Chart.1** Number of tests done/positive: adult in 2015

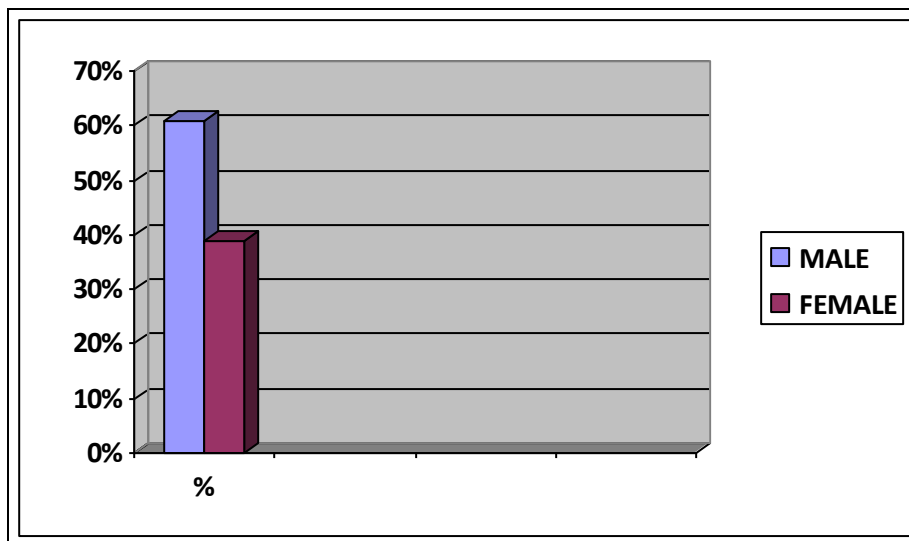**Chart.2** Number of tests done/positives: adult in 2016

Chart.3 Number of tests done/positive: children in 2015

Chart.4 Month wise distribution in 2015 and 2016

Chart.5 Male: Female ratio

Table.1 Classification of dengue cases

CLASSIFICATION	CASES	PERCENTAGE
DENGUE FEVER	164	73%
DENGUE HAEMORRHAGIC FEVER	60	27%
DENGUE SHOCK SYNDROME	0	0%

In this study increased Dengue virus activity was seen during the North East monsoon period (November to January) with peak in the month of December (Chart - 5). The incidence of Dengue was higher following rainfall. Studies by Hati *et al.,*[11] and Gunasekaran *et al.,* [9]also showed that most of the cases had occurred in post monsoon season, which coincides with this study. Another study by John Victor *et al.,*[13], 2007, showed a seasonal distribution which was more during June to December, which is also similar to this study analysis and shows that the prevalence of Dengue infection is more after rainy season. Decreased incidence of cases in the year 2016 may be attributed to very little rainfall in 2017. In our study IgM capture ELISA has proved to the best confirmatory test by eliciting 100% which goes in favour with the study by Karina Billote –Domingo *et al.,* which showed 96% of sensitivity for the ELISA.

In the present study it was found that 137 out of 224 (61%) of the affected population were males and 87 out of 224 (39%) were females

(Chart 6). The common age group of patient presenting with Dengue infection was 16 to 30 years. Ukey *et al.,*[19] study showed male preponderance and 15 to 30 years age group was highly affected with Dengue which is consistent with my study results. The Delhi outbreak in 2003 also presented with the similar statistical data as in this present study.

Emmanuel Bhaskar *et al.,* showed proportion of male and female were equal which differs from the present study results. The male preponderance could be because the men travel more and do more outdoor work hence exposed to the vector more.

In the present study fever (100%) was the most common symptom presenting symptoms followed by myalgia (87%) and headache (68%) (Table 4). In the study conducted by M Emmanuel Bhaskar *et al.,* (2010) showed fever 100% followed by myalgia 68% and headache

67%. This is in coincidence with the present study.

According to WHO guidelines the Dengue cases were classified and in the present study Dengue fever cases were found to be 72.5%, Dengue Hemorrhagic fever cases were 27.5%, and no evidence of any case of Dengue Shock Syndrome (Table 1). A study by Manoj kumar *et al.,*[14], New Delhi showed an incidence of Dengue fever in 75% of cases and Dengue hemorrhagic fever in 21.9% cases which is in coincidence with the present study. Usme - Ciro *et al.,*[15], 2008 study shows 10% of cases were Dengue Hemorrhagic fever / Dengue Shock Syndrome. This discrepancy of results with the present study may be due to different places (Geographical distribution). 2363 samples were further analysed for the confirmation of IgM positivity by IgM capture ELISA technique and it was found that 224(9.4%) cases were positive. In a study by Paramasivan *et al.,*[17] showed 52% positivity for Dengue virus specific IgM antibodies In our study IgM capture ELISA has proved to the best confirmatory test, which goes in favour with the study by Karina Billote –Domingo *et al.,* which showed 96% of sensitivity for the ELISA.

Currently diagnosis by advanced Technology is creating revolution. As per the present study, early detection of Dengue infection can be done by detecting the serology. Dengue cases were more during the month of November to February in the post monsoon season which can be useful to public health authorities to plan special preventive strategies. Dengue fever (72.5%) and Dengue haemorrhagic fever (27.5%) were present and there was no case with Dengue shock syndrome, as per WHO guidelines. Classification helps in knowing the type of infection prevalent in Thiruvannamalai. The Dengue IgM seropositivity among the suspected cases indicates active Dengue virus activity. Increase in the probable secondary infection especially in a country like ours where multiple serotypes are prevalent raises concern over probable increase in the incidence of the more serious DHF/DSS.

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