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Original Article

Clinical and Serological Characteristics of Japanese Encephalitis, Dengue, and Chikungunya in Individuals with Acute Febrile Illness at a Tertiary Care Hospital in the Andaman and Nicobar Islands – Retrospective Study

Malarvizhi M[1], Aishwarya P[2], Dr. Shahina Mustaqim[3], Dr. Ruby Thomas[4], Damodharan P[5], Dr. Dilpreet Kaur Chani[6]
[1-6]Viral Research and Diagnostic Laboratory (VRDL), Department of Microbiology, Andaman and Nicobar Islands Institute of Medical Sciences (ANIIMS), Sri Vijaya Puram, Inda.

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Corresponding Author:

Malarvizhi M
Email id:
malarvizhi07@gmail.com

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ABSTRACT

Background: The early diagnosis and surveillance of acute febrile illness in tropical regions are complicated by the overlapping clinical features of arboviral infections, including Japanese Encephalitis (JE), Dengue, and Chikungunya.
Objective: To identify the distribution, clinical features, and serological profiles of JE, Dengue, and Chikungunya in patients with acute febrile illness via ELISA diagnoses at the Virus Research and Diagnostic Laboratory (VRDL).
Methods: This retrospective hospital study included 194 patients with sudden febrile illness at VRDL in the Andaman and Nicobar Islands. Demographic and clinical data were collected, and serum samples were tested for JE, Dengue, and Chikungunya IgM antibodies using capture ELISA kits; OD values were compared between seropositive and seronegative groups, and associations with clinical features were analyzed using chi-square or Fisher's exact test.
Results: Seropositivity was found in 27 patients (13.9%): Dengue 12 (6.2%), Chikungunya 10 (5.1%), JE 6 (3.1%); dual positives in 3 (JE–Chikungunya 1; JE–Dengue 2). Mean OD values were higher in seropositive versus seronegative samples for all viruses ($p < 0.001$). JE was associated with seizures, altered sensorium, headache, and fever >5 days ($p \leq 0.046$). Chikungunya correlated with myalgia ($p = 0.004$), headache ($p = 0.022$), and arthralgia. Dengue had few predictors, only fever >5 days was significant ($p = 0.040$).
Conclusion: Dengue was the most common arboviral infection, followed by Chikungunya and JE. Neurological features strongly suggest JE, while musculoskeletal symptoms favor Chikungunya.

Keywords: *Japanese Encephalitis, Dengue, Chikungunya, acute febrile illness, arboviral surveillance.*

INTRODUCTION

Acute febrile illness is one of the most common clinical presentations in tropical and subtropical regions and continues to pose a significant diagnostic and public health challenge. Among the infectious causes, viral infections, particularly arboviral diseases, account for a major proportion of undifferentiated fever cases. In India and other Southeast Asian countries, Japanese Encephalitis (JE), Dengue, and Chikungunya are important mosquito-borne viral infections contributing to significant morbidity and occasional mortality (Shanmugam et al., 2022; WHO, 2025; Saady et al., 2025). Dengue is the most widely distributed arboviral infection globally, with a steadily increasing incidence and substantial hospitalization burden in endemic regions (Weaver and Lecuit, 2015). Chikungunya has re-emerged in multiple outbreaks in India, causing prolonged morbidity due to severe arthralgia. Japanese Encephalitis, although less frequent, remains the leading cause of viral encephalitis in Asia and is associated with severe neurological complications and high case fatality rates (Bhatt et al., 2013; Velyutham et al., 2026; Ali, 2026).

These infections have a similar transmission cycle and frequently exhibit similar symptoms like fever, headache, nause a, and myalgia, despite variations in clinical severity. (Simmons et al., 2012; Althouse et al., 2021). This nonspecific clinical presentation makes early differentiation difficult in routine clinical practice. Neurological manifestations may suggest JE, while musculoskeletal symptoms may indicate chikungunya; however, significant clinical overlap is common (Kok et al., 2023).

In resource-limited settings, clinical diagnosis alone may lead to misclassification and delayed management. Therefore, laboratory confirmation is essential for accurate diagnosis and surveillance. The establishment of Virus Research and Diagnostic Laboratories (VRDLs) under the National Viral Disease Surveillance Programme has strengthened diagnostic capacity in India by enabling standardized detection of viral pathogens (ICMR, 2024; Velayudhan et al., 2025).

Available diagnostic modalities include rapid immunochromatographic tests, Enzyme-Linked Immunosorbent Assay (ELISA), and polymerase chain reaction (PCR). While rapid tests provide rapid bedside applicability, they have limited sensitivity. PCR offers high diagnostic accuracy but is limited by cost, infrastructure requirements, and reduced sensitivity in the post-viremic phase. Serological testing may also demonstrate reduced sensitivity during the early viremic phase prior to detectable IgM antibody production (Yang et al., 2004). In contrast, ELISA-based detection of virus-specific IgM antibodies is widely used due to its sensitivity during the acute phase, cost-effectiveness, and scalability. Additionally, ELISA provides semi-quantitative assessment through optical density (OD) values, making it suitable for epidemiological studies. Hence, ELISA remains the preferred diagnostic tool in VRDL settings (Romero et al., 2022; Cheng et al., 2023).

Although individual studies exist for JE, Dengue, and Chikungunya, comparative analyses within a single VRDL-based cohort remain limited. Integrated evaluation of clinical features and laboratory confirmation is essential for improving early diagnosis and strengthening surveillance. In this context, the present study was undertaken to analyze VRDL data from patients with suspected viral febrile illness. The VRDL functions as a referral diagnostic centre for the detection and confirmation of viral infections, including Japanese Encephalitis (JE), Dengue, and Chikungunya. The objectives were to determine the distribution of JE, Dengue, and Chikungunya infections, evaluate the utility of ELISA-based serological testing in differentiating these infections, and assess their clinical associations.

MATERIALS AND METHODS

Study Design and Setting

This hospital-based retrospective study was conducted at a Viral Research and Diagnostic Laboratory (VRDL) established under the scheme of Setting up of Nation-wide Network of Laboratories for Managing Epidemics and National Calamities. The study included patients presenting with sudden febrile illness during the study period.

Study Population

A total of 194 patients of all age groups and both sexes, clinically sudden febrile illness, were included in the study. Patients with incomplete clinical data or inadequate serum samples were excluded from the analysis. The study sample represented all eligible patients presenting during the study period.

Sample Collection and Processing

Peripheral venous blood samples were collected under aseptic precautions from all enrolled patients. Serum was separated by centrifugation and stored under appropriate conditions until serological testing was performed in the VRDL.

Clinical Data Collection

Demographic details such as age and sex were recorded using a structured proforma. Clinical features including fever, chills, rigor, headache, nausea, vomiting, myalgia, arthralgia, abdominal pain, retroorbital pain, and neurological manifestations (seizures, altered sensorium, and neck rigidity) were documented. Some patients exhibited more than one neurological manifestation. Duration of fever was also recorded for all patients.

Laboratory Investigations

Serological Testing by ELISA

Serological diagnosis for Japanese Encephalitis, Dengue, and Chikungunya was performed using commercially available IgM antibody capture ELISA kits approved for VRDL use, following the manufacturer's instructions. These assays were used to detect virus-specific IgM antibodies in patient serum samples, enabling laboratory confirmation of acute infections. The diagnostic panel included JE IgM capture ELISA, Dengue IgM capture ELISA, and Chikungunya IgM capture ELISA. Each test were performed according to standardized protocols recommended by the respective manufacturers- NIV, Pune to ensure consistency and reliability of results.

Optical density (OD) values were recorded using an ELISA reader. Results were interpreted based on predefined kit-specific cut-off values provided by the manufacturer. Based on these criteria, each sample was categorized as either positive or negative for the respective viral infection.

Quality Control and External Quality Assurance
The VRDL participated in External Quality Assurance (EQA) and quality control testing conducted by the Resource Centre for Virus Research and Diagnostic Laboratories, National Institute of Virology (NIV), Pune. ELISA performance for Japanese Encephalitis, Dengue, and Chikungunya IgM assays was periodically assessed using blinded panel samples provided through the VRDL network.

The laboratory demonstrated satisfactory concordance with reference results provided by NIV Pune, supporting the reliability and consistency of ELISA-based serological testing during the study period.

Statistical Analysis
Data were entered into Microsoft Excel and analyzed using Statistical Package for the Social Sciences (SPSS) version 25.0. Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were expressed as frequencies and percentages.

Associations between categorical variables were assessed using Chi-square test or Fisher's exact test where appropriate. Mean OD values between seropositive and seronegative groups were compared using independent sample t-test.
A p-value < 0.05 was considered statistically significant.

RESULTS
Study Population and Demographics
A total of 194 patients with acute febrile illness were included in the study. The study population included 112 males (57.7%) and 82 females (42.3%). The mean age was 32.8 ± 17.6 years, ranging from 1 year to ≥70 years, indicating representation across pediatric, adult, and elderly age groups.
The age distribution showed maximum representation in the 21–40 years age group (35.1%), followed by 41–60 years (22.7%), 11–20 years (16.5%), 0–10 years (14.4%), and >60 years (11.3%) (Table 1).

Table 1. Demographic Characteristics of Study Population (n = 194)

Variable	Frequency/Value
Total patients	194
Male	112 (57.7%)
Female	82 (42.3%)
Mean age ± SD	32.8 ± 17.6 years
Age range	1–≥70 years
0–10 years	28 (14.4%)
11–20 years	32 (16.5%)
21–40 years	68 (35.1%)
41–60 years	44 (22.7%)
>60 years	22 (11.3%)

Clinical Presentation
Fever was the most common presenting symptom, observed in 178 patients (91.8%). Other frequently reported symptoms included nausea (66.5%), headache (60.8%), chills (45.3%), and myalgia (37.1%). Arthralgia (14.4%), Abdominal pain was noted in 21.1% of patients.

Neurological manifestations were less frequent but clinically significant and included altered sensorium (3.6% seizures (2.6%), and neck rigidity (3.1%). Some patients exhibited multiple neurological manifestations.
The mean duration of fever was 4.6 ± 2.8 days (range: 1–20 days), and approximately 33% of patients had fever lasting more than 5 days (Table 2).

Table 2. Distribution of Clinical Symptoms Among Study Participants

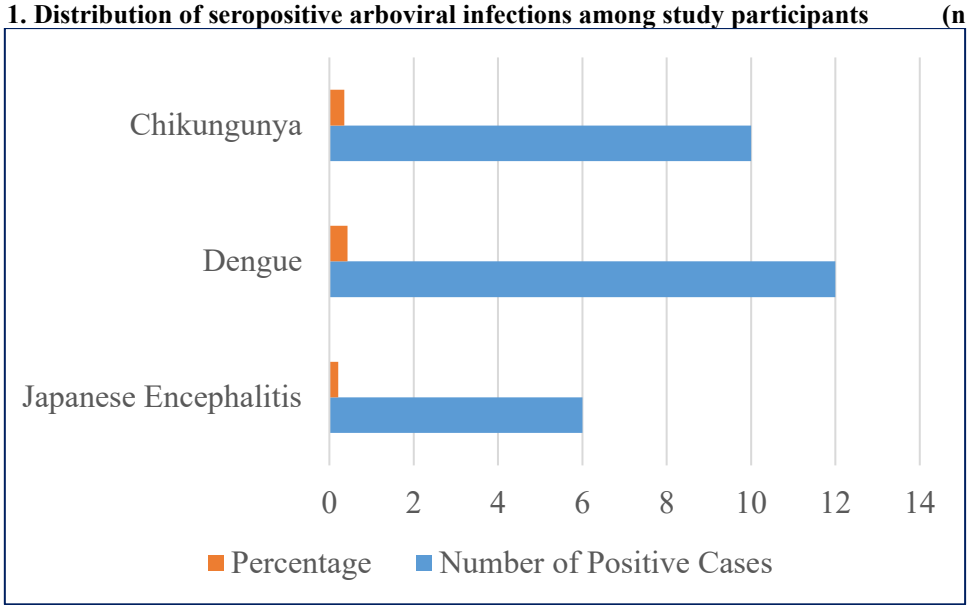
Clinical Feature	Frequency (%)
Fever	178 (91.8%)
Headache	118 (60.8%)
Nausea	129 (66.5%)
Chills	88 (45.3%)

Myalgia	72 (37.1%)
Arthralgia	28 (14.4%)
Abdominal pain	41 (21.1%)
Altered sensorium	7 (3.6%)
Seizures	5 (2.6%)
Neck rigidity	6 (3.1%)

Laboratory Findings

Serological testing revealed positivity for Japanese Encephalitis in 6 patients (3.1%), dengue in 12 patients (6.2%), and chikungunya in 10 patients (5.1%). One patient demonstrated dual seropositivity for JE and chikungunya and two patients confirmed dual seropositivity for JE and Dengue. Thus, a total of 27 patients (13.9%) showed seropositivity for at least one arboviral infection. No triple co-infections were observed (Table 3). The distribution of seropositive arboviral infections is shown in **Figure 1**.

Figure 1. Distribution of seropositive arboviral infections among study participants (n = 194)



All infections demonstrated significantly higher mean optical density (OD) values in positive cases compared to negative cases ($p < 0.001$), indicating clear differentiation between seropositive and seronegative samples.

The VRDL also demonstrated satisfactory performance in external quality assurance assessments conducted by NIV Pune for Dengue, Japanese Encephalitis, and Chikungunya IgM ELISA assays, supporting the reliability of the serological results obtained in the present study.

Table 3. Serological Positivity and Optical Density (OD) Comparison

Infection	Positive n (%)	Negative n (%)	Mean OD Positive ± SD	Mean OD Negative ± SD	p-value
Japanese Encephalitis	6 (3.1%)	188 (96.9%)	0.52 ± 0.05	0.13 ± 0.06	< 0.001
Dengue	12 (6.2%)	182 (93.8%)	0.29 ± 0.10	0.12 ± 0.06	< 0.001
Chikungunya	10 (5.1%)	184 (94.9%)	0.27 ± 0.09	0.12 ± 0.06	< 0.001

Association Between Clinical Features and Japanese Encephalitis

Bivariate analysis demonstrated significant associations between neurological manifestations and JE infection. Headache ($p = 0.03$), seizures ($p < 0.001$), altered sensorium ($p < 0.001$), and fever duration >5 days ($p = 0.046$) were significantly associated with JE positivity, whereas fever itself was not statistically significant ($p = 0.51$).

Neurological manifestations, particularly seizures and altered sensorium, demonstrated the strongest clinical association with JE positivity.

Association Between Clinical Features and Dengue

Dengue infection demonstrated comparatively weaker clinical associations. Most symptoms were not statistically significant. However, fever duration >5 days was significantly associated with dengue positivity ($p = 0.040$).

Association Between Clinical Features and Chikungunya

Chikungunya infection demonstrated a distinct musculoskeletal profile dominated by myalgia. Myalgia ($p = 0.004$), headache ($p = 0.022$) and arthralgia were significantly associated with infection, while fever duration >5 days showed borderline significance ($p = 0.09$).

DISCUSSION

Acute febrile illness remains a major clinical challenge in tropical and subtropical regions because of its diverse etiologies and overlapping clinical manifestations. In the present study, VRDL, ANIIMS evaluated the distribution and clinical correlates of three important arboviral infections—Japanese Encephalitis, Dengue, and Chikungunya—using a VRDL-based diagnostic approach with ELISA confirmation (Galate et al., 2016; Endy, 2020; Deshpande et al., 2023).

In our study, dengue was the most frequently detected infection (6.2%), followed by chikungunya (5.1%) and Japanese Encephalitis (3.1%). This distribution is consistent with epidemiological trends in endemic regions where dengue remains the dominant arboviral infection because of widespread transmission and circulation of multiple serotypes. Chikungunya, though less frequent, continues to re-emerge in periodic outbreaks, while JE remains relatively less common but clinically significant because of its neurological severity (Debnath et al., 2026; Saxena et al.2019; Paixão et al., 2018; Awal and Swu, 2024)).

The clinical profile of patients demonstrated that fever was the predominant symptom, followed by headache, nausea, chills, and myalgia. However, these symptoms were largely nonspecific and overlapped across infections, reinforcing the diagnostic challenge in differentiating arboviral diseases based solely on clinical presentation. Neurological manifestations such as seizures and altered sensorium were strongly associated with JE, consistent with its neurotropic nature and propensity to cause encephalitis (Chaoji et al., 2026; Robert et al., 2025).

Bivariate analysis demonstrated that JE had the strongest association with neurological manifestations, particularly seizures and altered sensorium.

In contrast, dengue infection showed limited distinguishing clinical predictors, with prolonged fever demonstrating statistical significance. This reflects the nonspecific febrile presentation commonly observed in dengue, especially during early illness.

Chikungunya infection demonstrated a distinct musculoskeletal profile, particularly myalgia, which showed significant association with infection. This finding aligns with previous literature describing chikungunya as a debilitating arthralgia-associated illness. Headache also showed a significant association, although less pronounced than myalgia (Imad et al., 2021; de Lima Cavalcanti et al., 2022).

Serologically, ELISA-based testing demonstrated clear differentiation between seropositive and seronegative samples across all three infections, highlighting its utility in confirming acute viral infections and supporting surveillance activities. The reliability of ELISA-based diagnosis in the present study was further supported by satisfactory performance in external quality assurance assessments conducted under the VRDL network by NIV Pune. Regular participation in EQA programmes strengthens diagnostic accuracy, standardization, and surveillance quality across VRDL laboratories (Klumpp-Thomas et al., 202; Faizo et al., 2021; White et al.m 2026).

The detected presence of dual seropositivity might indicate serological cross-reactivity, a sustained antibody response, or actual co-infection, especially since molecular confirmation was lacking.

The use of VRDL-based diagnostic infrastructure in this study highlights its importance in strengthening viral surveillance and improving diagnostic accuracy in endemic regions. Integration of clinical assessment with laboratory confirmation enhances early detection and appropriate disease categorization.

SUMMARY

This study evaluated 194 patients with suspected viral febrile illness using VRDL-based ELISA diagnostics. Dengue was the most common infection, followed by Chikungunya and Japanese Encephalitis. Fever was the predominant presenting symptom, although significant overlap in clinical features was observed across infections. Neurological manifestations were strongly associated with JE, whereas musculoskeletal symptoms were more characteristic of chikungunya. Dengue demonstrated comparatively weaker clinical associations. ELISA-based serological testing demonstrated significant

differentiation between seropositive and sero negative samples, confirming its utility in routine surveillance and laboratory diagnosis.

Significance of this study

The findings of this study will help identify the clinical and serological profile of JEV, DENV, CHIKV in patients with acute febrile illness. Understanding the prevalence of JEV, DENV, CHIKV and their clinical association is essential for making public health policies.

The transmission of JEV in Andaman and Nicobar Islands is possible due to the abundance of JE vectors, especially in Nicobar district where the vector host is contact is present, though not detected/suspected for the past two decades. This study is stresses on the need for increasing serological surveillance of JE in ANI.

This study will provide baseline evidence for surveillance programs especially in JEV including vaccination and other preventive interventions, identify the hotspot, community for implementation of public health measures.

CONCLUSION

Arboviral infections such as Japanese Encephalitis, Dengue, and Chikungunya remain important causes of acute febrile illness with overlapping clinical manifestations that complicate early clinical diagnosis. Japanese Encephalitis showed strong association with neurological manifestations, whereas Chikungunya was predominantly associated with musculoskeletal symptoms. Dengue was the most frequently detected infection but lacked distinctive clinical predictors.

Participation in external quality assurance programmes further strengthens the reliability and standardization of diagnostic testing within the VRDL network. Integration of clinical assessment with increasing standardized serological diagnostics is essential for timely diagnosis, patient management, and strengthening arboviral surveillance in endemic regions.

LIMITATIONS

This study has certain limitations. First, it was a single-centre hospital-based study, which may limit the generalizability of findings to the broader community population. Second, diagnosis was based solely on IgM ELISA, which may not reliably distinguish recent from past infections in certain cases. Third, molecular confirmation using PCR was not performed, which could have improved diagnostic accuracy and reduced the possibility of false-positive serological results. Additionally, the relatively small number of JE-positive cases limited advanced statistical analysis. Finally, longitudinal follow-up was not conducted, restricting assessment of clinical outcomes and disease progression.

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