

MOLECULAR DETECTION OF JAPANESE ENCEPHALITIS VIRUS 5' UNTRANSLATED REGION IN SEROLOGICALLY REACTIVE PAEDIATRIC CASES FROM SOUTHERN CHHATTISGARH, INDIA

Nikita Sherwani¹, Neha Singh^{2*}, Rebeka Ekka³, Sharad Deshmukh⁴, Aparna Sahu⁵, Debashish Samal⁶, Rani Soni⁷

¹Department of Microbiology, Pt. J.N.M. Medical College, Raipur, Chhattisgarh, India. Email: dnikita.sherwani@gmail.com

^{2*}Department of Microbiology, Pt. J.N.M. Medical College, Raipur, Chhattisgarh, India. Email: nehaashishsingh2015@gmail.com

³Department of Microbiology, Pt. J.N.M. Medical College, Raipur, Chhattisgarh, India. Email: eve.cool.breez@gmail.com

⁴Department of Microbiology, Lt. Baliram Kashyap Memorial Government Medical College, Jagdalpur, Chhattisgarh, India. Email: srdeshmukh66@gmail.com

⁵Department of Microbiology, Pt. J.N.M. Medical College, Raipur, Chhattisgarh, India. Email: appysahu16@gmail.com

⁶Department of Microbiology, Lt. Baliram Kashyap Memorial Government Medical College, Jagdalpur, Chhattisgarh, India. Email: debashish15488@gmail.com

⁷Department of Microbiology, Lt. Baliram Kashyap Memorial Government Medical College, Jagdalpur, Chhattisgarh, India. Email: ranisoni.01011990@gmail.com

ABSTRACT

Background: Japanese encephalitis virus (JEV), a mosquito-borne flavivirus, remains an important cause of acute encephalitis syndrome (AES) in India. Molecular detection of viral RNA provides direct evidence of infection and also constitutes the first step toward genomic sequencing and genotype characterization. However, the short and low-level viraemic phase of JEV frequently limits the sensitivity of reverse-transcription polymerase chain reaction (RT-PCR), particularly when specimens are collected after the appearance of virus-specific IgM antibodies.

Objectives: To evaluate molecular detection of the conserved 5' untranslated region (5'UTR) of JEV in serum specimens from pediatric patients reactive for JEV-specific IgM and to describe their demographic, clinical and serological characteristics.

Methods: A descriptive laboratory-based investigation was done on ten pediatric serum specimens obtained between March 6 and July 13, 2026 from patients in the Bastar, Dantewada, and Kondagaon districts of Chhattisgarh, India. All specimens tested positive for JEV-specific IgM using the NIV JEV IgM ELISA kit. Molecular testing for JEV RNA was then performed at Pt. J.N.M. Medical College, Raipur, using the DiAGSure® JEV Detection Kit, which targeted the JEV 5'UTR region. Demographic variables, clinical presentation, AES status, epidemiological history, ELISA optical density values, and RT-PCR results were all analyzed descriptively.

Results: The mean age of the patients was 5.45 years, with a median age of 4.75 years and a range of 3–11 years. Five patients were male and five were female. Six cases originated from Bastar, two from Dantewada and two from Kondagaon. Four patients (40%) fulfilled the recorded AES classification, while six (60%) did not. Convulsions or abnormal body movements were documented in four patients. The mean JEV IgM ELISA optical-density value was 1.50 ± 0.69 , with values ranging from 0.63 to 2.88. Despite IgM reactivity, JEV RNA targeting the 5'UTR was not detected in any of the 10 serum specimens. Thus, the observed molecular detection rate among the IgM-reactive samples was 0%.

Conclusion: JEV-specific IgM reactivity in these pediatric cases was not associated with detectable viral RNA in serum, according to a 5'UTR-targeted RT-PCR assay. The results demonstrate the diagnostic distinction between serological evidence of recent infection and direct molecular detection of circulating virus RNA. Negative serum RT-PCR results should not be used to rule out JEV infection, especially if the samples were acquired after the short viraemic phase. Early acute-phase serum, whole blood (validated), and CSF sample combined with serology may boost molecular yield. To assess the circulating JEV genotypes and genetic diversity in Chhattisgarh, prospective genomic surveillance using sequencing of RT-PCR-positive specimens will be necessary.

KEYWORDS: Japanese encephalitis virus; JEV; 5' untranslated region; 5'UTR; RT-PCR; molecular diagnosis; IgM ELISA; acute encephalitis syndrome; molecular epidemiology; Chhattisgarh

INTRODUCTION

Japanese encephalitis virus is an enveloped, positive-sense single-stranded RNA virus from the Orthoflavivirus genus of the Flaviviridae family. The genome is around 11 kb long and has a single open reading frame bordered by highly organized 5' and 3' untranslated regions. The viral genome encodes three structural proteins—capsid, premembrane/membrane, and envelope—as well as seven nonstructural proteins. The untranslated sections are crucial in viral translation, replication, and genome cyclization, and they contain relatively conserved sequence components that can be used for molecular detection. Japanese encephalitis continues to contribute to AES in India despite longstanding immunization and surveillance programmes. Recent national laboratory surveillance covering 2014–2023 demonstrated continuing JEV activity across India and emphasized the importance of strengthened laboratory diagnosis and pathogen surveillance. Studies from central India have similarly demonstrated continuing childhood encephalitis and JE activity.

The primary laboratory diagnostic of JE is the identification of JEV-specific IgM antibodies in serum or cerebrospinal fluid. In contrast, molecular techniques such as RT-PCR detect the viral genome directly, making them very specific when positive. However, viral RNA is frequently found in human blood in trace amounts. The World Health Organization surveillance guidance states that JEV genome detection in serum, plasma, or blood has poor sensitivity in clinically evident JE and that a negative molecular result should not be used to rule out the diagnosis.

JEV demonstrates significant genetic variety, therefore molecular surveillance is especially important. Five primary genotypes (GI-GV) have been described predominantly using envelope-gene nucleotide sequences. Genotype III has historically been predominant in India, although genotype I is becoming more common in other regions. A review of Indian molecular epidemiology highlighted the circulation and spatial alterations of GI and GIII, emphasizing the need for ongoing genomic surveillance. More recently, complete-genome sequencing of JEV from pigs and mosquito vectors in Assam revealed widespread genotype III viruses with detectable nucleotide polymorphisms. There is relatively little data on molecular JEV detection in the tribal-dominated districts of southern Chhattisgarh. The current work analyzed JEV RNA detection targeting the conserved 5'UTR among pediatric patients with JEV IgM reactivity and investigated the relationship between serological positive, clinical features, and molecular findings.

MATERIALS AND METHODS

Study design and period

This was a descriptive, laboratory-based observational study of JEV IgM-reactive paediatric specimens collected between March 6 and July 13, 2026.

Serological tests were conducted at the Late Baliram Kashyap Memorial Government Medical College in Dimrapal, Jagdalpur, Chhattisgarh. The specimens were then transferred under controlled cold-chain conditions at 2-8°C to Pt. J.N.M. Medical College in Raipur, Chhattisgarh, for RT-PCR-based molecular testing.

Study population

The study comprised ten paediatric patients whose serum specimens tested positive for JEV-specific IgM. Demographic, geographical, clinical, epidemiological, and laboratory data were collected from the laboratory dataset. Variables included age, gender, district, presenting clinical characteristics, documented AES status, existence of similar illness in the household/village/locality, history of travel within the previous 10 days, sample type, JEV IgM result, ELISA optical-density value, and JEV RT-PCR result.

Serological investigation

Serum specimens were analyzed for JEV-specific IgM using the NIV JEV IgM ELISA Kit, following the manufacturer's testing and interpretation instructions.

Individual optical-density measurements were maintained for descriptive analysis. Because the dataset did not include individual plate cut-off calculations or positive-to-negative ratios, absolute OD values were not interpreted as quantitative estimates of antibody concentration.

Molecular detection of JEV RNA

Serum samples were sent for molecular analysis and analyzed with the DiAGSure® JEV (Japanese Encephalitis Virus) Detection Kit. The molecular assay focused on the 5' untranslated region of the JEV RNA genome. Viral RNA amplification was assessed using the manufacturer's criteria and reported qualitatively as positive or negative. None of the specimens yielded detectable JEV RNA, therefore sequencing, nucleotide alignment, phylogenetic analysis, and genotype assignment were not possible.

Statistical analysis

The data were analysed descriptively. Continuous variables are presented as mean, standard deviation, median and range where appropriate. Categorical variables are presented as frequencies and percentages. Given the small sample size and exploratory nature of the study, formal inferential statistical comparisons were not considered appropriate.

RESULTS

Demographic characteristics

A total of ten JEV IgM-reactive pediatric cases were studied. The average age was 5.45 years, with a median of 4.75 years and a range of 3 to 11 years. Five patients (50%) were male and five (50%) were female. Geographically, six patients (60%) came from the Bastar district, two (20%) from Dantewada, and two (20%) from Kondagaon.

Table 1. Demographic and laboratory characteristics of the study population

Characteristic	Finding
Total patients	10
Mean age	5.45 years
Median age	4.75 years
Age range	3–11 years
Male	5 (50%)
Female	5 (50%)
Bastar district	6 (60%)
Dantewada district	2 (20%)

Characteristic	Finding
Kondagaon district	2 (20%)
Recorded AES status – Yes	4 (40%)
Similar illness in locality/household	2 (20%)
Travel during preceding 10 days	0
JEV IgM reactive	10 (100%)
JEV 5'UTR RT-PCR positive	0 (0%)
JEV 5'UTR RT-PCR negative	10 (100%)

Clinical profile

Fever was the most often documented systemic symptom. Convulsions and aberrant body movements were among the neurological signs.

Four patients (40%) obtained an AES designation in their study records. Four children had convulsions or odd body movements, and additional symptoms mentioned included vomiting, cold and cough, abdominal pain, back pain, and loss of appetite.

Two patients claimed a history of identical illness in their household, village, or neighborhood. There was no record of any travel in the previous ten days.

JEV IgM ELISA findings

All 10 specimens tested positive/reactive for JEV-specific IgM.

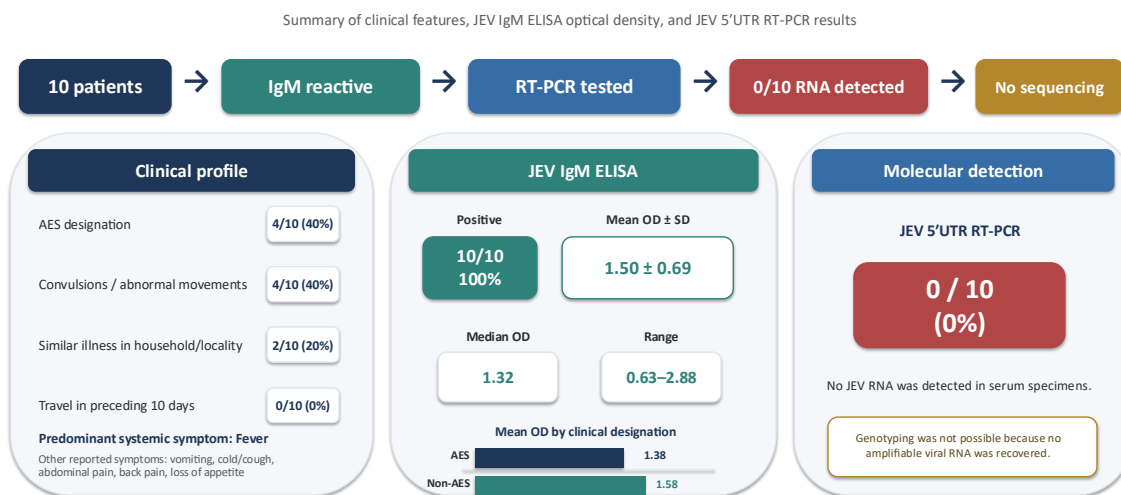
The average ELISA OD value was 1.50 ± 0.69 , with a median of 1.32 and a range of 0.63–2.88. The mean OD for the four AES cases was roughly 1.38, whereas the remaining six patients had a mean OD of about 1.58. Because of the small sample size and the fact that raw OD values are not standardized quantitative antibody titres, no inferential comparison was made.

Molecular detection of the JEV 5'UTR

All ten serum specimens had negative RT-PCR results for the JEV 5' untranslated genomic region. Thus, the molecular positivity among the JEV IgM-reactive specimens was: 0/10 (0%).

No specimen was available for further sequence-based genetic characterization because genotype identification necessitates the recovery of viral RNA adequate for amplification and sequencing.

Clinical, Serological and Molecular Findings in JEV IgM-Reactive Patients (n=10)



Note: Raw ELISA OD values are not standardized quantitative antibody titres; therefore, no inferential comparison was performed between AES and non-AES groups.

Figure 1. Clinical, serological, and molecular findings among JEV-suspected cases. The figure summarizes the clinical profile of the study participants together with JEV IgM ELISA and RT-PCR results. All 10 serum specimens were reactive for JEV-specific IgM, whereas RT-PCR targeting the JEV 5' untranslated region was negative in all specimens.

Table 2. Individual serological and molecular findings

Case	Age/Sex	District	AES	IgM ELISA OD	JEV RT-PCR, 5'UTR
1	7 y/M	Bastar	No	2.3150	Negative
2	4 y/M	Dantewada	Yes	1.9007	Negative
3	4 y/F	Bastar	Yes	0.9577	Negative
4	5 y/M	Kondagaon	Yes	1.1924	Negative
5	8 y/F	Bastar	No	2.8800	Negative

Case	Age/Sex	District	AES	IgM ELISA OD	JEV RT-PCR, 5'UTR
6	4.5 y/M	Kondagaon	No	0.6300	Negative
7	3 y/F	Bastar	No	1.1700	Negative
8	3 y/F	Bastar	No	0.9100	Negative
9	5 y/M	Dantewada	Yes	1.4512	Negative
10	11 y/F	Bastar	No	1.5934	Negative

DISCUSSION

This study found a distinct difference between serological and molecular indicators of JEV infection in a small group of pediatric patients from southern Chhattisgarh. Although all ten serum specimens tested positive for JEV-specific IgM, none yielded detectable JEV RNA when analyzed using RT-PCR targeting the 5'UTR.

This observation should not be taken as indicating that the serological results were always false-positive. Rather, it demonstrates a crucial aspect of JEV pathogenesis and laboratory diagnosis: in humans, viraemia is typically modest and temporary, but the antibody response becomes detectable as the illness progresses. WHO surveillance recommendations expressly acknowledge the poor sensitivity of genome detection in blood and emphasize that negative PCR or virus isolation results do not rule out JE in a clinically suspected patient.

As a result, the timing of specimen collection is anticipated to play an important role in determining molecular yield. Several children in the current dataset had been sick for multiple days, one had a two-week history, and another had symptoms for around a month. By the time JEV IgM is readily identifiable, circulating viral RNA may have dropped below the analytical detection limit. The lack of precise onset-to-blood-collection intervals for each case precluded a clear assessment of this association.

The observation is also consistent with the wider diagnostic literature emphasizing the complementary roles of molecular and serological methods. Modern diagnostic approaches to JE include RT-PCR, IgM capture ELISA, virus isolation, neutralization assays and emerging molecular platforms, but no single assay provides optimal sensitivity throughout the entire course of disease.

The prevalence of young children in the current dataset is epidemiologically significant. National and regional Indian studies continue to show a significant burden of JE and other infectious causes of AES in pediatric populations. National VRDL network data from 2014 to 2023 indicated consistent JEV detection across India, emphasizing the importance of ongoing diagnostic surveillance.

The geographic distribution is also notable. The majority of the cases in the current dataset were from Bastar, with others from Dantewada and Kondagaon. These districts have large rural, woodland, and agricultural landscapes that can support mosquito-vertebrate transmission ecosystems. However, the current dataset excluded vector density, pig exposure, vaccination status, rice-field proximity, and environmental variables. As a result, these data alone cannot be used to draw conclusions about ecological risk.

From a molecular-genetic standpoint, the 5'UTR is an appealing amplification target because it contains functionally significant and conserved nucleotide sequences involved in viral RNA replication and translation. Nonetheless, detection of a conserved genetic target differs from genotyping. JEV genotypes are often determined using sequence data, notably from the envelope gene or broader genomic areas.

Recent Indian research show why sequence-based surveillance is vital. Datey and colleagues studied JEV molecular epidemiology in pigs from Odisha, Assam, and Manipur and discovered that genotype III was the most common, as well as specific amino-acid changes in envelope sequences. Pegu and colleagues subsequently sequenced the genomes of JEV from pigs and mosquitos in Assam, identifying genotype III viruses with unique nucleotide polymorphisms. Indian molecular epidemiological assessments have also highlighted the spread of GI and GIII, as well as the regional worry over genotype alterations.

More recent vector surveillance has provided evidence for genotype I circulation in Assam, further illustrating the dynamic molecular epidemiology of JEV in India. Such findings make prospective sequencing from central and eastern Indian regions, including Chhattisgarh, particularly important.

An important practical relevance of the current findings is that molecular investigations should ideally focus on materials collected as soon as feasible following symptom onset. Collection of carefully handled CSF and associated serum specimens may improve laboratory interpretation. If JEV RNA is identified, the envelope gene should be amplified or whole-genome sequenced to determine genotype, nucleotide alterations, amino-acid variations, and phylogenetic links with Indian and neighboring Asian JEV strains.

The present data therefore provide a useful pilot framework for a larger molecular surveillance programme for JEV in Chhattisgarh rather than evidence for a specific circulating genotype.

Molecular-Genetic Implications

A future extension of this study should include early acute-phase sampling as well as sequence-based characterisation. RT-PCR-positive specimens could be amplified for the whole envelope gene, followed by Sanger sequencing or, if viral load allows, whole-genome next-generation sequencing. The sequence readings could then be aligned with GenBank's representative JEV GI-GV reference genomes. Phylogenetic reconstruction could reveal the relationships between Chhattisgarh strains and previously described Indian GI and GIII lineages. Comparative study could also reveal nucleotide polymorphisms and non-synonymous amino acid alterations in the envelope and non-structural proteins.

This strategy is particularly relevant because genomic studies from India have demonstrated ongoing JEV genetic evolution and persistence of GIII as well as expansion of GI.

LIMITATIONS

The primary limitations were the limited sample size, retrospective use of available laboratory data, and the lack of precise test collection time for all patients. Only serum specimens were provided; no matched CSF specimens were included. Viral load and Ct values were unavailable since all RT-PCR results were negative. Vaccination history and thorough epidemiological exposure information were also missing.

Most crucially, the lack of PCR-positive samples precluded viral sequencing, phylogenetic reconstruction, and JEV genotype identification. As a result, this study should not be interpreted as evidence for the prevalence of any specific JEV genotype in Chhattisgarh.

Raw ELISA OD values were available, but plate-specific cut-off information was not included in the dataset. Consequently, OD values were used only for descriptive purposes and should not be considered quantitative antibody titres.

CONCLUSION

Pediatric JEV IgM-reactive cases from Bastar, Dantewada and Kondagaon districts demonstrated no detectable JEV RNA in serum by an RT-PCR assay targeting the 5' untranslated region.

The data support the complementary, rather than interchangeable, roles of serological and molecular testing in Japanese encephalitis. When serum RT-PCR is used after the transient viraemic phase, it has limited efficacy to exclude JE, whereas IgM testing demonstrates the emerging humoral immune response.

Molecular epidemiology surveillance requires early specimen collection and prospective testing. RT-PCR-positive samples should be subjected to envelope-gene sequencing or whole-genome sequencing to determine circulating JEV genotypes, genetic substitutions, and phylogenetic connections in Chhattisgarh.

DECLARATIONS

Ethical approval: No./MC/Ethics/2024/180 Raipur, dated 21/12/25

Consent to participate: Yes

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Data availability: The data supporting the findings are available from the corresponding author on reasonable request, subject to institutional and ethical requirements.

Authors' Contributions: Nikita Sherwani: Conceptualization, supervision, study oversight, methodology, review and editing of the manuscript. Neha Singh: Conceptualization, methodology, molecular investigation, data interpretation, supervision, coordination between participating centres, manuscript preparation, review and editing, and corresponding author responsibilities. Rebeka: Data collection, clinical and laboratory data compilation, literature review, and assistance in sample collection. Aparna Sahu: Molecular laboratory work, data entry and compilation, laboratory coordination, and assistance in data analysis. Sharad Deshmukh: Supervision of serological testing, clinical and laboratory coordination at the Jagdalpur study site, and critical review of the manuscript. Debashish Samal: Serological investigation, data collection and verification, study-site coordination, and critical review of the manuscript. Rani Soni: Serological laboratory work, specimen processing and documentation, data compilation, and review of the manuscript. All authors contributed to the interpretation of the findings, reviewed the final manuscript, and approved the version submitted for publication.

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