RESEARCH PAPER

Public Health Laboratory Surveillance and Diagnosis of Japanese Encephalitis: Time to Revisit

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Objective: We assessed detection of recent Japanese encephalitis virus infection using recommended strategy.

Methods: Cross-sectional community-based study conducted in 12 villages in Kushinagar, Uttar-Pradesh, India in 2012-13. Recent infection with Japanese encephalitis virus in 239 healthy children aged 1-15 year was detected using a combination of serology and molecular methods.

Results: 24 (10%) children showed recent infection; 2 by serology and 22 by molecular method. Symptomatic cases were estimated as 626 in Kushinagar against reported 139 in all age groups across the state.

Conclusion: Lower positivity using recommended serology suggests major gap in existing surveillance and diagnostic protocols and estimation of burden of Japanese encephalitis.

Keywords: Japanese encephalitis, Laboratory diagnosis, Surveillance.

The diagnosis of Japanese encephalitis (JE) is based on single positive IgM enzyme-linked immunosorbent assay (ELISA) in cerebrospinal fluid (CSF) or serum of suspected cases of Acute encephalitis syndrome (AES). Isolation of JE virus or detection of viral RNA from CSF and serum is not recommended for diagnosis and surveillance, given the transient presence and low yield [1-3]. In recent years, JE has expanded to new areas but reportedly declined as a proportion of AES cases [4]. This has been attributed to public health interventions, particularly JE vaccination. Arguments for impact of JE vaccination are rarely backed by effective vaccination strategies or credible evidence on vaccination coverage [5]. Further, analyses of performance characteristics of available JE diagnostics show limitations, especially in test sensitivity and specificity [6]. A pressing public health question then is whether the apparent decline in incidence of JE is real or an artefact of the inability of current diagnostics to detect true cases of JE.

The objective of this analysis was to compare the ability of serology and molecular methods to detect acute JE infection (asymptomatic and symptomatic).

METHODS

This study reports on the analysis of data collected for a larger study (EcoHealth) to identify sources, pathways and drivers that influence JE transmission in Kushinagar, a high endemic Northern Indian district in 2012-13. A cross-sectional descriptive study was conducted in Kushinagar between July 2012 and February 2013. JE specific IgM antibody prevalence has been reported to be around 10% in South Indian children [7]. Kushinagar being high endemic, we assumed this as 15%. Minimal sample size for random sample at 95% confidence level, with absolute precision of 5%, was computed as 196 children.

Multistage sampling was used to identify study villages. Based on retrospective analysis of JE/AES incidence, data blocks were stratified into high, medium and low burden tertiles of endemicity. One block was selected randomly from each strata followed by random selection of four villages – two with and two without pigs, from each block, totalling 12 villages.

Using systematic random sampling, 5% village households were sampled. Whole blood and serum was collected from all healthy children aged 1-15 years in sample households. Samples were screened for JE virus-specific IgM using antibody capture ELISA (IgM MAC ELISA) [8]. Samples negative for anti-JEV IgM were tested for JE virus RNA by real time reverse transcriptase Polymerase chain reaction (rRT-PCR) in peripheral blood mononuclear cells [9]. To rule out post-vaccination IgM antibodies, children vaccinated against JE in last three months were excluded. Total symptomatic cases of JE

during 2012 transmission season were estimated by applying symptomatic: asymptomatic ratio of 1:200, as reported earlier for India [7].

Samples were stored and tested at Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow. Ethical clearance from PHFI's Institutional Ethics Committee and Health Ministry's Screening Committee was received, and written informed consent was obtained from adult respondents.

RESULTS

Out of 239 healthy children from 12 villages, 24 (10%; 19 males) showed evidence of recent infection. Out of 24 children, only two were positive for recent infection by IgM MAC-ELISA, and rest by rRT-PCR. Children from all age groups (***Table* I**), study blocks and in 9/12 (75%) villages tested positive for recent infection with JE virus. The number of symptomatic JE cases in 2012 in Kushinagar was estimated at 626 (assuming above 10% positivity in children 1-15 years of age as representative of recent JE virus infection activity, 125,155 asymptomatic cases in a projected population of 1,246,573 children under 15 and symptomatic: asymptomatic ratio of 1:200).

DISCUSSION

In this study conducted in a high endemicity district, we observed low positivity for recent JE infection using IgM MAC-ELISA. This is consistent with recent assessment of currently available ELISA tests [6,10]. Since the study focused on healthy children in contrast to earlier assessments of only AES cases, higher prevalence rate was expected, given the large number of expected asymptomatics for every symptomatic case. Lower prevalence rates obtained were therefore likely underestimates of infection load due to low test-sensitivity, especially among unvaccinated younger cohorts where previous exposure and protection was less likely.

TABLE I AGE-WISE DETECTION OF RECENT INFECTION WITH JAPANESE ENCEPHALITIS VIRUS BY TWO METHODS

Age	*No.*	*PCR*	*ELISA*
1	2	0	0
2	9	2	0
3	11	3	0
4	19	0	0
5	22	0	0
6	19	2	0
7	25	2	1
8	24	3	0
9	14	0	0
10	25	3	0
11	14	3	0
12	19	0	0
13	16	1	0
14	19	3	0
15	1	0	1
Total	239	22	2

Majority of recent JE virus infections in the study were detected by rRT-PCR on peripheral blood mononuclear cells. Presence of JE virus RNA in these cells has been identified as marker of recent infection and latent infection that could be a source of transmission and clinical disease in ensuing weeks or months [11,12]. Clinical and public health significance of this phenomenon needs further enquiry. Higher positivity for recent infection revealed by molecular methods compared to recommended strategy of serological tests also calls for revisiting existing surveillance and diagnostic protocols. Although PCR may not be a feasible option, especially in field conditions, its planned deployment needs consideration. This is important to help accurately estimate true burden [13], especially in context of circulation of newer genotypes and their potential impact on antigenic confirmation that could affect sensitivity of currently available serodiagnostics [14].

We estimated 626 symptomatic JE cases in Kushinagar in 2012, while the state of UP reported 139 confirmed cases during the same transmission season. Nationally, number of confirmed JE cases (reported) stood at 745 [15]. This extrapolation needs further validation through wider studies. It highlights potentially large undiagnosed and/or unreported burden of JE, questioning the recently reported declining trend of JE as a cause of AES in Gorakhpur region [4,5].

Findings from the study highlight the possibility of a large gap in estimation and understanding of JE burden in Northern India. The inadequately sensitive diagnostics currently in use needs urgent attention, along with revisiting of recommended laboratory diagnosis and surveillance strategy. In the absence of true estimates and presence of alternate etiology narratives, public health programs run the risk of being sub-optimally informed, or at times, misinformed.

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Contributors: MK and TND: Conception and design, acquisition of data, analysis and interpretation of data, drafting the manuscript; ETR: acquisition of data, analysis and interpretation of data, drafting the manuscript; SC: conception and design, analysis and interpretation of data, drafting the manuscript.

WHAT THIS STUDY ADDS?

- Serology (IgM-MAC-ELISA) underestimates recent infection with Japanese encephalitis virus, in comparison to real time reverse transcriptase PCR.

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