

Letter to Editor

Asian Pacific Journal of Tropical Medicine

apjtm.org

doi: 10.4103/apjtm.apjtm_157_25

Waning neutralizing antibodies among vaccinated children in Japanese encephalitis endemic region

Hina Reem MS[1#], Neha Srivastava[1#], Ashok Kumar Pandey[1✉], Rohit Beniwal[1], Umaer Alam[1], Aman Agarwal[1], Vijay Kumar Srivastava[2]

[1]*ICMR–Regional Medical Research Centre, Gorakhpur, Uttar Pradesh, India*

[2]*Office of Additional Director of Health, Gorakhpur Division, Uttar Pradesh, India*

Japanese encephalitis (JE), a vector-borne disease caused by the Japanese encephalitis virus (JEV), remains a major public health concern in South and Southeast Asia[1]. JEV, a Flaviviridae family virus, is primarily transmitted by *Culex* mosquitoes, especially the *Culex vishnui* subgroup, which breeds extensively in rice fields[1]. Birds (Ardeidae family) act as natural reservoirs, while pigs serve as amplifying hosts, and humans are incidental hosts[1].

In India, JE was first reported in 1955, with Uttar Pradesh emerging as one of the most affected states[2]. Recurrent outbreaks since 1978 and massive outbreak of 2005 highlighted the urgent need of combating this disease[2], leading to introduction of mass immunization in 2006 and the integration of JE vaccines into the Universal Immunization Programme in 2014[2]. Additionally, initiatives such as enhanced surveillance, vector control, and public awareness campaigns like DASTAK (knock the door) have helped curb JE outbreaks[3], though waning vaccine-induced immunity remains a challenge.

Vaccination remains the cornerstone of JE prevention. India uses two vaccines—SA-14-14-2, a live attenuated vaccine introduced in 2006 with 94.5% efficacy, and JENVAC, an inactivated Vero cell-derived vaccine introduced in 2020[3]. While JENVAC, shows promise, its long-term efficacy and booster dose requirements remain to be evaluated[3].

During January-April 2024, Maharajganj district of eastern Uttar Pradesh, which shares the border with Nepal (other JE endemic country) reported 17 JE seropositive (anti-JE IgM positive in ELISA) cases. The cases were presented with fever (100%) with an onset of 4 (3-7) days [median (IQR)], seizures (46.2%), headache (38.5%) and vomiting (30.8%). Most cases were under 5 years of age (69.2%), median age of cases was 5 (3-6.5) years [median (IQR)] and 69.2% were males.

What made this outbreak unusual was its occurrence during the non-transmission season for JE. Additionally, the district practices biannual rice cultivation, unlike other parts of eastern Uttar Pradesh. This unique agricultural practice may sustain a high density of *Culex tritaeniorhynchus*, the primary vector for JE, contributing to continued transmission risk. All 17 cases were vaccinated with Chinese vaccine SA-14-14-2 as per district health record and vaccination card was verified at time of home visit. We investigated all cases for anti-JE antibodies, entomological positivity of virus and presence of other risk factors associated with JE transmission.

We recruited JE seropositive children and their guardians in the study after taking written informed consent. Guardian of 13 JE seropositive cases given consent to participate in the study. The study received ethical approval from the Institutional Human Ethics Committee (IHEC) of ICMR–Regional Medical Research Centre on 30 April 2025, Gorakhpur (No.RMRCGKP/EC/2024-25/6a.1). Blood samples were collected from 13 JE seropositive cases as well as their siblings. The siblings from the house of JE cases were included as controls to minimize the external confounding factors such as exposure to mosquitoes, living conditions and genetic predisposition. A total of 26 blood samples (13 from JE positive

[#]These authors contributed equally to the work.
[✉]To whom correspondence may be addressed. E-mail: ashokpandey10@gmail.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2025 *Asian Pacific Journal of Tropical Medicine* Produced by Wolters Kluwer-Medknow.

How to cite this article: MS Reem H, Srivastava N, Pandey AK, Beniwal R, Alam U, Agarwal A, et al. Waning neutralizing antibodies among vaccinated children in Japanese encephalitis endemic region. Asian Pac J Trop Med 2025; 18(8): 376-379.

Article history: Received 17 March 2025 Revision 5 May 2025
Accepted 21 May 2025 Available online 25 June 2025

cases and 13 from their siblings) were collected and tested for JE virus-specific antibodies using NIV JE IgM Capture ELISA Kit (Pune) and InBios JE IgG Capture ELISA kit (InBios International Inc., USA), following the manufacturer's instructions. The socio-demographic, epidemiological, and clinical information was recorded on standard case record proforma.

For entomological investigation, a total of 570 *Culex* mosquitoes were collected from residences of the JE case using handheld aspirators and identified using morphological keys. Mosquitoes were pooled in 19 pools for JE virus screening *via* PCR analysis. RNA extraction was done using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). For RT-PCR amplification, the Promega Access RT-PCR kit was used. The primers targeting the PrM/M region of the JEV genome were FLV For-1 (ATNACRCARTCRTCTCCRCT) and FLV Rev-2 (AGYGGAGAYGAYTGYGTNGT), producing a 140 bp amplicon was used. PCR cycling conditions included a reverse transcription step at 50 ℃ for 30 min, followed by initial denaturation at 94 ℃ for 3 min. Thirty amplification cycles were carried out with denaturation at 94 ℃ for 30 sec, annealing at 54 ℃ for 30 sec, and extension at 68 ℃ for 1 minute, with a final extension at 68 ℃ for 7 min. Amplified products were analysed on a 1.5% agarose gel stained with ethidium bromide.

The socio-demographic, clinical, epidemiological and laboratory testing data of cases and controls are presented in Table 1. 26 samples were tested for anti-JE IgM and IgG using ELISA. None of the samples tested positive for anti-JE IgM, while 2/26 (7.7%) cases and 4/26 (15.4%) samples from siblings were equivocal (borderline

Table 1. Socio-demographic, clinical, epidemiological characteristics of cases and controls recruited during Maharajganj Japanese encephalitis outbreak [*n* (%)].

Characteristics	Cases (*n*=13)	Controls (*n*=13)
Age group, years		
0-5	9 (69.2)	4 (30.8)
6-11	2 (15.4)	6 (46.2)
12-18	2 (15.4)	3 (23.1)
Age*, years	5 (3-6.5)	8 (3-12)
Sex		
Male	9 (69.2)	4 (30.8)
Female	4 (30.8)	9 (69.2)
Referral	13 (100.0)	-
Referred by		-
Registered Medical Practitioner	0 (0.0)	-
Government hospital	13 (100.0)	-
Quack	0 (0.0)	-
Mode of transport		
Bike	9 (69.2)	
Walk	2 (15.4)	-
Ambulance	1 (7.7)	
Auto	1 (7.7)	
Animal exposure	13 (100.0)	13 (100.0)
Pigs	1 (7.7)	2 (15.4)
Cattle	9 (69.2)	8 (61.5)
Cats	13 (100.0)	13 (100.0)
Other animals	13 (100.0)	13 (100.0)
Rodents inside house	13 (100.0)	13 (100.0)
Rearing animals	9 (69.2)	9 (69.2)
Pigs	0 (0.0)	0 (0.0)
Poultry	0 (0.0)	0 (0.0)
Cattle	2 (15.4)	1 (7.7)
Birds/ Pigeons	0 (0.0)	0 (0.0)
Any other animals	7 (53.8)	8 (61.5)
Type of house		
Brick	12 (92.3)	12 (92.3)
Wood	1 (7.7)	1 (7.7)
Stone	0 (0.0)	0 (0.0)
State of house		
Excellent	0 (0.0)	0 (0.0)
Good	0 (0.0)	0 (0.0)
Fair	12 (92.3)	12 (92.3)
Poor	1 (7.7)	1 (7.7)
Clothes drying behavior		
Inside house	5 (38.5)	6 (46.2)
Outside house	8 (61.5)	6 (46.2)
On the roof	0 (0.0)	0 (0.0)
On cloths line	0 (0.0)	1 (7.7)
On scrub vegetation	0 (0.0)	0 (0.0)
Defecation behavior		
Open defecation	4 (30.8)	4 (30.8)
Latrine inside house	3 (23.1)	4 (30.8)
Latrine outside house	6 (46.2)	5 (38.5)
Type of drinking water		
Swallow hand pump	9 (69.2)	9 (69.2)
Deep hand pump	4 (30.8)	4 (30.8)
Tap water	0 (0.0)	0 (0.0)
Fever	13 (100.0)	-
Fever duration, days*	4 (3-7)	-
Altered sensorium	0 (0.0)	-
Seizure	6 (46.2)	-
Single	2 (15.4)	-
Multiple	4 (30.8)	
Generalized	6 (46.2)	-
Focal	0 (0.0)	
Headache	5 (38.5)	-
History of rash	0 (0.0)	-
Abdominal pain	0 (0.0)	-
Vomiting	4 (30.8)	-
Episodes of vomiting*	2 (1-2)	-
Loose motions	1 (7.7)	-
Cough	2 (15.4)	-
Nasal discharge	0 (0.0)	-
Pain in limbs/joints	3 (23.1)	-
Eye pain/retro orbital pain	0 (0.0)	-
Similar illness in family/close contacts	2 (15.4)	-
Similar illness in village	3 (23.1)	-
Vaccination		
JE1	13 (100.0)	13 (100.0)
JE2	13 (100.0)	13 (100.0)
Measles	13 (100.0)	13 (100.0)
JE IgM positive	0 (0.0)	0 (0.0)
JE IgG positive	2 (15.4)	1 (7.7)

*: Data were expressed as median (IQR); JE: Japanese encephalitis; "-" indicates not present.

Table 1 continues in next column.

positive). Three (11.5%) children out of the 26 were found to be positive for anti-JE neutralizing antibodies (IgG). Among the 3 IgG-positive samples, 2/26 (7.7%) were cases, and 1 (3.8%) was one of the siblings, while 1/26 (3.8%) case was equivocal for IgG. No mosquito pools out of 19 were found to be positive for JE in RT-PCR.

While none of the samples tested positive for anti-JE IgM, only 3/26 (11.5%) cases were seropositive for anti-JE IgG antibodies, which indicate long-term immunity. This low seropositivity is concerning, given that the affected population resides in a JE-endemic area and had completed the recommended vaccine schedule. Entomological investigations also failed to detect JEV in mosquito pools through RT-PCR analysis, adding to the ambiguity surrounding the outbreak. These findings raise critical questions:

Why did 11 JE IgM-positive cases test negative for IgG if they had been recently infected? Why were protective JE IgG antibodies absent in vaccinated individuals?

If the true positivity of JE IgM ELISA results is questioned, the absence of JE IgG antibodies raises critical concerns about the effectiveness and durability of vaccine-induced immunity in the tested population. JE IgG positivity is expected in vaccinated individuals, as it reflects the presence of neutralizing antibodies generated in response to the vaccine. The lack of detectable protective antibodies in previously vaccinated children highlights the need to revisit current vaccination strategies.

The increasing reports of waning JE vaccine induced antibodies from India and other JE-endemic countries like China, Sri Lanka, and Nepal[4–7] with persistent vector presence, high transmission rates, and unique ecological factors, such as rice cultivation, is particularly concerning. A previous study conducted by our team in this JE-endemic region[4] observed a clear linear relationship between the number of years elapsed since vaccination and the decline in seropositivity rates for anti-JEV neutralizing antibodies (nAbs). In our analysis, nearly 98% of the community-recruited children, aged 0 to 10 years, were found to be negative for anti-JEV-specific IgG antibodies, highlighting a concerning trend of waning immunity over time[4]. Similarly, a study from China reported comparable patterns, showing a steady decline in both the seropositive rates and the titres of anti-JEV IgG antibodies and neutralizing antibodies in vaccinated children[5].

The Maharajganj outbreak study and previous studies from JE endemic regions observed the concerning reduction in antibody levels after SA-14-14-2 immunization which become more pronounced as the time since the last booster vaccination increased. These findings highlighted the potential vulnerability of vaccinated populations in JE-endemic regions. There is an urgent need to assess the durability of newly introduced vaccine (JENVAC)-induced immunity and explore the inclusion of booster doses in the JE vaccination schedule. India should also consider large-scale, community-based studies to evaluate immune responses among vaccinated populations and determine the feasibility of introducing booster doses.

Conflict of interest statement

The authors have declared no conflicts of interest

Acknowledgements

We extend our gratitude to Dr. Krishna Pandey, Director-in-Charge, ICMR-RMRC Gorakhpur, for his invaluable support and guidance during the course of this investigation. Our sincere thanks also go to the State Health Department of Uttar Pradesh for their collaboration and assistance in the field. We are deeply appreciative of the health and administrative officials of Maharajganj district for their unwavering support throughout this study. Finally, we wish to acknowledge the dedication and contributions of the Mr Nalin Kumar, Technician][and other technical and administrative personnel of ICMR-RMRC Gorakhpur, whose efforts were instrumental in the successful completion of this work.

Funding

The authors received no extramural funding for the study.

Data availability statement

All data are incorporated into the article.

Authors' contributions

AKP and NS contributed in the conception and study design. HRMS was involved in the field work and data collection. NS and UA was responsible of data curation. HRMS and RB were responsible of laboratory procedures. AKP and VKS were

responsible of resources management and general supervision of the work. NS and AA was responsible of data analysis. AKP, HRMS and NS wrote the first draft of the article which was subsequently revised by UA and approved by all co-authors.

References

[1] World Health Organisation. *Japanese encephalitis.* [Online]. Available from: https://www.who.int/news-room/fact-sheets/detail/japanese-encephalitis. [Accessed on 11 November 2024].

[2] Kulkarni R, Sapkal GN, Kaushal H, Mourya DT. Japanese encephalitis: A brief review on Indian perspectives. *Open Virol J* 2018; **12**: 121-130.

[3] Srivastava N, Deval H, Mittal M, Kant R, Bondre VP. The outbreaks of acute encephalitis syndrome in Uttar Pradesh, India (1978-2020) and its effective management: A remarkable public health success story. *Front Public Health* 2022; **9**: 793268.

[4] Preethi L, Alina MS, Chandran L, Asvin S, Jagadeesan M, Vijayakumar TM, et al. Duration of seroprotection of the live attenuated SA-14-14-2 Japanese encephalitis vaccine in children in India. *J Travel Med* 2023; **30**(2): taac147. doi: 10.1093/jtm/taac147.

[5] Wang R, Xie L, Gao N, Fan DY, Chen H, Wang PG, et al. Decreases in both the seroprevalence of serum antibodies and seroprotection against Japanese encephalitis virus among vaccinated children. *Virol Sin* 2019; **34**(3): 243-252. doi: 10.1007/s12250-019-00099-z.

[6] Wijesinghe PR, Abeysinghe MRN, Yoksan S, Yao TF, Zhou BL, Zhang L, et al. Immunogenicity of live attenuated Japanese encephalitis SA 14-14-2 vaccine among Sri Lankan children with previous receipt of inactivated JE vaccine. *Vaccine* 2016; **34**(48): 5923-5928.

[7] Turtle L, Brindle HE, Schluter WW, Faragher B, Rayamajhi A, Bohara R, et al. Low population Japanese encephalitis virus (JEV) seroprevalence in Udayapur district, Nepal, three years after a JE vaccination programme: A case for further catch up campaigns? *PLoS Negl Trop Dis* 2019; **13**(4): e0007269.

Publisher's note

The Publisher of the *Journal* remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Edited by Zhang Q, Lei Y, Pan Y