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Dengue serotype-specific seroprevalence among 5- to 10-year-old children in India: a community-based cross-sectional study

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SUMMARY

Background: Dengue surveillance data in India are limited and probably substantially underestimate the burden of disease. A community-based study was undertaken to assess the prevalence of dengue-specific immunoglobulin G (IgG) antibodies in children across India and to examine historical dengue exposure rates. Potential associations between socio-economic factors and dengue seroprevalence were also assessed (registered at ctri.nic.in: CTRI/2011/12/002243).

Methods: A convenience sample of 2609 healthy children aged 5–10 years was enrolled; these children were registered at or were living in the vicinity of eight centres located at six geographically distinct sites across India. Blood samples were drawn to test for the presence of dengue IgG antibodies using ELISA. Serotype-specific neutralizing antibody titres were measured in dengue IgG-positive children using dengue plaque reduction neutralization tests. Socio-demographic and household information was collected using a questionnaire.

Results: Overall, 2558/2609 children had viable samples with laboratory results for dengue IgG. Dengue IgG seroprevalence across all sites was 59.6% (95% confidence interval 57.7–61.5%): the lowest (23.2%) was in Kalyani, West Bengal, and the highest (80.1%) was in Mumbai. Seroprevalence increased with age. Multivariate analysis suggested associations with household water storage/supply and type of housing. Half of the subjects with positive IgG results presented a multitypic profile, indicating previous exposure to more than one serotype.

Conclusions: The overall dengue seroprevalence suggests that dengue endemicity in India is comparable to that in highly endemic countries of Southeast Asia. Additional prospective studies are required to fully quantify the disease burden, in order to support evidence-based policies for dengue prevention and control in India.

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1. Introduction

Dengue is caused by a mosquito-borne flavivirus that is endemic in tropical and subtropical countries, including India.[1] Sporadic outbreaks have been reported in India for over 200 years. The scale and severity of two major epidemics in the 1990s prompted the implementation of a number of strategies to aid the control and surveillance of dengue. In particular, a passive surveillance programme and publication of guidelines for dengue prevention and control was launched as an initiative of the National Vector Borne Disease Control Programme,[2] in collaboration with the existing Integrated Disease Surveillance Programme. Due to non-specific and often mild symptoms, dengue is significantly under-reported in nearly all countries. This is exacerbated in India, where dengue surveillance data are collected from only approximately 500 sentinel hospitals.[3] Studies using global or extrapolated data have quantified this under-reporting, and suggest that the dengue disease burden in India is likely to be the highest in the world.[3,4]

Dengue has spread from urban to rural areas of India in recent decades.[2,5] All four virus serotypes – DENV-1 to DENV-4 – have been documented in India, without a clear geographical distribution. Areas where serotypes co-circulate are increasing in number and scale.[2] Specifically, DENV-1, DENV-2, and DENV-4 were isolated during an outbreak of dengue fever in Vellore in 1963 and all four serotypes were isolated during another outbreak in the same city in 1968.[2] DENV-2 was the predominant serotype from the early 1970s to 2000, responsible for large epidemics in 1993 and 1996. DENV-3 was the predominant serotype in a 2003 outbreak and co-circulated with DENV-1 in 2006 in Dehli.[2] Delhi became hyperendemic for dengue, with all four serotypes isolated in 2003 and 2006.[2] No study to date has taken a nationally representative view of serotype distribution.

Cross-sectional, population-based, age-stratified seroprevalence data describe historical disease transmission intensity.[6,7] A seroprevalence study was undertaken to describe the prevalence of dengue-specific immunoglobulin G (IgG) antibodies and the infecting serotype profiles of positive samples, in children from eight sites across India.

2. Methods

2.1. Study design and centres

This was a community-based, descriptive, cross-sectional seroprevalence study and was conducted between January 2011 and October 2012 in healthy children (registered at ctri.nic.in: CTRI/2011/12/002243). A convenience sample of eight private or government medical colleges at six geographically distinct locations was selected (1) to provide a wide geographical distribution across India, (2) to represent rural and peri-urban areas, and (3) based on the recognized ability of the site to conduct epidemiological research. Overall, two sites were selected in New Delhi and Hyderabad, and one site each in Kalyani, Wardha, Mumbai, and Bangalore.

This study was conducted in accordance with the latest revision of the Declaration of Helsinki (Seoul, Korea, October 2008), guidelines for Good Epidemiological Practice,[8] and local regulatory requirements. The study protocol was approved by ethics committees at the study centres and by the Health Ministry Steering Committee (HMSC) of the Government of India.

2.2. Participants

Children, 5–10 years old, who were resident at the study sites, were eligible. This is an age at which blood sample collection is relatively straightforward. Furthermore, seroconversion, and thus the demonstration of age-specific variation in seroprevalence, was considered likely in this age group. Parents or legal guardians were invited to enrol children during routine household visits by community health workers. Written informed consent was obtained from the parents or legal guardians, and children aged 8–10 years also signed an assent form. Enrolment at the two sites in Hyderabad was school-based; parent–teacher meetings were held at randomly selected schools to explain the purpose of the study, and all eligible children at those schools were invited to participate. Permission was obtained from the District Education Officer to perform study visits, complete questionnaires, and collect blood samples from study participants on the premises of each school.

Assuming a dengue seroprevalence of 30%, a sample size of 323 participants at each site was calculated to ensure a precision of 5% for the two-sided 95% confidence interval (CI) around the seroprevalence point estimate.

2.3. Data and sample collection

Socio-demographic data (participant's demographic characteristics, household occupancy, water supply/storage, self-reported history of dengue or Japanese encephalitis (JE) virus infection, and education levels attained by the parents/guardians) were collected using a questionnaire. The questionnaire was administered by the health worker through interviews with the participant's parents or legal guardians during the first visit. Participants were asked to report to the affiliated centre for blood sample collection (5 ml) by a trained laboratory technician. The participant's height and weight were recorded using standard methods. Significant medical history, current or previous medical conditions, concomitant medication, recent vaccinations, and reasons for refusal of blood sampling, where relevant, were recorded.

Blood samples were left at room temperature for 1–2 h before centrifugation. Each serum sample was divided into aliquots and stored in 3-ml cryotubes: 0.5 ml for dengue IgG antibody assessment, 1 ml for dengue plaque reduction neutralization tests (PRNT), and 0.5 ml for JE IgG antibody detection. Serum samples were kept frozen at −20 °C or below until analysis.

2.4. Assays

Samples were sent to the Microbiology Department of the Maulana Azad Medical College (New Delhi) for analysis. Dengue IgG antibody levels were assessed using commercially available ELISA kits. The EL1500G kit (Focus Diagnostics, California, USA) was used for samples from the first two sites (New Delhi); however, due to supply issues, the E-DEN 10G kit (Panbio Diagnostics, Brisbane, Australia) was used for the other sites. A sensitivity analysis of the two dengue IgG-specific ELISA kits performed on 30 samples confirmed 100% concordance; data from all centres were thus pooled. JE IgG antibody testing by indirect ELISA was also performed using commercially available kits (InBios, Washington, USA). Dengue IgG-positive samples were sent to the Centre for Vaccine Development (Mahidol University, Thailand) for measurement of dengue serotype-specific neutralizing antibody titres using PRNT based on 50% or greater reduction in plaque counts ($PRNT_{50}$).[9]

Seropositivity for dengue and JE were defined according to the manufacturer's instructions. Seroprevalence was the percentage of seropositive participants.

For the interpretation of $PRNT_{50}$ titres, participants were classified as follows: 'naïve', if antibody titres were <10 (1/dil) for the four serotypes; 'monotypic', if antibody titres were ≥ 10 (1/dil) for only one serotype or if titres were ≥ 10 (1/dil) for

different serotypes, with a single serotype having a high titre (>80 (1/dil) titre, and ≥5 times higher than other titres); and 'multitypic', if antibody titres were ≥10 (1/dil) for different serotypes without a single predominant titre.

At each site, a designated clinical research associate performed periodic visits to monitor implementation. All serum samples were checked for quantity and storage temperature by a lot quality assurance method.

2.5. Statistical analysis

Descriptive statistics reported baseline characteristics and immunogenicity results. Associations between all demographic–socio-economic factors and dengue serostatus were assessed by univariate analysis using the Chi-square test or *t*-test (for age) and multiple logistic regression with backward selection (significant if the *p*-value is ≤0.05). JE serostatus was not included as a covariate due to possible cross-reaction between flavivirus antibodies. Statistical significance was considered at and below a *p*-value of 0.05. Statistical analyses were performed using SAS software version 9.1 (SAS Institute, Cary, NC, USA) and Stata v. 12.1 (StataCorp LP, College Station, TX, USA).

2.6. Role of the funding source

The sponsor participated in all operational aspects of the study, including data collection, statistical analyses, and the writing of the study report. The sponsor funded medical writing support for the development of this publication. The corresponding author had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

3. Results

3.1. Demographic characteristics of the study population

Overall, 2609 participants from eight health centres were enrolled in the study. A total of 18 participants were excluded, due to age <5 years or >10 years (*n* = 8) and/or assent form not signed (*n* = 13). Thus, 2591 participants were included, all of whom had a blood sample drawn; 1364 (52.6%) were female, and the mean age of all participants was 7.8 (standard deviation (SD) 1.6) years.

3.2. Socio-economic characteristics

The mean number of people living in the participants' households was 5.4 (SD 2.3), including 2.6 (SD 1.2) children under 15 years of age. Most (2170/2591; 83.8%) participants lived in a house, with 381 (14.7%) living in precarious lodgings; 1121 (43.3%) had an indoor piped public water supply, 2343 (90.4%) had water storage in the house, 1675 (64.6%) were connected to the public sewer, and 1339 (51.7%) had regular organized waste collection.

3.3. Medical history

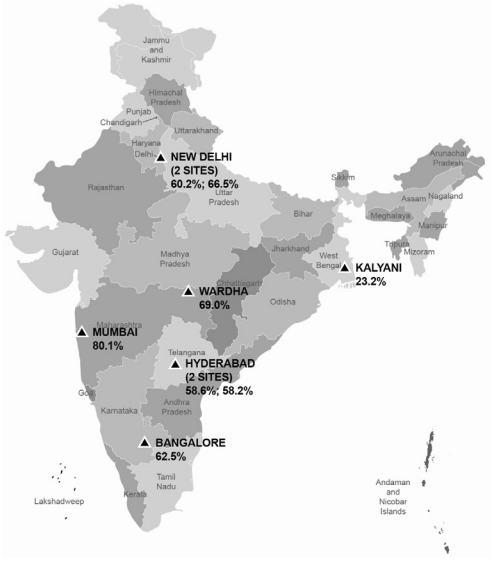
A notable medical history was recorded for 94 (3.6%) children; 61 (2.4%) children were undergoing at least one current treatment at the time of enrolment. A history of dengue or a family history of dengue was reported by 15 (0.6%) and 48 (1.9%) participants, respectively. No participants reported a history or family history of JE infection. Only one participant reported receiving JE vaccination.

3.4. Dengue IgG seroprevalence

Anti-dengue IgG results were available for 2558/2591 (98.7%) participants. Serology data were missing for 33 participants; 22 samples from a single site (Mumbai) could not be analysed due to haemolysed red blood cells. Overall, 1525/2591 (59.6%) participants were dengue seropositive, with similar prevalence in males and females. Six of the eight sites had dengue seropositivity ranging from 58.2% to 69.0%. The sites in Kalyani and Mumbai had the lowest (23.2%) and highest (80.1%) seroprevalence, respectively (Figure 1). Overall, dengue IgG seroprevalence increased with age, from 40.7% (95% CI 36.0–45.5%) in children aged 5 years to 73.4% (95% CI 67.9–78.5%) in 10-year-olds (Figure 2). At the Bangalore site, seroprevalence remained relatively stable across the age strata (varying from 58.8% in 7-year-olds to 70.9% in 8-year-old children).

3.5. Socio-economic characteristics associated with dengue seroprevalence status

In univariate analyses, children seropositive for dengue were found more likely to be from homes with more than two children ($p < 0.0001$), more likely to have water storage ($p < 0.0001$) and



Site	n/N	% IgG positive	95% CI
New Delhi 1	195/324	60·2	54·6–65·6
New Delhi 2	216/325	66·5	61·0–71·6
Kalyani	75/323	23·2	18·7–28·2
Wardha	223/323	69·0	63·7–74·0
Mumbai	241/301	80·1	75·1–84·4
Hyderabad 1	188/321	58·6	53·0–64·0
Hyderabad 2	185/318	58·2	52·5–63·7
Bangalore	202/323	62·5	57·0–67·8
Overall	1525/2558	59·6	57·7–61·5

CI=confidence interval; IgG=Immunoglobulin G; n=number of participants seropositive for dengue; N=total number of participants with blood samples per site

Figure 1. Dengue IgG seroprevalence by site.

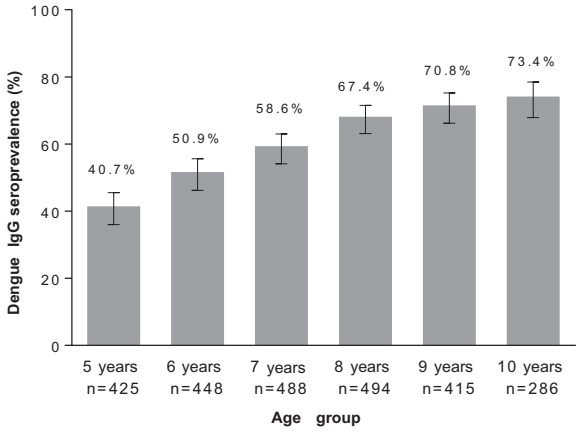


Figure 2. Percentage of participants with antibody titres ≥10 (1/dil) against dengue according to age group (all study sites). Error bars show 95% confidence intervals. n, number of participants with available results per age group.

indoor piped water from the public water supply ($p < 0.0001$), and less likely to be living in precarious housing ($p = 0.0048$) compared with dengue seronegative children (Table 1). Multiple logistic regression confirmed possible positive associations with household water storage (odds ratio (OR) 5.00, 95% CI 3.54–7.06) and indoor piped water from the public water supply (OR 1.49, 95% CI 1.19–1.85). Increasing participant age ($p < 0.0001$) and living in precarious lodgings compared to a house (OR 1.54, 95% CI 1.17–2.03) were also associated with dengue status. In terms of geography, Kalyani was associated with decreased exposure (OR 0.18, 95% CI 0.10–0.31), while Wardha (OR 1.49, 95% CI 1.07–2.08) and Mumbai (OR 2.49, 95% CI 1.70–3.65) had an elevated risk, in comparison with Delhi. The pseudo R^2 of the final model was 0.085.

3.6. Dengue serotype analysis

Of 1525 IgG seropositive participants tested, 1511 had $PRNT_{50}$ data available for all four serotypes. Of these, 1468 (97.2%) had antibody titres ≥10 for at least one serotype and 1205 (79.7%) had antibody titres ≥10 against all four serotypes. Nearly half (736/1511; 48.7%) had a multitypic antibody profile and 732/1511 (48.4%) had a monotypic profile. DENV-1, DENV-2, and DENV-3 were nearly equally represented among dominant serotypes in participants with monotypic profiles overall (Figure 3).

3.7. Japanese encephalitis IgG seroprevalence

Anti-JE IgG results were available for 2544 (98.2%) participants. Of these, 345 (13.6%; 95% CI 12.3–15.0%) participants were seropositive against JE, 327 (94.8%) of whom were also dengue seropositive (Table 2). JE seroprevalence ranged from 4.3% (95% CI 2.4–7.1%) in Kalyani to 20.5% (95% CI 16.2–25.3%) in Wardha.

4. Discussion

These findings demonstrate a high intensity of dengue transmission among children in India; more than 50% of the children had been infected at least once by the age of 6 years, results which are broadly consistent with existing, limited dengue seroprevalence data for adults and children in Chennai and Hyderabad.[10,11] All four serotypes were found to circulate, varying by geographic location. Nearly half of all participants had a multitypic dengue antibody profile. Dengue IgG seroprevalence increased with age at all but one study site, consistent with age-related cumulative exposure to dengue.[10] The exception in Bangalore could be related to epidemiological, behavioural, or environmental factors moderating exposure risk, such as the occurrence of large, infrequent outbreaks.

The observed level of dengue exposure was comparable to that reported in other highly endemic countries of Southeast Asia and Latin America: 56.2% for 4–9-year-olds in Yogyakarta, Indonesia (1995–1996),[12] 65% for 11-year-olds in Rayong, Thailand (2010),[13] 34.4% for under 7-year-olds to 70.5% in 14–16-year-olds from a primary health care facility in Sri Lanka (2013–2014),[14] 53% for under 7-year-olds and 88% by the age of 13 years among primary school children in southern Vietnam,[15] and 35.7% and 52.2% for 5–9 and 10–14 years age groups, respectively, in two localities in Mexico in 2011.[16] A higher seroprevalence was observed in a study in Managua, Nicaragua (2001–2003), where 80% of enrolled children were seropositive by 5 years of age.[17]

Considering these similarities in exposure history, it might be expected that rates of symptomatic, reported dengue are similar in India and other countries. In fact, there are huge disparities: from 2007 to 2011, India reported an approximate average annual incidence of 1.4 cases/100 000 population,[5] whereas case notifications in Cambodia, Malaysia, the Philippines, and Sri Lanka for 2011 were 119, 70.4, 134, and 135 per 100 000, respectively.[18,19] Despite their significant and often multitypic infection history, very few participants in the present study reported a history of dengue. Similarly low reporting was observed in the

Table 1
Demographic and socio-economic characteristics associated with dengue seroprevalence status

	Dengue IgG positive	Dengue IgG negative	*p*-Value (OR[a])
Demographic characteristics			
Overall, *n*	1525	1033	
Age, mean (SD) years	8.13 (1.56)	7.39 (1.56)	<0.0001[b,*]
Socio-economic characteristics			
Number of children living in the household (%)			<0.0001[c]
≤2 children	855 (56.07)	671 (64.96)	
>2 children	670 (43.93)	362 (35.04)	
Type of housing (%)			0.0048[c,*]
House	1290 (84.6)	855 (82.8)	(1.00)
Apartment	29 (1.9)	7 (0.7)	(2.16)
Precarious lodgings	206 (13.5)	171 (16.6)	(1.54)
Water storage in the house (%)	1465 (96.1)	845 (81.9)	<0.0001[c,*] (5.00)
Indoor piped public water supply (%)	721 (47.3)	380 (36.8)	<0.0001[c,*] (1.54)
Connected to public sewer (%)	959 (62.9)	688 (66.6)	0.0541[c]

IgG, immunoglobulin G; SD, standard deviation.
[a] The odds ratio (in parentheses) is provided for significant categorical multivariate results.
[*] $p < 0.05$ in multivariate analysis.
[b] *t*-test.
[c] Chi-square test.

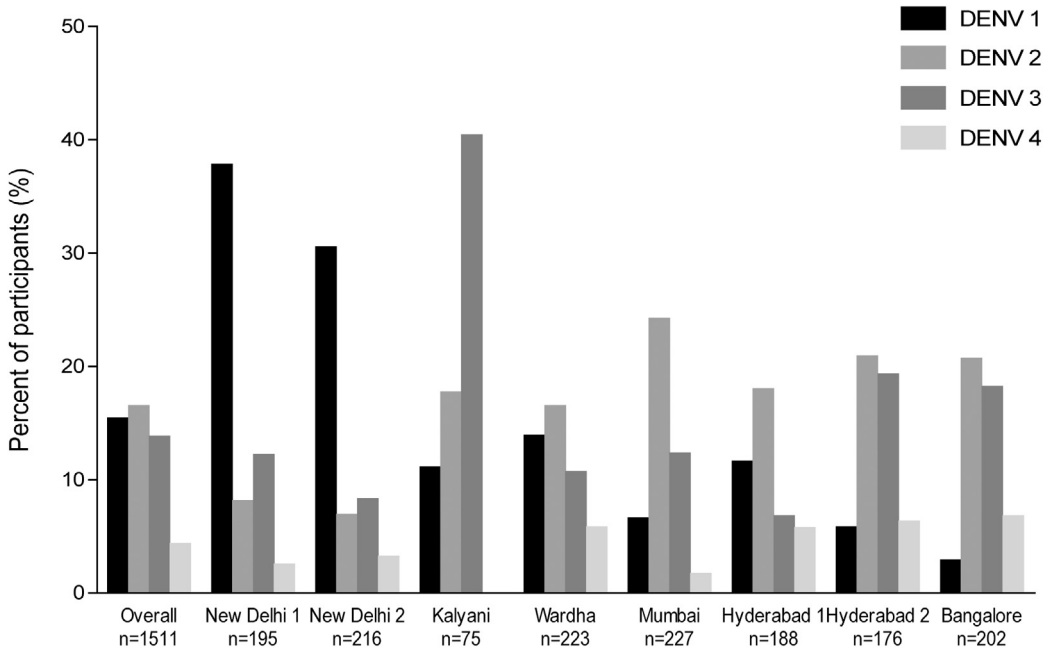


Figure 3. Proportion of participants with $PRNT_{50}$ results showing monotypic profiles, according to dominant serotype, by site and overall.

recent household-based study in Chennai,[10] in which 744/800 (93%) subjects were dengue IgG seropositive, but only 1% of participants reported a history of dengue. The present authors are unaware of virological or genetic factors that might disassociate infection history from the incidence of symptomatic disease; likely explanations include a lack of health-seeking for patients with apparent infection, lack of recognition of the disease, or misdiagnosis of dengue.[1,4] For these reasons, and because dengue surveillance reports are collected from only sentinel sites,[3] it must be assumed that dengue burdens reported in the routine surveillance system represent only a fraction of symptomatic episodes.

In the current study, serotype-specific analyses identified historical circulation of all four dengue virus serotypes at each site, with DENV-1 present in a high proportion of samples in New Delhi and DENV-3 in Kalyani. These serological findings in children are worrying: co-circulation of multiple serotypes is a population risk factor for severe dengue because it allows for sequential infection, and because secondary infection is a risk factor for severe disease.[20,21]

Multivariate risk factor analysis suggested relationships between water availability/storage practices and dengue infection risk, an association with biological plausibility due to the aquatic larval and pupal stages of the vector life-cycle. However, these results should be interpreted with caution because they were mainly driven by data from Kalyani and are thus highly susceptible to confounding with site-specific socio-demographic covariates, or other factors. After excluding Kalyani data from the multivariate analysis, only participant age remained significantly associated with dengue positivity. Furthermore, the determination coefficient (R^2) of the model was lower than 0.1, confirming the limited predictive value of these variables for dengue serostatus, in agreement with inconclusive/variable socio-economic drivers of dengue serostatus identified in other studies. In Chennai, univariate logistic regression showed a negative association with household income and no associations with other household factors.[10] Thai et al. (2005) found associations with littering in and around the home and the types of sanitary facilities in an initial univariate analysis, but these associations were not confirmed on multivariate analysis.[15] A community-based study of potential risk factors for dengue transmission in Venezuela found several household and socio-economic factors, including storing water and used tyres (univariate analysis), and crowding, household size, and living in a shack (multivariate analysis), to be associated with an increased risk of dengue infection.[22]

The seroprevalence of anti JE IgG antibodies was also measured in the participants in the present study. JE is endemic in some regions in India, particularly in the south and north-east;[23] however, during the current study period, none of the study sites were considered to be within a JE endemic area and none were in an area subject to routine JE vaccination. The observed seroprevalence of JE in the current study (13.6%; 95% CI 12.3–15.0%) confirms circulation of the virus, but is lower than that reported in a number of other studies on JE seroprevalence in endemic countries,[14,24,25] perhaps because these sites are located within less-endemic areas of India.

Cross-reaction between anti-flavivirus IgG antibodies has been documented[26] and cannot be excluded here from affecting the observed JEV or dengue IgG rates. The PRNT is a more specific assay and may be used to distinguish between cross-reactive and pathogen-specific responses. Encouragingly, 99% of dengue IgG-positive samples in the current study were also dengue PRNT-positive. However, the lack of JEV PRNT data and dengue PRNT in dengue-negative samples remains a limitation in conclusively addressing this risk.

Table 2
Prevalence of dengue and Japanese encephalitis IgG in participants with blood samples[a]

		Dengue IgG		
		n	Positive	Negative
JE IgG	*n*	2591	**1525**	1033
	Positive	**345**	**327**	18
	Negative	1794	801	990

JE, Japanese encephalitis; *n*, number of participants with results corresponding to the specified category.

[a] 33/2591 total samples had no results available for dengue IgG; 47 were inconclusive and 405 were missing for JE IgG.

Other limitations include the use of different dengue-specific IgG ELISAs at different sites, but the sensitivity test showing 100% concordance was reassuring. In this study, a convenience sample was selected, including some low-income settings, to provide geographical spread across India. However, sites were not randomly selected, subjects were consecutively recruited, and these data cannot be considered nationally or locally representative. As with other epidemiological studies, recall bias (during the questionnaire) and selection bias (e.g., self-selection of healthy subjects) cannot be excluded. Despite these sampling limitations, these data provide a first multi-centre view on dengue seroprevalence in India. The use of a single protocol and consistent methods between the sites strengthens the validity of the data.

In conclusion, high levels of dengue exposure were observed in Indian children, and age-stratified data describe transmission intensity at these locations. This information may inform dengue burden estimates and populate transmission models to assess the potential impact of prevention and control measures, including vaccination programmes.

Author contributions

SG, AC, and GF designed the study. SG, RS, NRRM, RCG, GRJ, EG, NS, MMS, and SO refined the study design, performed the study, and collected demographic and clinical data from study subjects, in the field. AC performed laboratory assays for dengue and JE virus in accordance with good laboratory practice. JN and AM analysed the data. JN provided an initial interpretation and worked to develop a manuscript outline. All authors interpreted the data with refinements and contributed to writing the manuscript. All authors critically reviewed the manuscript while in preparation and approved the final draft.

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Conflict of interest: JN, AM, and SO are employees of Sanofi Pasteur. AC, EG, MMS, RM, and RS have no conflicts of interest to declare.

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