International Journal of Infectious Diseases 54 (2017) 25–30

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

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

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ARTICLE INFO

*Article history:*
Received 7 October 2016
Received in revised form 27 October 2016
Accepted 27 October 2016

**Corresponding Editor:** Eskild Petersen, Aarhus, Denmark

*Keywords:*
Dengue
Seroprevalence
Children
India
Community-based study
Socio-economic factors

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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http://dx.doi.org/10.1016/j.ijid.2016.10.030

## 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 $\geq 10$ (1/dil) for only one serotype or if titres were $\geq 10$ (1/dil) for