Burden of dengue infection in India, 2017: a cross-sectional population based serosurvey


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Summary

Background The burden of dengue virus (DENV) infection across geographical regions of India is poorly quantified. We estimated the age-specific seroprevalence, force of infection, and number of infections in India.

Methods We did a community-based survey in 240 clusters (118 rural, 122 urban), selected from 60 districts of 15 Indian states from five geographical regions. We enumerated each cluster, randomly selected (with an Android application developed specifically for the survey) 25 individuals from age groups of 5–8 years, 9–17 years, and 18–45 years, and sampled a minimum of 11 individuals from each age group (all the 25 randomly selected individuals in each age group were visited in their houses and individuals who consented for the survey were included in the study). Age was the only inclusion criterion; for the purpose of enumeration, individuals residing in the household for more than 6 months were included. Sera were tested centrally by a laboratory team of scientific and technical staff for IgG antibodies against the DENV with the use of indirect ELISA. We calculated age group specific seroprevalence and constructed catalytic models to estimate force of infection.

Findings From June 19, 2017, to April 12, 2018, we randomly selected 17 930 individuals from three age groups. Of these, blood samples were collected and tested for 12 300 individuals (5–8 years, n=4059; 9–17 years, n=4255; 18–45 years, n=3976). The overall seroprevalence of DENV infection in India was 48.7% (95% CI 43.5–54.0), increasing from 28.3% (21.5–36.2) among children aged 5–8 years to 41.0% (32.4–50.1) among children aged 9–17 years and 56.2% (49.0–63.1) among children aged between 18–45 years. The seroprevalence was high in the southern (76.9% [69.1–83.2]), western (62.3% [55.3–68.8]), and northern (60.3% [49.3–70.5]) regions. The estimated number of primary DENV infections with the constant force of infection model was 12 991 357 (12 825 128–13 130 258) and for the age-dependent force of infection model was 8 655 425 (7 243 630–9 545 052) among individuals aged 5–45 years from 30 Indian states in 2017.

Interpretation The burden of dengue infection in India was heterogeneous, with evidence of high transmission in northern, western, and southern regions. The survey findings will be useful in making informed decisions about introduction of upcoming dengue vaccines in India.

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Research in context

Evidence before this study

We searched PubMed for estimates of seroprevalence of dengue infection in India on Dec 6, 2018, using the search terms “dengue”, “seroprevalence” and “India”. We identified 43 publications, of which eight reported seroprevalence of dengue infection. A systematic review and meta-analysis, which included seven of these studies, reported the seroprevalence of dengue in India as 56·9% (95% CI 37·5–74·4). Age-specific seroprevalence was reported by three studies. These studies reported that by the age of 9 years, 47·6–73·4% of children have developed antibodies against dengue. These studies were done on a conveniently selected sample or were limited to a few cities and hence the results could not be generalised. In this context, we did a cross-sectional survey among individuals aged 5–45 years to estimate the age-specific seroprevalence of dengue in India.

Added value of this study

Our study indicates a heterogeneous seroprevalence in different geographical regions in India with high level of dengue transmission in northern, western, and southern geographical regions, whereas low transmission was observed in northeast and eastern regions. In all regions, younger children had higher force of infection corresponding to suboptimal immunity in this age group. Our serosurvey also generated data about profile of dengue serotype specific neutralising antibodies in a subsample. In eastern and northeastern regions, where dengue seroprevalence was low, most of the infections were monotypic in nature; whereas in northern, western, and southern regions, most dengue infections were multitypic in nature.

Methods

Study design and participants

We did a cross-sectional, community-based survey in five geographical regions of India (north, east, west, south, and northeast; appendix) covering three age groups (5–8 years, 9–17 years, and 18–45 years) from 30 states. We adopted a multistage sampling design. We randomly selected three states from each geographical region (total 15 states; appendix). From each state, we selected four districts with the probability proportional to population size method (total 60 districts). We then randomly selected four clusters (two villages from rural clusters and two wards from urban clusters) from each district (total 240 clusters). From each cluster, we randomly selected one Census Enumeration Block (CEB). CEB is the area allotted to each census enumerator for carrying out decennial census operations and usually has 120–150 households. For all random selection, we did simple random sampling using computer generated random numbers.

Assuming 60% seroprevalence of dengue infection,14 relative precision of 10%, and design effect of 2, and for 95% CI, we required a sample size of 513 people (rounded to 528 [the nearest number divisible by the total number of clusters per region ie, 48]) per age group per region, with 11 individuals per age group per cluster. We assumed that about half of the randomly selected respondents would not be available for participation in the survey for reasons such as locked houses, selected individuals or their parents (in case of children) were not available at the time of survey or blood specimen collection, refusal to participate in the survey, or refusal to provide a blood specimen, or haemolysis of blood specimen. We therefore planned to select 22 people (rounded to 25) in
each age group. With the use of data for birth rates, infant mortality ratio (Sample Registration System, 2016 bulletin), and household size (Census of India, 2011), a minimum of 107 households were required to be enumerated to recruit at least 25 individuals in each of the three age groups (appendix). Among the enumerated population, age group was the only criteria for random selection.

The survey team, on reaching the identified cluster, appraised residents or local leaders about the purpose of survey, and enumerated households in the CEB residing for more than 6 months. During enumeration, all households were numbered and identification details of people residing in the households, including name, age, and sex were collected with the use of tablets with an android application developed for the survey. After completing enumeration, data were uploaded to the central server of the Indian Council of Medical Research-National Institute of Epidemiology (ICMR-NIE), Chennai.

All people enumerated in each of the three age groups from the cluster constituted the sampling frame. 25 people in each age group were randomly selected centrally with the use of an application developed for the survey. The survey team then visited all the selected individuals in their households and interviewed them to collect information about sociodemographic details, after obtaining consent or assent.

The Institutional Ethics Committees of ICMR-NIE and all the participating institutes approved the study protocol. Written informed consent from people aged 18 years and older, parental consent from parents of children aged between 5–17 years, and assent from children aged between 7–17 years was obtained before the survey.

Statistical analysis

We estimated weighted age group specific seroprevalence of dengue infection along with 95% CI for each geographical region using design weight and adjusting for non-response. We estimated the national seroprevalence based on regional prevalence. We constructed a Receiver Operator Characteristic Curve (ROC) to compare the sensitivity and specificity of IgG ELISA with PRNT90 titres to adjust the ELISA cutoff. We did the analyses using survey data analysis module in STATA SE version 13.0, SPSS Inc version 18.0, and R version 3.5.1 software.

We developed catalytic models to estimate the dengue force of infection, based on unweighted seroprevalence at different ages. FOI is defined as the rate at which susceptible individuals are infected. Since the indirect IgG ELISA cannot distinguish between primary and secondary dengue infections, the term FOI meant the annual risk of infection with any serotype among seronegative individuals. We fitted two different models

![Figure 1: Study profile](https://www.thelancet.com/lancetgh/e1067)

*In 15 clusters, the number of enumerated children was <25. †The houses were locked, hence the eligible person (who was randomly selected) could not be interviewed.
to our seroprevalence data: model 1 assuming a constant FOI, and model 2 assuming FOI varies with age (appendix).

Based on FOI estimated from the age-dependent model, we calculated seroprevalence among children aged 9 years (SP9) in different geographic regions and classified the transmission intensity as very low (SP9 <10%), low (SP9: 11–30%), moderate (SP9: 31–50%), high (SP9: 51–70%), and very high (SP9>70%). SP9 was calculated from the best fit catalytic model.24

We estimated the number of new dengue infections, based on the age specific population (2011 population, projected for 2017) for individuals aged between 5–45 years, and constant and age-dependent FOI. 15

Role of the funding source
The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
From June 19, 2017, to April 12, 2018, we enumerated 117,675 individuals from 240 clusters (118 rural and 122 urban; all clusters from two districts of NCT Delhi were urban) from 15 Indian states, of whom 77,640 were in the age group of 5–45 years. We randomly selected 17,930 individuals, of whom 2980 (16·6%) were not available for participation in the survey. Of the 14,950 individuals who were available for participation, 1213 (8·1%) refused to participate in the survey, 1405 (9·4%) refused to provide blood specimen, and 32 (0·2%) were excluded because their actual age was different than the age group for which they were randomly selected. Thus, data on 12,300 individuals were used for estimation of dengue seroprevalence (figure 1).

Of the 12,300 individuals enrolled, 4059 (33·0%) were in the age group of 5–8 years, 4265 (34·7%) were in the age group of 9–17 years, and 3976 (32·3%) were in the age group 18–45 years. Most participants belonged to Hindu religion (77·9%), 52·7% were women, and 50·7% were residents of rural areas. 8·2% had no formal education and 43·6% had a below poverty line card (table 1). About 74·5% participants reported that their households received piped water for drinking purposes.

Of the 500 sera tested for PRNT90, 215 (43%) had IgG antibodies against dengue. Considering PRNT90 as the gold standard, the cutoff of 11 PU for IgG antibodies had a sensitivity of 79·7% and a specificity of 88·8%. Based on the ROC curve; we chose the optimal cutoff of 15 PU for IgG antibodies against dengue (area under the curve 0·89 [95% CI 0·86–0·92]). This cutoff had a sensitivity of 77·6% and specificity of 94·4% (appendix).

Using the optimised cutoff, the overall seroprevalence of DENV infection in India was 48·7% (95% CI 43·5–54·0). The seroprevalence was highest in the southern (76·9%, [69·1–83·2]) region, followed by the western (62·3% [53·3–70·0]) and the northern (60·3% [49·3–70·0]) regions. The seroprevalence was lowest in the northeastern (5·0% [3·3–7·6]) region (table 2). The unweighted seroprevalence in 15 Indian states is given in the appendix.

The dengue seroprevalence increased with age (p<0·0001). The seroprevalence among children aged 5–8 years was 28·3% and ranged between 1·6% in the northeastern region and 47·0% in the northern region.
The prevalence increased to 41.0% among children aged 9–17 years and 56.2% among individuals aged 18–45 years. The overall seroprevalence was higher in urban (70.9%) than in rural areas (42.3%; p<0.001), while the seroprevalence was not different among men (50.9%) and women (47.5%; table 2). This pattern was consistent across all geographical regions.

Of the 500 sera tested, 233 (46.6%) had NAb titres of 10 or more against at least one serotype of DENV. 64 (27.5%) of the 233 had a monotypic and 169 (72.5%) had a multitypic antibody profile (table 3). Ten (43.5%) of 23 infected individuals in the eastern region and 19 (76%) of 25 in the northeastern region had monotypic dengue infection, whereas in the northern, western, and southern...
regions, only 13–27% infections were monotypic in nature. The distribution of serotype-specific antibodies indicated that the northern and eastern regions had predominantly DENV-1 and DENV-2 serotypes, the western and southern had DENV-3, DENV-2, and DENV-1 serotypes, and the northeastern region had DENV-3 serotype.

As per the constant FOI model, FOI varied between 0.07–0.09 in the southern, northern, and eastern regions, with SP9 ranging between 47–55%. Although WHO’s recommendations about Dengvaxia has changed to prevaccination screening or vaccination in areas with seroprevalence more than 80%, the findings of our serosurvey could be useful in optimising age group and geographical regions targeted for test and vaccination programmes. In India, seroprevalence was higher (>50%) among children aged 9–17 years or older individuals residing in the southern and northern regions.

Based on the FOI models, we estimated that during 2017, about 8.8–12.9 million primary dengue infections occurred among individuals aged 5–45 years from 30 Indian states. Assuming about 25% of these infections were clinical in nature, the number of clinical infections from the 30 states is estimated to be around 2.2–3.2 million. During 2017, the National Vector Borne Disease Control Programme reported 188,401 clinical cases of dengue from India.

The sociodemographic characteristics of the sample surveyed in our study were similar to the data from Census of India (2011) or the National Health and Family Survey-4 (2015–16), with respect to religion, caste, proportion of women, literacy of head of households, and water supply (appendix). However, only 8% of the study population was illiterate compared with 26% according to census data. This disparity could be because our study was restricted to individuals aged 5–45 years, 68.4% of whom were students.

In India, dengue seroprevalence was higher in urban than rural areas and these findings were consistent across all regions. However, in the northern, western, and southern regions where dengue seroprevalence was higher (>50%) among children aged 9–17 years or older individuals residing in the southern and northern regions.

### Table 4: Estimates of the force of infection, $R_0$ and SP9 for different geographical regions in India

<table>
<thead>
<tr>
<th>Region</th>
<th>5–45 years estimate (95% CI)</th>
<th>Age-dependent force of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern region</td>
<td>0.0109 (0.0105–0.1010)</td>
<td>0.0358 (0.0301–0.0783)</td>
</tr>
<tr>
<td>Northeastern region</td>
<td>0.0012 (0.0010–0.0122)</td>
<td>0.0015 (0.0012–0.0019)</td>
</tr>
<tr>
<td>Eastern region</td>
<td>0.0240 (0.0238–0.0242)</td>
<td>0.0240 (0.0238–0.0242)</td>
</tr>
<tr>
<td>Western region</td>
<td>0.0096 (0.0094–0.0100)</td>
<td>0.0235 (0.0232–0.0238)</td>
</tr>
<tr>
<td>Southern region</td>
<td>0.0065 (0.0062–0.0067)</td>
<td>0.0383 (0.0380–0.0385)</td>
</tr>
</tbody>
</table>

Estimates obtained from a model fit to dengue age-specific seroprevalence data. $R_0$ is basic reproduction number. SP9 is seroprevalence among children aged 9 years.

**Discussion**

This serosurvey was initiated based on the WHO’s initial recommendation of generating nationally representative seroprevalence data to guide decisions about introduction of Dengvaxia in India. The survey findings indicated that 49% of country’s population had been previously infected with DENV, although prevalence varied widely by region. The seroprevalence was lower in the northeastern and eastern regions, with an SP9 of less than 10%. The seroprevalence was higher in the northern, western, and southern regions, with SP9 ranging between 47–55%.
higher, 53–72% of the population from rural areas had evidence of dengue infection, indicating that dengue transmission is also frequent in rural areas as well.14–16 Studies have observed population growth, rapid urbanisation, globalisation, climate change, and ineffective mosquito control as the major drivers of dengue epidemic.17,18

Our serosurvey also generated data about the profile of dengue serotype-specific neutralising antibodies. In eastern and northeastern regions, where dengue seroprevalence was low, most infections were monocytic in nature; while in northern, western, and southern regions most dengue infections were multitypic in nature. Low seroprevalence of dengue infection in eastern and northeastern regions could also be attributed to lower proportion of multitypic infections in the region.

Although WHO recommends school-based sampling for dengue serosurveys,19 such surveys have some challenges in terms of variable school drop-out rates and low participation of private schools. Community-based design provided us an opportunity to enrol children studying in all types of schools and school drop-outs. The consent and assent process was also easier in community based surveys. Enumeration of entire CEB and random selection of individuals in each age group provided a probability-based sample for estimating seroprevalence in different regions of India. In our survey, we sampled individuals aged 5–45 years, whereas WHO recommends survey among children aged 5–18 years.20,21 Based on the simulation exercise, recommend that dengue serosurveys need to include children younger than 9 years in high transmission settings and older children and adults in low transmission settings. Because of the expected variation in dengue transmission across states in India, we decided to sample individuals from a wide age range of 5–45 years.

Our survey had some limitations. First, we calculated the sample size assuming uniform seroprevalence of 60% in different regions and age groups.22 Our sample size was probably not adequate for eastern and northeastern regions where seroprevalence was lower. Second, for logistical reasons, we could only do PRNT on 100 specimens from each region. Third, since IgG antibodies based on indirect ELISA cannot distinguish between primary and secondary dengue infections, we were not able to estimate the proportion of secondary infections. Fourth, our survey was designed to generate dengue prevalence estimates at the regional level. In the future, Dengvaxia or other candidate vaccines are likely to be introduced at the subnational or state level. Dengue transmission can vary substantially between areas in close proximity and FOI can differ substantially between districts within a state. Small surveys with a sufficient sample size would be useful to do at the state level to capture geographical heterogeneity within a state.23

In conclusion, our study indicates a heterogeneous seroprevalence in different geographical regions in India with a high level of dengue transmission in three of the five geographic regions in India. In all regions, younger children had higher force of infection corresponding to suboptimal immunity in this age group. The findings of our survey will be useful in making informed decisions about the introduction of newer dengue vaccines in the country.

Contributors
MVM was the principal investigator of the survey. MVM, PK, MSK, NG, and SMM conceived and designed the study. MVM, PK, MSK, SAK, RRA, PB, BD, SK, UM, SSM, SR, VS, DS, BVT, RKT, SB, GSG, PVML, CMM, PS, PKS, SKS, CPY, RK, SD, GST, CG, TDY, AJ, AS, DA, and PAK coordinated the field operations. GS and CPGK oversaw all laboratory procedures with the support of AB, RSR, ERD, and TK. RSa developed the application for the survey. PK, MSK, RSa, and MVM managed and analysed data. VSK developed the force of infection models and estimated the number of dengue infections. MVM drafted the first version of the manuscript and all authors contributed, reviewed, and approved this article.

Declaration of interests
We declare no competing interests.

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References
5 Kakkar M. Dengue fever is massively under-reported in India, hampering our response. BMJ 2012; 345: e8574.


