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

Manoj V Murhekar, P Kamaraj, Muthusamy Santhosh Kumar, Siraj Ahmed Khan, Ramesh Reddy Allam*, Pradip Barde*, Bhagirathi Dwibedi*, Suman Kanungo*, Uday Mohan*, Suman Sundar Mohanty*, Subarna Roy*, Vivek Sagar*, Deepali Savargaonkar*, Babasaheb V Tandale*, Roshan Kamal Topno*, Gajanan Sapkal, C P Girish Kumar, R Sabarinathan, Velusamy Saravana Kumar, Sailaja Bitragunta†, Gagandeep Singh Grover†, P V M Lakshmi†, Chandra Mauli Mishra†, Provash Sadhukhan†, Prakash Kumar Sahoo†, S K Singh†, Chander Prakash Yadav†, Asha Bhagat, Rashi Srivastava, E Ramya Dinesh, T Karunakaran, C Govindhasamy, T Daniel Rajasekar, A Jeyakumar, A Suresh, D Augustine, P Ashok Kumar, Rajesh Kumar, Shanta Dutta, G S Toteja, Nivedita Gupta, Sanjay M Mehendale*

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 Andriod 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=4265; 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 individuals 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.

Funding Indian Council of Medical Research.

Lancet Glob Health 2019

Published **Online** June 11, 2019 http://dx.doi.org/10.1016/S2214-109X(19)30250-5

See **Online/Comment** http://dx.doi.org/10.1016/S2214-109X(19)30249-9

*Contributed equally

†Contributed equally

Indian Council of Medical Research (ICMR)-National Institute of Epidemiology, Chennai, India (M V Murhekar MD, P Kamaraj MPhil, M S Kumar MPH, C P G Kumar PhD, R Sabarinathan BE, V S Kumar PhD, E R Dinesh MSc, T Karunakaran MSc, C Govindhasamy MSc, T D Rajasekar MSc, A Jeyakumar MPhil, A Suresh MA, D Augustine BA, P A Kumar BA); **ICMR-Regional Medical Research Centre, Northeast Region, Dibrugarh, India** (S A Khan PhD); **Science Health Allied Research Education India, Hyderabad, India** (R R Allam MBBS, S Bitragunta MAE); **ICMR-National Institute of Research in Tribal Health, Jabalpur, India** (P Barde PhD); **ICMR-Regional Medical Research Centre, Bhubaneswar, India** (B Dwibedi MD, P K Sahoo PhD); **ICMR-National Institute of Cholera and Enteric Diseases, Kolkata, India** (S Kanungo PhD, P Sadhukhan PhD, S Dutta PhD); **King George's Medical University, Lucknow, India** (U Mohan MD, C M Mishra MBBS, S K Singh MD); **ICMR-Desert Medicine Research Centre, Jodhpur, India** (S S Mohanty PhD, G S Toteja PhD); **ICMR-National Institute of Traditional**

Introduction

Dengue is the most rapidly spreading vector-borne disease globally. The Global Burden of Disease study[1] estimated that dengue accounted for 1·14 million (0·73 million–1·98 million) disability-adjusted life-years in 2013, with the southeast Asia region contributing 52% of the disease burden. India contributed to 34% of the 96 million apparent dengue virus (DENV) infections estimated to have occurred globally in 2010.[2] Most Indian states have been classified as having frequent or continuous risk of dengue transmission.[3] A meta-analysis[4] of published studies from India estimated a dengue case-fatality ratio of 2·6% (95% CI 2·0–3·4). Although dengue is a notifiable disease in India, studies and modelling estimates[5–8] suggest that the disease is grossly under-reported. Using surveillance data, WHO estimated that 12 484 dengue cases occurred in India in 2010, whereas 32 million apparent cases were estimated based on mathematical models.[2] Another study[6] reported that the actual number of cases in the country were 282 times the number reported by the national vector-borne disease control programme.

The dengue disease burden in India is poorly quantified.[4] Existing public health surveillance systems are not sensitive; mild febrile illnesses are less likely

Medicine, Belagavi, India (S Roy PhD)**; Postgraduate Institute of Medical Education and Research, Chandigarh, India** (V Sagar PhD, R Kumar MD, P V M Lakshmi MD)**; ICMR-National Institute of Malaria Research, New Delhi, India** (D Savargaonkar MBBS, C P Yadav PhD)**; ICMR-National Institute of Virology, Pune, India** (B V Tandale MD, G Sapkal PhD, A Bhagat MSc, R Srivastava MSc)**; ICMR-Rajendra Memorial Research Institute of Medical Sciences, Patna, India** (R K Topno MBBS)**; Department of Health and Family Welfare, Government of Punjab, Punjab, India** (G S Grover MD)**; and Epidemiology and Communicable Diseases Division, ICMR, New Delhi, India** (N Gupta PhD, S M Mehendale MD)

Correspondence to:
Dr Manoj V Murhekar, ICMR-National Institute of Epidemiology, Ayapakkam, Chennai 600077, India
mmurhekar@nieicmr.org.in

See **Online** for appendix

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.

Implications of all the available evidence

Evidence on seroprevalence of dengue infection would be useful for making informed decisions about the introduction of upcoming dengue vaccines in the country.

to be diagnosed and reported. The data from the private sector, where most patients seek care, largely remains untapped. Moreover, surveillance systems are not designed to capture subclinical infections, which account for about 75% of dengue infections.[9] No population-based studies have been done that estimate incidence of dengue in India.[4] Well designed population-based seroprevalence studies could provide information about dengue burden by age, sex, and region.

In India, case detection, case management, and integrated vector control are the main strategies for dengue prevention and control. Several dengue vaccine candidates are in different phases of development.[10] The first dengue vaccine, CYD-TDV (Dengvaxia), developed by Sanofi Pasteur, has now been recommended for use among individuals aged 9–45 years.[11] In 2016, WHO recommended introduction of this vaccine in geographical settings with high burden of disease, as indicated by dengue seroprevalence of 70% or higher. This recommendation was revised in 2018, with prevaccination screening and vaccination of people with past evidence of infection as the preferred strategy. If this strategy is not feasible, vaccination without individual screening could be considered in areas with a seroprevalence of 80% or higher by the age of 9 years.[12,13]

Very few studies, however, are available about dengue seroprevalence in India. These studies were either done on a conveniently selected sample[14] or were limited to a few cities.[15,16] Given the limitation of available data to support policy for introduction of a dengue vaccine, we did a nationally representative survey among individuals aged 5–45 years to estimate age-specific seroprevalence of dengue infections in India.

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),[17] and household size (Census of India, 2011),[18] a minimum of 107 households were required to be enumerated to recruit at least 25 individuals in each of the three age groups (appendix).[19] 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.

Procedures

A venous blood specimen of 3 mL was collected from all the consenting participants; the serum was separated at the nearest government health facility and transported to the respective implementing institutes under cold chain and stored at –20°C. At the end of the survey, sera were transported to the ICMR-NIE under cold chain.

All sera were tested for IgG antibodies against dengue with Panbio Dengue IgG indirect ELISA (Standard Diagnostics, Yongin-si, South Korea). Panbio units (PU) were calculated by dividing specimen absorbance by the cutoff value given by the manufacturer and then multiplying by 10. Samples were considered positive with a PU of more than 11, were considered negative with a PU of less than 9, and were considered equivocal with a PU between 9–11. Equivocal samples were retested with the same assay. Specimens that were equivocal on repeat testing were considered as negative.

Using systematic random selection with computer generated random numbers, the 500 randomly selected sera (100 from each geographical region) were tested by plaque reduction neutralisation test ($PRNT_{90}$) against four DENV serotypes at the ICMR-National Institute of Virology (Pune, India) according to the procedure described by Russell and colleagues (appendix).[20,21] $PRNT_{90}$ titre of 1:10 or more to at least one dengue serotype was considered seropositive. A monotypic response was defined by the presence of neutralising antibodies against only one DENV serotype, while concomitant detection of neutralising antibodies against more than one dengue serotype was considered as a multitypic response.

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 $PRNT_{90}$ 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.[22] FOI is defined as the rate at which susceptible individuals are infected.[22] 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.[23] We fitted two different models

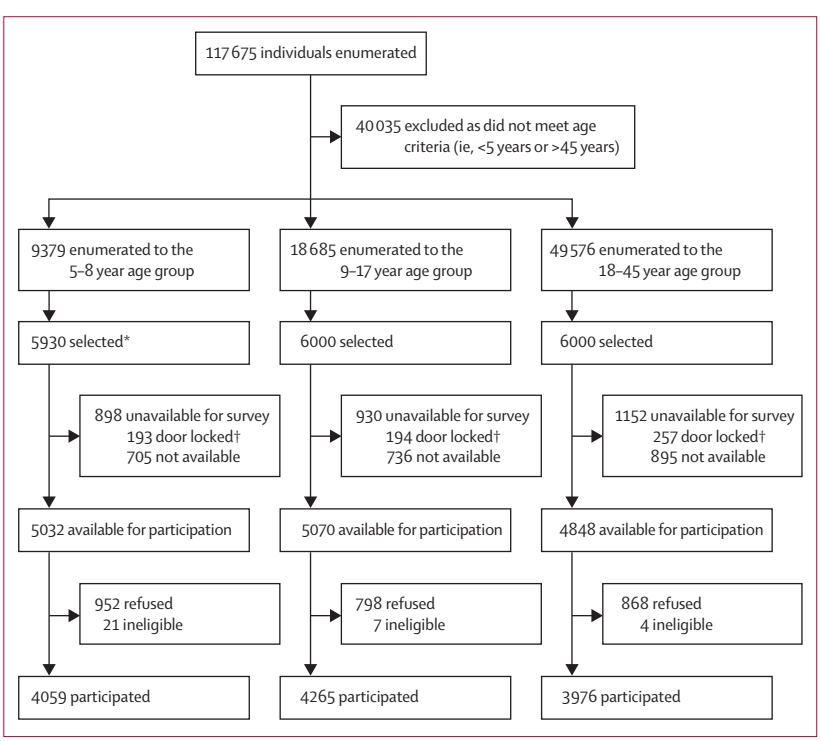


Figure 1: **Study profile**
*In 15 clusters, the number of enumerated children was <25. †The houses were locked, hence the eligible person (who was randomly selected) could not be interviewed.

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]

	Number of participants (n=12 300)
Age group, years	
5–8	4059 (33·0%)
9–17	4265 (34·7%)
18–45	3976 (32·3%)
Age, years	13 (8–23)
Sex	
Male	5813 (47·3%)
Female	6487 (52·7%)
Religion	
Hindu	9374 (77·9%)
Muslim	1254 (10·4%)
Christian	677 (5·6%)
Sikh	610 (5·1%)
Others	126 (1·0%)
Not mentioned	259
Caste	
General	3793 (31·5%)
Other backward caste	4373 (36·3%)
Scheduled caste	2306 (19·2%)
Scheduled tribe or nomadic tribe	1569 (13·0%)
Not mentioned	259
Education	
No education	1005 (8·2%)
≤5 years (primary school)	5276 (43·1%)
6–8 years (middle school)	2544 (20·8%)
9–10 years (secondary school)	1740 (14·2%)
11–12 years (higher secondary school)	1066 (8·7%)
Diploma or degree	598 (4·9%)
Don't know	13 (0·1%)
No data	58
Area of residence	
Rural	6237 (50·7%)
Urban	6063 (49·3%)
Duration of stay at this house, years	22 (12–40)
Have below poverty line card (n=12 039)	5249 (43·6%)

Data are n (%) or median (IQR).

Table 1: **Sociodemographic characteristics of the population surveyed**

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 12 300 sera tested, 5338 were positive for IgG antibodies against dengue (PU >11), with the weighted overall seroprevalence of 53·0% (95% CI 47·6–58·2; appendix).

Of the 500 sera tested for $PRNT_{90}$, 215 (43%) had IgG antibodies against dengue. Considering $PRNT_{90}$ 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% [55·3–68·8]) and the northern (60·3% [49·3–70·5]) 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.

	Northern region (n=2402)	Northeastern region (n=2360)	Eastern region (n=2486)	Western region (n=2336)	Southern region (n=2716)	All regions (n=12 300)
Age group, years						
5–8	794 (47·0% [33·7–60·7])	722 (1·6% [0·5 -5·1])	815 (5·4% [3·0–9·8])	768 (27·0% [17·5–39·1])	960 (46·4% [36·3–56·9])	4059 (28·3% [21·5–36·2])
9–17	826 (57·8% [41·0–73·0])	805 (1·2% [0·3–4·8])	874 (7·4% [4·2–12·6])	824 (48·5% [39·4–57·8])	936 (69·6% [56·7–80·0])	4265 (41·0% [32·4–50·1])
18–45	782 (64·4% [47·7–78·2])	833 (7·3% [5·0–10·5])	797 (25·4% [20·3–31·3])	744 (76·4% [67·6–83·4])	820 (84·0% [71·9–91·5])	3976 (56·2% [49·0–63·1])
Sex						
Male	1145 (59·5% [52·2–66·3])	1028 (8·7% [4·3–16·8])	1192 (24·4% [19·8–29·7])	1159 (63·7% [56·4–70·4])	1289 (75·9% [66·3–83·5])	5813 (50·9% [46·8–55·1])
Female	1257 (61·3% [46·7–74·1])	1332 (3·3% [1·4–7·9])	1294 (14·7% [11·1- 19·2])	1177 (61·5% [53·6–68·9])	1427 (77·7% [69·9–83·9])	6487 (47·5% [40·8–54·3])
Area of residence						
Rural	1117 (53·1% [38·1–67·5])	1196 (4·6% [2·8–7·5])	1280 (17·1% [13·3–21·7])	1229 (58·3% [49·7–66·5])	1415 (72·4% [62·3–80·6])	6237 (42·3% [36·0–48·9])
Urban	1285 (75·9% [64·7–84·4])	1164 (9·8% [5·6–16·6])	1206 (27·8% [20·6–36·2])	1107 (79·1% [72·3–84·6])	1301 (87·3% [79·6–92·4])	6063 (70·9% [64·3–76·6])
All age groups, years						
5–45	2402 (60·3% [49·3–70·5])	2360 (5·0% [3·3–7·6])	2486 (18·3% [14·8–22·4])	2336 (62·3% [55·3–68·8])	2716 (76·9% [69·1–83·2])	12 300 (48·7% [43·5–54·0])

Data are n (% [95% CI]), where n is the number of sera tested and % is the seroprevalence. An optimised cutoff was used and sera samples with ≥15 Panbio units were considered as positive.

Table 2: **Seroprevalence of IgG antibodies against dengue virus in different geographic regions of India, by selected sociodemographic characteristics**

	Northern region	Northeastern region	Eastern region	Western region	Southern region	All regions
Number positive for dengue neutralising antibodies	51 (21·9%)	25 (10·7%)	23 (9·9%)	64 (27·5%)	70 (30·0%)	233 (46·6%)
Monotypic	13 (25·5%)	19 (76·0%)	10 (43·5%)	13 (20·3%)	9 (12·9%)	64 (27·5%)
DENV-1	4	0	6	3	0	13
DENV-2	4	1	1	1	2	9
DENV-3	3	18	0	6	7	34
DENV-4	2	0	3	3	0	8
Multitypic	38 (74·5%)	6 (24·0%)	13 (56·5%)	51 (79·7%)	61 (87·1%)	169 (72·5%)
DENV-1 and DENV-2	4	0	3	2	2	11
DENV-1 and DENV-3	0	1	2	2	6	11
DENV-1 and DENV-4	0	0	0	0	0	0
DENV-2 and DENV-3	1	1	0	3	5	10
DENV-2 and DENV-4	1	0	1	0	0	2
DENV-3 and DENV-4	0	2	0	2	0	4
DENV-1, DENV-2, and DENV-3	17	2	5	12	29	65
DENV-1, DENV-2, and DENV-4	1	0	0	0	0	1
DENV-1, DENV-3, and DENV-4	1	0	0	5	4	10
DENV-2, DENV-3, and DENV-4	0	0	0	1	0	1
DENV-1, DENV-2, DENV-3, and DENV-4	13	0	2	24	15	54
Frequency of serotypes						
DENV-1	40	3	17	49	56	165
DENV-2	41	4	12	43	53	153
DENV-3	35	24	8	56	66	189
DENV-4	18	2	6	35	19	80

Data are n or n (%). 100 randomly selected sera from each geographic region were tested for PRNT.

Table 3: **Distribution of dengue serotype-specific neutralising antibodies by region in India, 2017**

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

	Constant force of infection, λ		Age-dependent force of infection					
	5–45 years estimate (95% CI)	Akaike information criterion	5–8 years estimate (95% CI)	9–17 years estimate (95% CI)	18–45 years estimate (95% CI)	Akaike information criterion	R_0 (95% CI)	SP9 (95% CI)
Northern region	0·05206 (0·0491–0·0551)	420·09	0·09061 (0·0810–0·1001)	0·03629 (0·0085–0·0641)	0·01367 (0·0013–0·0260)	146·891	2·49 (1·61–3·37)	53·3 (48·1–57·9)
Northeastern region	0·00232 (0·0018–0·0028)	136·236	0·00229 (0·00094–0·0036)	0·00315 (0·0013–0·0050)	0·00204 (0·00053–0·0046)	131·384	0·13 (0·05–0·22)	2·12 (0·87–3·32)
Eastern region	0·01110 (0·0100–0·0122)	191·674	0·00943 (0·0068–0·0121)	0·00438 (0·0075–0·0137)	0·01863 (0·0130–0·0242)	117·995	0·53 (0·45–0·82)	7·67 (6·00–10·5)
Western region	0·06375 (0·0601–0·0674)	204·407	0·07213 (0·0638–0·0805)	0·06387 (0·0312–0·0966)	0·03455 (0·0158–0·0533)	111·888	2·61 (1·69–3·52)	47·3 (41·8–52·3)
Southern region	0·08278 (0·0785–0·0872)	237·856	0·09605 (0·0869–0·1051)	0·04066 (0·00301–0·0783)	0·03348 (0·0117–0·0553)	105·858	3·48 (2·08–4·88)	55·4 (50·3–60·1)

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

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

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 0·002 in northeastern, 0·011 in the eastern, 0·052 in the northern, 0·064 in the western, and 0·083 in the southern region. These results imply that on average 0·23% of the susceptible population in the northeastern region, 1·1% in the eastern region, 5·07% in the northern region, 6·18% in the western region, and 7·94% in the southern region seroconverted every year. The FOI with the age dependent model among 5–8 years ranged between 0·07–0·09 in the southern, northern, and western regions, and 0·002–0·009 in the eastern and northeastern regions (table 4; figure 2A–E). The estimated transmission intensity, as measured through SP9, was very low in the northeastern and eastern regions, moderate in western region, and high in the northern and southern regions (table 4).

With the constant FOI model, we estimated that a total 12 991 357 (95% CI 12 825 128–13 130 258) primary dengue infections occurred among individuals aged 5–45 years from 30 Indian states covering five regions in 2017. The corresponding number for the age-dependent FOI model was 8 655 425 (7 243 630–9 545 052; appendix).

Discussion

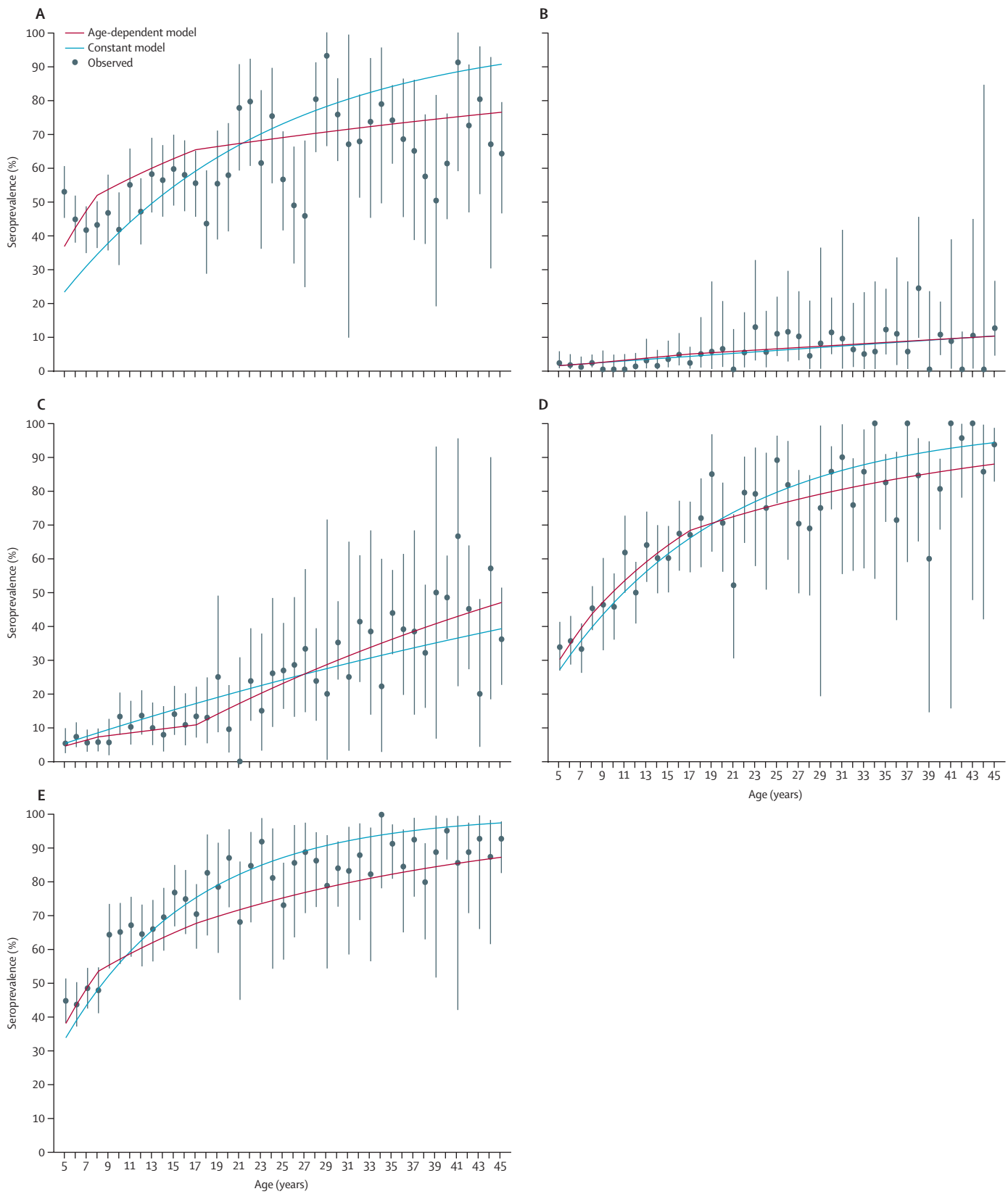
This serosurvey was initiated based on the WHO's initial recommendation of generating nationally representative seroprevalence data to guide decisions about introduction of Dengvaxia in India. The survey findings indicated that 49% of country's population had been previously infected with DENV, although 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%. Although WHO's recommendations about Dengvaxia has changed to prevaccination screening or vaccination in areas with seroprevalence more than 80%,[12,13] the findings of our serosurvey could be useful in optimising age group and geographical regions targeted for test and vaccination programmes.[25] 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,[2] 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.[26]

The sociodemographic characteristics of the sample surveyed in our study were similar to the data from Census of India (2011)[27] or the National Health and Family Survey-4 (2015–16),[28] 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

Figure 2: **Observed and model-predicted seroprevalence of dengue by age**
Data presented with 95% CIs. (A) Northern region. (B) Northeastern region. (C) Eastern region. (D) Western region. (E) Southern region.


A
B
C
D
E
Age-dependent model
Constant model
Observed
Seroprevalence (%)
0
10
20
30
40
50
60
70
80
90
100
5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45
Age (years)

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.[4,29] Studies have observed population growth, rapid urbanisation, globalisation, climate change, and ineffective mosquito control as the major drivers of dengue epidemic.[30,31]

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 monotypic 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;[9] 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. Imai and Ferguson,[25] 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.[14] 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.[25]

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, TDR, 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.

Acknowledgments

The study was funded by the Indian Council of Medical Research. We thank the Director General of the Indian Council of Medical Research for his support and encouragement for the study. We thank K Ramachandran for his critical inputs on survey design. MVM, MSK, and VSK thank Hannah Clapham for guidance in developing the dengue force of infection models and providing critical inputs on the model. We thank Vasna Joshua, T Magesh, Annamma Jose, G-H Arun, P Jayasree, K Gayathri, K Satishkumar, K Ramu, D Murugan, I Kalaimani, P Lourdu Stella Mary, V Ramachandran, G Elavarasu, S Satheesh Kumar, M Mohanraj, P Vaishnavi, S Ragupathi, D Nallathambi for technical support, and Sudipto Bora, Purvita Chowdhury, Varun Shahi, Satinder Bharti, Lakhwinder Singh, Rajat Chauhan, Suchandra Chowdhury, Arshad Shamim, and Pravin Suryakantrao Deshmukh for their support in field operations. We thank all the state and district health officials who supported and facilitated field operations. We thank all the study participants for taking part in the study.

References

1 Stanaway JD, Shepard DS, Undurraga EA, et al. The global burden of dengue: an analysis from the Global Burden of Disease Study 2013. *Lancet Infect Dis* 2016; **16:** 712–23.

2 Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. *Nature* 2013; **496:** 504–07.

3 Jentes ES, Lash RR, Johansson MA, et al. Evidence-based risk assessment and communication: a new global dengue-risk map for travellers and clinicians. *J Travel Med* 2016; **23:** taw062.

4 Ganeshkumar P, Murhekar MV, Poornima V, et al. Dengue infection in India: a systematic review and meta-analysis. *PLoS Negl Trop Dis* 2018; **12:** e0006618.

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

6 Shepard DS, Halasa YA, Tyagi BK, et al. Economic and disease burden of dengue illness in India. *Am J Trop Med Hyg* 2014; **91:** 1235–42.

7 Bagcchi S. Dengue surveillance poor in India. *Lancet* 2015; **386:** 1228.

8 Neuberger A, Turgeman A, Lustig Y, Schwartz E. Dengue fever among Israeli expatriates in Delhi, 2015: implications for dengue incidence in Delhi, India. *J Travel Med* 2016; **23:** taw003.

9 WHO. Informing vaccination programs: a guide to the design and conduct of dengue serosurveys. Geneva: World Health Organization, 2017. https://apps.who.int/iris/bitstream/handle/10665/252850/9789241512589-eng.pdf?sequence=1&isAllowed=y (accessed April 30, 2019).

10 Vannice KS, Durbin A, Hombach J. Status of vaccine research and development of vaccines for dengue. *Vaccine* 2016; **34:** 2934–38.

11 Hadinegoro SR, Arredondo-Garcia JL, Capeding MR, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. *N Engl J Med* 2015; **373:** 1195–206.

12 Wilder-Smith A, Hombach J, Ferguson N, et al. Deliberations of the strategic advisory group of experts on immunization on the use of CYD-TDV dengue vaccine. *Lancet Infect Dis* 2019; **19:** e31–38.

13 WHO. Dengue vaccine: WHO position paper—September 2018. *Wkly Epidemiol Rec* 2018; **93:** 457–76.

14 Garg S, Chakravarti A, Singh R, et al. Dengue serotype-specific seroprevalence among 5- to 10-year-old children in India: a community-based cross-sectional study. *Int J Infect Dis* 2017; **54:** 25–30.

15 Rodriguez-Barraquer I, Solomon SS, Kuganantham P, et al. The hidden burden of dengue and chikungunya in Chennai, India. *PLoS Negl Trop Dis* 2015; **9:** e0003906.

16 Mishra AC, Arankalle VA, Gadhave SA, et al. Stratified sero-prevalence revealed overall high disease burden of dengue but suboptimal immunity in younger age groups in Pune, India. *PLoS Negl Trop Dis* 2018; **12:** e0006657.

17 Government of India. Office of the Registrar General and Census Commissioner, Minstry of Home Affairs. SRS bulletin. Sample registration system, volume 50, no. 1. July, 2016. http://www.censusindia.gov.in/vital_statistics/SRS_Bulletin_2014.pdf (accessed May 20, 2019).

18 Government of India. Office of the Registrar General and Census Commissioner, Minstry of Home Affairs. HH-1 normal households by household size (census 2011). http://www.censusindia.gov.in/2011census/hh-series/HH-1/DDW-HH01-0000-2011.XLS (accessed May 20, 2019).

19 WHO. World Health Organization vaccination coverage cluster surveys: reference manual. Geneva: World Health Organization, 2018. https://apps.who.int/iris/bitstream/handle/10665/272820/WHO-IVB-18.09-eng.pdf?ua=1 (accessed April 30, 2019).

20 Russell PK, Nisalak A, Sukhavachana P, et al. A plaque reduction test for dengue virus neutralizing antibodies. *J Immunol* 1967; **99:** 285–90.

21 WHO. Guidelines for plaque reduction neutralization testing of human antibodies to dengue viruses. Geneva: World Health Organization, 2007. https://apps.who.int/iris/bitstream/handle/10665/69687/who_ivb_07.07_eng.pdf?sequence=1&isAllowed=y (accessed April 30, 2019).

22 Ferguson NM, Donnelly CA, Anderson RM. Transmission dynamics and epidemiology of dengue: insights from age-stratified sero-prevalence surveys. *Philos Trans R Soc Lond B Biol Sci* 1999; **354:** 757–68.

23 Tam CC, Tissera H, de Silva AM, et al. Estimates of dengue force of infection in children in Colombo, Sri Lanka. *PLoS Negl Trop Dis* 2013; **7:** e2259.

24 Flasche S, Jit M, Rodriguez-Barraquer I, et al. The long-term safety, public health, impact, and cost-effectiveness of routine vaccination with a recombinant, live-attenuated dengue vaccine (Dengvaxia): a model comparison study. *PLoS Med* 2016; **13:** e1002181.

25 Imai N, Ferguson NM. Targeting vaccinations for the licensed dengue vaccine: considerations for serosurvey design. *PLoS One* 2018; **13:** e0199450.

26 Government of India. Ministry of Health and Family Welfare. National vector borne disease control programme. Dengue/DHF situation in India. http://nvbdcp.gov.in/index4.php?lang=1&level=0&linkid=431&lid=3715 (accessed April 30, 2019).

27 Government of India. Office of the Registrar General and Census Commissioner, Ministry of Home Affairs. Population enumeration data 2011. http://www.censusindia.gov.in/2011census/population_enumeration.html (accessed April 30, 2019).

28 Government of India. Ministry of Health and Family Welfare. National Family Health Survey (NFHS-4) 2015–16. India fact sheet. http://rchiips.org/nfhs/pdf/NFHS4/India.pdf (accessed April 30, 2019).

29 Chakravarti A, Arora R, Luxemburger C. Fifty years of dengue in India. *Trans R Soc Trop Med Hyg* 2012; **106:** 273–82.

30 Struchiner CJ, Rocklov J, Wilder-Smith A, Massad E. Increasing dengue incidence in Singapore over the past 40 years: population growth, climate and mobility. *PLoS One* 2015; **10:** e0136286.

31 Gubler DJ. Dengue, urbanization and globalization: the unholy trinity of the 21(st) century. *Trop Med Health* 2011; **39** (suppl 4)**:** 3–11.