SARS-CoV-2 antibody seroprevalence in India, August–September, 2020: findings from the second nationwide household serosurvey

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Summary

Background The first national severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) serosurvey in India, done in May–June, 2020, among adults aged 18 years or older from 21 states, found a SARS-CoV-2 IgG antibody seroprevalence of 0·73% (95% CI 0·34–1·13). We aimed to assess the more recent nationwide seroprevalence in the general population in India.

Methods We did a second household serosurvey among individuals aged 10 years or older in the same 700 villages or wards within 70 districts in India that were included in the first serosurvey. Individuals aged younger than 10 years and households that did not respond at the time of survey were excluded. Participants were interviewed to collect information on sociodemographics, symptoms suggestive of COVID-19, exposure history to laboratory-confirmed COVID-19 cases, and history of COVID-19 illness. 3–5 mL of venous blood was collected from each participant and blood samples were tested using the Abbott SARS-CoV-2 IgG assay. Seroprevalence was estimated after applying the sampling weights and adjusting for clustering and assay characteristics. We randomly selected one adult serum sample from each household to compare the seroprevalence among adults between the two serosurveys.

Findings Between Aug 18 and Sept 20, 2020, we enrolled and collected serum samples from 29 082 individuals from 15 613 households. The weighted and adjusted seroprevalence of SARS-CoV-2 IgG antibodies in individuals aged 10 years or older was 6·6% (95% CI 5·8–7·4). Among 15 084 randomly selected adults (one per household), the weighted and adjusted seroprevalence was 7·1% (6·2–8·2). Seroprevalence was similar across age groups, sexes, and occupations. Seroprevalence was highest in urban slum areas followed by urban non-slum and rural areas. We estimated a cumulative 74·3 million infections in the country by Aug 18, 2020, with 26–32 infections for every reported COVID-19 case.

Interpretation Approximately one in 15 individuals aged 10 years or older in India had SARS-CoV-2 infection by Aug 18, 2020. The adult seroprevalence increased approximately tenfold between May and August, 2020. Lower infection-to-case ratio in August than in May reflects a substantial increase in testing across the country.

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Introduction

As of Sept 30, 2020, India reported the second highest number of COVID-19 cases in the world, amounting to nearly 6·3 million cases and more than 97 000 deaths.[1] Case reporting is influenced by strategies implemented for case finding, testing, and contact tracing, and might underestimate the true burden of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Population-based data can supplement case-based surveillance to inform public health measures. Population-based seroepidemiological studies are useful to measure the extent of SARS-CoV-2 infection and the effect of ongoing public health responses in controlling the pandemic.[2]

The first nationwide SARS-CoV-2 serosurvey in India was done in May–June, 2020, when the entire country

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See Online for appendix

Research in context

Evidence before this study

The seroprevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies is important to understand the transmission dynamics of the virus; estimate total infections, including mild and asymptomatic individuals who might not receive testing; and inform the possibility of transmission interruption through the depletion of susceptible individuals, if seroconversion is associated with robust immunity. We reviewed the evidence for the seroprevalence of SARS-CoV-2 available as of Sept 30, 2020, by searching the National Library of Medicine article database and *medRxiv* for preprint publications, published in English, using the terms "serology", "seroconversion", "serosurveillance", "seroepidemiology", "seroprevalence", "seropositivity", "SARS-CoV-2", and "COVID-19". Several studies describing the seroprevalence of SARS-CoV-2 had been done across various geographical areas, using different sampling and recruitment strategies, as well as a range of testing approaches. Most studies were limited to smaller subnational areas, few were representative of the population as a whole, and potential sources of bias included the method of participant selection, non-response rates, and misclassification resulting from test specificity, particularly when the prevalence was low. The first national SARS-CoV-2 serosurvey in India indicated an overall low seroprevalence among adults by May, 2020, and the majority of infections were in people living in urban areas, with an estimated 82–130 infections for every reported COVID-19 case.

Added value of this study

India represents one of the largest populations at risk of COVID-19 and as of Sept 30, 2020, had reported the second highest number of confirmed cases globally. Because of India's large size, geographical diversity, and population heterogeneity, it is difficult to understand the extent of transmission of SARS-CoV-2 using case-based surveillance data alone. Furthermore, Indian cities represent challenging conditions for COVID-19 control, with some of the world's highest population densities and contact rates. This population-based study represents seroprevalence at the national level, covering many areas across India's large expanse. Our findings indicate an overall seroprevalence of around 7% among individuals aged 10 years or older, with a tenfold increase in adult seroprevalence between May and August, 2020. We estimated that for every reported case of COVID-19 there were 26–32 infections, and the infection-fatality ratio in surveyed districts was 0·09–0·11%. We found no difference in seropositivity by age group, sex, or occupation. Our findings indicate a substantial transmission in rural areas, although seroprevalence continues to be higher in urban slum and non-slum areas. We also found evidence of seroconversion among those without symptoms or known exposure, highlighting the limitations of symptom-directed or exposure-directed testing.

Implications of all the available evidence

The increasing national seroprevalence in India suggests a growing epidemic moving from urban to rural areas, but most of the population remain susceptible to infection. Continued expansion of testing capacity and stringent application of infection control measures remain warranted. Further rounds of the national serosurvey are planned and should provide crucial information on the rate of seroconversion, informing overall public health strategy and action.

was under stringent lockdown, with the exception of conditional relaxation in areas deemed to be minimally affected.[3] It found a low seroprevalence of 0·73% (95% CI 0·34–1·13) among the general adult population aged 18 years or older.[4] Notably, this serosurvey found a high infection-to-case ratio (81·6–130·1 infections per reported COVID-19 case), suggesting the need for a further expansion of testing, and a low infection-fatality ratio (0·27–15·04 deaths per 10 000 infections). From June, 2020, onwards, India had various phases of relaxation of lockdown measures that varied across the states, depending on the local epidemic situation.[3]

We aimed to do a second national household serosurvey to measure changes in the epidemiology of SARS-CoV-2 infection, compare changes in population-based indicators for infection, and assess the effect of the public health response to the epidemic in India. The objectives of the second serosurvey were to estimate the nationwide seroprevalence of SARS-CoV-2 antibodies in the general population, including by age group, sex, area of residence, occupation, and COVID-19-related characteristics, and determine the trends in infections since the previous serosurvey.

Methods

Study design and participants

We did a cross-sectional serosurvey in the same 700 clusters (villages in rural areas and wards in urban areas) from 70 districts in 21 states across India that were included in the first nationwide serosurvey (appendix pp 4–8), between Aug 18 and Sept 20, 2020. Within each of the selected clusters, four random locations were selected. In each location, the survey teams chose a random starting point and visited a minimum of four consecutive households. The survey teams listed all household members aged 10 years or older who were permanent residents, and all eligible individuals present in the household at the time of the survey team visit were invited to participate. Individuals aged younger than 10 years and households that did not respond at the time of survey were excluded. From each random location, at least ten individuals were enrolled

in the serosurvey. By selecting ten clusters per district, a minimum of 400 individuals were enrolled from each district.

We obtained written informed consent from individuals aged 18 years or older, or assent from children aged between 10 and 17 years, with written informed consent from their parents or guardians, before the survey. The study protocol was approved by the Central Ethics Committee of Health Research of the Indian Council of Medical Research (ICMR) and the Institutional Human Ethics Committee of the ICMR National Institute of Epidemiology, Chennai.

Procedures

Eligible participants were interviewed to collect information about sociodemographic details, symptoms suggestive of COVID-19 since March 1, 2020 (eg, fever, cough, shortness of breath, sore throat, new loss of taste or smell, fatigue), exposure history to laboratory-confirmed COVID-19 cases, and history of COVID-19 illness using the Open Data Kit mobile phone application. 3–5 mL of venous blood was collected from each participant, and centrifuged serum samples were transported to ICMR National Institute of Epidemiology, Chennai under cold chain.

Participant serum samples were tested for the presence of SARS-CoV-2 specific IgG antibodies on the Abbott Architect i2000SR automated analyser using the Abbott SARS-CoV-2 IgG assay (Abbott Park, IL, USA) as per the manufacturer's instructions. This assay detects IgG antibodies against the SARS-CoV-2 nucleocapsid protein, and has a sensitivity of 100·0% and specificity of 99·6%.[5] The assay was calibrated with positive and negative quality controls before analyses. Assay results higher than or equal to the cutoff index value of 1·4 were interpreted as positive for SARS-CoV-2 antibodies. As a part of quality control, 10% of positive serum samples and an equal number of negative serum samples were re-tested using the same assay.

Statistical analysis

We described the characteristics of study participants as percentages, means, and SDs. We categorised the reported occupations into high-risk and low-risk categories, on the basis of the potential risk of exposure to a known or unknown COVID-19 case. For example, occupations such as health-care workers, police or security personnel, shopkeepers, bus or taxi drivers, or bank employees were considered as high-risk occupations; whereas, for example, farmers, retired employees, students, or information technology professionals were considered as being at lower risk of exposure. The information about occupation of the participants was captured as open-ended text and was categorised into high and low risk by the investigators. The data were analysed to estimate the seroprevalence of IgG antibodies against SARS-COV-2 with 95% CI, using a random-effects model to account for cluster sampling. To estimate the weighted seroprevalence, we calculated sampling weights as a product of the inverse of the sampling fraction for the selection of districts and the selection of villages or wards from each district. The weighted seroprevalence was further adjusted for the sensitivity and specificity of the assay.[6] We also calculated the seroprevalence by age group, sex, area of residence, and COVID-19-related characteristics of study participants. In the first serosurvey, only one adult aged 18 years or older was randomly selected from each household,[4] whereas in this second serosurvey, all consenting individuals aged 10 years or older were sampled. To compare the seroprevalence between the first and second nationwide surveys, we randomly selected one adult per household from the survey database, and estimated the adjusted seroprevalence among these adults. We calculated the infection doubling time among adults using the observed seroprevalence, and difference in time between the median survey dates of the two serosurveys.[7] We obtained a non-linear correlation coefficient by fitting polynomial curves for the IgG positivity and cumulative incidence of reported COVID-19 cases by districts.

For more on **Open Data Kit** see https://getodk.org/

To estimate the total number of SARS-CoV-2 infections among individuals aged 10 years or older, we multiplied the adjusted seroprevalence in the population aged 10 years or older in the selected 70 districts, by the total population of the entire country aged 10 years or older. We divided the estimated number of infections by the number of reported COVID-19 cases detected by RT-PCR or rapid antigen test, at 1 week (Aug 18, 2020) and 2 weeks (Aug 10, 2020) before the median survey date (Aug 25, 2020) to estimate the infection-to-case ratio. As the number of COVID-19 cases reported nationally are not specified by the type of tests (eg, positive by RT-PCR, positive by rapid antigen test, or negative by rapid antigen test but RT-PCR positive), it was not possible to adjust the total number of reported cases to account for the lower sensitivity of the rapid antigen test. We estimated the infection-to-case ratio at two different timepoints because studies indicate that IgG antibodies against SARS-CoV-2 start appearing between 7 and 14 days after symptom onset.[8]

We calculated the infection-fatality ratio for the 70 districts by dividing the number of deaths reported 3 weeks after symptom onset (assuming a 3 week lag time from infection to death),[9] by the estimated number of SARS-CoV-2 infections in the selected 70 districts. The data were analysed using STATA version 16.1, and R version 3.5.1 (appendix p 2).

Role of the funding source

The funder of the study was involved in reviewing the study design, writing of the manuscript, and the decision to submit the paper for publication. All authors had access to all the data in the study and had final responsibility for the decision to submit for publication.

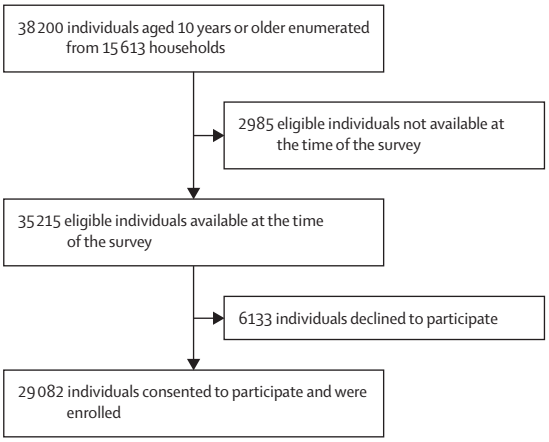

Figure 1: **Flowchart of participant enrolment**

	Participants (n=29 082)
Age, years	
10–17	3021 (10·4%)
18–44	16 663 (57·3%)
45–60	6630 (22·8%)
>60	2768 (9·5%)
Mean age, years (SD)	37·0 (16·4)
Sex	
Male	14 870 (51·1%)
Female	14 191 (48·8%)
Other	21 (0·1%)
Area of residence	
Rural	21 524 (74·0%)
Urban non-slum	4932 (17·0%)
Urban slum	2626 (9·0%)
Occupation with high risk of exposure to COVID-19 (n=29 033)	4263 (14·7%)
History of COVID-19-related symptoms since March 1, 2020	546 (1·9%)
Symptomatic individuals who sought medical care (n=545)	191 (35·0%)
Symptomatic individuals who were hospitalised (n=191)	31 (16·2%)
History of contact with a known COVID-19 case (n=29 044)	215 (0·7%)
Previously tested for COVID-19 (n=29 044)	747 (2·6%)
Previous positive COVID-19 test (n=747)	47 (6·3%)

Data are n (%) unless otherwise stated.

Table 1: **Participant characteristics**

Results

Between Aug 18 and Sept 20, 2020, we enumerated 38 200 individuals aged 10 years or older from 15 613 households in the 700 clusters. Approximately 26% of clusters were located in urban areas. 35 215 eligible individuals (92·2%) were available at the time of survey, of whom 29 082 (82·6%) consented to participate and were enrolled (figure 1).

Of the 29 082 survey participants, 16 663 (57·3%) were in the age group 18–44 years, 6630 (22·8%) were aged 45–60 years, 3021 (10·4%) were aged 10–17 years, and 2768 (9·5%) were aged older than 60 years (table 1). 14 191 participants (48·8%) were female, 21 524 (74·0%) were residing in rural areas, and 4263 (14·7%) had an occupation with a high risk of exposure to people potentially infected with COVID-19. 546 participants (1·9%) reported symptoms suggestive of COVID-19 since March, 2020, of whom 191 (35·0%) reported seeking medical care. 747 individuals (2·6%) reported having been tested for SARS-CoV-2 previously, of whom 47 (6·3%) previously had a positive COVID-19 test.

Of the 29 082 participants, 3135 tested positive for the presence of IgG antibodies against SARS-CoV-2; resulting in an unweighted seroprevalence of 10·8% (95% CI 9·8–11·8; table 2). The seropositivity across districts ranged from 0·5% (Palakkad in Kerala; Kullu in Himachal Pradesh) to 42·6% (Ganjam in Odisha; appendix pp 4–8). The weighted seroprevalence adjusted for test performance was 6·6% (95% CI 5·8–7·4).

Seroprevalence was lowest among children aged 10–17 years (5·4% [95% CI 4·5–6·4]), and highest among adults aged 18–44 years (6·9% [6·1–7·7]), but did not differ significantly between age groups, and was similar among males (6·7% [5·9–7·5]) and females (6·5% [5·7–7·3]; table 2). Seroprevalence was higher in urban slums (16·9% [12·9–21·7]) and urban non-slum areas (9·0% [7·1–11·3]) than in rural areas (5·2% [4·6–6·0]). There was no difference in seroprevalence between occupations categorised as high or low risk based on potential exposure to COVID-19. Among individuals who reported a history of symptoms suggestive of COVID-19, seroprevalence was 11·2% (8·8–14·3), compared with 6·5% (5·8–7·3) among individuals who did not report symptoms suggestive of COVID-19. However, among seropositive individuals, 99 (3·2%) reported a history of symptoms consistent with COVID-19 but 3029 (96·6%) reported no symptoms. Seroprevalence was higher among those who reported history of contact with a laboratory-confirmed COVID-19 case (12·7% [9·5–16·8]) and among those who had previously been tested for SARS-CoV-2 (11·8% [9·6–14·6]).

Individuals who were positive on previous SARS-CoV-2 testing had a higher seroprevalence (80·9% [64·5–90·7]) than those who tested negative (19·9% [15·4–25·2]) or were not aware of their result (8·6% [2·4–26·7]; table 2). Of the nine individuals with laboratory-confirmed SARS-CoV-2 infection but who were seronegative, the duration between PCR and serology testing was 1 day for two individuals, 8 days for one, 20–40 days for four, and more than 90 days for two. After excluding the three individuals with an interval of less than 2 weeks (to account for up to 2 weeks' delay in the development

of IgG antibodies between the date of laboratory confirmation and serological testing), 38 (86%) of 44 previous COVID-19 patients had IgG antibodies. The remaining six seronegative individuals were tested for COVID-19 because of their contact with a confirmed case, and none of them reported symptoms during illness. For two (33%) of these six seronegative individuals, the duration between laboratory confirmation and serological testing was more than 90 days.

Among 15 084 randomly selected adults (one from each household), 1696 were seropositive for SARS-CoV-2 IgG antibodies, resulting in an unweighted seroprevalence of 11·2% (95% CI 10·2–12·4). The weighted and adjusted seroprevalence among adults was 7·1% (6·2–8·2). Using an interval of 99 days between the two nationwide surveys, the infection doubling time was 30·2 days (95% CI 23·6–34·6).

We estimated that, among individuals aged 10 years or older, a cumulative 10 663 677 infections (95% CI 9 371 110–11 956 244) had occurred by Aug 18, 2020, in the selected 70 districts. Extrapolation to the entire country resulted in an estimation of 74 326 463 infections (65 317 195–83 335 732). Considering there were 2 339 112 reported COVID-19 cases by Aug 10, 2020, and 2 856 248 by Aug 18, 2020, we estimated there were 31·8 (95% CI 27·9–35·6) and 26·0 (22·9–29·2) infections per reported case by these respective dates. Among the 70 surveyed districts, there were 10 058 COVID-19 deaths by Aug, 31, 2020, and 11 358 deaths by Sept 8, 2020, with an infection-fatality ratio ranging from 9·43 (95% CI 8·41–10·73) to 10·65 (9·50–12·12) COVID-19 deaths per 10 000 infections. The seroprevalence had a positive non-linear correlation with the cumulative incidence of reported COVID-19 cases (correlation coefficient 0·702) in the selected 70 districts (figure 2).

	Participants tested, n	Seropositive participants, n	Unweighted seroprevalence, % (95% CI)*	Weighted seroprevalence, % (95% CI)†	Weighted seroprevalence adjusted for test performance, % (95% CI)‡
Overall	29 082	3135	10·8% (9·8–11·8)	7·0% (6·2–7·8)	6·6% (5·8–7·4)
Sex					
Male	14 870	1673	11·2% (10·2–12·4)	7·1% (6·3–7·9)	6·7% (5·9–7·5)
Female	14 191	1462	10·3% (9·3–11·4)	6·9% (6·1–7·7)	6·5% (5·7–7·3)
Other	21	0	..	..	..
Age, years					
10–17	3021	271	9·0% (7·7–10·5)	5·8% (4·9–6·8)	5·4% (4·5–6·4)
18–44	16 663	1820	10·9% (9·9–12·0)	7·3% (6·5–8·1)	6·9% (6·1–7·7)
45–60	6630	753	11·4% (10·1–12·7)	6·9% (6·1–7·9)	6·5% (5·7–7·5)
>60	2768	291	10·5% (9·0–12·3)	6·6% (5·6–7·7)	6·2% (5·2–7·3)
Area of residence					
Rural	21 524	1889	8·8% (7·8–9·8)	5·6% (5·0–6·4)	5·2% (4·6–6·0)
Urban non-slum	4932	672	13·6% (11·4–16·2)	9·4% (7·5–11·7)	9·0% (7·1–11·3)
Urban slum	2626	574	21·9% (17·7–26·6)	17·2% (13·2–22·0)	16·9% (12·9–21·7)
Occupation with high risk of exposure to COVID-19 (n=29 033)					
Yes	4263	519	12·2% (10·4–14·2)	6·9% (6·0–8·0)	6·5% (5·6–7·6)
No	24 770	2608	10·5% (9·6–11·5)	7·0% (6·2–7·8)	6·6% (5·8–7·4)
History of COVID-19-related symptoms since March 1, 2020 (n=29 045)					
Yes	546	99	18·1% (14·1–23·0)	11·6% (9·2–14·6)	11·2% (8·8–14·3)
No	28 499	3029	10·6% (9·7–11·6)	6·9% (6·2–7·7)	6·5% (5·8–7·3)
History of contact with a known COVID-19 case (n=29 044)					
Yes	215	57	26·5% (18·4–36·6)	13·0% (9·9–17·1)	12·7% (9·5–16·8)
No	25 013	2690	10·8% (9·8–11·8)	6·7% (6·0–7·5)	6·3% (5·6–7·1)
Not known	3816	381	10·0% (8·1–12·2)	8·3% (6·9–9·9)	7·9% (6·5–9·5)
Previously tested for COVID-19 (n=29 044)					
Yes	747	173	23·2% (18·5–28·6)	12·2% (10·0–14·9)	11·8% (9·6–14·6)
No	28 297	2955	10·4% (9·5–11·4)	6·2% (5·5–7·0)	5·8% (5·1–6·6)
Previous COVID-19 test result (n=747)§					
Positive	47	38	80·9% (64·5–90·7)	..	..
Negative	665	132	19·9% (15·4–25·2)	..	..
Not known	35	3	8·6% (2·4–26·7)	..	..

*Adjusted for clustering. †Weighted for sampling weights. ‡Adjusted for test performance as reported by manufacturer (sensitivity 100·0% and specificity 99·6%). §Weighted and adjusted seroprevalence not estimated because of small sample number.

Table 2: **Seroprevalence by demographic characteristics**

Discussion

Our findings from the second nationwide serosurvey indicate that nearly 7% of India's population aged 10 years or older had been exposed to SARS-CoV-2 infection by August, 2020, with an estimated 74 million infections. Seroprevalence did not differ by age group or sex, but was higher in urban areas, especially in the slums, than in rural areas.

Seroprevalence among adults increased by about ten times, from 0·7% in May, 2020, to 7·1% in August, 2020. All 70 surveyed districts showed a rise in IgG seropositivity between the two serosurveys, although the change was highly variable. Despite the study not being powered to provide reliable district-level estimates, some of the variation observed between districts matches the known context. For example, the largest increase in seropositivity was recorded in the Ganjam district, which also reported the highest number of COVID-19 cases in Odisha State, subsequent to migration of interstate and intrastate informal workers, and challenges in facility-based quarantine. Interstate migration of informal workers is also thought to explain the substantial rise in seroprevalence in all six districts in Bihar, and Kamrup Metropolitan district in Assam.[10] The increase in seropositivity among adults between the first and second serosurveys also indicates widespread infection in all districts, except for Palakkad and Kullu.

The seroprevalence of SARS-CoV-2 infection did not differ by age group or sex, indicating similar exposure and susceptibility between these groups. This absence of a significant difference was despite school closures and other non-pharmaceutical infection-control interventions (eg, washing hands, wearing masks, physical

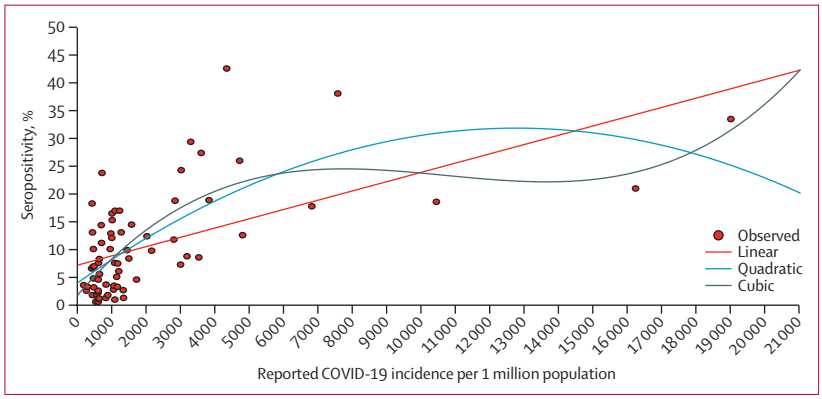

Figure 2: **SARS-CoV-2 IgG seropositivity and incidence of reported COVID-19 cases per 1 million population by district, fitted with polynomial curves**
Points on the graph represent the 70 surveyed districts. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

distancing) during the survey period, suggesting household-level exposure of children and adults aged older than 60 years to other household members who are more mobile, socially active, and perhaps non-adherent to the prescribed non-pharmaceutical measures. Seroprevalence was reported to be similar across age groups in Brazil (first survey),[11] and Spain,[12] with the seroprevalence among adults aged older than 65 years being lower than in those aged 5–65 years in Santa Clara County, CA, USA,[9] and higher in older adults in Greece[13] and Iceland.[14] Children had a lower seroprevalence compared with adults aged younger than 60 years in the second survey in Brazil.[11] A similar seroprevalence by sex to that observed in our study has been reported from serosurveys in Santa Clara County[9] and Spain,[12] but a serosurvey in Geneva, Switzerland[15] showed a higher seroprevalence among males.

During June–October, 2020, a number of serosurveys have been done in various Indian cities or states (appendix p 11). The higher seroprevalence in urban slum and non-slum areas observed in our study is consistent with that of other serosurveys in densely populated urban areas, where the prevalence ranged from 7·8% to 51·5%. The seroprevalence was also higher in slum areas of Mumbai (54·1%) compared with non-slum areas (16·1%).[16] Although population density, coupled with high mobility and challenges in safe physical distancing and hand hygiene are the main drivers of spread of infection in urban areas, especially in urban slums,[17] our findings also indicate substantial transmission among the rural population later in the epidemic, by contrast with the first serosurvey.[4] Transmission is likely to increase further in these rural areas in the coming months, emphasising the need to implement non-pharmaceutical interventions, as well as strengthening health-care facilities for the effective management of infections.[18]

One in nine individuals who reported no COVID-19-related symptoms were seropositive for SARS-CoV-2 IgG antibodies (adjusted seroprevalence 6·5% [95% CI 5·8–7·3]), indicating asymptomatic seroconversion among the general population in India. Seroconversion was also documented among individuals without a history of known contact with a COVID-19 case, and among those without any previous SARS-CoV-2 testing. These data support the expansion of testing strategies to include individuals who do not have known exposure or symptoms.[19] Only 3% of seropositive individuals reported symptoms suggestive of COVID-19, highlighting the limitations of symptom-directed testing and the importance of universal prevention methods.

Among the laboratory-confirmed patients with COVID-19 identified in our survey, only 81% of patients had SARS-CoV-2 IgG antibodies. The reasons for absence of IgG antibodies in recovered COVID-19 individuals might be due to poor B-cell response,[20,21] false-negative testing, or waning immunity over time.[22]

The laboratory infrastructure for the diagnosis of COVID-19 has been rapidly built up from one laboratory in January, 2020, to 1511 laboratories by August, 2020.[23] With the addition of rapid point-of-care antigen-detection tests and the expansion of testing criteria, test capacity and use saw further growth, resulting in more than 34 million tests having been done as of Aug 21, 2020.[23–25] The decrease in infection-to-case ratio from 82–131 in May, 2020, to 26–32 in August, 2020, is a consequence of the growth of testing outpacing the growth of infection rate. The ratio of estimated infections to reported cases in Brazil was 10·3, based on the serosurveys done in May–June, 2020.[11]

Population-based seroprevalence data are useful in understanding the current and future course of the COVID-19 pandemic. The overall seroprevalence of less than 10% in India indicates that a large proportion of the population remains susceptible to SARS-CoV-2 infection. The transmission of infection is expected to continue in most states in India until the herd immunity threshold is achieved, either by natural infection or vaccination. Although this threshold is unknown, most estimates place it at higher than 50% of the population.[26] Heterogeneity in individual susceptibility or exposure to infection, pre-existing immunity in the population, and use of non-pharmaceutical infection-control interventions might alter the required prevalence for herd immunity.[27–29] The infection doubling time at the national level was estimated to be 30·2 days (95% CI 23·6–34·6). Assuming the same rate of infection continued, the required herd immunity threshold could be estimated to be reached as of November–December, 2020. However, the duration of persistence of IgG antibodies[14] and memory B cells, and the contribution and durability of cell-mediated immunity against SARS-CoV-2 is still uncertain.[29] It is pertinent to note that the reported number of COVID-19 cases in India has been declining since October, 2020.

The infection-fatality ratio indicates the probability of death among those infected. In our study, the infection-fatality ratio ranged from 0·09% to 0·11%. A systematic

review and meta-analysis of published studies on COVID-19 as of July, 2020, indicated an infection-fatality ratio of 0·68% (95% CI 0·53–0·82).[30] Another study based on the seroprevalence data from 51 locations indicated substantial variation in infection-fatality ratios, ranging from 0·00% to 1·54%, with a median of 0·23%.[31] The lower infection-fatality ratio in our study could be accounted for by several factors, including the completeness of death reporting, variation in the prevalence of comorbidities, and the age structure of the population.[32,33] Due to the absence of age-stratified death data from these 70 districts, and as the study was not powered for age-stratified seroprevalence, we could not calculate age-stratified infection-fatality ratios.

Our study has several limitations. The representation of children aged 10–17 years in the surveyed sample was lower than the census-based age distribution in India. According to Census of India projections, about 14% of the population are aged 10–17 years,[34] whereas 10·4% of the study population were aged 10–17 years. The under-representation of children and over-representation of adults in the survey could lead to overestimation of the true seroprevalence, if we expect a real difference in the risk of exposure to SARS-CoV-2 across age groups. Although the required sample size was achieved, about 17% of the eligible population declined to participate in the survey. If this non-response was not at random, then this could introduce selection bias. Individuals who declined to participate were more likely to be male and younger than 60 years of age (appendix p 9). We adjusted our weighted seroprevalence estimate as per the manufacturer specified sensitivity (100·0%) and specificity (99·6%) of the Abbott SARS-CoV-2 IgG assay. According to an external evaluation, the sensitivity is reported as 92·7% and the specificity as 100·0%.[35] Adjusting for these figures, our estimated overall seroprevalence was 7·6% (95% CI 6·7–8·4; appendix p 10). In the first nationwide serosurvey, we used a laboratory assay which detected IgG antibodies against whole cell antigen, and positive serum samples were re-tested with an assay that detects antibodies against the S1 domain of the spike protein of SARS-CoV-2, to improve the specificity of testing.[4] In this second serosurvey, we used a laboratory assay which detected IgG antibodies against the nucleocapsid protein of the virus. Although we used different assays in the two serosurveys, we adjusted the seroprevalence to account for each assay's sensitivity and specificity. Additionally, as antibodies to the nucleocapsid protein of SARS-CoV-2 virus have been shown to reduce over time,[36] we might have underestimated the seroprevalence and number of infections. For the same reason, we might have underestimated the true difference in seroprevalence between the two serosurveys, as we used antibody assays for different viral proteins. Finally, we might have overestimated the infection-to-case ratio by using COVID-19 cases reported at 1 week and 2 weeks before the median date of survey for all clusters. About half of the 700 clusters were surveyed within the first 8 days of the study period. The remaining clusters were surveyed over the next 3 weeks, and the number of cases reported from these clusters at 1 or 2 weeks before the actual date of survey would have been higher.

In conclusion, our findings indicate that nearly one in 15 individuals aged 10 years or older were exposed to SARS-CoV-2 in India by Aug 18, 2020. Although the seroprevalence among adults increased approximately tenfold between May and August, 2020, a large proportion of the population remains susceptible to SARS-CoV-2. These findings, in combination with the first national serosurvey and other serosurvey data, give a clear picture of the epidemic in India; there is high seroprevalence in urban slums, as well as non-slum urban areas, and seroprevalence is now increasing in the vast rural areas of the country. Although the epidemic was successfully contained to the cities at the outset, the current general trend presents many forthcoming challenges. We recommend continued expansion of testing capacity to improve the infection-to-case ratio, especially in districts with high seroprevalence but low case reporting; continued application of interventions to control transmission of the virus; and health facility planning for increased caseloads throughout the country, with particular focus in rural areas. Finally, we recommend further rounds of the national serosurvey, to continue providing strategic insight into the epidemiology of the SARS-CoV-2 pandemic, and to inform public health action.

Contributors

TB, JWVT, and MSaK did the literature search. MVM, TB, KR, MSaK, NS, DCSR, and BB did the study design. SSe, RSa, SA, RB, SDB, AKB, JB, VC, DD, AKD, KRD, GRD, SMSK, MSuK, AL, MM, AMa, SSM, CR, AT, DKB, ASC, FD, IH, AK, SK, JSK, GGJNL, AMi, ARN, GVP, MAQ, SeS, RKS, KS, VKS, PKS, PS, RaS, DSV, AV, and SP did the data collection. CPGK and KS did the laboratory investigations. VS, JWVT, MVM, TB, RSa, and MSaK did the data analysis. MVM, TB, KR, NS, DCSR, and BB did the data interpretation. MVM, TB, VS, JWVT, and MSaK accessed and verified the data. MVM, TB, JWVT, NS, VS, and MSaK wrote the first draft of the manuscript. All authors approved the final version of the manuscript.

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Declaration of interests
We declare no competing interests.

Data sharing
A subset of the key anonymised individual participant data collected during the study, along with a data dictionary, is available upon request to the corresponding author, after approval of a proposal with a signed data access agreement.

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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 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.

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

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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

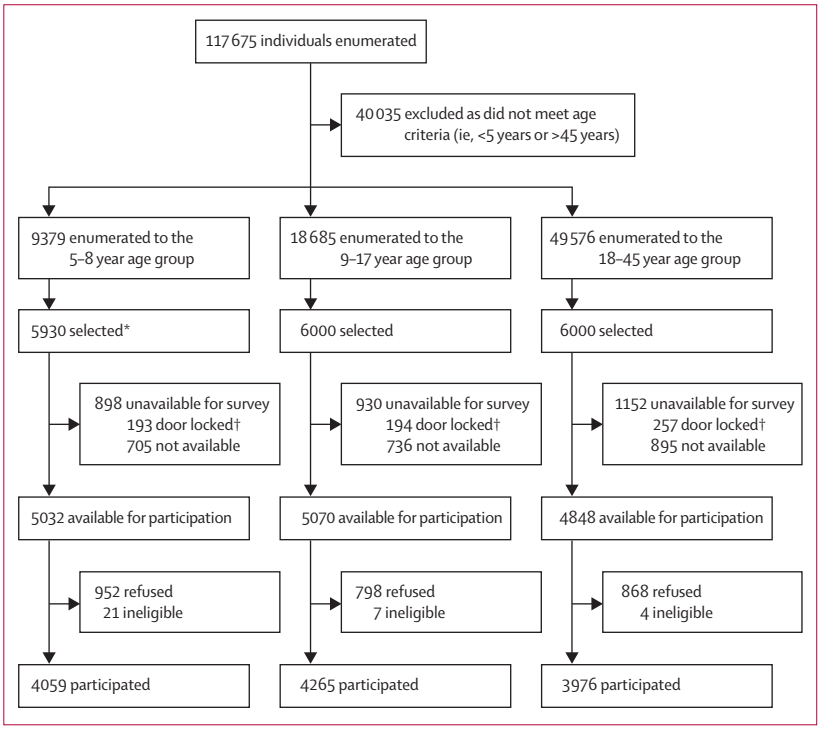


Figure 1: **Study profile**
*In 15 clusters, the number of enumerated children was <25. †The houses were locked, hence the eligible person (who was randomly selected) could not be interviewed.

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

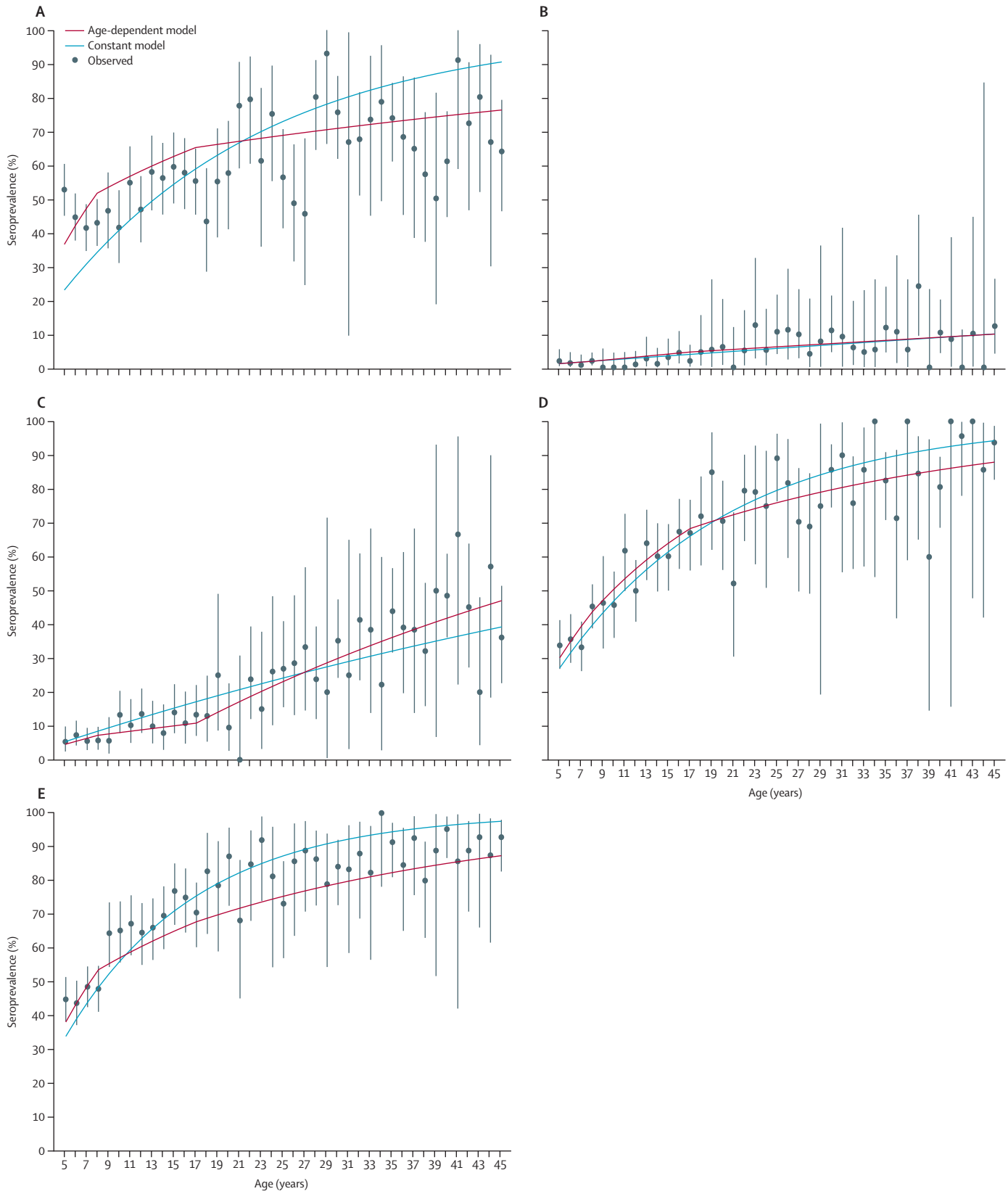
This serosurvey was initiated based on the WHO's initial recommendation of generating nationally representative seroprevalence data to guide decisions about introduction of Dengvaxia in India. The survey findings indicated that 49% of country's population had been previously infected with DENV, although 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.

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