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Seroprevalence of Dengue Infection Using IgG Capture ELISA in India, 2017–2018

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Abstract. We conducted a nationally representative population-based survey in 60 districts from 15 Indian states covering all five geographic regions during 2017–2018 to estimate the age specific seroprevalence of dengue. Of the 12,300 sera collected, 4,955 were positive for IgG antibodies against dengue virus using IgG Indirect ELISA indicating past dengue infection. We tested 4,948 sera (seven had inadequate volume) positive for IgG antibodies on indirect ELISA using anti-dengue IgG capture ELISA to estimate the proportion of dengue infections with high antibody titers, suggestive of acute or recent secondary infection. Of the 4,948 sera tested, 529 (10.7%; 95% CI: 9.4–12.1) were seropositive on IgG capture ELISA. The proportions of dengue infections with high titers were 1.1% in the northeastern, 1.5% in the eastern, 6.2% in the western, 12.2% in the southern, and 16.7% in the northern region. The distribution of dengue infections varied across geographic regions, with a higher proportion of infections with high antibody titer in the northern and southern regions of India. The study findings could be useful for planning facilities for clinical management of dengue infections.

Dengue is a major public health challenge worldwide. The Global Burden of Disease Study estimated that there were about 100 million dengue infections and 40,000 deaths in 2017.¹ India accounted for nearly one-third of the dengue cases reported globally in 2010.² The age-standardized incidence rate in India was 4,072.9 dengue cases per 100,000 population.¹ Population-based sero-epidemiological studies are useful to measure the disease burden due to dengue virus (DENV) in the community.³ These studies estimate the proportion of population positive for IgG antibodies against DENV. Commercial dengue IgG ELISAs can measure past or recent infections.^{3,4} Indirect ELISA measures the past infection, and the cutoff value for detection of IgG antibodies against DENV is set lower than the capture ELISA. On the other hand, in capture ELISA the cutoff value is higher, reflecting titers observed in recent or secondary dengue infections.^{3–5} Capture IgG and IgM capture ELISA was recommended to differentiate primary and secondary dengue virus infection among patients with acute illness by calculating IgM/IgG ratio.⁶ Recently population based serosurveys used dengue IgG capture ELISA to estimate the proportion of participants with high antibody titers observed in a recent or secondary infection.^{4,5} Knowledge about the burden of acute or recent secondary infections would be useful for program managers to identify areas with recent transmission, epidemic preparedness, establish facilities for critical care, train health care professionals, and thereby reduce mortality due to dengue.⁷

In 2017–2018, we conducted a nationally representative population-based survey to estimate the age-specific seroprevalence of dengue in India.⁸ The weighted seroprevalence of IgG antibodies against DENV, as detected by IgG indirect

ELISA, was 48.7% (95% CI: 43.5–54.0).⁸ The presence of IgG antibodies detected through IgG indirect ELISA could be due to past dengue infection, whereas IgG antibodies detected through capture ELISA indicates acute or recent secondary dengue infections.^{3,4} We tested the sera positive for IgG antibodies by indirect ELISA to estimate the proportion of dengue infection with high antibody titers in India.

The population-based serosurvey was conducted across five geographic regions (north, northeast, east, south, west) of India covering three age groups (i.e., 5–8, 9–17, and 18–45 years). In each region, three states were selected randomly. From each state, four districts were selected by probability proportional to population size. A map showing the districts where the survey was conducted is given in the supplemental material. Four clusters (two from urban and two from rural areas) were selected randomly from the list of villages/wards in each district. One census enumeration block (CEB) was selected randomly from each cluster. In India, during decennial census operations, an enumerator is allotted one CEB, which has about 120–150 households. All households in the CEB were enumerated, and information about all household members, including their age and sex, were collected. We randomly selected 25 individuals from each age group (5–8, 9–17, and 18–45 years) from the population enumerated during our survey.⁸

Serum samples were first tested for IgG antibodies against DENV using IgG indirect ELISA. Samples positive for IgG indirect ELISA based on the adjusted cutoff were tested with anti-dengue IgG-capture ELISA (Cat no. 01PE10; Panbio, Panbio Diagnostics, Brisbane, Australia).⁸ The cut-point of this capture assay was set at higher level (> 22 Panbio Units) compared with indirect ELISA (11 Panbio Units) to detect high level of IgG antibodies observed in acute or recent secondary infections.^{3,4,9} Anti-dengue IgG Panbio units were calculated by dividing the sample absorbance by the cutoff value and then multiplying this value by 10. A value of > 22 Panbio units

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was used to identify acute or recent secondary infection, a value of < 18 Panbio units was considered negative, and values between 18 and 22 Panbio units were considered equivocal.⁹ Sera with values between 18 and 22 Panbio units were retested, and specimens that were equivocal on repeat testing were considered as negative. As per the kit insert, the assay had 80.9% sensitivity for secondary dengue, 87.1% specificity for primary dengue, and 100% specificity for endemic negatives.⁹

The data were analyzed to describe the proportion of individuals with dengue infection by age group, sex, geographic region, and area (urban/rural). We also compared the socio-demographic characteristics of individuals with past dengue infection (positive for IgG antibodies on indirect ELISA but negative on capture ELISA) and dengue infection with high antibody titer (positive for IgG antibodies on indirect as well as capture ELISA) using logistic regression analysis.

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 and 17 years, and assent from children aged between 7 and 17 years was obtained before the survey.

The sero-survey was conducted in 240 clusters (118 rural, 122 urban; all clusters from two districts of National Capital Territory Delhi were urban) from 60 selected districts of 15 Indian states spread across all five geographic regions of India. We collected blood specimens from 12,300 individuals (5–8 years: 4,059; 9–17 years: 4,265; and 18–45 years: 3,976). Of the 12,300 sera tested, 5,338 samples were positive for IgG antibodies using DENV indirect IgG ELISA with the manufacturer recommended cutoff of > 11 Panbio units. Of the 12,300 samples, we tested a subset of 500 samples (100 from each geographic region) for the plaque reduction neutralization test (PRNT). Based on the receiver operator characteristic curve that was constructed to compare the sensitivity and specificity of IgG ELISA with PRNT 90 titers, we adjusted the ELISA cutoff from 11 Panbio units (as recommended by the manufacturer) to 15 Panbio units. This revised cutoff had a sensitivity of 77.6% and specificity of 94.4%. Of the 4,955 sera positive for IgG antibodies against DENV using IgG indirect ELISA with > 15 Panbio units, we tested 4,948 sera with anti-dengue IgG-capture ELISA.⁸ The quantity of seven sera was inadequate.

PREVALENCE OF DENGUE INFECTION USING IGG CAPTURE ELISA

Of the 4,948 sera tested, 529 (10.7%; 95% CI: 9.4–12.1) were seropositive on IgG capture ELISA. Of the total dengue infections, the proportions of dengue infections positive on IgG capture ELISA were 1.1% in the northeastern, 1.5% in the eastern, 6.2% in the western, 12.2% in the southern, and 16.7% in the northern region. The proportion of dengue infections positive on IgG capture ELISA in different Indian states is given in the supplemental table. The dengue infections with high antibody titer were higher in urban as compared with rural areas ($P = 0.0252$), whereas the proportion was not different by sex ($P = 0.4538$) (Table 1). The proportion of dengue infections was also different by age group ($P = 0.0049$).

TABLE 1

Proportion of dengue infection positive on IgG capture ELISA among individuals with past exposure to dengue, by age group, sex, geographic region, and location, India, 2017–2018

Age group (years)	No. positive/total (%; 95% CI)					
	North (N = 1,307)	Northeast (N = 91)	East (N = 397)	West (N = 1,360)	South (N = 1,793)	Overall (N = 4,948)
5–8	55/357 (15.4, 10.2–22.6)	0/10	0/49	16/287 (5.6, 3.5–8.6)	58/444 (13.1, 9.1–18.4)	129/1,147 (11.2, 8.8–14.3)
9–17	86/436 (19.7, 12.9–28.9)	0/13	1/98 (1.0, 0.1–8.0)	34/475 (7.1, 4.5–11.2)	86/641 (13.4, 9.6–18.3)	207/1,663 (12.4, 9.8–15.6)
18–45	77/514 (15.0, 10.6–20.7)	1/68 (1.5, 0.2–9.7)	5/250 (2.0, 0.9–4.5)	35/598 (5.8, 4.0–8.5)	75/708 (10.6, 7.9–14.0)	193/2,138 (9.0, 7.4–10.9)
Sex						
Female	117/690 (17.0, 13.3–21.3)	0/53	4/202 (2.0, 0.8–4.9)	50/718 (7.0, 5.1–9.3)	118/963 (12.2, 9.9–15.1)	289/2,626 (11.0, 9.5–12.7)
Male	101/617 (16.4, 12.3–21.5)	1/38 (2.6, 0.3–17.9)	2/195 (1.0, 0.2–4.2)	35/642 (5.4, 3.7–7.9)	101/830 (12.2, 9.7–15.1)	240/2,322 (10.3, 8.8–12.2)
Area						
Rural	61/495 (12.3, 7.1–20.5)	1/54 (1.8, 0.3–11.2)	1/174 (0.6, 0.1–4.0)	35/588 (5.9, 3.9–9.0)	93/863 (10.8, 7.9–14.6)	191/2,174 (8.8, 6.9–11.2)
Urban	157/812 (19.3, 15.5–23.9)	0/37	5/223 (2.2, 1.0–5.1)	50/772 (6.5, 4.7–8.8)	126/930 (13.5, 10.9–16.7)	338/2,774 (12.2, 10.5–14.1)
Overall	218/1,307 (16.7, 13.3–20.7)	1/91 (1.1, 0.1–7.3)	6/397 (1.5, 0.7–3.2)	85/1,360 (6.2, 4.8–8.0)	219/1,793 (12.2, 10.2–14.6)	529/4,948 (10.7, 9.4–12.1)

TABLE 2
Factors associated with dengue infection positive on IgG capture ELISA among individuals with past exposure to dengue, India, 2017–2018

Variable	Dengue infection (positive on capture ELISA)* (N = 529)	Dengue infection (positive on indirect ELISA)† (N = 4,419) (%)	Odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Age group (years)				
5–8	129 (24.4)	1,018 (23.0)	1.3 (1.0–1.6)	
9–17	207 (39.1)	1,456 (33.0)	1.4 (1.2–1.8)	
18–45	193 (36.5)	1,945 (44.0)	1	
Sex				
Female	289 (54.6)	2,337 (52.9)	1.1 (0.9–1.3)	
Male	240 (45.4)	2,082 (47.1)	1	
Region				
Northeast/east‡	7 (1.3)	481 (10.9)	1	1
North	218 (41.2)	1,089 (24.6)	13.8 (6.4–29.4)	12.9 (6.0–27.6)§
West	85 (16.1)	1,275 (28.9)	4.6 (2.1–10.0)	4.4 (2.0–9.5)§
South	219 (41.4)	1,574 (35.6)	9.6 (4.5–20.4)	9.2 (4.3–19.7)§
Area of residence				
Urban	338 (63.9)	2,436 (55.1)	1.4 (1.2–1.7)	1.4 (1.2–1.7)
Rural	191 (36.1)	1,983 (44.9)	1	1

* Based on presence of IgG antibodies against dengue virus (DENV) on capture and indirect ELISA.

† Based on presence of IgG antibodies against DENV on indirect ELISA.

‡ Very few samples were positive; hence, the data were merged for these two regions.

§ Adjusted for age group and area of residence.

|| Adjusted for age group and region.

FACTORS ASSOCIATED WITH DENGUE INFECTION WITH HIGH ANTIBODY TITER

Individuals with evidence of dengue infection positive on IgG capture ELISA were more likely to be aged < 18 years (unadjusted odds ratio for children aged 5–8 years: 1.3, 95% CI: 1.0–1.6; for children aged 9–17 years: 1.4, 95% CI: 1.2–1.8). Such individuals were more likely to be from urban areas (adjusted odds ratio [AOR]: 1.4; 95% CI: 1.2–1.7) and to belong to the northern (AOR: 12.9; 95% CI: 6.0–27.6), western (AOR: 4.4; 95% CI: 2.0–9.5), or southern region (AOR: 9.2; 95% CI: 4.3–19.7) of India than northeastern or eastern regions (Table 2).

Our study findings indicate that about 10% of individuals with past exposure to DENV had high levels of IgG antibodies. High levels of IgG antibodies as detected by capture ELISA are suggestive of acute or recent secondary dengue infection.^{4,5} The proportion of dengue infections positive on IgG capture ELISA varied widely across geographic regions, with < 2% of dengue infections in eastern and northeastern regions, whereas the proportions in the northern, western, and southern regions were higher. Individuals with dengue infection were more likely to be aged < 18 years and to reside in urban areas of northern, western, and southern regions of the country.

A serosurvey conducted in the city of Pune in Western India reported that 11% of dengue infections were positive on dengue capture ELISA.⁵ Another serosurvey done in south Indian city of Chennai found that 19% of those who showed prior exposure to dengue virus were positive on IgG capture ELISA.⁴ The reason for a lower proportion of 12% observed in our study for the southern region could be due to inclusion of both rural and urban areas.

Our study findings revealed higher odds of dengue infections with high antibody titer in urban areas and in northern, western, and southern regions of India, confirming the higher endemicity of dengue as well as co-circulation of multiple dengue virus serotypes in these areas.^{10,11} In India, although dengue infection is endemic in all states, the seroprevalence of dengue infection was found to be higher in northern, western,

and southern regions as well in urban areas.⁸ This study also reported nearly three-fourths of individuals with past dengue infections having multi-typic neutralizing antibody profile on PRNT.⁸ Children were found to have higher odds of dengue infection with high antibody titers.

Our study had certain limitations. First, we did not test the samples for IgM antibodies against dengue because the survey was conducted among healthy individuals and during low transmission months in some districts. As a result, we could not reliably classify individuals with high antibody titers as detected on IgG capture ELISA into acute or recent acute secondary infection. Second, we did not collect information about previous hospitalizations due to dengue. We were therefore not able to comment about severity of illness among individuals who had high levels of IgG antibodies on capture ELISA.

In conclusion, the distribution of dengue infection varied across geographic regions, with a higher proportion of dengue infections with high antibody titers from northern India. The study findings could be useful in planning facilities for clinical management of dengue infections in different geographic regions in India.

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