Indian J Med Res 152, July & August 2020, pp 48-60
DOI: 10.4103/ijmr.IJMR_3290_20

Quick Response Code:

Prevalence of SARS-CoV-2 infection in India: Findings from the national serosurvey, May-June 2020

Manoj V. Murhekar[Ω], Tarun Bhatnagar[1], Sriram Selvaraju[4], Kiran Rade[10], V. Saravanakumar[2], Jeromie Wesley Vivian Thangaraj[1], Muthusamy Santhosh Kumar[1], Naman Shah[14], R. Sabarinathan[2], Alka Turuk[11], Parveen Kumar Anand[16,*], Smita Asthana[17,*], Rakesh Balachandar[22,*], Sampada Dipak Bangar[23,*], Avi Kumar Bansal[19,*], Jyothi Bhat[27,*], Debjit Chakraborty[28,*], Chethana Rangaraju[29,*], Vishal Chopra[32,*], Dasarathi Das[35,*], Alok Kumar Deb[28,*], Kangjam Rekha Devi[36,*], Gaurav Raj Dwivedi[20,*], S. Muhammad Salim Khan[37,*], Inaamul Haq[37,*], M. Sunil Kumar[33,*], Avula Laxmaiah[38,*], (Major) Madhukar[39,*], Amarendra Mahapatra[35,*], Anindya Mitra[34,*], A.R. Nirmala[30,*], Avinash Pagdhune[22,*], Mariya Amin Qurieshi[37,*], Tekumalla Ramarao[40,*], Seema Sahay[24,*], Y.K. Sharma[15,*], Marinaik Basavegowdanadoddi Shrinivasa[5,*], Vijay Kumar Shukla[15*], Prashant Kumar Singh[18,*], Ankit Viramgami[22,*], Vimith Cheruvathoor Wilson[4,*], Rajiv Yadav[27,*], C.P. Girish Kumar[3], Hanna Elizabeth Luke[6], Uma Devi Ranganathan[7], Subash Babu[8], Krithikaa Sekar[4], Pragya D. Yadav[25], Gajanan N. Sapkal[26], Aparup Das[a,†], Pradeep Das[b,†], Shanta Dutta[c,†], Rajkumar Hemalatha[d,†], Ashwani Kumar[e,†], Kanwar Narain[f,†], Somashekar Narasimhaiah[g,†], Samiran Panda[h,†], Sanghamitra Pati[i,†], Shripad Patil[j,†], Kamalesh Sarkar[k,†], Shalini Singh[l,†], Rajni Kant[m,†], Srikanth Tripathy[n,†], G.S. Toteja[o,†], Giridhara R. Babu[31], Shashi Kant[12], J.P. Muliyil[9], Ravindra Mohan Pandey[13], Swarup Sarkar[11], Sujeet K. Singh[41], Sanjay Zodpey[42], Raman R. Gangakhedkar[11], D.C.S. Reddy[21] & Balram Bhargava[@] for India COVID-19 Serosurveillance Group[#]

[1]ICMR School of Public Health, [2]Division of Epidemiology & Bio-Statistics, [3]Laboratory Division, [Ω]ICMR-National Institute of Epidemiology, [4]Divisions of Epidemiology, [5]Clinical Research, [6]HIV/AIDS, [7]Immunology, [8]NIH-ICER (International Centers for Excellence in Research) Program, [n]ICMR-National Institute for Research in Tuberculosis, Chennai, [9]Independent Consultant, Vellore, Tamil Nadu, [10]WHO Country Office for India, [11]Division of Epidemiology & Communicable Diseases, [@]Indian Council of Medical Research (DHR), Ministry of Health & Family Welfare, [12]Centre for Community Medicine, [13]Department of Biostatistics, All India Institute of Medical Sciences, New Delhi, [14]Jan Swasthya Sahyog, Bilaspur, [15]Directorate Health Services, Raipur, Chhattisgarh, [16]Division of Bio-Statistics, [o]ICMR-National Institute for Implementation Research on Non-Communicable Diseases, Jodhpur, Rajasthan, Divisions of [17]Epidemiology & Biostatistics, [18]Preventive Oncology, [l]ICMR-National Institute of Cancer Prevention & Research, Noida, [19]Division of Epidemiology, [j]ICMR-National JALMA Institute for Leprosy & Other Mycobacterial Diseases, Agra, [20,m]ICMR-Regional Medical Research Centre, Gorakhpur, [21]Independent Consultant, Lucknow, Uttar Pradesh, [22]Division of Clinical Epidemiology, [k]ICMR-National Institute of Occupational Health, Ahmedabad, Gujarat, [23]Divisions of Epidemiology & Biostatistics, [24]Social and Behavioural Research Sciences, [h]ICMR-National AIDS Research Institute, [25]Maximum Containment Laboratory, [26]Diagnostic Virology Group, ICMR-National Institute of Virology, Pune, Maharashtra, [27]Division of Communicable Diseases, [a]ICMR-National Institute of Research in Tribal Health, Jabalpur, Madhya Pradesh, [28]Division of Epidemiology, [c]ICMR-National Institute of Cholera & Enteric Diseases, Kolkata, West Bengal,

[*]All Nodal Officers contributed equally (names given in alphabetical order)
[†]All Institute Directors contributed equally (names given in alphabetical order)
Supplementary material available from *http://www.ijmr.org.in/article.asp?issn=0971-5916;year=2020;volume=152;issue=1;spage=48;epage=60;aulast=Murhekar*

[29]Division of Advocacy, Communication & Social Mobilisation, [30]Lady Willingdon State TB Centre, Government of Karnataka, [31]Indian Institute of Public Health, [g]National Tuberculosis Institute, Bengaluru, Karnataka, [32]State TB Training & Demonstration Centre, Patiala, Punjab, [33]State TB Training & Demonstration Centre Thiruvananthapuram, Kerala, [34]State TB Training & Demonstration Centre Ranchi, Jharkhand, [35,i]ICMR-Regional Medical Research Centre, Bhubaneswar, Odisha, [36]Division of Enteric Diseases, [f]ICMR-Regional Medical Research Centre, Northeast Region, Dibrugarh, Assam, [37]Department of Community Medicine, Government Medical College, Srinagar, Jammu & Kashmir, [38]Division of Public Health Nutrition, [d]ICMR-National Institute of Nutrition, Hyderabad, Telangana, [39]Division of Clinical Medicine, [b]ICMR-Rajendra Memorial Research Institute of Medical Sciences, Patna, Bihar, [40]State TB Cell, Vijayawada, Andhra Pradesh, [e]ICMR-Vector Control Research Centre, Puducherry, [41]National Centre for Disease Control & [42]Indian Institute of Public Health, Delhi, India

Received August 1, 2020

***Background & objectives*: Population-based seroepidemiological studies measure the extent of SARS-CoV-2 infection in a country. We report the findings of the first round of a national serosurvey, conducted to estimate the seroprevalence of SARS-CoV-2 infection among adult population of India.**

***Methods*: From May 11 to June 4, 2020, a randomly sampled, community-based survey was conducted in 700 villages/wards, selected from the 70 districts of the 21 States of India, categorized into four strata based on the incidence of reported COVID-19 cases. Four hundred adults per district were enrolled from 10 clusters with one adult per household. Serum samples were tested for IgG antibodies using COVID Kavach ELISA kit. All positive serum samples were re-tested using Euroimmun SARS-CoV-2 ELISA. Adjusting for survey design and serial test performance, weighted seroprevalence, number of infections, infection to case ratio (ICR) and infection fatality ratio (IFR) were calculated. Logistic regression was used to determine the factors associated with IgG positivity.**

***Results*: Total of 30,283 households were visited and 28,000 individuals were enrolled. Population-weighted seroprevalence after adjusting for test performance was 0.73 per cent [95% confidence interval (CI): 0.34-1.13]. Males, living in urban slums and occupation with high risk of exposure to potentially infected persons were associated with seropositivity. A cumulative 6,468,388 adult infections (95% CI: 3,829,029-11,199,423) were estimated in India by the early May. The overall ICR was between 81.6 (95% CI: 48.3-141.4) and 130.1 (95% CI: 77.0-225.2) with May 11 and May 3, 2020 as plausible reference points for reported cases. The IFR in the surveyed districts from high stratum, where death reporting was more robust, was 11.72 (95% CI: 7.21-19.19) to 15.04 (9.26-24.62) per 10,000 adults, using May 24 and June 1, 2020 as plausible reference points for reported deaths.**

***Interpretation & conclusions*: Seroprevalence of SARS-CoV-2 was low among the adult population in India around the beginning of May 2020. Further national and local serosurveys are recommended to better inform the public health strategy for containment and mitigation of the epidemic in various parts of the country.**

Key words Antibody - COVID-19 - ELISA - IgG - India - SARS-CoV-2 - seroepidemiology - seroprevalence - serosurveillance

In India, the first case of COVID-19 was reported on January 30, 2020[1]. As of June 20, 2020, 395,048 laboratory-confirmed cases and 12,948 deaths were reported from India. There is a wide variation in the reporting of cases across the States/Union Territories and across the districts within each State[2]. The case reporting is based on the testing of individuals by real-time reverse transcription-polymerase chain reaction (RT-qPCR). Laboratory capacity for testing, health-seeking behaviours and testing strategy in terms of who gets tested, influence the numbers reported. Furthermore, the current testing criteria, which prioritize the allocation of testing capacity, will miss many asymptomatic and mild infections.

Knowledge about the true extent of infection is critical for an effective public health response

to COVID-19. Facility-based surveillance efforts, though useful to understand the trend of infection in sentinel populations, are not population representative. Population-based seroepidemiological studies are therefore, recommended to measure the extent of spread of infection in an area and recommend containment measures accordingly[3,4]. The WHO has recommended three types of seroepidemiological studies: (*i*) cross-sectional surveys, most appropriate after the peak transmission is established; (*ii*) repeated cross-sectional investigation in the same geographic area to establish trends in an evolving pandemic; and (*iii*) longitudinal cohort study with serial sampling of the same individuals[5]. For India, being in the early stages of the pandemic at the time of study, the Indian Council of Medical Research (ICMR) adopted the option of repeated cross-sectional surveys. The results of the first cross-sectional serosurvey conducted with the objectives of estimating the seroprevalence for SARS-CoV-2 infection among the adults in the general population and determining the socio-demographic factors associated with SARS-CoV-2 infection in the country are described here.

Material & Methods

The details of this national serosurvey procedure are given elsewhere[6]. Briefly, the survey to estimate the seroprevalence of SARS-CoV-2 infection in the general population was conducted among individuals aged 18 yr or more in selected representative 736 districts in India. Districts were categorized into four strata according to the incidence of reported COVID-19 cases per million population (zero, low: 0.1-<5, medium: 5-10, high: >10) as on April 25, 2020. At least 15 districts were randomly selected from each stratum (Supplementary Table available from *http://www.ijmr.org.in/articles/2020/152/1/images/IndianJMedRes_2020_152_1_48_294807_sm7.pdf*).

The ICMR Central Ethics Committee on Human Research approved the survey protocol. Written informed consent was obtained from the participants, and the test results were communicated to them.

Sampling design and sample size: A multistage cluster sampling design was used. A sample size of 5,929 (rounded to 6,000) was calculated per stratum of districts to estimate one per cent seropositivity, with 40 per cent relative precision, 95 per cent confidence interval (CI) and design effect of 2·5. Four hundred individuals were selected from each district. In each district, 10 clusters (village in rural areas and ward in urban areas) were selected by probability proportion to population size. In each cluster, four random locations were selected. A random starting point was selected from each location and all contiguous households were visited until 10 eligible individuals were enrolled. One adult was selected from each household following the Troldahl-Carter-Bryant Grid method[7].

Survey procedure: The survey was conducted from May 11 to June 4, 2020. The survey team visited the selected households and briefed them about the survey objectives and process involved. After obtaining written informed consent, information on basic demographic details, exposure history to laboratory-confirmed COVID-19 cases and symptoms suggestive of COVID-19 in the preceding one month was collected using an Open Data Kit application (*https://getodk.org/*). Trained phlebotomists collected 3-5 ml of venous blood from each participant. Serum was separated after centrifugation in a local health facility and transported to the laboratories in the designated ICMR institutes under cold chain.

Laboratory procedure: Serum samples were tested for the presence of IgG antibodies against COVID-19 using commercial ELISA (COVID Kavach-Anti-SARS-CoV-2 IgG Antibody Detection ELISA, M/s Cadila Healthcare Limited, Ahmedabad). The assay detects IgG antibodies in the serum/plasma, which bind to the SARS-CoV-2 virus whole cell antigen. The manufacturer reported no cross-reactivity with other viruses in the serum from real-time RT-qPCR-confirmed patients of influenza A (H1N1) pdm09, influenza A (H3N2), human coronavirus OC43, rhinovirus, respiratory syncytial virus, influenza B, parainfluenza type 4, hepatitis B virus, hepatitis C virus, as well as serum with IgG antibodies against dengue and chikungunya. The sensitivity and specificity of the assay were 92.4 and 97.9 per cent, respectively[8].

Testing procedures were followed as per the manufacturer's instructions. For each plate, samples with optical density (OD) value more than the cut-off value and positive/negative (P/N) ratio more than 1.5 were considered as positive. Samples with OD value of 10 per cent ± ranges of the cut-off were considered to be indeterminate. The P/N ratio was defined as the ratio of average OD value of the positive control divided by the average OD of the negative control. The cut-off OD value was calculated as the average OD value of negative control +0.2.

Serum samples with indeterminate results were repeat tested with COVID Kavach ELISA. Those with indeterminate results on repeat testing also were considered as negative. All serum samples showing positive results with COVID Kavach ELISA were serially tested with Euroimmun SARS-CoV-2 ELISA (IgG) (Euroimmun AG, Germany). This kit uses S1 domain of the spike protein of SARS-CoV-2 expressed recombinantly in the human cell line HEK 293 and has a sensitivity and specificity of 93.8 and 99.6 per cent, respectively, as per the kit insert[9]. Additional data submitted for the registration to the U.S. Food and Drug Administration (FDA) describe the specificity of 100 per cent (95% CI: 95.4-100) in an independent clinical validation study (n=80) and 99.5 per cent (95% CI: 99.1-99.9) among pre-COVID banked adult serum samples (n=1195)[10]. For quality assurance, one per cent of negative serum samples were randomly selected from each stratum and tested with COVID Kavach-Anti-SARS-CoV-2 IgG Antibody Detection ELISA.

A positive infection was defined as an adult whose serum sample was found to be positive upon testing with Euroimmun ELISA subsequent to being positive by COVID Kavach ELISA. It is assumed that seropositive status indicates prior infection with SARS-CoV-2.

Data analysis: The frequency of characteristics of the survey participants was described. The reported occupations were categorized into high and low risk considering the potential risk of exposure to known or unknown COVID-19 case. The serial sensitivity and specificity of our sequential testing were calculated using the following formulae:

Serial sensitivity=sensitivity of Kavach×sensitivity of Euroimmun

Serial specificity=specificity of Kavach+(1−specificity of Kavach)×specificity of Euroimmun.

The serial sensitivity and specificity calculated using the sequential testing of positive results were 86.67 and 99.99 per cent, respectively, and were used to adjust the seroprevalence[11].

The seroprevalence of SARS-CoV-2 infection along with the 95 per cent CI was estimated for each of the four strata using appropriate sampling weights and taking into account the sampling strategy used for the survey. Sampling weights were calculated as a product of inverse probabilities of selection of districts in the stratum, selection of clusters in each district and selection of individuals in each cluster. The stratum seroprevalence and 95 per cent CI were calculated using the survey data analysis module in the STATA software (StataCorp LLC, TX, USA). The final prevalence estimates were adjusted for the serial IgG test characteristics[12,13]. The estimates across the strata were pooled to calculate the overall national prevalence with 95 per cent CI[14]. The adjusted stratum-specific seroprevalence was applied to the total adult population in each stratum, projected for the year 2020 using 2011 census data (*https://censusindia.gov.in/2011census/population_enumeration.html*), to estimate the number of infections in each stratum and overall infections[14].

Factors associated with IgG seropositivity: Individuals who were seropositive for SARS-CoV-2 infection were compared with those who were seronegative to identify socio-demographic factors associated with IgG positivity using logistic regression analysis. Odds ratio (OR) with 95 per cent CIs were calculated with the adjustment of each factor for its known confounders, if any.

Estimated infection ratios (*IFR*): The published literature indicates that the IgG antibodies against SARS-CoV-2 infection start appearing by the end of the first week after symptom onset and most cases are IgG positive by the end of second week[15]. We therefore, considered the number of reported RT-qPCR-confirmed COVID-19 cases by May 3 and 11, 2020 (respectively, 15 days and one week before the initiation of serosurvey on May 18 in at least half of the clusters) to estimate the plausible range of infections. The infection to case ratio (ICR) was defined as the number of individuals with SARS-CoV-2 infection (as per the IgG detection) divided by the number of RT-qPCR cases of COVID-19 reported by the date of sample collection from the ICMR laboratory database. Assuming a three-week lag time from infection to death[16], we considered the reported number of deaths in the districts included in the serosurvey by May 24 and June 1, 2020 to estimate the plausible range of the infection fatality ratio (IFR)[17]. The number of infections was estimated only in the surveyed districts for each stratum for calculating stratum-specific IFR.

Results

A total of 30,283 households were visited from 700 clusters in 70 districts across the four strata (Table I). About one-fourth (n=181, 25.9%) of the

surveyed clusters were from urban areas. A total of 28,000 individuals consented to participate. The response rate in different strata ranged from 86.9 to 95.9 per cent. Nearly half (n=13,552, 48.5%) of the survey participants were aged between 18 and 45 yr and 51.5 per cent (n=14,390) were female. In all, 18.7 per cent of the participants had an occupation with a high risk of exposure to potentially infected persons (Table II).

Four hundred and eighty six individuals (1.7%) reported a history of respiratory symptoms in the preceding one month, of whom, 44.7 per cent (n=217) sought medical care and 30.9 per cent (n=67) of those who sought care were hospitalized. One hundred and fifty one (0.5%) individuals reported a history of contact with a COVID-19 case and 70 (0.3%) reported that they were tested for COVID-19 any time before the survey. One person had been diagnosed positive (Table II).

Of the 28,000 individuals initially tested by COVID Kavach ELISA, 256 were classified as

Table I. Districts and number of individuals surveyed

Stratum	Total number of districts	Number of districts/ cities	Number of clusters selected	Number of clusters in urban area (%)	Number of households visited	Number of individuals enrolled (%)
Zero cases*	233	15	150	17 (11.3)	6301	6014 (95.4)
Low*	229	22	220	45 (20.5)	9202	8822 (95.9)
Medium*	84	16	160	49 (30.6)	7340	6380 (86.9)
High*	190	17	170	70 (41.2)	7440	6784 (91.2)
Total	736	70	700	181 (25.9)	30,283	28,000 (92.5)

*Based on the incidence of reported COVID-19 cases in the ICMR laboratory database as on April 25, 2020. Low: 0.1-<5, medium: 5-10, high: >10 per million

Table II. Characteristics of study participants in different strata of districts

Characteristics	Stratum*				Overall (n=28,000)
	Zero (n=6,014)	Low (n=8,822)	Medium (n=6,380)	High (n=6,784)	
Age (yr)					
18-45	3,234 (53.8)	4,302 (48.9)	2,611 (41.1)	3,405 (50.3)	13,552 (48.5)
45-60	1,844 (30.7)	3,031 (34.4)	2,310 (36.4)	2,340 (34.6)	9,525 (34.1)
>60	930 (15.5)	1,468 (16.7)	1,431 (22.5)	1,019 (15.1)	4,848 (17.4)
Missing data	6	21	28	20	75
Mean age±SD	43.4±15.4	45.1±15.0	48.3±15.2	44.6±14.8	45.3±15.2
Sex					
Male	2,964 (49.3)	4,300 (48.9)	3,209 (50.5)	3,041 (44.9)	13,514 (48.4)
Female	3,037 (50.6)	4,493 (51.0)	3,140 (49.4)	3,720 (55.0)	14,390 (51.5)
Others	7 (0.1)	9 (0.1)	5 (0.1)	6 (0.1)	27 (0.1)
Missing data	6	20	26	17	69
Occupation with high exposure	1,186 (19.7)	1,501 (17.1)	1,142 (18.0)	1,397 (20.7)	5,226 (18.7)
History of respiratory symptoms in last 30 days	131 (2.2)	156 (1.8)	109 (1.7)	89 (1.3)	486 (1.7)
Sought medical care for respiratory symptoms	70 (53.0)	68 (43.6)	51 (46.8)	28 (31.5)	217 (44.7)
History of hospitalization	24 (34.3)	24 (35.3)	15 (29.4)	4 (14.3)	67 (30.9)
History of contact with COVID-19 case	88 (1.5)	46 (0.5)	6 (0.1)	11 (0.2)	151 (0.5)
Ever tested for COVID-19 by RT-qPCR	6 (0.1)	11 (0.1)	16 (0.3)	37 (0.5)	70 (0.3)

Values given as n (%) except otherwise stated. *Based on incidence of reported COVID-19 cases as per the ICMR laboratory database. RT-qPCR, real-time reverse transcription-polymerase chain reaction

positive and 69 as indeterminate. On repeat testing of the indeterminate serum samples by COVID Kavach ELISA, 34 turned positive. Finally, 157 of these 290 were detected positive using the Euroimmun ELISA. The overall unweighted seroprevalence was 0.56 per cent (95% CI: 0.48-0.66%). The unweighted prevalence of IgG antibodies against SARS-CoV-2 was 0.47 per cent (95% CI: 0.31-0.67%) in the stratum with zero reported COVID-19 cases, 0.48 per cent (95% CI: 0.34-0.64%) in the stratum with low incidence, 0.74 per cent (95% CI: 0.54-0.98%) in the stratum with medium incidence and 0.59 per cent (95% CI: 0.42-0.80%) in the stratum with high incidence. The weighted prevalence of infection after adjusting for the serial sensitivity and specificity of the two ELISA tests in the respective strata was 0.68 per cent (95% CI: 0.42-1.11%), 0.62 per cent (95% CI: 0.43-0.89%), 1.03 per cent (95% CI: 0.44-2.37%) and 0.72 per cent (95% CI: 0.44-1.17%). The pooled adjusted prevalence of SARS-CoV-2 infection was 0.73 per cent (0.34-1.13%) at the national level (Table III). The *post facto* design effect was 1.9.

Factors associated with IgG positivity: As compared to the seronegative individuals, the individuals positive for IgG antibodies were more likely to be male (OR: 1.47; 95% CI: 1.07-2.02), have an occupation with a higher risk of exposure to potentially infected persons (adjusted OR: 1.39; 95% CI: 0.96-2.02) and reside in urban slums (OR: 1.90; 95% CI: 1.23-2.94) (Table IV).

Burden of SARS-CoV-2 infection: Applying the stratum-specific adjusted prevalence of IgG antibodies to the total population of adults in 2020, we estimated a cumulative 6.46 million (3.82-11.1 million) infections in India by May 3, 2020 (Table V). The infection to case ratio was 81.6 (95% CI: 48.3-141.4) up to May 11 and 130.1 (95% CI: 77.0-225.2) up to May 3, 2020 considering a total of 79,230 and 49,720 COVID-19 cases reported in India by the respective dates. The IFR per 10,000 infections on May 24 ranged between 0.18 (95% CI: 0.11-0.29) in zero stratum and 11.72 (95% CI: 7.21-19.19) in the high stratum districts. IFR per 10,000 infections as on June 1 ranged between 0.27 (95% CI: 0.17-0.44) in zero stratum and 15.04 (95% CI: 9.26-24.62) in the high stratum districts (Table V).

Discussion

The findings of the first national population-based serosurvey indicated that 0.73 per cent of adults in India were exposed to SARS-CoV-2 infection, amounting to 6.4 million infections in total by the early May 2020. The seroprevalence ranged between 0.62 and 1.03 per cent across the four strata of districts.

Population-based estimates of seroprevalence provide information about the state of the epidemic in the country. A dashboard of seroepidemiological data available from 22 countries estimated the pooled seroprevalence to be 4.76 per cent, ranging from 0.65 Zero cent in Scotland to 26.6 per cent in Iran[18]. These surveys used different types of serologic tests including lateral flow immunoassay using capillary blood (rapid test), ELISA, Luciferase immunoprecipitation system assay, immunochromatography and chemiluminescence[18,19]. The findings of our survey indicated that the overall seroprevalence in India was low, with less than one per cent of the adult population exposed to SARS-CoV-2 by mid May 2020. The low prevalence observed in most districts indicates that India is in early phase of the epidemic and the majority of the Indian population is still susceptible to SARS-CoV-2 infection. It is, therefore, necessary to continue to implement the context-specific containment measures including the testing of all symptomatics, isolating positive cases and tracing high risk contacts to slow transmission and to prevent the overburdening of the health system[20].

Table III. Seroprevalence of IgG antibodies against SARS-CoV-2 infection in different strata of districts

Incidence of reported COVID-19 cases (stratum)	Number of individuals tested	Number positives	Per cent (95% CI)		
			Unweighted prevalence	Weighted prevalence*	Adjusted prevalence**
Zero	6,014	28	0.47 (0.31-0.67)	0.60 (0.37-0.97)	0.68 (0.42-1.11)
Low	8,822	42	0.48 (0.34-0.64)	0.55 (0.38-0.78)	0.62 (0.43-0.89)
Medium	6,380	47	0.74 (0.54-0.98)	0.90 (0.39-2.06)	1.03 (0.44-2.37)
High	6,784	40	0.59 (0.42-0.80)	0.63 (0.39-1.02)	0.72 (0.44-1.17)
Overall	28,000	157	0.56 (0.48-0.66)	0.64 (0.30-0.99)	0.73 (0.34-1.13)

After applying *sampling weights and clustering; **adjusting for test performance. CI, confidence interval

Table IV. Socio-demographic risk factors associated with IgG positivity

Socio-demographic characteristics	IgG positive	IgG negative	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Age (yr)	(n=157)	(n=27,768)		
18-45	68 (43.3)	13,484 (48.6)	1.00	
46-60	62 (39.5)	9,463 (34.1)	1.30 (0.92-1.84)	
>60	27 (17.2)	4,821 (17.3)	1.11 (0.71-1.74)	
Sex	(n=157)	(n=27,774)		
Male	91 (58.0)	13,423 (48.3)	1.47 (1.07-2.02)	
Female	66 (42.0)	14,324 (51.6)	1.00	
Others	-	27 (0.1)	-	
Area of residence	(n=157)	(n=27,843)		
Urban slum	25 (15.9)	2,496 (9.0)	1.90 (1.23-2.94)	
Urban non-slum	23 (14.6)	4,694 (16.9)	0.93 (0.59-1.46)	
Rural (village)	109 (69.4)	20,653 (74.1)	1.00	
Occupation with higher risk of exposure to potentially infected persons	(n=155)	(n=27,668)		
Yes	41 (26.5)	5,185 (18.7)	1.56 (1.09-2.23)	1.39 (0.96-2.02)*
No	114 (73.5)	22,483 (81.3)	1.00	

Values shown as n (%). *Adjusted for age, sex, area of residence

Table V. Estimated number of infections and infection fatality ratio (IFR) by strata of districts

Stratum of districts	Estimated number of infections in all districts (95% CI)	Estimated infections in surveyed districts	Deaths (May 24, 2020)	Deaths (June 1, 2020)	IFR (per 10,000 infections) 95% CI	
					May 24, 2020	June 1, 2020
Zero	856,062 (528,744-1,397,395)	109,872	2	3	0.18 (0.11-0.29)	0.27 (0.17-0.44)
Low	1,817,118 (1,260,259-2,608,443)	212,885	15	22	0.70 (0.49-1.02)	1.03 (0.72-1.49)
Medium	1,518,367 (648,623-3,493,718)	391,941	54	97	1.38 (0.60-3.23)	2.47 (1.08-5.79)
High	2,276,841 (1,391,403-3,699,866)	289,143	339	435	11.72 (7.21-19.19)	15.04 (9.26-24.62)

As per the present survey findings, the prevalence of infection in the general population was not different across different strata of districts categorized on the basis of the level of PCR-based case reporting. The level of seropositivity to SARS-CoV-2 detected in the stratum of districts with zero cases could be on account of two reasons. First, the stratification of districts was done based on the reported number of COVID-19 cases as on April 25, 2020. The serosurvey in the 15 districts of these strata was conducted during May 11 to June 4, 2020 after a median interval of 23 days (range: 16-40). During this period, as per the ICMR laboratory database, three districts had reported COVID-19 cases at least two weeks before the initiation of survey and thus were no longer reporting zero cases. Second, there could be under-detection of COVID-19 cases in the zero stratum districts on account of low testing as well as poor access to the testing laboratories. In four of the 15 districts in this stratum, COVID-19 testing laboratory was not available at the district headquarters and the samples were transported to the State headquarter hospitals for diagnosis. The present findings of seropositivity in the strata of districts with zero to low incidence of COVID-19 cases underscores the need to strengthen surveillance and augment the testing of suspected cases in these areas.

The estimated seroprevalence is a function of the sensitivity and specificity of serological tests. Adequate thresholds for sensitivity and specificity are influenced by the prevalence of infection. As was done in our study, the use of two tests in a sequential manner under the condition of positive result on both the tests would

lead to an overall increase in the specificity at the cost of lowering of sensitivity[11,13]. The sequential use of COVID Kavach and Euroimmun ELISA allowed us to potentially reduce the false positive to as low as 0.01 per cent by obtaining a serial specificity of 99.99 per cent (if the independence between the tests is high). However, the serial sensitivity was reduced to 86.67 per cent that resulted in a slight increase in the false negatives, resulting in a potential underestimation of seroprevalence. Testing with greater specificity is preferred in a low prevalence setting such as ours to minimize the large number of false positives[21].

Serosurveys provide important estimates of the total number of infections in the country. Based on the overall adjusted seroprevalence of 0.73 per cent and reported number of COVID-19 cases, it was estimated that for every RT-qPCR confirmed case of COVID-19, there were 82-130 infections in India. The high infection to case ratio in India could be on account of the prioritization of testing among symptomatics or the variability in testing rates across the States[22]. The IFR reflects the societal cost of achieving SARS-CoV-2 herd immunity through infection. Calculation of IFR is dependent on an accurate reporting of deaths and the number of estimated infections. Considering that the death reporting in India is incomplete, and differences in access to testing facilities across districts necessary for declaring the COVID-19 confirmed deaths, the present IFR is likely an underestimate. While the overall IFR based on the serosurvey findings was much lower than that reported from Santa Clara County, USA (0.12-0.2%)[16], Iran (0.08-0.12%)[23], Brazil and Spain (1%)[24], the IFR from the high-stratum districts, where reporting is assumed to be more complete, was similar to those reported above. In addition to the completeness of death reporting, the heterogeneity in IFR can also be explained by the differences in age structure of the population, access to healthcare facilities, quality of care and variation in the prevalence of comorbidities[24,25].

The present serosurvey had certain limitations. First, the seroprevalence estimates had wide confidence intervals across all the strata of districts. The sample size was calculated assuming a minimum seroprevalence of one per cent across all strata. Our sample size was underpowered to precisely estimate the lower prevalence observed in the strata of districts with low incidence of reported COVID-19 cases. However, our sample size was adequate to estimate the seroprevalence in other strata. The estimate of infection to case ratio also had low precision as a result. These baseline results will help improve sample size estimations in future rounds of serosurveys. Second, the study participants were interviewed to collect information about history of the symptoms for the preceding month. However, as the presence of IgG antibodies reflects exposure to SARS-CoV-2 since the beginning of the epidemic, we were not able to estimate how many seropositive individuals ever had probably COVID-19 symptoms. Due to only a few observations, it was not possible to associate prior RT-qPCR testing, hospitalization or contact status with the seropositivity. Third, errors in serological testing, especially due to the test specificity, can affect prevalence estimates, particularly when the prevalence is low. We sought to improve the test specificity by confirming positives detected in the general population using a separate test with a different antigen. However, both ELISAs use the same mechanism, serology, and the Euroimmun ELISA antigen, which is solely a recombinant domain of the primary immunogenic component of the virus, is a subset of the whole virion, which is used by Kavach ELISA. Thus, positive test results will be conditionally dependent between the two. The degree of dependence is unknown, but this assumption creates an upward bias in our prevalence estimate. The seroprevalence of 0.73 per cent was estimated assuming that the two tests are completely independent. The seroprevalence could be as low as 0.26 per cent (considering sensitivity of COVID Kavach and specificity of Euroimmun ELISA) assuming that the two tests are completely dependent. However, as the dependence between the two ELISAs is unlikely to be complete, serial testing would improve the serial specificity to some degree. In the worst case scenario of complete dependence between the two tests, the conclusions of the study that in the beginning of May 2020, there was limited spread of SARS-CoV-2 infection across India, remained the same. Fourth, with emerging data about the highly clustered nature of SARS-CoV-2 transmission, our estimates could be biased. By selecting only a single individual per household, we may be underestimating the prevalence as transmission would be expected to be higher within the household. We may also underestimate prevalence if our selection missed clusters with higher prevalence including those among most of the metropolitan cities. Only Chennai and Bengaluru were included in the serosurvey on account of the random selection process.

In conclusion, the findings of the serosurvey indicated a low prevalence of SARS-CoV-2 infection

in the general population in India in early May 2020. As most of the population remains susceptible to infection, our public health strategy needs to plan for an inevitable increase in transmission. Repetition of the population-based serosurvey can better inform changes in the extent and speed of transmission and help evaluate the potential impact of containment strategies over time in different parts of the country. Seroprevalence estimates conducted later in the epidemic, or in the settings with higher prevalence, will provide more robust infection to case and infection to fatality ratios. It is further recommended to establish the district-level facility-based sentinel serosurveillance to systematically monitor the trend of infection in the long term to inform local decision-making at the lowest administrative unit of public health response towards the COVID-19 epidemic in the country.

***Acknowledgment*:** Authors acknowledge the field supervision and support provided by the WHO-India, Ministry of Health and Family Welfare, Government of India, State and District Health officials, and Primary Healthcare staff in planning and conduct of the serosurvey.

***Financial support & sponsorship*:** Financial support provided by the Indian Council of Medical Research, New Delhi, is duly acknowledged.

***Conflicts of Interest*:** None.

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For correspondence: Dr Manoj V. Murhekar, ICMR-National Institute of Epidemiology, Ayapakkam, Chennai 600 077, Tamil Nadu, India
e-mail: mmurhekar@nieicmr.org.in

India COVID-19 Serosurveillance Group

Andhra Pradesh Team: Shanta A., Dheeraj Tumu, U. Venkateswarlu, Bharath J., Seetahal Varma, Vijaya Kumari, G.G.J.N. Lakshmi, Venkata Prasad, K. Naga Bhushanam, K. Ramu, K. Sindhuja, M. Prasad Rao, R.J.K. Hemanth, S.N. Prasad, K. Appalanaidu, V.V. Savitrammadevi, T. Govindarao, Govind, B. Avinash, A. Sandeep, A. Kiran Kumar, G. Vijaya, B. Ramesh, Banka Jagadeesh, G. Appalanaidu, C. Narayanamma, S. Suguna, V.A. Appalacharulu, G. Tamada, Ramesh, K. Syam Sundar Rao, M. Kranthi Kumar, Priyanka, J. Ranikosala, M. Siva Prasad, Prasad, V. Naga Lalitha, P. Siva, Rajkumar, Thanuja, Prasad, V. Karuna Sri, N. Srinivasa Rao, P. Jeevan, Jayalaksmi, Keerthi Battu, Prasad, Ch Vijayalakshmi, Jayalaksmi, Susila, R. Vijayalakshmi, G. Leela Sri, Sudharani, Vamsi, Manjushalini, Shafi, Suvarna, S. Sazeera Banu, Vijaya Sekhar, Vasanthi, Padma Narendra, Seenaiah, S. Sazeera Banu, Vijaya Sekhar, Suresh, Giri, J. Ramesh, D. Ravikumar, M. Rajasekhar, G. Vijay Kumar, A. Sreekanth, B. Vinay, Ch Pallivi, Sarath V., Srihari R. Kalyan, U. Sabarinath

Assam Team: Gautam Borgohain, Mohib Chandra Dekaraja, Mridul Bharati Nath, Ankumoni Saikia, Arup Deka, Bhaskar Das, Nabanita Sharmah, Pratul Sharmah, Ekparana Hazarika, Dhurba Baruah, Madhurjya Changkakoti, Saraswati Kaushik, Syedu Semin Islam, Nazia Mehzabin, Nagen Sarma, Ganesh Deori, Nakul Shyam, Jirjar Phura, Clif Pator, Biren Chandra Tumung, Joydhon Timung, Bhaskarjyoti Bharali, Dondo Gogoi, Montu Shyam, Thaneswar Teron, Jimmy Paul Kerkatta, Bhabajyoti Borah, Dhurbajyoti Pathak, Gokul Brahma, Utpal Sarmah, Pankaj Kumar Das, Lekhraaj Gautam Chetry, Jamanjyoti Sarmah, Anjan Hazarika, Moniruth Zaman, Liladhar Brahma, Raju Brahma, Beauty Boro, Premtush Muru, Anju Rabha Hazarika, Malika Bhuyan, Monika Hazarika, Bikash Bora, Madhuchand Rabha, Tamanna Choudhury, Seema Das, Amal Kalita, Abanti Das, Sanjib Saikia, Jyotish Kalita, Sahjahan Ali, Kishor Kumar Sharma, Chandi Keot, Debojit Deka, Nilima Hembrom, Nandeswar Hazarika, Abani Barman Birwajit, Kumar Bora, Sanjeev Engti Kathal, Bhaiti Engti, Parikhit Gogoi, Lek Chetry, Jyotish kalita, Subroto Bhattarjee, Bhagya Deka, Arup Das, Kalpana Devi, Kasturi Dutta, Fredrick Daimari, Netra Kamal, Chakrabarty, Rranaki Puma, Pranjal Bora, Bipul Mech, Pranab Kumar Sarmah, Nebedita Bharali, Sanjib Kumar Rajguru, Rutheshvardhan Burru, Agastin Kerketa, Amir Sohail Khan, Amlan Jyoti Bora, Bibek Saikia, Bidya Pegu, Boidujya Rai Gogoi, Chandrasmita Sarmah, Daisy Gogoi Thapa, Gamuk kutum, Gunin Mili, Jitumoni Saikia, Krishna Kadka, Mirnal Ngatey, Moon Saikia, Munna Sarkar, Pankaj Phukan, Parmanada Upadhyay, Prahlad Das, Pranjal Das, Rimpi Konwar, Rituraj Borgohain, Ritwik Dutta, Ronjali Doley, Santanu Kakoty, Tapan Kalita, Tikendra Gogoi, Tikendrajit Das, Jiban Saikia, Pullab Das, Mahanta Gogoi

Bihar Team: B.P. Subramanya, Gitika Shankar, Dileep Kumar, Anand Gautam, Susheel Gautam, Adarsh Varghese, Kunnal Kuvalekar, Naveen Mandal, Kumar Gautam, Sanjeev Gupta, Ujjwal Prakash, Sahdeo Mandal, Kumar Ayush, Saurav Kumar, Santosh Kumar, Rahul Kumar, Ranjeet Kumar, Paras Kumar, Kumar Mandal, Satish Thakur, Amit Kumar, Amit Lakra, Ashish Kumar, Binod Kumar, Amit Ranjan, Prateek Raushan, Vikash Kumar, Mumtaz Alam, Amrendra Kumar, Baijnath Rai, Alok Kumar, Sudarshan Kumar, Sakaldeep Kumar, Ajit Kumar, Aaditya Panday, Umesh Kumar, Dhirendra Kumar, Abhay Kumar, Sanjeet Kumar, Bhoop Dhakar, Kamlesh Kumar, Alupt Kumar, Vikash Kumar, Kundan Kunal, Vikash Roy, Sushil Kumar, Vivek Kumar

Chhattisgarh Team: Gaurav Parihar, Manish, P. Vijay, Rochak Saxena, Varun, Prashant, Shitij Khapade, Pranit, Anshuman Chaudhury, Archana Nagwanshi, Sunil Kumar Pankaj, Irshad Khan, Rahul Roy, Nand Kumar Sahu, Gulshan Sahu, Kunj Bihari Patel, Devadas Joshi, Nand Kumar Modi, Pekhan Kumar Sahu, Rajesh Kumar Soni, Dev Kumar Sahu, Vivek Jaiswal, Hemant Kumar Bawanthade, Mahesh Gopal Patel, Bhoopendra Kumar, Avadh Ram Baghel, Champuram Ratre, Vokesh Kumar Yadu

Gujarat Team: A.M. Kadri, Harsh N. Bakshi, Pranav G. Patel, R.S. Kashyap, Arthur Mcwan, Anand Santoke, Monark Vyas, Pankaj Nimavat, S.A. Aarya, Chirag Ramesh Chandra Modi, Praveen L. Asari, Pankaj Nimawat, Shabbir Ali Dedhrotiya, Yagvalky Jani, Aniket Rana, Jitendra Patel, Swapnil B. Shah, Hasmukh Vaghsinh Parmar, Arthur Mcwan, Hardik Nakshiwala, Vaidehi Gohil, Jagdish Patel,

H.V. Parmar, Parulben Patel, Jigneshbhai Tadvi, Piyushbhai Parasar, Vinodbhai Valvi, Jagdishbhai Padvi, Hardik Gavit, Krishnaben Joshi, Hasmukhbhai Variya, Chiragbhai Bhil, Dharmendra Rathva, Dhawal Patel, Divyaben Zala, Jigneshbhai Patel, Mayurbhai Vasava, Manmitbhai Solanki, Darshnaben Patel, Chetnaben Chaudhari, Aartiben Rathva, Riyaben Mistry, Nikiben Bhau, Jyotsnaben Bariya, Tejasbhai Patel, Krunalbhai Darji, Kartikbhai Prajapati, Rahulbhai Rohit, Kum Babita Roy, Pareshbhai M. Parmar, Virendrasinh V. Zala, Manojbhai Balabhai Bhagora, Brijeshbhai Rameshbhai Sutariya, Pareshbhai V. Patel, Hemantbhai D. Kalasva, Jigneshbhai B. Patel, Yashvantbhai N. Nayak, Hiteshbhai B. Patel, Pragneshkumar R. Modi, Sonaben Lakhiyabhai Bumbadiya, Rekhaben Kantibhai Parmar, Kokilaben J. Parmar, Nitaben Dharmendrabhai Patel, Varshaben Patel, Nitaben Bharatbhai Prajapati, Shardaben Jayantibhai Vankar, Chetnaben Bharatbhai Bhatiya, Jagrutiben Jitendrasinh Chauhan, Alkaben Maheshbhai Chamar, Divya Chhaganbhai Patel, Ravisinh Chauhan, Nimisha Patel, Yash Lalitbhai Shah, Misha Patel, Parul Pankajbhai Parmar, Harsha Sadat, Puja Patel, Girish Maneklal Shah, Partapsinh Chaturbhai Taviyad, Vasudev Javalabhai Paragi, Raginiben Jivangir Gosai, Krutikaben Anilbhai Rana, Imtiyazbhai Rasulbhai Shaikh, Madhuben Khushalbhai Mahera, Bhavikaben Surendrabhai Patel, Rajendrabhai Babubhai Patel, Prakashakumar Vadilal Patel, Sangitaben Somabhai Patel, Geetaben Motibhai Patel, Hemprabha Gumansinh Baria, Pratapbhai Bhikhabhai Pagi, Bharatbhai Punambhai Rana, Jinalben Harshadbhai Patel, Archanaben Parsing Pandavi, Dilipbhai Jivsinh Baria, IshavarSinh Jasvantsinh Rathod, Sharmishtha Somabhai Patel, Sunitaben Kasiram Solanki

Jammu and Kashmir Team: Tasnim Syed, Haseena Mir, Shazia Khan, Sahila Nabi, Nazia Khaki, Iqra Nisar, Tanzeela Bashir Qazi, Shahroz Nabi, Misbah Ferooz Kawoosa, Iram Sabah, Abdul Aziz Lone, IshtiyaQ Sumji, Afnan Showkat, Mudassira, Arif, Arsalaan, Asif, Sheema, Javaid, Suraiyya, Humaira, Jahangeer, Muhammad Akram, Sameena, Syed Bilal, Feroz Ahmad, Tufail Ahmad, Mushtaq Ahmad, Abdul Rashid, Farooq Ahmad

Jharkhand Team: Rajeev Ranjan Pathak, Amarendra Kumar, Anoob Razak, Valema Deogam, Mrityunjay, Shekhawat Hussain, Pramod Kumar, Sunil Kumar Singh, Tarun Joshlakra, Ashok Kumar, Sobhna Toppo, Sharan, Buka Oroan, Kamlesh Oroan, Suraj Mahto, Ajay Kerketta, Anushil Anand, Viresh Kumar Mishra, Abdul Kalam, Azad Raushan Raj, Aman Gupta, Puja Kachhap, Jyoti Anant, Alok Kumar, Soni Khatun, Mukesh Kumar Agrawal, Pratima Kumari, Vikash Kumar Sinha, Mamta Kachhap, Prakash K., Jayram Mehta, Swagata Lakshmi Tarafdar, Sudhanshu Munda, Nilesh Kumar

Karnataka Team: Kiran K., Sarika Jain, Kumar M.V., H.P. Arundathi Das, Ranganath R., Vivekanand Reddy, Nischit K.R., Hamsaveni G., Swathi S. Aithal, Hemanth Kumar N.K., S. Shantharaju, N. Vijayalakshmi, S. Somashekharayya, Hiremath, Charanraj Rao, Ravi Kumar M.T., Neelkanthayyaswamy I. Hiremath, Srikantha Y.G., Hariprashanth R., Prasanna M.N., Lal Kumar R., Bhuvaneshwari R., Ragapriya R., Dineshkumar B., Praveen B. Pujar, Ullera Ashoka, Sunil A.N., Umar Farooq M. Dalwai, Narasimharaju N., Bheema Zakeer Hussain, Lakshmikanth Sankara, Laxmikant Shrimant Dhanshetti, Santhosh M.S.

Kerala Team: Rakesh P.S., Srinath Ramamurthy, Vinod Kumar V.G., Suja Aloysius, Anitha A.K., Sharath G. Rao, Nikilesh Menon Ravikumar, Arun Raj, Akhila Pradeep, Abhirami M.R., Shilna A., Nikhilamol T., Anumol Raju S., Asitha A.S., Manoj M., Sarath T.S., Sindhya R., Jaicy G., Neethu Sugathan, Sumi K., Peneena Varghese, Banupriya K., Anupranam M.P., Prakash Jaison V., Vishnu Raj B.S., Venoth V.S.

Madhya Pradesh Team: Praveen K. Bharti, Pushpendra Singh, Suyash Shrivastav, Anil Kumar Verma, R.K. Saxena, Shivendra Mishra, Mahavir Khandelwal, Sunita Parmar, Seema Jaiswal, Praveen Jadhiya, Bal Krishan Tiwari, Jitendra Kumar, Priyanka Singore, Santosh Kumar Patkar, Monu Sen, Rekha Prajapati, Lipi Jain, Hemant Singh Thakur, Priyanka Birha, Pratipal Singh, Vikram Bathri, Sanjay, Prahalad Kumar Soni, Ashish Patel, Ashok Solanki, Jyothi Ahirwar, Ashok Kumar Gupta, Geeta Devi, Manjeeta, Shashikant Tiwari, Hemant Pancheshwar, Mahendra Kumar Jain, Ganesh Damor, Ramswaroop Uikey, Akansha Kushram, Vivek Patel, Bhagwansingh Patil, Shashibhushan Dubey, Yogendra Morya, Shashank Kesharwani, Himmat Singh Kewat, Pushpendra Singh Rajput, Surendra Kumar Jhariya, Sandip Sharma, Hari Burman, Amirullah Khan, Shorabh Bhadoriya, Ramesh Prajapati, Sheetal Sariyam

Maharashtra Team: Padmaja Jogewar, Archana Patil, Anupkumar Yadav, Rushikesh Rajkumar Andhalkar, Salil Patil, Shahanara Valwalkar, Swati Salunkhe, Seema Nair, Deepthy Benoy, Asmita Kadhe, Rushikesh Mane, Sandip Bharaswadkar, Sandip Shinde, Rahul Dwiwedi, Pradip N. Murambikar, Sandip Sangale, Sunil D. Pote, Chetan Khade, Dhakane, Nagoji S. Chavan, Dilip Potule, Prakash Nandapurkar, Rahul Rekhawar, Radhakishan Pawar, Ashok Thorat, Sanjay Suryavanshi, Balaji Shinde, Nilkqnth Bhosikar, Vipin Itankar, Amol Gaikwad, Shivshakti Pawar, Dhupal Girigosavi, Sanjay Salunkhe, Satish Ghatage, Abhijit Choudhary, Abhijit Raut, Sujata Joshi, Madhav Thakur, Deepak Mungalikar, Balasaheb Nagargoje, Shankarao Deshmukh, Mujib Sayyad, Hema Ramesh Vishwakarma, Nyutan Rajkumar Wankar, Rahul Bapurao Arke, Pritam Balasaheb Marodkar, Inayatulla Mahamad Husen, Archana Ganpat Gaikwad, Pramod Ananat Jamale, Namrata Hajari, Aditya Ashok Bengle, Jagdeep Pralahd Bansode, Sumeta Nilkanth Dhete, Akshay Ramesh Phulari, Sunil Balkrishna Shirke, Aakansha Chaudhari, Vivek Uttamrao Yengade, Padmakar Gurunath Kendre, Suraj Shivaji Rakhunde, Bhagwan Munjabhav Harkal, Avinash Madhukar Shinde, Amit Pralhadrao Patil, Prathamesh Shivaji Chavan, Ajit Balu Buchude, Anil Vyankat Rathod, Dhiraj Prakash Panpatil, Tejas Hetendrakumar Phule, R.N. Ahire, Sahane A., S. Dange, D.S. Motkar, B.N. Sanap, Vilas Latpate, A.B. Labade, A.N. Pathan, Ranjit Bhor, Dnyandev Sangale, D.B. Aher, Ganesh Gunjal, Pankaj Deshmukh, Chaudhatri J., Shahane, B.S. Darade, J.K. Phate, Pallavi More, Kalpesh Patil, Satish Mahajan, Wasim Haidar Shaikh, Harish Patil, Gajanan Gadri, Pooja Mahajan, Sunil Patil, Nana Borse, Satish Mahajan, Jayant Nehate, Bhagyashri Kambale, Gautam Gayakwad, Samir Somkul, Dipak Pohekar, Sunil Mahajan, Jivandas Meshram, Rajendra

Deshmukh, Baban Zagade, Vitthal Sanap, Amol Patki, Sachin Jadhav, S. Deshpande, R.S. Jadhav, Shivaji Rathod, Santosh Hajare, Pawal Y.J., Ashish Waghamare, Mohan M. Galande, Pund, Sanap, Jadhav Gundprasad, Jyotiba Kale, Nagargoje, Kranti Gholve, L.D. Kulkarni, Priyanka Chavhan, Choudhari, K.B. Kachve, Kalidas Walivadikar, Varsha Kale, Omprakash Navghare, Shamshul Hudda, Jyoti Kale, Atul Kulkarni, Pornima Bhise, S.N. Sayyad, S.D. Bansode, Ritesh Chavan, Amol Patil, Nitin Desai, Patel, Kalyani Wadkar, Manjusa Dhage, Rani Ballal, Jyoshna Patil, V.V. Kulkarni, Sachine Chavan, Ashwini Patil, Mahesh Shinge, Laxman Gejage, Asharani Anuse, Akshay Mali, Ankita Javalekar, Kumare, Kadtan, Bharat Bagal, Shrimati Ramdhane, Sudhir Kandharkar, Deshmane, S.B. Gaikwad, Asohk Waddewar, Sudarshan Admankar, V.R. Methekar

Odisha Team: Nutan Dwibedi, Spandan Bhanjadeo, Sushree Sukanya Samantray, Sagarkanta Pradhan, Sadruddin Khan, Kahnu Charan Sahoo, Satyabrata Rout, Dinabandhu Padhan, Subrat Kumar Nayak, Janaki Biswal, Manas Bhoi, Jeevan Kumar Mohanta, Rojalin Das, Nirupama Sahoo, Ashish Kumar Mohapatra

Punjab/Haryana Team: Alok Kumar, Priyanka Agarwal, Srinivasan Selvamani, Ashrafjit Chahal, Kamal Paul Rakesh Sarpal, Ramesh Kumar, Sudesh Sahota, Harpreet Bains, Suchitra, Gaurav Kumar, Pankaj Sharma, Wilson Masih, Gurmeet Singh, Gurpreet Singh, Karanvir Ghosal, Davinderdeep Kaur, Jyoti, Harwinder Singh, Manpreet Kaur, Pardeep Kaur, Kalpna

Rajasthan Team: Suman Sundar Mohanty, Suresh Yadav, Ramesh Kumar Sangwan, Vikas Dhikav, Ramesh Kumar Huda, Elantamilan D., Mahendra Thakor, Rakesh Vishwakarma, Azmat Khan, Mohd Arif Baig, Anirudh Tiwari, Rajneesh Kumar, Trilok Kumar, Balwant Manda, G.S. Deval, Pooranlal Meena, J.P. Bunkar, Narrottam Sharma, R.S. Bharti, Satish Kumar Mishra, Bheron Singh Jatav, Sharwan Kumar,Sadav Khan,Mohan Meena, Praveen Baghel, Krishan Kumar,Javed Khan, Krishan Kumar, Rana Ram, Raghunath, Manohar Singh, Pardeep Singh Jodha

Tamil Nadu Team: Nivethitha K., Ezhilarasan, Shreejaa Varrier, Aby Robinson, Joe Daniel, Bharani Anbalagan, Banuchandar K., Arvindh, Kameswaran D., Kirankumar, Gowtham Raj M., Vigneshwar, Aravindan, Sudha, Sowmiya, Umeshkrishna, Elango, Dheepalakshmi, Prakash, Arunkumar, Manikanda Prabu, Suresh, Naveen, Saravanan, Raghavan, John Arokyadoss Y., M. Magesh Kumar, M. Karthikesan, P. Kumaravel, Vasudevan, Anbarasan, Ramesh Kumar, A. Gomathy, R. Vijaya Prabha, I. Kalaimani, P. Lortu Stella Mary, D. Ashok Kumar, S.A. Ravindhra, Rakeshkumar Yadav, T. Ravichandran, R. Hari Krishnan, R. Gopinath, C. Prabhakaran, S. Gomathy, N. Santhanakumar, Udhayakumar, Ranjithkumar, Murugesan, Navaneethapandiyan, Rajmohan, Aravindh Babu, Selvendiran, Tamil Mani Devi, Nandhakumar, G. Preethi, Chandrabalu, Akshitha, Satham Hussain, Hari Vignesh, Sentrayan, Suresh, Senbagavalli, Chandrakumar, Ponmalaiselvan, Sundaramoorthy, Thirumalai, Manikumar, Venkatesh

Telangana Team: Pucha Uday Kumar, B. Dinesh Kumar, J.J. Babu, N. Arlappa, I.I. Meshram, G.M. Subba Rao, J.P. Devraj, M.V. Surekha, R. Ananthan, Mammidi Raja Sriswan, M. Mahesh Kumar, B. Santosh Kumar, P. Raghavendra, D. Anwar Basha, Blessy Prabhu Priyanka, D. Teena, G. Sarika, Mane Kumar, B. Raju Naik, Ronald Rose, Adepu Rajesham, Sneha Shukla, K. Jayakrishna, Md. Shahed Ali, Arroju, Purnachandar, K. Rahul, Nagender Babu, K.S. Ravi, B. Deepak Kumar, N. Anjaiah, R. Laxman, N. Hanmanthu, G.V. Raji Reddy, M. Pydiraju, Sai Kumar, Narasimhulu, P. Sreenu, K. Sree Ramakrishna, Chandrababu, Srinivas Reddy, G.L.A. Stephen, Tulja, Raghunatha Babu, Sailaja, C. Sai Babu, P. Sunu, B. Satyanarayana, Bhavani, Aruna, Srinivas, Sheela, Nancharamma, Roja, Venkataramana, Jhansi, Rani, Swaroopa, Vijayalaxmi, Anitha, Tulsi

Uttar Pradesh Central/Himachal Pradesh/Uttarakhand Team: Haribhan Singh, Ravinder Kumar, Rajesh Guleri, Sushil Chander, Satyavrat Vaidya, Raman Sharma, Ashwini Yadav, Vikas Sabharwal, Pankaj Singh, Manu Jain, Manoj Bahukhandi, Ramesh Kunwar, Ashish Gusain, Arjit Kumar, Ravindra Nath, Ashwini Yadav, Dhruv Gopal, R.C. Pandey, Prashant Upadhyay, Shishir Puri, Archana Srivastava, Gautam Ranjan, Vineet Kumar Shukla, R.K. Gautam, Kishan Kumar, Nandan Kumar Mishra, Simran Kaur Bhojwani, Dechen Yangdol, Upendra Singh, Amit Kumar Yadav, Mohit Tiwari, Shivani Yadav, Rahul Kumar, Harshit Kumar, Basudev Singh, Deepak Babu, Sushil Kumar Pal, Mohit Kumar Sharma, Gopal Prasad, P. Vaidivel, Maneesh Kumar, Rahul Gond, Bitesh Kumar, Prabhat Kumar, Hariom Kushwaha, Mohammad Gani Afridi, Nishtha Verma, Rakesh Kumar Sharma, Uday Singh Kushwaha, Veer Vishal, Saurav Yadav, Satya Prakash, Navneet Rajput, Raju Kashyap, Mahaveer Chaudary, Iftikhar Uddin, Sunny Sharma, Santosh Kumar, Kushwah, Akhalesh, Himanshu Parashar, Sapna Yadav

Uttar Pradesh East Team: Rajeev Singh, Kamran Zaman, Ashok Kumar Pandey, Madhu Gairola, Vinay Dange, Ghanshyam Singh, Atul Kumar Singhal, Prakash Agrawal, Satish Chandra Singh, Amit Mohan Prasad, Ramesh Chandra Pandey, Birendra Panchal, Vishal Yadav, Mukesh Kumar Mishra, Sonal Rajput, Jaibardhan Siddharth, Rohit Baghel, Rashmi Yadav, Ayushi Yadav, Punit Kumar, Abhishek Kumar Mishra, Akash Kushwaha, Deepak Kumar, Vinod Kumar, Ranjeet Singh, Vipul Kumar, Vijay Kumar Prasad

Uttar Pradesh West Team: Akhileshwar Sharda, Vijaya, Bharat, Anand, Sunil Dohre, Hari Dutt, Samrat, Dilip Singh, Vijay, Naresh, Vinay, Akash, Deshdeepak Gautam, Brijesh, Swati Singh, Shaurabh Kumar, Narendra Kumar, Sonu Yadav, Rahul Yadav, Manisha, Sheena, Himalaya, Raju, Mevaram, Jagrat, Shah Alam, Rezwan, Preeti, Arvind, Aseem, Sonu, Krishnalata, Pravesh, Himachal, Lalit Kumar, Rifakat Hussain, Ravi Shankar, Renu Choudhury, Natwarlal, Praveen Kumar, Himanshu Rawal, Kailash Chandra, Rajendra Pal, Sattu Sain, Susheel Kumar, Md. Mumtazir, Vinit Chouhan, Surajban, Anil, Sandeep, Sanjay, Pradeep

West Bengal Team: Malay Kumar Saha, Debottam Pal, Falguni Denath, Subrata Biswas, Suman Kanungo, Bipra Bishnu, Amitava Sarkar, Pritam Roy, Arup Chakrabarty, Abhijit Dey, Pallav Bhattacharya, Amlan Datta, Shubhadeep Bhuniya, Aniket Chowdhury, Subhendu Roy,

Sanjiv Jha, Shyamal Saren, Jagannath Sarkar, Puran Sharma, Subarna Goswami, Prakash Mridha, Nitai Mondal, Dilip Biswas, Samudra Sengupta, Somnath Mukherjee, Aatreye Chakraborty, Debashis Roy, Rabiul Islam Gayen, Santanu Nandy

National Centre for Disease Control: Himanshu Chauhan, Tanzin Dikid, Sanket Kulkarni, Aakash Shrivastava

ICMR-NIE Team: T. Karunakaran, Annamma Jose, R. Sivakumar, K. Vasanthi, K. Kalaiyarasi, S. Dhanapriya Vadhani, T. Magesh, E.B. Arun Prasath, R. Pradeepa, Sauvik Dasgupta, Josephine Pradhan, Arya Vinod, Elizabeth Varghese, M.P. Sarath Kumar, Ponnaiah Manickam, Amanda Rozario G.A., Beula Margrate, D. Augustine, D. Sudha Rani, Jasmine Farzana, Keerthana G. Kiruthika, Michaelraj E., Priyanka S., Roopavathi Ongesh, V. Vettrichelvan, D. Chokkalingam, H. Dinesh Kumar. **ICMR-NIV Team:** Anita Aich, Rajlaxmi Jain. **ICMR-NIRT Team:** Anuradha Rajamanickam, N. Pavan Kumar, Himanshu Singh Chandel, Sravanan Munisankar, Gokul Raj Katha Muthu, Harishankar Murugesan, Suganthi Chittibabu, Anbarasu Deenadayalan, D. Madhavan, Y. John Arockiadoss, M. Mahesh Kumar, G. Gnanamoorthy, Muthukumaravel S., Sakthivel A., Vaishnavi S., Esther Nirmala Mary J., Shakila V., Arul Nancy P., Karthikesan, Kumaravel, Kalaichelvi, Silambu Chelvi K., Angayarkanni B., Anbalagan S., Sathyamurthi P., Madheswaran A., Mangaiyarkarasi, Syed Nisar R.K., Inbanathan A., Sangeetha A., Karthika C., Purushothaman K., Tamilarasan V., S. Suresh, A. Yuvaraj, A. Harish. **ICMR-VCRC Team:** Sankari Thirumal, S. Muthukumaravel, S. Vaishnavi, Esther Nirmala Mary, Sakthivel, V. Sakila. **ICMR:** Nivedita Gupta, Priya Katriyal

Supplementary Table. Districts for the serosurvey by strata based on incidence of reported COVID-19 cases

Stratum	District selected in the stratum
Zero	Vizianagaram, Pakur, Beed, Ganjam, Bijapur, Balrampur, Kabeerdham, Gonda, Karbi Anglong, Udalguri, Kullu, Latehar, Chitradurga, Rayagada, Alipurduar
Low	Alipurduar, Parbhani, Nanded, Madhubani, Simdega, Koraput, Purnia, Rajsamand, Bareilly, Jangoan, Begusarai, Jalor Garhwal, Kurukshetra, Kamareddy, Unnao, Mau, Kamrup Metropolitan, Muzaffarpur, Sabar Kantha, Gurdaspur, Bankura, Jhargram
Medium	24 Paraganas South, Pulwama, Tiruvannamalai, Sangli, Ahmad Nagar, Arwal, Thrisur, Gwalior, Auraiya, Jalgaon, Ernakulam, Nalgonda, Ludhiana, Surguja, Palakkad, Medinipur East
High	Coimbatore, Chennai, Buxar, Ujjain, Dausa, Gautam Buddha Nagar, Patiala, Krishna, Sri Potti Sriramulu Nellore, Jalandhar, Saharanpur, Jyotiba Phule Nagar, Narmada, Mahisagar, Bangalore, Gulbarga, Dewas