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

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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. 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. 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 accordingly3,4. 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 individuals5. 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 elsewhere6. 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 method7.

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, respectively8.

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:

\[
\text{Serial sensitivity} = \text{sensitivity of Kavach} \times \text{sensitivity of Euroimmun}
\]

\[
\text{Serial specificity} = \text{specificity of Kavach} \times (1 - \text{specificity of Kavach}) \times \text{specificity of Euroimmun}.
\]

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

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

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 per cent in Scotland to 26.6 per cent in Iran18. These surveys used different types of serologic tests including lateral flow immunoassay using capillary blood (rapid test), ELISA, Luciferase immunoprecipitation system assay, immunochromatography and chemiluminescence18,19.

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 symptomatic, isolating positive cases and tracing high risk contacts to slow transmission and to prevent the overburdening of the health system20.

| Incidence of reported COVID-19 cases (stratum) | Number of individuals tested | Number positives | 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
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

| Socio-demographic characteristics | IgG positive | IgG negative | Crude odds ratio (95% CI) | Adjusted odds ratio (95% CI) |
|----------------------------------|-------------|-------------|--------------------------|---------------------------|
| Age (yr)                         |             |             |                          |                           |
| 18-45                            | (n=157)     | (n=27,768)  |                          |                           |
| 68 (43.3)                        | 13,484 (48.6) | 1.00        |                          |                           |
| 46-60                            | (n=157)     | (n=27,764)  |                          |                           |
| 62 (39.5)                        | 9,463 (34.1) | 1.30 (0.92-1.84) |                       |                           |
| >60                              | (n=157)     | (n=27,774)  |                          |                           |
| 27 (17.2)                        | 4,821 (17.3) | 1.11 (0.71-1.74) |                       |                           |
| Sex                              |             |             |                          |                           |
| Male                             | (n=157)     | (n=27,743)  |                          |                           |
| 91 (58.0)                        | 13,423 (48.3) | 1.47 (1.07-2.02) |                       |                           |
| Female                           | (n=157)     | (n=27,752)  |                          |                           |
| 66 (42.0)                        | 14,324 (51.6) | 1.00        |                          |                           |
| Others                           | (n=157)     | (n=27,774)  |                          |                           |
| Area of residence                |             |             |                          |                           |
| Urban slum                       | (n=157)     | (n=27,843)  |                          |                           |
| 25 (15.9)                        | 2,496 (9.0)  | 1.90 (1.23-2.94) |                       |                           |
| Urban non-slum                   | (n=157)     | (n=27,774)  |                          |                           |
| 23 (14.6)                        | 4,694 (16.9) | 0.93 (0.59-1.46) |                       |                           |
| Rural (village)                  | (n=157)     | (n=27,818)  |                          |                           |
| 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                              | (n=157)     | (n=27,784)  |                          |                           |
| 41 (26.5)                        | 5,185 (18.7) | 1.56 (1.09-2.23) |                       | 1.39 (0.96-2.02)* |
| No                               | (n=157)     | (n=27,766)  |                          |                           |
| 114 (73.5)                       | 22,483 (81.3) | 1.00        |                          |                           |

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

| 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) |
lead to an overall increase in the specificity at the cost of lowering of sensitivity. 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.

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. 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%) and Iran (0.08-0.12%) in the high-stratum districts, where reporting is assumed to be more complete, 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.

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.

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### 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, Pumia, 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, Suruga, Palakkad, Medinipur East |
| High    | Coimbatore, Chennai, Buxar, Ujjain, Dausa, Gautam Buddha Nagar, Patiala, Krishna, Sri Potti Sriramulu Nellore, Ilandhar, Saharanpur, Jyotiba Phule Nagar, Narmada, Mahisagar, Bangalore, Gulbarga, Dewas |