Original Article

Assessing the Burden of COVID-19 among Children Aged 6-14 Years in Karnataka, India: A Cross-sectional Survey

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Abstract

Background: India experienced three coronavirus disease (COVID-19) waves, with the third attributed to the highly contagious Omicron variant. Before the national vaccination rollout for children above 6, understanding severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) immunoglobulin G (IgG) positivity in the pediatric population was essential. This study aims to assess the burden of Covid-19 infection and to estimate the seroprevalence in children aged 6 to 14 years in the state of Karnataka. **Material and Methods:** We surveyed 5,358 children aged 6-14 across Karnataka using 232 health facilities, from June 6 to 14, 2022. We determined the sample size using the PPS (Population Proportional to Size) technique and employed cluster sampling. We tested all participants for SARS-CoV-2 IgG with an enzyme-linked immunosorbent assay (ELISA) kit and SARS-CoV-2 RNA with reverse transcription-polymerase chain reaction (RT-PCR). We sequenced samples with a cycle threshold (CT) value below 25 using whole genomic sequencing (WGS). **Result:** We found an adjusted seroprevalence of IgG at 75.38% statewide, and we found 0.04% of children RT-PCR positive for COVID-19. We determined a case-to-infection ratio of 1:37 and identified the SARS-CoV-2 strains as Omicron, BA.5, and BA.2.10. **Conclusion:** The study showed a high seroprevalence of IgG among children with low active infection. Omicron, BA. 5, and BA. 2.10 variants were detected through WGS.

Keywords: Anti-SARS-CoV-2 IgG, COVID-19 antibody testing, Karnataka, pediatric serosurvey

Introduction

The term "seroprevalence" refers to the proportion of the population having antibodies to an infectious pathogen. The Indian Council of Medical Research (ICMR) has already conducted four rounds of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) serosurveys in selected districts all over the country. In the fourth national serosurvey conducted for children above 6 years, nearly 67.6% of India's population developed antibodies against the infection and the seroprevalence in children aged 6-9 years.[1] To contain the spread of infection, multi-phased lockdowns were imposed globally and schools were kept closed.[2] In Karnataka too, the schools were kept closed for the whole lockdown period and reopened in November 2021 in partial or full capacity. The State recorded 2,23,750 pediatric cases (6 to 14 years of age) till June 14 and a total of 64 deaths in this age group.

The presence of immunoglobulin G (IgG) against SARS-CoV-2 is more accurate to understand the real prevalence of pediatric coronavirus disease (COVID-19) infection, rather than solely real-time polymerase chain reaction (RT-PCR) testing.[3] The

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Government of India (GoI) relied on repeated population-level evidence from serosurveys to understand the infection burden in the general population. Based on the recommendation, two rounds of serosurveys were conducted in Karnataka which excluded the pediatric population.

No previous surveys were conducted on the pediatric age group (6-14 years), especially since most of them had not been vaccinated. By the end of March 2022, we had vaccinated 44% of children aged 12-14 with their first dose.[4] Following recommendations from the GoI and State Covid-19 Technical Advisory Committee (TAC), the Department of Health and Family Welfare initiated the serosurvey for this age group.

The aim and objectives of the survey were as follows:

Aim: The study aimed to assess the burden of COVID-19 infection and determine the seroprevalence in children aged 6 to 14 years. The primary objectives included estimating the prevalence of seropositivity (anti-SARS-CoV-2 IgG), assessing the incidence of both symptomatic and asymptomatic COVID-19, determining the rate of reinfections, and identifying the strain of SARS-CoV-2 through whole genome sequencing (WGS).

Methods

The Integrated Disease Surveillance Programme's State Surveillance Unit, within the Department of Health and Family Welfare Services (DHFWS), conducted a pediatric serosurvey across Karnataka. They collected data and samples in all districts from June 6 to 14, 2022.

Ethical approval

The Institutional Ethics Committee (IEC) of the Bangalore Medical College & Research Institute reviewed and approved the study (BMCRI/PS/15/2022-23) on 22.04.2022.

Study setting

Community-based survey - In Karnataka, each of the 30 districts was included as a survey unit. In Bengaluru's city corporation area, all the eight zones (East, West, South, Bommanahalli, Dasarahalli, Mahadevpura, RR Nagara, and Yelahanka) were included as separate survey units. Hence, there were a total of 38 survey units. Two-hundred and thirty-two survey centers were identified involving 146 general hospitals of all the talukas, 30 district hospitals, 56 health facilities/hospitals including referral hospitals in the 8 zones of Bangalore city to collect the blood and throat and nasal swab samples under the supervision of a pediatrician. Cluster sampling was followed at the community level. Children aged between 6 and 14 years were included in the study. Given that the IgG testing kit cannot discern between antibodies resulting from natural infection versus vaccination, the analysis excluded vaccinated children. Additionally, we excluded children who were critically ill, those reluctant to provide samples, or those who did not offer informed consent or assent.

Sample size

Sample size calculation Based on limited data, we assumed that 50% of children aged 6-14 had been infected by the pandemic until February 2022. We set the study at a 95% confidence level with a 5% permissible error. With a design effect of 3, we targeted a sample size of 4,611. After factoring in a 10% non-response error, we adjusted the sample size to 5,072. The desired sample size from each of these 38 units was obtained using the PPS (Population Proportional to Size) sampling technique and a cluster sampling technique was followed for recruitment of the participants at the field level.

Study Duration: The population-based fieldwork was carried out from June 6 to 14, 2022, for conducting interviews at the household level and collection of laboratory samples at the health facilities by mobilizing the participants.

Sample collection and laboratory testing

We adhered to ICMR and GoI protocols for sample collection, cold chain transport, and laboratory analysis. After obtaining informed consent, we collected nasopharyngeal/oropharyngeal swabs (NPS/OPS) and serum samples from children. We diagnosed active infections using RT-PCR tests through the state's ICMR-approved laboratory network. The cycle threshold (CT) value, indicative of the viral load in a sample, is inversely related to the virus amount: a higher CT suggests a lower viral load and vice versa. Following ICMR-India's INSACOG guidelines, we prioritized samples with a CT value of 25 or less for WGS.[5] Positive samples with a CT below 25 underwent WGS at Bangalore Medical College and Research Institute to identify the strain. For IgG antibody testing, we collected 2 ml of venous blood, separated the serum at health facility labs, and transported it in a cold chain to designated District Public Health Laboratories or Microbiology departments where needed. We detected SARS-CoV-2 specific IgG antibodies using the KAVACH KARWA enzyme-linked immunosorbent assay (ELISA) KIT, with 100% specificity and 93% sensitivity.[6]

Data collection

We informed participants and their parents/guardians about the survey's purpose, and the samples required, and used screening questions. We secured written consent from parents, verbal assent from children aged 6-11, and written assent from those over 12. We recorded each participant's demographic details, COVID-19 exposure, past respiratory symptoms, and clinical history. Additional details, including exposure to, confirmed COVID-19 cases and recent symptoms, were logged into a study-specific web application, which was linked to samples using ICMR specimen referral forms. We input RT-PCR results into the ICMR test-data portal and IgG antibody test results directly from the labs into the web app. From a consolidated participant list, we extracted data based on subcategories, age, gender, and location to estimate IgG prevalence and active infections. We communicated test results to relevant healthcare facilities, providing further instructions and recommendations for follow-up.

Statistical analysis

We analyzed data from our web application using STATA software (version 17) by the manufacturer StataCorp LLC.

Results, expressed in proportions, were tabled. IgG prevalence and active infection rates were determined from the respective sampled populations. Adjusted prevalence was calculated using the test's sensitivity, specificity, and the Rogan-Gladen formula from Karnataka's initial serosurvey.[7,8] We calculated the odds ratio for IgG seroprevalence among subcategories. The case-to-infection ratio was derived from RT-PCR confirmed cases in children aged 6-14 as of June 14, 2022 in Karnataka.

Participants were categorized based on RT-PCR results and IgG antibody presence:

- New Infection—RT-PCR positive, IgG negative.
- Reinfection—Positive for both RT-PCR and IgG.
- Old Infection—RT-PCR negative, IgG positive.
- Not Infected—Negative for both RT-PCR and IgG.

Results

Sample: Of the 5,358 participants enrolled across Karnataka, 5,253 (98.04%) had valid IgG and RT-PCR test results. Further, 5,329 (99.45%) had valid IgG test outcomes while 29 (0.55%) were rejected. Similarly, 5,278 (98.5%) had valid RT-PCR test outcomes, 57 (1.0%) samples were rejected, and 23 (0.5%) had inconclusive RT-PCR test outcomes.

IgG prevalence: The ELISA KIT (KAVACH KARWA) for anti-SARS-CoV-2 IgG had 100% specificity and 93% sensitivity. As of June 14, 2022, the study's adjusted seroprevalence for IgG in Karnataka was 75.38% (95% CI: 74.23-76.54). With 3,736 positive results from 5,329 valid IgG tests, the crude IgG prevalence stood at 70.11% [Table 1].

Active infection: Among the valid 5,278 RT-PCR results, two were found to be positive, one symptomatic from Udupi district, and one asymptomatic from Chitradurga district, and hence, the crude active infection rate was 0.04%.

WGS results for RT-PCR positive individuals revealed the following strains of SARS-CoV-2: The symptomatic COVID-19-positive individual (n = 1) was infected with the **BA.5 Omicron variant**, while the asymptomatic COVID-19 positive individual (n = 1) had the **BA.2.10 Omicron variant**.

Stratifications The seroprevalence of IgG antibodies was 77.83% in females, compared to 73.02% in males. Additionally, urban non-slum residents had a higher seroprevalence at 77.96%, in contrast to rural residents at 71.98% [Table 1].

Case-to-Infection ratio (CIR)

The case-to-infection ratio is a statistical measure that represents the ratio of confirmed cases of a particular disease to the estimated total number of infections. It provides insight into the proportion of infections that are detected and reported as cases. Considering the age group of 6-14 years as of June 14, 2022, there were 2,23,750 COVID-19 RT-PCR confirmed cases reported in Karnataka. The Projected Mid-Year Population of 2022 for the 6-to-14-year age group as per the GoI was 1,08,90,285. Thus, the case-to-infection ratio was calculated using reported RT-PCR confirmed cases to the estimated infection-based adjusted seroprevalence of IgG against SARS-CoV2 (75.38%) in the study group. In the age group of 6 to 14 years, it was estimated that there were 37 infected individuals for every RT-PCR confirmed case detected as of June 14, 2022.[7,9]

Incidence of reinfection: An analysis of the burden of infection in the study group showed that despite three waves of Covid-19 in the state, 29.75% remained "uninfected," 0.02% were reinfected, and 0,02% were newly infected [Table 2].

Discussion

Our study was the first to identify the percentage of children between 6 and 14 years who had SARS-CoV-2 antibodies (IgG positive) in Karnataka state and those actively infected as indicated by positive RT-PCR results. A systematic review and meta-analysis study on the COVID-19 showed that acute SARS-CoV-2 infections in children were typically asymptomatic or paucisymptomatic, and life-threatening consequences are uncommon.[10] ICMR conducted three rounds of COVID-19 serosurveys, in May, August, and December in the year 2020, in Chitradurga, Bengaluru Urban, and Davangere districts of

Table 1: Seroprevalence of IgG antibodies against SARS-CoV-2 in Karnataka

Category	Sub-Category	Types	Valid Results for IgG (*n*=5,329)	%-IgG against SARS-CoV-2* (95% CI)	Odds ratio
State	Karnataka	Crude	5329	3736/5329	
		Adjusted		75.38 (74.23-76.54)	
Demography	Age	6-8 years	1654	68.52 (66.28-70.76)	1
		9-11 years	1908	79.07 (77.24-80.89)	1.58 (1.37-1.82)
		12-14 years	1767	77.83 (75.89-79.77)	1.49 (1.29-1.72)
	Gender	Female	2618	77.83 (76.24-79.42)	1.24 (1.10-1.39)
		Male	2711	73.02 (71.35-74.69)	1
	Resident Type	Rural	2045	71.98 (70.04-73.93)	0.8 (0.69-0.92)
		Urban-Non-Slum	1673	77.96 (75.98-79.95)	1.04 (0.89-1.22)
		Urban-Slum	1611	77.02 (74.97-79.08)	1
Participant condition at the time of the survey		Asymptomatic	4635	76.81 (75.60-78.03)	1
		Symptomatic	694	65.85 (62.32-69.38)	0.63 (0.54-0.75)

*Adjusted for sensitivity and specificity of antibody testing kit

Table 2: Categorization of subjects based on the results of RT-PCR and IgG antibodies against SARS-CoV-2

Categories	Result of test	*n*	Percentage
Cat 1 (New Infection)	RTPCR +ve and IgG -ve	1	0.02%
Cat 2 (Reinfection)	RTPCR +ve and IgG +ve	1	0.02%
Cat 3 (Old Infection)	RTPCR -ve and IgG +ve	3688	70.21%
Cat 4 (Not Infected)	RTPCR -ve and IgG -ve	1563	29.75%
Total Valid Result for IgG and RT-PCR		5253	100.00%

Karnataka, which showed a seroprevalence of 21.7%, 30.1%, and 26.3% in these districts, respectively, in the third round. The fourth nationwide serosurvey conducted by ICMR in June-July 2021 in the aforementioned districts estimated that 57.2% (95% CI 55.0% to 59.4%) and 61.6% (95% CI 59.8% to 63.3%) had antibodies against SARS-CoV-2 among children aged 6–9 years and 10–17 years, respectively.[1] In September 2020 and January 2021, the state executed two serosurveys targeting adults 18 years and older. These surveys disclosed IgG seropositivity rates of 16.8% and 15.6%, respectively.[7,9] A cross-sectional study conducted in Paschim Medinipur, West Bengal, during August 2020 showed an average total seropositivity rate of 4.15% among asymptomatic 458 general population and 30 previously RT-PCR positive cases.[11] During the same period in India, a nationwide household survey showed a seroprevalence of 5·4%, in the age group of 10-17 years.[12] In a study conducted in urban wards of Hyderabad in January 2021, the seropositivity among children in the age group of 10-19 was 54.6%.[13] A study conducted in the slums of Bangalore in April 2021 showed overall seropositivity of 20.3%.[14] Whereas a study conducted in the rural district of south India after the second wave showed high seroprevalence (45.8%) of COVID-19 infection among children below 18 years.[15] Similar studies conducted in rural and urban areas of NCR of Delhi, Bhubaneswar, Gorakhpur, and Agartala during March to June 2021, showed 55.7% of seropositivity in 2-17 years children.[16] A study conducted in Kerala showed a seroprevalence of 41.7% among children between 5 and 18 years during August to October 2021.[17] These periodic serosurveys show the increasing prevalence of antibodies against SARS-CoV-2 in the population especially in the pediatric age group. The global serosurvey study from January 2020 to April 2022, highlighted the multi-fold increase in the seropositivity after the second wave and based on seroprevalence, the global estimates of infections were much higher than reported cases captured by surveillance systems.[18]

With nearly one year after the ICMR study and post the third wave of COVID-19 in January-February 2022, it was assumed that the seroprevalence of IgG against SARS-CoV-2 in the community would be high due to increased community transmission. The present study confirmed this assumption with the overall adjusted IgG seroprevalence of 75.38% in the unvaccinated pediatric age group. In a study conducted in Chile, in December 2021 to compare the SARS-COV-2 IgG positivity in vaccinated and non-vaccinated children aged 6-18 years, it was found that those who were not vaccinated had IgG positivity of 26.4% compared to 96.1% among those vaccinated with Pfizer/BioNTech.[19]

It is expected that higher seropositivity develops herd immunity, as reflected in Dharavi, Maharashtra, which did not show a surge in infections in the COVID-19 second wave. To understand herd immunity, a study was conducted to correlate the seroprevalence and the infection rate in the slums, which showed high seropositivity in the slums of Dharavi.[20] The present study also showed higher seropositivity in urban areas, as in previous serosurveys conducted during August 2020 when there was a partial lockdown across Karnataka.[21]

The government of India established INSACOG on December 30, 2020, as a forum under the Ministry of Health and Family Welfare to research and track COVID-19 viral variation and genome sequencing in India. The Government of India introduced the WGS dashboard as a part of special surveillance in the Integrated Health Information Platform (IHIP), where all metadata of the WGS results are uploaded. Karnataka IHIP data recorded 22336 samples referred for WGS testing from July 2021 till June 14, 2022. Out of these, 7,499 Variants of Concern of SARS CoV-2 were detected of which Omicron (BA.2) was the predominant strain (39.51%).

With a constant mutation from the beginning of the pandemic, the prevalent strain during March to June 2021 was seen to be the Delta variant.[22] The BA.4 and BA.5 variants of Omicron were initially identified in South Africa in January and February 2022. Subsequently, these lineages have been identified in various other regions worldwide and have been reported in multiple countries.[23] Notably, these two newer Omicron lineages (BA.4 and BA.5) have quickly supplanted the previously dominant BA.2 variant in many countries.[23] Similar findings were seen in this pediatric serosurvey where the results are in line with the global trend.

Conclusion

Our study highlighted that 75.38% of children between 6 and 14 years showed SARS-CoV-2 seropositivity in Karnataka in June 2022. Meanwhile, a mere 0.04% were actively infected, translating to a case-to-infection ratio of 1:37. Approximately, 25% of the children did not develop the antibodies even after the three waves of COVID-19, making them vulnerable to infection during any surge of cases in the future. In the scenario of the dwindling practice of COVID-19 Appropriate Behaviors (CAB), a robust vaccination drive should be strongly considered. BA.2.10 and BA.5 lineage of SARS-CoV-2 were found during the study which turned out to be a clinically mild infection and did not require hospitalization.

Recommendation

A robust vaccination drive is strongly recommended as almost 25% of the children did not develop the antibodies even after the three COVID-19 waves leaving them vulnerable to infection during any future surge in cases. RT-PCR testing and regular WGS should continue as a part of ongoing surveillance

given the repeated COVID-19 waves as it is imperative for recognizing prevalent and emerging strains of the virus.

Limitation of the study

Even though there was a lot of public awareness raised about COVID-19 due to the pandemic's worldwide character, recall bias in recalling the history of past exposure cannot be completely ruled out.

Relevance of the study

This study reflected the indirect burden of COVID-19 in the community especially in the pediatric age group, who largely remained unvaccinated even after three waves of infection in India. Due to the nationwide lockdowns and schools being closed, the exposure of particularly pediatric age group was limited to their households, friends, and surroundings including the neighbors. It allowed assessing the transmissibility of the virus within the unvaccinated children between 6 and 14 years. The study showed that almost 75% of the children were infected in the three waves of COVID-19 which showed high transmissibility of the infection in the community.

Data sharing

The data is accessible to researchers upon formal request to the Commissioner, Health and Family Welfare Services, Government of Karnataka.

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Conflicts of interest

There are no conflicts of interest.

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