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**Research Article** 

Serosurveillance

#### Serosurveillance of COVID-19 among unvaccinated children aged 10-17 in Sikkim, North-Eastern India: A cross-sectional analysis

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**Introduction:** Serological studies offer valuable insights into the burden of diseases, especially among vulnerable populations. This preliminary serological survey for COVID-19 was conducted among unvaccinated children aged 10-17 years in both urban and rural areas of East Sikkim, India, following the second wave of the pandemic. Conducting the study after the peak of transmission was deemed appropriate for a one-time cross-sectional investigation, to assess the prevalence of COVID-19 in the region.

**Methods:** This cross-sectional study was conducted in 10 randomly selected urban areas and 9 rural areas within Gangtok Municipal Corporation. The survey team consisted of an accredited social health activist (ASHA), an Anganwadi worker, and a laboratory technician. Antibody levels were estimated using the Cobas-e411 equipment and Roche's Elecsys Anti-SARS-CoV-2 immunoassay reagent, which detects both IgM and IgG antibodies in human serum/plasma.

**Results:** A total of 1,104 participants were enrolled in the study. The overall seroprevalence rate was 58.24% (n=643), with urban areas contributing 39.24% (n=433) and rural areas contributing 19% (n=210). Seroprevalence did not significantly differ between genders, with 56.27% (n=269) of males and 59.74% (n=374) of females testing positive for SARS-CoV-2 antibodies. A significant difference was observed between symptomatic and asymptomatic participants, with seroprevalence rates of 71.25% and 57.22%, respectively, indicating higher antibody prevalence in symptomatic individuals. Among the seropositive participants, only 9% were symptomatic, while 91% were asymptomatic. The case-to-infection ratio (CIR) was 1:8, and the case-fatality rate was zero for children aged 10-17 in this region.

**Conclusions:** Although seroprevalence has been increasing nationally, a portion of the population remains seronegative. The case-to-infection ratio suggests that many infections go undetected or unreported. This data highlights that the actual number of COVID-19 infections was significantly higher than the number of confirmed cases reported by testing. Continued monitoring and targeted public health measures are essential to address COVID-19.

Keywords: Anti-SARS-CoV-2, Cross-sectional study, Serological studies, 10-17 years unvaccinated children

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#### Introduction

Our study aims to assess the burden of COVID-19 infection among unvaccinated children aged 10-17 years in Sikkim, providing a crucial understanding of the impact of the pandemic on this vulnerable group. The global coronavirus pandemic of 2019 (COVID-19) was responsible for over 622,389,418 confirmed cases and 6,548,492 deaths worldwide until mid-October 2022 [1]. During this time, when the world was trying to cope with the catastrophic effects of the COVID-19 pandemic, COVID-19 vaccination became a critical tool for controlling and preventing infection [2].

Sikkim, one of the smallest states in Northeast India, reported its first COVID-19 case on May 23, 2020, nearly four months after Kerala reported the first case in India on January 27, 2020 [3],[4]. Sikkim witnessed the first wave of COVID-19, with the highest number of cases (1299) in September 2020(IDSP state data).

The second wave surged in early April 2021, slightly later than in other parts of India, resulting in 7,364 cases(IDSP state data). In Sikkim, there was a 155% increase in new COVID-19 cases and a 49% rise in deaths during the second wave, compared to the first wave [5].

Despite the government's rapid and efficient response to the COVID-19 emergency by establishing molecular diagnostic labs, not all contacts of COVID-19 cases volunteered for testing. This reluctance was due to social stigma, fear of isolation or quarantine, and associated social or economic limitations. Consequently, relying solely on recorded cases could be misleading to see the burden of infection in the population.

We aim to assess the burden of COVID-19 infection among unvaccinated children aged 10-17 years by serological testing of the antibody against the SARS-CoV-2. Serological testing of unvaccinated groups shows the level of community vulnerability to COVID-19. As per the WHO population-based age-stratified sero epidemiological investigation protocol for COVID-19, the timing of the study after the peak of transmission of the epidemic waves is appropriate for one-time cross-sectional investigations [6]. Therefore, in this study, we conducted a serological survey from July to August 2021, following the peak in Sikkim in April 2021.

Antibody levels estimated were using the Electrochemiluminescence model Cobas -e411 equipment and Roche's Elecsys Anti SARSCoV2 Immunoassay reagent for in vitro quantitative detection of antibody (IgM + IgG). The studyalso estimated this group's infection-to-case ratio and the case fatality rate. This community-based serological survey among the 10-17-year age group, who were not vaccinated for COVID-19 in the Gangtok Municipal Corporation (urban and rural areas), provides a crucial preliminary investigation of seroprevalence in Sikkim. The findings of this study will significantly contribute to our understanding of the impact of COVID-19 on unvaccinated children in Sikkim.

## Materials and method

The cross-sectional study, conducted from July 6 to August 4, 2021, in randomly selected ten wards and nine rural areas of Gangtok municipal corporation, was meticulously designed to assess the burden of COVID-19 infection among unvaccinated children aged 10-17 years. The thoroughness of our serological testing of the antibody against the SARS-CoV-2inGangtok municipal corporation (urban and rural)of Sikkim, and the estimation of the infection-to-case ratio in this vulnerable group of the population, ensures the validity and reliability of our findings. The Census of 2021 projected population and a list of wards under Gangtok Municipal Corporation were taken for the assessment[8].

The sample size was planned at 1000 children (600 urban + 400 rural) with 95% confidence intervals (CI) and 5% error. The survey team comprisinga local accredited social health activist (ASHA), an Anganwadi worker, a local auxiliary midwife, a senior technical officer, and a laboratory technician from the Biochemistry department of STNM hospital was formed. Initially, orientation and training were provided to the respective survey teams regarding a random selection of houses, the process of approaching the family, obtaining parent consent, and motivating children (10-17 years) to volunteer. The survey team randomly selected households, and one child aged 10-17 years was chosen from each household to compile a list of those who consented. The volunteers who agreed to participate were then called to a local public place (ICDS centre, primary health subcentre, Panchayat Bhawan, and schools) in batches for sample collection.

According to COVID protocols, children and accompanying parents were grouped in batches in the collection centre. They were briefed about the motive of the survey, immunity against COVID-19, interpretation of positive and negative tests, the situation of having /not having antibodies in the blood, and vulnerability of COVID-19 infection concerning the new delta variant that was detected in Sikkim. Once they were willing to participate, informed consent and a questionnaire were collected.

Blood (2ml) was collected with all aseptic measures and was transported to the Biochemistry department at Sir Thutob Namgyal Memorial Hospital (STNM). Antibody level was estimated using the Electrochemiluminescence model Cobas e411 equipment and Roche's Elecsys Anti SARSCoV2 Immunoassay reagent for in vitro quantitative detection of antibody (IgM + IgG).

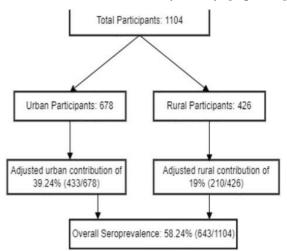
It measures antibodies against SARS-Cov-2 S-spike protein receptor binding domains (RBD) with strong Neutralizing capacity with double antigen sandwich principle. This test is intended as an aid to assess the adaptive immune response to SARS-Cov2 spike protein and has 100% sensitivity and 99.8% specificity, which is approved by the FDA(USA), WHO, CDC, and ICMR- India. [9]

Result interpretation was done as per the manufacturer guidelines, < 0.8 U/ml - Negative for -SARS-CoV-2antibody and  $\geq$  0.8U/ml - Positive for SARS-CoV-2antibody. Reports of the test were conveyed telephonically within three days of the sampling to the volunteer's guardian.

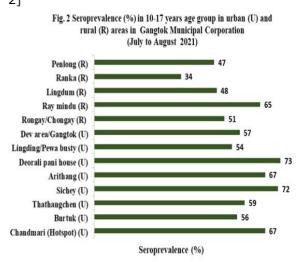
Statistical analysis: Collected demographic and clinical data from participants and entered it into Microsoft Excel. Correlation / P value analysis was done using SPSS version 21 using Chi-square or student T-test as applicable. Case to infection ratio in the surveyed region - estimated taking the cumulative reported cases in the last four months. Case fatality rate calculated with the reported number of deaths in children 10-17 years from last year in the surveyed region.

#### Results

In this study, 1104 unvaccinated children aged 10-17 years participated. Of 1104 participants, 678 (61%) were from urban and 426 (39%) were from rural areas. The total seroprevalence rate was 58.24% (n=643), with an urban contribution of 39.24% (n=433) and a rural contribution of 19% (n=210). [Figure 1]



Amongst the urban areas, seroprevalence observed ranged from 54% - 73.25 %, and in rural areas, seroprevalence varied from 33.8% - 65.3%. [Figure 2]



There was no significant difference in seroprevalence between genders, with 56.27% (n=269) of males and 59.74% (n=374) of females testing positive for antibodies against SARS-Cov-2. Additionally, the distribution was consistent across the age range of 10 to 17 years.

Community: The highest seropositivity was observed among participants from other ethnic groups at 68.18% (n=45), followed by the scheduled caste group at 61% (n=61), the Nepali community at 58.60% (n=286), and the scheduled tribe community at 55.77% (n=251). The differences in seropositivity among these ethnic groups were not statistically significant (P>0.05). [Table 1]

Table 1: Demographic characteristics:	Seroprevalence of	COVID-19	among	unvaccinated	children	aged	10-17
years in Gangtok Municipal Corporation	, Sikkim.						

	Characteristics	Number Tested	Number Positive	Crude Seroprevalence % (CI %)	P value			
1	Total Participants	1104	643	58.24(55.33 - 61.15)				
	Urban	678	433	63.86(60.12 - 67.49)	<0.0001			
	Rural	426	210	49.29(44.45 - 67.49)				
2	Gender							
	Male	478	269	56.27(51.70 - 60.78)				
	Female	626	374	59.74(55.78 - 63.61)				
3	Age in years							
	10-11 Years	232	128	55.17(48.53 - 61.68)				
	12 -13 years	325	189	58.14(52.58 - 63.51)				
	14- 15 years	317	186	58.67(53.04 - 64.15)				
	16 - 17 years	230	140	60.86(54.27 - 67.22)				
Ļ	Community/Ethnicity							
	Nepali	488	286	58.6(54.90 -63.02)				
	Schedule Tribe	450	251	55.77(51.05 - 60.43)				
	Schedule Caste	100	61	61(50.73 -70.60)				
	Others	66	45	68.18(55.56 - 79.11)				
;	House Holds							
	Owned	660	331	50.15(46.27 - 54.03)				
	Rented	437	307	70.25(65.72- 74.50)	<0.0001			
;	Number of rooms in the house							
	1	77	60	77				
	2	314	183	58	<0.001			
	>3	711	400	56.3				
,	COVID symptoms and seropositivity (partici	pants n=1104)						
	Symptomatic	80	57	71.25(60.05-80.82)	<0.014			
	Asymptomatic	1024	586	57.22 (57.22-60.28)				
	Symptoms requiring medical attention		2/57	3.5				
;	COVID-19 Symptoms in seropositive group (	n=643)						
	Symptomatic		57	8.86(6.78-11.33)				
	Asymptomatic		586	91.13(88.67 - 93.22)	<0.0001			
)	H/O contact with COVID							
	Yes	24 (2.17%)	13	54.16(32.82 - 74.45)				
	No	1063 (97%)	619	58.23(55.20 - 61.22)				
	Don't Know	17 (1.73%)	11	64.7(38.33 - 85.79)				
0	H/O COVID Test in last 60 Days							
	Yes	10 (0.9%)	7	77.77(39.99- 97.19)				
	No	1094 (99%)	636	58.08(55.10 - 61.03)				
.2	Total seropositive	1104	643	58.24 (55.27 - 61.17)				
13	Total Seronegative	461/1104		41.75(38.83 - 44.74)				

No correlation was observed between COVID-19 seropositivity and conditions like family type/size and number of toilets in house. Seropositivity rates among children varied significantly based on their living conditions. Children residing in rented houses had a seropositivity rate of 70.25% (n=307), compared to those residing in their own houses with 50.15% (n=331).

Additionally, seropositivity rates were 77% for children living in one-room homes, 58% for two-room homes, and 56.3% for those with three or more rooms. These differences were statistically significant (P < 0.001). COVID-19 symptoms were recorded in only 7.2% (n=80) participants, whereas 92.7% (n=1024) were asymptomatic in the last 60 days of the testing.

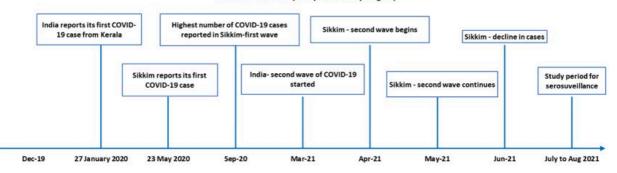
Among those who exhibited symptoms, 71.25% (57 out of 80) tested positive for antibodies, with only two requiring medical attention. In the seropositive group, 91.13% (586/643) of children were asymptomatic, and 8.86% (57/643) were symptomatic (P value <0.00001). Among the seropositive participants (n=643), 96% (n=619) had no known contact with COVID-19 patients, while 2% (n=13) reported contact with COVID-19 patients or relatives. Of those reporting contact, only individuals from urban areas (10 out of 13) underwent laboratory diagnostic (RTPCR) testing. Among these ten individuals, six tested positive, and four tested negative.

The average antibody level was 153.35 U/ml (ranging from 0.83 to  $\geq$  250U/ml). Age-wise distribution of average antibody levels observed was  $\geq$  250 U/ml in 12-15 years old seropositive participants, followed by 16-17 years (246.9 U/ml) and 10-11 years (149 U/ml).The case-to-infection (seropositive) ratio was 1: 8.03 to 8.13, and the case fatality rate was Zero. No death was reported in this age group in the last survey in the region.

#### Discussion

As per the WHO population-based age-stratified sero epidemiological investigation protocol for COVID-19, the timing of the study after the peak of transmission of the epidemic waves is appropriate for one-time cross-sectional investigations. [6] This study was done right after the second wave in the state, so the timing was appropriate for the serosurveillance.[Figure 3] The total seroprevalence rate was 58.24% (n=643), with an urban contribution of 39.24% (n=433) and a rural contribution of 19% (n=210). The seroprevalence is similar in a study conducted among children 10-17 years of age during the same time frame, where antibodies against SARS-CoV-2 tested was 61.6% by the fourth Indian Council of Medical Research (ICMR) survey in 70 districts (June- July 2021)[10]. The first survey conducted in May-June 2020 among adults aged 18 years or older found the infection rate to be 0.73% nationally[11]. In the second round, nearly one in 15 (6.6%) people above the age of 10 was found to be exposed to the virus till August 2020[11].

Figure 3: COVID 19 in Sikkim (2019-2021): Key milestones and trends from the first case to decline, compared to major milestones in India (Study Period: July-August)



The third sero survey, conducted between December 17, 2020, and January 8, 2021, found 21.4% of those aged ten and above infected with the virus[12]. Although seroprevalence has been increasing since the first survey conducted by ICMR, a portion of the population remains seronegative. As in our study, the prevalence observed is 58.24% among participants aged 10-17 years, leaving 42% still susceptible to infection. The highly transmissible Delta variant of COVID-19 was detected in samples sent by Sikkim for genome sequencing in June 2021[13]. This variant has been identified as the primary cause of the second wave of COVID-19 infections in the country, significantly increasing daily case counts and death numbers.

The rapid spread of infections in urban areas, where people live nearby, coupled with the presence of the Delta variant, may have led to the widespread transmission among adults and children, despite children having a lower concentration of ACE2 the entry point for the virus[14]. Earlier serosurveys indicated higher seroprevalence in urban slums and urban non-slum areas than in rural areas [11].

The present study's findings are consistent with those of other studies; the urban areas' seroprevalence is higher (63.86%) than the rural areas (49.29%). This low seroprevalence in the rural areas may be due to effective lockdowns in surrounding rural areas.

These restricted movements in rural setups may have contributed to the slow spread of infection among the children who are primarily home-bound due to the complete closure of schools and entertainment establishments. There is no notable difference in seroprevalence between genders and age groups in the present study, which is consistent with findings from previous studies [12].

Unlike UK, US and Chicago studies (on seropositivity/severity of COVID-19) in adults, no ethnic association of seropositivity was observed in unvaccinated 10-17-year-old children in this study [15]. The correlation of seropositivity in children living in one room (77%), two rooms (58%) and three or more rooms (56.3%) was significant. Overcrowding in fewer rooms with common sharing of toilets and improper ventilation may be contributing factors. This finding is consistent with study conducted in refugee camps in Bangladesh [16].

A higher level of antibody ( $\geq$  250U/ml) was observed in children in the 12-15 years group, implying more robust immunity in pubertal and adolescent age, which makes them less vulnerable to severe COVID-19 infection[17]. A recent study on natural antibodies and their neutralizing capacity in variants of SARS-CoV2 infection is encouraging as this natural antibody was found to neutralize many variants of SARS-CoV2 [17,18].

Limitations of the study include the possibility that the sample may not accurately represent the rural demographics of hilly regions of Sikkim, where 70-80% of the population resides. There may be selection bias, as the ASHA workers, being residents, might have included only familiar and easily accessible individuals in the sample pool. Additionally, this study reports on COVID-19 seroprevalence at a single point, so comparisons and changes in seroprevalence over time could not be assessed.

# Conclusions

This study found no significant differences in seroprevalence between genders and age groups, mirroring previous research. Despite effective lockdown measures, urban areas exhibited higher seroprevalence than rural areas, likely due to higher population density and movement. Children aged 12-15 exhibited higher antibody levels, indicating stronger immunity in pubertal and adolescents. The presence of natural antibodies that neutralize various SARS-CoV-2 variants offers an encouraging outlook for immunity in children. Continued monitoring and targeted public health measures are essential to address the remaining susceptible population and mitigate the spread of infection.

# What does this study add to existing knowledge?

This study contributes to the existing knowledge by providing critical insights into the seroprevalence of COVID-19 among unvaccinated children aged 10-17 years in a relatively isolated setting like Sikkim. A large proportion of seropositive children (91.13%) were asymptomatic, which suggests that many children may have been infected without showing symptoms, thus underlining the importance of serosurveillance in understanding the actual burden of infection.

The study observed stronger antibody levels in the 12-15 age group, suggesting that pubertal and adolescent children may have a more robust immune response, which could offer them greater protection against severe infection. The study did not find significant ethnic differences in seroprevalence, unlike studies from Western countries, suggesting that in the Sikkimese context, ethnic background may not be a significant determinant of COVID-19 exposure or infection in children.

This study is an important contribution to understanding how COVID-19 affected unvaccinated children in a developing country context, especially in regions with unique demographic and geographic challenges. The findings underscore the importance of continuous serosurveillance and public health interventions to protect vulnerable groups such as children in both urban and rural settings.

**Ethical consideration:** Ethical clearance was obtained from the institutional ethics committee of Sir Thutob Namgyal Memorial Hospital, vide order no 02/IEC/STNM/221. Permission was also obtained from the Health and Family Welfare Department, Government of Sikkim, to carry out the study and publish the results.

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