## **Original Research Article**

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# Assessment of Anti-SARS CoV-2 seroprevalence in habitants of Assam (AASSHA): report of the first serosurvey in Assam

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#### **ABSTRACT**

**Background:** Today, there is a pressing need to identify the proportion of people immune to the infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) so that public health policies can be formulated accordingly for the ongoing COVID-19 pandemic. Keeping this in mind, we designed a serosurvey in Assam with aims to estimate the prevalence of infection as well as the infection to case ratio of the novel coronavirus in Assam.

**Methods:** A total of 9 districts belonging to three different strata of districts were randomly selected for the study. In these selected districts, blood samples were collected from a sample of population and were checked for the antibodies (IgG type). Those testing reactive for the mentioned antibodies were considered to have been infected ever before the onset of the study.

**Results:** A total of 2390 study subjects were tested for the presence of antibodies against the SARS-CoV-2. The proportion of people harboring antibodies against the infection was found to be 23.7 percent.

**Conclusions:** The serosurvey revealed that the proportion of people having antibodies was lower than that required for attaining herd immunity levels in a population. The case to infection ratios reveal that there is a large chunk of population who didn't know about their infection.

Keywords: Serosurvey, COVID-19, Assam, SARS-CoV-2

#### **INTRODUCTION**

The world today has almost come to a standstill because of a virus wreaking havoc in the form of a pandemic of COVID-19, which reinstates the might of an invisible enemy as against the most evolved species on earth. As on 28th September 2020, more than 33 million confirmed cases of COVID-19 were reported, including close to 1 million deaths worldwide. As on 29th of September 2020, 6.1 million people in India were affected. Out of this number the contribution of Assam is more than 1.7 lakh.

More than ever before, today, there is a pressing need to identify the proportion of people immune to this infection

so that public health policies can be formulated accordingly for the ongoing COVID 19 pandemic.<sup>4</sup>

Owing to the fact that the virus is a novel one, there have been non-uniform testing strategies, case definition as and disease presentations. This has led to missing out on many cases from reporting due to various factors. In this situation, population based sero-surveys that measure anticoronavirus antibodies provide a relatively reliable method of estimating rates of infection as well as the progression of the disease. This has been acknowledged by the World Health Organization (WHO) and they have suggested different types of studies to find out the seroprevalence for COVID-19 in different populations.

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The various seroprevalence studies available in the public domain are mostly done in developed countries with external grant aids. There is scanty evidence of studies on population-based seroprevalence in Assam.

Therefore, this study is conceived in an attempt at finding the seroprevalence of the infection in the north-eastern state of Assam in India. Also, there is a difference in the prevalence of infection in general population and certain groups of people like health care workers, bank employees, security personnel, etc.<sup>7</sup>

This study also aims to include high risk population so that their results can also be obtained.

## Objectives of the study

Primary objectives were to obtain the rate of infection of SARS-CoV-2 in the people of Assam, to determine the descriptive of the people showing seropositivity for the infection and to determine the infection to case ratio in the study population.

Secondary objectives were to estimate the prevalence of infection among high risk groups of population, and to formulate recommendations based on the observations of the study for control and containment measures.

### **METHODS**

## Type of study

The study conducted was a population based cross sectional study.

## Study area

The area of the study conducted was Assam.

## Study population

The study population consisted of all people 5-65 years old residing in Assam.

## Study duration

The duration of the study was from 23rd August 2020 to 26th September 2020.

## Sample size

According to an update by Indian Council of Medical Research (ICMR) the seroprevalence was found to be around 0.73% in India.8 Assuming the prevalence of infection as 0.73%, 95% confidence interval and an absolute error of 1 %, the required minimum sample size obtained was 279 per stratum. Considering a design effect of 1.5, the sample size came to be 419 per stratum. So, the minimum sample size calculated was 1257.

#### Sampling method

Sampling design

The districts of Assam were ranked according to the cases reported per million population. The population data was obtained from the census 2011 database and the number of reported cases was obtained from the state health portal available at https://covid19.assam.gov.in/. The reported cases from the 6 (six) districts formed after 2011 viz. Majuli, West Karbi Anglong, Charaideo, Bishwanath, Hojai and Mancachar and South Salmara were clubbed with their parent districts for the ranking. The districts were then ranked from 1 to 27 according to the number of cases on 1st August as obtained from the state health portal of Assam (Table 1).

Table 1: The districts were then divided into three strata as given.

Strata	Rank
High case load districts	1 to 9
Medium case load districts	10 to 18
Low case load districts	19 to 27

Then 3 districts from each stratum were selected randomly. So, we had 9 districts selected randomly from the three strata. In the next stage, sample collection camps were organized, mostly at the district headquarters, as per the convenience of the district administration of the selected districts and every possible effort was made to obtain a representative sample of population in the camps held for sample collection. Along with the subjects according to the above mentioned method, we also made an effort to include a few high risk groups as recommended by the ICMR, viz. healthcare workers, security personnel, police and paramilitary forces, press corps, etc.

## Study procedures

The core team coordinated with the district administration and facilitated the conduct of camps in various districts. The field team visited camps and briefed the participants about the survey objectives and processes involved. After obtaining written informed consent, information on basic demographic details, history of exposure to laboratory-confirmed COVID-19 cases, symptoms suggestive of COVID-19 in the preceding one month and clinical history were recorded. Trained phlebotomists in the field team collected 3-5 ml of venous blood from each consenting participant. The blood was transported to the laboratory in Medicity, Guwahati, maintaining cold chain, where centrifugation of the samples was done and they were tested.

## Laboratory procedure

Serum samples were tested for the presence of IgG antibodies against SARS-CoV-2 using commercial chemiluminescence immunoassay (CLIA) (ADVIA

Centaur SARS-CoV-2 IgG) (ADVIA Centaur XP Immunoassay systems, Siemens Healthcare Diagnostics Inc. Tarrytown, NY, USA). The assay detects the presence of IgG antibodies binding to the whole cell SARS-CoV. The manufacturer reports no cross-reactivity with any other known virus. The manufacturers claim that the sensitivity and specificity of the tests were 99.89% and 100% respectively after 2 weeks of infection.

The testing procedure strictly followed the manufacturer's instructions. 0.5 to 20 index was reported. For each plate, samples with optical density (OD) of more than 1 were considered reactive or positive for antibodies. The ones with OD value below 1 were considered non-reactive/negative. A positive infection was defined as a subject whose serum sample was found to be positive in the CLIA. It is assumed that seropositive status indicates prior infection. For quality control, ADVIA Centaur COV2G QC was used every day before running the test samples as recommended by the manufacturer.

#### Inclusion criteria

All consenting people between 5 and 65 years of age were included in the study.

#### Exclusion criteria

People who do not consent to participate in the study, people <5 years or >65 years, bedridden or seriously ill patients and pregnant women were excluded from the study.

## Statistical analysis

The data was entered in Microsoft Excel spread sheets and analyzed using statistical software statistical package for the social sciences (SPSS ver. 16). The frequency of characteristics of the survey participants is described. The reported occupations were classified as low risk and high risk depending upon the potential risk of exposure to known or unknown cases of COVID-19 cases.

Individuals who were seropositive were compared with the seronegative people and associated factors were identified. Infection rates were identified based on the assumption that the antibodies start appearing in the serum after 1 week of infection and most of the people infected would have the antibodies by the end of second week. The data used for comparison of the rates of infection is thus taken as the rates during the first week of August. Factors associated with seropositivity of the study subjects were determined using binary logistic regression and odds ratio (OR) was obtained with 95% confidence intervals for each factor.

## Ethical considerations

Ethical clearance was obtained from Institutional Ethics Committee, Assam Downtown University, Guwahati. Written informed consent was obtained from the study participants and only the participants giving consent were included. Interviews took place as per the convenience of the participants to ensure privacy. All data was stored securely under the investigator's responsibility, with a focus on ensuring the participant's confidentiality. The final report is based on aggregate data without any identifying information.

#### **RESULTS**

A total of 2390 subjects participated in the study. The district-wise distribution of study participants can be viewed from Table 2.

The mean age of the study participants was found to be 39.08 years (±11.91 years). There were 70.2% males. Most of the people participating in the study were urban. 29.2% (697) of study population were engaged in a high risk occupation. 11.1% (266) of the study subjects had some or the other comorbidity. Table 3 shows the demographic characteristics of the study participants. 285 (11.9%) of the study subjects reported of symptoms of COVID-19 since the disease was identified in India. 318 (13.3%) subjects had a history of contact with a suspected case of COVID-19. 420 (17.6%) subjects had a history of ever been quarantined for COVID-19. Only 5.5% (132) subjects had a history of travel among the subjects included in the study.

The people in India, in general, have been following various restrictions due to myriad of factors including IEC activities by the government and also legislations that were put in place under the epidemic act. Data regarding COVID appropriate behavior shows that, majority of subjects were following COVID appropriate behavior. Overall 95.5% people wore mask consistently, 66.9% of the study subjects avoided crowded places, 62.8% avoided unnecessary travel, and 79.5% subjects showed no discrimination against people who were infected with COVID 19. Table 4 represents the COVID appropriate behavior by the study participants.

The seroprevalence was found to be 22.4% in high case load districts, 27.6 % in medium case load districts and 23.5% in low case load districts. The pooled prevalence was found to be 23.7%. The seroprevalence in different strata is represented in Table 5.

The seroprevalence was found to be highest among medium case load stratum and lowest in high case load stratum. However, this difference in seroprevalence was not found to be statistically significant.

The only factors found to be statistically significant as associated factors with seropositivity were presence of any symptom of COVID in past 1 month with an OR of 0.39 (CI 0.30-0.52) (p=0.000) and having been quarantined for COVID-19 with OR 0.58 (CI 0.45-0.75) (p=0.000) as obtained by binary logistic regression technique.

Table 2: District-wise participation of the study subjects according to their sex.

Stratum	District	Male	Female	Total
High	Kamrup Metro	524 (74.4)	180 (25.6)	704
	Dibrugarh	179 (66.3)	91 (33.7)	270
	Nagaon, Hojai	301 (71.2)	122 (28.8)	423
Medium	Sonitpur, Bishwanath	69 (45.4)	83 (54.6)	152
	Dhubri, Mancachar South	98 (71.5)	39 (28.5)	137
	Salmara			
	Barpeta	90 (43.5)	117 (56.5)	207
	Sibsagar, Charaideo	173 (85.2)	30 (14.8)	203
Low	Kamrup	110 (74.3)	38 (25.7)	148
	Chirang	134 (91.8)	12 (8.2)	146
Overall		1678 (70.2)	712 (29.8)	2390

Figures in parentheses are row-wise percentages.

Table 3: Characteristics of study participants.

Characteristic	Stratum			Overall (n=2390)	
Characteristic	High (n=1397)	Medium (n=496)	Low (n=497)	Overall (II=2390)	
Age (years)					
5-15	30 (2.2)	3 (0.6)	0	33 (1.4)	
16-25	229 (16.4)	30 (6.1)	28 (5.6)	287 (12)	
26-35	430 (30.8)	97 (19.6)	131 (26.4)	658 (27.5)	
36-45	371 (26.6)	175 (35.3)	145 (29.2)	691 (28.9)	
46-55	206 (14.8)	141 (28.4)	147 (29.6)	494 (20.7)	
56-65	131 (9.4)	50 (10.1)	46 (9.3)	227 (9.5)	
Mean age (SD)	37.99 (12.3)	38.82 (9.5)	43.24 (10.6)	38.97 (11.8)	
Sex					
Male	1004 (71.9)	257 (51.8)	417 (83.9)	1678 (70.2)	
Female	393 (28.1)	239 (48.2)	80 (16.1)	712 (29.8)	
Occupation					
High risk	423 (30.3)	189 (38.1)	85(17.1)	697 (29.2)	
Low risk	974 (69.7)	307 (61.9)	412 (82.9)	1693 (70.8)	
History					
COVID symptoms	186 (65.3)	45 (15.8)	54 (18.9)	285	
Travel	80 (5.7)	33 (6.7)	19 (3.8)	132	
Quarantine	293 (21)	29 (5.8)	98 (19.7)	420	
Co-morbidity	153 (11)	73 (14.7)	40 (8.0)	266	

Figure in parentheses represent percentage within strata or column-wise percentage.

Table 4: COVID appropriate behavior of the study participants.

Desirable behavior	Stratum			
Desirable behavior	High	Medium	Low	Overall
Physical distancing	1249 (89.4)	448 (90.3)	347 (69.8)	2044 (85.5)
Hand-washing	1363 (97.6)	451(90.9)	476 (95.8)	2290 (95.8)
Covering mouth and nose while coughing or sneezing	1208 (86.5)	321 (64.7)	270 (54.3)	1799 (75.3)
Not spitting in public places after eating paan or khaini or gutkha	1292 (92.5)	445 (89.7)	476 (95.8)	2213 (92.6)
Avoiding unnecessary travel	838 (60)	268 (54)	395 (79.5)	1501 (62.8)
Wearing of mask consistently	1365 (97.7)	442 (89.1)	475 (95.6)	2282 (95.5)
Not discriminating against people with COVID-19	1000 (71.6)	426 (85.9)	475 (95.6)	1901 (79.5)
Not engaged in spreading of false information	1347 (96.4)	464 (93.5)	493 (99.2)	2304 (96.4)
Avoiding crowded places	874 (62.6)	304 (61.3)	422 (84.9)	1600 (66.9)

Figures in parentheses are the percentage within the stratum.

Table 5: Seroprevalence of IgG antibodies against SARS-CoV-2 infection in different strata of districts.

Stratum	Number of samples tested	Number of positives	Prevalence (%age)
High	1397	313	22.4
Medium	496	137	27.6
Low	497	117	23.5
Overall	2390	567	23.7

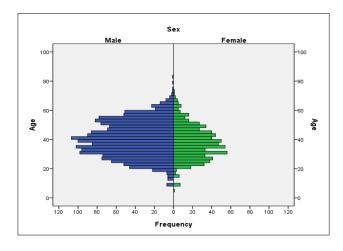


Figure 1: Population pyramid of the study participants.

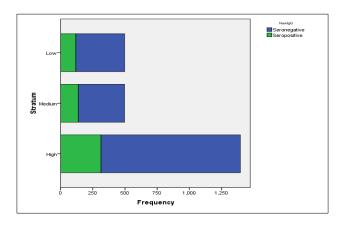


Figure 2: Bar graph representing the seroprevalence in different strata of districts.

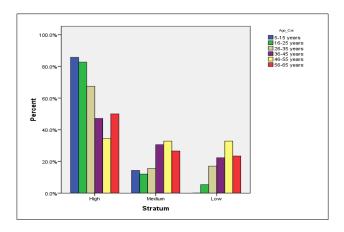


Figure 3: Age distribution of the seropositive subjects.

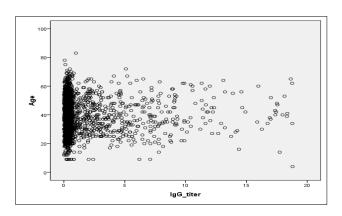


Figure 4: Scatter diagram showing the scatter of IgG titre by age of the study participants.

#### DISCUSSION

The findings of our study suggest that 23.7 percent of people in Assam were exposed to SARS-CoV-2 infection till mid-August. According to 2011 census data, the total population of Assam is 3,11,69,272. This translates to a number of 85,09,211 infections in Assam till the aforementioned period. However the data from the state portal showed that only 76,875 cases were reported till 17th of August.<sup>3</sup> Even as on 1st October 2020 (6 weeks after the estimation time) the total number of cases reported stand at only 1,81,000. This reinstates the fact that a large number of cases are being missed out due to various factors. The seroprevalence ranged from 22.4% to 27.6% across different strata of districts in Assam and this difference in seroprevalence was not found to be statistically significant. The highest seroprevalence was found in the district of Chirang (37.7%) and lowest in Sivasagar (9.4%). However, nothing conclusively can be commented on these figures as our study does not have enough power to do analysis and comment on district-level prevalence of seropositivity. Nevertheless, there can be multitude of factors responsible for this. The testing rates, testing strategies, infection rates as well as representation of general population in our sample collection camps could result in such differences. However, it is worth mentioning that the seroprevalence rates point towards a huge number of infections being missed out which raises an alarm.

The seroprevalence found by ICMR in their study done in May 2020 indicated a seroprevalence of 0.73%. Our study indicates that the seroprevalence has increased and this indicates that the epidemic is gaining momentum in Assam and that we are still far from attaining herd immunity which is optimistically presumed to be attained when

seroprevalence of SARS-CoV-2 antibodies is at least 50%. <sup>10</sup> There are 2 ways to increase the seroprevalence: by vaccination and by natural infection. However the latter method has far reaching consequences and hence in today's scenario when our health system is already exhausted and vaccine availability is also not there, prevention is the best thing that can be done. Therefore, we suggest that the current measures of context specific containment measures including ramping up of the testing facilities as well as isolating cases along with contact tracing and testing are important for slowing down the epidemic.

There has been seen a difference in positivity rates in different strata, although not statistically significant. This could be due to variations in testing rates in different districts. The camp attendees and their characteristics across different strata may be attributable to these differences observed. This emphasizes the need to augment surveillance activities, contact tracing and scale-up testing of suspected cases in all the districts.

The serosurveys give us an estimate of the total burden of disease in a community. From our study findings and the data from the state health portal, we can come to the conclusion that for every case of COVID-19 infection detected till one week before the study commenced, there were 111 undetected cases in the state of Assam. This infection to case ratio of 111:1 is comparable to the national statistic of 82 to 130 as found by ICMR.<sup>6</sup> This infection to case ratio can be attributed to the fact that the testing strategy applied in Assam is mostly concentrated among the symptomatic cases and till August rapid antigen tests (RAT) was not used profoundly. Also, it is worth mentioning that the infections with SARS-CoV-2 do not always result in a symptomatic case.

The number of deaths reported in Assam till 16th August 2020 was 189 and the infection fatality rate (IFR), as estimated from this value and our study, stands at a figure of 0.002%. However, it is pertinent to mention that for accurate calculation of IFR, we need good reporting of deaths including proper cause of death. Added to it, the presentation of the disease, which is mostly asymptomatic, has resulted in underreporting of cases of COVID-19, and hence the IFR is expected to be hugely underestimated. It was found by a study that only 13.7 % deaths were reported and 0.9% of registered deaths reported were medically certified. That is why, not much can be concluded from the IFR.

## **CONCLUSION**

The findings from our study suggest that the seroprevalence of SARS-CoV-2 antibodies in Assam is 23.7% ranging from 9.4% to 37.7% in different districts. There is a large chunk of population who didn't know about their infection. This may be attributed to factors like, asymptomatic and atypical presentations of the infection, variable testing strategy or limitations in the number of

tests done. The seroprevalence data further reiterates that herd immunity is a far reaching goal, at least for the moment in Assam. Until an effective vaccine comes up and a substantial proportion of people are vaccinated, this cannot be considered feasible for us. The context specific containment measures and ramping up of the testing facilities to facilitate early recognition and isolation of cases and tracing and testing their contact remains the only feasible solution in current times. There needs to be more stringent legislation for violators and more thrust to the information, education and communication measures for raising awareness among masses to deal with the behavioral exhaustion seen in the masses.

#### Recommendations

Further studies are recommended for assessing the stage of the epidemic and the seroprevalence in near future so that the trend can be monitored and proper actions taken at appropriate times.

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